Social recognition and telencephalic binding sites of oxytocin in a solitary and a social Otomyine species

By

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Social recognition and telencephalic binding sites of oxytocin in a solitary and a social Otomyine species

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Declaration

I declare that the dissertation, which I hereby submit for the degree Master of Science at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signature: …….

Date: …….

Ethics statement

The author, whose name appears on the title page of this dissertation, has obtained, for the research described in this work, the applicable research ethics approval (University of Pretoria Animal Ethics Committee EC003-13).

The author declares that he/she has observed the ethical standards required in terms of the University of Pretoria's Code of ethics for researchers and the Policy guidelines for responsible research.

Disclaimer

The chapters from this thesis have been prepared to serve as stand-alone manuscripts for publication purposes in addition to forming a single coherent thesis. Therefore, unavoidable overlap and repetition may occur between chapters.

Abstract

This study examined the sociality of two phylogenetically closely related otomyine, murid rodent species that display differences in social behaviour in the wild. A fundamental characteristic of sociality in mammals is the ability to recognise conspecifics and discriminate between familiar and unfamiliar animals. In rodents, olfactory cues serve as the main source of such recognition and has been linked to dopaminergic reward centres in the brain, structures and regions responsible for short and long term memory, as well as neural processes involved in reducing stress. The neuropeptide, oxytocin, is produced by hypothalamic cells and can act as a neurotransmitter. Recent work has linked these neural, telencephalic structures (the nucleus accumbens, amygdala and hippocampus) to social recognition and oxytocin. Oxytocinergic receptor density is greater in such regions in social, monogamous or gregarious rodents, whereas it is much less in solitary species. Experimental studies have found mechanistic links between oxytocin function and social recognition and discrimination in laboratory mice and rats. However, no known study has tested social recognition and discrimination in wild-caught, non-typical model species in conjunction with a description of their oxytocinergic neuroanatomy. This justified my study to investigate whether the social-living, gregarious, colonial ice rat, *Otomys sloggetti robertsi*, show similar oxytocin receptor binding to other social species, and whether it has the ability to recognise conspecifics and discriminate between familiar and unfamiliar animals. Similarly, I investigated and compared these traits in a solitary, phylogenetically closely related species, the vlei rat, *Otomys auratus*.

Neither sexes of both species showed social recognition abilities based on olfactory cues. This was surprising, as social-living ice rats were predicted to display recognition abilities. Interestingly, female vlei rats showed the ability to discriminate between a familiar and novel conspecific. The results suggest that vlei and ice rats exhibit social recognition flexibility, while

social discrimination demonstrated by solitary female vlei rats may provide adaptive advantages in the wild. The impaired social recognition and discrimination observed by ice rats may be explained by their temporal flexibility in social behaviour in the wild. Colonial living and social tolerance by ice rats may indicate phenotypical plasticity, or 'social flexibility', to harsh ecological constraints.

In contrast, the neuroanatomy of vlei and ice rats reflects their wild behaviour. Neural oxytocin receptor binding sites, identified using ligand-binding receptor autoradiography, were more intense in the nucleus accumbens, islands of Calleja, claustrum, indusium griseum, prefrontal cortex, insular cortices, extended amygdala, bed nuclei of the stria terminalis and hypothalamic nuclei of the ice rats, compared to that of the vlei rats. The overall patterns of neural oxytocin receptor (OTR) binding in ice rats are similar to that found in social voles, while that of vlei rats and solitary voles are comparable, particularly the binding intensities observed in the lateral septum. The brains of the vlei rat had OTR binding in the medial habenula and dentate gyrus, which was absent in the ice rat brains. Similarly, OTR binding was only detected in the subfields of hippocampus, intermediodorsal and rhomboid thalamic nuclei in the brain of the ice rats. As predicted from their social behaviour in the wild, the telencephalic OTR binding of the two species reflected their socially disparate behaviour, similar to other studies. Based on the lack of extreme differences in behavioural data, and various similarities in oxytocinergic receptor binding sites in the telencephalic structures, I suggest that a continuum of oxytocinergic effects on social, group-living behaviour of these related species may exist in this otomyine group. The differences in neuropeptidergic circuitry in these two species contributes further to our understanding of evolutionary neuroendocrinology of sociality.

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Chapter 1: General Introduction

Social behaviour

Many different ecological and evolutionary factors influence the behaviour of mammals. By studying neuro-chemical processes, development and endocrinology together with ethology, possible mechanistic links can be made in an attempt to explain the range of behaviour (e.g. aggression, mating, intraspecific interactions and sociality) that are exhibited by animals. Understanding the social behaviour of animals requires an integrative approach where both ultimate and proximate mechanisms are linked to explain observed behavioural phenomena (Blumstein *et al.* 2010).

Social behaviour encompasses the study of same-sex altruistic behaviour, cooperative behaviour and maternal care, persistent bonds between mother and offspring, and long term bonds between mating pairs. The definition of sociality is complex, and many mammals interact socially only during some activities, yet may not have lasting reproductive social structures and persistent social behavioural characteristics. In terms of reproductive behaviour, social structure can be categorised as 'eusocial' (highest level of sociality), 'cooperative breeding', 'social', 'colonial or gregarious', or 'solitary' (lowest level) based on the mating system employed by a species (Sherman *et al.* 1995; Burda *et al.* 2000).

A species can be defined as being truly social, or 'eusocial', when there is a division of labour, a temporal overlap of various ages and life stages within one group, and cooperative parental care (Mitchener, 1969; Burda *et al.* 2000). However, various species display some of these social behavioural traits, but do not necessarily meet all the requirements of eusociality. Still, it is important to define the differences that such species may have from each other, both on a proximate and an ultimate level, in order to better understand social behaviour. As suggested

by Blumstein *et al.* (2010), better model organisms in natural populations should be used to study the proximate factors affecting social behaviour, which can be combined to understand the ultimate factors affecting sociality. By studying, for example, the neuroendocrine processes that regulate and influence social behaviour in two related species, an integrative explanation about the mechanistic links to ecological and evolutionary factors that determine sociality can be obtained (Blumstein *et al.* 2010).

The ecological, evolutionary and physiological characteristics of closely related rodent species, that display differences in sociality, have been studied in species such as mole-rats and voles. Naked mole-rats from eastern Africa live in colonies and display a eusocial system, whereas a closely related species, the Cape mole-rat, maintains a solitary lifestyle and are averse to novel animals of the same species showing marked xenophobia (Bennett and Faulkes, 2000). Similarly, prairie voles are monogamous social animals that form long lasting pair bonds, whereas the closely related montane voles, occurring in montane areas, are solitary (Demas *et al*. 1997).

Social behaviour is not always distinct and species may exhibit a range of social-type behavioural actions such as huddling (Beery and Zucker, 2010), group-living (Beery *et al.* 2008), gregarious behaviour and tolerance towards each other (Willan, 1990), or even social flexibility within a species (Schradin and Pillay, 2005; Schoepf and Schradin, 2012). It is worth studying these ethological mechanisms to assess the degree of sociality. Certain species are eusocial, whereas others can even display spatial and temporal dichotomies in social behaviour in the same population and colony (Hinze *et al.* 2013). This provides further support for the role that environmental and ecological factors have on shaping the social behaviour of mammalian species.

By examining the ecological, environmental, and physiological influences on the social behaviour of non-typical model species (in conjunction with their evolutionary history), we can obtain a better understanding of the biology of social behaviour. The present study attempts to contribute to this line of research by investigating the differences in two linked proximate factors - that is, social recognition ability and neuroendocrinological circuitry - between two phylogenetically closely related murid rodent species inhabiting vastly different environments.

Social recognition

Function in social behaviour

A fundamental requirement of social behaviour is the ability of an animal to be able to recognise and remember different conspecifics. In this context major urinary proteins (MUPs) and pheromones have been implicated in social recognition in rodents which have poor sight and mainly sense other animals by olfactory recognition (Bielsky and Young, 2004). These chemical messengers, from individual odours in the atmosphere, are received by the animal and target chemosensory receptor neurons in the olfactory epithelium and vomeronasal organ; the olfactory bulbs in the forebrain subsequently receive signals from the vomeronasal organ (Bluthe and Dantzer, 1993; Mateo, 2004).

The neural processes of the olfactory bulbs are linked to the hypothalamic regions involved in social affiliative bond formation (Fig 1.1). The afferents of the vomeronasal organ project to the accessory olfactory bulb (AOB) and those of the olfactory epithelium to the main olfactory bulb (MOB), which, in turn, send stimuli to other parts of the brain which influence areas

involved in social behaviour such as the medial amygdala and the lateral septum (LS) (Bielsky and Young, 2004).

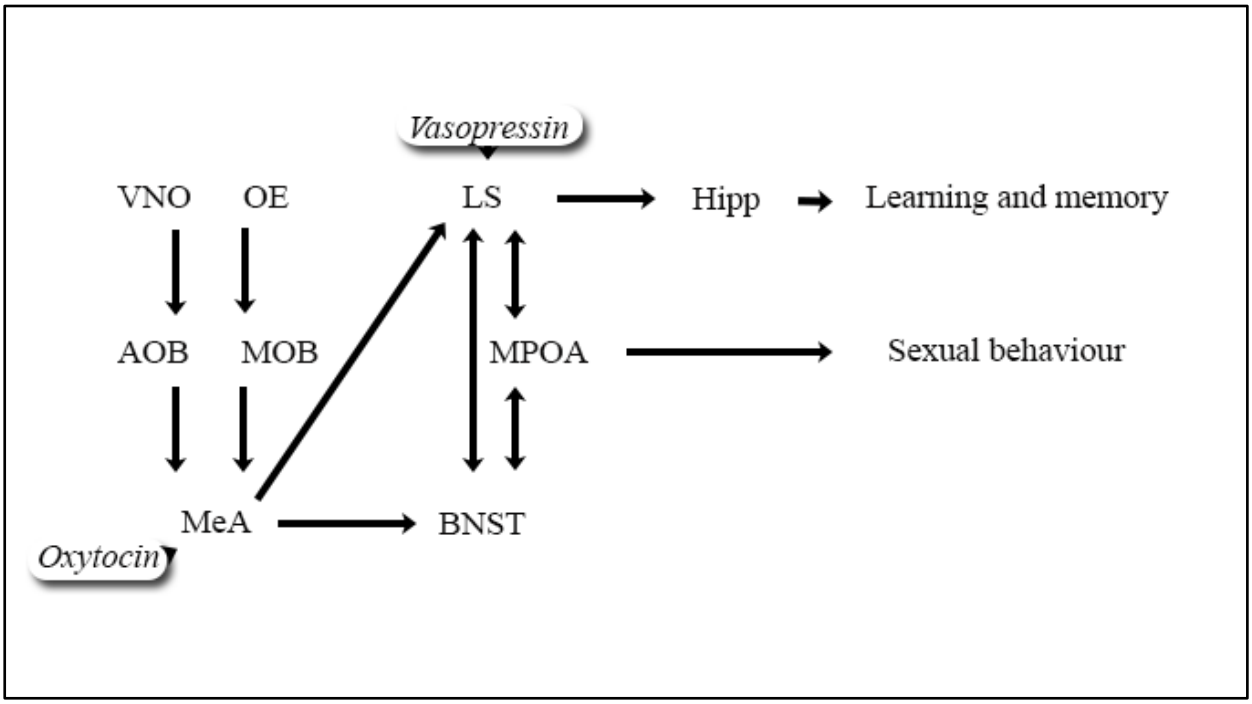

Figure 1.1: Representation of oxytocin- and vasopressinergic neural pathways regulating social recognition and social memory in rats. AOB: Accessory Olfactory Bulb, AVP: Arginine-Vasopressin, BNST: Bed Nucleus of the Stria Terminalis, Hipp: Hippocampus, LS: Lateral Septum, MeA: Medial Amygdala, MOB: Main Olfactory Bulb, MPOA: Medial Preoptic Area, OE: Olfactory Epithelium, OT: Oxytocin, VNO: Vomeronasal Organ. Source: Adapted from Bielsky and Young, 2004, p. 1571 © Elsevier Inc.

The ability to recognise conspecifics disappears where olfactory sensing is inhibited experimentally, either chemically, surgically or genetically, in various species which normally show recognition ability (Bielsky and Young, 2004; Spehr *et al.* 2006; Tobin *et al.* 2010). Inhibition of the olfactory function subsequently prevents the oxytocin binding necessary for social bond formation (Dluzen *et al.* 1998).

In studies where neuropeptide binding sites are inhibited (for example by deletion of the genes coding for oxytocin or vasopressin), experimental subjects lack the ability of social recognition

(Ferguson *et al.* 2000). Although both oxytocin and vasopressin are involved in social behaviour, the emphasis of this thesis is on oxytocin, thus discussions will focus on oxytocin. Oxytocin knock-out mice (where genes that transcribe neuropeptides are removed) lack the ability to recognize conspecifics (Winslow and Insel, 2002; Crawley *et al.* 2007), and chemical inhibition of vasopressin (a neuropeptide homologous to oxytocin) binding attenuates social discrimination abilities in rats (Landgraf *et al.* 1995). However, this is rapidly restored upon administration of intracerebroventricular (icv) oxytocin (Ferguson *et al.* 2000; 2001) and vasopressin (Ferguson *et al.* 2002; Albers, 2012) or the transfer of vasopressin receptors genes (Landgraf *et al.* 2003), respectively. Oxytocin infusion activates receptors in the olfactory bulbs of rats, restoring and intensifying social recognition ability (Dluzen *et al.* 2000) and aggregating behaviour (Mooney *et al.* 2014).

The binding sites for oxytocin are also associated with the main olfactory and accessory olfactory bulb in the brain (Brennan and Kendrick, 2006) (Fig. 1.1). Since the neural processing of olfactory cues is critical to social memory, social behaviour is closely related to olfactory sensing in mammals and olfactory recognition is necessary for social memory of rodents (Ferguson *et al.* 2000). Therefore, social recognition plays a vital role in social affiliation and social behaviour in mammalian species (McEwen, 2004; Van der Kooij and Sandi, 2012).

The Social discrimination paradigm as a test for Social recognition

Social recognition ability, a fundamental requirement of social behaviour, can be investigated in rodents with an elegant experiment that tests the preference to conspecifics under controlled conditions (Kaidanovich-Beilin *et al.* 2011). Traditionally, the habituation-dishabituation paradigm was employed, whereby a decrease in investigative behaviour towards a conspecific

implies the ability to learn the identity of the conspecific, i.e. its social memory. Conversely, there will be a greater investigative duration to a newly introduced, stranger conspecific (Matochik, 1988; Engelmann *et al.* 1995; Ferguson *et al.* 2002; Moy *et al.* 2004; Crawley *et al.* 2007). A lack of a significant decrease in investigation duration to the first conspecific, as exposure events increase, together with no increased investigation duration of the stranger, indicates an impaired ability to recognise the first as a familiar conspecific (Thor and Halloway, 1982; Ferguson *et al.* 2002).

The possibility, however, exists that the animal simply becomes disinterested in the first conspecific, and the interest in a novel animal may influence the statistical viability of the data, and an alternative paradigm to test social recognition has been developed – the social discrimination paradigm (Crawley *et al.* 2007).

In the social discrimination paradigm, testing determines whether the subject animal investigates a stranger conspecific for a longer duration than a control empty compartment, but the effects of novelty or disinterest are eliminated by a second session, where two conspecifics - the first familiar and a novel stranger - are introduced simultaneously (Engelmann *et al.* 1995). This tests the basic ability of the subject to recognise the first animal as a conspecific, as well as tests whether it can discriminate between a newly introduced, unfamiliar conspecific, thereby strengthening statistical power of the results and conclusions that can be made from the data. (Ferguson *et al.* 2002; Crawley *et al.* 2007). Animals with a propensity for social memory, consequently being able to recognise conspecifics, will tend to investigate the novel, unfamiliar stranger for a longer period of time, while the absence of a difference in investigation duration between novel and familiar stimulus animals indicates impaired sociability (Crawley *et al.* 2007). This procedure allows for social recognition to be studied

during a simple two session test, reducing repetition, minimizing confounding factors and can also be used when studying social recognition of sexually mature males and females, since the habituation-dishabituation paradigm can only be used for juvenile males (Ferguson *et al.* 2002).

Social recognition in rodents

The link between neuropeptides and their effect on social recognition ability has been studied in laboratory rats and mice (Bielsky and Young, 2004), but few studies have been conducted on the social recognition abilities in wild populations of non-model species. Recent studies on the neuroendocrinological differences between social and solitary species that are closely related, have revealed fascinating results (Kalamatianos *et al*. 2010). Even intraspecific neuropeptidergic differences have been investigated. For example, African striped mice, show differences in their neuropeptide circuitry in subjects from two different populations inhabiting extreme habitats, and subsequent behavioural differences are also shown to be correlated with these differences in circuitry (Schradin *et al*. 2013).

Although group behaviour such as huddling and pair-bonding have been extensively studied, social recognition – the fundamental 'starting point' of rodent social behaviour – has not been investigated or correlated with neuroendocrinological circuitry involved in social behaviour.

Influence of neuropeptides on social behaviour

Not only have differences in social behaviour been found in populations within the same species inhabiting different habitats (for example striped mice), but further links with the neuroendocrinological aspects of such behaviour have been determined. The neuroendocrine function of social behaviour of African striped mice from an arid region was investigated

(Schradin *et al.* 2013) and a greater number of arginine vasopressin (a similar protein to oxytocin) neurons was found in subjects which showed more 'social' behaviour based on reproductive status. Breeding males and philopatric males had more vasopressin neurons in brain regions associated with social behaviour than did their solitary conspecifics.

A study conducted on eusocial and solitary mole-rats have found marked differences in such neural circuitry, particularly of vasopressin (VP) and oxytocin (OT) neurons (Kalamatianos *et al.* 2010). The study also described the regions which were stimulated by these neuropeptides. Oxytocin receptor density has been shown to be correlated with huddling behaviour of same-sex meadow voles (Beery and Zucker, 2010). Therefore, sexual and non-sexual social behaviour are influenced by neuropeptidergic systems in these species.

It is clear that the differences in social behaviour can be observed between individuals of the same populations with different neural circuitries and/or circulating hormones (Schradin *et al.* 2013; Beery and Zucker, 2010); those of the same species with different neural circuitries from different environments (Schradin *et al.* 2012); as well as those of closely related species with genetically determined differences in neuroendocrinology (Kalamatianos *et al*. 2010). The description of the neuroendocrinological circuitry of a new non-typical model species can contribute to understanding the neural basis of social behaviour. Living in similar habitats, two similar species may retain similar distributions of neuropeptide receptors and neuropeptidergic cells, and display similar social behaviour. It may be postulated, then, that closely related species, sharing very similar morphological and physiological attributes, but exhibiting different social behaviour and occurring in different habitats, will display differences in these neuropeptidergic receptor distributions.

Oxytocin in mammals

In mammals, the main function of oxytocin (OT) is to stimulate uterine wall contraction during birth and lactation (Bielsky and Young, 2004). However, neuropeptides also act as neurotransmitters in the brain. The neuropeptide is produced by magnocellular neurons and parvocellular neurons in the accessory nuclei of the hypothalamus – the supraoptic (SON) and paraventricular (PVN) nuclei and the bed nuclei of the stria terminalis (BNST). These neurons project their axons to various sites throughout the brain. The parvocellular neurons project into the circulatory system of the posterior pituitary gland and secretes OT into the anterior pituitary gland as a hormone or as a neurotransmitter in other regions of the brain (Bielsky and Young, 2004; McEwen, 2004). Here OT plays an important role in the regulation of social behaviour in mammals.

Chemistry of hormones

Each oxytocin molecule is simple in structure, composed of only nine amino acid residues (Fig. 1.2). It has a disulphide bond between two of the amino acid residues, giving the linear molecule a ring-like cyclic peptide characteristic (McEwen, 2004). Oxytocin differs from vasopressin in the amino residues at the third and eighth amino acid position. These differences lead to differences in structural conformation and thereby functionality and chemical specificity of the hormone.

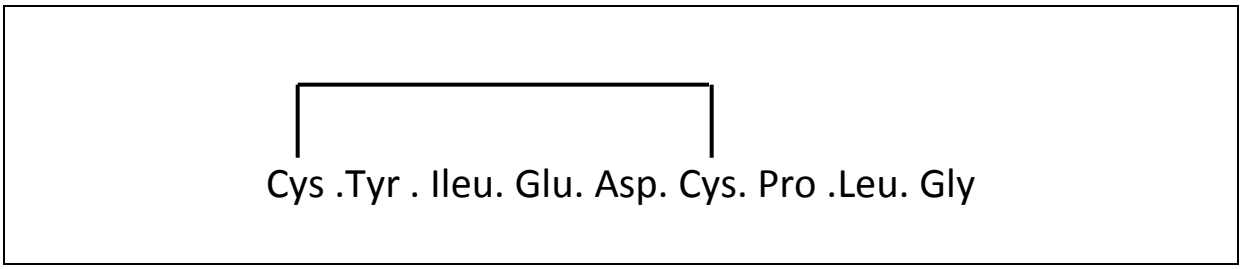

Figure 1.2: Proposed structure of oxytocin, a nonapeptide neurotransmitter. The black line represents a disulphide bond between the two cysteine residues (Adapted from du Vigneaud *et al.* 1953).

Functions of neuropeptides in mediating social behaviour

The OT producing cells in the SON transport the hormones to the posterior pituitary gland (Hatton, 1990). Those in the PVN project to the median eminence (ME), which is an intermediary to the posterior pituitary gland, and OT is released in circulation to reach the uterine wall cells. The parvocellular cells that produce OT in the PVN project to the external zone of the median eminence, where OT is secreted into the pituitary portal system to stimulate the release of anterior pituitary hormones (Swanson *et al*. 1980). The OT-ergic cells in the suprachiasmatic nucleus (SCN) project to the thalamic PVN and several other hypothalamic nuclei. The parvocellular cells that produce OT also project to regions in the brain (e.g. BNST, the ventral hippocampus and amygdala) to act as neurotransmitters and stimulate certain functions in the brain by projecting their terminal fibres into the limbic system of the forebrain (McEwen, 2004).

Oxytocin and vasopressin assert their effect on target cells in the brain by means of ligand-receptor binding. Oxytocin binds to membrane receptors in the brain, and vasopressin binds to membrane V1a receptors in neurons in the brain specifically (Barberis and Tribollet, 1996). Binding in areas associated with reward have been shown to be stimulated by oxytocin. The

nucleus accumbens, prefrontal cortex and ventral pallidum specifically bind these peptides during, or following sex, stimulating the feeling of reward (Young and Wang, 2004).

Eusocial mole-rats have a great number of oxytocinergic cells in the nucleus accumbens (Rosen *et al.* 2008; Valesky *et al.* 2012) and monogamous prairie voles that show preferential social behaviour towards long term partners, have noticeable binding in the nucleus accumbens, suggesting that oxytocin binding in this region stimulates pair-bonding (Demas *et al*. 1997). Parental care is also regulated by oxytocin in prairie voles (Lim and Young, 2006; Olazabal and Young, 2006; Olazabal, 2014). The oxytocin receptors in the medial amygdala and BNST have been shown to be involved in social recognition of conspecifics (Bielsky and Young, 2004), and those for VP in the lateral septum and amygdala, and these regions have afferents that innervate the prefrontal cortex and nucleus accumbens (Landgraf *et al.* 2003).

Receptor binding and stimulation by oxytocin in the brain of mammals is complex and many structures may also reciprocate stimuli. The medial amygdala and lateral septum project to the hippocampus; the medial preoptic area, BNST and olfactory bulbs all contain OT fibres and terminals, all of which are involved in maternal bonding to offspring, as shown for example in maternal bonds in sheep (Levy *et al.* 1995; Ferguson *et al.* 2001; Levy *et al.* 2004; Young and Wang, 2004; Brennan and Kendrick, 2006; Olazabal and Young, 2006b). Extensive research into the neural circuitry of oxytocin and same-sex affiliative behaviour in prairie voles show that OT and VP receptors concentrate in the nucleus accumbens (Beery and Zucker, 2010). It is important to note that the hippocampus, which is involved in memory, receives projections from the lateral septum which itself is stimulated by OT and VP (Kogan *et al.* 2000; McEwen, 2004).

Tests to identify functions

Briefly, immunohistochemistry techniques are used to identify neurons that secrete and are stimulated by OT and VP (Rosen *et al.* 2008). These techniques involve raising antibodies against a specific antigen, labelling these antibodies and then binding these labelled antibodies to the antigen found in the soma, axons and terminal fibres, after which they can be stained to be visualized (Oosthuizen *et al.* 2008). Where brain sections are incubated in radioactively labelled peptides, these can then be exposed to radioactively sensitive film (see methods, Chapter 3) and receptors appear as darker areas under normal photomicrographic measurement (Kalamatianos *et al.* 2010).

Study species

Studying the neuroendocrinological circuitry involved in social behaviour, together with the social recognition ability of non-model mammalian species from wild caught populations, will provide a better understanding of the underlying functions of social behaviour (Blumstein *et al.* 2010). Ideally, these species should exhibit differences in social or prosocial behaviour, be either closely related or from the same species and/or from habitats with different environmental and ecological factors. Two such species from the murid rodents are the subjects of this study – gregarious, colonial ice rats and anti-social, solitary vlei rats.

Otomyine rodents

There are 23 species in the murid rodent sub-family Otomyinae that are endemic to Southern-Africa (Taylor *et al.* 2009). Most of the *Otomys* spp. in southern Africa occur in the moist, eastern and southern parts, whereas *Parotomys* spp. occurs in the dry semi-desert in the southwest (Skinner and Chimimba, 2005). One species, the ice rat, *Otomys sloggetti*, which

has, in some cases, been subdivided into subspecies, occurs exclusively in the afro-montane regions of the Drakensberg while the other *Otomys* spp. are found in mesic, wetland environments (Skinner and Chimimba, 2005).

Morphologically, the vlei rats (*Otomys* spp.), are very similar, varying only slightly in size and outer appearance, and have similar physiological characteristics, especially their thermal regulatory systems (Richter *et al*. 1997). Most otomyine rats exhibit antisocial, solitary lifestyles, except for *Otomys angoniensis* and *Otomys unisulcatus*, which may occur in small familial groups, but are primarily solitary (Skinner and Chimimba, 2005); and *Otomys sloggetti*, which are colonial (Willan, 1990; Hinze *et al*. 2013).

Vlei rats (*Otomys auratus*) have been separated from the more southern occurring vlei rats *(Otomys irroratus)* (Engelbrecht *et al.* 2011; Nengovhela, 2014) and both follow an entirely solitary lifestyle (Davis, 1973). However, ice rats, also known as Sloggett's vlei rat (*Otomys sloggetti* spp.), have been observed to live in subterranean colonies and display gregarious behaviour in communal nest chambers. This behaviour has presumably evolved from ecological drivers during harsh, cold winter conditions and favourable soft soil allowing for underground burrowing (Hinze *et al*. 2013). It has been suggested that the evolutionary factor that drives the altitudinal distribution limits of ice rats, from the other otomyine species in southern Africa, is competitive exclusion by vlei rats (Richter *et al.* 1997), rather than morphological or physiological adaptation to the harsher environment.

Ice rats

Morphology and biology

These red-brown, herbivorous, laminate-toothed, murid rodents, (Schwaibold and Pillay, 2003), weigh between 120 g and 140 g when mature (Hinze, 2005; G. Göldner, pers obs.) and have short tail to body ratios compared to other otomyine species (Skinner and Chimimba, 2005) (Fig 1.3). Although they occur in a temperate climate, ice rats are not physiologically adapted to colder temperatures compared to other temperate zone rodents, since they show elevated metabolic rates and high evaporative water loss traits shared with species occurring in lower, warmer habitats (Richter *et al*. 1997).

They also share similar morphological features with closely related warm climate species in the genus, for example vlei rats (*Otomys auratus)* and Angoni vlei rats (*Otomys angoniensis)*, such as having small fat reserves with dense fur, and do not exhibit the morphology of other hibernating rodents of alpine climates (Rymer *et al.* 2007). It has been suggested that as an adaptation to the cold climate of their habitat, they only give birth to 2-3 offspring and one litter per breeding season (Hinze, 2005). However, four wild caught females in this study were observed to have at least 4 pups, while two had 5 pups (G. Göldner, pers. obs.).

Social behaviour

Ice rats (Sloggett's vlei rat, *Otomys sloggetti robertsi*) are thought to be social, according to their group-living lifestyle (Willan, 1990) (or at least prosocial or colonial/gregarious and possibly communal, based on the definitions by Burda *et al.* (2000)), as multiple reproductive adults have been found in the same burrow system (G. Göldner, pers. obs.). Willan (1990) reported pair bonding in ice rats, which was disputed by Hinze (2005), whom in turn concluded

them to be promiscuous breeders, although a detailed study of their reproductive behaviour has not been conducted to date.

Hibernation does not occur in this species, but instead ice rats are behaviourally adapted to their climate and huddle together in groups in their communal underground burrows and nests (Schwaibold and Pillay, 2006) during cold surface conditions. These colonies consist of several males and females (Hinze 2005) and offspring remain with parents after weaning (Willan, 1990).

In captivity, ice rats show tolerant behaviour towards familiar animals (Willan, 1990; G. Göldner, pers. obs.), but spatial dichotomy in social behaviour has been observed (Hinze *et al.* 2013). During surface foraging, ice rats interact aggressively, yet group together and show gregarious behaviour within burrows and nests.

Location and habitat

Ice rats are endemic to the Drakensberg Mountains of Southern Africa in the sub-alpine phytogeographic belt at elevations above 2000m, and up to 3200m (Skinner and Chimimba, 2005; Hinze *et al.* 2013; this study). Almost all behavioural and ecological studies conducted on ice rats have been from populations and samples in the eastern Drakensberg Mountains of Southern Africa. In this study, animals were sampled from a location in the southern Drakensberg Mountains at an elevation of 2772 – 3200m.

Figure 1.3. Communal/Colonial Ice rat or Sloggett's vlei rat, *Otomys sloggetti robertsi*.

Photo by M. Oosthuizen

Figure 1.4: Ice rat habitat and study site at Tiffindell. Photo by G. Göldner

The ice rat population in this study is located at Tiffindell Ski Resort in the Southern Drakensberg Mountains, Eastern Cape Province, South Africa (30°39'09.3"S 27°55'32.2"E; Fig. 1.4). This site is in the transitional vegetation zone between the Sub-Alpine (1830-2865 m) and Alpine belt (2866-3353m) and the vegetation type is classified as Southern Drakensberg Highland Grassland (Mucina and Rutherford, 2006). The climate is characterized by cold, dry winters with snowfall and frost, and daytime temperatures throughout the year not exceeding cool-temperate conditions (Mucina and Rutherford, 2006). The estimated average annual precipitation ranges between 1000 and 2000mm (Nel and Sumner, 2008), with summer temperatures of 14-26ºC and winter temperatures of 0-18ºC, although cold conditions can occur anytime (Rosen *et al.* 1999). While the main resort is located at elevation of 2720m, ice rats are found from the lower regions (2700m) up to the higher section of the slopes (2820m) (G. Göldner pers. obs).

Colonies are identified by the presence of burrow openings (holes) in the steep sloping sections of the grassland ski slopes and surrounding vegetation and burrow entrances in rocky crags (Hinze *et al*. 2013; this study, see fig. 2.3 chapter 2)

Vlei rats

Morphology and biology

The vlei rats, *Otomys auratus*, while similar, are larger than ice rats, with a greater tail to body length ratio, dark brown fur and also have large eyes and ears and are distinct from Angoni vlei rats by lacking a feint lighter ring around the eye (Davis, 1973) (fig. 1.5). This allows them to be distinguished from sympatrically occurring Angoni vlei rats, in addition to genetic species identification. Vlei rats (formerly *Otomys irroratus)* have recently been reclassified into two separate species, *Otomys irroratus* – found in the south-western parts of southern Africa - and

Otomys auratus – found in the northern, eastern and central parts (Nengovhela, 2014; Engelbrecht *et al.* 2011). The species in this study has been identified as *O. auratus* by independently testing skull morphometrics (Nengovhela, 2014) and genetic analysis and will be further referred to in this manuscript as 'vlei rats'.

Adults of this species range from 102-206 g (Davis, 1973) but the largest male in this study weighed 220g. They are similar to ice rats in having dense fur, large eyes and laminate teeth (Skinner and Chimimba, 2005), and short hair under their upper lip (G.Göldner, pers. obs), as well as small and few fat pads and renal adaptations for warm, mesic climates (Hinze, 2005). Davies (1973) reported vlei rats breed between August and May, coinciding with warmer periods in the southern African climate, but pregnant females were wild caught at the same site in this study during June, extending the breeding season (G. Göldner, pers.obs.).

Social behaviour

Vlei rats are never seen to engage in social behaviour in the wild other than aggression or sexual behaviour (Davis and Meester, 1981). Very little intraspecific interaction occurs in the wild and strong intrasexual aggression is displayed during laboratory tests (Davis, 1973; G. Göldner pers. obs.) with the absence of sexual behaviour, suggesting an antisocial, isolated lifestyle (Davis, 1973). Although pups cling to nipples from birth, mother-infant aggression quickly ensues after pups are weaned as mother-offspring bonds do not form and laboratory animals do not seem to recognise conspecifics in unfamiliar environments (Davis, 1973). Vlei rats display aggressive territoriality and antisocial tendencies, living a solitary life and individuals are highly aggressive towards conspecifics (Davis, 1973).

Location and habitat

Vlei rats are widely distributed across southern Africa and occur in mesic habitats (marshes or wetlands) (Skinner and Chimimba, 2005) in the warmer grassland biome (Fig. 1.6). They live in thick grasses on saturated soil near water in wetland areas and occasionally on floating vegetation (Davis, 1973; G. Göldner pers. obs.). Nests are shallow, bowl shaped, very small, simple and constructed from grass bedding very close to water.

The habitat of vlei rats within the reserves occurs very close to water (Davis, 1973) or predominately on floating vegetation growing in saturated soil of a wetland (G. Göldner pers. obs.). Tunnel-like formations, or 'runs' are made when animals feed on thick 'woven' grasses near water by cutting each grass shoot to get to the higher grass leaves (see chapter 2, fig. 2.1, 2.2).

The study site for collection of specimens from this species was Rietvleidam Nature Reserve and the Bullfrog Pan (Fig. 1.6) in the Highveld Grassland biome of South Africa (Mucina and Rutherford, 2006) and the climate is characterized by warm, wet summers (14-30°C) and dry winters (-2-20^{o}C) with natural fires and frost (Marais, 2004). The Rietvlei Nature Reserve (elevation 1480m) is a wetland area (marsh) with mean annual precipitation of 724 mm and the vegetation type of the specific habitat of vlei rats within the nature reserve is classified as low-lying Grassland Community and Grassland Communities on Andesitic lava (Marais, 2004). The source of the Ses Myl Spruit river, which supplies water to the Rietvlei Dam (Venter *et al.* 2003) is located near Benoni, where the Bullfrog Pan is located (elevation 1642m). The pan is surrounded by wetlands and has a mean annual rainfall of 850mm (Welling, 2009).

Figure 1.5: Solitary Vlei rat, *Otomys auratus.* Photo by M. Oosthuizen

Figure 1.6: Vlei rat habitat in saturated soil and floating vegetation (top) and wetland study site at Bullfrog Pan (bottom).

Aims

The aim of this study was to establish whether the two phylogenetically related, socially behaviourally distinct, otomyine species, namely ice rats (*Otomys sloggetti robertsi)* and vlei rats (*Otomys auratus),* demonstrate possible differences in social recognition abilities required as a fundamental attribute of sociality, and to investigate differences in neuroendocrine circuitry of neuropeptides known to regulate social behaviour.

Various studies have been conducted on the differences in neuropeptidergic distribution in the brains of social mammals and related solitary species, and evidence exists for the role of oxytocin and vasopressin in regulating these differences (Van der Kooij and Sandi, 2012; Young and Wang, 2004). However, few have investigated the link between species-specific neuroendocrinology determined by genes, and behavioural traits necessary for social behaviour to even be possible, i.e. social recognition. While laboratory studies on mice and rats have focussed on providing evidence of neuropeptidergic regulation of social recognition (Gheusi *et al.* 1994; Ferguson *et al.* 2000; Engelmann *et al.* 2011), no studies on wild species from natural populations have been conducted. This study therefore gives a novel description of the differences in neural distribution of the neuropeptide, oxytocin (involved in regulating social behaviour) of two phylogenetically related, but behaviourally socially dissimilar species, as well as investigates if they indeed possess social recognition abilities.

Chapter 2

The aim of this chapter was to determine whether the ice rat and the closely related, vlei rat have the ability to recognise conspecifics under controlled conditions and to compare this ability between the species.

It was predicted that ice rats would show the ability to distinguish between a familiar and an unfamiliar conspecific, when tested in the social discrimination paradigm. However, I anticipated that vlei rats would lack the ability, or have impaired social recognition ability, when tested under the same conditions.

Chapter 3

The aim of this chapter was to identify and compare the species-specific, telencephalic binding sites for oxytocin in the brain of wild caught ice rats and vlei rats. The chapter describes the distribution patterns of oxytocin receptors in the brain of the two species.

It was expected that ice rats would have strong oxytocin receptor binding in the nucleus accumbens, the lateral septum, amygdala and hippocampus.

It was expected that vlei rats would have a low or mild receptor binding in the same regions than the ice rats responsible for social behaviour.

Chapter 4

The results and conclusions from the previous chapters are reviewed under current literature of studies focussing on similar brain regions and the regulation of social behaviour by oxytocin and social recognition.

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Chapter 2: Social recognition and preference for social novelty in solitary and social species of Otomyine rodents

Abstract

The ability to recognise and distinguish a familiar from an unfamiliar animal, is an important part of the formation of social bonds in mammalian species. Olfactory discrimination is necessary for social memory of rodents, a fundamental characteristic of social behaviour. Using the social discrimination paradigm, this study investigated whether social recognition and discrimination exists in an observed social/gregarious murid rodent, the ice rat (*Otomys sloggetti robertsi*), and a closely related, solitary/antisocial rodent, the vlei rat (*Otomys auratus*). Exploratory behaviour was investigated to assess the general activity and level of investigation as a control for interspecific comparisons. Ice rats were generally more active than vlei rats, but displayed greater durations of anxiety behaviour. Both male and female vlei rats, as well as male and female ice rats, failed to discriminate between a stimulus animal and an empty cup, suggesting impaired social recognition abilities. A lack of social recognition and discrimination was predicted for the solitary vlei rats, and the males conformed to this assumption, but female vlei rats showed the ability to discriminate between a familiar and novel conspecific. Ice rats, unexpectedly, lacked social discrimination and recognition abilities. The results from this study suggest that vlei and ice rats exhibit social recognition flexibility. Social discrimination demonstrated by female vlei rats may provide adaptive advantages in the wild. The impaired social recognition and discrimination observed in ice rats may be explained by their temporal flexibility in social behaviour in the wild. Harsh ecological constraints may have allowed ice rats to adopt a gregarious, subterranean social lifestyle, which may not truly be prosocial, rather displaying phenotypical plasticity. Vlei rats, which occur in warm, wet, mesic environments with abundant resources, have adopted a solitary lifestyle, but females retained a social discrimination ability.

Introduction

Social memory is a necessary aspect of complex social behaviour in animals that form intra-specific bonds. The ability to recognise and distinguish a familiar animal from an unfamiliar one is an important part of the formation of social bonds in mammalian species (Thor and Halloway, 1982; Ferguson *et al.* 2002; Mateo, 2004; McEwen, 2004; Brennan and Kendrick, 2006; Albers, 2012). This ability enables social affiliative behaviour such as altruism, pair-bonding, maternal bonding and same-sex lasting relationships to be established (Brennan *et al.* 1990; Bielsky and Young, 2004; Levy *et al.* 2004; Olazabal, 2014) and it also facilitates inbreeding avoidance (Pusey and Wolf, 1996).

Primates and many higher mammals rely primarily on visual and physical cues to recognise individuals (Ferguson *et al.* 2002), however, the main olfactory and accessory olfactory systems have been demonstrated to be directly involved in the recognition of individual rodents such as the spiny mouse (Matochik, 1988), Norway rats (Carr *et al.* 1976) and the house mouse (Ferguson *et al.* 2000; Kogan *et al.* 2000). The vomeronasal system modulates social recognition in rats (Engelmann *et al.* 1995; Brennan and Kendrick, 2006) and the olfactory system has been linked to the neuro-anatomical pathways that are responsible for social affiliative bond formation (Bluthe and Dantzer, 1993; Ferguson *et al.* 2002; McEwen, 2004; Albers, 2012; Van der Kooij and Sandi, 2012). Chemical substances from individual odours in the atmosphere (that an animal receives by olfactory sensing) stimulate the vomeronasal organ to convert these stimuli into a neurochemical message, which in turn is received by the olfactory bulbs (Bluthe and Dantzer, 1993; Mateo, 2004). The neural processes of the olfactory bulbs are linked to the hypothalamic regions involved in social affiliative bond formation, specifically the binding sites for the neuropeptides oxytocin and vasopressin (chapter 3) which facilitate social memory (Ferguson *et al.* 2001). This is shown, for example, where oxytocin

knock-out mice (where genes that transcribe neuropeptides are removed) lack the ability to recognize conspecifics (Winslow and Insel, 2002; Crawley *et al.* 2007), and chemical inhibition of vasopressin binding attenuates social discrimination abilities in rats (Landgraf *et al.* 1995). Studies where olfactory sensing was inhibited experimentally, either chemically, surgically or genetically, have revealed an inability to recognise conspecifics (Bielsky and Young, 2004; Spehr *et al.* 2006; Tobin *et al.* 2010). The neural processing of olfactory cues is critical for social memory, therefore social behaviour is closely related to olfactory sensing in mammals and olfactory discrimination is necessary for social memory of rodents (Ferguson *et al.* 2000; McEwen, 2004; Van der Kooij and Sandi, 2012).

Understanding the social behaviour of animals requires an integrative approach where both ultimate and proximate mechanisms are linked to explain observed behavioural phenomena (Blumstein *et al.* 2010). Various taxa have been studied to try to elucidate the mechanisms of social behaviour and neuro-endocrinological studies have been linked to behavioural experiments of social recognition. Wild-type strains of laboratory rats and mice, homologous to social species with intact recognition abilities, have shown the ability to recognise conspecifics in social recognition experiments (Kaidanovich-Beilin *et al.* 2011). Ethological studies have identified social or gregarious behaviour in many wild social rodent species (Jarvis *et al.* 1994), and antisocial and aggressive behaviour in solitary species have also been described (Davis, 1973). Recent studies into the neural anatomy of social species targeting the distribution and binding of oxytocin and vasopressin in the brain of these mammals has provided important insights into the dichotomy of being either solitary or social (Kalamatianos *et al.* 2010). It is therefore predicted that field captured social species should show similar social recognition abilities to rats in laboratory studies and consequently solitary species would show similar social recognition abilities to knock-out rats as other species of rodents have

shown differences in oxytocin and vasopressin binding and distribution in the brain (Ferguson *et al.* 2002; Kalamatianos *et al.* 2010).

Conducting qualitative studies in the 'total presence or absence' of social behaviour together with quantitative studies in the differences in neuro-anatomical chemistry between closely related species may improve our understanding of the evolution of social behaviour (O'Connell and Hofmann, 2012). Closely related species may display varying social behaviour, such as solitary Cape mole rats (Bennett and Jarvis, 1988; Bennett and Faulkes, 2000) and eusocial naked mole rats (Jarvis, 1981; Faulkes and Bennett, 2001). Living in different environments may influence the divergence in social behaviour of such similar species (Jarvis *et al.* 1994). Even intraspecific differences in social behaviour may occur due to environmental and habitat differences, for e.g. arid living striped mice are more gregarious than those inhabiting mesic environments (Schradin and Pillay, 2004; Schradin *et al.* 2012). Environmental factors, such as rainfall and temperature, food abundance and habitat type may all influence social behaviour of these animals by affecting the onset and duration of their breeding season, territory size, response to ambient temperature to keep cool or warm, choice in and availability of nesting sites and also their population density (Schradin and Pillay, 2005). The neural networks regulating social behaviour are similar in related taxa (O'Connell and Hofmann, 2012), therefore interspecific differences in social behaviour can be studied in more detail in order to reveal the underlying mechanisms.

The difference in social organization of two closely related otomyine rat species provides an opportunity to study social behaviour. Members of the genus *Otomys* are widely distributed throughout South Africa, however, detailed information on the social behaviour of these species is limited (Skinner and Chimimba, 2005) (see Chapter 1).

Overall, the aim of the study was to determine whether the observed 'social'/gregarious murid rodent species (*Otomys sloggetti robertsi)* and the closely related, solitary/antisocial species (*Otomys auratus*) are able to recognise and discriminate between conspecifics. Specifically, the study aimed to 1) assess the general activity related to exploratory behaviour of each social recognition experiment; 2) investigate the social affiliation of the two species by comparing the investigative behaviour between an unfamiliar conspecific and a control object and 3) determine if the two species show the ability to discriminate between familiar and unfamiliar individuals, which is an essential trait for social behaviour in mammals (Thor and Halloway, 1982; Ferguson *et al.* 2002; Mateo, 2004; Brennan and Kendrick, 2006; Albers, 2012). I hypothesize that ice rats would show increased social recognition abilities when compared to vlei rats and investigate the novel, unfamiliar stranger for a longer period of time than the familiar stranger, while vlei rats would show no difference in investigation duration between novel and familiar stimulus animals.

Materials and Methods

Animal collection and location

Adult male and female rats of both species were collected using Sherman live traps (H. B. Sherman Traps, Inc. Tallahassee, Florida, U.S.A.) baited with a mixture of peanut butter and oats, since both species are exclusively vegetarian (Skinner and Chimimba, 2005). Vlei rats were trapped during the austral winter and spring (August to October) at Bullfrog Pan (26°08'20.8"S 28°19'23.1"E) in the Gauteng Province of South Africa. The reserve is located in the Highveld Grassland biome of South Africa (Mucina and Rutherford, 2006) and the climate is characterized by warm, wet summers (14-30°C) and dry winters (-2-20^{o}C) with

natural fires and frost (Marais, 2004). The Bullfrog Pan (elevation 1642m) is surrounded by wetlands and has a mean annual rainfall of 850mm (Welling, 2009).

Since the habitat of vlei rats within the reserves occurs very close to water or predominately on floating vegetation (Fig. 2.1.a; G. Göldner pers. obs.) growing in saturated soil of a wetland, the presence of animals was first determined by locating 'runs' in thick grass, as described in (Davis, 1973). These 'runs' are tunnel-like formations made when animals feed on thick 'woven' grasses near water by cutting each grass shoot to get to the higher grass leaves (Fig. 2.1, 2.2). Traps were opportunistically placed along these grass 'runs' in order to intercept vlei rats directly. Where possible, two traps were placed facing opposite directions inside a 'run' when there was a high presence of activity, as indicated by freshly chewed 5cm grass cuttings and moist faeces (Fig 2.1.b, 2.1.b), in order to further maximize trapping success. Traps were checked daily at dawn and dusk when activity is known to be at a maximum (Davis, 1973), then at intervals of 2 to 3 hours throughout the day and more frequently during very hot, very cold or rainy days.

a)  b) 

Figure 2.1: Habitat of vlei rats showing **a)** floating vegetation and grass on saturated soil in a wetland and **b)** grass 'runs' containing chewed grass cuttings and fresh faeces, which indicate presence of vlei rats.

a)  b) 

Figure 2.2: Location of trap placement in a) thick grassy habitat of vlei rats on saturated soil. Nests (b) occur underneath a thick grassy carpet in wetlands.

Ice rats were trapped during summer (January) at Tiffindell Ski Resort in the Southern Drakensberg Mountains, Eastern Cape Province, South Africa (30°39'09.3"S 27°55'32.2"E). This site is in the transitional vegetation zone between the Sub-Alpine (1830-2865 m) and Alpine belt (2866-3353m) and the vegetation type is classified as Southern Drakensberg Highland Grassland (Mucina and Rutherford, 2006). The climate is characterized by cold, dry winters with snowfall and frost, and daytime temperatures throughout the year not exceeding cool-temperate conditions (Mucina and Rutherford, 2006). The estimated average annual precipitation ranges between 1000 and 2000mm (Nel and Sumner, 2008), with summer temperatures of 14-26^{o}C and winter temperatures of 0-18^{o}C (Rosen *et al.* 1999), although cold conditions can occur anytime. While the main resort is located at an elevation of 2720m, ice rat habitat is found from the lower regions (2700m) up to the higher section of the slopes (2820m) (G. Göldner pers. obs.).

Presence of ice rats was determined by locating burrow openings (holes) in the steep sloping sections of the grassland ski slopes and surrounding vegetation (Fig. 2.3.a) and within burrow

entrances in rocky crags (Fig. 2.3.b) (Hinze *et al*. 2013). Single traps were placed directly in front of burrow entrances and covered with thick dried grass to prevent overheating in harsh sunlight during clear days and to insulate the metal trap during rainy and cold days. Where high activity was observed near or in burrows by the presence of grass cuttings and fresh faeces (similar to vlei rats; see previous section) more traps were set to maximize trapping success at that burrow system. Traps were checked daily from dawn to dusk, when activity is at a maximum (Hinze and Pillay, 2006), and at intervals of 2 to 3 hours throughout the day and more frequently during very hot, very cold or rainy days.

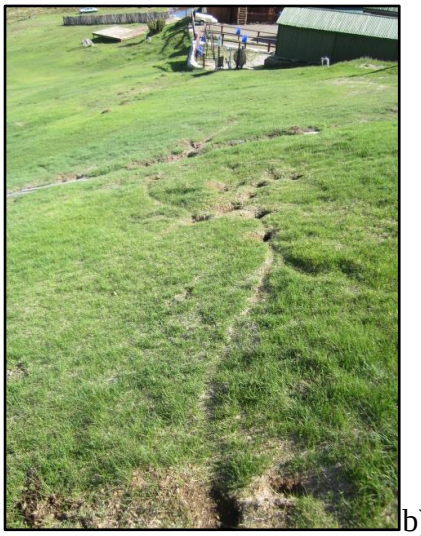
a)  b) 

Figure 2.3: Burrow entrances of ice rats are located a) on steep sloping grasslands floating and b) between steep sloping mountain rocks near grasses. Similar to vlei rat habitat, grass 'runs' containing chewed grass cuttings and fresh faeces, which indicate presence of ice rats.

After collection of animals, vlei rats were individually housed (cage size = 25x40x40cm), preventing olfactory and visual contact, and acclimated to laboratory conditions at 25ºC on a 12 hour light-dark cycle for ten days at the Department of Zoology and Entomology, University

of Pretoria. Vlei rats have been shown to acclimate quickly (Davis 1973) and so do ice rats (Hinze, 2005) and the protocol recommends this minimum period (Kaidanovich-Beilin *et al.* 2011). They were provided with abundant soft sawdust, nesting material, dried grasses, fresh water and fed *ad libitum* on a daily diet comprising fresh lettuce and sweet potatoes, supplemented with mouse pellets, fresh grasses, carrots and apples (Davis 1973). Due to the remote location of trapping, ice rat specimens were housed individually (to prevent olfactory contact prior to experimentation) and acclimated in a quiet room on location, prior to experimentation, with similar conditions to the laboratory and every attempt was made to minimize disturbance. The diet of the ice rats comprised of fresh grasses, supplemented with floral parts of local vegetation, apples and carrots. All experiments were conducted with approval of the University of Pretoria Animal Ethics Committee (EC003-13) and specimens were collected duly authorised (CPF6-0101; CRO157/14CR).

Behavioural tests

To assess the sociability (also known as social recognition and social affiliation (Dantzer *et al.* 1987)) and preference to social novelty (also known as social discrimination (Engelmann *et al.* 2011) and social memory (Ferguson *et al.* 2000)), each animal was subjected to a two-session experiment, as well as an experiment to assess general exploratory behaviour. All experiments were conducted between 6 am and 6 pm when both ice (Hinze, 2005) and vlei rats (Davis, 1973) have been shown to be active (according to the daily time budget analysis) and video footage was analysed with EthoLog 2.2 (Ottoni, 2000; Software published by E.B. Ottoni 1995-1999). During this study, no differences were assumed between behaviour for the experiments conducted at different times of day based on activity budgets recorded previously (Davis, 1973; Hinze, 2005), therefore, the data were analysed collectively and time of day was eliminated as a confounding factor.

Exploratory behaviour

In order to assess the general activity and level of investigation, exploratory behaviour was investigated. This serves as a control for interspecific comparisons to make sure that one species is not generally less active and therefore may appear to show less interest in new animals during the subsequent sociability experiments. Each animal was allowed to freely explore a 29x39x29cm compartment (Figure 2.4) for 10 minutes and behaviour was recorded using a standard video camera. Behavioural actions were defined and then categorised as Exploration, Walking, Grooming, Jumping and Anxious behaviour (Table 2.1) and these categories were compared between the two species as a general indication of overall behaviour which might influence the social experiment.

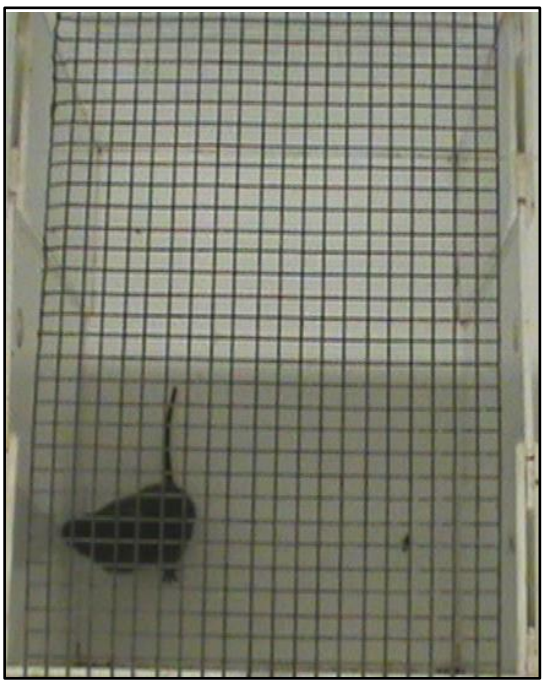


Figure 2.4: Experimental apparatus used in the exploratory control experiment.

Social recognition and discrimination

Preceding the social recognition tests, behavioural actions were defined (Kaidanovich-Beilin *et al.* 2011), whereby different behavioural actions were observed in order to accurately identify these actions during experimentation and distinguish investigative behaviour (Table 2.1). These actions included walking, sniffing, grooming, 'freezing' (no movement longer than 3 seconds), fighting, investigative posture and unusual behaviour such as jumping. Investigative behaviour in this experiment was defined as direct contact sniffing of the wire-mesh containment cup, close following of the control animal inside the cup and stretching of the body with directed sniffing towards the containment cup in an area 3-5 cm around the cup (Table 2.1).

Table 2.1: Ethogram of observed and recorded behavioural actions during social discrimination experiments for this study.

Category	Action	Description
Investigative	Sniffing	Direct contact sniffing of the wire containment cup, close following of the control animal inside the cup and stretching of the body with directed sniffing towards the containment cup in an area 3-5 cm around the cup.
Exploration	Sniffing	Sniffing of maze walls, partition walls, maze floor.
Grooming	Licking	Animal licks fur in a directed movement. Also licks paws and washes head repeatedly.
	Scratching	Fast jerking movement of hind legs with claws moving across fur.
Orientation	Walking	Animal moving one foot in front of the other.
	Rearing	Animal pausing momentarily and lifts forelimbs off the ground.
	Jumping	Animal moving from one position to the next (close in proximity) by quick airborne movements for a brief second.
	Climbing	Animal uses fore and hind limbs to grab hold of walls, wire or edges of the maze supporting itself.
Stationary	Sleeping	Relaxed with eyes closed or head tucked down, neck pulled in towards body, limbs folded and close to body. Whiskers still.
	Resting	Stationary animal, neck not extended, or extended, may be standing or sitting. Not visibly disturbed or alert. Whiskers still.
	Freezing	Animal is stationary for longer than 3 seconds without any other action. Whiskers moving.
Aggression	Aggressive display	Body is stiff and rigid directed towards other animal. Tail is straightened and stiff. Body held forward, nose towards other animal.
	Threat call	Loud, repeated chatter.
	Chasing	Actively moves abruptly and intently behind other animal, biting at it and calling threateningly
	Biting	Reaches at other animal with mouth and teeth, retreating quickly.
	Fighting	Biting at other animal, jumping, aggressive threatening calls, and tail flicking quickly.
Anxiety	Fearful call	High pitched, short squeak.
	Tail-flick	Animal visibly shaking, fur standing upright, tail flicking quickly. Eyes widened.

The social discrimination paradigm was used to ascertain social memory and sociability (Engelmann *et al.* 1995; Crawley *et al.* 2007) using a rectangular maze divided into three similar compartments, large enough to allow unrestricted movement of the test animal. The three equally sized compartments (29x39x29cm) were separated from each other with an opaque division, which can be opened to allow an animal free access (Fig 2.5). The left and right compartments were first isolated with partitions and empty weighted wire-mesh containment cups were placed in the centre of each compartment, after which the test animal (T) was placed in the centre compartment (Mid) and allowed to explore it and habituate for ten minutes (as described in Kaidanovich-Beilin *et al.* 2011). This initial habituation is to allow the animal to acclimate to the conditions of the maze.

During the first session (Session 1: Social recognition), a control animal of the same sex (Control A/First stranger) was placed under the wire-mesh containment cup in one of the outer compartments (systematically alternated between test animals) and the partitions removed (Fig. 2.5), allowing the test animal (T) to move freely and explore all three compartments (Moy *et al.* 2004). The wire-mesh containment cup allowed free air flow between the control animal and the test animal to not restrict olfactory cues, while keeping a physical separation between them, preventing fighting and injury to animals.

The duration and number of direct investigative contacts between the test animal and the control animal, as well as between the test animal and the empty containment cup was recorded for 15 minutes (as initial pilot tests showed animals were slow to begin exploring). In addition, the number and duration of entries into each of the three compartments (all four paws in compartment) was recorded. Finally, the total duration in each compartment was calculated.

After the first session, the partitions were replaced, isolating each compartment and a second control animal (Control B/Novel stranger) of the same sex as the first control animal (Control A/ Same stranger) was placed in the previously empty wire containment cup (Kaidanovich-Beilin *et al.* 2011). The partitions were then removed allowing the test animal to once again move freely during a second session (Session 2: Social discrimination) and all previously mentioned variables were recorded for another period of 15 minutes to compare behaviour of the test animal. The entire procedure was performed on male and female test animals for each species. The social discrimination paradigm was employed in this second experiment due to the more robust results and control parameters as suggested by Engelmann *et al.* (1995).

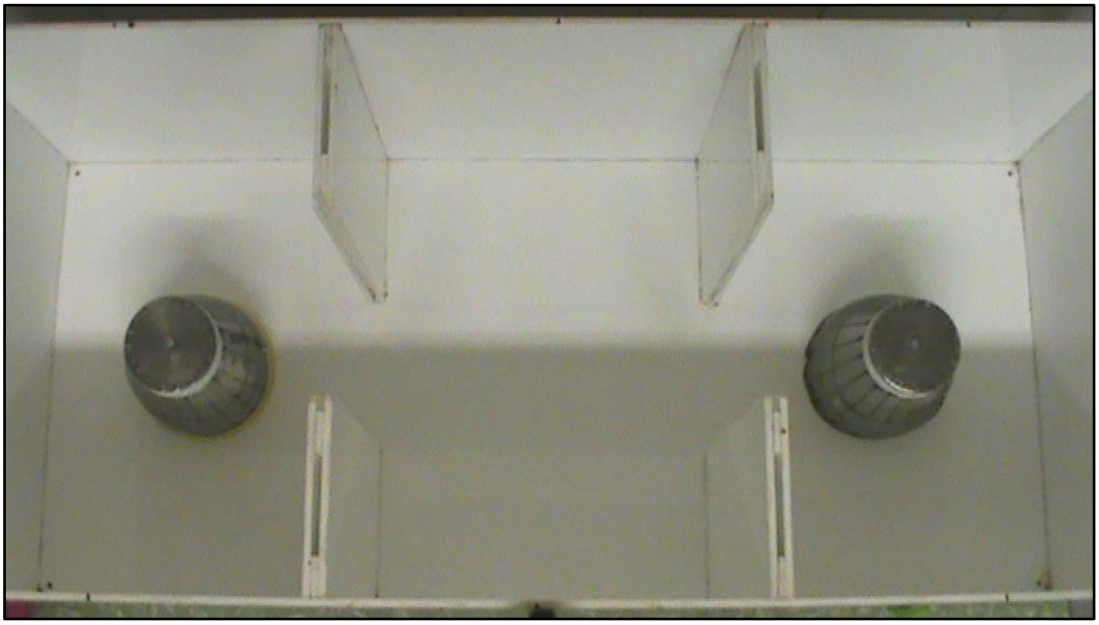


Figure 2.5: Experimental apparatus used in the sociability and preference for social novelty experiment.

Data analysis

Exploratory behaviour was analysed for 15 vlei rats (6 male, 102±23g; 9 female, 117±20g) and 26 ice rats (14 male, 104±36g; 12 female, 106±37g). Data were tested for normality using

Shapiro-Wilk tests and normality plots before being analysed for significant differences using generalized linear models with a gamma distribution between sexes within species and between species. Models with a significant Wald Chi-square statistic were selected; subsequent Wald Chi-square tests compared fixed effects within models for significance. Differences between the behaviour of males of both species and females of both species respectively were compared as well as between males and females within each species.

For the social recognition and discrimination experiment, 16 vlei rats and 39 ice rats were initially tested and the 4 stranger animals were not tested as any data from their social interaction would be compromised from continued exposure to various test animals. Each test animal was given an equal opportunity to investigate and explore all three compartments freely in each session and consequently data from subjects that did not enter the compartment with the control animal in session 1 were regarded as unusable and excluded from the analysis as no comparable data would be available for analysis. Therefore, results are presented for vlei rats (males n=6; females n=9) and ice rats (males n=14; females n=12) that showed suitable activity during both sessions. The variables for each session between both species and sexes were compared for significant differences using a generalized linear mixed effects modelling approach with a three-way comparison of sex, species and compartment with repeated measures on the testing session, and a two-way comparison with sex and compartment, as well as sex and species. Models, with significant *F*- values, and subsequent *t*-test values, were used to determine if fixed effects (sex/compartment/species/session) significantly affected the data. A generalized linear model with a gamma distribution was used to analyse the total duration and the mean time spent in a compartment per entry between sexes within species for a given test session (as data did not meet the assumptions of mixed models). Models with a significant likelihood ratio chi-square statistic were selected and subsequent Wald Chi-square tests used

to compare fixed effects within models for significance ($p<0.05$). All data were analysed in IBM SPSS Statistics Version 23 (IBM Corporation).

Results

General exploratory behaviour of novel environment

The time spent engaged in exploration differed between ice rats and vlei rats ($\chi^2 = 6.423$, df = 1, $p = 0.011$). A significant interaction of species with sex ($\chi^2 = 8.310$, df = 3, $p = 0.040$) indicated no significant differences in exploration between males of the two species ($p = 0.359$), but female vlei rats explored the environment more than female ice rats ($p = 0.007$) (Fig. 2.6). Male vlei rats did not spend significantly less time exploring than female vlei rats ($p = 0.253$) and no sex differences in exploration duration were observed within ice rats ($p = 0.505$).

No difference in the duration of walking around the cage was observed between the two species ($\chi^2 = 0.135$, df = 1, $p = 0.714$), and the time spent walking did not significantly differ within each species with respect to sex ($\chi^2 = 0.257$, df = 3, $p = 0.968$) (Fig. 2.6). There was no difference between species in time walking by males ($p = 0.848$) and females ($p = 0.737$). Male vlei rats did not spend significantly more or less time walking than did female vlei rats ($p = 0.813$) and male ice rats also showed no significant difference in walking duration than female ice rats ($p = 0.860$).

Species differences were observed in grooming behaviour ($\chi^2 = 4.501$, df = 1, $p = 0.034$). Male vlei rats spent significantly more time grooming than male ice rats ($p = 0.019$), but female vlei rats did not groom significantly more than female ice rats ($p = 0.575$). No significant species interaction with sex ($\chi^2 = 6.538$, df = 3, $p = 0.088$) was observed and there were no differences

in grooming duration between male and female vlei rats (p = 0.080) and male and female ice rats (p = 0.959).

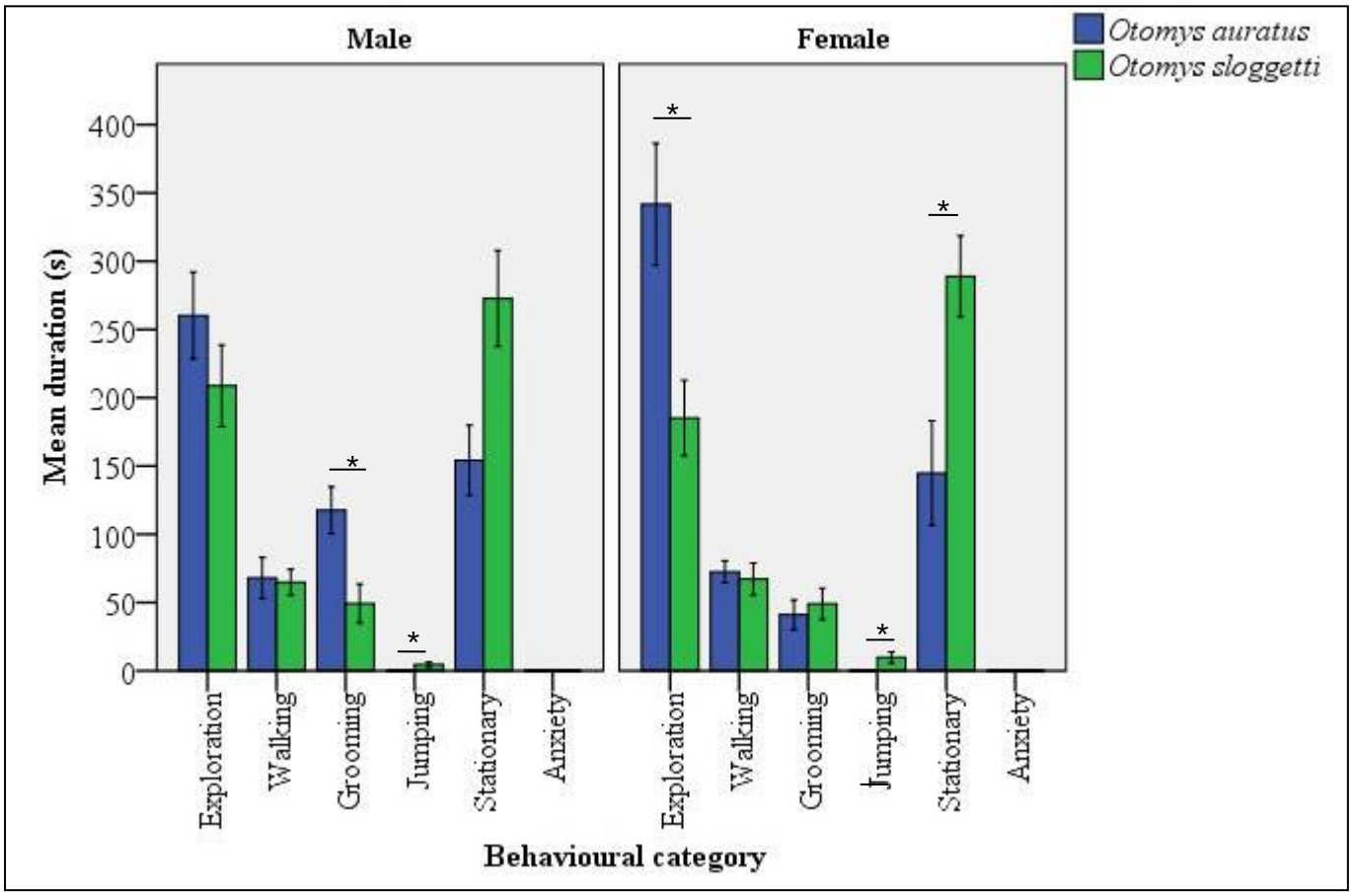
Overall, ice rats spent significantly more time remaining stationary than vlei rats (χ^2 = 6.893, df = 1, p = 0.009) and there was no significant interaction of species with sex on stationary behaviour (χ^2 = 7.105, df = 3, p = 0.069). Male vlei and ice rats did not show differences in time spent stationary (p = 0.096), but female ice rats remained stationary longer than female vlei rats (p = 0.043). Vlei rat males and females did not display differences in stationary behaviour (p = 0.873) and ice rats also showed no sex differences (p = 0.844).

Vlei rats did not display any jumping behaviour, but ice rats showed some jumping behaviour (Table 2.2; Fig. 2.6). There was no significant difference in jumping between male and female ice rats (χ^2 = 2.354, df = 1, p = 0.125). No anxiety behaviour was observed by any vlei rats nor male ice rats (Table 2.2), but female ice rats appeared slightly anxious.

Table 2.2: Summary of exploratory behaviour for vlei rats (*Otomys auratus*) and ice rats (*Otomys sloggetti*). Mean time of each activity is given in seconds ±SE.

			Behavioural category					
Species	Sex	n	Exploration	Walking	Grooming	Jumping	Stationary	Anxiety
Otomys auratus	Male	6	260.20 ±31.75	68.03±15.04	117.62±17.21	0	154.16±25.67	0
	Female	9	341.73 ±44.73	72.48±8.03	41.02±11.02	0	144.77±38.25	0
Otomys sloggetti	Male	14	208.75 ±29.93	64.82±9.58	49.22±14.32	4.49±1.82	272.72±35.11	0
	Female	12	185.16 ±27.61	67.17±11.69	48.98±11.41	9.76±4.02	288.87±29.69	.06±.06

Figure 2.6: General exploratory behaviour of vlei rats and ice rats in a novel environment in this study. Means±SE



Social recognition (session 1) and preference for social novelty (session 2)

The comparison of variables between the two sessions and compartments are summarised in Table 2.3. The presence of subjects in each compartment is presented (number of entries, time in compartment and mean duration in compartment) and then the investigative behaviour to each containment cup (number of contacts, duration of contact and mean time per contact). Results are presented for each species separately to compare variables between compartments in each session and then between species.

Number of entries into each compartment

Vlei rats

There were no significant differences in the number of entries into each compartment in either session by the male vlei rats ($F_{3,148} = 2.335$, $p = 0.074$). The number of times female vlei rats entered a compartment in either session did not significantly differ from each other ($F_{3,148} = 1.810$, $p = 0.148$) (Figure 2.7). Males did not differ from females in the number of entries in any of the compartments (Session 1: Empty cup: $F_{1.148} = 0.001$, $p = 0.970$; First stranger; $F_{1,148} = 0.956$, $p = 0.330$; Session 2: Same stranger: $F_{1.148} = 1.660$, $p = 0.200$; Novel stranger: $F_{1.148} = 0.052$, $p = 0.820$).

Ice rats

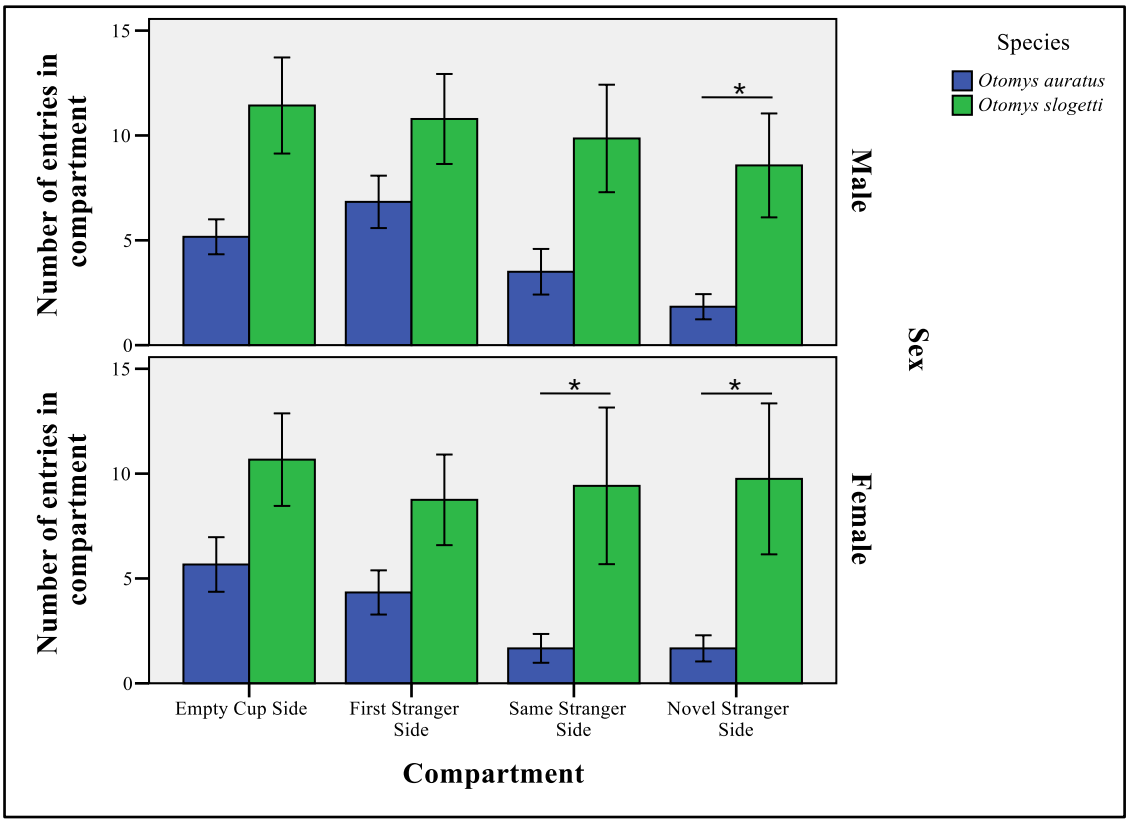
Male ice rats made equal numbers of entries into the two compartments for both experimental phases ($F_{3,148} = 1.526$, $p = 0.210$), and so did female ice rats ($F_{3,148} = 0.736$, $p = 0.532$) (Figure 2.7). There were no differences in numbers of entries between males and females in any of the compartments (Session 1: Empty cup: $F_{1.148} = 0.025$, $p = 0.874$; First stranger: $F_{1,148} = 0.536$, $p = 0.465$; Session 2: Same stranger: $F_{1.148} = 0.254$, $p = 0.615$; Novel stranger: $F_{1.148} = 0.032$, $p = 0.858$).

Species differences

When comparing the two species, ice rats made more entries into the different compartments than vlei rats ($F_{1,148} = 15.064$, $p < 0.001$). No significant differences in the number of entries into either compartment were found in the first session of the experiment for males (Empty cup: $F_{1,148} = 2.102$, $p = 0.149$; First stranger: $F_{1,148} = 0.741$, $p = 0.391$) or females (empty cup: $F_{1,148} = 11.972$, $p = 0.162$; First stranger: $F_{1,148} = 2.223$, $p = 0.138$). However, during the second session, male ice rats made a similar number of entries into the compartment with the same stranger to vlei rats ($F_{1,148} = 2.873$, $p = 0.092$) and they entered into the compartment containing the novel stranger significantly more times than the vlei rat males ($F_{1,148} = 5.084$, $p = 0.026$).

Female ice rats made more entries than vlei rats into both the compartment with the same stranger ($F_{1,148}$ = 7.184, p = 0.008) and the compartment with the novel stranger ($F_{1,148}$ = 6.454, p = 0.012)(Figure 7).

Figure 2.7: Number of entries into each compartment by ice and vlei rats during the social recognition and preference for novelty tests. The empty cup and first stranger were investigated during the first session and during the second session a novel stranger was presented with the same stranger. Means ±SE.



Total time in compartment

Vlei rats

Significant differences in the time spent in each compartment by vlei rats were found in the overall model (χ^2 = 24.357, df = 7, p = 0.001), but neither male (p = 0.933) nor female (p = 0.131) vlei rats spent significantly more or less time in the compartment containing the stranger

during the first session (Fig. 2.8). Also, there were no differences in time spent in the compartments between male and female vlei rats (Empty cup: p = 0.830, First stranger: p = 0.146). However, during the second session, female and male vlei rats each showed differences in total time spent in the different compartments overall (χ^2 = 11.269, df = 7, p = 0.127). Female vlei rats explored the compartment containing the novel stranger for a significantly longer time (Fig. 2.8) than that containing the first stranger (p = 0.031). No significant difference was found in the total exploration duration of the compartments containing the novel stranger and that containing the first stranger by the male vlei rats (p = 0.953) (Fig. 2.8). There were no sex differences in the time in the compartments with the empty cup (p = 0.104) or the first stranger (p = 0.736).

Ice rats

The time spent in the compartment with the stranger differed significantly from the one with the empty cup by the ice rats (χ^2 = 24.357, df = 7, p = 0.001). The duration between compartments did not differ for female ice rats (p = 0.063), but male ice rats spent significantly more time in the compartment with the empty cup (p <0.0001; Fig. 2.8). Also, there were no differences in time spent in the compartments between male and female ice rats (Empty cup: p = 0.260, First stranger: p = 0. 413). No significant differences in the time spent in the compartments was found by the ice rats during the second session (χ^2 = 11.269, df = 7, p = 0.127) and the duration in the compartments by the female ice rats (p = 0.628) or the male ice rats (p = 0.532) (Fig. 2.8). There were no sex differences in the time spent with the same (p = 0.789) or novel strangers (p = 0.406).

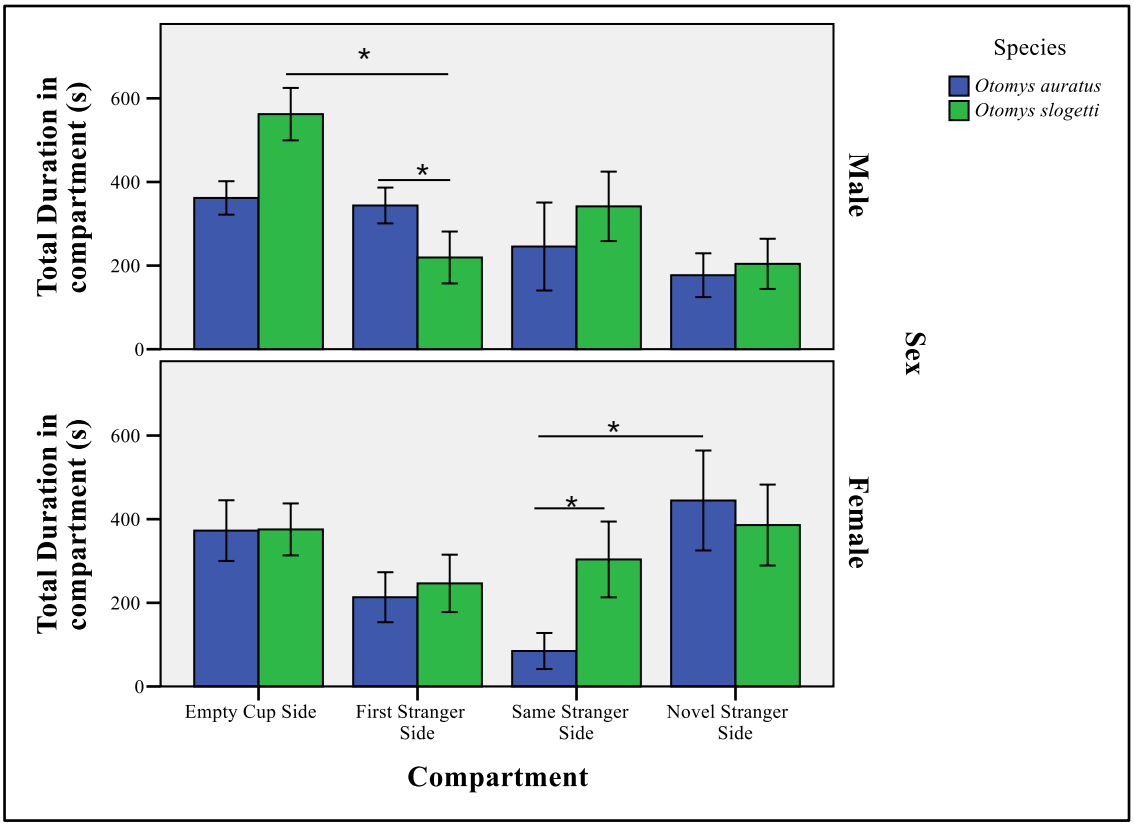
Species differences

The time spent in the compartments by the two species during the first session did not differ significantly when comparing the two species overall (χ^2 = 0.904, df = 1, p = 0.324). The time spent in the compartments by each sex was significantly different between the two species (χ^2

= 24.357, df = 7, p = 0.001). No significant species differences were found in the time spent in the compartment by males (empty cup: p = 0.502) or females (empty cup: p = 0.991; first stranger: p = 0.905), but male ice rats spent a shorter amount of time with the first stranger than vlei rat males (p = 0.024) (Fig. 2.8).

During the second session the time spent in each of the two compartments did not differ significantly between species overall (χ^2 = 2.227, df = 3, p = 0.136). There was also no significant differences in the time spent in each compartment when sex was considered (χ^2 = 11.269, df = 7, p = 0.127). No species differences were found between the time spent by the males with the same stranger (first stranger) (p = 0.666) or with the novel stranger (p = 0.992). However, female ice rats spent more time with the same stranger than did the vlei rat females (p = 0.023; fig. 2.8). No significant species differences in the time spent with the novel stranger by the females were found (p = 0.719).

Figure 2.8: Total time spent in each compartment by ice and vlei rats during the social and preference for social novelty tests.



Number of contacts

Vlei rats

No significant differences in the number of direct contacts by male vlei rats ($F_{3,148}$ = 1.158, p = 0.328) (Fig. 2.9) with the cups between the compartments in both sessions were observed. Although the number of contacts with the cups in both sessions differed significantly overall for the female vlei rats ($F_{3,148}$ = 5.761, p = 0.001), it did not differ significantly (fig. 2.9) between the empty cup and first stranger (t = 0.699, p = 0.486). During the second session female vlei rats only investigated the novel animal a significantly greater number of times (t = 2.657, p = 0.009) than the same stranger (fig. 2.9). There were no sex differences in either compartment (Empty cup: $F_{1,148}$ = 0.141, p = 0.707; First stranger: $F_{1,148}$ = 1.756, p = 0.187; Same stranger: $F_{1,148}$ = 3.084, p = 0.081; Novel stranger: $F_{1,148}$ = 0.001, p = 0.979).

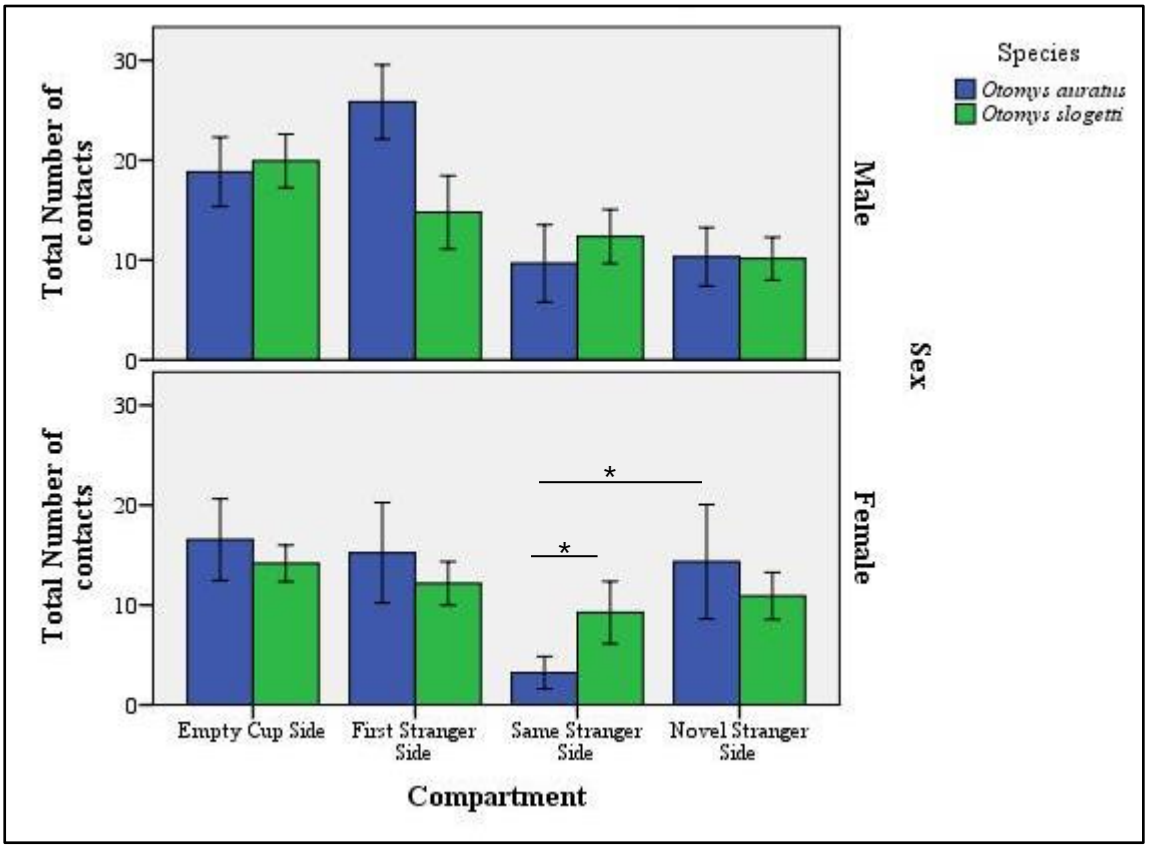
Ice rats

The number of contacts with the containment cups made by the male ($F_{3,148}$ = 1.839, p = 0.143) or the female ice rats ($F_{3,148}$ = 1.128, p = 0.340) did not differ for both sessions (Fig. 2.9). No sex differences in the number of contact events by the ice rats were found in either session (Empty cup: $F_{1,148}$ = 1.027, p = 0.312; First stranger: $F_{1,148}$ = 0.034, p = 0.854; Same stranger: $F_{1,148}$ = 0.690, p = 0.408; Novel stranger: $F_{1,148}$ = 0.053, p = 0.818).

Species differences

The number of times animals directly investigated the cups did not differ significantly between species for both sessions ($F_{1.148}$ = 0.049, p = 0.825). There were no significant differences between males of the two species for the number of contacts in the first session (empty cup: $F_{1,148}$ = 0.076, p = 0.783; first stranger: $F_{1,148}$ = 1.747, p = 0.188) (Fig. 2.9) or females (empty cup: $F_{1,148}$ = 0.024, p = 0.877; first stranger: $F_{1,148}$ = 0.009, p = 0.923). During the second session, males of the two species did not show differences in the number of investigative contacts (Fig. 2.9) (same stranger: $F_{1,148}$ = 0.163, p = 0.687; novel stranger: $F_{1,148}$ = 0.047, p = 0.828), but female ice rats investigated the same stranger more than did female vlei rats ($F_{1,148}$ = 4.783, p = 0.030). There were no species differences in the number of contacts with the novel stranger by the female rats ($F_{1,148}$ = 0.005, p = 0.943).

Figure 2.9: Number of investigative contacts with wire-mesh containment cups by ice and vlei rats during the social recognition and preference for social novelty tests.



Total duration of contact

Vlei rats

There were no differences in the total direct investigation time between cups for the male vlei rats ($F_{3,148}$ = 1.763, p = 0.157), but there were for female vlei rats ($F_{3,148}$ = 6.893, p <0.0001). Vlei rats showed no significant differences in total direct investigation time (Fig. 2.10) between the empty cup and stranger 1 (males: t = 0.697, p = 0.487; females: t = 0.543, p = 0.588) during the first session and between the same stranger and novel stranger by the males (t = 0.966, p = 0.335) (fig. 2.10) during the second session, but the total duration of investigation of the novel animal by vlei rat females (t = 2.725, p = 0.007) was significantly higher than that spent investigating the first stranger. No sex differences were observed between the total

investigation time with the containment cups by the vlei rats in both sessions (Empty cup: $F_{1,148}$ = 0.015, p = 0.902; First stranger: $F_{1,148}$ = 1.374, p = 0.243; Same stranger: $F_{1,148}$ = 3.134, p = 0.079; Novel stranger: $F_{1,148}$ = 0.167, p = 0.683).

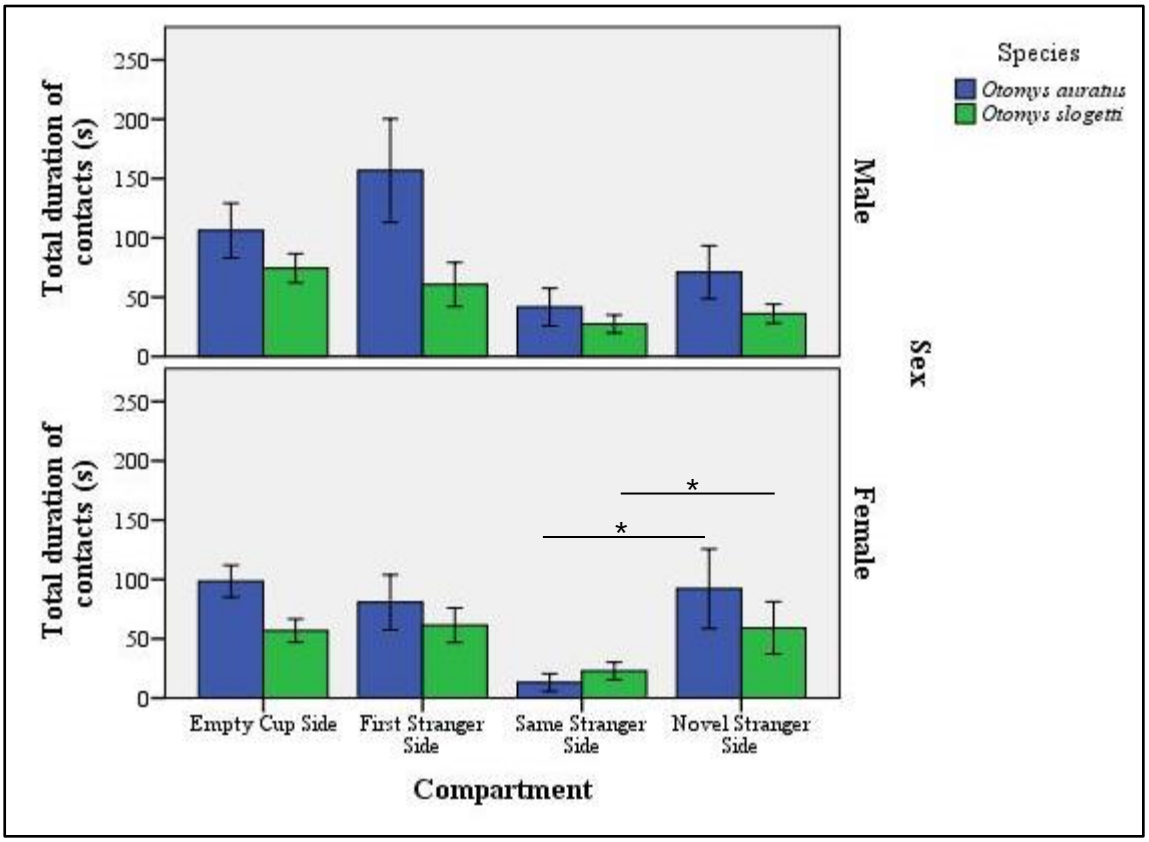
Ice rats

The duration of investigation of cups by male ($F_{3,148}$ = 2.720, p = 0.047) and female ice rats ($F_{3,148}$ = 3.363, p = 0.020) differed between some compartments and sessions (Fig. 2.10). Ice rats spent similar durations investigating the containment cups during session 1 (males: *t* = 0.759, p = 0.449; females: *t* = 0.154, p = 0.877,) and so did ice rat males during the second session (t = 0.821, p = 0.413). However, during the second session, female ice rats investigated the novel stranger significantly longer than the first stranger (t = 2.058, p = 0.041). No sex differences were observed between the total investigation time with the containment cups by the ice rats in both sessions (Empty cup: $F_{1,148}$ = 0.549, p = 0.460; First stranger: $F_{1,148}$ = 0.014, p = 0.905; Same stranger: $F_{1,148}$ = 0.145, p = 0.704; Novel stranger: $F_{1,148}$ = 1.378, p = 0.242).

Species differences

Vlei rats spent more time investigating the containment cups than ice rats overall ($F_{1,148}$ = 4.312, p = 0.040). However, there were no species differences in the duration of contact with the cups during the first session by the males (empty cup: $F_{1,148}$ = 0.428, p = 0.514; first stranger: $F_{1,148}$ = 2.431, p = 0.121) or females (empty cup: $F_{1,148}$ = 1.409, p = 0.237 first stranger: $F_{1,148}$ = 0.340, p = 0.561) (fig. 11) and no species differences in the duration of contact with the cups by males (same stranger: $F_{1,148}$ = 0.633, p = 0.428; novel stranger: $F_{1,148}$ = 1.405, p = 0.238) or females (same stranger: $F_{1,148}$ = 2.531, p = 0.127 novel stranger: $F_{1,148}$ = 0.810, p = 0.369) were found during the second session.

Figure 2.10: Duration of investigative contacts with wire-mesh containment cups by ice and vlei rats during the social recognition and preference for social novelty tests.



Mean duration of contacts

Vlei rats

The mean duration of contacts with the cups in both sessions was similar for male vlei rats ($F_{3,148}$ = 0.645, p = 0.588), but not for females ($F_{3,148}$ = 8.641, p < 0.001). The mean time each of the two containment cups were investigated (Figure 11) in session 1 by vlei rats did not differ significantly (male: *t* = 0.062, p = 0.951; female: *t* = 0.607, p = 0.545) and neither did it for session 2 (males: t = 0.514, p = 0.608; females: t = 1.619, p = 0.107) (Fig. 2.11). There were no sex differences in the mean duration of contact by vlei rats during the first session (Empty cup: $F_{1,148}$ = 0.315, p = 0.575; First stranger: $F_{1,148}$ = 0.002, p = 0.963), but males had

greater mean duration of contact event with the same stranger than females during session 2 (Same stranger: $F_{1,148} = 4.937$, $p = 0.028$; Novel stranger: $F_{1,148} = 1.799$, $p = 0.182$).

Ice rats

The mean duration spent investigating each containment cup by female ice rats ($F_{3,148} = 0.962$, $p = 0.412$) and males ($F_{3,148} = 1.539$, $p = 0.207$) did not differ between compartments in either session (Fig. 2.11). No sex differences were observed between the mean investigation time with the containment cups by the ice rats in both sessions (Empty cup: $F_{1,148} = 0.032$, $p = 0.859$; First stranger: $F_{1,148} = 1.332$, $p = 0.250$; Same stranger: $F_{1,148} = 0.879$, $p = 0.350$; Novel stranger: $F_{1,148} = 0.132$, $p = 0.717$).

Species differences

The mean duration of contact events to containment cups in both sessions was similar for the two species overall ($F_{1,148} = 0.646$, $p = 0.423$). There was no significant species difference in the mean duration of contact events with the cups by the males (empty cup: $F_{1,148} = 0.517$, $p = 0.473$; first stranger: $F_{1,148} = 2.028$, $p = 0.157$; same stranger: $F_{1,148} = 0.939$, $p = 0.334$; novel stranger: $F_{1,148} = 0.153$, $p = 0.696$). The mean duration of contact events by the females did not differ between species during the first session (empty cup: $F_{1,148} = 1.711$, $p = 0.193$; first stranger: $F_{1,148} = 0.241$, $p = 0.624$) or by the males with the novel stranger during the second session ($F_{1,148} = 0.715$, $p = 0.399$), but female ice rats had a higher mean duration of contact events with the same stranger than did the female vlei rats ($F_{1,148} = 5.855$, $p = 0.017$).

Figure 2.11: The average duration of investigation contacts per animal by ice and vlei rats during the social recognition and preference for social novelty tests.

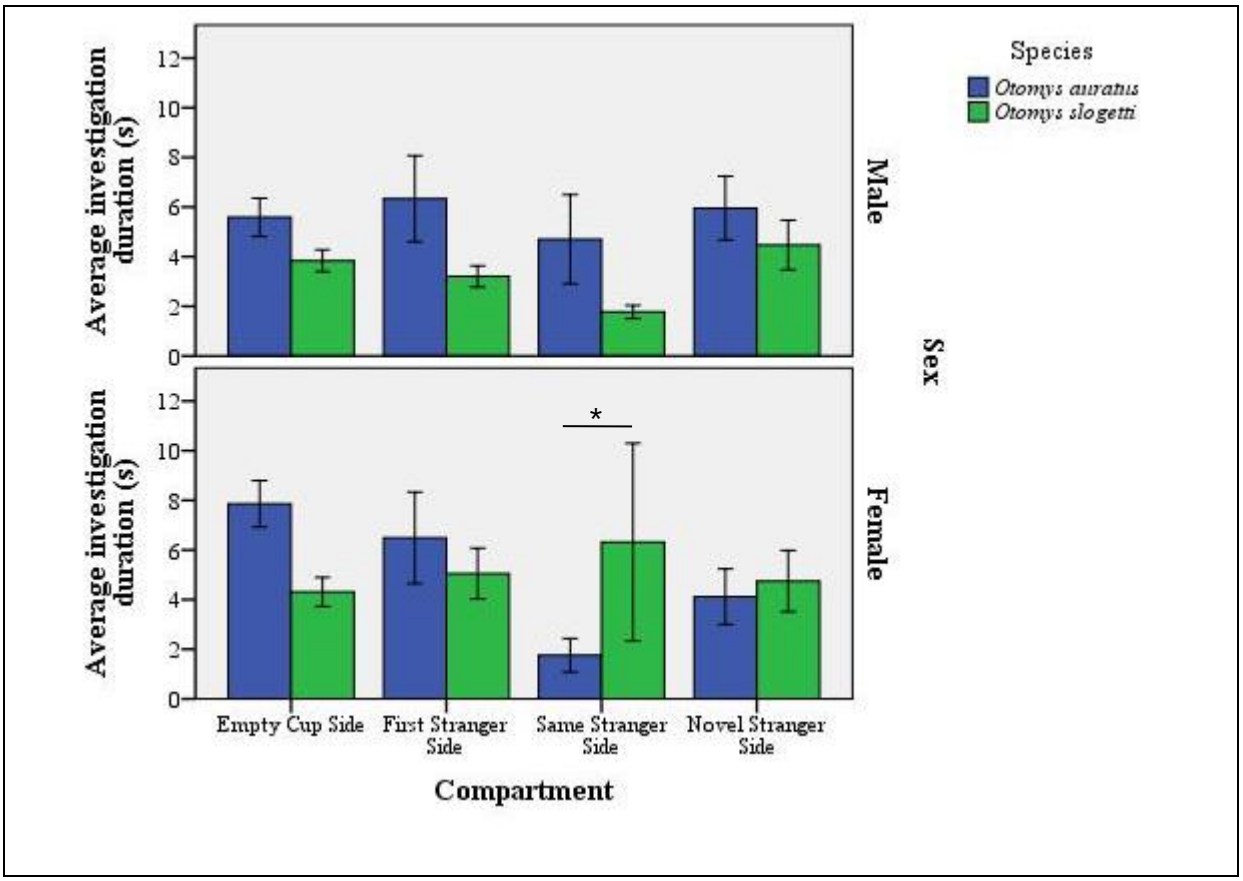


Table 2.3: Summary means of recorded behavioural variables for the social recognition and social discrimination experiments for vlei rats (*Otomys auratus*) and ice rats (*Otomys sloggetti)*(mean ±SE).

Species	Sex	Compartment	n	Number of entries in compartment (entries)	Total Duration in compartment (s)	Total Number of contacts (contact)	Total Duration of contacts (s)	Mean Duration per contact (s/contact)
Otomys auratus	Male	Empty Cup	6	5.17 ±.83	361.80 ±39.99	18.83 ±3.48	106.16 ±23.13	5.58 ±.78
		First Stranger	6	6.83 ±1.25	343.74 ±42.80	25.83 ±3.72	156.82 ±43.70	6.33 ±1.74
		Same Stranger	6	3.50 ±1.09	245.61 ±105.18	9.67 ±3.88	41.67 ±15.95	4.70 ±1.80
		Novel Stranger	6	1.83 ±.60	176.99 ±52.40	10.33 ±2.93	71.09 ±22.37	5.95 ±1.28
	Female	Empty Cup	9	5.67 ±1.30	372.72 ±72.55	16.56 ±4.09	98.50 ±13.40	7.86 ±.94
		First Stranger	9	4.33 ±1.05	213.38 ±59.80	15.22 ±5.03	80.73 ±23.26	6.49 ±1.84
		Same Stranger	9	1.67 ±.69	84.77 ±43.12	3.22 ±1.62	13.16 ±7.44	1.76 ±.67
		Novel Stranger	9	1.67 ±.62	444.70 ±119.50	14.33 ±5.73	92.12 ±33.56	4.12 ±1.12
Otomys sloggetti	Male	Empty Cup	14	11.43 ±2.29	562.19 ±62.68	19.93 ±2.68	74.51 ±12.23	3.83 ±.44
		First Stranger	14	10.79 ±2.15	219.38 ±62.05	14.79 ±3.67	60.71 ±18.53	3.21 ±.43
		Same Stranger	14	9.86 ±2.56	341.64 ±83.04	12.36 ±2.70	27.39 ±7.59	1.77 ±.26
		Novel Stranger	14	8.57 ±2.48	204.16 ±59.99	10.14 ±2.13	36.05 ±8.06	4.47 ±1.00
	Female	Empty Cup	12	10.67 ±2.21	375.52 ±62.14	14.17 ±1.83	56.79 ±9.83	4.31 ±.59
		First Stranger	12	8.75 ±2.16	246.51 ±68.61	12.17 ±2.17	61.39 ±14.48	5.05 ±1.02
		Same Stranger	12	9.42 ±3.73	303.75 ±90.61	9.25 ±3.12	22.87 ±7.34	6.32 ±3.99
		Novel Stranger	12	9.75 ±3.60	385.94 ±96.77	10.92 ±2.36	59.14 ±22.04	4.75 ±1.24

Discussion

Since social behaviour is intricately tied to the ability to recognise conspecifics, this study investigated how social discrimination underlies the apparent disparity in observed social behaviour between gregarious ice rats and solitary vlei rats. Very few studies have tested the social recognition abilities of wild social and solitary species, with only a few investigating gregarious and huddling behaviour, therefore the present study provides novel insight into the social behaviour of wild caught, phylogenetically related species.

Vlei rats

Vlei rats are solitary and very aggressive towards each other in the laboratory (Davis, 1973; G. Göldner, pers. obs.) as well as exhibiting strict territoriality (Pillay, 1990). They have a promiscuous mating system (Davis and Meester, 1981) and lack parental care after weaning (Davis, 1973; Skinner and Chimimba, 2005). Pups cling firmly to the maternal nipples during weaning, are ignored when they become detached, often fight a few days after birth and have even been observed to resort to cannibalism in the laboratory (G. Göldner, pers. obs.).

Both male and female vlei rats failed to discriminate between a stimulus animal and an empty cup in any of the parameters tested. This result suggests a decrease in social motivation and this was predicted for the solitary vlei rats. Similarly, male vlei rats did not demonstrate a preference for social novelty when presented with a familiar and a novel conspecific. A lack of differentiation was also observed in laboratory mice and rats lacking the oxytocin gene; these animals are antisocial, aggressive towards each other and fail to form pair bonds (Moy *et al.* 2004; Van der Kooij and Sandi, 2012). Thus, the impaired sociability and lack of social novelty of male vlei rats is in agreement with the observed solitary behaviour of the species. Interestingly, female vlei rats showed more variable and unexpected results. Despite showing

decreased social motivation by not distinguishing between unfamiliar animals and an empty cup, they appear to be able to discriminate between familiar and novel animals in the amount of time spent in the vicinity of the novel animal and the number and duration of contacts. This result was not anticipated, as other rodent strains that do not show social recognition, usually also do not show social discrimination (Moy *et al.* 2004).

Despite the fact that solitary species are associated with a decrease in social behaviour, social recognition and discrimination abilities vary widely with species and certain aspects thereof demonstrate a more fluid, flexible function of social recognition in solitary rodents. Some solitary species such as the red squirrel can recognise same-sex conspecifics (Vache *et al.* 2001). Vache *et al.* (2001) suggested that, in species that show high intra-specific aggression (such as red squirrels and vlei rats), social discrimination may enable animals to avoid unnecessary fights. Non-gregarious female Virginia opossums also retained the ability to distinguish between individual odours transferred to disks, but they could not discriminate male odours (Holmes, 1992). Solitary tuco-tucos showed reduced aggression towards familiar conspecifics in paired tests and it was suggested that this may prevent costly interactions with familiar conspecifics (Zenuto, 2010). Individual recognition may also allow animals to distinguish parasitized individuals, as rodents have been shown to use social odours to identify infected individuals, recognise and evade them (Kavaliers *et al.* 2005).

In addition, many biological factors are known to influence social behaviour. For example, meadow vole females show seasonal variation in their degree of sociality, they are solitary and territorial during summer months and lack long term affiliative same-sex bonds, whereas social huddling increases during winter months (Beery and Zucker, 2010). Maternal care is another example of temporal behavioural changes, virgin or pregnant rats avoid or attack pups, but with

the onset of nest building they develop an increased social motivation towards pups (Numan 1994 from Insel 2003). In male prairie voles, their life stage dictates whether they can discriminate between females, paired males can recognise females while bachelor males cannot (Blocker and Ophir, 2015).

It is highly probable that hormones may also influence social recognition abilities in rodents. Since social recognition is regulated by neural regions affected by olfaction in other rodent species, including voles, mice and rats (Albers, 2012), it is plausible that hormonal changes in these regions may affect their social recognition abilities. Oxytocin release, which influences and attenuates social recognition in rats (Bielsky and Young, 2004), is dependent on oestrogens. Oestrogen treatment regulates the production of OT and the density of OTRs in regions such as the amygdala (Choleris *et al*. 2009), a structure essential to social recognition (Demas *et al*. 1997; Ferguson *et al.* 2001). Although every attempt was made to ensure non-pregnant females were used during this study, it is possible that other hormones may also affect social behaviour. Social discrimination may be important for female vlei rats during the weaning period of young. Indeed, increased social tolerance has been found in some mammals when the oxytocin levels are elevated (Beery and Zucker, 2010; Mooney *et al.* 2014).

Consequently, while vlei rats exhibit marked solitary, anti-social behaviour, the results from this study suggest that social recognition and discrimination abilities are indeed flexible. The evolutionary benefits of a basic discrimination ability may be to initiate or increase maternal behaviour, or to reduce aggressive interactions. Social recognition and discrimination may not always be evident, and appear to be species-specific according to the specific life history of the species in question.

Ice rats

Ice rats are gregarious, living in small groups of up to 17 animals (Hinze, 2005). Ice rats also breed promiscuously (Hinze, 2005) and pups cling to the maternal nipples during weaning. Interestingly, female ice rats take pups back after a separation of around 24 hours, however it is unclear whether they can recognise their own pups, since a female was observed to readily accept a pup from another female (G. Göldner, pers. obs.). Spatial dichotomy in social behaviour has been reported for ice rats, being tolerant and gregarious in underground burrows, but aggressively competitive while foraging aboveground (Hinze *et al.* 2013).

Surprisingly, male and female ice rats did not show a significant differentiation between the side containing the unfamiliar rat compared to the empty cup for both presence and investigation time. In fact, male ice rats actually preferred to spend more time with the empty cup, suggesting impaired social recognition abilities. This result was unexpected since the social rodent species has previously been found to spend significantly more time with the unfamiliar conspecific, and investigate it significantly more, compared to the empty cup (Moy *et al.* 2004; Kaidanovich-Beilin *et al.* 2009; Engelmann *et al.* 2011; Van der Kooij and Sandi, 2012). Where social olfactory recognition abilities were inhibited by neural lesions or chemical methods, increased aggression and reduced social behaviour such as bond formation or huddling occurred (Demas *et al.* 1997).

In congruence with the sociability experiment, ice rats also did not show a preference for social novelty. Female ice rats showed an apparent discrimination ability in one variable, however this effect disappears when the investigation durations are averaged. Although these results are unusual for an apparently gregarious species, social responses to different challenges are very diverse across species. Some gregarious rodent species, such as beach voles, choose same-sex

odours over control cotton-swabs during non-breeding seasons, but meadow voles (which are essentially solitary) do not (Ferkin, 1990). These socially gregarious beach voles show social tolerance, although it may be a function of an inability to disperse to other areas, similar to the habitat restriction of ice rats in montane regions. Species such as great gerbils show social tolerance by females with temporal differences in aggression and group formation: females are solitary when food is limited and mortality is increased, but share territories with female kin under favourable conditions (Randall *et al.* 2005). Also, female meadow voles vary their sociality with season, from summer to winter; they more tolerant towards conspecifics during winter (Beery and Zucker, 2010). Likewise, ice rats may be more socially tolerant during winter, and they display increased gregarious behaviour during winter (Hinze, 2005, Hinze *et al.* 2013). For this study, animals were captured and studied during summer, hence the behaviour may differ from that during winter.

Prior experience of conspecifics may also influence social discrimination. For example, in hamsters, social recognition ability was intact when male hamsters were raised together regardless of relatedness, and when related males were raised in a different litter, subjects could not recognise them (Todrank *et al.* 1999). While ice rats in this study were captured from different burrow systems, it cannot be excluded that they could have had prior contact somewhere in their lives in the environment. Therefore, although unlikely, prior experience of individuals aboveground, or in other burrows, may have affected their motivation to investigate the test animals.

It was suggested that the observed spatial dichotomy in the social behaviour of ice rats (Hinze *et al.* 2013) ensues as a result of limited resources under harsh conditions and competition. Thus, a more solitary lifestyle would be more adequate, but mutualistic social behaviour also

benefits individuals during cold, wet conditions. Social flexibility has been shown in striped mice (Schradin *et al.* 2012), where ecological constraints affect the prevalence of social grouping behaviour. Under conditions of reduced resources and harsh, arid conditions, striped mice aggregate and are tolerant towards conspecifics in nests (similar to ice rats in underground burrows), but in mesic environments with abundant resources, they display intense territoriality and antisocial, solitary behaviour (Schradin and Pillay, 2005; 2004). The aridity food distribution hypothesis (AFDH) postulates that animals occur in groups due to scarce food resources, such as in arid regions (Jarvis *et al.* 1994), and has been used to explain group living in striped mice (Schradin *et al.* 2012). This hypothesis may also be appropriate to explain the gregarious behaviour in ice rats. They inhabit montane environments, with harsh ecological conditions during winter, and scarce food resources, which I propose is analogous to the arid environments faced by social flexible striped mice, and I suggest that ice rats may display a similar social flexibility.

As far as ice rat social flexibility is concerned, I may speculate that ice rats will show social flexibility when exposed to differing extreme simulated environmental conditions in a controlled laboratory setting, such as comparing laboratory raised ice rats in simulated mesic environment conditions with those raised in simulated harsh, cold and wet montane environment conditions.

Species differences

Despite apparent species differences in sociality, the social recognition abilities of these two species appear to be fairly similar. Interestingly, the solitary vlei rat females show more social discrimination than the supposedly ‘social’ ice rats. Still, ice rats appear to be more tolerant

towards conspecifics when in their underground tunnels and nests. This increased tolerance may not be reserved for familiar animals, but may extend to any individual encountered, thus negating the need to recognise and remember specific individuals (including young). On the other hand, solitary vlei rats are aggressive towards any individual encountered. The discrimination ability of female vlei rats may be as a result of hormones related to maternal care. High oestrogen and oxytocin levels towards the end of pregnancy, and afterwards, increase social motivation and recognition (Bielsky and Young, 2004, Numan 1994 from Insel 2003). Vlei rats have an extended breeding season of up to 9 months (excluding the winter months of May-July), and may produce up to three litters per season (Davis, 1973). Since experiments were performed during spring and summer, females have a high probability of being pregnant or suckling young during this time period. This could be a potential explanation for the increased social discrimination displayed by vlei rat females compared to ice rat females.

Although the duration and number of direct contacts in the respective compartments did not differ for the two species, both male and female ice rats made many more entries into the different compartments during all stages of the experiment. The increased activity of ice rats upon exposure to other animals during the experiment may be stress or anxiety related. Although stress hormones were not measured in this experiment, ice rats showed less investigative behaviour compared to vlei rats during the initial general exploratory phase of the experiment. Stress is known to reduce investigative behaviour and also impair social discrimination (De Vries *et al.* 1996, Bourin *et al.* 2007, Anacker *et al.* 2011). The greater attention to investigation observed in vlei rats may then be attributed to them being more relaxed and not merely moving from one compartment to the other. Vlei rats started investigating the containment cups immediately upon commencement of the experiment, while

ice rat males remained stationary for an initial period, and female ice rats were stationary for extended periods of time inside the different compartments without investigating the containment cups or the compartment.

Conclusion

A lack of social recognition and discrimination was predicted for the solitary vlei rats, and while the males conformed to this assumption, female vlei rats show the ability to discriminate between a familiar and novel conspecific. A number of factors may be responsible for the increased social motivation in the females, including hormonal profiles that are associated with maternal care. The absence of social recognition and discrimination in ice rats was surprising, since this species is more gregarious than the vlei rat. Since other species have been found to show seasonal variation in sociality, it may be plausible to suggest that ice rats would show increased sociality during the cold winter months when they would benefit from huddling. In addition, stress may decrease their motivation to investigate, however stress hormones have not been measured in this study, thus no conclusive inferences can be made.

Harsh ecological constraints may have promoted ice rats to adopt a gregarious, subterranean social lifestyle while vlei rats, which occur in warm, wet, mesic environments with abundant resources, have adopted a solitary lifestyle. Neural regions responsible for social behaviour (which are linked to social recognition neural circuitries) are highly conserved (O'Connell and Hofmann, 2012), thus the selection pressure exerted on the social behaviour of *Otomys* spp. in this study may yet drive ice rats to adapt a more social behavioural organisation. Nonetheless, it is possible that ice rats and vlei rats do not possess inherent sociability and social structures,

but may possess social flexibility as defined by Schradin *et al.* (2012), yet further intraspecific comparisons are needed for this to be established.

Overall, the results from this study suggest that both vlei and ice rats exhibit flexibility in terms of social recognition abilities, as vlei rat females are able to distinguish between conspecifics and ice rats possess temporal flexibility in their social behaviour by exhibiting social dichotomy in their natural habitat.

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Chapter 3: Telencephalic binding sites of oxytocin in a solitary and a social Otomyine species

Abstract

Social mammals exhibit distinct differences in their neural binding of oxytocin, when compared to solitary mammals. Vlei and ice rats (two otomyine species) provide non-typical model animals to study the neuroendocrinology of social behaviour. Multiple, reproductively active, adult ice rats occur colonially underground and are socially tolerant towards conspecifics. In contrast, the closely related vlei rats are aggressively territorial and exhibit a solitary lifestyle. Compared to social mammals, solitary species have been found to have a reduced binding affinity for oxytocin in the telencephalic regions responsible for reward stimuli and memory formation, which contribute to social behaviour. Using ligand-binding receptor autoradiography, neural oxytocin receptor (OTR) binding sites in the two otomyine species were identified. Compared to the vlei rat, OTR binding of the ice rat was more intense in the nucleus accumbens, islands of Calleja, claustrum, indusium griseum, prefrontal cortex, insular cortices, extended amygdala, bed nuclei of the stria terminalis and hypothalamic nuclei. However, both species displayed similar intensities of binding in the lateral septum. In the medial habernula and dentate gyrus, OTR binding was only present in the brain of the vlei rat. Binding occurred exclusively in the hippocampal subfields and intermediodorsal and rhomboid thalamic nuclei in the brain of the ice rat. The overall patterns of neural OTR binding in ice rats and vlei rats is similar to that found in social and solitary voles. In some aspects, although, there are also similarities in the neural OTR binding of the ice rats and vlei rats. The patterns of telencephalic OTR binding of the two species may reflect their socially disparate behaviour. However, a continuum of oxytocinergic effects on social, group-living behaviour of related species may exist in this otomyine group. The differences in neuropeptidergic circuitry in these two species contributes further to our understanding of neuroendocrinology of sociality.

Introduction

The ability of mammals to form social relationships depends on a large variety of factors determined by evolutionarily derived genetic attributes that lead to neurological, endocrinological and ultimately behavioural expression of social or prosocial behaviour. There is evidence that mammals that form social bonds, such as mother-offspring bonds (Levy *et al.* 1995), same-sex affiliative bonds (Beery and Zucker, 2010) and mating pair-bonds in monogamous species (Young and Wang, 2004), exhibit marked differences in the distribution and intensity of the telencephalic binding sites for various neuropeptides that contribute to the formation of these bonds compared to non-social, solitary mammals (Kalamatianos *et al.* 2010; Olazabal, 2014).

Oxytocin and vasopressin are neuropeptides secreted not only from the posterior pituitary, but also at sites within the brain associated with social behaviour (Landgraf *et al.* 2003; Olazabal and Young, 2006a; Kalamatianos *et al.* 2010). These important neuropeptides regulate behaviour such as pair-bonding, maternal behaviour, altruism and other social interactions between conspecifics (Bielsky and Young, 2004; McEwen 2004). Furthermore, the distribution of oxytocin and vasopressin receptors in the brain has been linked to the formation of specific relationships in many mammals. In mammalian species, especially rodents, that have been described as gregarious, prosocial, social or at least colonial, the distribution of oxytocin receptors has been shown to be more intense in neural regions responsible for affiliative behaviour; yet it seems that the density of neuronal processes capable of releasing oxytocin at the relevant site is not the key variable (Young and Wang, 2004).

The neuroanatomical pathways involved in the prosocial or social behaviour of several rodent species have been studied. Oxytocinergic immunoreactive neurons have been found in the

paraventricular nucleus, supraoptic nucleus and preoptic area, as well as in the septum, bed nucleus of the stria terminalis and nucleus accumbens in the eusocial, naked mole rat (*Heterocephalus glaber)* (Rosen *et al.* 2008). Certain cortical areas such as the hippocampus subfields, indusium griseum and amygdala also contain oxytocin-positive processes (Kalamatianos *et al.* 2010). Oxytocinergic processes are found in the paraventricular and supraoptic nuclei as well as the preoptic area in the eusocial Ansell's mole rat *(Fukomys anselli)* too (Valesky *et al.* 2012). Meadow voles, which are sexually promiscuous and display same-sex social behaviour (measured as increased huddling behaviour), have concentrations of oxytocinergic processes in similar regions, including the central amygdala, nucleus accumbens, certain nuclei of the hypothalamus and lateral septum (Beery and Zucker, 2010).

In some species, such as monogamous prairie voles (*Microtus ochrogaster),* increased duration of huddling over pups is positively correlated with oxytocinergic receptors in the nucleus accumbens. This huddling behaviour is suppressed, following intra-cerebroventricular injection of an oxytocinergic antagonist, thereby providing support that these regions play an important role in mother-offspring bonding (Lim and Young, 2006).

It is clear that the amygdala and the hippocampal areas are also important for social behaviour, specifically for social memory, as injections of oxytocin into the amygdala of oxytocin knock-out mice rescues social recognition abilities and therefore social aggregating type behaviour (Bielsky and Young, 2004). However, the neural circuitry of social behaviour is a complex process and the distribution of oxytocin immunoreactive neurons indicates where this peptide is produced, and how it is transported, but it is important to determine the exact location in the brain stimulated by these neurons.

In order to further study the mechanisms and circuitry of social behaviour and the link to neuropeptides, the binding of oxytocin to neurons with oxytocin receptors also needs to be identified, as oxytocin is a ligand neurotransmitter that binds and stimulates certain areas in the brain. It was shown that, although the oxytocin secreting neuron distribution in areas such as the nucleus accumbens, lateral septum and paraventricular and supraoptic nuclei may be similar, it is the distribution of specific binding sites on oxytocin reactive neurons that is directly linked to social behaviour in voles (Insel and Young, 2000). Promiscuous and solitary montane and meadow voles, while displaying similar immunoreactive patterns of oxytocin in hypothalamic regions and hippocampal areas to social and huddling/gregarious prairie voles, display lower concentrations of oxytocin binding receptors in regions such as the caudate putamen, nucleus accumbens and lateral septum (Lim and Young, 2006; Olazabal and Young, 2006b).

The ventral pallidum and amygdaloid areas also have greater oxytocin binding intensity in social species of voles and mole rats, compared to closely related solitary species of the respective taxa (Insel and Young, 2000; Olazabal and Young, 2006a). In the eusocial naked mole- rat, the oxytocin binding is much higher than in the closely related, solitary Cape mole rat in these areas (Kalamatianos *et al*. 2010). Furthermore, the piriform cortex and islands of Calleja, as well as other areas, have been found to bind oxytocin in rats and mice showing social behaviour (Olazabal, 2014) and colonial South American tuco-tucos show increased binding in the amygdaloid areas, compared to closely related solitary species (Beery *et al.* 2008).

Experimental studies have revealed oxytocin and vasopressin to be intimately linked to social behaviour and social recognition. Oxytocin knock-out mice lack the ability to recognize

conspecifics (a vital ability to social interaction), but this is rapidly restored upon administration of intra-cerebroventricular oxytocin (Ferguson *et al.* 2001; 2000). Regions such as the lateral septum, medial amygdala, cortical amygdala and the bed nuclei of the stria terminalis in the telencephalon of rats appear to be associated with social memory (Bielsky and Young, 2004). In addition, the binding sites of oxytocin and vasopressin are also associated with the main olfactory and accessory olfactory bulb (Brennan and Kendrick, 2006). Therefore, social behaviour is closely related to olfactory sensing in mammals such as rodents (Ferguson *et al.* 2000), since the neural processing of olfactory cues is critical to social memory.

Species that lack lasting social relationships and complex social structures have reduced binding for oxytocin (Rosen *et al.* 2008) in the telencephalon and low densities of distribution of the oxytocinergic and vasopressinergic fibres innervating neural regions responsible for reward stimuli (e.g. nucleus accumbens, amygdala, stria terminalis) and memory formation (e.g. lateral septum and hippocampus) (Ferguson *et al.* 2001; Olzabal and Young, 2006a; Olzabal, 2014). Social and aggregating species, such as the Ansell's mole rat (Valesky *et al.* 2012), naked mole rat (Mooney *et al.* 2014) and meadow vole (Demas *et al.* 1997; Beery and Zucker, 2010), in contrast, have a greater intensity of oxytocin binding in these regions.

Members of the genus *Otomys* are widely distributed throughout South Africa; however, detailed species information on their social behaviour is lacking (Skinner and Chimimba, 2005). Interspecies variations in social behaviour exist between the species; vlei rats, *Otomys auratus*, display aggressive territoriality and antisocial tendencies (Davis, 1973), whereas ice rats, *Otomys sloggetti robertsi*, huddle together during cold periods and display mild sociality (Schwaibold and Pillay, 2006). A possible explanation of the species diversification of social behaviour may be provided by the aridity food distribution hypothesis (AFDH). The AFDH

postulates that animals occur in groups due to scarce food resources, such as in arid regions (Jarvis *et al*. 1994), and has been used to explain group living in striped mice (Schradin *et al.* 2012). This hypothesis may also be appropriate to explain the gregarious behaviour in ice rats. They inhabit montane environments, with harsh ecological conditions during winter, and scarce food resources, which I propose is analogous to the arid environments faced by social flexible striped mice, and I suggest that ice rats may display a similar social flexibility. While work has been done on the link between neuropeptides and sociality in rodents, marked differences appear to be evident in the exact brain regions of oxytocin binding sites between rats, mice and other rodents (Van der Kooij and Sandi, 2011). The different social organization of these two species provides an opportunity to study neurological oxytocin expression as well as reception in otomyine species.

The aims of the study are to identify and compare telencephalic binding sites for oxytocin in the brain of the observed social/gregarious murid rodent ice rats (*O. sloggetti robertsi)* and the closely related, solitary/antisocial vlei rats (*O. auratus*), by determining how the distribution and intensity of telencephalic oxytocin receptor binding differs between the two species.

Materials and Methods

Animal collection and location

Adult male (n = 11; body mass = 126.43 ± 4.66 g) and female (n = 11; body mass = 124.55 ± 7.24 g) vlei rats (*O. auratus)* were trapped using Sherman live traps (H. B. Sherman Traps, Inc. Tallahassee, Florida, U.S.A.) baited with a mixture of peanut butter and oats since the species are exclusively vegetarian (Davis, 1973). Trapping occurred during the austral autumn and winter (April to July) at the Rietvleidam Nature Reserve (25°52'23.9"S 28°16'42.4"E) and

during winter and spring (August to October) at Bullfrog Pan (26°08'20.8"S 28°19'23.1"E) in the Gauteng Province of South Africa. Traps were opportunistically placed along grass 'runs' in order to intercept vlei rats directly (Chapter 2). Traps were checked daily between dawn and dusk when activity is known to be at a maximum (Davis, 1973), at intervals of 2 to 3 hours throughout the day and more frequently during very hot, very cold or rainy days.

Specimens of the social ice rats (*O. sloggetti)* (male: n = 5; body mass = 115.2 ± 28.39 g; female: n = 9; body mass = 134.43 ± 7.10 g) were trapped in the southern Drakensberg Mountains (30°39'09.3"S 27°55'32.2"E) during summer (November). Single traps were placed directly in front of burrow entrances and covered with thick dried grass to prevent overheating in harsh sunlight during clear days and to insulate the metal trap during rainy and cold days.

After collection, all animals were sexed and the sexual maturity determined, after which they were individually housed at the Department of Zoology and Entomology, University of Pretoria, provided water and fed on an *ad libitum* daily diet composed of fresh lettuce and sweet potatoes, supplemented with mouse pellets, apples, carrots and fresh grasses. These animals were used in a companion study (see chapter 2) in order to assess social recognition abilities.

Oxytocin receptor ligand binding autoradiography

The animals were euthanized with an overdose of isofluorane anaesthetic and the brains were immediately removed and frozen on dry ice and stored at -80^{o}C. All killing was conducted with approval of the University of Pretoria Animal Ethics Committee (EC003-13). Using a cryostat, the telencephalon of each animal (vlei rats: 6 males, 6 females; ice rats: 3 males, 5 females) was cut into 12µm serial coronal sections, were mounted on Superfrost Plus slides (Sigma-Aldrich, Poole, United Kingdom) and divided in six rostrocaudal anatomical levels. Sections

were allowed to air-dry at room temperature before being frozen at -80^{o}C (Kalamatianos *et al.* 2010).

Ligand binding autoradiography was used to identify oxytocin receptor binding sites, as described in Olazabal and Young (2006a) for both sexes. All sections were briefly (approximately 2.5 min) immersed in an ice cold 0.1% paraformaldehyde (PFA) in 0.1M phosphate buffered saline solution (PBS) (pH 7.4) and then pre-incubated for 5 minutes in a 50mM Tris-HCl pH 7.4 solution. After air-drying for 1 hour, each slide mounted with sections was immersed in 500µL of a 50mM Tris-HCl (pH 7.4) mixture containing 50pM ^{125}I-Ornithine Vasotocin analog [NEX 2540 50UC; (d[CH_2]$_5$[Tyr(Me)^2,Thr^4,Orn^8,[^{125}I]Tyr^9-NH_2]-vasotocin;^{125}I-OVTA, 2200 Ci/mmol] (Perkin Elmer), 10 mM magnesium chloride and 0.1% bovine serum albumin (RIA grade, fraction V, Sigma) and 0.05 % Bacitracin and incubated at room temperature for 2 hours. Following incubation sections were rinsed for 5 minutes with an ice cold solution of 50mM Tris-HCl (pH 7.4) and 10 mM magnesium chloride four times and then briefly dipped in ice cold Milli Q Water. Finally, slides were air dried with cold air and then exposed to BioMax MR ^{125}I-sensitive film (Kodak, Rochester, NY) with ^{125}I-autoradiographic microscale standards (Amersham Biosciences) for 5 days in a dark room. In order to control for non-specific binding, one of the series of sections was incubated in 1 µM non-radioactive OTR-selective ligand [Thr^{4}Gly^7] oxytocin or oxytocin (Sigma-Aldrich Ltd.) to eliminate competition of ^{125}I-OVTA binding.

Anatomical localisation

After radioactivity was no longer detectable from sections as monitored by a Geiger counter, slides were hydrated by sequential immersion in 100% ethanol for two 5 minute sessions, 95% ethanol, 70% and 50% ethanol for 2 minutes each and then distilled water for 2 minutes in

order to be immersed in a 0.5% cresyl violet acetate solution for 2 minutes to allow Nissl-body staining of neural cells (Paxinos and Watson, 2007). Sections were then dehydrated in 50%, 70%, and 90% ethanol for 2 minutes each and immersed in 100% ethanol twice for 5 minutes, before finally being soaked in a xylene solution twice for 5 minutes. Cover slips were placed on the slides using a xylene-based dibutyl phthalate mounting medium.

Image analysis

Using a precision illuminator (Northern Light R95, Interfocus Imaging, Cambridge, United Kingdom), a microscope (Nikon Eclipse E600, Interfocus Imaging, Cambridge, United Kingdom), and a MicroPublisher 5.0 camera (Interfocus Imaging), autoradiographs and photomicrographs were taken using MCID Core software (Interfocus Imaging). Brightness and contrast settings were kept constant and the area was selected so that the largest section just fit the capture field.

Autoradiographic signals were qualitatively compared by matching anatomical regions (using neuroanatomical nomenclature and distribution of Paxinos and Watson (2007)) and corresponding Nissl-stained sections by graphical overlay in Adobe Photoshop CS6 13.0 (Adobe Systems Inc., Mountain View, CA). Signals in the basal ganglia (nucleus accumbens and caudate putamen), olfactory system (islands of Calleja), cortical region (prefrontal cortex and piriform cortex), indusium griseum, cingulate cortex, choroid plexus, interstitial nucleus of the posterior limb of the anterior commissure, dentate gyrus, septal nuclei, extended amygdala and hippocampus (Kalamatianos *et al.* 2010) were scored on a scale of four intensities: ‘absent’, ‘mild’, ‘moderate’ and ‘strong’.

Results

Neural autoradiographic signals for oxytocin receptor binding in vlei and ice rats

The oxytocin receptor (OTR) binding sites in vlei and ice rats were qualitatively compared for similarities and differences in both distribution and intensity. The subjects of this study were all adult male and female individuals. Although all sections from all brains were compared, representative coronal sections for an individual of each species are presented at six levels (Fig. 3.1 and 3.2: A-L) containing the relevant structures.

Nucleus accumbens and basal ganglia

The OTR binding signal in the nucleus accumbens core and shell was very strong and densely distributed in the telencephalon of the ice rats (Fig 3.1, Table 3.1). In contrast, a very weak signal (and absent in most animals) was detectable dorsal to the ventral pallidum and near the major islands of Calleja in the shell of the nucleus accumbens of the vlei rats (Fig 3.2, A-D). OTR binding was observed in the islands of Calleja in both species, with very strong binding in ice rats, but weak to mild signals in vlei rats (Fig 3.3). The olfactory tubercle had strong signals of OTR in the telencephalon of the ice rats, with moderate signals in the vlei rats. No sex differences were found in the distributions and intensities of OTR binding in the nucleus accumbens for either species.

There was strong OTR binding in the ice rats for the lateral stripe of the striatum, whereas a mild signal was evident in the vlei rats (Fig. 3.1,3.2: E-H, Table 3.1). No OTR binding was observed in the caudate putamen or globus pallidus of the vlei rats; however, ice rats had diffuse, mild to strong OTR binding throughout the rostro-caudal extent of the caudate putamen, as well as strong signal in the globus pallidus (Fig 3.1, E-H). There was mild OTR

binding present in the dorsal part of the claustrum of vlei rats, whereas this binding was strong in the ice rats (Fig 3.1).

Cortex and Indusium griseum

Both species showed OTR binding in the prefrontal cortex (dorsal peduncular cortex). This signal was moderate in vlei rats, but was very strong in ice rat brains (fig 3.1,3.2:A-B; Table 3.1). While OTR binding in the indusium griseum was found in both species, it was only present in the more rostral regions of the vlei rats, displaying moderate signal at level 1 and 2 (Fig. 3.2:A-D). OTR binding was much stronger in the ice rat brain throughout the rostro-caudal extent of the indusium griseum (Fig 3.1:A-J).

In both species OTR binding was detected in the insular cortex (dorsal, granular and agranular) (Fig.3.1, 3.2). The binding was very strong throughout the rostro-caudal regions of the brain of the ice rats, but only moderate in the vlei rats. In the brains of the ice rat, there was marked, albeit diffusely distributed, OTR binding in the primary and secondary somatosensory cortex (S1ULp, S2)(Fig.3.1:E; Table 3.1). However, this binding signal was absent in the vlei rats. Very mild OTR binding was observed in the dorsal intermediate entorhinal cortex (DLEnt) of the vlei rats (Fig. 3.2), with none in the ice rats. Mild binding was found in the intermediate endopiriform nucleus (IEn) of the ice rats, with none in the vlei rats.

Septal nuclei

OTR binding was present in the lateral septal nuclei, extending from the rostral (dorsal, intermediate and ventral) parts, to the caudal parts, in both species. Signal intensity was very strong in both species in this region throughout the telencephalon (Table 3.1). There was also

strong OTR binding in the septohippocampal, septofimbrial and septohypothalamic nuclei in both species (Fig 3.1:E-H; 3.2:C-H).

Extended amygdala

Both otomyines displayed OTR binding in the extended amygdala, including the central, medial, basolateral and cortical areas (Fig. 3.1;3.2:C-L). Vlei rats showed moderate signal intensity in the central amygdala, with weak or mild signal of OTR binding towards the caudal end of the central amygdala (Fig. 3.2. K-L). There was moderate signal in OTR binding in the basolateral and medial nuclei of the amygdala in the brain of vlei rats. Ice rats displayed very strong OTR binding throughout the central amygdala, with moderate signal in the basolateral and medial parts, as well as the cortical posterior parts (Table 3.1).

In both species, strong OTR binding was observed within the bed nucleus of the stria terminalis, in the dorsal, lateral and medial parts (fig 3.1;3.2) throughout the rostro-caudal extent. The interstitial nucleus of the posterior limb of the anterior commissure (IPAC) had a very intense and strong OTR binding in the ice rats, but only mild to weak signal in the vlei rats.

Hippocampus

The hippocampus of ice rats had a very marked and intense signal for OTR binding in the CA1, 2, and 3 subfields, whereas vlei rats did not. A very weak, signal was observed in the CA1 and CA3 fields of the hippocampus of vlei rats (Fig. 3.2:I-L). There was some mild to weak OTR binding observed in the granular layer of the dentate gyrus (Fig. 3.2:I-L) of vlei rats, but no signal was observed in the dentate gyrus of the ice rats (Table 3.1).

Hypothalamus

Both species had strong signals in the medial preoptic nuclear area (fig 3.1; 3.2: I-L) as well as moderate OTR binding in the horizontal band of the diagonal limb of the hypothalamus (HDB). There was marked moderate signal in the ventromedial hypothalamic nucleus (VMH) and arcuate hypothalamic nucleus (Arc) of the vlei rats, which was absent in the ice rats (Table 3.1). The vlei rat brains also showed mild OTR binding in the ventrolateral preoptic nucleus (VLPO), the medial parvocellular part of the paraventricular hypothalamic nucleus (PaMP), and the dorsomedial hypothalamic nucleus (DMD), which was also absent from the ice rat. There was mild signal for OTR in the suprachiasmatic nucleus in the ice rat hypothalamus, which was not observed in the vlei rat.

Thalamus and other regions

OTR binding signal was intense in the paraventricular thalamic nucleus throughout the anterior and posterior parts, in both species. The intermediodorsal thalamic nucleus (IMD) and rhomboid thalamic nucleus (Rh) showed strong OTR binding in the brain of the ice rat (Fig 3.1:I-L); the signal in these regions was absent from the vlei rat brain (Fig 3.2). Conversely, there was a distinct signal for OTR binding in the medial habenula (MHb) in the vlei rats, but this signal was also absent from the ice rats (Table 3.1). OTR binding was only present in the bed nucleus of the accessory olfactory tract (BAOT) of the vlei rat brains (Fig 3.2, I-J).

Abbreviations used in autoradiographs (other abbreviations are given in the text)

ACo	**Anterior cortical amygdaloid nucleus**
AcbC	**accumbens nucleus, core**
AcbSh	**accumbens nucleus, shell**
AID	**agranular insular cortex, dorsal part**
AIP	**agranular insular cortex, posterior part**
AIV	**agranular insular cortex, ventral part**
Arc	**arcuate hypothalamic nucleus**
BAOT	**bed nucleus of the accessory olfactory tract**
BLA	**basolateral amygdaloid nucleus, anterior part**
BLV	**basolateral amygdaloid nucleus, ventral part**
BMA	**basomedial amygdaloid nucleus, anterior part**
CA1	**field CA1 of the hippocampus**
CA2	**field CA2 of the hippocampus**
CA3	**field CA3 of the hippocampus**
CeA	**central amygdaloid nucleus, anterior part**
CeC	**central amygdaloid nucleus, capsular part**
CeL	**central amygdaloid nucleus, lateral division**
CPu/CP	**caudate putamen (striatum)**
DCI	**Dorsal part of claustrum**
DI	**dysgranular insular cortex**
DLEnt	**dorsal intermediate entorhinal cortex**
DMD	**dorsomedial hypothalamic nucleus, dorsal part**
DP	**dorsal peduncular cortex**
GI	**granular insular cortex**
GP	**globus pallidus**
HDB	**nucleus of the horizontal limb of the diagonal band (hypothalamic nucleus)**
ICj	**islands of Calleja**
IEn	**dorsal endopiriform nucleus**
IG	**indusium griseum**
IMD	**intermediodorsal thalamic nucleus**
IPAC	**interstitial nucleus of the posterior limb of the anterior commissure**
LaDL	**lateral amygdaloid nucleus, dorsolateral part**
LSD	**lateral septal nucleus, dorsal part**
LSI(1)	**lateral septal nucleus, intermediate part**
LSS	**lateral stripe of the striatum**
LSV	**lateral septal nucleus, ventral part**
MeAV	**medial amygdaloid nucleus, anteroventral part**
MePD	**medial amygdaloid nucleus, posterodorsal part**
MePV	**medial amygdaloid nucleus, posteroventral part**
MHb	**medial habenular nucleus**
MnPO	**median preoptic nucleus**
NA	**nucleus accumbens**
PaMP	**paraventricular hypothalamic nucleus, medial parvicellular part**
PFC	**prefrontal cortex**
PMCo	**posteromedial cortical amygdaloid nucleus**
PV	**paraventricular thalamic nucleus**
PVA	**paraventricular thalamic nucleus, anterior part**
PVP	**paraventricular thalamic nucleus, posterior part**
Rh	**rhomboid thalamic nucleus**
S1ULP	**Somatosensory cortex, primary**
S2	**somatosensory cortex, secondary**
SFi	**septofimbrial nucleus**
SHi	**septohippocampal nucleus**
SHy	**septohypothalamic nucleus**
st	**stria terminalis**
STLD	**bed nucleus of the stria terminalis, lateral division, dorsal part**
STLP	**bed nucleus of the stria terminalis, lateral division, posterior part**
STLV	**bed nucleus of the stria terminalis, lateral division, ventral part**
Tu	**olfactory tubercle**
VDB	**nucleus of the vertical limb of the diagonal band (hypothalamic nucleus)**
VLPO	**ventrolateral preoptic nucleus**
VMH	**ventromedial hypothalamic nucleus**
VP	**ventral pallidum**

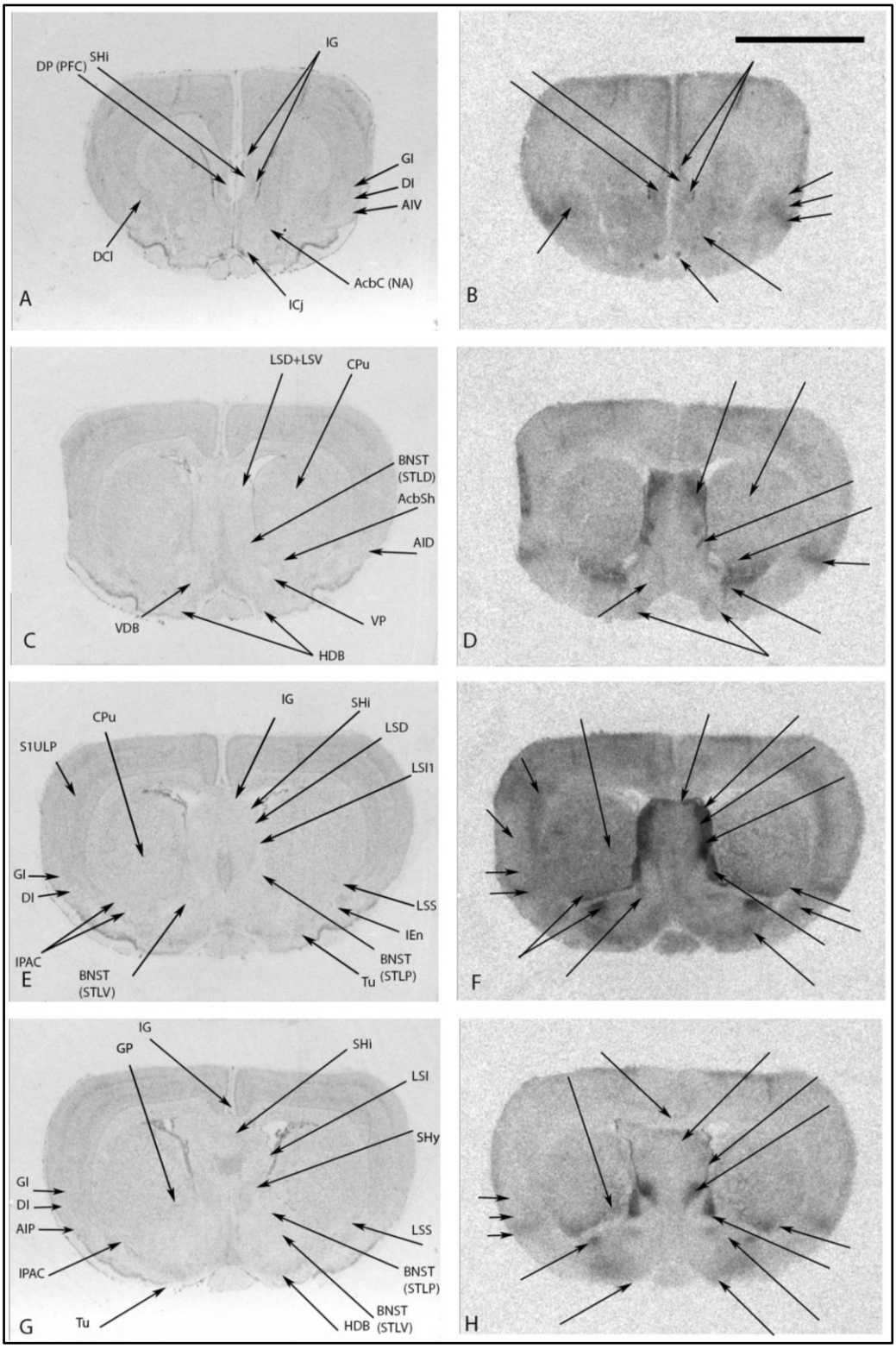

Figure 3.1: Photomicrographs of representative coronal brain sections ice rats showing Nissl-staining (left images) and corresponding Oxytocin receptor (OTR) binding (right images) using ^{125}I-OVTA. Scale bar = 5mm (see abbreviation list on previous page, for fig. 3.1 and 3.2)

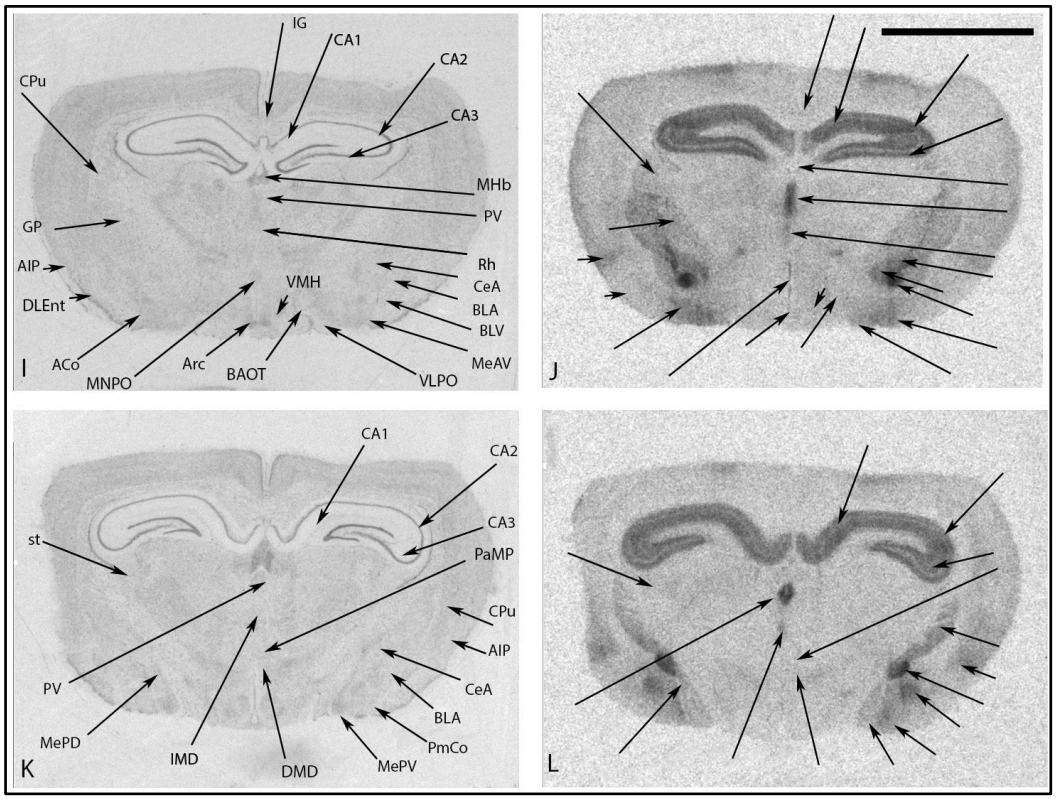

Fig 3.1: Continued

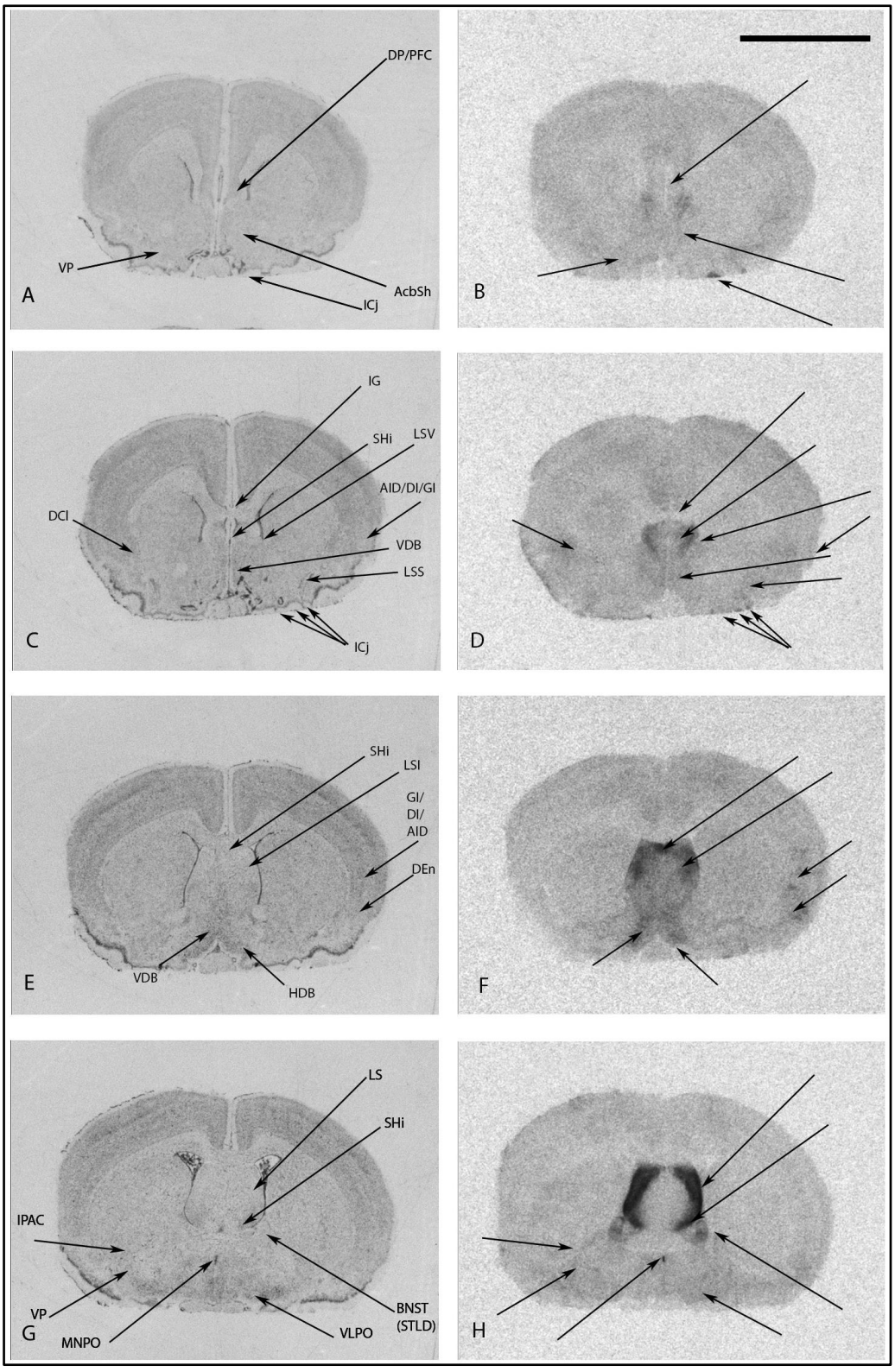

Figure 3.2: Photomicrographs of representative coronal brain sections of vlei rats showing Nissl-staining (left) and corresponding Oxytocin receptor (OTR) binding (right) using ^{125}I-OVTA. Scale bar = 5mm

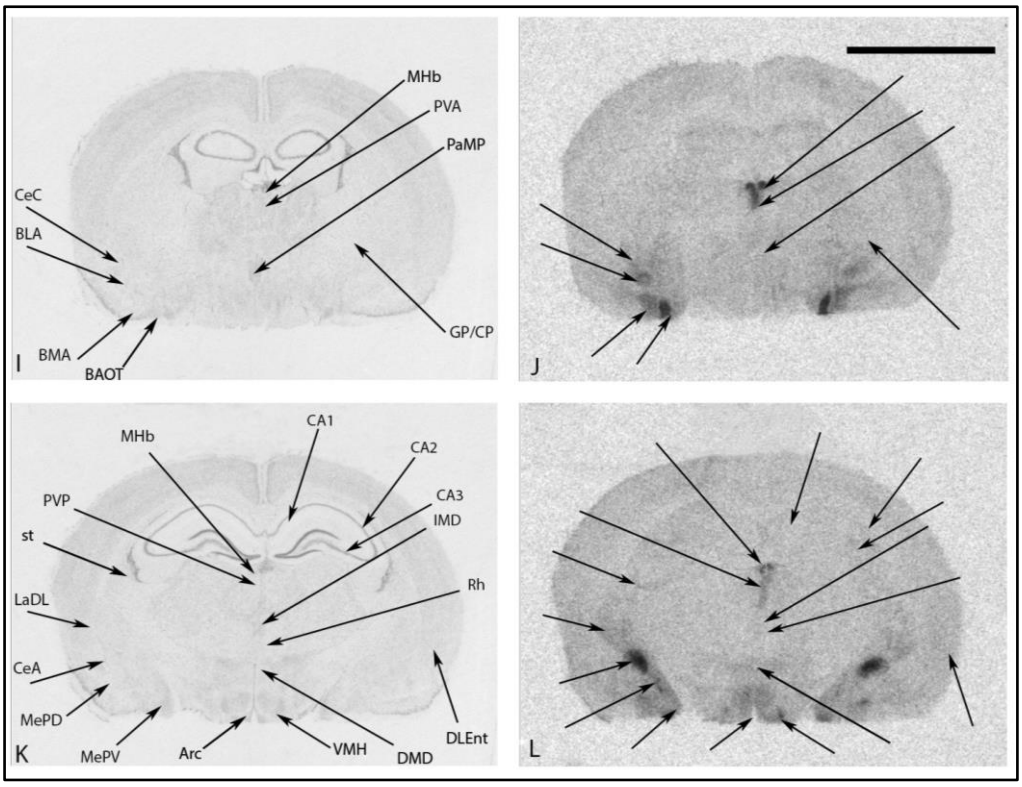

Figure 3.2: Continued

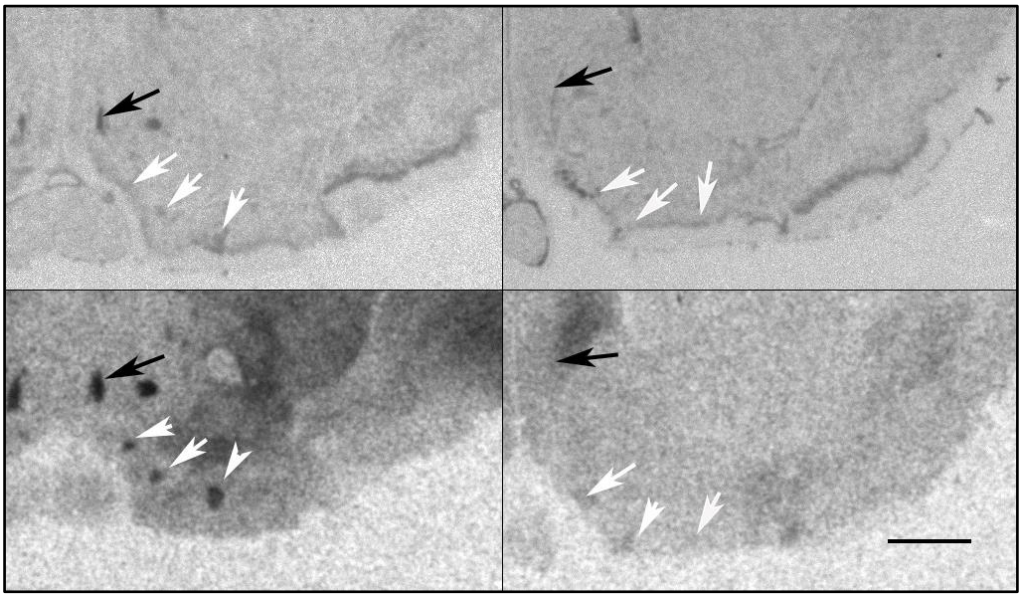

Figure 3.3: Photomicrographs of rostral sections of ice (upper and lower left) and vlei rats (upper and lower right). Marked differences in the Islands of Calleja Major (Dark arrows) and minor (light arrows) exist between the two species. Scale bars = 1 mm

Table 3.1: Semi-quantitative OTR binding of some of the most prominent structures observed in *Otomys auratus* and *Otomys sloggetti* brains. Intensity scores are designated as: – absent; * mild; **moderate; *** strong. NM=not measured. See text for further details.

Neural area	Description	Vlei rat	Ice rat
Olfactory system			
BAOT	Base nucleus of the accessory olfactory tract	***	-
AOP	Anterior olfactory nucleus, posterior	*	NM
ICj	Islands of Calleja	*	***
Cortical Areas			
PFC	Prefrontal cortex (infralimbic region)	*	***
Pir/En	Piriform cortex/Endoperiform nucleus	-	*
Insular	Dorsal, granular and agranular cortices	**	***
S1ULp/S2	Somatosensory cortices	-	**
Basal ganglia			
NA	Nucleus accumbens	-	***
CPU	Caudate putamen	-	**
VP	Ventral pallidum	-	***
Limbic system			
LS	Lateral septum	***	***
BnST	Bed nucleus of stria terminalis	**	***
IPAC	Interstitial nucleus of the posterior limb of the anterior commissure	-	***
CeA	Central nucleus of the amygdala	**	***
BLA	Basolateral nucleus in the amygdala	**	**
PostA	Posterior amygdala (medial/cortical)	*	*
CA1	Hippocampus, field 1 of Ammons Horn	-	***
CA3	Hippocampus, field 3 of Ammons Horn	-	***
DG	Dentate gyrus	*	-
Thalamus			
Midline/PVA	Paraventricular thalamus	***	***
Other thalamus	Rhomboid nucleus, Medial Habenula	***	-
Hypothalamus			
IG	Induseum griseum	*	*
HDB	Horizontal limb of the diagonal band	**	-
MPOA	Medial Preoptic area	***	***
VMH	Ventromedial hypothalamus	***	-
Arc	Arcuate nucleus of the hypothalamus	**	-
SHi, SHy	Septo-hippocampal, -hypothalamic nuclei	***	***

Discussion

The present study compares OTR binding in the brains of ice rats and vlei rats. The aim of the study was to describe and identify differences between these two species that show opposing social behavioural characteristics. However, upon further review, it seems that ice rats may not be truly social, but merely tolerant of each other in underground burrows during harsh winter conditions, since they show differences in tolerance and aggression spatially and temporally toward conspecifics (Hinze *et al*. 2013). Nevertheless, ice rats do have a more gregarious lifestyle than vlei rats, and the OTR binding sites in the brains of the two species are discussed in the light of these behavioural differences.

Nucleus accumbens

Ice rats were found to have intense and extensive OTR binding in the nucleus accumbens (NA) throughout its extent in both males and females. In contrast, OTR binding in the nucleus accumbens of vlei rats is largely absent apart from a relatively weak signal in the shell of the NA. The nucleus accumbens has been linked to monogamous pair bonding as well as maternal bonding behaviour in various rodent species (Young and Wang, 2004) and other mammal species such as sheep (Levy *et al.* 1995) and primates (Chang *et al.* 2012). Monogamous prairie voles show a greater number of OTR binding sites than polygamous montane voles (Insel and Shapiro, 1992; Olazabal and Young, 2006a), which lack pair bonding behaviour. Similarly, the eusocial naked mole-rat exhibits much greater OTR binding in the nucleus accumbens compared to the solitary, promiscuous Cape mole-rat (Kalamatianos *et al.* 2010) and peripheral administration of oxytocin increases huddling and investigative behaviour in naked mole-rats (Mooney *et al*. 2014). Same-sex voles that show increased huddling and tolerance behaviour towards conspecifics, have greater OTR binding in the nucleus accumbens (Beery and Zucker, 2010), providing support for the role that the OT plays in same-sex social behaviour. In the

nucleus accumbens of laboratory rats and mice OTR binding is low (Tribollet, 1992; Bales and Perkeybile, 2012; Beery *et al*. 2008). Therefore, this extensive body of evidence is consistent with the current findings of greater OTR binding in the NA of the socially gregarious ice rats compared to the almost absent OTR binding in the solitary vlei rats.

Vlei rats show very little socio-sexual contact, except during mating (Davis, 1973); therefore, low or absent OTR binding in the nucleus accumbens is to be expected. The more intense OTR binding in the NA of the ice rats compared to the vlei rats in the present study can be associated with the increased tolerance and gregarious behaviour in their underground communal nests and burrows (Hinze, 2005). Maternal bonding and altruistic parental care has also been linked to OTR binding in the NA, for example in voles (Olazabal and Young, 2006a) and in rats (Febo *et al.* 2005). By experimentally inhibiting OT expression, spontaneous maternal behaviour in prairie voles can be impaired, which, in turn, can be restored by infusion of OT in the nucleus accumbens, showing that maternal behaviour is correlated with OTR density in the NA shell (Olazabal and Young, 2006a).

In this study, a pup from a female ice rat with 5 pups was successfully accepted by a female that had a litter of only three pups. However, this behaviour may be as a result of the increase in circulating OT, since OT has been shown to increase social tolerance during gestation and lactation (Bielsky and Young, 2004). The mere presence of OTR binding may not necessarily indicate binding of OT in the brain, and the OT receptors in the NA of ice rats may indeed be vestigial. However, this seems not to be the case, as gregarious behaviour and social tolerance underground is indeed evident in the ice rat species, and no social tolerance whatsoever has been observed in the adult vlei rats, both in this study or previously. Therefore, I suggest that the greater OTR binding in the NA of ice rats helps to explain their gregarious/prosocial

behaviour, and this, together with the antisocial, aggressive behaviour to conspecifics of the vlei rats and their corresponding low level of OTR binding, provides support for the role of OT-ergic effects in the NA and social behaviour.

Islands of Calleja

As in the nucleus accumbens, much stronger OTR binding is observed in the ICj of the ice rat compared to the solitary vlei rats in the present study. The major and minor islands of Calleja have been linked to the dopaminergic system, which is also reciprocally linked to the nucleus accumbens and the ventral tegument (Ridray *et al.* 1998). The markedly stronger signal of OTR binding in both the major and minor Islands of Calleja in the ice rats may thus indicate that OT would have a more intense dopaminergic effect in those brain regions. A similar pattern is observed in the nucleus accumbens and ICj of other solitary and social rodents (Beery *et al.* 2008). However, stronger OTR binding was observed in the Islands of Calleja in polygamous deer mice than in monogamous California mice (Insel *et al.* 1991) and laboratory rats (essentially promiscuous) also have strong OTR binding in this region (Kremarik 1995; Gimpl and Fahrenholz, 2001). Also, with decreased socio-typical behaviour, an increase in OTR binding of the ICj was found in other species (Ridray *et al*. 1998, Schwartz *et al*. 1998).

In contrast, in eusocial naked mole-rats and solitary Cape mole-rats, as well as monogamous prairie voles and polygamous montane voles, similar intensities of OTR binding in the ICj minor and major were found (Kalamatianos *et al.* 2010; Olazabal and Young, 2006a). Furthermore, both social tuco-tucos and non-social Patagonian tuco-tucos lack OTR binding in the ICj (Beery *et al*. 2008). Kalamatianos *et al*. (2010) suggests that diffusion of OT in these regions, rather than OT-receptors in the ICj, may explain the observed social disparity between the two mole-rat species. As previously mentioned, in rats, the major and minor islands of

Calleja are connected with other areas in the brain (Fallon *et al.* 1978, Fallon 1983; Kalamatianos *et al.* 2010) that do show a differential expression of OTR binding in ice and vlei rat species. Therefore, the functioning of OT in the islands of Calleja is indeed species-specific as no clear pattern or trend in OTR expression in the ICj can be extrapolated to mammalian species, at least in rodents. It is possible that ice rats and vlei rats may not actually show extreme differences in social behaviour, and rather exhibit social differences on a continuum. The lack of major differences in the OTR binding of the ICj reflects this, as both vlei and ice rats showed OTR, albeit at different intensities.

Basal ganglia

Stronger OTR binding was found in the striatum of ice rats when compared to vlei rats. This was expected since the striatum is a transitional region between the amygdala and nucleus accumbens (Veinante and Freund-Mercher, 1997), in which greater OTR binding has been shown in social species (Olazabal and Young, 2006b). Diffuse, strong to mild OTR binding was observed in the caudate putamen (CPu) of the ice rats, whereas it was lacking in the vlei rats. While no detectable OTR binding was found in the caudate putamen of eusocial mole-rats or solitary Cape mole rats (Kalamatianos *et al.* 2010), it has been found in monogamous prairie voles, but not polygamous montane voles (Olazabal and Young, 2006b; Beery *et al*. 2008). Similar to the effect of OT in the nucleus accumbens, greater OTR binding in the CPu has been found to correlate with an increase in cooperative parenting in rodents (Olazabal and Young 2006b). The results from the present study may provide further support for the function of OT in the gregarious behaviour of ice rats and the overall function of the CPu in social behaviour, although many species-specific differences occur and further mechanistic experimental studies need to be conducted before the exact functioning of the CPu in social behaviour can be established.

While it was expected that the ventral pallidum would show greater OTR binding in the ice rats, OTR was only strong in a region known as the globus pallidus, and absent in the vlei rats. The globus pallidus (GP), extends to the ventral pallidum and also receives projections from the olfactory tubercle. It can also be considered part of the extended amygdala and OTR binding in this region of prairie voles has been found, compared to solitary voles (Lim *et al*. 2004). The strong OTR binding signal in the olfactory tubercle of the ice rats, with moderate signal in the vlei rats, and the strong signal in the globus pallidus of the ice rats, appears to indicate an apparent prosocial circuitry of OT in the ice rats, similar to that of prairie voles, although this is speculative without further experimental study. Interestingly, OTR binding is present in the GP of humans (Loup *et al.* 1991) and prolactin binding occurs in this region in oestrogen treated, ovariectomised rats (Pi and Grattan, 1998).

OTR binding was found in the claustrum of both vlei rats and ice rats, the latter having a much stronger signal. Claustrum OTR binding has rarely been found, but arginine-vasopressin (a neuropeptide involved in social behaviour) binding has been shown in the claustrum of guinea pigs (Tribollet *et al*. 1992). The claustrum seems to be related to the sensorimotor function of chewing and linked to sexual stimulation (Edelstein and Denaro, 2004). It is also a transitional area between the insular cortex, especially the region responsible for somatic and visceral effects, and the caudate putamen and amygdala (Edelstein and Denaro, 2004). Therefore, there may be a link between OTR binding in the claustrum and somatic and/or sensory function in the insular cortex. Since suckling of young stimulates OTR binding (Febo *et al.* 2005), it may be concluded that the functioning of OT in suckling and maternal-offspring behaviour is coordinated by the claustrum and its role in social behaviour. Ice rats may have stronger maternal social behaviour than vlei rats, but this needs to be investigated directly.

Indusium griseum and Cortex

In ice rats, OTR binding was seen throughout the rostro-caudal extent of the indusium griseum, whereas it was only present in the more rostral regions in the vlei rats. While the exact function of this structure is not yet clear, OTR binding in the indusium griseum of eusocial mole-rats is similar to that observed in the ice rats, but solitary mole-rats do not have any binding sites there (Kalamatianos *et al*. 2010). The OTR binding in the indusium griseum of tuco-tucos resembles the binding pattern of the vlei rats, but occurs in equal intensities in social and solitary tuco-tucos. Binding in the indusium griseum is absent in laboratory mice, but has been found in the brains of social Syrian hamsters (Beery *et al*. 2008). Although OTR binding in the indusium griseum does not reflect social behavioural differences (it is not consistently found in social mammalian species) it may indicate some degree of variation across a spectrum of OTR-mediated social behavioural traits. Beery *et al* (2008) concluded that a range of environmental factors can affect the group-living gregarious nature of species, and therefore the role and function of OT, and consequently, the distribution of OTR in the brain, may vary across different species. Since the indusium griseum is stimulated by afferents from the olfactory bulbs (Kunzle, 2004), this may indicate that olfactory cues, are more pronounced in social memory formation, and to a degree, subsequent social bond formation in ice rats, and not in vlei rats.

Stronger OTR binding was observed in the ice rats than the vlei rats in the infralimbic region of the prefrontal cortex (dorsal peduncular cortex). The infralimbic, as well as the prelimbic areas, have links to olfactory structures such as the olfactory tubercle; the prefrontal cortex has projections to the raphe nuclei, which in turn exerts a serotonergic effect in the cerebral cortex related to impulse behaviour in rats (Heidbreder and Groenwegen, 2003), Such impulse

behaviour includes flexibility in spatial and visual discrimination, responses to environmental stimuli, as well as integrating 'goal-directed initial responses' (mediated by the prelimbic cortex) with learnt behaviour from repetitive habituation (Heidbreder and Groenwegen, 2003). Such learnt behaviour may include social recognition related to prosocial behaviour. Therefore, I speculate that olfactory sensing could mediate impulsive reactions via this infra- and prelimbic/prefrontal cortex axis. Olfactory stimuli include attraction towards a mate in gregarious ice rats, or a same-sex conspecific, for example, and this would not be the case in solitary vlei rats. This is supported by the observation that stronger OTR binding has also been found in the prelimbic region of social prairie voles compared to solitary montane voles (Insel and Shapiro, 1992) and thus OT in ice rats may play a role in cerebral processing, as would be expected in a prosocial animal.

Other Cortical areas

Ice rats exhibit strong OTR binding in the dorsal, granular and agranular insular cortices, while the vlei rat shows very mild-moderate binding. The insular cortex is structurally similar to the amygdala and has projections to many of the regions already discussed and also the primary and secondary somatosensory areas, amygdala, prefrontal cortex, olfactory bulb, hippocampus, entorhinal cortex and motor cortex (Nagai *et al.* 2007). Interestingly, the marked OTR binding in the somatosensory cortices of the ice rats was completely absent in the vlei rats. The insular cortex processes visceral sensory information, as well as pain, olfactory, visual, auditory and tactile information (Nagai *et al.* 2007), and has a function in the discrimination of sensory information (Berman *et al.* 1998). It is also critical for memory formation and recall of specifically previously encountered reward stimuli (Cardinal *et al.* 2002). Therefore, it is conceivable that enhanced gregarious behaviour can be related to the ability to recall social conspecifics, as OT has been linked to social memory in rodents (Ferguson *et al*. 2000). From

a literature review, I found in no other wild rodent species has OTR binding been observed in this region and the potential function of OT in ice rats may be interesting to explore.

While it was expected that ice rats would exhibit OTR binding in the piriform cortex, OTR binding was absent in the piriform cortex in the sections investigated, but was present in the intermediate endopiriform nucleus. No binding was observed in these areas in the vlei rat. Kalamatianos *et al.* (2010) speculated that the OT function in the piriform cortex is involved in suckling and maternal behaviour in eusocial mole-rats, and the endopiriform nuclei have been implicated in connections between the piriform cortex and amygdala, processing stress and anxiety related information (Goldstein *et al*. 1996). Therefore, it is conceivable that OT induces an anxiety reducing effect in this region. The presence of OTR in ice rats, and not in vlei rats, is in accordance with the perceived tolerant, i.e. less anxious, behaviour that may arise when in an underground, gregarious social situation in nests and burrows. This is also supported by the greater intensity OTR binding found in social species of tuco-tuco's and social prairie voles, compared to their solitary counterpart species (Beery *et al.* 2008).

Septal nuclei

OTR binding is detected in similar intensities in the lateral septum of both species in the present study. This is in contrast to lower OTR binding found in a solitary species of tuco-tuco when compared to a social species (Beery *et al*. 2008; Beery and Zucker, 2010) and, similarly, non-social species of voles have more OTR binding than social species (Insel and Shapiro, 1992). However, OT function in the lateral septum seems to be species-specific and cannot be generalised. OTR binding is absent from both eusocial and solitary mole-rats (Kalamatianos *et al*. 2010). This may indicate that vlei rats and ice rats are more similar to one another than other social and solitary related species are to one another, an assertion that is further supported by

the similarities in certain regions previously discussed. Ice rats may indeed be less prosocial than previously thought (Hinze, 2005), and show more similarities to solitary vlei rats.

OTR binding is present in both species in the septohippocampal and septofibrial nuclei. These nuclei are involved in transient memory formation (Brito *et al.* 1990). Similar OTR binding was observed in guinea pigs (Tribollet *et al*. 1992), indicating that OT also has an impact in social behaviour of less social species.

Extended amygdala

Although OTR binding was present in both species in the central amygdaloid nucleus, as well as in other amygdaloid nuclei, binding was more intense in ice rats compared to vlei rats. The effect of the central amygdaloid nucleus can be viewed as the sum of the other interconnected amygdaloid nuclei (Pitkänen *et al.* 1997). This region has been shown to be involved in inhibiting stress and anxiety (Choleris *et al.* 2007), as well as the medial amygdala, which is also involved in processing social recognition (Choleris *et al.* 2007; Van der Kooij and Sandi, 2012; Ferguson *et al*. 2001). The medial amygdala also projects to the BNST, a site associated with maternal behaviour (Febo *et al*. 2005) and greater maternal investment in young (Campbell *et al*. 2009), thus it was surprising that the solitary vlei rats species also showed strong OTR binding in the BNST, similar to the ice rats.

OTR binding is greater in the central amygdaloid nucleus in social tuco-tucos (Beery *et al*. 2008) and eusocial mole-rats (Kalamatianos *et al*. 2010), but similar in social prairie voles and solitary montane voles (Insel and Shapiro, 1992). In addition, greater OTR binding is observed in the medial and cortical nuclei in social mole rats (Kalamatianos *et al*. 2010) and prairie voles (Beery *et al*. 2008), compared to their solitary counterparts. The mere presence of OT receptors

in the amygdala does not necessary imply functionality of OT in that area and OT immunoreactive processes need to be present to confirm this. Conversely, the fact that both species in this study exhibit similar distributions of OTR in the amygdala could indicate similar effects of OT on anxiety inhibition and maternal behaviour, further supporting the working hypothesis that these species are more similar in social behaviour than previously thought.

Hippocampus

Ice rats exhibited strong OTR binding in the hippocampus, whereas it was virtually absent in vlei rats, apart from in the dentate gyrus. The hippocampus, which has been associated with memory formation, is reciprocally linked to the amygdala, with the CA1 subfield of the hippocampus projecting to - and containing afferents of - the central amygdaloidal nucleus (Kishi *et al*. 2006). A large amount of OTR expression has been found in the CA3 subfield of rats, which is also connected to the medial amygdala (Ferguson *et al.* 2000) and the hippocampus has been shown to be involved in social memory (Lee *et al*. 2009; van Wimersma Greidanus and Maigrat, 1996; Feinberg *et al.* 2012).

The functionality of hippocampal stimulation by OT varies across species. OTR binding is absent in a social and a solitary vole species (Insel and Shapiro, 1992), however OTR binding has been found in the hippocampus of meadow voles, essentially a solitary species (yet seasonally 'socially' flexible), which was enhanced when an intracranial injection of OT was administered (Beery and Zucker, 2010). However, it has been found to be much stronger in eusocial mole-rats than solitary mole-rats (Kalamatianos *et al*. 2010), as well as in more socially vocal singing mice (Campbell *et al*. 2009). In both studies it was suggested that OT plays a role in the spatial memory formation of the species, increasing their fitness. Hippocampal OTR binding is effectively absent in the vlei rats, which inhabit mesic

environments with abundant resources spread across large areas. I speculate that increased spatial memory in ice rats may provide an adaptive advantage in their more restricted, harsher montane environment. Since space is limiting factor there, especially during winter conditions when individuals huddle and live communally underground, increased social memory related to spatial memory would increase the adaptive fitness of the species.

The brains of vlei rats exhibited OTR binding in the dentate gyrus, and this has only been found in solitary mole-rats (Kalamatianos *et al*. 2010), where the authors suggested that it may be involved in memory elimination. Vlei rats have a more exposed lifestyle (they do not have the protection of underground burrows) where they move quickly aboveground, compared to ice rats that have been observed to remain still and relaxed in open, exposed fields. I propose the significance of this relating to the dentate gyrus is that vlei rats may have less need for spatial memory and even benefit from such a memory eliminating system, thereby spending less effort and energy on spatial memory. A novel OTR binding site, not previously reported in other rodent species, was in the medial habenula (MHb) in the vlei rats. Although binding in the MHb was absent in the ice rats, the exact function of this structure remains unclear.

Hypothalamus and thalamic structures

OTR binding was present in the horizontal limb of the diagonal band (HDB of hypothalamic nuclei) of both species, although feint (considered absent in table 3.2) for the ice rats. The signal was absent in the vertical band (VDB) of ice rats, but present in vlei rats. Similar OTR binding signal patterns were observed in social and solitary tuco-tucos, (Beery *et al.* 2008). The medial preoptic area has been shown to induce social recognition (Popik and Van Ree, 1991) and maternal behaviour (Pedersen *et al*. 1994), facilitated by OT function. Despite being solitary, tuco-tuco mothers still tolerated pups, and the increased maternal behaviour,

facilitated by OT, may explain the vlei and ice rat results for the medial preoptic area. The strong OTR binding in the hypothalamic nuclei of the vlei rats is similar to that found in laboratory rats, where oestrogen increased OTR binding (Johnson *et al*. 1989), and stronger hypothalamic OTR binding was also observed in non-social montane voles (Insel and Shapiro, 1992). This is expected, as OT binding in the hypothalamic nuclei is involved in social and maternal behaviour, and supports the greater hypothalamic signal results found for the vlei rats. Furthermore, OTR binding is absent in the VMH, PaMP and DMD of the ice rats, and this is unexpected for a social species. However, OTR binding in the hypothalamic paraventricular nucleus (PV, PVP, PVA) has been linked to reduced stress in rats (Gray *et al*. 1989; Windle *et al*. 2004). It follows thus that I expected that ice rats would show stronger OTR binding in these areas, but since they did not, the current results may suggest increased stress in the ice rats. The stronger OTR binding in these areas of the vlei rat are difficult to explain and requires further investigation, but species and taxon specific variations may show these structures to be more complex.

Interestingly, the strong OTR binding in the intermediodorsal thalamic nucleus (IMD) and rhomboid thalamic nucleus (Rh) of the ice rats, was absent in the vlei rats. These structures are connected to the amygdala and are involved in the reduction of anxiety and stress (Gray *et al*. 1989). The two species in this study showed similar OTR binding in the paraventricular thalamic nucleus, which has been linked to reducing stress as well as regulating maternal behaviour in sheep (Da Costa *et al*. 1996). This may be explained by both species exhibiting maternal behaviour, as evidenced by pup-licking in solitary vlei rats (G. Göldner, pers. obs; Davis, 1973) and ice rats (G. Göldner, pers. obs; Willan, 1990). Furthermore, this reduction in stress may be enhanced in ice rats, possibly contributing to their huddling and communal grooming underground (Hinze, 2005). However, some social species such as tuco-tucos (Beery

et al. 2008) and prairie voles (Olazabal and Young, 2006a) have greater OTR binding in this region, which provides further support for the continuum of OT functionality in maternal behaviour and social tolerance amongst rodent taxa. Interestingly, a related species, *Otomys unisulcatus,* is the only otomyine rodent that supplements nipple-clinging maternal behaviour with bouts of mouth-carrying (Pillay *et al.* 1993), and also has been shown to occur in occasional familial groups at nest sites. It is conceivable that *O. unisulcatus* may exhibit social tolerance and gregarious behaviour that is intermediate between that observed in vlei and ice rats.

Conclusion

The overall patterns of OTR binding in the ice rats and vlei rats of the present study are similar to that found in social and solitary voles. However, some OTR binding occurred in regions not previously documented in other species. OTR binding was present in brain regions associated with social behaviour, olfactory and spatial sensory processing, as well memory processing. This may shed some light on the evolution and adaptive benefits of socially tolerant behaviour that is modulated by neuropeptides and may provide some support for the role of the aridity-food hypothesis in the formation and explanation of gregarious and group living in mammals.

While interesting, correlation does not imply causation, and more mechanistic experimental studies need to be performed on the functioning of OT on social behaviour in these two wild caught, non-typical model species. Although the ice rat shares many of the patterns of OTR binding observed in documented social rodent species, these patterns also appear very similar to that of its solitary cousin, the vlei rat, in some aspects. It is therefore concluded that there may exist a continuum of the effects that oxytocin has on the social and prosocial, or gregarious

and group living behaviour of closely related mammal species. Still, the differences in neuropeptidergic circuitry in these two species from markedly opposing habitats contribute to the understanding of neuroendocrine systems in sociality.

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Chapter 4: General Discussion

Social recognition plays a vital role in the formation and maintenance of social relationships in mammals. Specifically, rodents rely on a subclass of urinary pheromones known as Major Urinary Proteins (MUPs) and the Major Histocompatibility Complex (MHC) to distinguish between individuals (Brennan and Kendrick, 2006; Spehr *et al.* 2006). These chemical messengers stimulate signals in sensory neurons related to the olfactory regions in the brain, which, in turn have afferents to specific hypothalamic and telencephalic neuronal structures that have receptors for neuropeptides (Choleris *et al.* 2009). Therefore, the neuropeptides, oxytocin and vasopressin, play an important and intriguing role in social recognition, the cornerstone of social behaviour and sociality in mammals.

In this study, I investigated and compared the sociality of two otomyine, murid rodent species - the solitary vlei rat and the social-living ice rat. For this purpose, I used a behavioural paradigm based on the innate olfactory investigative behaviour of rodents. Using a social discrimination experiment, I determined whether differences existed in social recognition ability and preference for social novelty between both species. This work was complimented with a description of the oxytocin neural binding sites in these two species, as it was expected that their social behavioural differences would be reflected in neuroanatomy of these binding sites. A number of studies have aimed to establish mechanistic links between social behaviour and oxytocin function in the brain. For example, by experimentally manipulating oxytocinergic genetic differences (Ferguson *et al.* 2000; Winslow and Insel, 2002; Bielsky and Young, 2004), social recognition and discrimination abilities can either be attenuated or inhibited. By enhancing neuropeptide action by intracerebroventricular administration of oxytocin (Ferguson *et al.* 2002) and oxytocin infusion (Dluzen *et al.* 2000) in the brain, social recognition can also be altered. Therefore, I proposed to establish if social-living ice rats show social recognition and discrimination and whether their neuroanatomy reflected oxytocinergic

receptor binding similar to that found in other social-living, eusocial or gregarious species. In addition, I aimed to compare the solitary vlei rats with the ice rats and determine whether they lack social recognition and discrimination abilities, as well as displayed oxytocin receptor binding similar to other solitary, antisocial rodent species.

From previous work and the observed gregarious behaviour of ice rats, *Otomys sloggetti robertsi*, it was thought that this species might have a prevalence for social living (Willan, 1990). Closely related vole species show differences in pair-bonding behaviour related to social behaviour, such as prairie voles that have been shown to exhibit monogamous and social behaviour compared to polygamous montane voles (Olazabal and Young, 2006). In the African striped mouse, social flexibility can occur within individuals occurring in different ecotopes (Schradin *et al.* 2012). Schradin *et al.* (2012) found that those displaying solitary, anti-social, promiscuous behaviour occur in mesic environments with abundant resources, whereas those which are gregarious, socially tolerant and exhibit social behaviour occur in arid environments with limited resources. The aggregation of multiple male and female adult ice rats in the same burrow system and underground nests in the wild (Hinze *et al.* 2013; pers. obs.) justified the need to investigate possible features that these animals may possess. One such feature, which may contribute to their social-type lifestyle, is social recognition.

As I predicted, vlei rat males did not possess an ability to recognise or discriminate same-sex conspecifics in the experiments performed (Chapter 2). Surprisingly, female vlei rats did exhibit some form of social discrimination, but female ice rats did not. Hinze (2005) concluded that ice rats are less social than previously postulated since they show solitary-like behaviour above-ground during bouts of foraging. Furthermore, the present results show that the males of both species, also failed to exhibit social recognition abilities. This supports my notion that

vlei rats lack social recognition and are indeed solitary, territorially aggressive animals by nature, while it provides new insight into the properties of the behaviour of ice rats.

In summary, ice rats have an elementary social inclination, as shown by my data, and the social dichotomy demonstrated by Hinze (2005) supports this conclusion. However, social behavioural differences still occur between the two species. Hence, I suggest that the origin of these differences may rather lie in their physiology and the constraints the environment places on their behavioural characteristics on a temporal and spatial scale. In other words, ice rats may display social tolerance in response to environmental pressures, but, given the opportunity, may actually be similar to solitary vlei rats in terms of their social behaviour. I suggest that they rather be flexible in terms of their social behaviour, as reported for the striped mice (Schradin *et al.* 2012). Ice rats may possess this 'social flexibility' within the species, with their social behaviour adapting to differing environments, but this requires further investigation.

The neuroendocrinology of OTR binding sites in my study provides further support for the role of oxytocin in sociality, since the differences observed in these two species are similar to those reported from previous studies for socially disparate groups. There are some transitional characteristics in the intensity and distribution of OTR binding neural structures, as well as the social recognition capability of the two species. In terms of reproductive behaviour, the social structure of ice rats would be categorised as 'social' or 'colonial or gregarious', and that of vlei rats as 'solitary', based on the mating system employed by the species (Sherman *et al*, 1995; Burda *et al*. 2000). From the results of my study, I speculate that this may provide support that both ice and vlei rats occupy intermediate niches on the sociality spectrum/continuum. This is further supported by the fact that a closely related arid-living otomyine species, *Otomys unisulcatus*, may live either solitarily or in familial groups (Skinner and Chimimba, 2005), and

thus has transitional (or possibly 'flexible') social characteristics. Overall, ice rats may still retain characteristics relating to social behaviour of their solitary counterparts, but have in turn evolved neural circuitry which manifests as gregarious behaviour in adaptive response to montane environmental constraints.

Since social recognition is intricately linked to oxytocinergic systems in the brain, it was feasible to establish a correlation in these traits in the two species (Chapter 3). In eusocial mole-rats, oxytocinergic systems have been extensively studied (Rosen *et al.* 2008) and compared to that of solitary Cape mole-rats, *Georychus capensis* (Kalamatianos *et al.* 2010). My results from the neuroendocrinological study of the two otomyine species supported the observed behaviour in the wild, but it did not necessarily correlate with the social recognition abilities found in Chapter 2. Oxytocin receptors were present in ice rat brain regions that function in memory processing **(**for example the hippocampus**)**, as well as reward, pair-bonding (for example the nucleus accumbens**)** and spatial stimuli; all contributing to social behaviour and social recognition (Bielsky and Young, 2004), while vlei rat brains lacked these. Many similarities were found with OTR distributions of monogamous prairie voles in the ice rat brain, which provides a neuropeptidergic basis for gregarious behaviour in ice rats.

As was expected, when compared to the vlei rats, ice rats showed more intense receptor binding of oxytocin in regions relating to social behaviour such as the nucleus accumbens, lateral septum and amygdala. The difference in results of the two species were very similar to the oxytocin receptor distribution differences found in monogamous prairie voles and polygamous montane voles (Insel, 2010). Although there were differences in social recognition abilities between sexes in the behavioural study of Chapter 2, no observable sex differences occurred in neuro-circuitry of oxytocin receptors within each species. This was unexpected, as

oxytocinergic systems have been shown to display sexual dimorphism in some species, especially in the area of the nucleus accumbens of laboratory rats (*Rattus norvegicus)* (Bielsky en Young, 2004). The conclusions made from Chapter 2 would seem to support the notion that there should be an intraspecific sex difference in neuroanatomy in these species, but no such difference was found. While every attempt was made to ensure accuracy in the behavioural assays, sex and species differences other than social recognition ability may have affected the ability and motivation of test subjects to investigate conspecifics. Therefore, further studies to investigate social memory is advised. Spatial tests should also be included to ensure control of spatial memory in such experiments, as differences in OTR binding were found in the somato-sensory region (linked to spatial memory) in the brains of the ice rats.

While this study did not specifically detect social recognition abilities in ice rats, female ice rats appeared to exhibit characteristics of social recognition vital to social behaviour. Their social-type behaviour is also supported by the OTR binding in the brains. The differences of such binding distribution with that of vlei rats, a solitary otomyine species, also supports this. It is worth mentioning that the OTR binding sites were not statistically compared and quantified, and it is suggested that this should be done to get a more accurate impression of ice and vlei rat neuroanatomy. Also, describing and quantifying oxytocin producing neurons and fibres in these regions may provide clues as to the distribution of the hormone in the brain itself. This will reveal if the OTR binding sites in the ice rat brain are indeed functioning in social behaviour or merely vestigial. In addition to this, mechanistic studies can be performed where oxytocin can be inhibited experimentally (Ferguson *et al.* 2000; 2002) and retesting ice and vlei rats to ascertain social recognition abilities is advised.

Due to the fact that the behavioural data casts doubt that ice rats are more socially adept than their vlei rat cousins, I suggest that the gregarious behaviour observed in ice rats is merely a response to environmental constraints, and not necessarily an inherent predisposition towards sociality. The behavioural data supports prior work on ice rats that ascertained that their gregarious lifestyle is related to their response to environmental constraints during winter when resources are limiting, as evidenced by the social dichotomy they exhibit both spatially and temporally (Hinze *et al.* 2013). However, the neurohistological data confirms there to be distinct differences in brain structure previously observed in many other rodent and mammal species with disparate social behaviour (Beery *et al.* 2008; Kalamatianos *et al*. 2010; Anacker and Beery, 2013).

The neuroendocrinological differences between these two socially disparate species also provides support for the conclusion that differences in brain structure and function have occurred, specifically with the oxytocinergic and social recognition systems, that have influenced and regulated social behaviour. Therefore, my study has provided additional support for the functionality of oxytocin in social recognition**.** Studying the different features relating to social recognition and oxytocinergic neural circuitry, and describing it in this study, provides clues into the phylogenetic correlates of social behaviour. My study provides a novel investigation into how social recognition and oxytocinergic systems affect, or determine, social characteristics in mammals.

The two socially diverse species in this study did not possess social recognition abilities, however, the neuroendocrinological data support the functionality of oxytocinergic systems and binding sites in the regulation of group-living, and prosocial, behaviour. My results show similarities, but also differences, with oxytocin-impaired laboratory rats and mice, which lack

social recognition. The neuroanatomy of ice rats and vlei rats revealed similarities (particularly the lateral septum and amygdala) to other rodent species such as voles, tuco-tucos and mole rats (Anacker and Beery, 2013). However, some neural regions were completely unique to these species (for example, the medial habernula, insular cortex, sensory cortex and globus pallidus, see Chapter 3) and provide even more intriguing novel insights into the complexity that the oxytocinergic system has on social recognition and, ultimately, sociality. Therefore, while this study provides novel information regarding the differences in neural structure between two previously unstudied species, further investigation into the mechanistic links of otomyine social behaviour, social recognition and oxytocin needs to be conducted.

Interestingly, while collecting ice rats for this study, vlei rats were found to inhabit the same burrow systems occupied by ice rats (on several occasions, both vlei rats and ice rats were caught at the same burrow entrance). I suggest an exciting study would be to sample from a population of vlei rats found at Tiffindell and vlei rats from a mesic habitat. Since vlei rats are solitary, antisocial and aggressive towards conspecifics, such a study may well elucidate any social flexibility in this species caused by the different ecological factors, as hypothesised by the AFDH and is evident from the social flexibility of striped mice.

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