PAIR BONDING, THE PREFERRED MATING TACTIC OF *MICROTUS OCHROGASTER*, INDUCES CHANGES IN EXPRESSION OF AGGRESSION AND SOCIAL COGNITION, BUT NOT RECEPTOR DISTRIBUTION

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Abstract:

This thesis is comprised of 1 published paper (Chapter III), 1 paper in the peer review process (Chapter II) and 1 supportive chapter (Chapter IV). Chapters I and V contain the introduction and general conclusions respectively.

In Chapter II of this dissertation we consider the natural mating tactic of the prairie vole. We review the literature that contains controversial views on the "preferred" mating tactic exhibited by voles, versus the mating tactic taken on when "making the best of a bad situation." In addition, we address how the expression of aggression changes with pair bonding and familiarity in male voles. In Chapter III, we demonstrate that social cognition can be context dependent as we show that the life stage of the male prairie vole (bonded or not) affects his ability or interest in recognizing female conspecifics. In Chapter IV, we evaluate whether the behavioral shifts that we see as a result of pair bonding are mirrored with neural receptor distribution changes. In Chapter V, we summarize the major findings of this dissertation.

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Preface

This dissertation is comprised of five chapters, where Chapters I and V are the Introduction and Conclusion respectively. Chapter II has been submitted for publication to Animal Behavior, and awaits the completion of the peer review process. Chapter III has been published in Animal Behavior. Chapter IV is an unpublished study that serves as a supportive chapter.

CHAPTER I

INTRODUCTION

While humans have taken note of animal behavior for centuries, the actual field of ethology emerged at the beginning of the 1900's. Before that, Darwin was one of the earliest scientists who believed that behavior could be programmed (instinct) (Darwin, 1982) and that animals adapted behaviorally, just as they did physically. Later, Pavlov discovered conditioning (Pavlov, 1927), which Karl von Frisch used to demonstrate that bees could see both color and UV light, and that this ability determined the flowers they chose to feed from (von Frisch, 1956). But it was Konrad Lorenz, and his persistent observation of animals within their own environments (Lorenz, 1935/1937), that ultimately established the foundation for the emerging field of ethology. Since then, those ideals, along with additions from other early ethologists, have become the foundation of our field (Gould, 1982).

Now ethology allows us to study many types of behavior. Social behavior in particular, presents a unique field in which to investigate the diverse interactions between organisms. It includes affiliative, aggressive, reproductive and communicative behaviors. This dissertation examines numerous social behaviors exhibited in a social organism, the prairie vole, *Microtus ochrogaster*. This introductory chapter will briefly review various topics that provide literature relevant to introducing the subsequent chapters. The remainder of this chapter will briefly summarize Chapters 2, 3, and 4.

Mating Tactics: Monogamy

Mating tactics, or methods utilized by organisms to maximize fitness, are often highly evolved behaviors. In a given species, they are usually driven by one (reproduction limiting) sex

(Bateman 1948; Trivers 1972); typically the female, as she dedicates more resources toward the offspring during development, than the male. In this scenario, it is usually males that must compete for females (Andersson 1994). This dynamic is common to most mating systems, including monogamy. Monogamy can be defined generally as a form of exclusivity between mates (be that in a sexual or social relationship) (Dewsbury, 1987). It is most common among birds and is thought to have evolved through any number of avenues (including as a method to mate guard or avoid cuckolding in males).

There are certain characteristics that are thought to best set the stage for the manifestation of monogamy. Among them is decreased environmental potential for polygyny (EPP, Emlen & Oring, 1977). EPP is associated with a male's ability to monopolize or defend a greater number of resources/mates. When the potential for polygyny is high, the probability for animals to engage in any form of monogamy is low (Emlen & Oring, 1977). When the EPP decreases, the likelihood of monogamy increases, presumably a corollary of when there is decreased availability of mates (Emlen & Oring, 1977). Mate availability may be decreased through geographic dispersal (creating a male to female ratio of 1:1), or through temporal distribution (i.e. female estrous synchrony, creating a similar male to female ratio of 1:1, but in regard to sexual availability) (Emlen & Oring, 1977). EPP also decreases when there is a greater requirement for parental care, specifically paternal care (Emlen & Oring, 1977). In these scenarios, the benefit of mating multiply is negated by the decreased fitness associated with altricial young who would not survive with maternal care alone. All of these characteristics together, may set the stage for monogamy.

Monogamy can be sub-divided into two different forms: types I and II (Kleiman, 1977). Type I, or facultative monogamy, occurs when a species exists at such a low density, that mate availability necessitates single mating (Kleiman, 1977). Type II, or obligate monogamy, develops when the female of a species cannot rear young alone (Kleiman, 1977). It is highly associated with decreased sexual dimorphism, delayed sexual maturation in the offspring of a pair, and males that exhibit considerable paternal care (Kleiman, 1977). As stated before, monogamy need not refer to mating

behavior per se and may refer to a sexual or social relationship (Dewsbury, 1987). In sexual monogamy, the pair mates exclusively with one another; this is rare (Dewsbury, 1987). Social monogamy is much more common and is characterized by two mates who share living quarters and rear offspring together (Dewsbury, 1987). It is worth noting, that despite the type of monogamy that characterizes the species in general, there may be intraspecific differences such that some animals may engage in other mating tactics that deviate from the mode. It is this variability in the expression of a mating tactic on which evolution may operate. Similarly, variation in mating decisions provides an opportunity to study social behavior, mating systems and the evolution of these phenomena.

Prairie Vole Model

Many organisms are used to study social behavior and monogamy, but one in particular, has emerged as a good mammalian model, the prairie vole (*Mictrotus ochrogaster)* (Dewsbury, 1987; Carter, et al., 1995; Insel and Young, 2001). Prairie voles are small rodents, weighing approximately 40g, and inhabit the grasslands of central United States and Canada (Tamarin, 1985). A prairie vole nest typically contains a male, a female and their offspring (Getz and Hoffman, 1986; Getz et al., 1981, 1993; Carter et al., 1995). Prairie voles are among the few mammalian species that are characterized as monogamous, exhibiting multiple social behaviors that make them ideal for studying social behavior.

Prairie voles are socially monogamous, and appear to exhibit obligate monogamy. Individuals of a monogamous pair may have several sexual partners (e.g. Wolff et al., 2002), with no assumptions on the exclusivity of mating (Gowaty, 1996). Here, social monogamy is characterized as two animals that **1)** affiliate preferentially with each other, **2)** demonstrate 'selective aggression', and **3)** each provide parental care (LJ Young and Wang, 2004; KA Young and Wang, 2008).

These behaviors characterizing monogamy are strongly influenced by two signaling molecules, oxytocin (OT), and arginine vasopressin (AVP) The widely studied neuropeptides OT and

AVP are broadly involved in social behavior, including learning and memory (Hamburger-Bar et al., 1987) and sexual behavior (Argiolas et al., 1988, 1989; Carter et al., 1995). More pertinent though is their involvement in the formation of pair bonds (Williams et al., 1992; Winslow et al., 1993).

Social Recognition

Social recognition, at the most basic level, is the ability to recognize conspecifics, and it is accomplished through various mechanisms. For example, humans visually differentiate between faces to recognize others (Kanwisher et al. 1997, O'Craven & Kanwisher 2000). Many other mammals, however, depend on olfaction to differentiate between conspecifics. Rodents, including prairie voles, fit into this group. The olfactory system in rodents is composed of an extensive network of neural regions, and it is further developed in these animals than it is in animals that do not recognize one another via olfaction. It has been shown that scents, especially pheromones, stimulate the olfactory system and regulate recognition through various areas of the brain, including the lateral septum and medial amygdala (reviewed Choleris et al. 2009). These two areas, in particular, have been shown to play an important role in social recognition in rodents.

Social recognition allows rodents to make important social ties between themselves and conspecifics. For example, it may allow animals to differentiate between family and non-family (Carter & Keverne, 2002), early in life, helping them to avoid incest, and subsequently decreased fitness. In addition, territorial animals can differentiate between strangers and neighbors (Choleris, Kavaliers, & Pfaff, 2004), allowing them to appropriately defend their territories, and avoid wasting energetic resources on aggression toward non-threatening neighbors. Social recognition has previously been studied in non-social rodents, like rats. More recently, ethologists have begun studying recognition in social animals, like the prairie vole. This allows us to investigate a particularly social behavior, in a social species. For example, after prairie voles pair bond, mate and produce offspring, it becomes especially important for them to differentiate between their mate/social

partner and offspring, and others. This social species, allows us to research how social recognition changes through various behaviors, and at multiple life stages.

Social Behavior Network

The brain is complex, with various neuronal regions and numerous neurotransmitters working in concert to regulate all social behavior. Over time, multiple brain areas have been implicated in the activation and regulation of social behaviors, leading to the development of the idea of the social behavior network. Newman (1999), posed that the social behavior network was made up of a number of brain regions, that are interconnected, including the medial amygdala, the lateral septum, the medial preoptic area, the anterior hypothalamus, the ventromedial nucleus, the ventrolateral hypothalamus, the midbrain periaqueductal gray and the tegmentum. This social behavior network is similar to other brain networks (i.e. the limbic system), in that it is composed of various regions that are interconnected, and have projections to other areas of the brain. The social behavior network fits the criteria Newman suggested are necessary to represent a brain network. These criteria include interconnectivity amongst each of the brain regions, presence of gonadal receptors on neurons in each of the regions, and evidence that each region regulates/activates multiple social behaviors (Newman, 1999). Social behaviors that are regulated by this network include courtship, copulation, aggression, territoriality, and parental behavior.

Input into, and output from, this social behavior network is diverse, leading to the manifestation of diverse behavior. The presence of gonadal receptors, indicates that these brain regions, and subsequently related social behaviors, are all under the influence of steroidal hormones. In addition, Newman (1999) suggested that the sensitivity of receptors and neuronal connections change throughout the lifetime of an organism, with changing environments. Sexual maturation, experience, learning, reproductive cycles, disease and aging, were all posed to cause long-term changes in the social behavior network, which manifest as changes in behavior (Newman, 1999). Similarly, sensory stimuli, in the more immediate environment, were posed to lead to short-term

modifications in the social behavior network. Newman ultimately suggested that these factors that solicited change in the network have varying impacts on social behavior, and that the greatest impact on the network (and thus behavior), varied by species, based on their genes and evolution.

Prairie voles represent an ideal organism to study social behavior, and exhibit various modifications in behavior throughout their life cycles. As the social behavior network is known to regulate many of these behaviors, and to change throughout the lifetime of an organism based on environmental changes, it presents a model for analyzing neural changes in this social species.

Chapter II

Chapter II of this dissertation considers, and makes strides toward answering a question regarding prairie vole mating tactic. Though prairie voles are considered socially monogamous, scientists have found that in nature, prairie voles exhibit two different mating tactics (Getz et al. 1993). In one of the tactics, "residency," prairie voles exhibit stereotypical, socially monogamous behaviors. In the other tactic, "wandering," animals have larger home ranges than "residents," and these home ranges often overlap with the territories of other prairie voles. These single voles presumably "wander" these territories taking advantage of copulation opportunities that arise (Getz et al. 1993). There are two schools of thought in regard to prairie vole fitness. One hypothesis posits that that residency, or the more common monogamous prairie vole behavior, is the better mating tactic. An alternative hypothesis is that wandering is actually the better mating tactic, evidenced by better lifetime breeding success, and larger body size (Solomon and Jacquot, 2002). Chapter 2 investigates this controversy, and establishes through controlled trials that prairie voles will chose to pair bond, rather than taking advantage of the opportunity to mate multiply.

Chapter III

Chapter III of this dissertation investigates the effects of pair bonding on social cognition in male voles. I argue that social recognition is vital, not only to pair bonding, but to "pair bonding-associated behaviors". Recent work has shown that males can discern between male conspecifics, but not females (Zheng et al. 2013). Because it should be important for males to distinguish between mates and strangers, especially with respect to mate and pup guarding, I propose that there must be a shift in behavioral cognition with pair bonding. Chapter 3 investigates this hypothesis, and shows that social recognition is indeed dependent on social context, and that pair bonding either leads to increased cognitive ability, or perhaps increased effort/interest in differentiating between female conspecifics.

Chapter IV

Chapters II and III establish that pair bonding is the optimal mating tactic in prairie voles, and that pair bonding, facilitates an increase in social recognition of female conspecifics. Indeed many behaviors emerge or are altered after pair bond formation. Because many of these behaviors are controlled or modified by the action of OT or AVP or their receptors in a number of forebrain structures I asked whether pair bonding facilitates a shift in neural receptors responsible for these affiliative behaviors. Chapter IV analyzes neurohormones in the brain associated with social behavior expression, in pair bonded and single voles, to determine whether OT and AVP receptor profiles are modified with pair bonding.. I showed that pair bonded and single males exhibit no differences in neural receptor distribution, suggesting that if OT and AVP changes are associated with changes in behavior, it is at the level of the peptide (synthesis or release), not the receptor.

CHAPTER II

PAIR BONDING, THE "PREFERRED" MATING TACTIC

Preface

With some modifications, this chapter was submitted to Animal Behavior September 10, 2015, Blocker, TD & Ophir, AG. "Settling down or just settling? Male prairie voles form pair bonds even in the presence of multiple receptive females." On December 28, 2015 the editor requested a major revision, with mostly positive reviews. We are in the process of revising the manuscript for re-submission.

Abstract

Pair bonds are the cornerstone of a monogamous relationship. When individuals of the same species engage in monogamy and promiscuity (i.e., alternative reproductive tactics) it can be difficult to determine which tactic confers greater fitness since measures of fitness can be difficult to ascertain. However, in these circumstances, whether animals preferentially establish pair bonds can reveal decisions that presumably reflect the animals assessment of how to best maximize reproductive success. In nature, the majority of prairie voles (*Microtus ochrogaster*) establishes pair bonds and engages in social monogamy while a minority of individuals remains single and presumably mates promiscuously. To determine which of these two tactics is preferred, I provided single male prairie voles simultaneous access to two sexually receptive females for 24 hours and then subsequently tested males in partner preference tests with each female independently contrasted with a novel female. I aimed to determine if males would form a pair bond with one, both, or none of the original females. I found that males formed pair

bonds with only one female.. I also investigated male- and female-initiated aggression and found that during the bonding process, males were more aggressive with females that they did not ultimately bond with. In the partner preference tests, males showed more aggression toward unfamiliar females than familiar females. Mismatches in male- and female-initiated aggression suggest that aggressive interactions may be perpetuated more by males than females. Taken together, my data demonstrate that under conditions that are ideal for forgoing bonding and mating multiply, males choose to establish a pair bond, suggesting that selective pressures may have facilitated bonding by males.

Introduction

A mating system may be best considered as a collection of individual reproductive tactics that animals within a population adopt at a given time (Clutton-Brock 1989; Emlen & Oring 1977; Shuster & Wade 2003). As such, the reproductive decisions that animals make will define tactics, and the most common tactic (i.e., the mode) can be used to characterize the system overall. From this perspective, mating systems should be somewhat plastic and variable. In some instances, common patterns within the variability of tactics for a population or species will emerge and may lead to the evolution of one or more alternative reproductive tactics (Oliveira et al. 2008). In these cases, each alternative tactic is associated with its own set of reproductive costs and benefits.

Frequently, when alternative mating tactics are identified, there is often a more common, or bourgeois, tactic (Oliveira et al. 2008). The common tactic may be associated with greater reproductive success and therefore considered the ‘preferred’ tactic (Brockmann, 2001; Gross, 1996). When this is the case, the alternative is usually imposed by some limitation and characterized as making ‘the best of a bad job’ (Eberhard, 1982; Gross & Repka, 1998a, b). Alternatively, the less common tactic might be ‘preferred’ (Watters, 2005; Young et al., 2013). This should occur when it is associated with greater reproductive success but it is also costly to

sustain. As a result, only a few animals are able to engage in this behavior, and the others are relegated to a tactic that garners some fitness at a lower cost, but at a relatively lower rate of reproductive success. In other contexts, the net pay-offs of two tactics might balance out such that each option is equally good, and each is associated with its own set of costs and benefits (Ryan et al. 1992; Shuster & Wade 1991). The balance can take place over time (e.g., one tactic accrues greater reproductive success within a single breeding season or cycle, but life-time reproductive success for each tactic is equivocal) or can be biased by a changing environmental context (e.g., a volley between two ecological contexts that each favor one of the two tactics).

Measures of reproductive success usually provide the best way to disambiguate which tactic is 'preferred', but reproductive success can also be very difficult to quantify (both from a theoretical and practical point of view; (Arnold & Wade 1984; Byerly & Michod 1991; Clutton-Brock 1988)). Another way to address this question is to experimentally observe what choices animals make. For example, in a variable mating system (in which some individuals are monogamous and others are polygynandrous), determining if individuals will choose to establish pairs or mate multiply when they have the option to do either can reveal their natural predispositions and speaks to the selective pressures that shaped behavior in that system.

Although monogamy is a common mating system in many taxa, it is relatively rare among mammals (Kleiman 1977). Indeed, most mammals are polygynous and fit the "classic story" that males maximize reproductive success through multiple mating partners (Bateman 1948; Kleiman 1977; Trivers 1972). Social monogamy (a demographic and close sociospatial relationship between a pair, that does not assume exclusive mating (Reichard & Boesch 2003)) accounts for most of the instances of mammalian monogamy. Perhaps the best-known example of non-human mammalian monogamy is the prairie vole, *Microtus ochrogaster* (Carter 1998; Carter & Getz 1993; Carter & Keverne 2002; Getz et al. 1981; Getz et al. 1993; Insel et al. 1995; Insel & Young 2001; Young & Wang 2004; Young et al. 2005). Research in the lab and field has demonstrated that male and female prairie voles will form strong social preferences with each

other (i.e., pair bond), appear to co-defend relatively small and highly convergent areas of space (territories), and will each contribute fairly equally to offspring care (Getz & Hofmann 1986; Getz et al. 1993; McGuire et al. 2013; Wang & Insel 1996; Wang & Novak 1994; Williams et al. 1992; Winslow et al. 1993; Wolff 1985). However, both males and females also engage in a non-monogamous mating tactic known as 'wandering', and that paired (a.k.a., 'resident') males and females will engage in extra-pair copulations under naturalistic conditions (Getz et al. 1993; McGuire & Getz 2010; Ophir et al. 2008b; Solomon & Jacquot 2002; Solomon et al. 2004).

Support for whether the monogamous resident or non-monogamous wanderer tactics are associated with greater reproductive success has been mixed. Some evidence has indicated that monogamous residents are associated with greater fitness (measured over a single breeding cycle), and that selection appears to have eliminated variation in the neural phenotype associated with pair bonding thereby predisposing prairie voles to form pairs (Ophir et al. 2008b; Ophir et al. 2008c; Phelps & Ophir 2009). Evidence to the contrary is based on data from field studies that have shown that lifetime reproductive success (measured over two or more breeding cycles) is equivocal (Solomon & Jacquot 2002). Similarly, a laboratory study focused on female behavior, demonstrated that females readily engage in multi-male mating when given access to multiple males over a 24 h period (Wolff et al. 2002), supporting the notion that 'wandering' (i.e., living singly and mating promiscuously) may be a preferred tactic.

Although the evidence appears to support the idea that females prefer to adopt a promiscuous tactic when given the chance (Wolff et al. 2002), it is unclear which tactic males prefer. In this study, I asked if single male prairie voles choose to form a bond or forgo bonding in the presence of multiple females. I also asked if they would mate multiply when given the chance. To test this, I gave males access to two sexually receptive females for 24 hours and determined if they demonstrated a preference for one of the two females. I also determined if males mated with one or both of them. Next, I tested males in a 'partner preference test' with each of these females (each contrasted with a novel female) to determine if males demonstrated a

partner preference (i.e., pair bond) for one, both, or none of the females. Finally, because males become selectively aggressive after forming bonds, I assessed male aggression directed toward females in each of the two phases of the experiment.

Materials and Methods

Animals

All animals used in this study were from the F2 generation within a breeding colony derived from wild stock originally trapped in Champagne-Urbana, Illinois. At weaning (21 days), offspring were separated into same-sex litters and housed in polycarbonate cages (29 x 18 x 13 cm) lined with Sani-chip bedding and provided nesting material. No animals in this experiment were raised in isolation. Water and rodent chow (Rodent Chow 5000, Harlan Teklad, Madison, WI, USA) were provided *ad libitum* and animals were maintained on a 14:10 hr light:dark cycle (lights on at 0600) with ambient temperature maintained at 20±2°C. All procedures were approved by the Institutional Animal Care and Use Committee of Oklahoma State University (AS 09-6). All animals included in this study were sexually naïve adults ($\geq$ 50d) and unrelated to other animals to which they were exposed during the experiment.

Phase I: Multi-Female Mating and Pair Bond Formation Test

To determine if sexually mature single males would mate with multiple females, I exposed male subjects (N = 12) to two novel females. I measured mating behavior and behaviors indicative of pair bond formation (see below). Prior to experimentation, I induced sexual receptivity in stimulus females by exposing them for 48 hours to soiled bedding and nesting material from unfamiliar males that were unrelated to the subject males (Carter et al. 1980; Dluzen et al. 1981; Richmond & Stehn 1976).

Males were placed in a three chamber apparatus (60 x 50 x 40cm) consisting of a neutral chamber (20 x 50 x 40cm), and two smaller adjacent chambers (each 30 x 25 x 40cm) (***Figure 2.1***). Novel females were tethered in each of the adjacent chambers. Tethering involves using a plastic zip-tie as a collar connected to a light-weight chain attached to the apparatus, and does not inhibit animals from normal activities (e.g., moving, eating, or mating) (Ophir et al. 2007; Wolff & Dunplap 2002). Females were given a 20-minute acclimation period to adjust to the collars following tethering and observed during this period for discomfort and distress.

After the acclimation period, males were placed in the apparatus in the neutral chamber and recording began. Animals resided in this apparatus for 24 h. Food and water were provided *ad libitum* within both of the females' chambers and within the neutral chamber, so that males were not required to enter either chamber. This design allowed males to move and interact freely with each female, while limiting interactions between females.

I video recorded Phase I with a Sony SR-120 camcorder (Sony, New York City, NY, USA) placed approximately 1 m away from the front wall of the apparatus. Recordings were scored using Observer XT software (Noldus Information Technology, Leesburg, VA). Specifically, I counted mating bouts (defined as mounting followed by intromission), time spent in each chamber, time spent in side-by-side contact, number of aggressive events, and initiator of aggressive events (male-to-female and female-to-male aggression). In addition, male and female aggression quotients were calculated to take into account the number of aggressive interactions per the amount of time spent in each respective chamber (number of aggressive events / time spent in the chamber containing that female x 100). Although the full 24 hours of the test was recorded, I only scored the first 3 hours of every trial, along with the first ten minutes of every third subsequent hour. This scan sampling allowed us to get a representation of male behavior over the 24-hour period. I referred to a female from the pair as the 'preferred' female if males spent twice as much time with her than with the other ('non-preferred') female.

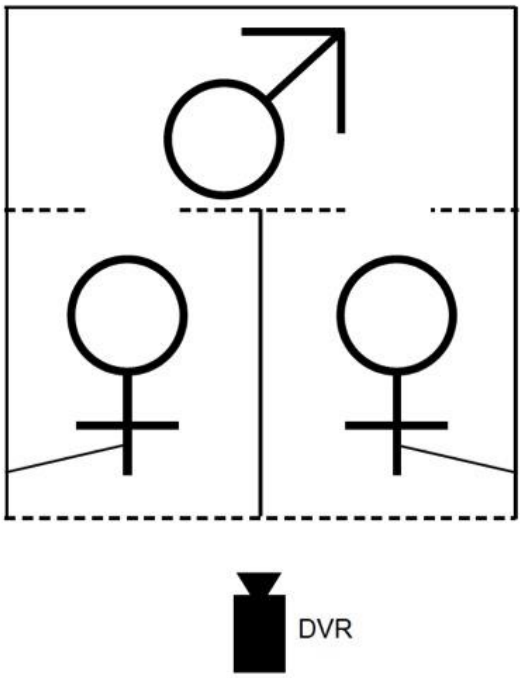

Figure 2.1: Overhead schematic of the testing apparatus. The camera (DVR; digital video recorder) was located at the midpoint of the apparatus and oriented horizontally. Solid lines = opaque Plexiglas; dashed lines = transparent Plexiglas; large break in dashed lines = open doorways; thin line to females represent tethers attached to walls.

Phase II: Partner Preference Tests

I next wanted to determine if males would demonstrate a partner preference for either of the two stimulus females after 24 h of co-habitation. Williams et al. 1992, demonstrated that 24 hours of cohabitation, even without mating, is sufficient to establish a pair bond. To assess the potential bonds established during Phase I, males participated in a series of two 3-hour 'partner preference' tests. Note that I refer to this test as the 'partner preference' test because this is what it has been called in the literature, but I did not assume that either of the familiar females were actually partners with the male. It was my intent to use this test to determine if either of the two females from Phase I should be considered a bonded 'partner'. I defined a female as a 'partner' if the male spent more than twice the amount of time with the female from Phase I over the novel female with which she was presented. This is a more conservative criterion than the convention

definition, which defines a 'partner preference' based on a male spending a majority of time with one of two females (Carter et al. 1995; Carter & Getz 1993; Insel & Hulihan 1995; Insel et al. 1995; Williams et al. 1992).

The first of two partner preference tests was held in the afternoon immediately following the morning that Phase I ended. Each partner preference test followed a similar design as Phase I, but was comprised of one of the females from Phase I, and a novel stimulus female. To avoid the confounding variable of familiarity and scent, partner preference apparatuses were cleaned with soap and water between each test. In addition, during Phase II, the female from Phase I was placed in the opposite chamber to the one that she inhabited during Phase I, while the novel stimulus female was placed in the chamber that previously housed the familiar female (***Figure2.2***). The second partner preference test was conducted exactly the same except it used the second female from Phase I, and a different unfamiliar stimulus female. Both stimulus females used in the partner preference tests were unrelated to the Phase I females and focal males. The experimenter was blind to whether the females from Phase I were either the 'preferred' or 'non-preferred' female. The order of the two partner preference tests was randomized (using right and left chamber placement from Phase I for randomization).

Three hour recordings were made using a Sony SR-120 camcorder (Sony, New York City, NY, USA) placed approximately 1m away from the front wall of the apparatus. The three-hour recordings were scored using Observer XT software (Noldus Information Technology, Leesburg, VA). Observers were blind to female identity and placement. I quantified the same behaviors as described in Phase I (mating bouts, time spent in each chamber, time spent in side-by-side contact, initiator of aggressive events, number of aggressive events, and aggression quotients).

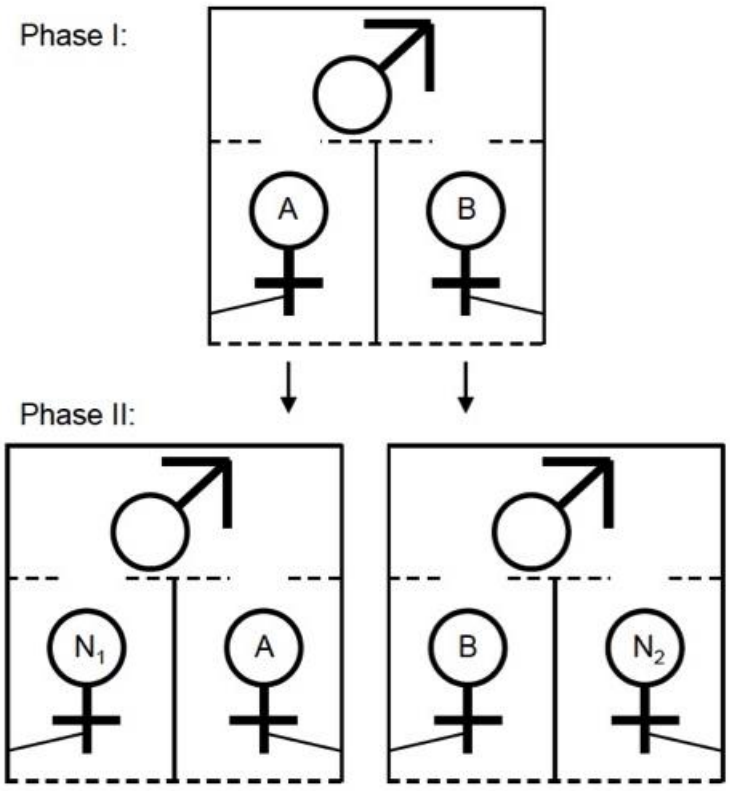

Figure 2.2: Experimental design. Phase I: Initial exposure of male to two sexually receptive females, lasting 24 hours. Phase II: Subsequent partner preference tests contrasting each female from Phase I (A and B) with a unique novel female (N_1 and N_2).

Results

Phase I: Multi-Female Mating and Pair Bond Formation Test

To assess affiliation, mating and aggression in Phase I, I began by assessing single males' responses to multiple sexually receptive females by focusing on measures of affiliation and sexual contact. One male was excluded from analysis (N = 11) due to experimenter error. All males preferred one female of the two, and ten of eleven males spent at least twice as much time in side-by-side contact with one female over the other (Exact binomial test, (two-tailed) P = 0.01), significantly more often than would be expected by chance. Not surprisingly, males spent significantly more time in side-by-side contact with preferred females, compared to non-preferred

females (two-tailed Wilcoxon signed rank test: $W = 66$, $N = 11$, $P = 0.001$, ***Figure 2.3a***). Also not surprisingly, males spent more time in the chamber containing the preferred females ($W = 66$, $N = 11$, $P = 0.001$, ***Figure 2.3b***).

I quantified the number of mating bouts to assess mating. Only three of eleven males mated. All three males that I observed mating, only mated with females that I later determined to be preferred females. Because so few males were observed mating, I did not find a difference in the number of mating bouts with either preferred or non-preferred females (Exact binomial test, (two-tailed) $P = 0.23$; two-tailed Wilcoxon signed rank test: $W = 6$, $N = 11$, $P = 0.25$, ***Figure 2.3c***). However, I note that the males that were observed mating engaged in several (29, 89, and 133) mating bouts.

I first investigated aggression by comparing the number of aggressive encounters the male initiated with each female, and the number of aggressive encounters initiated by each female toward the subject male. By this measure, males initiated the same number of aggressive encounters toward preferred and non-preferred females ($W = -12$, $N = 11$, $P = 0.51$, ***Figure 2.4a***), and females initiated the same number of aggressive encounters toward males ($W = 15$, $N = 11$, $P = 0.53$, ***Figure 2.4b***). However, because males spent more time in proximity to preferred females than non-preferred females, the probability of engaging in any interaction was higher with preferred females. I therefore normalized aggression toward each female based on the time spent in the chamber containing a female (i.e., number of aggressive encounters / total time in chamber x 100). Analyzing the data in this way revealed that males were relatively more aggressive toward non-preferred females than preferred females ($W = -39$, $N = 11$, $P = 0.02$, ***Figure 2.4c***), and that non-preferred females were more aggressive back ($W = -46$, $N = 11$, $P = 0.04$, ***Figure 2.4d***). Although aggression initiated by both females was not significantly different from the aggression that males initiated toward females, females tended to initiate more aggressive encounters in both absolute ($W = -39$, $N = 11$, $P = 0.09$) and relative aggressive encounters ($W = -38$, $N = 11$, $P = 0.10$).

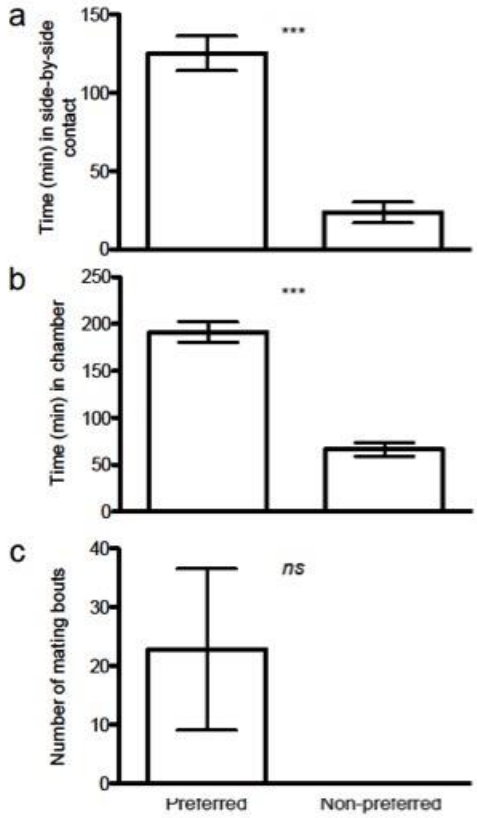

Figure 2.3: **A)** Mean ± SE time (in minutes) that single males spent in side-by-side (SxS) contact with each sexually receptive female in 24 hours. Females with which males spent more time in SxS contact were called the 'preferred' females; females with which males spent less time in SxS contact were called the 'non-preferred' females. **B)** Mean ± SE time (in minutes) that single males spent in the chamber housing each sexually receptive female in 24 hours. **C)** Mean ± SE number of observed mating bouts in which single males participated with each sexually receptive female in 24 hours. Note that only 3 of 11 males were observed mating. *** $P \leq 0.001$; *ns* = not significant.

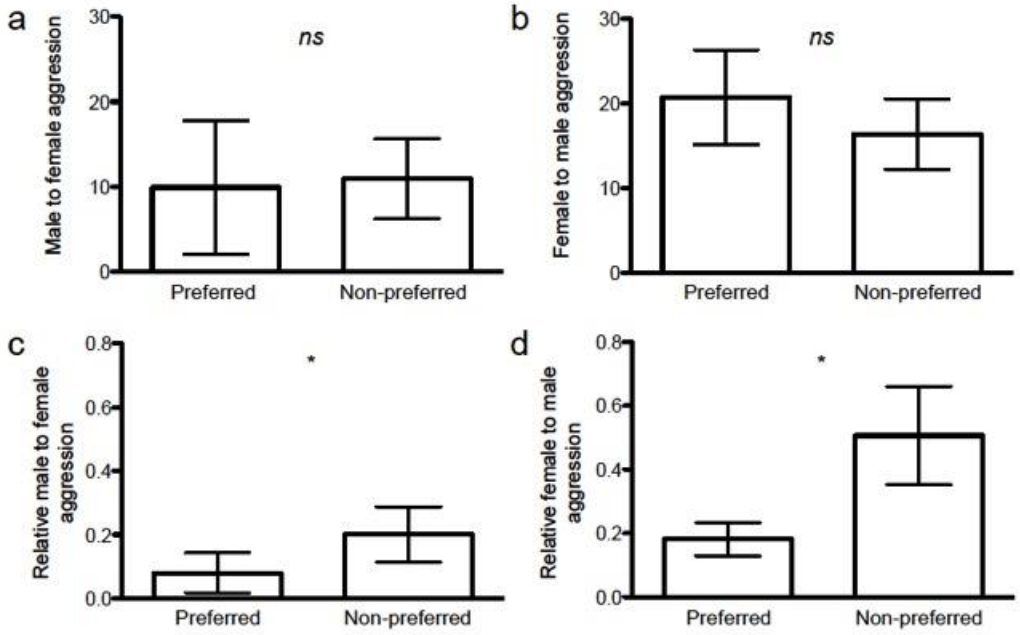

Figure 2.4: **A)** Mean ± SE number of observed aggressive events that single males initiated toward the 'preferred' and 'non-preferred' females (see text for definitions) in 24 hours. **B)** Mean ± SE number of observed aggressive events that the 'preferred' and 'non-preferred' females initiated toward the males in 24 hours. **C)** Mean ± SE relative aggression (number of aggressive events / time in the chamber containing the female x 100) that single males initiated toward the 'preferred' and 'non-preferred' females in 24 hours. **D)** Mean ± SE relative aggression that the 'preferred' and 'non-preferred' females initiated toward the males in 24 hours. * $P \leq 0.05$; *ns* = not significant.

Phase II: Partner Preference Tests

In Phase II I used partner preference tests to evaluate whether males showed evidence for a pair bond with either the preferred or non-preferred female when each was independently contrasted with a novel female. Several possible outcomes were possible. For example, considering that males had the opportunity to interact and (presumably) mate with both sexually receptive females in Phase I, if males are predisposed to mate multiply then they should show no

evidence for a pair bond with either female. On the other hand, if males are predisposed to form pair bonds when possible, then they should demonstrate a partner preference for only one of the two females (presumably the preferred female from Phase I). Yet another possible outcome is that males might show a general predisposition to interact with females from Phase I based on familiarity. In this case, males might either preferentially affiliate with familiar over unfamiliar individuals (a preference for familiar females), or vice versa (a preference for novel females), regardless of if the females from Phase I were preferred or non-preferred. To test these potential outcomes, I compared the time in side-by-side contact with the preferred female vs a sexually receptive novel (unfamiliar) female, and with the non-preferred female vs a sexually receptive novel female. As before I also compared aggression (absolute and relative) for each Phase II partner preference test. No mating bouts were observed in either 3-hour test.

Our results indicate that males demonstrated a partner preference for preferred females from Phase I (two-tailed Wilcoxon signed rank test: $W = 46$, $N = 11$, $P = 0.04$, ***Figure 2.5a***), but not for non-preferred females ($W = 6$, $N = 11$, $P = 0.83$, ***Figure 2.5b***). Similarly, males spent more time in the chamber with his preferred female over a novel female ($W = 46$, $N = 11$, $P = 0.04$, ***Figure 2.5c***) but not for non-preferred females compared to novel females ($W = -14$, $N = 11$, $P = 0.58$, ***Figure 2.5d***). These data indicate that familiarity does not produce preferences (males did not consistently prefer Phase I females to the novel females with which they were contrasted). More importantly, these results indicate that even when given access to two sexually receptive females, males will form bonds with only one female, and this bond persists over different testing bouts (Phase I and Phase II).

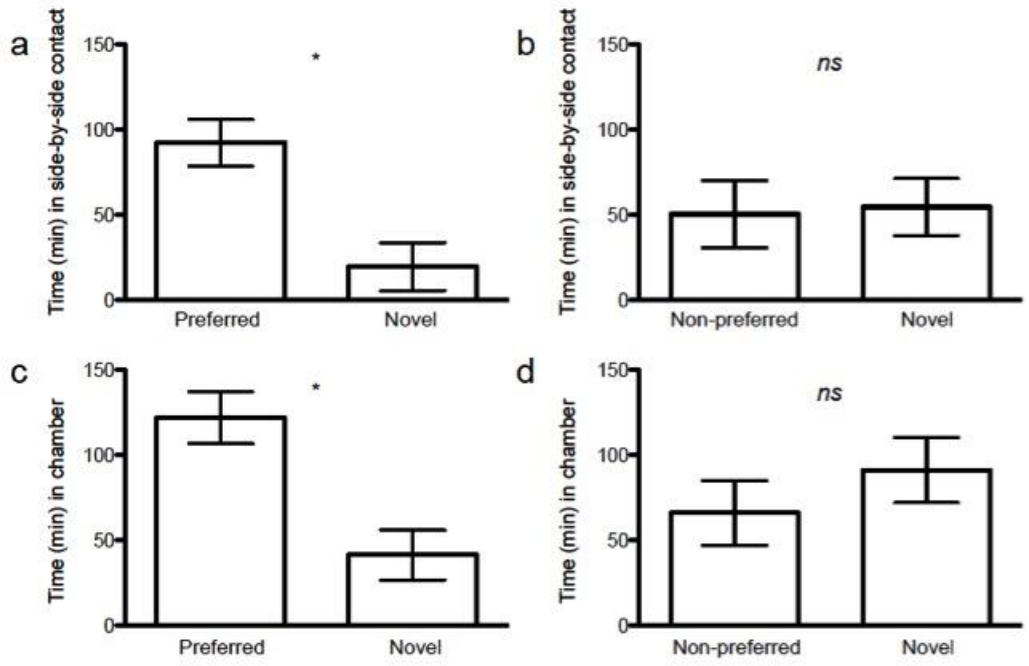

Figure 2.5: **A)** Mean ± SE time (in minutes) that single males spent in side-by-side (SxS) contact with the preferred female or a sexually receptive novel female in a 3 hour partner preference test. **B)** Mean ± SE time (in minutes) that single males spent in SxS contact with the non-preferred female or a sexually receptive novel female in a 3 hour partner preference test. **C)** Mean ± SE time (in minutes) that single males spent in the chamber housing the preferred or novel female. **D)** Mean ± SE time (in minutes) that single males spent in the chamber housing the non-preferred or novel female. * $P \leq 0.05$; *ns* = not significant

Next I compared the number of aggressive encounters initiated by males for the partner preference test comparing the preferred female with a novel female, and the non-preferred female with a novel female. Like during Phase I, actual numbers of aggressive encounters did not significantly differ between paired and novel females (W = -12, N = 11, P = 0.35, ***Figure 2.6a***). However unlike before, males initiated significantly more aggression with novel females over the non-preferred females (W = -42, N = 11, P = 0.02, ***Figure 2.6b***). When I accounted for the total amount of time that males spent in the vicinity of females in each of these choice tests (time in chamber), I found that males initiated significantly more aggression with each novel female over

either the preferred or non-preferred female (Preferred female: W = -30, N = 11, P = 0.04; Non-Preferred female W = -37, N = 11, P = 0.03, ***Figure 2.6d-e*** respectively). Furthermore, the degree of actual aggression and relative aggression males directed toward preferred and non-preferred females did not differ (Mann Whitney U Test; Actual Aggression: U = 60, P = 1.0; Relative Aggression: U = 59.5, P = 0.97, ***Figure 2.6c, f***). These results indicate that although males were more aggressive toward non-preferred females in Phase I, male aggressive behavior toward non-preferred females appeared to be diminished in Phase II. The results also show that familiarity appears to influence male initiated aggression, with males engaging in more aggressive encounters with unfamiliar (novel) females.

Consistent with the male-initiated aggression, females in the preferred v novel preference test initiated the same number of aggressive encounters toward males (W = -22, N = 11, P = 0.35, ***Figure 2.7a***), but the novel females initiated more relative aggression compared to preferred females (W = -58, N = 11, P = 0.007, ***Figure 2.7d***). Similarly, there was a trend indicating that novel females initiated more relative aggression to males compared non-preferred females, however, this was not significant (N = 11, P = 0.10; ***Figure 2.7b, e*** respectively). Finally, preferred and non-preferred females did not initiate aggression toward the male differently (Mann Whitney U Test; Actual Aggression: U = 55.5, N = 11, P = 0.76; Relative Aggression: U = 57.5, N = 11, P = 0.87, ***Figure 2.7c, f***). Taken together, the data indicate that male aggression and female aggression appear to be matched, suggesting that males likely drive aggressive encounters.

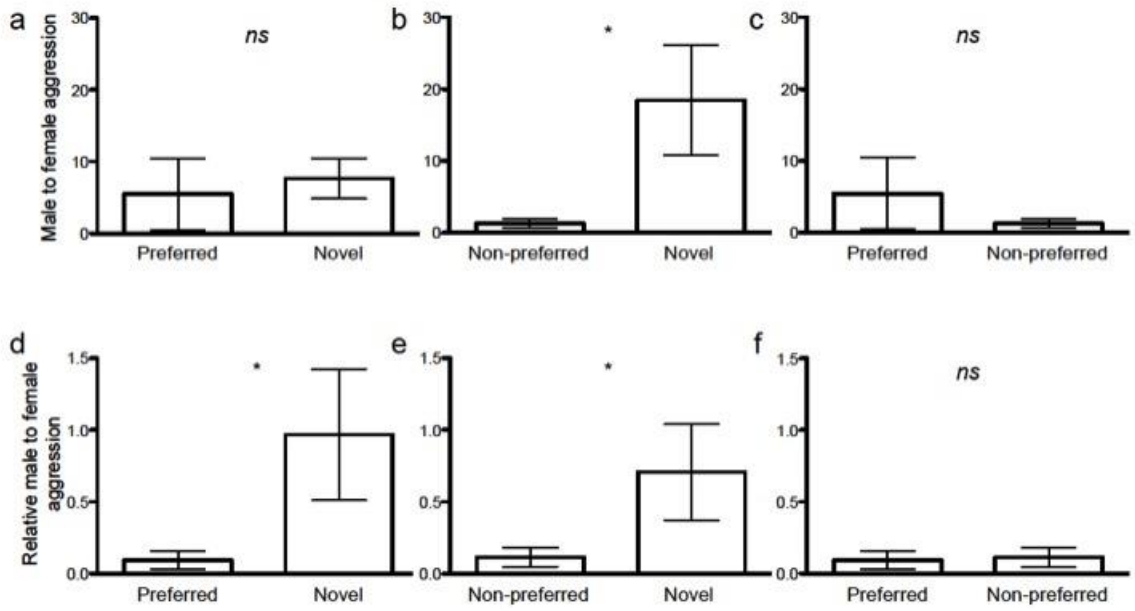

Figure 2.6: **A)** Mean ± SE number of observed aggressive events that single males initiated toward the preferred female or a sexually receptive novel female in a 3 hour partner preference test. **B)** Mean ± SE number of observed aggressive events that single males initiated toward the non-preferred female or a sexually receptive novel female in a 3 hour partner preference test. **C)** Mean ± SE number of observed aggressive events that single males initiated toward the preferred or non-preferred females in their respective 3-hour partner preference tests. **D)** Mean ± SE relative aggression (number of aggressive events / time in the chamber containing the female x 100) that single males initiated toward the preferred female or a sexually receptive novel female in a 3 hour partner preference test. **E)** Mean ± SE relative aggression that single males initiated toward the non-preferred female or a sexually receptive novel female in a 3 hour partner preference test. **F)** Mean ± SE relative aggression that single males initiated toward the preferred or non-preferred females in their respective 3-hour partner preference tests. * $P \leq 0.05$; *ns* = not significant.

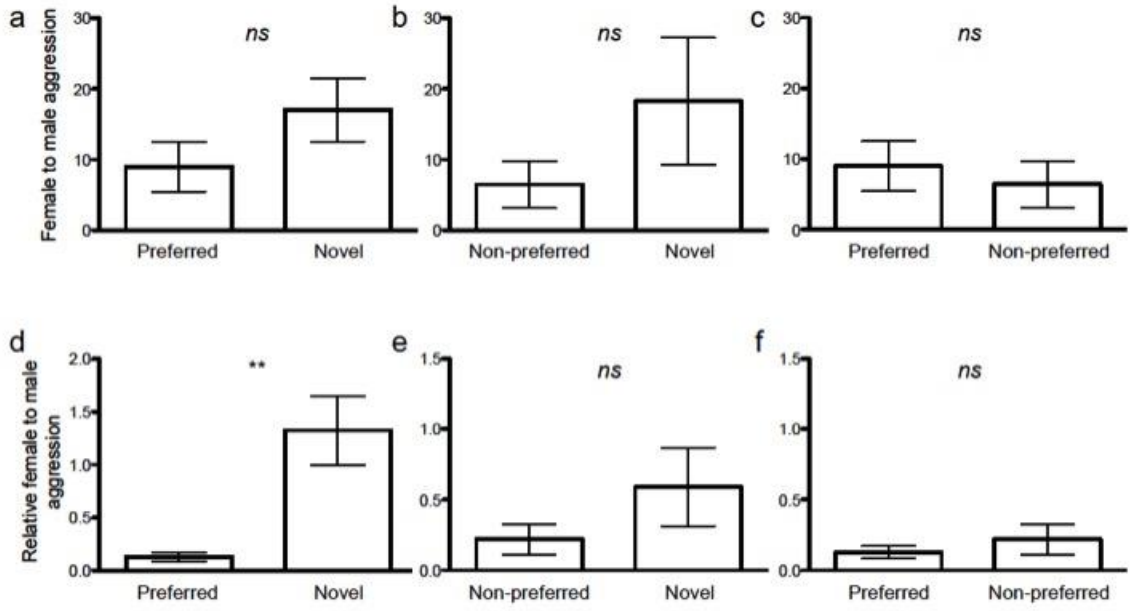

Figure 2.7: **A)** Mean ± SE number of observed aggressive events that the preferred female or a sexually receptive novel female initiated toward single males in a 3 hour partner preference test. **B)** Mean ± SE number of observed aggressive events that the non-preferred female or a sexually receptive novel female initiated toward single males in a 3 hour partner preference test. **C)** Mean ± SE number of observed aggressive events that the preferred or non-preferred females initiated toward single males in their respective 3-hour partner preference tests. **D)** Mean ± SE relative aggression (number of aggressive events / time in the chamber containing the female x 100) that the preferred female or a sexually receptive novel female initiated toward single males in a 3 hour partner preference test. **E)** Mean ± SE relative aggression that the non-preferred female or a sexually receptive novel female initiated toward single males in a 3 hour partner preference test. **F)** Mean ± SE relative aggression that the preferred or non-preferred females initiated toward single males in their respective 3-hour partner preference tests. ** $P \leq 0.01$; *ns* = not significant.

Discussion

I have demonstrated that prairie vole males appear to preferentially form pair bonds, even when conditions are ideal for forgoing bonding and mating multiply without social restriction.

Furthermore, I have shown that during the bonding process (Phase I), males demonstrate more aggression toward females with which they do not bond than the females with which they ultimately do bond. Males also show more aggression toward unfamiliar females than familiar females (Phase II).

Are Male Prairie Voles Predisposed to Bond?

(Ophir et al. 2008c) demonstrated that bonded ('resident') males fertilized more embryos (with or without their partner) than non-bonded 'wanderers'. They also showed that neural mechanisms in brain structures that are necessary and sufficient for forming pair bonds were ubiquitous in animals regardless of if they formed a bond or not. These results led to the conclusion that the pair bonded 'resident' tactic was the preferred route to maximize reproductive success, an idea supported by others (McGuire & Getz 2010). However, some have argued that remaining single is a preferred route toward maximizing reproductive success (McGuire et al. 2013; Solomon & Jacquot 2002). This idea is certainly in line with classic theory indicating that males maximize reproductive success though mating multiply (Andersson 1994; Bateman 1948; Kleiman 1977; Trivers 1972). It is also supported by the observation that non-bonded wanderers had larger body sizes (Solomon & Jacquot 2002), indicating that wanderers might be more competitive. Nevertheless, our lab has not found this body size relationship in past studies, and tended to find the reverse (Ophir et al. 2008c). Furthermore, (Ophir & DelBarco-Trillo 2007) showed that not only did females prefer males with larger ano-genital distances (AGD; a marker of competitiveness in many rodent species) in the lab, but paired 'resident' males had larger AGDs than single 'wanderers' under semi-natural field conditions. These conflicting results have made it difficult to determine which, if either, tactic is most competitive and/or best for maximizing fitness.

In the context of a discussion considering fitness consequences, is it important to remember that bonding and mating are not interchangeable concepts and that pair bonding does

not preclude individuals from mating with individuals outside the pair. For example, bonded males investigate sexually receptive females more than their mates (Parker et al. 2011; Rodriguez et al. 2013). Moreover, although none of the males in my study were observed mating with a female other than the one with whom they ultimately bonded, some proportion of males and females (approximately 25% by earlier measures) engage in extra-pair mating (Ophir et al. 2008b). So although males appear to preferentially affiliate with only one female initially, this behavior may be transient and it is unclear how long this potential partner fidelity persists after the bond has been safely established (but see Resendez & Aragona (2013).

Nevertheless, bonding is an inherent and necessary step toward residency. The data from the current study showed that males opted to form bonds and did not appear to mate with more than one female during or shortly after the bonding process. I believe this is supportive of the idea that males actively choose to bond when given the opportunity. Whether this is a means toward maximizing reproductive success (regardless of if it is achieved with partners) remains an open question. It is important to point out that the data from previous studies in semi-natural outdoor enclosures discussed above were limited in that they focused on a single reproductive cycle and did not account for survival of offspring (Ophir et al. 2008c). It is currently unclear if forming bonds and engaging in the resident tactic has greater lifetime reproductive success over single wanderers. Some data appear to support the idea that residents and wanderers have equal reproductive success over a longer time-scale (three breeding cycles; Nancy Solomon, personal communication), raising questions about whether or not wanderers are actually making the 'best of a bad job' (Solomon & Jacquot 2002). In the absence of more conclusive fitness data, and in the context that male prairie voles appear to prefer to pair bond with one female, I believe the majority of evidence supports the notion that residency (which by definition requires bonding) is a preferred tactic and that male prairie voles have been selected to form bonds. However more work is necessary to fully substantiate this hypothesis.

What Does Aggression Tell Us About the Bonding Process?

Winslow et al. 1993, showed that male prairie voles are highly aggressive toward strangers - but not their partners - once the pair bond has been established. In contrast they demonstrated that males are relatively unaggressive with strangers before they form a bond. In addition, it has been shown that the expression of selective aggression occurs within two weeks of pair bond formation (Aragona et al. 2006, Gobrogge et al. 2007). My data indicate that as the pair bond formed, males were relatively more aggressive toward non-preferred females compared to preferred females (i.e., the females I later determined were pair bonded with the males). This is particularly interesting and potentially suggests that selective aggression is initiated during the earliest stages of pair bond formation. It is also interesting to note that non-preferred females were more aggressive toward males than preferred females were toward males. This raises an important set of questions: 1) were males responding to female initiated aggression, 2) were females responding to male initiated aggression, and 3) did the heightened aggression impact male pair bond formation?

It is certainly worth asking whether increased aggression from the non-preferred female, drove male avoidance, and thus subsequent pair bonding with the less aggressive female (the preferred female). Unfortunately, it is difficult to disambiguate these possibilities based on the data I have. Though we cannot exclude the possibility that females are initiating the aggression, female sexual receptivity was induced, presumably making females receptive to males.. In further support of this idea, males were initially more aggressive toward non-preferred females than preferred, and female aggression matched (non-preferred > preferred). However, in the second phase of experimentation, as male aggression appeared to shift toward the novel female, novel female aggression toward the male was greater (though this was only a trend in comparison to the non-preferred female). All the while, preferred and non-preferred aggression toward and from the male did not differ in the second phase, indicating diminished aggression toward and from the

non-preferred female. If female aggression drove male aggression, and the non-preferred female's aggression prompted the male to bond with the preferred female, I would expect no relative changes in non-preferred female aggression across phases I and II. This was not the case. In fact, non-preferred female aggression diminished, while novel female aggression was greater in comparison. This pattern directly corresponds to male aggression, suggesting that male aggression drives female aggression, and making female-initiated aggression less likely to be the force driving males to pair bond with the alternate female.

Why are Males More Aggressive to Unfamiliar Females?

Our study showed that bonded males are more aggressive toward unfamiliar females. Although I found that familiarity does not produce preferences, my results are consistent with others that indicate that familiarity decreases aggressive behavior in prairie voles (Firestone et al. 1991). Indeed, animals often prefer familiarity and/or avoid unfamiliarity (e.g., Sheldon 1969). Several possibilities could explain why male prairie voles reacted more aggressively toward unfamiliar females. For example, females are usually more likely to commit infanticide (Wolff 1993) and an aversion to unfamiliar females might represent an instinct to defend offspring. The fact that these males did not yet have offspring makes this explanation questionable. The aggressive behavior directed toward unfamiliar females might simply be a consequence of territorial behavior. Indeed, bonded males will attack unfamiliar conspecifics. However, it seems strange that males would interact aggressively with individuals that potentially represent extra-pair mating opportunities. This is particularly true given that female prairie voles prefer affiliative males and avoid aggressive males (Ophir et al. 2008a). Aragona et al. (2006) argued that such behavior is regulated by changes in the proportion of DA receptors in the brain and helps reinforce and stabilize pair bonds in early stages. That said, males were relatively less aggressive with the non-preferred (familiar) females, further indicating that maybe males are less averse to interacting with familiar individuals, even if they are not a partner. Considering that under natural

conditions males are likely to be familiar with their neighbors, or at least their odors, an unfamiliar individual might represent a larger threat than the possible benefit of a mating opportunity is worth. If true, I would expect that the probability that males would engage in extra-pair mating with familiar neighbors over unfamiliar neighbors would be greater.

Possible Insight into the Evolution of Monogamy in Prairie Voles

At face value, my data show males prefer to bond. Females, on the other hand (up to 55%), appear to readily engage in multi-male mating when given access to multiple males over a 24 h period (Wolff et al. 2002). These combined results may shed light on why social monogamy might have evolved in prairie voles. Decades of work aimed toward understanding sexual selection has led to the belief that one sex limits the rate of reproduction, which causes an imbalance in the number of offspring each sex can produce (Bateman 1948; Trivers 1972). Males are believed to benefit most from mating with multiple females, and intense competition among males for access to females emerges as a consequence (Andersson 1994). Competition for resources (either the females directly or things that females desire like access to high quality territories, etc.) is energetically demanding and thus only a subset of males will successfully gain access to multi-female mating (Clutton-Brock & Vincent 1991; Emlen & Oring 1977; Shuster & Wade 2003). One route to (social) monogamy may evolve when the energetic costs of defending resources (either through resource holding potential for resources that females desire, or mate guarding the females themselves) becomes too great to effectively fertilize those females (Clutton-Brock 1989; Clutton-Brock & Vincent 1991; Emlen & Oring 1977; Komers & Brotherton 1997; Orians 1969; Ostfeld 1985, 1990; Reichard & Boesch 2003; Shuster & Wade 2003). In this context males will reduce effort from attempting to monopolize several females, to sustaining just one. Forming a bond presumably increases the probability that a male will sire some proportion of offspring, although this will obviously depend on the male's individual ability to mate guard (refs Op cit.). This behavior represents a shift in the mating system away from

polygyny toward monogamy. Interestingly, this theory suggests that male tactics provide the pressure toward monogamy, since the benefits of mating with multiple males to females will not be heavily curtailed by the benefits to males for ensuring paternity with a single partner. There are obviously other equally valid routes for monogamy to evolve (e.g., need for bi-parental care, extreme ecological constraints on males and females, etc. (Clutton-Brock 1989; Clutton-Brock & Vincent 1991; Emlen & Oring 1977; Gubernick & Teferi 2000; Komers & Brotherton 1997; Orians 1969; Ostfeld 1985, 1990; Reichard & Boesch 2003; Shuster & Wade 2003; Wolff & Macdonald 2004). However, these paths to monogamy do not predict that males should benefit from bonding while benefits to females from either bonding or remaining single should be equivocal (i.e., 'male-imposed monogamy'). The evidence showing that females readily mate multiply in the lab (Wolff et al. 2002) while males will choose to pair bond (current study), hints that monogamy in prairie voles may have followed this path. Retrospectively replicating the steps of evolution in this (or any) species is impossible, and it is well understood that males may in fact choose to mate multiply in different circumstances (i.e., in the wild, an environment where their pair bonded female cannot "witness" their infidelity). However, looking for behavioral tendencies and predispositions, particularly when male and female behaviors are misaligned, may provide insight into the process. Prairie voles are clearly a good example of social monogamy, however, it is not clear how or why this mating system might have evolved; the data from this study provides the basis for some speculation on these fundamental questions.

Acknowledgements

I **thank Asad Rehman and Josh Garner** for their help collecting behavioral data from video recordings. This work was supported by funding from the National Science Foundation Graduate Research Fellowship to T.D.B. (2010103297) and by the Eunice Kennedy Shriver National Institute of Child Health and Human Development to A.G.O. (HD065604).

CHAPTER III

PAIRED, BUT NOT SINGLE MALES, RECOGNIZE FEMALES

Preface

With some modifications, this chapter is reprinted from Animal Behavior, Vol 108, Blocker, TD & Ophir, AG, "Social recognition in paired, but not single male prairie voles," pages 1-8. Copyright 2015. The paper was submitted to Animal Behavior on November 6, 2014. It was initially accepted January 6, 2015, and its final acceptance was June 10, 2015 (MS. Number A14-00898R).

Abstract

Social recognition, or the ability to recognize conspecifics, is an integral component of behaviour that underlies many much larger behavioural suites. For example, monogamous pair bonding is relatively meaningless if an individual cannot recall with whom the bond was with. The prairie vole, *Microtus ochrogaster*, is a socially monogamous rodent, well known for its long-term pair bonds between males and females. Although previous work has shown that bonded males reliably spend more time with their pair-mate over an unfamiliar female, recent work has demonstrated that single male prairie voles do not discriminate between females. This discrepancy raises the important question: do paired males distinguish between nonmate females? I asked whether pair bonding alters the expression of social recognition in male voles by comparing social recognition of single and pair bonded males using the habituation/dishabituation paradigm. I found that pair bonded, but not single male prairie voles showed social recognition of (nonmate) females, suggesting a shift in cognitive behaviour after pair bond formation. This

difference was not due to differences in motivation to engage in social exchanges, as males attempted to contact unfamiliar females at similar levels. Based on these data, I speculate that the stage of life (single or bonded) influences the ability to recognize, or perhaps the attention given to recognition of, same- and opposite-sex conspecifics.

Introduction

Social recognition is a fundamental cognitive ability that contributes to most aspects of behavioral biology. Social recognition can be thought of as the ability to process and subsequently make use of social information, enabling animals to discriminate among conspecifics and socially interact based on past experiences. Without this ability an animal would be unable to differentiate between kin and potential mates, neighbors and intruders, dominant and submissive conspecifics, healthy or diseased individuals, or their own versus another's offspring (Carter & Keverne, 2002; Choleris, Kavaliers, & Pfaff, 2004; Colgan, 1983; Kavaliers, Choleris, Agmo, & Pfaff, 2004). The role of social recognition in these behaviors has obvious significant implications for fitness.

A pair bond between two individuals forms the foundation for a monogamous relationship. Such a bond requires much more than simply positive affiliative behavior directed towards the pair-mate. The need to identify and discriminate a mate from other conspecifics is crucial if an animal is going to establish a bond that lasts longer than a single encounter. Therefore, although it may not often be discussed in this way, social recognition is a necessary component of monogamous relationships.

Social recognition has been studied in a number of species. However, much of the attention has focused on traditional laboratory rodents, like mice (*Mus musculu*s) and rats (*Rattus norvegicus*), and indeed significant progress towards understanding the mechanisms that underlie social recognition and some of the contexts in which social recognition emerges has been gained

(Bielsky, Hu, Szegda, Westphal, & Young, 2004; Choleris et al., 2003; Ferguson, Aldag, Insel, & Young, 2001; Ferguson, Young & Insel, 2002; Kogan, Frankland, & Silva, 2000). Rodents primarily use olfaction to recognize conspecifics, rather than by visual means, as in humans. In addition, several studies have implicated the neuromodulators OT and AVP (and their respective receptors OTR and V1aR), in a brain region called the lateral septum, as necessary for social recognition (Bielsky, Hu, Ren, Terwilliger, & Young, 2005; Everts & Koolhaas, 1999; Ferguson et al., 2002). Unfortunately, these species do not form monogamous pair bonds.

Prairie voles, *Microtus ochrogaster*, are particularly useful in studies of social behavior because they are socially monogamous (Carter, 1998; Carter, DeVries, & Getz, 1995; Getz, McGuire, Pizzuto, Hofmann, & Frase, 1993; McGuire, Getz, Bemis, & Oli, 2013; Ophir, Phelps, Sorin, & Wolff, 2008; Solomon & Crist, 2008). Although some males and females in the population are known to engage in extrapair mating (Ophir, Phelps, et al., 2008; Solomon, Keane, Knoch, & Hogan, 2004; Wolff, Mech, Dunlap, & Hodges, 2002), most prairie voles nest in pairs and share in offspring care (Getz & Carter, 1996; Wolff et al., 2002). Furthermore, much has been learned in the past 20 years regarding their neurobiology and social behavior (Carter, 1998; Carter et al., 1995; Ophir, Wolff, & Phelps, 2008; Resendez & Aragona, 2013; Solomon et al., 2009; Young & Wang, 2004; Young, Young, & Hammock, 2005), and recently they have been studied for their social investigation and recognition (Ophir, Zheng, Eans, & Phelps, 2009; Zheng, Foley, Rehman, & Ophir, 2013). For example, Zheng, Foley, et al. (2013) showed that social recognition in male voles differs across social contexts. Of particular note, was that single male prairie voles showed social recognition of other males, but not of females. Zheng, Foley, et al. (2013) proposed that this difference in behavior might relate to an emphasis on the relevance of knowing male identity (perhaps to establish and defend a territory) over female identity (initiating courtship leading to a bond may be equally good for any available female at this stage of life). Furthermore, it is plausible that a male's skill at defending his home range (which might rely on discriminating between neighboring and competing males) has the power to impact his

desirability to females, thereby increasing the potential importance of establishing territories for single males.

This difference in social recognition of males and females raises a larger question: if social recognition is indeed necessary for social monogamy, do male prairie voles differentiate between females at all? Social recognition of female conspecifics should be particularly relevant to male prairie voles that have pair bonded. Indeed, pair bonded males show a characteristic partner preference for their mate over other females (Williams, Catania, & Carter, 1992) and selective aggression towards strangers (Young, Liu, & Wang, 2008; Young & Wang, 2004), strongly suggesting that males are able to at least discriminate between their partner and other females. However, it is unclear whether males are able to distinguish among nonmate females or if bonding induces a change in the expression of social recognition among nonmate females. In this study, I ask whether pair bonding alters the expression of female social recognition among male prairie voles. Specifically, I test the hypothesis that pair bonded males will demonstrate social recognition. To test this hypothesis, I compared the ability of single and pair bonded males to discriminate between unfamiliar females using the habituation/dishabituation paradigm.

Materials and Methods

Animals

All animals used in this study were from the F2 generation within a breeding colony derived from wild stock originally trapped in Champagne-Urbana, Illinois, U.S.A. At weaning (21 days), offspring were separated into same-sex litters and housed in polycarbonate cages (29 18 13 cm) lined with Sani-chip bedding and provided nesting material. No animals in this experiment were raised in isolation. Water and rodent chow (Rodent Chow 5000, Harlan Teklad, Madison, WI, U.S.A.) were provided ad libitum and animals were maintained on a 14:10 h light:dark cycle (lights on at 0600 hours) with ambient temperature maintained at 20 ± 2 C. This

study was approved by the Institutional Animal Care and Use Committee of Oklahoma State University (AS 09-6). All animals included in this study were sexually naïve adults (60 days of age) and unrelated to other animals to which they were exposed during the experiment.

Behavioral Testing

Twenty-eight adult sexually naïve males were ear-tagged and randomly divided into two experimental groups: pair bonded and single. All testing occurred between 0700 and 1600 hours and was semi-randomized such that each day an equal number of pair bonded and single males were tested, alternating pair bonded first, versus single first each day, and alternating between the two groups when greater than 2 animals were tested..

Pairing and Partner Preference Tests

Before establishing a pair bond between animals assigned to the pair bonded group, I induced sexual receptivity in the females by exposing them for 48 h to soiled bedding and nesting material from an unfamiliar male that was unrelated to the female and the focal male (Carter, Getz, Gavish, McDermott, & Arnold, 1980; Dluzen, Ramirez, Carter, & Getz, 1981; Richmond & Stehn, 1976). Next, I co-housed males assigned to the pair bonded group with females for 24 h to establish a pair bond (Williams et al., 1992; Winslow, Hastings, Carter, Harbaugh, & Insel, 1993). Notably, Williams et al. (1992) demonstrated that 24 h of cohabitation, even without mating, is sufficient to establish a pair bond. I confirmed that a pair bond had been established using a partner preference test (Williams et al., 1992) immediately after the period of cohabitation. Males were placed in a three-chamber apparatus (60x50x40cm) consisting of a neutral chamber (20x50x40 cm), and two smaller adjacent chambers (each 30x25x40 cm) (see Ophir & DelBarco-Trillo, 2007). The female with whom a ‘paired’ male had just been housed was tethered in one of the adjacent chambers and a novel female was tethered in the other. Prior to their involvement in this test, novel females were also induced to be sexually receptive as

described above. This design allows the male to move and interact freely with each female, while limiting the interactions between females. Tethering, which involves using a plastic zip-tie as a collar connected to a light-weight chain attached to the apparatus, does not inhibit animals from normal activities (e.g. moving, eating or mating; Ophir, Phelps, Sorin, & Wolff, 2007; Wolff & Dunplap, 2002). After 3h, males were returned to their home cages with their original pair-mate. I quantified time spent in side-by-side contact with each female to determine which female subject males preferred. A pair bond was defined as when a male spent at least twice as much time in contact with the paired female over the stimulus female (Carter et al., 1995; Carter & Getz, 1993; Insel & Hulihan, 1995; Insel, Preston, & Winslow, 1995; Williams et al., 1992).

Focal males assigned to the single group remained in their home cages with a single male sibling during the pair bonding period. To ensure that pair bonding alone would account for behavioral differences, single males also underwent a choice test akin to the partner preference tests. Single males were presented the same pairs of females that served as stimuli for a male assigned to the pair bonded group. Female pairs were reused only once to test a male serving in the single group and a male serving in the paired group. I counterbalanced the order of which male (single or paired) was first across the experiment.

Partner preference tests were recorded using a Sony HDR- XR200V camcorder (Sony, New York, NY, U.S.A.) placed approximately 1m above the apparatus. Videos were scored using Observer XT software (Noldus Information Technology, Leesburg, VA, U.S.A.). To use videos in Observer XT, the .mov files the recorder produced were converted into .mpg files using Quicktime X (Apple Inc, Cupertino, CA, U.S.A.). Video observers were blind to the mating status of the male (paired or single).

Partner Preference Test Analyses

I analyzed partner preferences in two ways. First, I used ANOVA to compare the time that single and paired males spent with each female. Comparing the data in this way allowed us to

determine whether bonded males demonstrated a preference for partners and whether they were indeed bonded. It also allowed us to determine whether single males demonstrated a preference for either a bonded or a receptive single female. Single males might be expected to systematically spend more time with unbonded females over bonded females if bonded females become less receptive after bonding. Although this analysis revealed the magnitude of time spent in side-by-side contact with each female type, it did not allow assessment of whether single males showed a preference for a particular female (regardless of type). For example, single males may prefer one female over the other without respect to whether the females are bonded. To address this question, I took the difference in time that bonded and single males spent with their preferred female (the female with whom males spent more time) and their nonpreferred female. This comparison allowed us to determine (1) the degree to which males spent time with their preferred females and (2) whether single males spent more time with one female over the other (regardless of whether she was single or bonded).

I tested all groups to determine whether they were normally distributed using the D'Agostino & Pearson normality test to determine which statistical test to use. When the data were not normally distributed, I transformed the data using log transformations before performing ANOVA, or I used nonparametric tests (e.g. Wilcoxon signed-ranks test). When the data were normally distributed, I used Student's t tests and one-sample t tests to compare between groups or to compare whether means differed from zero (respectively).

Social Recognition Tests

I tested males for social recognition 1 day after serving in the partner preference test. The tests were performed in a custom-made apparatus made of 1.3 cm thick transparent acrylic plastic (20 40 28 cm; see Zheng, Foley, et al., 2013). The long walls of the apparatus were different heights, enabling us to position a mirror at a 45 angle, and allowing us to simultaneously capture a top down and side view by recording from the side. The apparatus had four square openings,

two along the long back wall and one on each of the short walls. The openings could be fitted with either acrylic square panels to close each opening, or a 'presentation chamber'. Presentation chambers were boxes made of transparent acrylic, fitted with a hinged lid and a front face that had 13 holes (1.3 cm diameter). These holes allowed for auditory and olfactory information transfer between the stimulus animal and the focal animal, while preventing direct contact. The apparatus was dusted with a thin layer of Sani-chip bedding. Trials were recorded using a Sony HDR-XR200V camcorder placed approximately 1m away from the front wall of the apparatus.

To test for social recognition, I used the habituation/dishabituation paradigm (Thor & Holloway, 1981; Winslow & Camacho, 1995). This test works on the assumption that olfactory inspection decreases as test animals become increasingly familiar with conspecifics (habituation), but that olfaction will increase when test animals are presented with a novel conspecific (dishabituation) (Thor & Holloway, 1981). In practice, a focal animal is exposed repeatedly to a stimulus animal during a 'habituation phase', and then exposed to an unfamiliar animal during a single 'dishabituation phase' test. A difference is expected between the time a focal animal investigates the unfamiliar animal during the dishabituation phase and the time it investigates the familiar animal at the end of the habituation phase (Thor & Holloway, 1981). A lack of difference is interpreted as an inability to discriminate between conspecifics (Ferguson et al., 2002).

To initiate the trial, focal males were placed in the apparatus for 30 min, to allow them to acclimate. Stimulus animals were also placed in their presentation chambers for 30 min prior to their first presentation. Stimulus animals were sexually mature and sexually naïve females. All animals serving in each trial were unrelated to each other. To begin the habituation phase, focal males were placed in the center of the test chamber and covered with a white PVC cylinder (15.24 cm height, 7.62 cm in diameter). Next, the acrylic panel was removed from one opening along a short wall and replaced by the presentation chamber containing the first stimulus female. The white cylinder was then removed, and the focal male was allowed to move freely in the testing chamber for 5 min. The focal male was then covered with the white cylinder and returned

to the center of the test chamber, and the presentation chamber was removed and replaced with an acrylic panel. Stimulus animals within the presentation chambers were moved far enough away to prevent visual, olfactory or auditory interactions during this 15 min intertrial interval. During the intertrial interval, the white cylinder was removed and the focal male was allowed to move freely. This procedure was repeated five times in direct succession, such that the test lasted a total of 115 min from start to finish (including time to acclimate). I used the same stimulus female for the first four presentations (P1-P4); on the final presentation (P5) I used a new unfamiliar stimulus female.

I measured the time that focal males investigated stimulus females in the presentation chambers. Inspection was defined as the time when a focal male's head was within 2 cm of the presentation chamber with his nose directed towards the holes. I also measured the time that the focal male spent attempting to contact females in the presentation chambers. I defined attempted contact as the time when the focal male bit or clawed at the presentation chamber interface. Time spent investigating was interpreted as the focal male's attempt to gather olfactory information about the identity of the stimulus animal (Zheng, Foley, et al., 2013). Time spent attempting to contact stimulus females was interpreted as the focal male's motivation to interact physically and socially with the stimulus female (Zheng, Foley, et al., 2013).

As described above, videos were converted into .mpg files and scored using Observer XT software. Video observers were blind to the status of the male, and to the phase of the experiment (P1-P5).

Social Recognition Score and Analyses

I compared the difference between the time that focal males investigated stimulus animals presented in the first and final presentation of the habituation phase (P1 and P4, respectively) with the time spent investigating stimulus animals presented in dishabituation phase (P5) using ANOVA. While this comparison was important for demonstrating that the pattern of

inspection differed within and between paired and single males, it did not account for potential confounds introduced by variability in total investigation time. I therefore also calculated a 'social recognition score' by dividing the difference in inspection time of the novel stimulus from the familiar stimulus by the sum of the total inspection time for these presentation periods using the following equation: ((P5 -P4)/(P5 + P4)). The larger the social recognition scores, the greater the degree of social recognition. A social recognition score of zero means that no social recognition was detected. These scores allowed us to compare between groups, and to evaluate whether the animals demonstrated any social recognition at all (by comparing these scores to zero). To confirm that males did not fatigue during the recognition test, I compared 'novel animal recognition scores' (inspection) during the first presentation of the habituation phase (P1) and during the dishabituation phase (P5), ((P5 -P1)/(P5 + P1)), with the expectation that these scores should be zero. I used similar comparisons to analyse attempted contact data.

As with my partner preference data, I tested the data from all groups to determine whether they were normally distributed prior to analysis. When the data were not normally distributed, I log transformed the data before performing ANOVA, or I used nonparametric tests (e.g. Mann Whitney U test, Wilcoxon signed-ranks test). When the data were normally distributed, I used Student's t tests and one-sample t tests to compare between groups or to compare whether means differed from zero (respectively).

Results

Excluded Animals

Of the 12 males from the 'single' group, one male was excluded from the analyses because he remained in a corner of the apparatus opposite the presentation chamber during the social recognition test and did not participate in the trial. I excluded a second male from this group because the behavioral recording during the social recognition test was corrupted in the

conversion process. To be considered pair bonded, a male had to spend twice as much time in side-by-side contact with his female mate than with the sexually receptive novel female. Of the 12 males from the pair bonded group, one male did not meet this criterion and was excluded from analysis. Thus, my final sample sizes were 11 pair bonded males and 10 single males.

Partner Preference Test and Male Mating Status

To begin, I confirmed partner preference formation by comparing time spent in side-by-side contact with bonded females versus novel stimulus females. Because these data were not normally distributed, I log transformed the data (after adding a constant, 1, to account for values of zero). A two-factor ANOVA revealed that both main effects were significant (male mating status: $F_{1,19} = 14.16$, P= 0.001; female type: $F_{1,19} = 6.41$, P = 0.02). However, these effects were entirely driven by a significant interaction between female type (bonded female versus novel female) and male mating status (paired or single) (ANOVA: $F_{1,19} = 8.07$, P = 0.01). I also found that pair bonded males spent significantly more time in contact with their partners than with the stranger (two-tailed Wilcoxon signed-ranks test: W = 66, N= 11, P = 0.001; ***Figure 3.1a***), whereas single males showed no preference for either a paired or sexually receptive single female (W= 4, N= 10, P= 0.81; ***Figure 3.1a***). Raw (untransformed) data are presented in ***Figure 3.1a*** to show the actual amount of time that males spent in contact with each female, but the pattern of transformed data across groups was the same (data not shown).

I also calculated the difference in time that males spent in contact with their preferred and nonpreferred females, and compared this measure between paired and single males. In the case of these difference scores, a positive value means males spent more time with the 'preferred female' and a value of zero means males spent equal time with each female. Single males showed no difference in time spent with either female (one-sample t test from zero: $t_9 = 0.07$, P= 0.95; ***Figure 3.1b***), whereas paired males spent more time with their preferred (bonded) females (one-sample t test: $t_{10} = 5.60$, P = 0.0002; ***Figure 3.1b***). Moreover, paired males spent significantly

more time with their preferred female than single males (Student's t test: $t_{19} = 3.58$, P= 0.002; ***Figure 3.1b***).

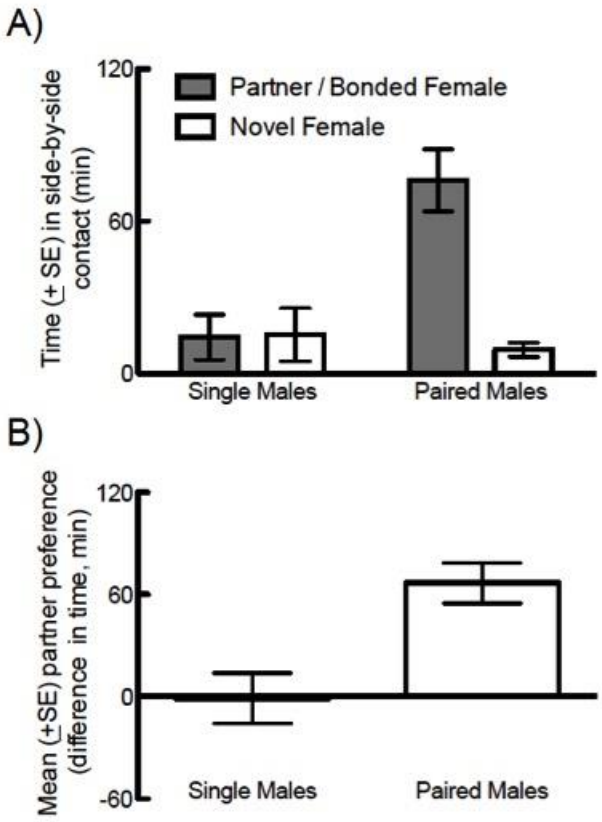

Figure 3.1: **A)** Mean ± SE time (in minutes) that pair bonded (paired) and single males spent in side-by-side contact with paired (shaded bars) and novel (open bars) females. **B)** Mean ± SE difference in time (in minutes) males spent in side-by-side contact between paired and novel females.

Social Recognition Test and Social Inspection

Social recognition data were not normally distributed and were therefore log transformed prior to analysis. However, raw (untransformed) data are presented in ***Figure 3.2a*** to show the actual time spent engaged in inspection.

Many studies employing the habituation/dishabituation paradigm have demonstrated that the critical measure is the change in inspection time between the fourth presentation (P4, final

presentation of the familiar stimulus female) and the fifth presentation (P5, the first presentation of the novel stimulus female). Social discrimination between the familiar and unfamiliar stimulus animal is confirmed if animals increase their inspection time of the novel stimulus animal (Ferguson et al., 2002). I found a significant interaction, indicating that paired males increased the time they spent investigating the novel female, whereas single males did not ($F_{1,19}$ = 9.43, P= 0.006). A main effect of presentation ($F_{1,19}$ = 13.03, P= 0.002), but not male mating status ($F_{1,19}$ =-0.11, P= 0.75), was also significant. Analyses of the raw (untransformed) data were consistent with these results (data not shown).

I next compared the social recognition scores of paired and single males. Social recognition scores with values of zero indicate that males inspected stimulus animals equally whereas positive values indicate that males increased their inspection of the novel stimulus animals in the dishabituation phase. Social recognition scores were normally distributed. Paired males had significantly greater social recognition scores than single males, indicating that paired males clearly showed social recognition, whereas single males did not (unpaired t test: t_{19} =3.083, P= 0.006; ***Figure 3.2b***). Single males' social recognition scores did not differ from zero (one-sample t test: t_9= 0.39, P= 0.71), but paired males' social recognition scores did (one-sample t test: t_{10}= 5.69, P= 0.0002), supporting the notion that paired males discriminate between females, but single males do not.

To ensure that differences that I observed between presentations were not the result of fatiguing interest for inspecting females, I calculated a novel female inspection score. The novel female inspection score evaluated the differences in time that males inspected stimulus females when they were unfamiliar (i.e. presentation 1 (P1) and presentation 5 (P5); see Methods). Novel female inspection scores were normally distributed. Pair bonded males and single males showed no difference in their novel female inspection scores (Student's t test: t_{19}= 0.50, P= 0.62), and neither score differed from zero (one-sample t test: single males: t_9= 0.63, P= 0.54; paired males:

t_{10}= 0.03, P= 0.98), indicating that paired and single males investigated unfamiliar females similarly (***Figure 3.2c***).

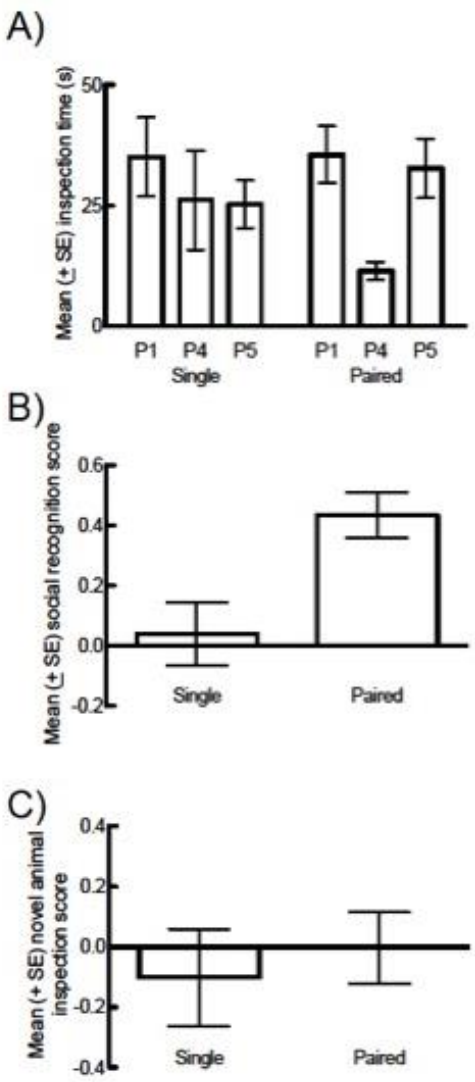

Figure 3.2: **A)** Mean ± SE time (in seconds, non-transformed) that pair bonded and single males spent inspecting stimulus females during presentations 1 and 4 in the habituation phase (P1, and P4) and presentation 5 during the dishabituation phase (P5). **B**) Mean ± SE inspection score ([P5 – P4] / [P5 + P4]) for single and paired males. **C**) Mean ± SE novel animal inspection score ([P5 – P1] / [P5 + P1]) for single and paired males.

Social Recognition Test and Attempted Contact

Whereas inspection is thought to reflect information gathering used to identify a conspecific, attempted contact is an estimation of the social motivation of the focal animal. This measure is important if evidence for recognition is not detected (i.e. single males), because if a lack of inspection reflects a lack of social motivation, then it is difficult to assess whether a group of animals is unable or unwilling to discriminate between conspecifics. I therefore compared attempted contact of paired and single males. Again, data were log transformed prior to analysis because they were not normally distributed, but raw (untransformed) data are presented in ***Figure 3.3a*** to show the actual time spent engaged in attempted contact. Attempted contact did not differ between the fourth and final presentation ($F_{1,19}$= 1.86, P=0.17), between single or paired males ($F_{1,19}$ =0.53, P= 0.48), or across these factors (interaction: $F_{1,19}$ = 0.34, P= 0.71). Untransformed data also showed no differences in attempted contact across these groups (data not shown).

I calculated an attempted contact score (ACS) for the fourth and final presentation and a novel animal attempted contact score (NACS) for the first and final presentation (similar to the social recognition score and the novel animal inspection score described above). The data for attempted contact scores were not normally distributed, but those for novel animal attempted contact scores were. Paired and single males did not differ in their attempted contact scores (Mann Whitney U test: U =45, N1= 11, N2= 10, P= 0.50; ***Figure 3.3b***) or novel animal attempted contact scores (t_{19}= 0.14, P = 0.91; ***Figure 3.3c***), and none of these values differed from zero (one-sample Wilcoxon signed-ranks test: single ACS: W= 25, P= 0.23; paired ACS: W= 2, P= 0.97; one-sample t test: single NACS: t_9= 0.50, P= 0.63; paired NACS: t_{10}= 1.07, P= 0.31), indicating that no change in attempted contact across all major comparisons for either single or paired males was detected. Taken together, these results indicate that single and paired males did not differ in their social motivation, reinforcing the interpretation that the difference between single and paired males is indeed a function of social recognition.

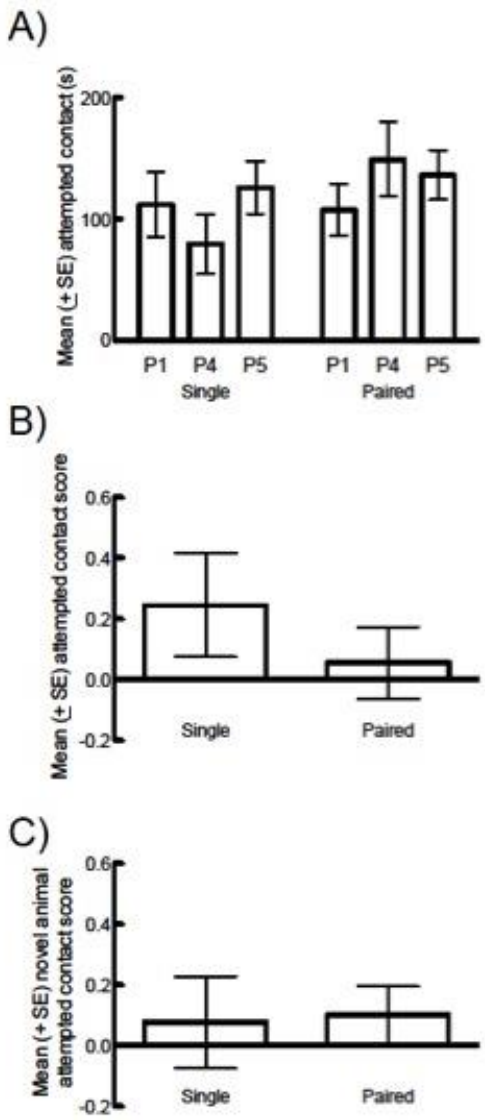

Figure 3.3: A) Mean ± SE time (in seconds, non-transformed) that pair bonded and single males spent attempting to contact stimulus females during presentations 1 and 4 in the habituation phase (P1, and P4) and presentation 5 during the dishabituation phase (P5). B) Mean ± SE attempted contact score ([P5 – P4] / [P5 + P4]) for single and paired males. C) Mean ± SE novel animal attempted contact score ([P5 – P1] / [P5 + P1]) for single and paired males.

Discussion

Our results indicate that pair bonded male prairie voles show social recognition of females, but single males do not. The latter result replicates part of what Zheng, Foley, et al. (2013) reported: single male prairie voles show social recognition of other males, but not of females. My results enrich this story, indicating that mating status influences male prairie vole responses to social contexts in which learning social identity about females is possible.

Our results suggest that the transition from being single to being pair bonded is associated with a difference in social recognition among male prairie voles. Failing to demonstrate social recognition in the habituation/dishabituation test, as was the case for single males, is commonly interpreted as evidence for a deficit in recognition (Ferguson et al., 2002). Note, however, that an inability to detect social recognition does not necessarily indicate an animal's inability to discriminate between conspecifics. Therefore, it is possible that single males may be capable of discriminating between females but choose not to. Nevertheless, it is peculiar that single males would not behave in a way that demonstrates their ability to distinguish between females, when they do this for males (Zheng, Foley, et al., 2013) or after forming a pair bond (the current study). One possibility is that single animals do not show social discrimination because they are not sufficiently motivated to interact with opposite-sex conspecifics. However, this interpretation seems unlikely for two reasons. First, males are usually highly motivated to interact with females, particularly sexually receptive females. Second, attempted contact (an estimation of social motivation, which may include sexual or nonsexual motivation) was the same across males regardless of whether they were single or bonded, and attempted contact was the same across all presentation periods within single and paired males. These results suggest that the differences in social inspection (the basis of my recognition measurements) have little to do with differences in social motivation, and inspection is most likely an indicator of information gathering. Another perspective is that paired animals become more sensitive to sensory cues necessary to

discriminate between unfamiliar females. Indeed, it is plausible that the overall experience of bonding (including increased behavioral interaction with a female, and any possible neurochemical changes resulting from the bonding experience that could alter perception, attention or other aspects of behavior) could enhance the ability of paired males to distinguish between unfamiliar females. Whether the current results represent an enhancement of social recognition ability among paired males or deficiencies among single males, this effect appears to be robust (Zheng, Foley, et al., 2013; present study).

Why should bonded males demonstrate evidence for social recognition of females, when single males do not? Zheng, Foley, et al. (2013) argued that their observation that single males discriminate between males but not females supports the hypothesis that establishing territories and tracking male conspecifics is particularly relevant for single males. They speculated that territorial behavior appears to be important for single males in nature, and therefore tracking male identity would be necessary. On the other hand, since single males have not yet become attached to a female, tracking female identity should be relatively less important. Although this hypothesis is plausible, more research is needed to fully test this idea. My results lend some support to this hypothesis, since an implicit assumption of this hypothesis is that the specific identity of females should be comparably more relevant and salient to paired males. This is because paired males, at a minimum, must be able to discriminate between their partner and other females. Several studies have demonstrated that paired males can indeed discriminate between their partner and a novel female, since the highly replicated partner preference test requires such discrimination (e.g. Insel et al., 1995; Williams et al., 1992; Winslow et al., 1993). What has heretofore been less clear is whether this transfers to two unfamiliar females. One possibility is that bonded males discriminate between their mate and nonmates, but not among nonmate females. My results indicate that this is not the case. Perhaps being bonded increases the relevance of unfamiliar female identity, such that paired prairie vole males, which engage in extra-pair mating (Ophir, Phelps, et al., 2008; Wolff et al., 2002), benefit by differentiating between females that are not

their partners. Such an ability might help paired males distinguish between and identify neighboring females that offer the greatest opportunities for extra-pair copulations.

I have identified a potential shift in cognitive ability (i.e. social recognition) between single and paired male prairie voles. This change in behavior also accompanies other important behavioral shifts that follow pair bonding. Once bonding has occurred, males become selectively more affiliative towards mates (Carter, Witt, Thompson, & Carlstead, 1988), selectively aggressive towards strangers (Getz, Carter, & Gavish, 1981; Winslow et al., 1993) and more parental towards offspring (Bamshad, Novak, & Devries, 1994; Terleph, Jean-Baptiste, & Bamshad, 2004). Because selective aggression is often associated with mate and/or pup guarding (Clutton-Brock, 1989; Kleiman, 1977) and paired males become aggressive to unfamiliar individuals of both sexes (Winslow et al., 1993; Huang & Ophir, n.d.), the pressure for paired males to discriminate between unfamiliar females may go beyond the benefits associated with identifying extra-pair mating opportunities (as mentioned above). Another plausible benefit may result from identifying whether neighboring females are likely to commit infanticide (Hausfater & Hrdy, 1984; van Schaik & Janson, 2000).

In contrast to the aforementioned behaviors that differ between paired and single male prairie voles, pairing does not appear to alter discrimination between a male's own pups or pups from other breeders. In fact, studies that have investigated changes in paternal behavior have shown that both bonded and single male prairie voles will accept and care for unfamiliar pups (Bamshad et al., 1994; Lonstein, 2002; Terleph et al., 2004). Despite the fact that so many behaviors are distinctly different between paired and single males (such as increased paternal behavior by paired males; Bamshad et al., 1994; Terleph et al., 2004), bonding is not associated with recognition of the pups for which paired males are providing more care. Taken together, the ability of paired and single male prairie voles to demonstrate social recognition appears to be tremendously nuanced and dependent on the context (i.e. features of the individuals being assessed and doing the assessing). Absence of social discrimination for pups aside, forming a pair

bond appears to significantly alter behavioral responses in many domains, and may affect the importance of, and reaction to, many forms of social information.

Young et al. (2005) suggested that social recognition is necessary for pair bond formation. Indeed, if males are unable to remember the identity of the females with which they have become attached, then those pair bonds are meaningless. I extend this idea by suggesting that social recognition is an essential cognitive ability for monogamy. The current study is the first to investigate within- species differences in social recognition within the context of mating status. I speculate that the social and environmental pressures associated with monogamy enhance the need to rely on, and attend to, female identity, an idea consistent with hypotheses presented elsewhere (Ophir, Gessel, Zheng, & Phelps, 2012; Ophir, Wolff, et al., 2008; Phelps, Campbell, Zheng, & Ophir, 2010; Zheng, Larsson, Phelps, & Ophir, 2013) and the data presented here. My results encourage consideration that changes in social context (forming bonds or remaining single) may accompany changes in cognitive decisions. Indeed, cognitive changes in social behavior have often been underappreciated, but offer great insight and may provide new directions in which to investigate animal behavior.

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CHAPTER IV

BEHAVIORAL SHIFTS ARE NOT REFLECTED IN NEURAL RECEPTOR DISTRIBUTION

Preface

This chapter currently stands as a supportive chapter for this dissertation.. The goal of this project was to analyze the prairie vole brain for changes in neural receptor distribution in response to the behavioral shifts associated with pair bonding. The negative findings make publication unlikely, however, the value of establishing that these phenotypes are stable at these different life stages is significant.

Abstract

Prairie voles, *Microtus ochrogaster*, are commonly studied for their expression of multiple forms of social behavior, and provide scientists with an avenue to investigate the neural mechanisms driving such behavior. They exhibit affiliative behavior toward mates, selective aggression toward strangers, social recognition of conspecifics, and parental care to offspring. All of these behaviors present, or are enhanced with the formation of a pair bond between mates. Many experiments have been done to elucidate the mechanisms driving these behaviors, with emphasis placed on oxytocin (OT) and arginine vasopressin (AVP), two neurohormones/neuropeptides that are often implicated in modulating social behavior. Less studied however, are their receptors, and how changes in their distribution impact behavior. As

these neurohormones have been highly correlated with social behaviors known to present, or increase with changes in bonding status, I questioned whether changes in the less studied OT or AVP receptors (OTR and V1aR, respectively) may account for these shifts in behavior. Comparing recently pair bonded and single males, I analyzed OTR and V1aR densities in areas of the brain linked to social behavior. Results showed no significant differences between pair bonded and single males, indicating that shifts in social behavior are not driven by changes in receptor distribution.

Introduction

As shown in nature, and in this dissertation, social context, in the form of prairie vole (*Microtus ochrogaster*) pair bonding, impacts the behavior and cognition of male voles (See Chapter 3). Specifically, prairie vole pair bonding is accompanied by shifts in a suite of social behaviors, all of which contribute to the maintenance of the monogamous mating tactic. For example, prairie voles exhibit more affiliation toward a partner after pair bonding, which is evident in side-by-side contact spent with partners compared to strangers.(Carter et al., 1988). Males exhibit selective aggression, which is, increased aggression toward male and female conspecifics, but not toward their bonded mate (Getz et al., 1981; Winslow et al. 1993). This behavior is thought to assist in mate and pup guarding (Bamshad et al., 1994; Terleph et al., 2004). Finally, as this dissertation shows, pair bonding alters the expression of social recognition in male prairie voles, assisting them in differentiating between their own mates and other female conspecifics (Blocker & Ophir, 2015).

Pair bonding, along with many social behaviors, has been shown to have complex neural mechanisms in the social behavior network of the brain (Young et al., 2008). Two neurohormones in particular, oxytocin (OT) and agrginine vasopressin (AVP), are well-studied modulators of this network (i.e., Goodson, 2005). Modulation of neuron activity is commonly regulated by changes in signaling molecule concentrations, changes in neural receptor

distribution, or changes in affinity of the two for one another. Regulation of neurohormone activity differs with the type of neurohormone in question.

Pair bonding, and the associated social behaviors, are regulated by both neurohormone and receptor activity. Some of the early studies in prairie vole social behavior showed that increases in OT and AVP serve to prompt pair bonding and affiliative behavior in voles (Winslow et al., 1993; Williams et al., 1994). Similarly, antagonizing their receptors, OTR and V1aR, prevents pair bond formation, even in the presence of infused OT and AVP (Winslow et al., 1993; Williams et al., 1994; Insel & Hulihan, 1995; Cho et al., 1999). The relevance that receptor distribution plays in the exhibition of prairie vole social behavior is best displayed in early studies that show that, it is differences in receptor distribution, and not neurohormone levels, that accounts for the distinct mating systems exhibited in prairie voles and the closely related polygynous meadow vole (*M. pennsylvanicus*) (Insel & Shapiro, 1992; Insel et al., 1994, Young et al., 2001, Lim & Young, 2004). Furthermore, studies in transgenic mice showed affiliative behavior resembling that of prairie voles after the gene encoding V1aR was replaced with the prairie vole V1aR gene, highlighting the significance that receptor expression plays in the exhibition of social behaviors (Young et al., 1999).

As pair bonding is followed by shifts in expression of this suite of social behaviors, and these behaviors have been closely tied to OT and AVP activity, I predict that a shift in behavior should be propagated by a similar neural shift. To that end I investigated whether pair bonding, a behavior that is integral to prairie vole monogamy, and that triggers the expression of behaviors that are central to the monogamous mating strategy, instigates its effects through changes in OTR and V1aR distribution.

I chose to investigate neurohormone receptor distribution, as opposed to neurohormones themselves because pair bonding is not the end of a behavioral shift, but the beginning, which leads to manifestations of social behavior and cognition that are integral to the long term maintenance of the pair bond. OT and AVP are peptide neurohormones that are produced via

gene transcription and translation, synthesized as prepropeptides and stored in vesicles (Brady et al., 2012). They require enzymatic processing once there, before they can be released. After reuptake of OT and AVP, they must first be broken down in vesicles and transported back to the cell body before they can be recycled (refs Op cit.). However, although their production and recycling is slow, the vesicular storage of OT and AVP allows for their quick release. Taken together, OT and AVP might have the ability to initiate changes in behavior relatively quickly, but their availability is limited by slow synthesis, processing, and recycling. Thus release may not be the best way to regulate changes in long-term behavior.

Similar to their signaling molecules, OTR and V1aR synthesis depend on gene transcription and translation (Brady et al. 2012). Their removal requires intracellular withdrawal from neuronal membranes, followed by vesicular breakdown (refs Op cit). Ultimately, their production is slow, but once changed, they would be relatively stable, potentially lending toward maintenance of behavior. In addition, contrary to most neurotransmitter receptors, OTR and V1aR have an extremely high binding affinity for their neurohormones (in the nanomolar range) (refs Op cit). This high affinity translates to a potential for changes, even small ones, in receptor distribution, to cause vast differences in neuronal activity, which may explain the degree of behavioral plasticity seen in prairie voles across varying life stages.

OT and AVP have been shown to regulate many of the behaviors of the social behavior network (Goodson, 2005), including affiliation, selective aggression, parental care, and social recognition. I, therefore, expect that OTR and V1aR undergo a similar shift after pair bonding. Presumably long-term behavior would be better modulated through increased expression of receptors, rather than through neurohormones themselves, because these behaviors are necessary through the lifetime of the vole, and neurohormone activity is fleeting. To evaluate the hypothesized neural shift that occurs with pair bonding, and correlates with multiple changes in behavior, I compared the brains of single and recently pair bonded male prairie voles.

Specifically, I analyzed OTR and V1aR distribution between these two groups, comparing receptor distribution in many of the brain nuclei associated with the social behavior network.

Materials and Methods

Animals

All subjects were from the F2 generation within a breeding colony derived from wild stock originally trapped in Champagne-Urbana, Illinois. At weaning (21 days), offspring were separated into same-sex litters and housed in polycarbonate cages (29 x 18 x 13 cm) lined with Sani-chip bedding and provided nesting material. No voles in this experiment were raised in isolation. Water and rodent chow (Rodent Chow 5000, Harlan Teklad, Madison, WI, USA) were provided *ad libitum* and animals were maintained on a 14:10 hr light:dark cycle with ambient temperature maintained at 20±2°C. All procedures were approved by the Institutional Animal Care and Use Committee of Oklahoma State University. All animals were sexually naïve and unrelated to other animals to which they were exposed during the experiment.

Pairing and Partner Preference Tests

Twenty-eight adult sexually naïve males were ear-tagged, and randomly divided into two experimental groups: pair bonded and single.

Before establishing a pair bond between animals assigned to the pair bonded group, I induced sexual receptivity in the females by exposing them for 48 hours to soiled bedding and nesting material from an unfamiliar male, unrelated to the female and the focal male (Carter et al. 1980; Dluzen et al. 1981; Richmond & Stehn 1976). Next I co-housed males assigned to this group with females for 48 hours to establish a pair bond (Williams et al. 1992; Winslow et al. 1993). I confirmed that a pair bond had been established using a partner preference test (Williams et al. 1992). Males were placed in a three chamber apparatus (60 x 50 x 40cm) consisting of a

neutral chamber (20 x 50 x 40cm), and two smaller adjacent chambers (each 30 x 25 x 40cm) (see (Ophir & DelBarco-Trillo 2007)). The female with whom a male had just been housed ('paired') was tethered in one of the adjacent chambers and a novel female was tethered in the other. This design allows the male to move and interact freely with each female, while limiting the interactions between females. After three hours, males were returned to their home cages with their original pair-mate. I recorded time spent in side-by-side contact with each female to determine which female he preferred. A pair bond was defined as when a male spent at least twice as much time in contact with the paired female over the stimulus female (Insel et al. 1995). Focal males assigned to the single group remained in their home cages with male siblings during the pair bonding period. To ensure that pair bonding alone would account for neural receptor differences, single males also underwent a choice test akin to the partner preference tests. Single males were presented the same pairs of females that served as stimuli for a male assigned to the pair bonded group. Female pairs were used only once to test a male serving in the single group.

Tissue Extraction and Autoradiography

Upon completion of the behavioral analyses, experimental males were euthanized with CO_2 gas. Brains were extracted and flash frozen on powdered dry ice, then stored at -80° C. Later, brains were coronally sectioned into four sets of 20μm thick slices, mounted on SuperfrostPlus slides (Fisher Scientific) and stored at -80° C. Using autoradiography, I visualized the OT receptor (using the ornithine vasotocin analog, ([^{125}I]-OVTA); NEX 254, PerkinElmer; Waltham, MA) and the V1a receptor (using vasopressin(Linear), V-1A Antagonist (Phenylacetyl1, 0-Me-D-Tyr2, [^{125}I-Arg6]); NEX 310, PerkinElmer; Waltham, MA). To process tissues, sections were lightly fixed in 0.1% paraformaldehyde (4° C) for 2 min, washed twice in 1x Tris-HCl (pH 7.4, 4° C) for 10 min, and incubated in either 40pM [^{125}I]-OVTA or 50pM ^{125}I-labeled AVP (Linear), V-1A antagonist for 90 min at room temperature. Next, I washed the slides at RT in a series of 5 min baths of 1X Tris-HCl (pH 7.4) with $MgCl_2$ followed by a final wash in

1X Tris with $MgCl_2$ for 30 min (50mM Tris, 100 mM $MgCl_2$), and then rapidly air dried them. I then exposed the radioactive sections to film for four (OTR) or three (V1aR) days; differences in the length of time on film is representative of the ligand's radioactive decay at the time of use. I converted optical density to receptor density using ^{125}I-labeled standards (American Radiolabeled Chemicals; St. Louis, MO). I digitized films on a Microtek ArtixScan M1 (Microtek, Santa Fe Springs, CA) and then measured optical densities using NIH ImageJ Software. I measured OTR in the prefrontal cortex (PFC), anterior, medial and posterior insular cortex (ICa, ICm, ICp), nucleus accumbens (NAcc), septo-hippocampal nucleus (SHi), lateral septum (LS), caudate-putamen (CPu), central amygdala (CeA), basolateral amygdala (BLA), hippocampus (Hi), and the intermedial dorsal, centromedial, centrolateral thalamic nuclei (combined, IMD-CM-CL). I measured V1aR in the ventral pallidum (VPall), lateral septum (LS), the medial, lateral and ventral regions of the bed nucleus of the stria terminalis (BSTm, BSTl, BSTv), anterior hypothalamus (AH), suprachiasmatic nucleus (SCN), paraventricular nucleus of the hypothalamus (PVN), the lateral dorsal, medial dorsal and the ventral posterior thalamic nuclei (LDTh, MDTh, VPTh), retrosplenial cortex (RSC), central amygdala (CeA), medial amygdala (MeA), and the ventromedial hypothalamus (VMH).

I calculated receptor density by first converting optical density to disintegrations per minute (dpm), adjusted for tissue equivalence (TE; for 1 mg in the rat brain), by using a log function to fit curves generated by radiographic standards. I measured optical density for each structure of interest three times (once on a series of three brain sections, bilaterally). I also measured nonspecific binding on each section by measuring the background levels of fiber tissue (bilaterally) (which do not express either peptide receptor in prairie voles) on each of the same sections measured. The values for each structure converted to dpm / mg TE using microscale standards (American Radiolabelled Chemicals, St. Louis MO), averaged, and adjusted to represent specific binding by subtracting nonspecific binding from total binding for each area.

Results

Neural Analysis

Vasopressin and oxytocin receptor density was similar in all structures of the brain I examined for paired and single males (one-way ANOVA, V1aR: all F $\leq$ 4.64, all Ps $\geq$ 0.12; OTR: all Fs (x) $\leq$ 2.70, all Ps $\geq$ 0.16; see ***tables 4.1 & 4.2***). I did not perform corrections for multiple corrections (e.g. Bonferroni) because none of the differences was significant. To evaluate whether overall (total brain) V1aR and OTR density differed between groups, I added

Brain Region	PB V1aR µ±SE	Single V1aR µ±SE	P-value
Ventral Pallidum	816.77 ± 76.3	768.36 ± 97.6	0.51
Lateral Septum	544.48 ± 100.3	649.15 ± 83.9	0.42
Medial BNST	177.28 ± 11.1	209.37 ± 20.9	0.12
Lateral BNST	235.06 ± 20.5	262.61 ± 28.8	0.3
Ventral BNST	935.5 ± 80.4	854.81 ± 66	0.32
Anterior Hypothalamus	403.43 ± 76.6	490.01 ± 122.6	0.42
Paraventricular Nucleus	791.38 ± 110.7	770.10 ± 150.7	0.61
Suprachiasmatic Nucleus	115.35 ± 19.9	108.45 ± 74.4	0.92
Lateral Dorsal Thalamus	2070.21 ± 370.6	2228.8 ± 401.2	0.94
Medial Dorsal Thalamus	869.78 ± 173.2	1129.12 ± 283.3	0.59
Posterior Cingulate	491.22 ± 139.5	742.08 ± 209.8	0.4
Ventral Posterior Thalamus	529.13 ± 105	495.49 ± 149.6	0.66
Central Amygdala	533.03 ± 60.2	446.79 ± 50.7	0.61
Medial Amygdala	388.43 ± 38.4	391.95 ± 45.7	0.88
Ventral Medial Thalamus	590.25 ± 33.7	554.05 ± 89.5	0.79
Total Brain Avg	9908.61 ± 194.8	10101.16 ± 200.4	0.9

Table 4.1: Mean ± SE V1aR density throughout the brain of pair bonded and single males. Degrees of Freedom (DF): DF1: 1, for all brain regions. DF2: 18, for VPall, LS, BNSTm, BNSTl, BNSTv, AH, PVN, and SCN. DF2: 17, for LDTh, MDTh, and PCing. DF2: 16, for VPTh, CeA, MeA, VMH

Brain Region	PB OTR µ±SE	Single OTR µ±SE	P-value
Prefrontal Cortex	508.81 ± 79.6	367.42 ± 46.8	0.16
Anterior Insular Cortex	286.02 ± 35.9	262.07 ± 51	0.69
Nucleus Accumbens	394.43 ± 59.2	297.86 ± 58.7	0.25
Septal Hippocampus	696.69 ± 183.3	754.29 ± 249.1	0.85
Lateral Septum	315.65 ± 48.5	269.53 ± 69.6	0.58
Caudate Putamen	95.01 ± 23.6	83.32 ± 21.2	0.71
Medial Insular Cortex	258.15 ± 31.7	205.06 ± 38.4	0.29
Central Amygdala	490.68 ± 82.8	494.76 ± 43.3	0.97
Basolateral Amygdala	559.72 ± 85.3	562.21 ± 60.5	0.98
Hippocampus	275.24 ± 61	248.53 ± 58.6	0.75
Posterior Insular Cortex	197.41 ± 28.5	155.49 ± 19.8	0.25
IMD/CM/CL combined	104.47 ± 15.7	77.76 ± 18.5	0.27
Total Brain	4245.86 ± 39.1	3778.29 ± 52	0.39

Table 4.2: Mean ± SE OTR density throughout the brain of pair bonded and single males. Degrees of Freedom (DF): DF1: 1, for all brain regions. DF2: 18 for ICa, NAcc, SHi, LS, CPu, ICm, CeA, BLA, Hi, ICp, IMD/CM/CL. DF2: 17, for PFC.

receptor distributions across all brain regions and compared paired and single males. Similarly, neither total brain V1aR ($F_{16} = 0.794$, P = 0.90) nor total brain OTR ($F_{16} = 0.032$, P = 0.39) were significantly differences (see ***tables 4.1 & 4.2*** and ***Figures 4.1 &4.2***). These data indicate that formation of a pair bond does not alter nonapeptide receptor expression patterns in male prairie voles.

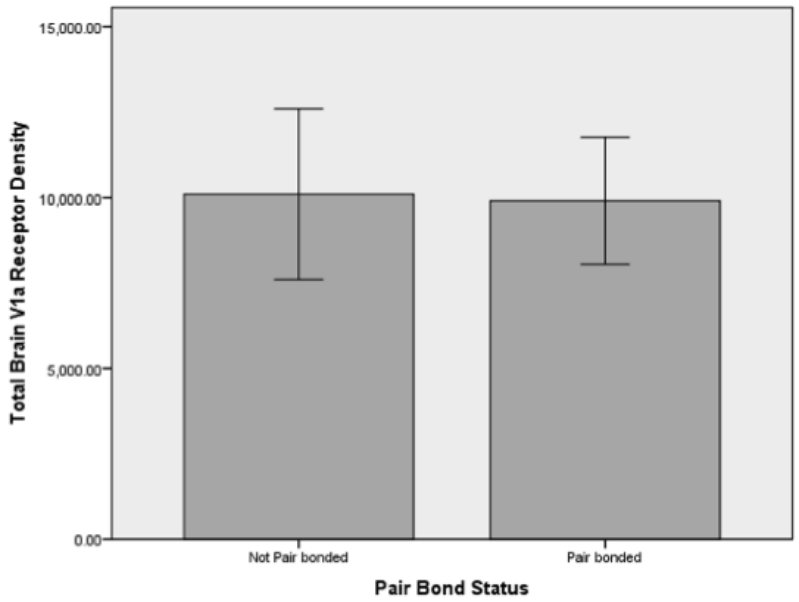

Figure 4.1: Mean ± SE total brain V1aR density in pair bonded and single males.

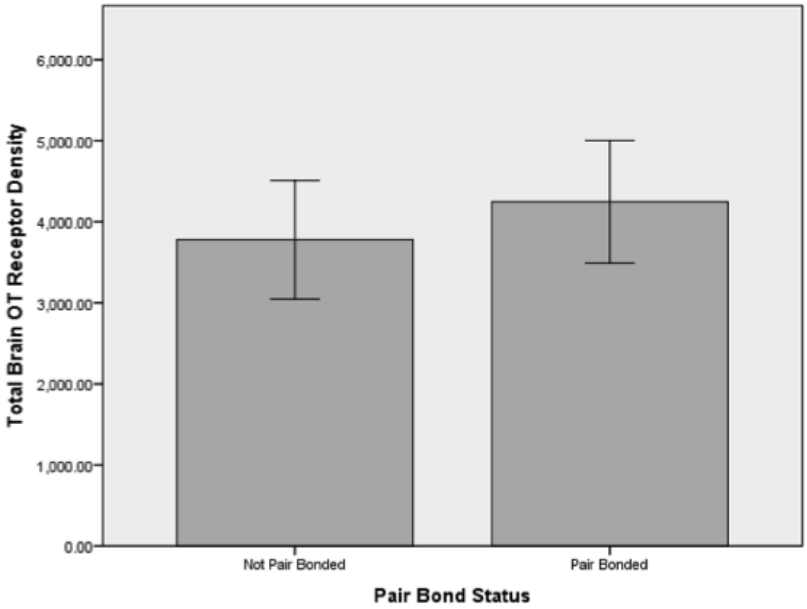

Figure 4.2: Mean ± SE total brain OTR density in pair bonded and single males.

Discussion

My results show that there is no difference between pair bonded and single males in the expression of OTR and V1aR, receptors of neurohormones that are vital to the expression of

social behavior. Even more, my results suggest that the behavioral shift associated with pair bonding is not regulated by receptor distribution. This is surprising because OT and AVP play significant roles in many of the social behaviors that are triggered by pair bonding (not to mention their roles in pair bonding itself) and all of these behaviors require long-term expression.

It may be possible that, despite their slow production and recycling, neurohormones themselves serve to modulate and maintain these behaviors. In fact, Ketterson and Nolan (1999) pose the hypothesis that behavioral changes at the individual level are regulated by hormones, while behavioral changes at the evolutionary level are most expected to occur via hormone receptors. Thus, day-to-day fluctuations in the life of an organism, for example, intraspecific variations in the expression of sociality, are driven by changes in neurohormone release. In contrast, branching in phenotypes of behavior, particularly on an evolutionary scale, might be propagated via phenotypic differences in receptors. A prime example of this is the interspecific differences seen in mating tactics between prairie voles and meadow voles (see Introduction).

Despite my initial prediction that receptors should capture the differences in nonapeptide modulation of bonding, day-to-day alterations in the peptides themselves might explain varying expression of behavior. If this is true, then there are several possible ways in which paired and single males may differ. For example, inhibition of extracellular proteases responsible for OT/AVP breakdown would serve to increase their availability in brain tissue. Similarly, increases in the enzymes responsible for processing of prepropeptides in the vesicles, could also increase available OT/AVP for release. Both of these methods would function to increase these reserves of peptide, and potentially behavior. However increasing their levels is not the only mechanism by which behavioral differences might emerge. In fact, many neuropeptides are known to be paired with more conventional neurotransmitters in their storage vesicles (Brady et al. 2012), leading to their release simultaneously upon neuron stimulation. Because these neurotransmitters would also act on post-synaptic neurons, they could provide a method by which a single neuron may have varying effects on surrounding neurons, and subsequently on behavior. An ideal example of this

would be AVP and corticotrophin releasing hormone (CRH), which are known to be stored and released together (Brady et al. 2012). Interestingly, both of these neuromodulators have been implicated in prairie vole social behavior (Devries et al. 1995; Carter et al. 1995, Carter 1998).

As I just argued, because neither V1aR nor OTR differed in any region of the brain I examined, it may seem reasonable to assume that modulation of behavior should be attributed to changes in neurohormone levels. However, there is evidence that social behavior is modified via changes in receptor expression in prairie voles. Specifically, selective aggression is expressed in prairie voles after pair bonding and is induced by AVP release in the anterior hypothalamus of male voles after pair bonding (Gobrogge et al., 2009). Pair bonding increased AVP-V1aR binding in the anterior hypothalamus (AH) and medial preoptic area (mPOA) of male voles occurs two weeks after pair bonding, which appears to be related to an increase in V1aR expression in pair bonded, but not single, males (Gobrogge et al., 2009). Aragona et al. (2006), provides additional support that changes in behavior are brought about through changes in receptor distribution. They showed that up-regulation of dopamine receptors in the nucleus accumbens was responsible for selective aggression exhibited by male prairie voles two weeks after the formation of pair bonds.. The results of these studies not only indicate that at least V1aR expression in the AH and mPOA should differ between paired and single males, they also raise an important point with respect to my study. The time at which the brains are analyzed relative to bonding may be crucial for detecting bond-induced changes in nonapeptide receptor expression. Here I analyzed vole brains for changes in OTR and V1aR distribution after only 48h of contact. Although research has shown that a pair bond is established in as little as 24h (Williams et al. 1992; Winslow et al. 1993), it is likely that this pair bond is activated by increases in OT and AVP release. It is within reason to assume that if pair bonding triggers behavioral changes via modification of OTR and V1aR, that the time necessary to propagate that change (through increased gene expression, resulting in increased protein receptor production, followed by transfer of the receptor to the neuronal membrane) would take longer than 48h. If this is true, future work should take into

consideration the timing of brain harvesting and DNA transcription/translation, and examine OTR and V1aR expression at a later time point.

Taken at face value, my results indicate that receptor expression is stable. Despite known involvement of OT and AVP in social behavior, my results suggest that sensitivity to these neurohormones does not change after pair bonding. If there is, in fact, no change in receptor expression after pair bonding, then the vast behavioral plasticity seen in prairie voles across life stages, and in varying social environments, must be driven by a different mechanism. Ultimately, though phenotypic expression of receptors appears stable, this dissertation emphasizes an important theme: social behavior is complex, and is regulated by numerous neural mechanisms, many of which are likely interrelated. Further research is required to elucidate the questions raised by this study.

CHAPTER V

GENERAL CONCLUSIONS

In summary, this dissertation shows that the preferred mating tactic in prairie vole males is "residency." Even when given the opportunity to forego pair bonding, male prairie voles will pair bond, and will not take advantage of the opportunity to mate multiply, at least in a 24h period. This research suggests that male prairie voles have been selected to pair bond over mating promiscuously without social ties. In addition, I have shown that males exhibit greater aggression toward non-bonded females than bonded females, and as greater aggression toward non-familiar females than familiar females. This implies that familiarity in any form relates to decreased expression of aggression. This is congruent with previous work suggesting that males exhibit selective aggression toward strangers, but not mates (Getz et al., 1981; Winslow et al. 1993), and that this is likely related to territorialism and mate/pup guarding (Clutton-Brock, 1989; Kleiman, 1977).

This dissertation presents data that is the first to show that cognitive ability/expression changes with pair bonding. Previous research showed that social context impacts social recognition in male prairie voles (Zheng et al. 2013). Males were shown to recognize other males, but not females in social recognition tests. My work shows that as the social context changes, and social cognition of females becomes more relevant to male life stage, social recognition of females appears. Taken with previous research from my lab (Zheng et al. 2013), my findings suggest that pair bonding serves as a shifting point for male prairie vole cognition. Whereas recognition of males is more likely to be relevant prior to pair bonding (perhaps in an effort to

defend territory and resources from other males) recognition of females becomes more important after pair bonding.

It is reasonable to expect that behavioral and cognitive shifts should be mirrored in the nervous system. My experiment failed to show the expected shifts in receptor distribution associated with post-pair boding behavioral changes. However, it is worth noting that such shifts may not occur within the 48hour period tested. Increasing the time after bonding to compare bonded and single voles may reveal differences that relate to pair bond maintenance. Nevertheless, OTR and V1aR are unlikely to directly account for the behavior differences that immediately emerge following bonding. Peptide dynamics (synthesis/release/breakdown) or other mechanisms all together are more likely to account for these differences.

Taken together, this dissertation adds to a great body of research investigating the prairie vole mating system, reinforcing and supplementing what previous research has shown. First, I showed that male prairie voles preferentially bond; emphasizing that pair bonding is likely the natural, if not optimal, mating tactic of prairie voles. Second, I showed that male recognition of conspecifics changes after pair bonding, adding social recognition to the suite of behaviors that are changed with pair bonding, and complementing the rhetoric that behavioral shifts may prompt maintenance of the pair bond. Finally, I showed that it is unlikely that shifts in neurohormone receptor distribution prompt the behavioral changes seen with pair bonding, supporting the idea that variation in behavior during the lifetime of an organism is more likely to be due to changes in hormones, rather than receptor distribution.

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VITA

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