FEMALE MATING PREFERENCE PREDICTS THE MALE'S REPRODUCTIVE SUCCESS IN RATS

THESIS

Presented to the Graduate Council of
Texas State University-San Marcos
in Partial Fulfillment
of the Requirements

for the Degree

Master of ARTS

by

Anastasia M. Benson, B.A.

San Marcos, Texas
May 2007

DEDICATION PAGE

This thesis manuscript is dedicated to my father, Jeffrey Lee Benson. Even when times were rough, he never let me give up. May he rest in peace.

ACKNOWLEDGEMENTS

I would like to thank Dr. Fay Guarraci for her continued support, academically, financially and personally, throughout this process and Dr. Maha Foote for her academic guidance and foresight in the project.

I am very thankful for Dr. Reiko Graham and Dr. Natalie Ceballos who chaired and sat on my committee, respectively. Their constant support, guidance, and reminders were key in the completion of this manuscript.

Cecil Harkley and Lauren King at the University of Texas at Austin have been instrumental in the completion of this project, particularly the genetic analysis, and I thank them for that.

I would also like to thank Abby Diehl, Elizabeth Joyce, Jennifer Lovell, Jennifer Cohn, and Jose Lopez for their work on the behavioral data required for this thesis.

Finally, I would like to thank Phil Brenner and Kathy, Rick, Kristina, Tori, and Lissa Benson for personally supporting me throughout this project and for always being willing to lend a hand or bring some late night coffee.

This manuscript was submitted on April 24, 2007.

TABLE OF CONTENTS

Page

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

CHAPTER 1

INTRODUCTION

Over the past fifty years, researchers have extended the literature to include the neurochemical mechanisms of erection, ejaculation, and other sexual responses and are currently examining the mechanisms of sexual motivation, sexual stimulation, and mate choice (Pfaus, Kippin, & Coria-Avila, 2003). Sexual behavior plays an important role in the physical and mental wellness of human beings (Pfaus et al., 2003). Although ethical considerations, impracticality, or lack of sufficient technology often limit researchers' ability to study human sexual behavior (Pfaus et al., 2003), animal models have been useful for understanding and treating sexual dysfunction in humans (Wyllie, 2005). The usefulness of any animal model depends on fully understanding the animal's neurobiological or behavioral rules that underlie sexual responses (Pfaus et al., 2003).

It has long been known that within a species, sexual selection contributes to the evolution of sex differences (Darwin, 1871). There are a variety of behaviors within a species' sexual selection, including competition and mate choice; both of which can lead to copulatory activity (Andersson, 1994). There are two main types of competition between same-sex individuals for mates (i.e., intrasexual competition) and competition among potential partner (i.e., intersexual choice). The most common type of mate preference for most animals is male-male competition for a female and female selectivity

of a mating partner (Andersson, 1994). Although males will compete for a female, a female is more selective in choosing her mate than is a male. Female have evolved to provide resources and care for the offspring and thus have a larger degree of investment in the successful outcome of the offspring (Clutton-Brock & Vincent, 1991). As a result, females exhibit greater selectivity in their choice of mating partners. The present study will use a rodent model to explore the possibility that a female's mate preference can influence the male's chance of siring offspring (i.e., reproductive success).

Rodents are a polygynandrous species, meaning that both the females and the males of the species mate with more than one member of the opposite sex during a reproductive cycle (Calhoun, 1962). Interestingly, even though the female rat will mate with multiple males in a single reproductive cycle, recent findings suggest that female rats exhibit a preference for a particular male (Ferreira-Nuno, Morales-Otal, Paredes, & Velazquez-Moctezuma, 2005; Lovell et al., 2006). In one study that allowed female rats to pace their sexual stimulation simultaneously, female rats consistently spent more time with one particular male during the mating tests (Ferreira-Nuno et al., 2005). Specifically, Ferreira-Nuno and colleagues found that while the females exhibited a preference for a particular male, the preference changed throughout the female's estrous cycle. Furthermore, copulatory activity only occurred during proestrus and there was no consistently of preference across the females.

Despite the fact that Ferreira-Nuno and colleagues (2005) did not see a consistent mate preference across females for four mating tests, Lovell and colleagues (2006) did see a consistent preference across seven weeks. The disparities can be attributed to methodological differences including various levels of estrous cycle when mated, the

number of males available for mating, and the mating apparatus. Lovell et al. (2006) used OVX females administered artificial hormones to reflect proestrus while Ferreira-Nuno et al. tested naturally cycling females throughout their estrous cycle and did not explicitly examine the relationship between preference during proestrus. In addition, Ferreira-Nuno allowed the females to mate with four males simultaneously and Lovell used only two, but both suggested that certain males are more successful at attracting potential mates than others. Having additional mating choices for the female might have not caused a change in the preference as Ferreira-Nuno suggested; instead, their apparatus might have caused the female to display a preference. The apparatus used in their study did not allow the female to fully escape any of the males because her individual compartment was relatively small compared to the individual male compartments allowing the males to constantly reach her. However, Lovell used an apparatus that would allow the female to fully escape the male and still found a consistent preference.

Though Ferreira-Nuno did not see a consistent preference across females, when Lovell allowed multiple females are each mated with the same pair of males, the females as a whole consistently preferred the same male about 70% of the time. Furthermore, consistent with previous studies (French et al., 1972), this preference remained consistent over a 4-week testing period. In addition, French et al. (1972) showed that when given a choice to pace mate between two males, the female's preference for a male remained stable throughout the duration of the five-month testing period.

Although female rats prefer one male when given the opportunity to mate with multiple males simultaneously, the traits or characteristics of the male that result in the

preference are unknown, behavioral or genetic traits may contribute to the appeal of some male rats. These attractive traits could include the male's odor, his physical appearance (e.g., tail length, coat color or texture), and/or his aggressive nature. Certain traits that female rats find appealing may be associated with fitness of offspring (Jennions & Petrie, 2000).

The current study has implications for understanding sexual behavior in the laboratory setting and in the wild because the female's preference for a particular male will affect the mating behaviors she displays. Rodent mating behavior is used to study, for example, the different effects of drugs on the brain, stress on the reproductive cycle, and various factors of sexual behavior and motivation, One area in which the current literature is lacking is the impact of a female's mating behavior on the differential reproductive success of her mating partners. The present study will use a rodent animal model to explore the possibility that a female's mate preference can influence the male's chances of siring offspring (i.e., reproductive success).

CHAPTER 2

ANIMAL MODEL CORRELATES OF HUMAN BEHAVIOR

When choosing an animal model correlate of human behavior, several factors are important to consider: the animal's physical size, ease of testing, an optimal testing environment, and predictive validity (for review see Pfaus et al., 2003). Testing large animals in sexual behavior experiments is not practical except in field studies for reasons such as housing and apparatus storage. So smaller animals including rats, hamsters, ferrets, and prairie voles are used in a laboratory environment instead to enhance the ease of testing. Having an optimal testing environment is also necessary when deciding on an animal correlate of human behavior. Rats, for example, are a social species and will mate in a variety of circumstances and in the presence of a human (Calhoun, 1962). Using an animal that displays their natural mating habits when in the presence of humans is essential when choosing a correlate to human behavior because it allows the researcher to observe the animal's natural behavior. Finally, using a paradigm that consistently yields the same behavior from the animal is critical when choosing an animal correlate, because it enhances the experiment's predictive validity.

In the laboratory setting, there are two methods to measure sexual behaviors in rats: non-paced mating and paced mating behavior (Pfaus et al., 2003). In a non-paced mating paradigm, the female and male are placed in a single compartment with no exit or

entrance (Erskine, 1989). In contrast, paced mating paradigms allow the female to control the receipt of sexual stimuli by the male by approaching and withdrawing from the male at her leisure. Paced mating behavior, but not non-paced, is a consistent and observable behavior (Erskine, 1989). Furthermore, females exhibit pacing behaviors in the wild (Calhoun, 1962). Therefore, a paced mating paradigm is more ecologically valid and has greater predictive validity when compared to the non-paced paradigms. Both of these methods will be discussed in more detail in subsequent chapters.

CHAPTER 3

PACED AND NON-PACED MATING BEHAVIOR

Traditionally, researchers have placed rats of both sexes into a single compartment to study their sexual interactions (Erksine, 1989). However, after studying rodent behavior in their natural habitat, this method was found to be ecologically invalid because it does not reflect the female's mating habits in the wild. Converging evidence suggests that females, instead of males, regulate their sexual interactions with multiple mates by retreating into burrows (i.e., pacing) between mating encounters (Calhoun, 1948; McClintock & Adler, 1978; McClintock, Anisko, & Adler, 1982; Robitaille & Bovet, 1976). When mating, if a sexually receptive female is given the opportunity, she will approach and withdraw from a sexually vigorous male, thereby controlling the timing of the receipt of sexual stimulation (i.e., mounts, intromissions, and ejaculations) (Erksine, 1985). The female's approaches and withdrawals create a pattern of contact with the male called pacing. Pacing occurs throughout mating in response to the stimuli provided by the male. Specifically, pacing is directly related to the intensity of the coital stimulation received immediately prior to the solicitation, with the rate of approaches toward the male decreasing as the intensity of the stimulus increases (Erksine, 1985).

In contrast to a non-pacing paradigm, paced mating paradigms allow the female to pace her receipt of sexual stimulations while in the laboratory setting and thus represents

a more ecologically valid method of study. However, independent of methodology used to study paced mating, allowing the female to engage in paced mating behavior reflects a semi-natural mating condition because it allows the female to control the number and timing of her sexual contacts by approaching and withdrawing from the male, thus controlling the mating encounter (McClintock & Adler, 1978). There are a multitude of paradigms used to measure paced mating behavior in female rats including requiring the female to press a lever in order to gain access to the male (Matthews et al., 1997), mating in a bi-level chamber so the female can move between levels to pace (Pfaus, Smith, & Coopersmith, 1999), and by having the male tethered to limit radius of movement during sexual interactions (Broekman, de Bruin, Smeenk, Slob, & van der Schoot, 1988). While these paradigms allow the female to pace, apparatus design inhibits the inter-experimenter reliability of measuring the female's pacing elements, making it difficult to quantify the female's behaviors. Although all pacing methods have proven to be effective measures of paced mating, this study will use a fourth model: a multi-compartment, single-level paced mating model (Paredes & Vazquez, 1999).

Furthermore, contrary to paradigms that use single compartments where females are not allowed to pace their receipt of sexual contact, paradigms that allow female pacing ensure optimal fertility (Coopersmith & Erskine, 1994). Specifically, the litter size of females that were allowed to pace their mating behaviors was significantly greater than the females who were not allowed to pace (Coopersmith & Erskine, 1994). In addition, Erskine (1989) found that a minimum of five paced sexual stimulations are necessary to induce the physiological changes (i.e., increase in the release of pituitary luteinizing hormone) required for pregnancy or pseudopregnancy in the female.

Furthermore, sexual stimulations received when the female was not allowed to pace were not as effective in inducing the necessary physiological changes.

Paced mating is not only advantageous for fertility, it is also rewarding for the female rat. Female rats that are able to pace freely develop a conditioned place preference for the environment where they received the sexual stimulations, while females that were unable to pace their contacts did not (Paredes & Alonso, 1997). This conditioned placed preference was disrupted when naloxone, an opioid receptor antagonist, was injected (Paredes & Martinez, 2001) indicating that paced mating behavior produces a neurochemical reward state. In addition, only female rats that are allowed to freely pace their sexual interactions developed a place preference for the compartment that contained the sexual receptive male (Martinez & Paredes, 2001; Paredes & Vazquez, 1999). Moreover, studies have shown that mating without pacing can have aversive consequences for females. For example, females do not exhibit sexual receptivity after a brief period of uninterrupted mounting by the male when the female cannot escape (Hardy & DeBold, 1971). These aversive consequences are greatly reduced when the female is allowed to pace her sexual contacts (Hardy & DeBold, 1972). If the female is allowed to leave the male as she pleases, the experience is then positively reinforcing and the chances of successful conception are increased. Collectively, these advantages make using a paced mating paradigm an exceptional model for the present study's purpose.

The present study uses a multi-compartment method because it allows the female to pace the receipt of coital stimulations (i.e., control the sexual interactions) on a single level platform while limiting, but not restricting, the male's ability to mate with the

female (Paredes & Vazquez, 1999). This method combines pacing and conditioned place preference methods in order to reduce the possible aversive consequences associated with mating and increase the likelihood of detecting the motivational effects of coital interaction in female rats (Paredes & Vazquez, 1999). Using a dual- or tri-chamber apparatus, the female mates at her leisure by escaping from the male's chamber to her own chamber through a small hole that only the female can fit through (Erskine, 1985; Erksine, 1989).

CHAPTER 4

COMPONENTS OF FEMALE MATING BEHAVIOR

The full repertoire of female mating behavior that is observed in both a natural and laboratory setting is represented by the presence of both receptive behaviors and solicitation behaviors, in combination with the female controlling the number and timing of her sexual contacts (Beach, 1976; Erskine, 1989). Receptive behavior in a female rodent is defined by the lordosis posture, a dorsoflexion of the female rat's back in response to a mount by a male rat (Beach, 1976). Both naturally cycling females in proestrous and ovariectomized (OVX), hormone-primed females display the lordosis position when mounted by a male (Beach, 1976). One method used to increase receptivity involves the subcutaneous injection of 10μg of estradiol benzoate 48 hours prior to testing and 1.0mg of progesterone four hours prior to each mating test. This method causes the OVX female to produce high levels of receptivity and pacing (Zipse, Brandling-Bennett, & Clark, 2000).

Solicitation behaviors are defined as the species-typical behaviors that are exhibited by the estrous female when engaging in copulatory activity with a male (Erskine, 1989). Female rats engage in soliciting behaviors such as hopping, ear wiggling, and darting (Erskine, 1989). Collectively, the display of receptive and

solicitation behaviors while in the presence of a male signals the intensity of estrous responsiveness or the female's willingness to mate (Beach, 1976). Similarly, if a female rat does not display these receptive and solicitation behaviors, her willingness or motivation to mate is concluded to be attenuated (Beach, 1976). Finally, female rats display solicitation behaviors in the presence of a non-responsive male or when mating with a sub par male, which seems to stimulate mating behavior in these males (Whishaw & Kolb as cited in Erskine, 1989) again illustrating the female's ability to control her coital interactions.

Furthermore, the size of the mating chamber affects the type and frequency of solicitation behaviors displayed by the female (Erskine, 1989). The female's approaches and withdrawals from the male are less frequently seen in this type of laboratory environment because the female is forced into close proximity of the male. However, this finding is not an indication that the female is unwilling to display this behavior, but instead suggests that her spatial restrictions have limited her behavior. Females are more likely to exhibit ear wiggling and hopping when in a small test chamber (Erskine, 1985). This display of increased hopping and darting suggests that these behaviors are exaggerated forms of the approaches and withdrawals that are more likely seen in a larger environment (Erskine, 1985).

CHAPTER 5

MEASURING FEMALE SEXUAL MOTIVATION

As discussed, a female rat's sexual behavior is composed of easily definable motor patterns (e.g., approaches, withdrawals, and hops) that are only displayed when participating in a copulatory act (Erskine, 1989). Researchers use a variety of measurements to calculate a female rat's sexual motivation in the laboratory setting. One model used to measure sexual motivation is a sexual partner preference paradigm. In this paradigm, the female's motivation is measured by her ability to be conditioned to a particular place using a sexual incentive such as a sexually active male (Pfaus et al., 2003). Although sexual partner preference paradigms are the main paradigm for measuring female sexual motivation, three behaviors are indicators of her motivation: lordosis, solicitation behaviors, and pacing behaviors. Females display the lordosis position when they are sexually receptive and as such indicates that the female is receptive to vaginal penetration.

The frequency of the female's solicitation behaviors also represents a female's sexual motivation (Pfaus et al., 2003). Solicitation behaviors (ear wiggles, hops, and darts) are displayed when the female is willing to mate. The increased presence of these behaviors is taken as an increased desire to initiate sexual activity.

The eagerness of the female to seek sexual contact also reflects a female's sexual motivation and is measured by the latency to return to a male rat after the receipt of sexual stimulation (i.e., the contact-return latency) (Meyerson & Lindstrom, 1973). Contact-return latency is calculated as the duration of time that passes between a female leaving the male's compartment after receiving a sexual stimulation and when she reenters the male's compartment (Erskine, 1989). Shorter contact-return latencies represent that the female is returning to the male quickly, and indicates a female's motivation to mate. The reverse is said for a longer contact-return latency. Another pacing behavior used as a measure of sexual motivation is how often the female leaves after receiving a sexual stimulation. This is seen in the frequency that the female leaves the male's compartment after sexual contact is termed the percentage of exits (Erskine, 1985; Guarraci, Mergroz, & Clark, 2004). Because the female's pacing is an indication of her sexual motivation, and a female's preference for a particular male reflects an increase in her sexual motivation, these measurements of sexual motivation are used to determine the female's preference for each male in a multi-male mating preference test (Ferreira-Nuno et al., 2005; Lovell et al., 2006).

CHAPTER 6

PARTNER PREFERENCE

Though researchers have previously looked at preferences during coital interactions, they have not included paced mating behavior before because it allows the experimental female to choose between two different stimulus animals (male and/or female); one that is sexually active and one that is not (Avitsur & Yirmiya, 1999; Paredes & Alonso, 1997; Paredes & Vazquez, 1999). While the combination of stimulus animals used for partner preference can vary depending on the type of sexual responses being observed, the stimulus animals often used for examining female sexual responses are a sexually experienced male and a castrated male or female (Avitsur & Yirmiya, 1999). Because one of the stimulus animals is not sexually active, the experimental animal can spend time with the animal of choice with or without solicitation of sexual stimulation. If, for example, the experimental female chooses to spend time with a sexually active male over a sexually inactive stimulus animal, she is soliciting sexual stimulation, thus displaying sexual motivation (Avitsur & Yirmiya, 1999). The partner preference paradigm allows the female to control the sexual stimulations because the male is limited in mobility and as such, used to measure female sexual behaviors and motivation (Avitsur & Yirmiya, 1999).

However, because females mate with multiple sexually active males in the wild and have competition from other females, paradigms that allow for such mate choice are more accurate assessments of the full repertoire of rodent sexual behaviors (Calhoun, 1962). In addition, because females can mate with more than one male, they are able to receive sexual stimulations, including ejaculations, from multiple males during the same mating cycle (Calhoun, 1962; Robitaille & Bovet, 1976). Therefore, a female is able to receive multiple ejaculations from multiple males so multiple sperm plugs within a short time period.

To better reflect this additional aspect of the natural mating environment, a multi-male mating preference test is used (Ferreira-Nuno et al., 2005; Lovell et al., 2006). In a multi-male mating preference test, a Plexiglas compartment is divided into three equivalent chambers by two removable dividers (i.e., a tri-chamber apparatus). Each of these dividers has a hole in the bottom corners that allows the female to leave the male at her own freewill. With a black Plexiglas covering over the dividers to hide the chamber contents from the other chambers, a male is placed in each of the two outer chambers and a female in the middle chamber. At the beginning of testing, the black coverings are removed and the female is allowed to roam freely across the three chambers via the holes. This method allows the female to mate with multiple males simultaneously, to leave as she pleases, and for the males to roam and mate freely in their compartment all while the researcher measures the duration and type of contact the female receives from each male. When using a multi-male mating paradigm, monitoring her actions with each male is crucial in understanding how the male interacts with the female because the female's

approaches and withdrawals from the male influence the type of subsequent stimulation she will receive.

CHAPTER 7

FEMALE REGULATION OF SEXUAL STIMULATIONS

Solicitation behaviors displayed by the female stimulate male mating behavior and increases the likelihood that the male will achieve an intromission (Erksine, 1985). McClintock and Adler (1978) reported that approximately 90% of intromissions were preceded by the female pacing her approach to the male, while only 35% of intromissions occurred after the male approached the female. The female's ability to withdraw from the male between intromissions lengthens the latency between receiving intromissions. Females tested in large arenas receive intromissions at a slower rate (every 2.9 minutes) than females who are tested in an environment where they cannot leave the male (every 0.8 minutes) (McClintock & Adler, 1978). In addition, females were more likely to solicit mounts from a male as the male approached an ejaculation (McClintock, Anisko, & Adler, 1982). In summary, females appear to regulate the type of sexual stimulation they receive from males by an increase in their solicitation behaviors.

Though ejaculations are necessary for impregnation, a female's solicitation of the appropriate number of intromissions is also a crucial part of the reproductive process (Adler, 1969; Wilson, Adler, & Le Boeuf, 1965). Wilson et al. (1965) found that nine of ten females who received four or more intromissions prior to an ejaculation became pregnant while only 22.2% of the females who received less than four intromissions

initiated and/or maintained a pregnancy. Furthermore, multiple pre-ejaculatory intromissions are necessary for sperm transport and blastocyst implantation in the uterine wall (Adler, 1969). Therefore, the number of intromissions prior to the ejaculation directly influences the female's ability to become pregnant.

Receiving the appropriate number of intromissions is critical in pregnancy initiation; however, the quantity of intromissions the female receives also negatively affects the male's likelihood of siring offspring in a multi-male mating environment (Adler & Zoloth, 1970). Subsequent intromissions can serve to dislodge sperm plugs from a previous ejaculation (Hart, 1983; Toner, Attas, & Adler, 1987). In other words, multiple intromissions must precede the ejaculation but subsequent intromissions must not follow too closely after an ejaculation in order for impregnation to occur. Adler and Zoloth (1970) reported that when a female receives five intromissions within 15 minutes after receiving an ejaculation, there is an inhibitory effect on sperm transport. Particularly, when the female received five intromissions from the second male, it was enough to reduce the number of sperm found in the female's uterus from the first male and reduced the number of uterine implantation sites. Thus, in a competitive environment, a male can essentially reverse the effects of a previous male if the female allows him to acquire an intromission soon enough.

CHAPTER 8

SPERM COMPETITION: EFFICACY OF PLUGS AND TRANSPORT

The number of sperm present in each sperm plug drops dramatically the more frequently a male copulates; beyond the sixth ejaculation, the sperm plug does not contain enough sperm to impregnate a female (Adler & Toner, 1986; Toner & Adler, 1985). Together these results suggest that a male's success in siring offspring in a multiple-partner copulation encounter relies on him being the last to mate with the female and for the ejaculation to occur at the beginning of his copulatory experience independent of sperm competition (Moore & Wong, 1992). Moreover, because the male's mating order and copulation frequency depends on the frequency and timing of the female's visits, the female indirectly controls the male's chances of reproductive success.

Male rivalry for the female can also continue from the external environment into the female's genital tract in the form of sperm competition (Birkhead & Moller, 1998), thus mating order and frequency of copulation are not the only factors that determines a male's reproductive success. Sperm competition occurs when a female that is near ovulation mates with more than one male and receives multiple ejaculations; the success of each male's sperm in this situation is measured by which male sires more offspring (Birkhead & Moller, 1998). In mammals, sperm competition occurs when ejaculations from multiple males overlap in the reproductive tract of a single female during a single

oestrus cycle and the sperm compete for fertilization of the ova (Dean, Ardlie, & Nachman, 2006). Coria-Avila, Pfaus, Hernandez, Manzo and Pacheco (2004) allowed female rats to mate with two males with different ejaculation intervals. They found that the male who ejaculated second sired more pups if he was allowed to mate with a female immediately after she received an ejaculation from the first male. However, the first male had the fertility advantage if the second male was introduced to the female five or 10 minutes after a female received an ejaculation from the first male. Thus, the second male only has an advantage in multi-partner copulation when the second male begins copulating within minutes of the first male's ejaculation.

In support of this, Moore and Wong (1992) found that when two male rats are allowed to each ejaculate, the male who ejaculated second had a greater chance of fathering offspring but only when he mated immediately after the first male. If the males were allowed to ejaculate in a noncompetitive environment, (i.e., not immediately succeeding each other) there was no difference in the number of offspring. These findings suggest that in order to gain an advantage in reproductive success, the successful male must mate immediately after the previous male. Therefore, the mating order and the interval between ejaculations may play a role in paternity when the mating intervals use a paced mating paradigm. However, both of these studies identified paternity from the pups' pigment coloration at birth and did not use DNA analysis.

DNA fingerprinting has recently been used to further investigate the relationship between mating order and timing between ejaculations and its effects on paternity. In to determine paternity of pups sired by two potential fathers. When female rats were allowed to mate with one male, and then a second male either 30 minutes or six hours

later. Mating order was found to significantly effect sperm competition in the genital tract because the male that ejaculated last, regardless of the duration, had the tendency to sire more pups (Shimmin, Sofronidis, Bowden, & Temple-Smith, 1995). This is inconsistent with previous attempts to analyze the relationship between mating order and reproductive success because the male who ejaculated second sired more pups (Shimmin et al., 1995) despite, as previously found, a duration long enough to give the first male the reproductive advantage (Coria-Avila et al., 2004; Moore & Wong, 1992). Though all three studies identified that mating second is optimal, Shimmin and colleagues contradicted previous studies because the second male has a reproductive advantage regardless of the latency to mate.

After Moore and Wong (1992) found that the male's mating order influenced his reproductive success, they suggested that the second male's advantage may have occurred because he displaces the first male's sperm plug before sperm transport can occur. Sperm transport operationally refers to the movement of the sperm from entering the female reproductive system to penetrating the egg's cell wall. Sperm transport is believed to be dependent on three main factors: number of intromissions, duration of female immobility at ejaculation, and the fit of the sperm plug (Toner, Attas, & Adler, 1987). However, sperm transport is most strongly predicted by the sperm plug's fit (Toner et al., 1982). Therefore, because the number of intromissions is the major predictor of a good fitting sperm plug, they are indirectly the best predictor for sperm transport (Toner & Adler, 1986).

However, intromissions can also serve to dislodge sperm plugs from a previous ejaculation (Hart, 1983; Toner, Attas, & Adler, 1987). Consequently, if each successive

male dislodges the previous male's sperm plug via intromissions, then the last male to copulate will sire the most offspring. To reiterate, in the present study, if the female's preference affects the male's mating order, his reproductive success will be influenced accordingly because the sperm plug's fit is a function of the intromissions that the female solicits (Erksine, 1985).

Although the female cannot directly influence sperm competition or sperm transport, her mating behaviors prior to the male's ejaculation indirectly influence the male's reproductive success. For example, if the female mates with a second male immediately following the first male's ejaculation, the first male loses his reproductive advantage. Thus a female's preference for a male and her corresponding behaviors toward him are likely to affect the preferred male's ability to achieve reproductive success.

The present study used 31 experimentally naïve females across three experimental conditions to ensure that the female's previous sexual experience and hormonal condition do not interfere with her preference for a male. Using both naturally cycling in proestrous and OVX, hormone-primed females, this study shows that the female's hormone condition does not affect her preference for a particular male or mating behavior as a whole. In addition, the OVX, hormone-primed females were either sexually naïve or sexually experienced to illustrate that previous sexual experience also does not interfere with a female's mating preference. Of the experimental females, eight naturally cycling females were mated with a pair of males (until each ejaculated) and then carried a litter to term. Using DNA extracted from these rats, the current experiment will determine if a female's mating preference during multi-male paced mating influences the male's chance

of siring pups. Because females display a preference for a male while mating and because a female rat's behavior during paced mating are associated with increases in reproductive success, it is hypothesized that, for evolutionary reasons, mate preference will affect the male's offspring success rate. Specifically, it was hypothesized that the preferred male would sire more offspring than the non-preferred male regardless of the order of ejaculation.

CHAPTER 9

METHOD

Subjects

A total of 71 Long Evans rats (*Rattus norvegicus*) were used for this study. Thirty-one experimentally naïve female rats (200-300 g) were used as experimental animals across three groups: 1) sexually naïve, naturally cycling rats in proestrous (n = 11); 2) sexually naïve, hormone-primed, OVX rats (n= 11); and 3) hormone-primed, OVX rats tested one week prior after sexual receptivity testing (n = 9). Thirty sexually experienced males (400-600 g) and 10 sexually experienced females (200-250 g) were used as stimulus animals during each of the mating tests to ensure sexual receptivity in the experimental animals. Stimulus animals were used to encourage behavior from the experimental females and their behavior was not measured. All rats were obtained from Harlan Sprague-Dawley (Indianapolis, IN) and were housed in hanging plastic cages with aspen wood-shavings covering the floor.

The male rats were pair housed upon arrival and remained in these pairings for the duration of the experiment. The male housing pairs were also the pairs used for mating. The experimental female and pups were housed three per cage until birth, and then housed by family. Temperature and humidity were monitored, and the lights were kept

on a reversed 12:12 hour cycle (lights off at 10:00 a.m.). All experimental procedures were conducted under red light during the dark cycle. Food and water were available *ad libitium* in the home cages. All rats were weighed weekly. All animal care guidelines were followed from the United States Public Health Service (*Guide for the Care and Use of Laboratory Animals*), Public Health Services (Public Health Services, 1996) and monitored by the Southwestern University Institutional Animal Care and Use Committee.

Surgery

Twenty of the experimental female rats were ovariectomized (OVX) under Nembutal (sodium pentobarbital, 50.0 mg/kg, i.p.) anesthesia one week prior to behavioral testing, following a pretreatment of atropine sulfate (2.05 mg) to reduce respiratory distress. The remaining 11 experimental female rats were left gonadally intact to observe their behavior under natural cycling conditions.

Hormone Treatment

All OVX rats ($n = 20$) received 10.0 μg of estradiol benzoate (EB) 48 hours prior to testing and 1.0 mg of progesterone (P) four hours prior to each mating test. All hormone injections were administered subcutaneously in the flank. These doses of EB and P have been shown to produce high levels of receptivity and paced mating behavior in OVX rats (Zipse et al., 2000). Both hormones were delivered in a sesame seed oil vehicle. All hormones were purchased from Sigma Chemical Company (St. Louis, MO).

Estrous Cyclicity

The 11 gonadally intact experimental female rats were monitored for one month to insure normal estrous cyclicity using vaginal cytology (Zipse et al., 2000). Vaginal

secretion samples were collected every morning at 8:00 a.m. and placed on a glass slide via a sterile plastic pipette filled with saline (Marcondes, Bianchi, & Tanno, 2002). Using a microscope, each rat was categorized as being in proestrous, estrous, metestrous, or diestrous. After the month of monitoring, the naturally cycling rats were mated in the afternoon at approximately 1:00 p.m. if the morning vaginal secretions determined the females to be in proestrous (Zipse et al., 2000). Estrous period was determined by the type of cell present in the sample: proestrous vaginal secretions consisted mainly of nucleated epithelial cells, estrous vaginal smears consisted of cornified non-nucleated cells, metestrous vaginal secretions consisted of equal proportions of round leukocytes, cornified, and nucleated epithelial cells, and diestrous samples consisted mostly of round leukocytes (Marcondes et al., 2002).

Acclimation

All rats were acclimated to the mating chamber on two separate sessions, each lasting 15 minutes. The mating chamber consisted of a three-section Plexiglas arena (101.0 cm long x 32.0 cm high x 37.0 cm wide) with wood shavings on the floor. The sections were divided by clear Plexiglass dividers with a 5.0 cm hole in the bottom corners so the female rats could roam freely across. During acclimation sessions, a single male rat was placed into each of the side compartments of the chamber and the males were tapped lightly on the nose if they attempted to go through the holes in the divider (Emery, 1986; Erksine, 1985). In contrast, the experimental and stimulus females were placed alone in the mating chamber and allowed to roam feely between the three compartments. Although the holes in the Plexiglas were designed so only the females could fit through, the males were small enough at the beginning of testing to also fit

through. Tapping of the male on the nose during the acclimation period conditioned the male to not leave his compartment through this hole.

Two-male Paced-mating Test

An opaque cover was attached to each divider (31.1 cm high x 36.2 cm wide) to enclose the experimental female in the middle chamber. The female was placed into the middle chamber for five minutes prior to testing so she could become acclimated to her surroundings, and the opaque covers prohibited the female from entering either of the adjacent side compartments that each contained one of a pair of cohabitating males.

Timing for the mating test immediately began when the opaque covers were simultaneously removed, thus allowing the female to roam freely between the three compartments. Once the female had received an ejaculation from both males and returned to both of their chambers, the test ended and the opaque covers were replaced. All three rats were then removed and returned to their home cages.

During each mating test the lordosis quotient (LQ), lordosis response (LR), type and timing of sexual stimulation (i.e., mount, intromission, ejaculation), solicitation behavior (i.e., hops, darts, ear wiggles), rejection behaviors (i.e., kicks, defensive postures), and the total mating test duration were all recorded. The LQ was calculated by dividing the number of LRs greater than two by 10 and the LR of the experimental female to each mount was scored on a 4-point scale (Hardy & DeBold, 1971; Hardy & DeBold, 1972). The contact-return latency and percentage of exits in response to each type of sexual stimulation were also calculated. Contact-return latency refers to the time elapsed between receiving a sexual stimulation, leaving the male rat's compartment and re-entering the male rat's compartment. If multiple sexual stimulations were received,

contact-return latency can only be calculated on the last stimulation received before the female exited the male rat's compartment. Percentage of exits is the likelihood that the female left the male rat's compartment following a sexual stimulation. The number of exits and entries into each compartment were also recorded; compartment entries were scored when all four paws of the experimental rat passed through the holes in the clear Plexiglas dividers. In addition, the percentage of total test time the female spent with each male was recorded. The male (left or right) that the female spent the greatest amount of time with was classified as the preferred male (Ferreira-Nuno et al., 2005). All mating tests were recorded with digital video cameras (Sony DCR-HC65) for off-line analysis of behaviors.

DNA Extraction

The experimental females in Group 1 who became impregnated during the multi-male mating test were allowed to carry the litter to term, each female being housed in an individual hanging plastic cage. Three naturally cycling females from Group 1 were not impregnated, but were in proestrous during mating because sexual receptivity was observed. Following birth of the pups (n = 95), all subjects (n = 119) were euthanized via an administration of sodium pentobarbital (400 mg/kg) and then perfused. Approximately two inches of the tail were removed and each placed in a 1.5 mL microcentrifuge tube for cold storage until needed for DNA extraction. Using a sterile razor blade for each sample, the hair from the tip of the proximal tail was shaven by hand and approximately 0.6 cm of this tail section was removed and weighed (25-30 mg). Excess hair on the sample caused the DNeasy Mini spin column to clog during the DNA purification process. DNA was obtained from euthanized Long Evans adult female (n =

8), adult male (n = 16), and their pups (n = 98) and then stored in labeled, individual 1.5 mL microcentrifuge tubes for cold storage until needed for DNA purification.

DNA Purification

DNA was isolated from the above collected tail samples using the QIAamp DNeasy kit following the manufacturer's protocol (Qiagen, Valencia, CA). Twenty μL of proteinase K and 180 μL of ATL buffer were added to each sample's microcentrifuge tube. The tail sections were then incubated in a 55°C water bath for a total six hours. The samples were removed from the bath every hour and vortexed at a low rpm to monitor and ensure complete lysis. Samples were then centrifuged for 15 seconds at 8,000 rpm in order to separate off any remaining hair follicles from the sample. The remaining sample was then pipetted into new 1.5 mL centrifuge tubes, and 4 μL of RNase A (100 mg/mL) was added to each.

Samples were then vortexed for 15 seconds and allowed to sit for two minutes at room temperature while 400 μL of 1:1 AL buffer-ethanol mixture was added to each tube. The samples were vortexed and then pipetted into DNEasy Mini spin columns. Spin columns were centrifuged for 120 seconds at 8,000 rpm, then for 150 seconds at 9,000 rpm, and then for 150 seconds at 10,000 rpm. The collection tube and precipitants were discarded and the DNeasy Mini spin column was placed into a new 2 mL collection tube. Five hundred μL of AW1 buffer was added to the column and centrifuged for 120 seconds at 8,000 rpm, 150 seconds at 9,000 rpm, and 150 seconds at 10,000 rpm.

The collection tube and precipitants were then discarded and the DNeasy Mini spin column was placed into a new 2 mL collection tube. Five hundred μL of AW2 buffer was added to the column and centrifuged for 180 seconds at 13,400 rpm. The

flow-through collection tube was discarded and the spin column placed in a new 2 mL microcentrifuge tube labeled as "Elution 1." Two hundred μL of AE buffer was added directly into each spin column, allowed to incubate for 60 seconds at room temperature and then centrifuged at 8,000 rpm for 60 seconds. The spin column was then placed in a new microcentrifuge tube labeled "Elution 2." Two hundred μL of AE buffer was added, and the sample incubated for 60 seconds at room temperature and then centrifuged at 8,000 rpm for 60 seconds. The microcentrifuge tube for both elutions were sealed, labeled, and stored in the freezer along with the remaining tail samples.

Paternity Analysis

Eighteen microsatellite markers (D1Cebr3, D1Cebr4, D1Cebr9, D2Cebr1, D3Cebr1, D3Cebr3, D3Cebr6, D4Cebr2, D4Cebr3, D5Cebr1, D6Cebr1, D7Cebr1, D9Cebr1, D10Cebr1, D11Cebr1, D13Cebr1, D16Cebr2, and D20Cebr1) were used to amplify loci on chromosomes 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 13, 16 and 20, respectively (Hinson, Hannah, Norris, Glass, & Klein, 2005; Giraudeau et al., 1999). Primers were custom synthesized at University of Texas at Austin and the forward primers labeled with fluorescent markers 6FAM, HEX, or NED (Applied Biosystems, Foster City, CA). The primers were resuspended in 1mL of water; the F-M13 tagged primers were diluted to 0.2μM (200μL) and the R primers and M13 were diluted to 2.0μM (200μL). In a 1.5 mL microcentrifuge tube, 2μL of the DNA sample, 1μL of the M13-F primer, 1μL R primer, 1μL M13 primer, 10μL master mix, and 5μL of water were added and PCR analysis was conducted using a 3-step cycle on a 96-well plate. Samples were then processed on the Applied Biosystems 3130XL and interpreted using the GeneMarker analysis software (SoftGenetics, State College, PA).

Behavioral Data Analysis

Mate preference was determined by the amount of time the female spent with each male. The male with whom the female spent the most time with was classified as the preferred mate. Because the operational definition of a male's preferred status depended on the amount of time spent with the two males in a given pair, any statistical test comparing the percentage of time spent with the preferred male to percentage of time spent with the non-preferred male was inflated and a direct consequence of the operational definition. Therefore, the female's preference for a male was measured using a preference ratio (Lovell et al., 2006). A preference ratio was calculated for each male: time spent with preferred male/time spent with preferred male + time spent with the non-preferred male. To determine the magnitude of the preference, these ratios were compared to 0.50 (i.e., the chance that randomly the female would spent time with the male) using a one-sample *t*-test

A number of male characteristics were also evaluated to explore the possible influence that male sexual behaviors can have on the female's preference. Hit rate [number of intromissions/(number of intromissions + number of mounts)*100] and inter-intromission intervals (III) were calculated. The male's latency to mount, intromit and ejaculate, and the number of ejaculations were also recorded. In order to determine if the preferred male had significantly different characteristics in each group that could have influenced paternity results, the above mentioned calculations and characteristics were analyzed independently via independent *t*-tests.

Genetic Data Analysis

Of the eight families that DNA was extracted from, two families had DNA concentrations too low to yield a PCR product and were excluded from analysis at this time. During paternity analysis, after each rat's allelic differences were identified, an independent t-test was used to determine if the preferred or non-preferred male for each pair sired significantly more pups than the other male for each pair. Analysis was run for each pair of males and then another independent *t*-test was used after the data were collapsed across all families. The ejaculation timing order and the preference of each male was then compared to the number of pups the male sired in the litter using an ANOVA.

CHAPTER 10

RESULTS

Behavioral Results

Two female rats (one each from Group 2 and Group 3) were not receptive and therefore their data were discarded and not used in any statistical analyses. Independent *t*-tests showed that there were no significant differences between the sexually naïve and sexually experienced OVX females for any mating behavior (all t's <1.3) and these groups were therefore collapsed into one group and compared to the naturally cycling female rats. All behavioral measures collected during the two-paced-mating test were analyzed with a repeated measure ANOVA. The rat's hormone condition (i.e., naturally cycling versus OVX) was used as the between subjects factor and preference (i.e., preferred versus non-preferred) as the repeated measure factor.

Both naturally cycling and OVX females spent a greater percentage of time with the preferred male than with the non-preferred male. The average preference ratio for the naturally cycling rats (0.72(±0.04)] was significantly greater than 0.50 ($t(10)=5.88$, $p<.05$). The average preference ratio for the OVX females [0.72 (±0.02)] was also significantly greater than 0.50 ($t(17)=8.97$, $p<.05$). Independent of hormone condition, the experimental females spent, on average, 21.9% of the time with the preferred male

and only 7.4% of the time with the non-preferred male (see Figure 1).

Additionally, a significant main effect of preference on the latency to return to the male after intromissions was observed, ($F(1,25)=16.89, p<.05$). These results indicate that the experimental rats returned faster to the preferred male than to the non-preferred male after intromissions (see Figure 2 Top). There was also a significant main effect of preference on percentage of exits after intromissions, ($F(1,26)<10.17, p<.05$). This suggests that, independent of hormone condition, after the female received an intromission from the preferred male she was less likely to leave than if she received an intromission from the non-preferred male (see Figure 2 Bottom).

A significant main effect of preference on the number of intromissions received was also observed, ($F(1,27)=7.89, p<.05$). Females received more intromissions from the preferred male than the non-preferred male, independent of hormone condition (see Figure 3 Top). Similarly, while both males were allowed the opportunity to ejaculate with the female, only preferred males achieved any additional ejaculations. Specifically, seven of the 29 experimental females received two or more ejaculations from the preferred male, whereas the non-preferred male achieved no additional ejaculations. Thus, independent of hormone condition, the females received significantly more ejaculations from the preferred male than from the non-preferred male, ($F(1,27)=9.84$, $p<.05$).

A significant main effect of preference on the number of visits to the males was also observed, ($F(1,27)=4.59, p<.05$). Independent of hormone condition, the females visited the preferred male more frequently than the non-preferred male (see Figure 3 Bottom). Finally, a significant main effect of hormone condition was observed for the

number of visits to the males, ($F(1,27)=8.30, p<.05$). Specifically, naturally cycling rats entered both males' compartments more frequently than OVX rats did. No other significant main effects of preference, hormone condition, or interactions between hormone condition and preference were found for behaviors observed.

No differences in any of the male's characteristics (e.g., weight, hit rate, III, latencies) were observed between the preferred and non-preferred males.

Genetic Results

Using GeneMarker analysis software, the paternity for each pup was attempted to be identified but unable to be determined. These results showed that of the 18 markers analyzed, eight markers separated across five families could be used to identify paternity (see Table 1). However, to confidently determine paternity, five markers are needed for each parent set (Hinson et al., 2006). The remaining parent sets were homozygous for all markers tested and therefore paternity was unable to be determined.

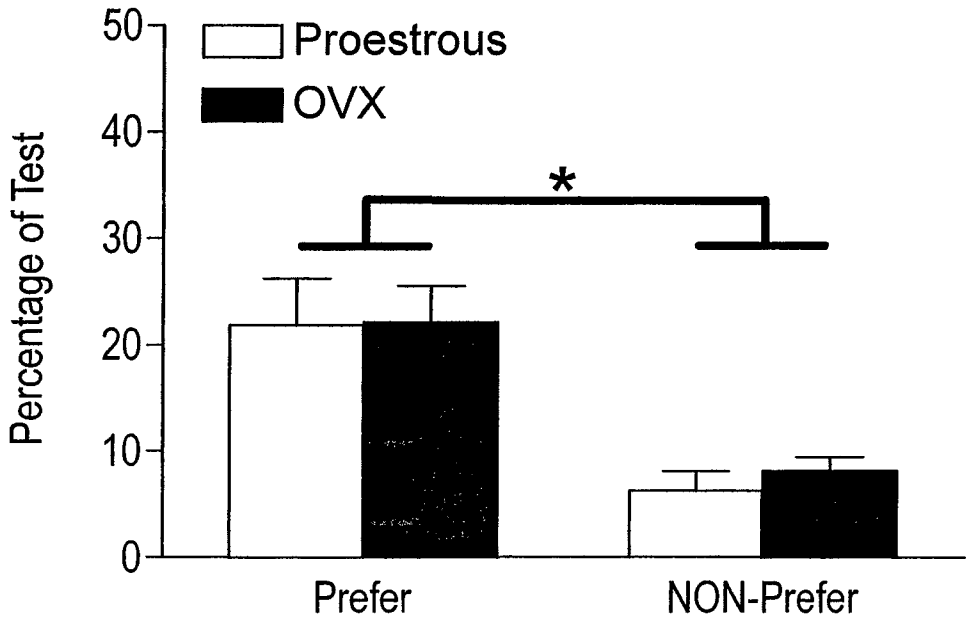

Figure 1. Hormone-primed OVX rats and naturally cycling rats tested in proestrous spent more time with their preferred male than their non-preferred male (proestrous n=11; OVX n=18). Data are expressed as means±SEM.

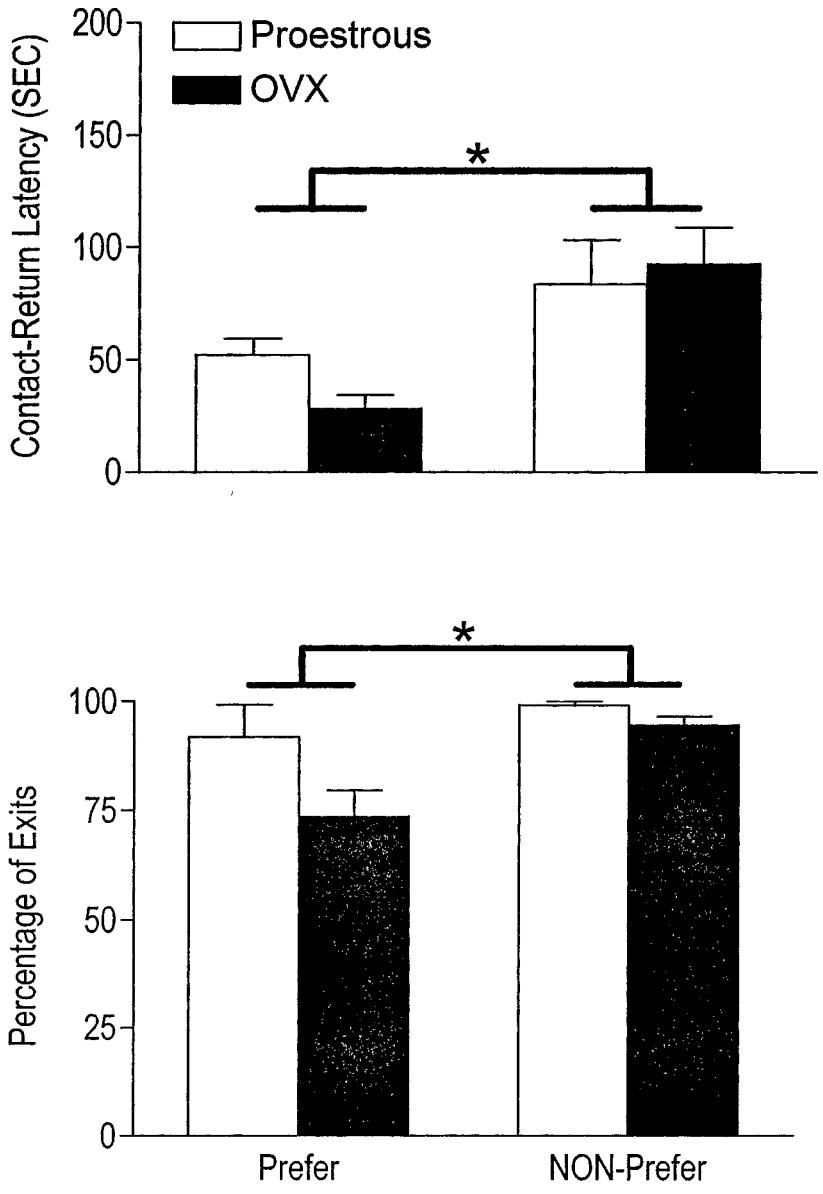

Figure 2. Hormone-primed OVX rats and naturally cycling rats testing in proestrous returned to their preferred male faster than their non-preferred male after intromissions (top: proestrous n=11; OVX n=18). In addition, hormone-primed OVX and naturally cycling rats were less likely to leave their preferred male than their non-preferred male after intromissions (bottom).

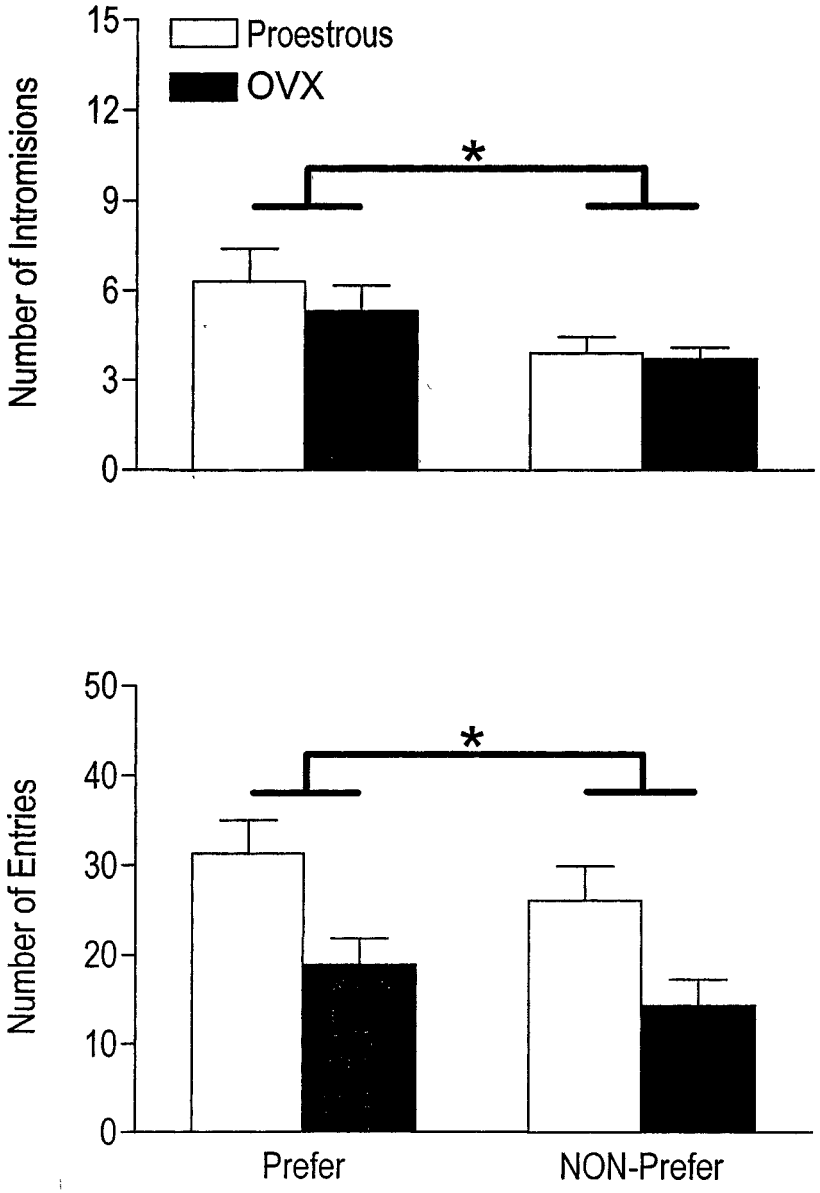

Figure 3. Hormone-primed OVX rats and naturally cycling rats tested in proestrous received more intromissions from their preferred male than their non-preferred male (top: proestrous n=11; OVX n=18). In addition, hormone-primed OVX and naturally cycling rats entered their preferred male rat's compartment more frequently than the non-preferred male rat's compartment (bottom). However, naturally cycling rats entered both male rat compartments more frequently than OVX rats (open bars different from closed bars, p<.05).

Table 1

Fragment Analysis of Mother and Father Sets

	Marker							
Parent Set	D1Cebr3	D1Cebr4	D1Cebr9	D2Cebr1	D3Cebr3	D4Cebr2	D4Cebr3	D5Cebr1
J12Mom	100	F	F	229	197	148	231/235	255/257
J12G1	100	F	F	239	185	148	235	257
J12G2	100/102	F	F	F	185	148	235	257
J13Mom	100	F	289/292	238	185/197*	148/162	231/235	257
J13Gig18	100	F	289	238	197*	148	231/235	255
J13Gig5	100/110	F	289	236	185*	148/162	235	255/257
J14Mom	118	313/320	F	F	197	148*	F	255/257
J14G28	100	313	F	236	185/197	148*	235	257
J14G30	100	313	F	236	197	162*	231/235	255/257
J16Mom	100	F	F	236/239	197	148/162	235	257
J16G3	100	F	F	236	197	F	235	257
J16G4	F	320	F	239	185/197	148	231/235	257
J20Mom	100	313/320	F	229*	197	162	235*	F
J20G14	100	313/320	F	239*	185/197	148	231*	257
J20G15	100	F	F	236*	197	F	235*	257
J21Mom	F	F	F	239	185/197*	148/162	231/235	255/257
J21G26	100	F	289	238	185*	148/162	F	257
J21G28	100	F	289	238	197*	148	F	257
J22Mom	100	F	F	F*	F	148	235	F
J22G34	F	320	289	236*	236	148	F	257
J22G34	100	F	289	238*	238	148/162	235	257

Total number of base pairs in each allele for each marker tested. One number indicates the subject is homozygous for the marker, and two numbers indicate the subject is heterozygous. Parent sets heterozygous enough to determine paternity are labeled with a *. The presence of a F indicates the sample failed.

Table 1 (cont'd)

Fragment Analysis of Mother and Father Sets

	Marker								
Parent Set	D6Cebr1	D7Cebr1	D9Cebr1	D10Cebr1	D10Cebr2	D11Cebr1	D13Cebr1	D16Cebr2	D20Cebr1
J12Mom	247	F	F	F	F	F	F	F	F
J12G1	259/261	F	F	F	F	F	F	F	334
J12G2	259/261	F	F	F	F	F	F	FF	
J13Mom	261	173*	270	194/196	218/221	384	241/249	246*	334
J13Gig18	261/265	170*	270	196	221	287	249	244*	F
J13Gig5	261/263	173*	270	194/199	221	284	235/243	246*	F
J14Mom	261	170	270	196	F	287	235/243	244/246	334
J14G28	261	170/174	270	196	F	284	F	244	334
J14G30	259/261	171/175	270	196	F	284	235/243	244	334
J16Mom	F	F	F	F	F	F	F	F	F
J16G3	261/263	F	F	F	F	F	F	F	F
J16G4	F	F	F	F	F	F	F	F	334
J20Mom	F	F	F	194/196	F	NO DNA	NO DNA	NO DNA	F
J20G14	263/265	172	270	196	F	F	F	246/246	334
J20G15	F	171/175	270	194/196	218	284	F	244	334
J21Mom	F	170	F	194/196	219*	F*	242	F	334
J21G26	261	172	270	196	221*	284*	242	299/303	334
J21G28	259/261	171/176	270	194/196	219*	287*	242/250	299	334
J22Mom	F	F*	F	F	F*	F	F	F	F
J22G34	F	172*	266/270	196	221*	284	242/250	246	334
J22G34	259/261	175*	270	196	218*	284	242/250	246	334

Total number of base pairs in each allele for each marker tested. One number indicates the subject is homozygous for the marker, and two numbers indicate the subject is heterozygous. Parent sets heterozygous enough to determine paternity are labeled with a *. The presence of a F indicates the sample failed.

CHAPTER 11

DISCUSSION

All female rats, regardless of hormone condition, spent significantly more time with their preferred male than the non-preferred male. On average, the female spent 21.9% of her time with the preferred male and only 7.4% of her time with the non-preferred male. These findings are consistent with previous research (Ferreira-Nuno et al., 2005) in that females display a mate preference when in a laboratory setting. In addition, the female's behavior while with each of the males differed based on her preference for that particular male. Specifically, the female was less likely to leave the preferred male than the non-preferred male after receiving an intromission. If the female did leave after receiving an intromission, she returned faster to the preferred male than to the non-preferred male. Collectively, the female's seeking behavior towards the two males suggests that the female's sexual motivation for the preferred male was higher than that for the non-preferred male (Drewett, 1973; Meyerson & Lindstrom, 1973).

The female received more intromissions and ejaculations from her preferred male than non-preferred male. This increase in the preferred male's ability to achieve sexual stimulation may be a function of the female's increased frequency of visits to his compartment. However, the female's decrease in latency following an intromission might be advantageous for the non-preferred male. Pregnancy initiation in pacing female

rats is likely a function of the contact-return latencies (Erksine, 1989). Therefore, if the female has a longer contact-return latency with the non-preferred male, the non-preferred male may be more successful at initiating pregnancy and siring offspring. Despite this, the increased contact-return latency does not accurately reflect the female's activity during the latency period. During this period between intromissions from the non-preferred male, the female was not necessarily sitting alone in her chamber, she was easily engaged in sexual behavior with the preferred male, thus negating the non-preferred male's supposed advantage.

Lastly, the female's hormone condition, naturally cycling or OVX and hormone-primed, did not interfere with the female's preference and that if given a choice, a preference for a particular male is seen regardless. This finding contradicts previous research that showed that naturally cycling in proestrus and OVX rats display different contact-return latencies following intromissions and mounts (Zipse et al., 2000). In contrast, this study found that naturally cycling and OVX females did not display significant differences in paced mating behaviors.

The present study's findings reinforce and extend those of previous multi-male mating paradigms. Consistent with the present study, Ferreira-Nuno and colleagues (2005) found that the female entered the preferred male's compartment more frequently than the non-preferred male's, particularly during proestrus. In their study, the majority of the sexual stimulations were achieved by the preferred male (83-94%) and the non-preferred male was rarely able to achieve any stimulation during the 15-minute testing period. The present study was able to extend the current literature by not restricting the allotted testing period and instead discontinuing the test only after both males had

achieved an ejaculation. This study also adds to the current literature that sexually receptive females that are naturally cycling, behave similarly to OVX hormone-primed females in mating conditions. Though there was a trend for the effect of preference to be more robust in OVX hormone-primed females, this could be attributed to the varying levels of hormones, estradiol and/or progesterone, in the naturally cycling females (versus the known concentration of hormone in the OVX group).

The continuous display of a preference by a female of a polygynandrous species is especially interesting, because logic leads one to believe that a promiscuous animal would not care or prefer one mate to another; yet female rats consistently exhibit a preference for a particular male over time (Lovell et al., 2006). The males of the species follow logic and do not exhibit a consistent preference over time. In fact, the male will mate with a female until they reach sexual satiety (Sachs & Meisel, 1988) which is, on average, five to eight ejaculations in an unlimited-time mating test. However, when a new female is placed in proximal vicinity, the previously satiated male will often immediately resume copulation with the new female (Bermant, Lott, & Anderson, 1968). This immediate recovery further illustrates the male's lack of preference of a mating partner. So why does the female exhibit a preference? If the female is attempting to "trade up" genetically by mating with a male who possess ideal traits, then theoretically the preferred male will sire more pups.

A preference for a particular male suggests that some males have certain characteristics that females consistently find attractive (Lovell et al., 2006). These attractive traits could include the male's odor, his physical appearance (e.g., tail length, coat color or texture), and/or his aggressive nature. Recently, researchers have learned

that the male's odor is not what causes the female to display a preference. McCracken, Lee, and Guarraci (2007) administered intranasal zinc oxide injections to render the females anomsic. Despite not being able to smell, the females still exhibited a male preference. However, zinc oxide only kills the epithelial lining of the nasal cavities, so the male's pheromones could still be involved

We believed that a female's preference for a particular male would increase his reproductive success. However, the paternity for each pup was unable to be determined. However, it is believed that the preferred male would sire more pups than the non-preferred male because perhaps the female prefers the male that is genetically more desirable (Jennions & Petrie, 2000). However, we were unable to confidently determine paternity in any of the eight families because the parents were homozygous for the same allele, thus making it a challenge to determine from which parent the pup received his/her allele. Genetic testing at the Harlan facility showed that the parents' original colony was outbred and heterozygous markers exist. That is, the parents for each offspring in the colony should not be closely related because the breeder has carefully monitored the mating occurrence of the population. In spite of this breeding, Harlan reports that the colony is currently over 60% homozygous for the markers tested. Thus for over 60% of the microsatellites tested the parents will have the same genotype or DNA sequence.

Currently, genetic analysis is underway at the University of Texas at Austin using the five Whitehead Institute markers recommended by Harlan Corporation after their analysis of the original colony. Unfortunately, it is believed that the primers are not annealing properly during the PCR process, so results are still pending. Future experiments could also use single nucleotide polymorphisms (SNPs) to determine

paternity. Nevertheless, this method will only examine one nucleotide on the gene versus an entire fragment that consists of a few hundred nucleotides, making it a high probability that the single nucleotide will be the same for all parents.

The results of the genetic testing were inconclusive due to the high frequency of homozygous alleles between parents. Nevertheless, it is important to note that the current findings emphasize important methodological consideration for future studies in this field. For instance, in the future, it would be more effective to determine the genetic sequence of the parents prior to mating in order to ensure that paternity could be easily determined. This could be done by using all parents from one colony and analyzing the microsatellites of the fathers beforehand or by using fathers from different breeding companies to decrease the percentage of homozygous alleles between the two potential fathers.

Furthermore, the current paternity study attempts to add to our prior understanding of sexual behaviors affect on a male's reproductive success because previous studies did not use DNA analysis to determine paternity. Instead, researchers would use males from two strains of rat and determine paternity from coat pigmentation (Adler & Zoloth, 1970; Coria-Avila et al., 2004; Moore & Wong, 1992). This method, though possibly accurate, is not ecologically valid because a female rat is unlikely to mate outside of her strain in the wild (Calhoun, 1962).

If the present study is able to determine if a female's mating preference influences the male's chances for reproductive success, then this study will provide better understanding into a female rodent's sexual motivation. However, other factors may contribute to the female's choice in her preferred male that are determined in the

intrauterine environment and not accounted for in this study. For example, while a rat pup is in utero, the male fetuses that are surrounded by female fetuses are less masculine as an adult due to the female hormone concentration surrounding it as a fetus (Clemens, Gladue, & Coniglio, 1978; vom Saal, 1979). Perhaps adult females can sense the level of aggression within the male, and chooses her mate based on this. These traits would be beneficial to the female because an aggressive male would be better suited to fight off attackers or gather resources for the pups. The pups' placement during the gestation period in the uterus may also affect the female's mate choice as an adult. Females that are surrounded by more females in utero are more attracted to males than a female that is surrounded by all males in utero, further altering the female's mate choice (Nelson, 2005). Future experiments should determine if the utero placement of the male affects the adult mate choice of females in multiple-male mating settings.

Maternal stress will also affect her litter's future sexual behavior, and thus mate preference. When pregnant rats are stressed, their male offspring produce less androgen, which negatively affects their mating behaviors in adulthood (Grisham, Kerchner, & Ward, 1991). In addition, these male rats also exhibit reduced aggression in adulthood (Ward, 1992 as cited in Nelson, 2005), which in turn, might decrease their chance of being preferred by a female for their aggression. Though maternal stress is believed to have been consistent across the animals, future experiments should use control groups to ensure that maternal stress does not influence the levels of androgens and the adult male's subsequent aggressive behavior in the study.

In summary, the present study examined the relationship between both a female's hormone condition and her previous sexual experience in regard to a preference for a

particular male. A female's hormone condition and previous sexual experience do not affect her preference because a consistent preference was displayed across the groups. Overall, female rats prefer to spend more time with one male than another when given the chance to mate with both simultaneously. In addition, the female is to less likely to leave the preferred male following an intromission, but will return faster to him if she does leave, thus indicating an increase in sexual motivation towards the preferred male. Finally, the presence of a consistent preference suggests that certain male rats may possess features that consistently attract females. Though paternity analysis was unable to determine if the female's preference for a male and subsequent sexual behaviors toward the preferred male increased his likelihood of reproductive success, future research exploring this stable preference will yield greater insight into the mechanism of mate preference, genetic or physical, in a polygynandrous species.

REFERENCES

Adler, N.T. (1969). Effects of the male's copulatory behavior on successful pregnancy of the female rat. *Journal of Comparative Physiological Psychology, 69*(4), 613-622.

Adler, N.T., & Toner, J.P. (1986). The effects of copulatory behavior on sperm transport and fertility in rats. *Annual New York Academic of Science, 474*, 21-32.

Adler, N.T., & Zoloth, S.R. (1970). Copulatory behavior can inhibit pregnancy in female rats. *Science, 168*(938), 1480-1482.

Andersson, M. (1994). Sexual selection. Princeton, NJ: Princeton University.

Avitsur, R., & Yirmiya, R. (1999). The partner preference paradigm: A method to study sexual motivation and performance of female rats. *Brain Research. Brain Research Protocols, 3*, 320-325.

Beach, F.A. (1976). Sexual attractivity, proceptivity, and receptivity in female mammals. *Hormones and Behavior, 7,* 105-138.

Bermant, G., Lott, D.F., & Anderson, L. (1968). Temporal characteristics of the Coolidge effect in male rat copulatory behavior. *Journal of Comparative and Physiological Psychology, 65*(3), 447-452.

Birkhead, T., & Moller, A. (1998). Sperm competition and sexual selection. San Diego: Academic Press Ltd.

Broekman, M., de Bruin, M., Smeenk, J., Slob, A.K., & van der Schoot, P. (1988). Partner preference behavior of estrous female rats affected by castration of tethered incentives. *Hormones and Behavior, 22*(3), 324-327.

Calhoun, J.B. (1948). The development and role of social status among wild Norway rats. *Anatomical Records, 101,* 694.

Calhoun, J.G. (1962). The ecology and sociology of the Norway rat. Bethesda: U.S. Department of Health, Education, and Welfare, Public Health Service.

Clemens, L.G., Gladue, B.A., & Coniglio, L.P. (1978). Prenatal endogenous androgenic influences on masculine sexual behavior and genital morphology in male and female rats. *Hormones and Behavior, 10,* 40-53.

Clutton-Brock, T.H., & Vincent, A.C. (1991). Sexual selection and the potential reproductive rates of males and females. *Nature, 351*(6321), 58-60.

Coopersmith, C., & Erksine, M.S. (1994). Influence of paced mating and number of intromissions on fertility in the laboratory rat. *Journal of Reproductive Fertility, 102*(2), 451-458.

Coria-Avila, G.A., Ouimet, A.J., Pacheco, P., Manzo, J., & Pfaus, J.G. (2005). Olfactory conditioned partner preference in the female rat. *Behavioral Neuroscience, 119,* 716-725.

Coria-Avila, G.A., Pfaus, J.G., Hernandez, M.E., Manzo, J., & Pacheco, P. (2004). Timing between ejaculations changes paternity success. *Physiology and Behavior, 80,* 733-737.

Darwin, C. (1871). The descent of man, and selection in relation to sex. London: John Murray.

Dean, M.D., Ardlie, K.G., & Nachman, M.W. (2006). The frequency of multiple paternity suggests that sperm competition is common in house mice (*Mus domesticus). Molecular Ecology, 15*, 4141-4151.

Drewett, R.F. (1973). Sexual behaviour and sexual motivation in the female rat. *Nature, 242*(5398), 476-477.

Emery, D.E. (1986). Effects of endocrine state on sociosexual behavior of female rats tested in a complex environment. *Behavioral Neuroscience, 100*, 71-78.

Erksine, M.S. (1985). Effects of paced coital stimulation on estrus duration in intact cycling rats and ovariectomized and ovariectomized-adrenalectomized hormone-primed rats. *Behavioral Neuroscience, 99*, 151-161.

Erskine, M.S. (1989). Solicitation behavior in the estrous female rat: A review. *Hormone and Behavior, 23*, 473-502.

Erksine, M.S. (2005). Learning about sex: Conditioning of partner preference: Theoretical comment on Coria-Avila et al. (2005). *Behavioral Neuroscience, 119,* 1136-1139.

Ferreira-Nuno, A., Morales-Otal, A., Paredes, R.G., & Velazquez-Moctezuma, J. (2005). Sexual behavior of female rats in a multiple partner preference test. *Hormone and Behavior, 47,* 290-296.

French, D., Fitzpatrick, D., & Law, O.T. (1972). Operant investigation of mating preference in female rats. *Journal of Comparative Physiological Psychology, 81,* 226-232.

Hardy, D.F., & DeBold, J.F. (1971). Effects of mounts without intromission upon the behavior of female rats during the onset of estrogen-induced heat. *Physiology & Behavior, 7*(4), 643-645.

Hardy, D.F., & DeBold, J.F. (1972). Effects of coital stimulation upon behavior of the female rat. *Journal of Comparative and Physiological Psychology, 78*(3), 400-408.

Hart, B.L. (1983). Role of testosterone secretion and penile reflexes in sexual behavior and sperm competition in male rats: A theoretical contribution. *Physiology and Behavior, 28,* 823-827.

Hinson, E.R., Hannah, M.F., Norris, D.E., Glass, G.E., & Klein, S.L. (2005). Social status does not predict responses to Seoul virus infection or reproductive success among male Norway rats. *Brain, Behavior, and Immunity, 20,* 182-190.

Giraudeau, F., Apiou, F., Amarger, V., Kaisaki, P.J., Bihoreau, M., Lathrop, M., Vergnaud, G., & Gauguier, D. (1999). Linkage and physical mapping of rat microsatellites derived from minisatellite loci. *Mammalian Genome, 10,* 405-409.

Grisham, W., Kerchner, M., & Ward, I.L. (1991). Prenatal stress alters sexually dimorphic nuclei in the spinal cord of male rats. *Brain Research, 551,* 126-131.

Guarraci, F.A., Megroz, A.B., & Clark, A.S. (2004). Paced mating behavior in the female rat following lesions of the three regions responsive to vaginocervical stimulation. *Brain Research, 999*(1), 50-52.

Jennions, M.D., & Petrie, M. (2000). Why do females mate multiply? A review of the genetic benefits. *Biological Research, 75,* 21-64.

Kippin, T.E., Samaha, A.N., Sotiropoulos, V., & Pfaus, J.G. (2001). The development of olfactory conditioned ejaculatory preferences in the male rat. II. Parametric manipulation of conditioning sessions number and duration. *Physiology and Behavior, 73,* 471-485.

Kippin, T.E., Talinakis, S., Schattmann, L., Bartholomew, S., & Pfaus, J.G. (1998). Olfactory conditioning of sexual behavior in the male rat (*Rattus norvegicus*). *Journal of Comparative Psychology, 112,* 389-399.

Lovell, J.L., Diehl, A., Joyce, E., Cohn, J., Lopez, J., & Guarraci, F.A. (2006). "Some guys have all the luck": Mate preference influences paced-mating behavior in female rats. *Physiology & Behavior,* In Press.

Marcondes, F.K., Bianchi, F.J., & Tanno, A.P. (2002). Determination of the estrous cycle phases of rats: Some helpful considerations. *Brazilian Journal of Biology, 62*(4A), 609-614.

Martinez, I., & Paredes, R.G. (2001). Only self-paced mating is rewarding in rats of both sexes. *Hormones and Behavior, 40*(4), 510-517.

Matthews, T.J., Grigore, M., Tang, L., Doat, M., Kow, L.M., & Pfaff, D.W. (1997). Sexual reinforcement in the female rat. *Journal of the Experimental Analyses of Behavior, 68*(3), 399-410.

McClintock, M.K., & Adler, N.T. (1978). The role of the female during copulation in wild and domestic Norway rats (*Rattus norvegicus*). *Behaviour, 67,* 67-96.

McClintock, M.K., Anisko, J.J., & Adler, N.T. (1982). Group mating among Norway rats: II. The social dynamics of copulation: Competition, cooperation and mate choice. *Animal Behavior, 30,* 410-425.

McCracken, S., Lee, M., & Guarraci, F.A. (2007, April). *Disruption of olfactory cues affects paced mating behavior, but not mate selection in the female rat.* Paper presented at the meeting of the Southwestern Psychological Association, Fort Worth, TX.

Meyerson, B.J., & Lindstrom, L.H. (1973). Sexual motivation in the female rat: A methodological study applied to the investigation of the effect of estradiol benzoate. *Acta Physiologica Scandinavica, 389*(Suppl. 1), 1-80.

Moore, C.L., & Wong, L. (1992). Copulatory behavior, reproduction, and sperm competition in two strains of male rats. *Physiology & Behavior, 51*(3), 569-573.

Nelson, R.J. (2005). *An Introduction to Behavioral Endocrinology* (3rd ed., pp. 156-157). Sunderland, MA: Sinauer Associates, Inc.

Paredes, R.G., & Alonso, A. (1997). Sexual behavior regulated (paced) by the female induces conditioned place preference. *Behavioral Neuroscience, 111,* 123-128.

Paredes, R.G., & Martinez, I. (2001). Naloxone blocks place preference conditioning after paced mating in female rats. *Behavioral Neuroscience, 115,* 1363-1367.

Paredes, R.G., & Vasquez, B. (1999). What do female rats like about sex? Paced mating. *Behavioural Brain Research, 105,* 117-127.

Pfaus, J.G., Kippin, T.E., & Coria-Avila, G. (2003). What can animal models tell us about human sexual response? *Annual Review of Sex Research, 14*, 1-63.

Pfaus, J.G., Smith, W.J., & Coopersmith, C.B. (1999). Appetitive and consummatory sexual behaviors of female rats in bilevel chambers. *Hormones and Behavior, 35*, 224-240.

Public Health Services, U.S. (1996). *Guide for the care and use of laboratory animals institute of laboratory animal resources commission on life sciences*. Washington, D.C.: National Academy Press.

Robitaille, J.A., & Bovet, J. (1976). Field observations on the social behaviour of the Norway rat, *Rattus norvegicus*. *Biological Behavior, 1*, 289-308.

Sachs, B.D., & Meisel, R.L. (1979). Pubertal development of penile reflexes and copulation in male rats. *Physiology and Behavior, 24*(2), 289-292.

Shimmin, G.A., Sofronidis, G., Bowden, D.K., & Temple-Smith, P.D. (1995). DNA fingerprinting to determine paternity in laboratory rat sperm competition experiments. *Electrophoresis, 16,* 1627-1632.

Toner, J.P., & Adler, N.T. (1985). Potency of rat ejaculations varies with their order and with male age. *Physiology and Behavior, 35*, 113-115.

Toner, J.P., & Adler, N.T. (1986). The pre-ejaculatory behavior of male and female rats affects the number of sperm in the vagina and uterus. *Physiology and Behavior, 36*(2), 363-367.

Toner, J.P., Attas, A.I., & Adler, N.T. (1987). Transcervical sperm transport in the rat: The roles of pre-ejaculatory behavior and copulatory plug fit. *Physiology and Behavior, 39*(3), 371-375.

vom Saal, F.S. (1979). Prenatal exposure to androgen influences morphology and aggressive behavior of male and female mice. *Hormones and Behavior, 12,* 1-11.

Wilson, J.R., Adler, N., & Le Boeuf, B. (1965). The effects of intromission frequency on successful pregnancy in the female rat. *Proceedings of the National Academy of Science USA, 53*(6), 1392-1395.

Wyllie, M.G. (2005). The hunt for pink "Viagra." *BJU International, 95*(1), 181-182.

Zipse, L.R., Brandling-Bennett, E.M., & Clark, A.S. (2000). Paced mating behavior in the naturally cycling and the hormone-treated female rat. *Physiology & Behavior, 70*, 205-209.

VITA

Anastasia Marie Benson was born in Plano, Texas, on December 16, 1982, the daughter of Kathryn Lynn Benson and Jeffrey Lee Benson. After completing her work at Prosper High School, Prosper, Texas, in 2001, she entered Southwestern University of Georgetown, Texas. During the summers of 2002 and 2003, she attended University of North Texas and Collin County Community College, respectively. She received her Bachelor of Arts from Southwestern University in May 2005. In August 2005, she entered the graduate program for Health Psychology at Texas State University-San Marcos. In November 2005, her first manuscript was published in *Pharmacology, Biochemistry and Behavior,* titled '"Coffee, Tea and Me": Moderate doses of caffeine affect sexual behavior in female rats.' During her two years at Texas State, she worked as a Graduate Teaching Assistant for the Psychology Department. She received her Master of Arts from Texas State in May 2007. She plans to attend medical school at Midwestern University Arizona College of Osteopathic Medicine in Glendale, Arizona beginning in August 2007.

Permanent Address: 3315 Preston Hills

Prosper, Texas 75078

This thesis was typed by Anastasia M. Benson.