Role of Context, Arousal, and Female Availability in the Conditioning of Sexual Behavior and Ejaculatory Preference in the Male Rat

Nafissa Ismail

A Thesis

in

The Department

of

Psychology

Presented in Partial Fulfillment of the Requirements
for the degree of Doctor of Philosophy at
Concordia University
Montréal, Québec, Canada

December, 2008

Your file Votre référence
ISBN: 978-0-494-45664-4
Our file Notre référence
ISBN: 978-0-494-45664-4

ABSTRACT

Role of Context, Arousal, and Female Availability, in the Conditioning of Sexual Behavior and Ejaculatory Preference in the male rat

Nafissa Ismail, PhD

Concordia University, 2008

Although male rats are known to display greater unconditional sexual arousal and mating preference to copulate with a novel female compared to a familiar one, research has shown that males given repeated copulatory trials in bi-level chambers with a random almond-scented female develop a conditioned ejaculatory preference (CEP) for females bearing this odor cue. These findings suggest that male rats can learn to associate cues with sexual reward and display preferences for partners that bear those familiar cues. Examination of the effect of copulation in pacing chambers bisected by a divider that either had one hole or four holes at the bottom that only allowed the female to cross, revealed that in 1-hole pacing chambers, females spend more time away from the male than in 4-hole pacing chambers. Males copulating in 1-hole pacing chambers displayed longer ejaculation latencies than those in the 4-hole condition. Interestingly, when animals were changed environments, the differences in ejaculation latencies were maintained suggesting that the pattern of copulation in these males had become conditioned by their initial environment of copulation. An examination of the preferred environment of copulation showed that males prefer to copulate in 4-hole over 1-hole pacing chambers perhaps because males can achieve their optimal rates of copulation in 4-hole chambers. However, contrary to what would be expected, males trained to copulate in 4-hole pacing chambers, with the same almond-scented female at every trial,

failed to develop CEP for their familiar female. Instead, it is the males that were trained to copulate in 1-hole pacing chambers that displayed CEP for their familiar female as opposed to the novel one. These findings suggest that copulation in environments in which the female withdraws away from the male for longer periods of time facilitate the development of CEP for a familiar scented female. An analysis of the components required for males to develop CEP for their familiar female revealed that copulation in 1-hole pacing chambers with the same female bearing an olfactory cue and of the same strain as their own are necessary conditions for males to display CEP for their familiar female. Examination of the brain areas involved in the development of CEP for a familiar female, using immunohistochemistry, demonstrated that males that developed CEP for their familiar female displayed significantly more Fos immunoreactivity in the ventral tegmental area (VTA) and in the arcuate nucleus of the hypothalamus (Arc). Pharmacological analyses of the neurochemical mechanisms important for the development of CEP showed that intact opioidergic, but not dopaminergic system, are necessary for the development of CEP in male rats. Examination of neural structures involved in the development of CEP revealed that the VTA and the Arc play an important role in the development of this preference. The present findings shed light on the effect of context on the development of male copulatory behavior and on the development of CEP for a familiar female and its underlying mechanism.

ACKNOWLEDGMENTS

First and foremost, I would like to thank my supervisor Dr. Jim Pfaus for giving me the opportunity to work in such a great lab. I am forever grateful to him for all the guidance, thoughtfulness, and support he has provided me with throughout my three years in this laboratory and throughout the redaction of my thesis. I thank him for his friendship and mentorship.

I am also grateful to Drs. Shimon Amir, Ann Clark, Paul Joyce and Jane Stewart for their time and for accepting to be on my examination committee.

I would also like to thank all my lab members for their help, friendship and encouragement especially, Dr. Genaro Coria-Avila, Dr. Sara Farrell, Michaela Georgescu, M. Dean Graham, Sherri Lee Jones, Mayte Parada, and Bita Sharifzadeh. A special thank you also goes to all the undergraduate students who have worked with me and helped me complete my laboratory work, especially Zina Al-Jabary, Fabienne Girard-Bériault, Jonathan Greggain, Amanda Knezevic, Ivonne Lachapelle, Shann Ménard, Ladan Mohebbinia, Saturo Nakanishi, Reuben Ostrofsky, Sylvia Serrano, Jessica Spape, and Yue Zhao.

I am also very grateful for the support that was provided to me by the CSBN staff: Steve Cabilio, Elizabeth Chau, Dave Monro, Heshmat Rajabi, Barry Robinson, Franc Rogan, and Phyllis Webster.

I would like to thank all my friends, Patrick Augustyniak, Dr. Fanny Botreau, Dr. Stephanie Bourgeon, Dr. Doug Caruana, Sabine Dhir, Alex Gavrila, Dr. Hélène Gelez, Valerie Harbour, Giovanni Hernandez, Suzanne Hood, Dr. Said Kourrich, Dr. Giovanna Paolone, Matt Quinlan, Laura Renteria Diaz, Rebecca Salomon, Lauren Seigal, Dr. Rob

Sorge, Stephanie Tobin, Michael Verwey, and all the others for their friendship, kindness, and support.

Finally, I would also like to take this opportunity, to thank the people I love the most, my family. My parents, Mumtaz and Nuruddin Ismail, my husband Aly, my brother Arif, and my sister Salima, have been so supportive, loving, and encouraging. There are no words to express how much I have gained from them. My family is my foundation. Without their help and their continuous motivation, I would have never made it so far. I love them and thank them with all my heart.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CONTRIBUTION OF AUTHORS

CHAPTER 1.

Experiments 1 and 2: Context-dependent acquisition of copulatory behavior in the male rat: Role of female availability.

Authors:

Nafissa Ismail: Collected most of the data, performed all the data analysis and was responsible for writing the paper.

Yue Zhao: Collected some of the behavioral data in Experiment 2.

James G. Pfaus: Author of the experimental design and helped with the writing of the manuscript.

CHAPTER 2.

Experiments 1, 2 and 3: Pacing conditions contribute to the conditioned ejaculatory preference for a familiar female in the male rat.

Authors:

Nafissa Ismail: Contributed to the design of the experiments, collected most of the data, performed all the data analysis and was responsible for writing the paper.

Hélène Gelez: Collected some of the behavioral data in Experiment 2.

Ivonne Lachapelle: Collected some of the behavioral data in Experiment 1.

James G. Pfaus: Contributed to design of the studies and helped with the writing of the manuscript.

CHAPTER 3.

Experiments 1 and 2: Effect of haloperidol treatment to females on female and male copulatory behavior and partner preference.

Authors:

Nafissa Ismail: Contributed to the design of the experiments, collected most of the data, performed all the data analysis and was responsible for writing the paper.

Clemence Laroche: Collected the behavioral data in Experiment 1.

Fabienne Girard-Bériault: Scored some of the behavioral data in Experiment 2.

Shann Ménard: Collected some of the behavioral data in Experiment 2.

Jonathan A. Greggain: Collected some of the behavioral data in Experiment 2.

James G. Pfaus: Contributed to design of the studies and helped with the writing of the manuscript.

CHAPTER 4.

The effect of paced copulation and strain conditioning on the development of partner preference.

Authors:

Nafissa Ismail: Contributed to the design of the experiment, collected most of the data, performed all the data analysis and was responsible for writing the paper.

Sherri Lee Jones: Collected some of the behavioral data.

M. Dean Graham: Collected some of the behavioral data.

Sarah R. Sylvester: Collected some of the behavioral data.

James G. Pfaus: Contributed to design of the study and helped with the writing of the manuscript.

CHAPTER 5.

Experiments 1 and 2: Naloxone, but not flupenthixol, disrupts the development of conditioned ejaculatory preference in the male rat.

Authors:

Nafissa Ismail: Contributed to the design of the experiments, collected most of the data, performed all the data analysis and was responsible for writing the paper.

Fabienne Girard-Bériault: Collected some of the behavioral data in Experiment 2 and scored some of the results.

Satoru Nakanishi: Collected some of the behavioral data in Experiment 2.

James G. Pfaus: Contributed to design of the studies and helped with the writing of the manuscript.

CHAPTER 6.

Experiments 1, 2, 3 and 4: Neuronal activation by conditioned odors and natural cues in male rats.

Authors:

Nafissa Ismail: Contributed to the design of the experiments, did most of the ICC, Performed all the counting and all the data analysis and was responsible for writing the paper.

Jessica Spape: Sliced and mounted all the brains for experiments 1 and 2 and did some of the ICC for Experiments 1, 2, 3 and 4.

Fabienne Girard-Bériault: Sliced and mounted all the brains for Experiments 3.

Amanda Knezevic: Sliced and mounted some of the brains and helped with some of the ICC for Experiments 4.

Zina El-Jabary: Sliced and mounted some of the brains for Experiment 4.

James G. Pfaus: Contributed to design of the studies and helped with the writing of the manuscript.

LIST OF ABBREVIATIONS

AOB	accessory olfactory bulb
Arc	arcuate nucleus of the hypothalamus
BLA	basolateral amygdala
CPU	caudate putamen
LHb	lateral habenula
LS	lateral septum
MHb	medial habenula
MOB	main olfactory bulb
MPOA	medial preoptic area
NAcccore	nucleus accumbens core,
NAccsh	nucleus accumbens shell,
PVN	paraventricular nucleus of the hypothalamus
Pircx	piriform cortex
PDMA	posterodorsal medial amygdala
SCN	suprachiasmatic nucleus
SON	supraoptic nucleus
VP	ventral pallidum
VTA	ventral tegmental area
VMH	ventromedial hypothalamus
Tu	olfactory tubercle

INTRODUCTION

"We have evolved a nervous system that acts in the interest of our gonads and one attuned to the demands of reproductive competition."

-- M. T. Ghiselin (1974)

"Erotic fetichism makes an idol of physical or mental qualities of a person or even merely of objects... because they awaken mighty associations with... sexual pleasure."

-- R. von Krafft-Ebing (1929)

It is often assumed that a single male can impregnate many females whereas a single female is usually impregnated by only one male. Thus male sexual behavior is said to be driven by a desire to spread the gene pool far and wide, with males being always "ready and willing" to copulate with many females, whereas female sexual behavior is driven by a desire to find the "right" mate and copulate with him under the "right" conditions (e.g., Buss, 2003). A humorous extension of this notion appeared on the internet in recent years depicting "The Male" as a machine with a simple on-off switch and "The Female" as a machine with a smaller on-off switch surrounded by an array of dials that have to be set just right. There is tempting face validity to this, if one assumes like Ghiselin, that sexual behavior serves reproduction and that gonadal steroids activate fixed patterns of sexual behavior and partner preference in a species. However, such assumptions must be tempered by the fact that sexual arousal and preference can be directed in many species toward objects or partners that cannot reproduce, as noted by

Krafft-Ebing in his landmark book on fetish development, *Psychopathia Sexualis* (1929), and with behavioral patterns that do not facilitate reproduction. Indeed, both Pavlovian and instrumental conditioning can alter patterns of sexual behavior and preference, especially during an animal's early sexual experience (Larsson, 1956; Pfaus, Kippin, & Centeno, 2001; Pfaus, Kippin, & Coria-Avila, 2003). Although rats have been described as "polygamous" in their mating strategy (Barnett, 1963), simple Pavlovian association of a neutral odor with sexual reward during their first sexual experiences leads males to display a subsequent ejaculatory preference for females bearing the odor, and leads females to solicit, pace, copulate, and receive ejaculations selectively from males that bear the odor. The ability of learning during a "critical early period" of sexual experience to alter patterns of sexual behavior and preference suggests that "dials" based on expectation of sexual arousal and reward exist. Even for males.

Human Sexual Behavior and the Role of Context

The human sexual response cycle consists of four interactive phases: Excitement, Plateau, Orgasm and Refractory phases (Masters & Johnson, 1966, 1970). Each of those phases is controlled by the sympathetic or parasympathetic branch of the autonomic nervous system along with interconnections with the central nervous system, and each define a set of relatively stereotyped copulatory behaviors that lead to orgasm or sexual reward and its aftermath (Pfaus, 1999). Although the human sexual response appears as a common flow of behavior from start to finish, the parameters of the behaviors are highly variable and are influenced by both genetic traits (e.g., temperament, personality type) and experiential factors, including the availability of partners, level of sexual arousal, and the intensity of sexual reward experienced or expected. Indeed, some relatively "fixed"

parameters of human sexual behavior, e.g., the ejaculation latency, can range widely from a few seconds to many hours within and between individuals (Waldinger et al., 2005). Indeed, the intensity of orgasm appears to be modulated by both the intensity of the tactile sexual stimulation experienced during copulation or masturbation and by the level of arousal at the time (Masters, Johnson, & Kolodny, 1983), and the reward value of orgasm in men following an extended period of copulation is much greater than in men that ejaculate too rapidly (Althof, 2006). Conversely, although orgasm typically accompanies ejaculation in men, it does not always do so (Buvat et al., 1985; Rosenbaum & Pollack, 1988). Small amounts of stress in men can enhance sexual arousal and desire, and lead to faster ejaculation, whereas high levels of stress tend to inhibit sexual arousal, desire, and orgasm (Bodenmann, Ledermann, Blattner and Galluzzo, 2006). In women, context and control play key roles in the ability of women to feel sexual arousal and desire, and to "give into" an orgasm (Basson & Brotto, 2003). A stressful context in which the woman has little control leads to a state of inhibited arousal, desire, and orgasm, whereas a context in which the woman has some degree of control of the initiation and rate of sexual activity leads to enhanced sexual responding. Interestingly, higher arousal under the right circumstances can also lead to an increased propensity of women to experience multiple orgasms, and potentially derive greater reward from sexual interaction. Thus, although the human sexual response appears as a relatively "fixed" pattern in men and women, the intensity and duration of each component is defined individually and modulated by both internal and external factors, including arousal and expectation.

Rat Sexual Behavior

As in humans, the sexual response of rats comprises a stereotyped, species-specific pattern. Female rats generally control the initiation and temporal patterning of copulation by soliciting sexual contact from males, and then pacing its rate by running away for brief periods before assuming a lordosis posture which allows the male to make sexual contact (Erskine, 1989; McClintock, 1984; Pfaus, Smith, & Coopersmith, 1999). Male rats typically chase females that solicit, and then mount and gain vaginal intromission. After several bouts of mounts with intromission, males ejaculate. This is followed by a refractory period of approximately 5 min, after which the male regains the ability to copulate again (Beach, 1956; Larsson, 1956; Pfaus, Mendelson, & Phillips, 1990; Sachs, 1978). With successive intromissions, females go into estrous termination, in which they display progressively fewer solicitations, impose more time between intromissions, and show increased bouts of fighting (Pfaus, Smith, Byrne & Stephens, 2000). With successive ejaculations, male rats show progressively longer refractory periods until they become sexually exhausted (Beach & Jordan, 1956; Larsson, 1956; Rodriguez-Manzo & Fernandez-Guasti, 1995).

Studies that utilize correlational and factor analyses of copulatory measures suggest that the sexual responses of rats are relatively "fixed" patterns that are displayed in different environmental contexts. For example, examination of the intercorrelations among measures of copulatory behavior led Beach (1956) to postulate the existence of two conceptually distinct sequential mechanisms that control sexual behavior in the male rat: a Sexual Arousal Mechanism that led to the initiation of copulation and an Intromission and Ejaculation Mechanism that summed the level of arousal built up during

each intromission to trigger ejaculation. Subsequently, Sachs (1978) used correlational and factor analyses to study male sexual behavior from three data sets derived from different laboratories and different testing conditions. His analysis derived four independent factors that accounted for over 80% of the variance for all measures. The factor that accounted for the largest proportion of variance (40%) was termed the *Copulatory Rate Factor*. This factor was loaded by the interintromission interval, the ejaculatory latency, and the absolute and relative postejaculatory interval (PEI). The factor that accounted for the second largest proportion of variance (16%) was called the *Initiation Factor*. This factor, reminiscent of Beach's *Sexual Arousal Mechanism* (1956) was loaded heavily by the mount and intromission latency, and moderately by the absolute and relative PEI. An equal amount of variance was accounted for by the *Hit Rate Factor*. This factor was loaded by the number of mounts and the intromission ratio (number of intromissions/number of mounts and intromissions). The fourth factor, termed the *Intromission Count Factor* accounted for 14% of the variance. This factor, reminiscent of Beach's *Intromission and Ejaculation Mechanism* (1956), was loaded by the number of intromissions and the relative PEI.

Using data from a single set of male rats over three successive copulatory tests, Dewsbury (1969) derived nearly the same set of factors as Sachs (1978), with the exception of the Intromission Count Factor. Although the similarity of the factors suggests that certain measures of copulatory behavior are relatively stable in male rats, there were several inconsistencies in the correlations among the measures and the factor loadings. It was impossible to determine whether the different number of subjects in each data set, or any difference in testing procedure, might have contributed to those

differences. Indeed, Sachs noted that none of the procedures took into account the role of the female rat's behavior. Use of the bilevel chamber for this kind of analysis allowed Pfaus, Mendelson, and Phillips (1990) to factor analyze normative measures of male sexual behavior in a situation in which female rats control the initiation and rate of copulation. That analysis found a nearly identical set of copulatory factors as Sachs (1978), but also detected a measure of anticipatory sexual behavior that comprised the conditioned level changing displayed by males in the 5-min period prior to the introduction of the female. This behavior formed its own factor, the *Anticipation Factor.* Together, these 5 factors accounted for over 95% of the variance for all measures. In this analysis, however, both intromissions and mounts loaded onto a *Mount Count Factor,* and not an *Intromission Count Factor*, as had been found by Sachs. This suggested that differences in the testing chambers, and more specifically in the ability of females to control the initiation and rate of copulatory contact, led to slight differences in the expression of male sexual behavior. This finding also suggests that sexual experience may alter the particular pattern of sexual activity in male rats.

Role of Sexual Experience

Sexual experience has pronounced effects on the sexual behavior of male rats (Drori & Folman, 1964; Gray, Smith, Dorsa & Davidson, 1981; Herz, Folman & Drori, 1969; Pfaus & Wilkins, 1995; Stone, 1922; Thor & Flanelly, 1977). For instance, numerous studies have shown that sexually inexperienced males take longer to initiate copulation in the presence of a sexually receptive female, display more mounts without intromissions, and have longer ejaculation latencies, compared to sexually experienced

males (Beach, 1942; Beach, 1956; Dewsbury, 1969; Larsson, 1956; Siegel, Nunez & Wade, 1981). Compared to sexually inexperienced males, sexually experienced males have larger testes (Drori & Folman, 1964), heavier penises (Herz, Folman, & Drori, 1969), lighter body weights (Siegel, Nunez, & Wade, 1981), and increased secretions from accessory sex glands (Drori & Folman, 1964). Sexual experience has also been shown to block the disruptive effects of anosmia (Thor & Flannelly 1977), castration, penile anesthesia, and the effect of the 5-HT-1A receptor agonist, 8-OH-DPAT (Centeno, Coopersmith, & Pfaus, 2001; Lisk & Heiman, 1980), penile deafferentation (Lodder, 1975), and age (Gray, Smith, Dorsa, & Davidson, 1981). Sexually experienced males prefer the odors of sexually receptive females over those of sexually nonreceptive females, whereas sexually inexperienced males do not show a significant preference (Carr, Loeb, & Dissinger, 1965; Carr, Loeb, & Wylie, 1966). Sexually naïve males are susceptible to the disruptive effects of novelty stress on copulation, whereas sexually experienced males are not (Pfaus & Wilkins, 1995). Copulatory experience thus provides a powerful disinhibitory influence on sexual behavior.

Environmental Context and Sexual Experience

The environment in which sexual experience is acquired affects the pattern of copulation. For instance, Pfaus and Phillips (1991) reported that males trained to copulate in bilevel chambers, ejaculate in an average of 250 sec, whereas males trained in circular open field arenas ejaculate in an average of 600 sec (Vega Matuszcyk, Larsson & Eriksson, 1998). These findings suggest that the environment in which sexual experience is acquired leads to the development of specific patterns of copulation that are stable

across time. Fadem & Barfield (1982) compared differences in the timing of male rat copulatory behaviors in unilevel chambers bisected with a divider that had holes at the bottom small enough to only allow the female to cross from one side to the other. Those bisected chambers, also used as pacing chambers to study female sexual behavior, restrict males from easily accessing the female and allow females to be in complete control the rate of copulation (Erskine, Kornberg & Cherry, 1989; Coria-Avila, Ouimet, Pacheco, Manzo & Pfaus, 2005; Paredes & Vasquez, 1999). Fadem and Barfield observed that males given access to chambers with a 1-hole divider displayed longer intervals between each intromission, longer ejaculation latencies, and longer post-ejaculatory intervals, compared to males tested in the chambers without the divider. Unfortunately, few other studies have varied the environmental context in which copulation occurs systematically in the study of male rat sexual behavior.

Conditioned Sexual Responses

Sexual responses can be modified by Pavlovian and operant contingencies in a variety of species, including humans. In general those modifications occur on appetitive measures of sexual arousal or desire, although a few studies have demonstrated modified consummatory copulatory responses.

Genital arousal. The study of parasympathetically-mediated genital sexual arousal is the most widely studied dependent measure of conditioning of sexual behavior in both human and animal literatures. In human studies, sexual arousal is defined as the measurement of blood flow to the genitalia: penile erection in men and vaginal pulse in women. In primates and rats, penile erections have been measured in response to

nonaccessible females (Nadler & Bartlett, 1997; Pomerantz, 1990, Sachs, Akasofu, Citron, Daniels, & Natoli, 1994; Sachs, 1995a). Penile erection induced by manual stimulation by the experimenter is also widely studied in rodents (Meisel & Sachs, 1994). However, the most common approach to examining sexual arousal in animal models has been to use the latencies to intromit and ejaculate.

Pavlovian conditioning can potentiate sexual arousal. For instance, Rachman (1966) and Rachman and Hodgson (1968) found that a pair of women's boots can elicit erection in men following pairing with erotic audiotapes that depicted a couple having sex. Moreover, McConaghy (1970; 1974) demonstrated that colored circles or squares previously paired with erotic videotapes or still pictures can induce erection in heterosexual and homosexual men. Kantorowitz (1978) further examined the nature of the association between the unconditioned stimulus and the conditioned arousal induced by still pictures. In this study, each subject was presented with three different slides that were paired with the plateau, refractory, and resolution stages of masturbation. During subsequent testing, the slide paired with the plateau phase produced an increase in penile erection, whereas the slide paired with the refractory phase produced a decrease in erection, and the slide paired with the resolution phase had no effect. Interestingly, those responses were still present whent tested 3 months later. To date, there is only one study that has examined Pavlovian conditioning of sexual arousal in women. In that study, researchers failed to find significant effects of conditioning on sexual arousal in women following the presentation of erotic films (Letourneau & O'Donahue, 1997). However, it was noted that the unconditioned stimuli (erotic films) only produced moderate levels of arousal whereas in studies with male subjects such stimuli produced high levels of

arousal. This suggests that there are sex differences in the level of arousal produced by different stimuli and that the failure to demonstrate conditioned arousal in women may have been due to the presentation of an ineffective unconditioned stimulus.

A number of studies have attempted to demonstrate instrumental control of sexual arousal in men and women. For example, Rosen, Shapiro, and Schwartz (1975) showed that men, who are given feedback and contingent monetary reinforcement, can learn to become sexually aroused in the absence of erotic stimuli. Men can also be instructed to suppress (Rosen & Kopel, 1977; Rosen, 1973) or increase (Reynolds, 1980) penile erection with feedback. However, in these studies, no learning effects were reported across trials. Similiarly, women can also be instructed to increase their vaginal pulse in the absence of erotic stimulation (Zingheim & Sandman, 1978) or decrease vaginal pulse in the presence of erotic stimulation (Cerny, 1978) but again, no learning effects were found.

Copulation. Several studies have demonstrated a clear influence of previous sexual experience in the speed of copulation in animals. Larsson (1956) found that ejaculation latency was reduced as a function of prior copulation. Dewsbury (1969) found similar effect of sexual experience on ejaculation latency, but also noted that mount and intromission latencies were also reduced. Similar results have been reported with mice (McGill, 1962b), cats (Michael, 1961), and guinea pigs (Valenstein & Goy, 1957). In mice, the number of mounts inappropriately directed towards the female's head decreased with sexual experience (McGill, 1962a). Thus, sexual experience appears to "automate" copulatory behavior through instrumental leaning and renders males less dependent on precopulatory behaviors to initiate or maintain sexual responding.

Penile stimulation received during intromission is a necessary unconditioned stimulus for the development of sexual behavior in male rodents. For instance, after repeated copulatory trials, male rats that are allowed to mount with intromissions displayed shorter latencies to copulate and shorter intermount intervals relative to males that are only permitted to mount without intromissions (Whalen, 1961). Kippin, Talianakis, and Pfaus (1997) examined the influence of ejaculation on the development of sexual behavior. In this study, male rats were allowed to either display intromissions but without ejaculation, intromit to one ejaculation, or two ejaculations, during 9 conditioning sessions. Results showed no differences between the groups on the intromission latency, ejaculation latency, intromission frequency, interintromission interval, and post-ejaculatory interval. Thus, these findings suggest that the penile stimulation received from intromission alone appears to be sufficient for the development of copulatory efficiency. Similar results were reported in the development of sexually-reinforced maze learning (Sheffield, Wulff, & Backer, 1951; Whalen, 1961). Interestingly, Hayashi and Kimura (1976) found that the latency to initiate copulation and to ejaculate was greatly reduced in sexually-naive male mice if they were allowed to observe a male and a female conspecific engaging in sexual behavior. Although it is not clear whether the sexually naïve males could also sense odors from the copulating pair, it is evident that penile stimulation received during intromissions are not the only important unconditioned stimulus in the development of copulatory behavior.

Learning can also affect copulatory behaviors in other ways. For example, Silberberg and Adler (1974) found that rats decreased the number of intromissions required to ejaculate if they were limited to seven intromissions per copulation session

using a negative punishment, whereas control rats showed no alteration in intromission frequency, suggesting that rats can learn to control their intromission frequency under a negative punishment schedule of responding. Similarly, rats that are allowed to copulate under an imposed operant requirement (Jowaisas, Taylor, Dewsbury & Malagodi, 1971) or with "enforced intervals" (Larsson, 1956) between each intromission produced an altered pattern of copulation in which males ejaculated with fewer intromissions.

Stimuli paired with sexual reward can also increase sexual arousal as reflected by copulatory rate measures. Zamble and colleagues (Zamble, Hadad, Mitchell, & Cutmore, 1985; Zamble, Mitchell, & Findlay, 1986) placed male rats in a holding cage and exposed them to a non-accessible receptive female on several training trials. On test trials, males that were previously placed into the holding cage prior to copulation displayed significantly shorter intromission and ejacuation latencies than males that were not exposed to this conditioned stimulus. Zamble and colleagues (1985) also found that second-order conditioned stimuli were effective at eliciting arousal and the strength of this conditioned reponse is maintained even after extinction. Moreover, male gouramies that are repeatedly exposed to a light paire with non-contact exposure to a receptive female, displayed significantly lower latencies to initiate copulation and lower levels of aggression towards females when the conditioned stimulus (light) was presented before access to a female (Hollis, Cadieux, and Colbert, 1989). Similar results have been demonstrated in Japanese quail. Male quails that receive repeated exposure to female quails artificially adorned with bright orange feathers prior to access to a receptive normal female quail displayed significantly shorter latencies to initiate copulation when the stimulus (adorned females) was present compared to when it was absent (Domjan,

O'Vary, & Greene, 1988). Pfaus, Kippin, and Centeno (2001) reported that somatosensory stimuli can also be used to condition sexual arousal. Male rats that were wearing an unattached harness jacket prior to sexual experience with receptive females displayed faster intromission and ejaculation latencies if tested with the jacket than without it.

Aversive conditioned stimulus can also modulate copulatory latencies. For example, injection of LiCl to male rats and hamster following copulation, resulted in an aversion to copulatory behaviors simply whereby males ceased to copulate (rats: Peters, 1983; hamsters: Johnston et al., 1978). It is important to note, however, that Emmerick & Snowdon (1976) failed to find these effects on copulatory behavior. The addition of a neutral stimulus (almond odor: Lawrence & Kiefer, 1987) or a component of scent marking (phenylacetic acid: Emmerick & Snowdon, 1976) facilitates the development of conditioned aversion to females. Finally, Sachs (1995) showed that LiCl injections paired with exposure to a female attenuated non-contact erections in male rats.

Lastly, conditioned stimuli of nonsexual nature paired with arousing or rewarding stimuli can also affect copulatory behavior. Fillion and Blass (1986) reported that adult male rats displayed shorter ejaculation latencies with receptive female bearing a neutral odor (lemon) associated with suckling during infancy than with receptive females not bearing the odor (see also Marr & Gardner, 1965). Contextual stimuli paired with drug administration also have effects on copulation. Mitchell and Stewart (1990) found that copulation in an environment previously paired with morphine injections facilitated the amount of female-directed behaviors in intact male rats and decreased the intromission latencies in castrated male rats. Mildly painful stimuli also appear to have an excitatory

effect on copulation in male rats. For instance, mild footshock delivered intermittently decreased intromission latency and postejaculatory refractory period (Barfield & Sachs, 1968). Mild tailshock or tail pinch can induce sexually inexperienced males to copulate (Caggiula & Elbergen, 1969). Moreover, the presentation of a conditioned stimulus previously paired with shock can turn sluggish males into stud males (Crowley, Popolow, & Ward, 1973).

Conditioned Place and Partner Preferences

Two paradigms are currently used to examine preferences in rats are conditioned place preference (CPP) and conditioned partner preference. The CPP paradigm measures approach responses to an environment previously paired with a reinforcing event (e.g., copulation to ejaculation). Conditioned partner preference measures approach responses to cues on a partner paired previously with a similar reinforcing event. Both responses are generally considered to reflect the reward value of the stimulus (odor, place cue), which feeds forward to augment sexual desire or other sexual responses. Using CPP, Martinez and Paredes (2001) demonstrated that male rats display preferences for environments in which they experience their optimal rate of copulation. In that experiment, males were given sequential copulatory experience in a pacing chamber with either a 1-hole divider or no divider. Following each 30-min experience, males were placed on one of two distinctive sides of a conditioned place preference (CPP) apparatus. On the final test, males were placed into the CPP apparatus and allowed to move freely between the two sides. Males spent more time on the side associated with copulation without the divider, suggesting that unobstructed access to the female was perceived as

being more rewarding compared to obstructed access. Development of a copulatory CPP is dependent on ejaculation, and can be abolished by treatment with the opioid receptor antagonist naloxone (Mehrara & Baum, 1990; Miller & Baum, 1987).

Kippin and Pfaus (2001) showed that ejaculation is also necessary for the development of a conditioned ejaculatory preference in male rats. However, although the reward state induced by ejaculation appears to be necessary in both CPP and conditioned ejaculatory preferences in male rats, the state alone may not be sufficient for conditioning to occur. In the study by Martinez and Paredes (2001), male rats ejaculated in both the unobstructed and obstructed conditions, but developed a preference only for the compartment associated with unobstructed access to the female. It may be the case that situational variables that include the availability of the female and/or the degree of sexual arousal induced by the particular copulatory condition, may be critical for the development of sexual reward. Finally, although it is clear that the environment in which sexual behavior is acquired affects the pattern of copulation displayed by male rats, it is not clear whether the patterns would be maintained in different environments.

The Role of Contextual Variables on Sexual Partner Preference.

Male rats are thought to be polygamous (Calhoun, 1962), as in the wild, they are often found chasing estrous females as a pack and competing to mate with the female (Robitaille & Bouvet, 1976). Interestingly, early life experiences seem to shape animals to recognize individuals of their own strain and selectively mate with them. In fact, D'Udine & Alleva (1983) showed that early postnatal exposure of male rats to a neutral odor smeared on their mother's teats and anogenital regions results in a preference to

ejaculation with estrous females bearing this neutral odor. Similarly, adult male rats show a partner preference for females bearing a lemon odor paired with somatosensory reward (brushing with a paintbrush after maternal separation) that was conditioned in an early perinatal period (Ménard et al., 2006). Moreover, mice raised by rat mothers attempt to copulate more with rats than with mice (Lagerspetz & Heino, 1970). Thus, despite evidence for polygamous mating strategies in rats, there is emerging evidence that male rats can learn to associate cues with sexual reward and display preferences for partners that bear those familiar cues.

In the studies mentioned above by Kippin and colleagues (2001, 2001a, 2001b), male rats were trained to associate a neutral odor (almond extract) painted on the neck and anogenital region of a sexually receptive female with copulation to ejaculation. Several control conditions were used. One was a non-paired condition in which males copulated with unscented receptive females and were given access to the almond odor on gauze in their home cages between tests. Another was a random-paired condition in which the odor was presented on the female every other trial (so that males had an equal number of trials with and without the odor). After the conditioning phase, males were tested for their partner preference in an open field where they were given the choice to copulate with either an almond-scented or an unscented receptive female. Males in the paired condition chose to ejaculate with the scented female first, and significantly more often, throughout the 30-min test compared with control males in non-paired or random-paired groups. However, males in the non-paired group ejaculated more frequently with the unscented female, also suggesting a similar preference for familiarity. As noted above, those findings indicated that male rats display a CEP for a female bearing a

familiar odor when this odor was paired with copulation. Subsequent studies showed that the post-ejaculatory state was the necessary unconditioned stimulus because males had to be in the presence of the scented female during this period to make the association (Kippin & Pfaus, 2001a).

Female rats can also learn to associate cues to sexual reward and develop preference for partners carrying these familiar cues. For example, following repeated copulation in pacing chambers with almond-scented males, females display significantly more solicitations towards almond-scented males than unscented males (Coria-Avila, Ouimet, Pacheco, Manzo & Pfaus, 2005). Interestingly, it appears that, in females, learning can also override the innate preference for assortative mating. Coria-Avila and colleagues (2006) showed that cues of different strains of males can induce conditioned partner preference for the strain of male associated with paced copulation, suggesting that sexual experience can override the innate preference for the same strain in females. It remains yet to be investigated whether learning can also override the innate preference for partners of their own strain in males.

Whether a male will display preferences towards "familiarity" or "novelty" may depend on female availability and accessibility. For instance, if males have easy access to many females, it would be advantageous for them to mate with a different female every time to increase their reproductive fitness. However, if access to females is limited, then it may be advantageous for the males to remain with the female they have already mated with and display preferences for this familiar female or for cues that reflect some familiarity. Differences in mating strategies have been reported in other rodents, for example, prairie and meadow voles (Winslow, Shapiro, Carter & Insel, 1993) that are

found in either sparsely or densely populated groups, respectively. Field studies have demonstrated that male prairie voles share their nests with their partner throughout the mating season (Getz & Hofman, 1986) and are actively involved in parental care; they are thus termed monogamous (Insel, Young, & Wang, 1997). In contrast, meadow voles are deemed polygamous because the males do not form pair bonds and display minimal parental care (Insel, Young, & Wang, 1997).

Rats seem to be able to display both polygamous-like and monogamous-like mating strategies depending on their initial sexual experiences, and in particular, the circumstances in which their initial experiences with sexual reward occur. The level of arousal that the male experiences during copulation may also play an important role during conditioning (Pfaus, Kippin, & Centano, 2001). The arousal level can be modulated by a number of factors, one being the environment in which males copulate (Borg, Esbenshade, Johnson, Lunstra, & Ford, 1992). For instance, environments in which males have restricted access to the female may increase the level of arousal as males anticipate future copulatory contact with the female (Amstislavskaya & Popova, 2004). There may well be an optimal level of arousal that supports copulatory performance and the development of CPP or CEP. Given that sexual experience and learning affect sexual arousal, sexual behavior and partner preference, it is obvious that certain brain areas are sensitive to these conditions and influence sexual responding.

Neural Structures and Neurochemicals Important in the Development of Partner Preference

Studies that examined the brain areas involved in sexual behavior used immunocytochemistry of the protein product (Fos) of the immediate early gene (c-fos). Following copulation, increases in Fos-immunoreactivity (Fos-IR) have been reported in the medial preoptic area (MPOA), the bed nucleus of the stria terminalis (BNST), the medial amygdala (MeA), and in the midbrain central tegmental fields in male rats (Baum & Everitt, 1992; Robertson, Pfaus, Atkinson, Matsumura, Phillips & Fibiger, 1991; Pfaus & Heeb, 1997; Wersinger, Baum, Erskine; 1993). Coolen and colleagues (1996) also reported Fos-IR in the MPOA, posteromedial bed nucleus of the stria terminalis (BNSTpm), posterodorsal medial amygdala (MEApd), and parvicellular subparafascicular nucleus (SPFp), following ejaculation. Some of these areas were also found to be activated following exposure to female estrous odors in the bedding. Kippin and colleagues (2003) reported Fos-IR in the MEApd, MPOA, accessory olfactory bulb, nucleus accumbens core (NAcc) and shell (NAcsh), ventromedial hypothalamus (VMH) and ventral tegmental area (VTA) following exposure to estrous odor in the bedding. Kippin and colleagues also examined the brain areas activated following exposure to a conditioned odor in bedding. In that study, males were trained to associate an almond odor on sexually receptive females with the postejaculatory reward state. Following exposure to the almond odor alone in the bedding, these males expressed more Fos-IR in the piriform cortex (Pir), NAcc, basolateral amygdala (BLA) and in the anterior region of the lateral hypothalamic area, suggesting that these areas are critical for the development of partner preference in males. The areas activated following exposure to the almond

odor alone differ from those activated by exposure to estrous odors, suggesting that different neural pathways are sensitive to these odors. More specifically, the conditioned odor activated main olfactory terminals, while the unconditioned estrous odors activated the accessory olfactory terminals.

Activation of the VTA following exposure to female estrous odors is particularly interesting because this area is rich in dopamine (DA) cell bodies that project throughout the limbic system (Fallon & Moore, 1978; Phillipson, 1979). The mesolimbic DA system mediates attention to reward-related cues (Robinson & Berridge, 1993) and also plays a role in the prediction of the occurrence of a reward (Schultz, 1998). DA has long been known to be important for male sexual motivation and behavior (Sachs & Meisel, 1988). Pfaus and colleagues (1990) have shown that DA is released increasingly in the nucleus accumbens and the striatum during copulation and DA release peaks around the time of ejaculation. Studies comparing monogamous Prairie voles to non-monogamous Meadow voles reported differences in the functioning of the mesolimbic dopamine reward pathways in the NAc and the striatum (Curtis, Stowe & Wang, 2003). Specifically, dopamine D2-receptor subtype within the NAc appears to be critical for the formation of pair bonds in prairie voles (Aragona, Liu, Curtis, Stephan & Wang, 2003; Gingrich, Liu, Cascio, Wang & Insel, 2000). For example, blockade of DA D2 receptors in the NAc disrupts copulation-induced partner preference (Liu & Wang, 2003; Young, Lim, Gingrich & Insel, 2001). The development of pair bond in monogamous Prairie voles is also mediated by oxytocin, a hormone that also acts as a neurotransmitter in the brain. It is found in neurosecretory cells in the SON and PVN. Oxytocin modulates the development of pair bonding via its receptors located in the NAc and the prefrontal

cortex (Young et al., 2004). For instance, female Prairie voles injected with oxytocin in the NAc develop pair bonds even in the absence of copulation (Young et al., 2001). Moreover, injection of an oxytocin antagonist directly in the NAc disrupts the development of pair bond following mating (Liu & Wang, 2003). The development of pair bond is analogous to the development of partner preference because its formation requires the association of cues to sexual reward induced by the first copulation (Young et al., 2001; Young et al., 2004). Interestingly, exposure to an almond odor previously paired with copulation to ejaculation, increases the number of Fos-IR cells in magnocellular neurons in the PVN (Ménard et al., 2005). Furthermore, systemic injection of oxytocin to male rats prior to their first sexual experience with an almond scented female increases the number of males that display conditioned ejaculatory preference (Gelez et al., unpublished observation). These findings suggest that oxytocin plays a role in the development of partner preference and it may be part of the mechanism through which conditioned cues become associated to sexual reward.

Arginine vasopressin is a peptide hormone that appears to play an important role in the development of pair bond and partner preference. Vasopressin is produced in the SCN, PVN and SON (Hammock & Young 2006). Though its primary effect is to increase water retention in the kidneys, it has also been found to facilitate the development of pair-bonding. For instance, systemic administration of vasopressin to males facilitates pair-bonding (Insel et al., 1995). However, administration of vasopressin to females failed to facilitate the development of partner preference, suggesting that, unlike oxytocin, the effect of vasopressin on pair-bonding is sex-specific. Thus, similar to oxytocin,

vasopressin may also play an important role in the development of conditioned partner preference in males.

The VTA and the mPOA are abundant in μ-opioid receptors. These receptors have been shown to mediate drug intake (Duvauchelle, Fleming & Kornetsky, 1996), food intake (Will, Franzblau & Kelley, 2003) and sexual behavior (Balfour, Yu & Coolen, 2004). Importantly, research has shown that ejaculation and the release of opioids are necessary for conditioning to occur (Miller & Baum, 1987; Mehrara & Baum, 1990). Agmo and Berenfeld (1990) showed that the development of conditioning can be effectively blocked by systemic injections of naloxone, a non-specific opioid antagonist. Similarly, Coria-Avila et al. (2008) showed that as sexually conditioned partner preference in female rats could be abolished if females were administered naloxone systemically during the conditioning phase. Together, those findings suggest that opioids are part of the neurochemical substrate of reward that underlies the development of conditioned preferences. Given the overwhelming evidence that male rats learn particular patterns of sexual activity during their first sexual experiences, associate cues with sexual reward, and come to direct their ejaculations toward females bearing those cues, it is likely that males are sensitive to contextual cues, neural "dials" that direct their sexual interest based on female availability, level of arousal and the anticipation of sexual reward.

Outline of Experiments

The general goal of this thesis was to examine the role of the context, arousal, and female availability, on the development of sexual behavior and conditioned ejaculatory

preference in male rats. In Chapter 1, behavioral experiments were carried out to investigate the development of male copulatory behavior following copulation in different pacing chambers and to see whether the copulatory pattern acquired in one condition would be carried over to another condition. In this chapter, we also examined the preference of male rats to copulate in 1-hole or 4-hole pacing chambers since copulation in these chambers led to different patterns of copulation and perhaps even different levels of arousal during copulation. In Chapter 2, a series of behavioral studies were conducted to investigate whether males can develop partner preference for a familiar female following copulation in pacing chambers that optimize the males preferred rate of copulation. We hypothesized that copulation in environments that allow males to copulate at their optimal rates would result in increased sexual arousal during copulation and would facilitate the association of female cues and sexual reward and would direct males to copulate and ejaculate with females bearing those familiar cues. In Chapter 3, we tested the preference of males to copulate with receptive females that are easily accessible during copulation while keeping the environment constant to examine whether male sexual arousal is dependent on female proceptive behavior or on simple penile stimulation. In Chapter 4, the importance of the strain of the female partner in the development of partner preference was investigated by pairing Long Evans males with either a Long Evans or Wistar female to examine whether conditioning in 1-hole or 4-hole pacing chambers can override the innate preference for assortative mating. In Chapter 5, the roles of brain opioid and dopamine systems in the development of CEP in males were investigated following systemic administration of receptor antagonists. Finally, in Chapter 6, brain regions involved in the development of partner preference for

a familiar female or for familiar cues associated with sexual reward were examined using Fos immunocytochemistry. In this study, males were exposed to their almond-scented female or to the almond odor alone behind a screen and brain activation was scored following these exposures. This study allowed brain areas important in the development of CEP for a familiar-scented female to be examined, so that the areas that are most sensitive to contextual variables during copulation could be evaluated.

CHAPTER ONE

The pattern of copulatory behavior in rats is influenced by sexual experience, the environment of copulation, the arousal level, and the availability and accessibility of the partner during copulation. Pacing chambers are often used to study female sexual behavior. These chambers consist of uni-level chambers bisected with a partition that has holes at the bottom that are small enough to only allow the female to cross and thus provide the females with control over the rate of copulation. However, differences in the pattern of male copulatory behavior in different pacing chambers are unknown. The first set of experiments examined the development of male copulatory behavior in pacing chambers bisected with a divider that has either 1 hole or 4 holes at the bottom, and whether the pattern would change if males were switched from one context to the other after baseline rates of sexual responding had been extablished. This study also examined whether the preference of males for copulation in the two conditions would be reflected in a conditioned ejaculatory preference for females associated with one of the two conditions.

Context-Dependent Acquisition of Copulatory Behavior in the Male Rat: Role of Female Availability

Nafissa Ismail, Yue Zhao, James G. Pfaus

Concordia University

RUNNING HEAD: ACQUISITION OF MALE COPULATION AND PREFERENCE

Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, Montréal, QC.

This research was supported by a grant from the Canadian Institutes of Health Research (MOP-74563) to JGP. The authors would like to thank Drs. Tod Kippin, Martha McClintock, and Raúl Paredes, for useful discussions. All animal procedures conformed to the guidelines of the Canadian Council on Animal Care, and were approved by the Concordia University Animal Research Ethics Committee. This paper is dedicated to the late Dr. Mary Erskine of Boston University, whose pioneering work into the role of context and female sexual motivation laid the groundwork for the current experiments.

Correspondence concerning this article should be addressed to James G. Pfaus, Ph.D., Center for Studies in Behavioral Neurobiology, department of Psychology, Concordia University, Montréal, QC H4B 1R6 Canada. E-mail: Jim.Pfaus@concordia.ca.

Citation: *Behavioral Neuroscience, 122,* (2008) 991-997

Abstract

The effect of the environment in which sexual experience is acquired was examined on patterns of male rat copulatory behavior. Males trained in a pacing chamber with a 4-hole partition had significantly shorter ejaculation latencies compared to males trained in chambers with a 1-hole partition. Those differences persisted when males were switched into the other pacing condition, suggesting that the pattern of copulation in these males had become "fixed". In the second experiment, males were trained to associate an almond odor with copulation in either the 1-hole or 4-hole condition. Males ejaculated preferentially with females associated with the 4-hole pacing condition. Copulatory behavior in male rats is sensitive to female availability, and females associated with greater availability are preferred.

Keywords: Ejaculation, Paced Copulation, Arousal, Context.

Introduction

Sexual behavior in the male rat is composed of a sequence of appetitive responses that bring males into proximity of females and consummatory responses such as pursuit of females, mounts, and vaginal intromissions that lead to ejaculation (Beach, 1942, 1956; Larsson, 1956; Pfaus, 1999; Pfaus, Mendelson & Phillips, 1990; Sachs & Barfield, 1970; Sachs, 1978; Toates, 1986; Sachs & Meisel, 1988). The expression of sexual responses is affected by both individual or genetic differences between males (e.g., Pattij, de Jong, Uitterdijk, Waldinger, Veening, et al., 2005), and by the particular sexual experience of each male (Drori & Folman, 1964; Gray, Smith, Dorsa & Davidson, 1981; Herz, Folman & Drori, 1969; Pfaus & Wilkins, 1995; Stone, 1922; Thor & Flanelly, 1977). For example, sexually inexperienced males take longer to initiate copulation in the presence of a sexually receptive female, display more mounts without intromissions, and have longer ejaculation latencies, compared to sexually experienced males (Beach, 1942; Beach, 1956; Dewsbury, 1969; Larsson, 1956; Pfaus & Wilkins, 1995; Siegel, Nunez, & Wade, 1981). Sexual experience thus provides a degree of instrumental learning which appears to "fix" motor patterns into stable, although individual, baselines of copulatory responding. Those motor patterns depend critically on the genitosensory feedback provided by intromissions and ejaculations, as males that mount but do not intromit (following penile anesthesia) do not develop baseline rates of copulatory responding relative to males that experience penile stimulation and ejaculatory reward (Whalen, 1961).

The environment in which sexual experience is acquired also affects the pattern of copulation. For instance, males trained to copulate in bilevel chambers take an average of

250 sec to ejaculate (Pfaus & Phillips, 1991), whereas males trained in circular open field arenas take an average of 600 sec to ejaculate (Vega Matuszcyk, Larsson, & Eriksson, 1998). Those data suggest that the environment in which sexual experience is acquired leads to the development of specific patterns of copulation that are stable once they are acquired. However, few studies have systematically examined this effect. In one such study, Fadem & Barfield (1982) compared differences in the timing of male rat copulatory behaviors in unilevel chambers bisected by a divider with a hole at the bottom big enough to allow the female to cross from one side to the other, but too small for the male to get through. Those chambers, also used more commonly as "pacing chambers" to study female sexual behavior (e.g., Coria-Avila, Ouimet, Pacheco, Manzo & Pfaus, 2005; Coria-Avila et al., 2006; 2008 a,b; Erskine, Kornberg & Cherry, 1989; Paredes & Vasquez, 1999), allow females to control the initiation and rate of copulation with males. Fadem and Barfield observed that males given access to chambers with the 1-hole divider displayed longer intervals between each intromission, longer ejaculation latencies, and longer post-ejaculatory intervals, compared to males tested in the chambers without the divider.

A subsequent study by Martinez and Paredes (2001) demonstrated that males prefer to spend time in environments associated with their preferred rate of copulation. In that experiment, males were given sequential copulatory experience in a similar pacing chamber with either a 1-hole divider or no divider. Following each copulatory trial, males were placed into one of two distinctive sides of a conditioned place preference (CPP) apparatus. On the final test, males were placed into the CPP apparatus and allowed to move freely between the two sides. Males spent more time on the side associated with

copulation without the divider, suggesting that unobstructed access to the female was perceived as being more rewarding compared to obstructed access with the divider. Given that ejaculation and opioid release are critical for the experience of sexual reward that leads to sexual CPP in male rats (Ågmo & Berenfeld, 1990; Mehrara & Baum, 1990; Miller & Baum, 1987), and given that male rats ejaculated in both conditions, the data of Martinez and Paredes suggest that ejaculation is necessary but not sufficient to induce sexual reward; situational variables that include the availability or accessibility of the female may be critical for the development of sexual reward. Indeed, castrated male rats can maintain copulatory responding if they receive unobstructed access to females that display high rates of solicitation and lordosis, whereas castrated males that receive obstructed access to those females or to females that do not display appetitive behaviors show a faster decline in copulatory responding (Madlafousek, Hliňák, & Beran, 1976). Similarly, sexually naïve males take far longer to develop baseline rates of copulation if they are presented with females that do not display appetitive behaviors (e.g., following priming with estrogen alone; Madlafousek & Hliňák, 1982). Thus, competent and preferred rates of copulatory behavior appear to become crystallized during early sexual experience and impact on the degree of sexual reward necessary for the induction of sexually conditioned place preference. These preferences seem to depend on the availability of the female and the intensity of her appetitive and consummatory sexual behaviors, which are associated with the experience of sexual reward.

Pacing chambers can be bisected by dividers that contain one or several holes. This changes the dynamics of pacing behavior in females as it alters the accessibility of the male (Coria-Avila et al., 2005; 2006). For example, females typically spend more

time away from the male in pacing chambers bisected with a 1-hole divider, relative to females in chambers bisected by a 4-hole divider. This occurs largely because the male restricts the female's return by obstructing the hole while she attempts to get through it. With the 4-hole divider, females typically direct the male toward one hole and dart through another open hole. This results in faster and potentially more efficient control of copulatory contact by the female. Interestingly, females show significant CPP for the 4-hole pacing situation relative to the 1-hole pacing situation (Coria-Avila et al. 2005), although they will show CPP for a 1-hole pacing situation relative to a non-paced situation in which the divider is removed (Paredes & Alonso, 1997; Paredes & Vasquez, 1999).

The present study had two goals. The first was to investigate the patterns of copulation acquired by males in pacing chambers that are bisected by either a 1-hole or a 4-hole divider, and to examine whether these patterns of copulation are maintained when the males are switched into the other environment. The two dividers give males different types of access to females, with the 1-hole providing relatively restricted access and the 4-hole providing easier access. We predicted that these two chambers would result in the acquisition of different patterns of sexual behavior in response to the availability of the female. The second goal was to use a conditioned ejaculatory preference paradigm (Kippin et al., 1998; Kippin & Pfaus, 2001a,b) to investigate the preference of males for females associated with the 1-hole versus 4-hole copulatory conditions. We predicted that females associated with greater availability would be more preferred than females associated with restricted access.

Experiment 1

Methods

Subjects and Surgery

40 male Long Evans rats, weighing approximately 300 g at the start of the experiment, were obtained from Charles River Laboratories (St-Constant, QC). Rats were housed in groups of five, in plastic solid floor cages, with free access to food (Purina Rat Chow) and water, and maintained on a 12-hour dark/light cycle (lights on at 8 pm) in a temperature-controlled room (21 ± 2°C). Behavioral testing was conducted in the dark phase of the cycle. Males were sexually naïve at the start of the experiment.

40 female Long Evans rats, weighing approximately 200 g at the start of the experiment, were obtained from the same supplier as the males and were housed under the same conditions as the males. Females were anaesthetized with a mixture of ketamine (50 mg/ml) and xylazine hydrochloride (4 mg/ml), at a ratio of 4:3, respectively, administered intraperitoneally in a volume of 1 ml/kg of body weight. Anaesthetized females were then bilaterally ovariectomized via a lumbar incision. All females were given a week of postsurgical recovery prior to sexual training. Following recovery, all females were placed in a pacing chamber with intact and sexually vigorous males during four sexual training trials. For all behavioral tests, sexual receptivity was induced by priming the females with an injection of 10 μg (sc in 0.1 ml of sesame oil) of estradiol benzoate (EB) 48 hrs and 500 μg (sc in 0.1 ml of sesame oil) of progesterone (P) 4 hrs prior to behavioral testing.

Apparatus

Copulatory conditioning trials took place in semicircular pacing chambers (38 cm x 60 cm x 38 cm) covered with bedding and bisected by a clear Plexiglas divider with either one hole or four holes cut into the bottom of the divider. These holes were large enough to allow the female to cross through but small enough to prevent the males from doing the same. All conditioning sessions were recorded on a DVD and subsequently scored using a PC-based behavioral observation program (Cabilio, 2007).

Procedure

The 40 naïve males were assigned randomly to two groups. One group was given nine copulatory trials with a random female at every trial in 1-hole pacing chambers and the other group was given nine copulatory trials with a random female at every trial in 4-hole pacing chambers. For all conditioning sessions, males were placed individually into a pacing chamber for 5 min, after which a female was placed into the chamber for 20 min. Females were primed with EB and P as described above. Conditioning trials occurred at 4-day intervals during the middle third of the rat's dark cycle following hormone priming. Four days following the 9^{th} conditioning trial, animals were given a 10^{th} copulatory trial but this time, they were switched so that 1-hole males were now placed in a 4-hole pacing chamber and 4-hole males were now placed in a 1-hole pacing chamber.

Behavioral Measures and Statistical Analyses

Latency and frequency data were recorded for all mounts, intromissions, and ejaculations. Criteria for sexual behaviors were those described by Sachs and Barfield (1970). Female solicitations (defined as a headwise orientation to the male followed by a

runaway, forcing the male to chase the female), hops and darts, lordosis magnitude (on a scale from 1 to 3, with 1 representing low magnitude, 2 representing moderate magnitude, and 3 representing high magnitude, as in Hardy & DeBold, 1972), and the time spent away from the male were also recorded. One-way analyses of variance (ANOVAs) were used to determine differences in the frequencies and latencies of copulatory behaviors among the two conditioning groups. The criterion for statistical reliability for all comparisons was set to $p < 0.05$.

Results

Figure 1 illustrates the average frequencies and latencies of male copulatory behaviors in 1-hole and 4-hole pacing chambers across the nine conditioning trials. Overall, male rats in the 1-hole pacing condition displayed significantly fewer intromissions ($F(1,350) = 6.730$, $p < 0.05$) and ejaculations ($F(1,350) = 21.340$, $p < 0.05$) compared to males in the 4-hole pacing condition. Males in the 1-hole condition also had longer intromission ($F(1,346) = 3.944$, $p < 0.05$) and ejaculation ($F(1,345) = 23.92$, $p < 0.05$) latencies during the acquisition of copulatory behavior. Figure 2 shows the ejaculation latency for the 1-hole and 4-hole males during all ten copulation trials. When males were switched to the other pacing condition on the last test day, differences in ejaculation latencies were maintained ($F(1,37) = 3.021$, $p < 0.1$). Figure 3a illustrates the average time that females in 1-hole and 4-hole pacing conditions spent away from the male. It can be seen in this figure that 1-hole females spent significantly more time away from the males as compared to 4-hole females ($F(1,345) = 22.024$, $p < 0.05$). To examine the influence of the time spent by the female away from the male, the ejaculation latency

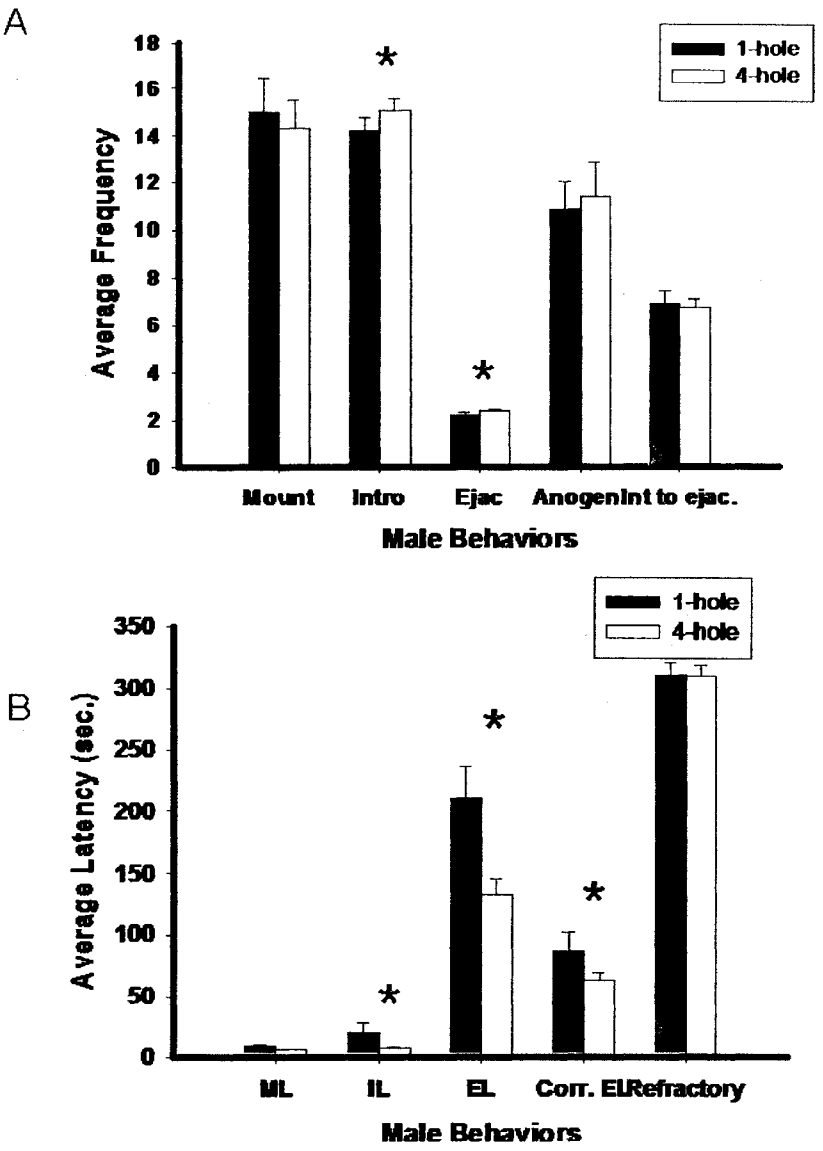

Figure 1: a) Average frequencies of male copulatory behaviors in 1-hole and 4-hole pacing chambers. (1-hole: n= around 19 males per trial, 4-hole: n= around 20 males per trial). b) Average latency of male copulatory behaviors in 1-hole and 4-hole pacing chambers. (1-hole: n= around 18 males per trial, 4-hole: n= around 17 males per trial).

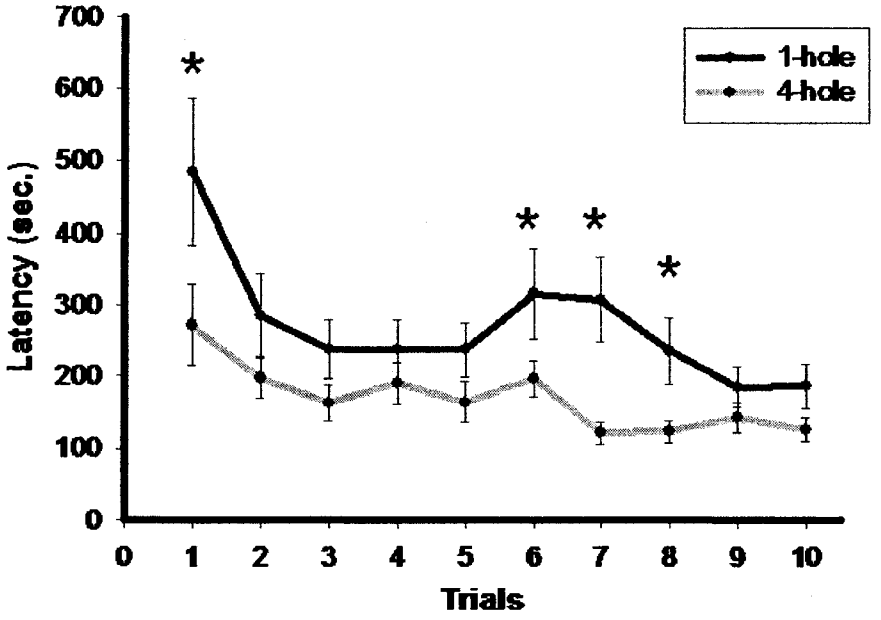

Figure 2: Ejaculation latency (sec.) for 1-hole and 4-hole males during all ten copulation trials. (1-hole: n= around 19 males per trial, 4-hole: n= around 20 males per trial).

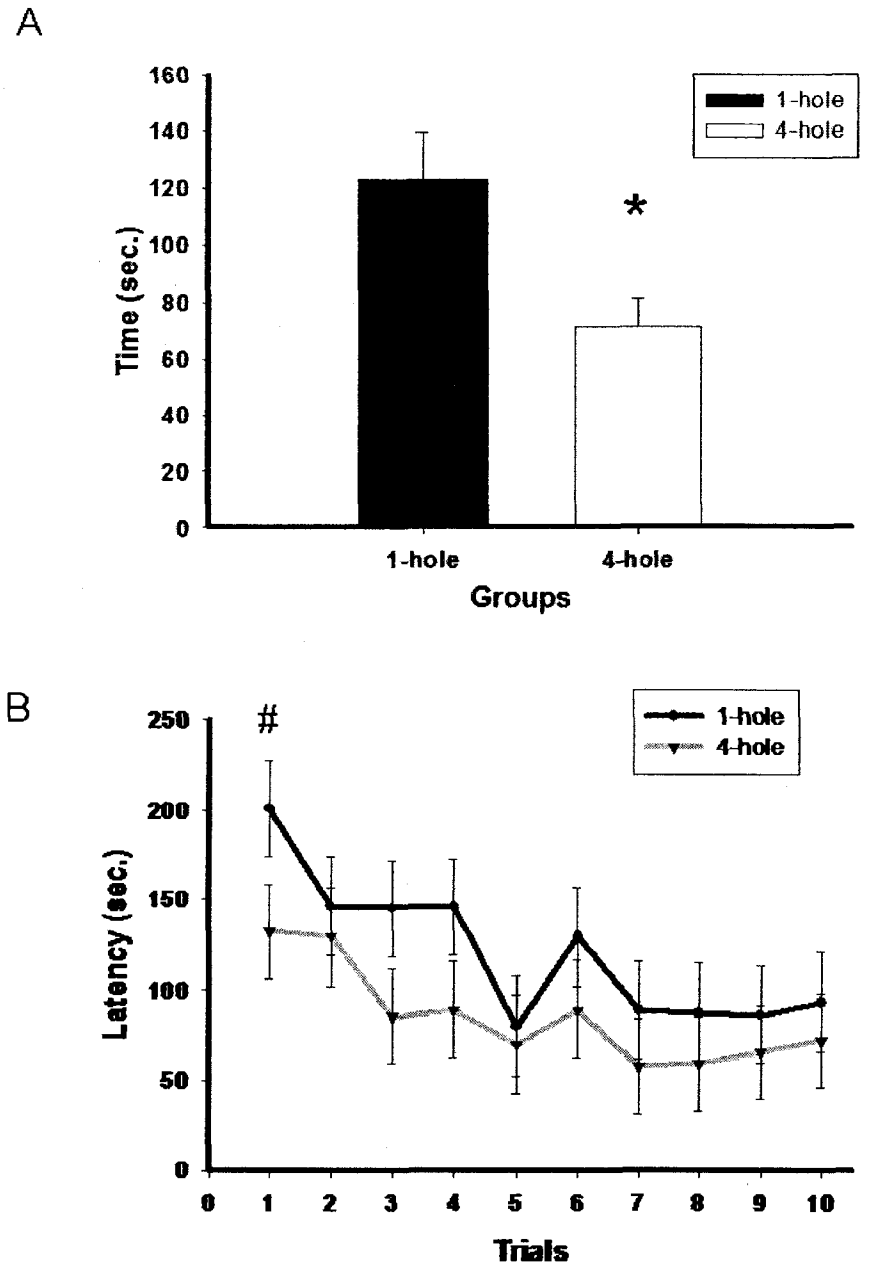

Figure 3: a) Average time (sec.) females spent away from males in 1-hole and 4-hole pacing conditions. (1-hole: n= approx. 19 females per trial, 4-hole: n= around 20 females per trial). b) Corrected ejaculation latency (sec.) for 1-hole and 4-hole males during all ten copulation trials. (1-hole: n= approx. 19 males per trial, 4-hole: n= around 20 males per trial).

of all males was corrected by subtracting the time the female spent on the other side of the divider. Figure 3b illustrates the average corrected ejaculation latency for 1-hole and 4-hole males during all ten copulation trials. As can be seen in this figure, following the correction, 1-hole males still displayed longer ejaculation latency than 4-hole males ($F(1,345) = 7.778$, $p < 0.05$.

Females in the 4-hole condition displayed significantly more lordosis magnitude 1, solicitations and hops and darts ($F(1,388) = 12.935$, $p < 0.05$, $F(1,388) = 14.259$, $p < 0.05$ and $F(1,388) = 16.146$, $p < 0.05$, respectively) compared to females in the 1-hole condition (Figure 4). No differences were observed between 1-hole and 4-hole females on lordosis magnitudes 2 and 3 ($F(1,388) = 0.015$, $p > 0.05$ and $F(1,388) = 0.956$, $p > 0.05$, respectively) (Figure 4).

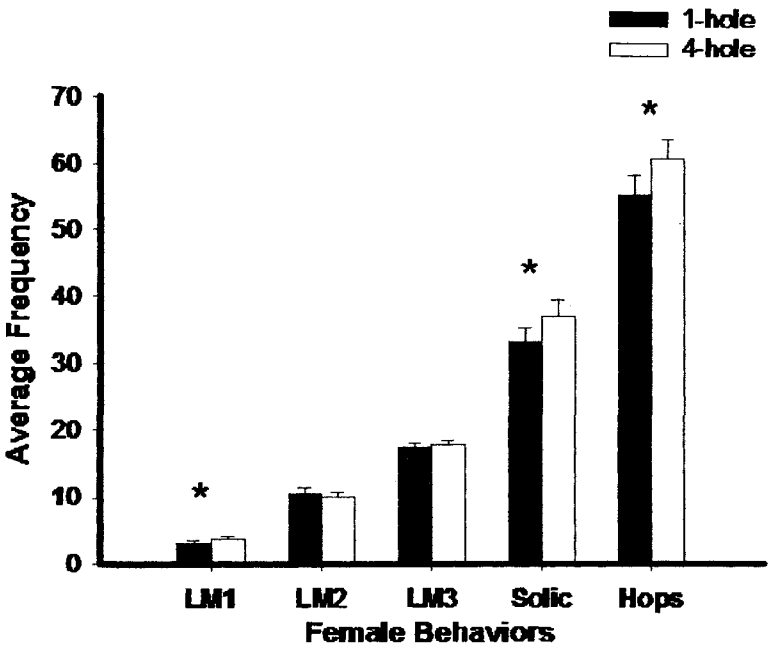

Figure 4: Frequency of female sexual behavior in 1-hole and 4-hole pacing chambers. (1-hole: n= around 19 females per trial, 4-hole: n= around 20 females per trial).

Experiment 2

Methods

Subjects

40 male Long Evans rats and 40 female Long Evans rats served as subjects in this experiment. Subjects were acquired from the same supplier and housed under the same conditions as subjects in Experiment 1. Females were ovariectomized and sexually trained in the same manner as in Experiment 1. During all conditioning sessions, females were scented with approximately 0.2 ml of almond extract (Club House, London, ON, Canada) applied to both the back of the neck and the anogenital area using a syringe.

Procedure

Odor conditioning phase: The 40 naïve male rats were divided into two groups. One group was given ten copulatory trials in 1-hole pacing chambers with a random almond scented female and in 4-hole pacing chambers with a random unscented female on alternate trials. The other group, which was the counterbalance of the previous group, was given ten copulatory trials in 4-hole pacing chambers with an almond scented female and in 1-hole pacing chambers with an unscented female also on alternate trials. This way, half of the rats associated scented females with 1-hole pacing condition and the other half associated scented females with 4-hole pacing condition. Males received a total of ten sequential conditioning trials (five in each pacing condition). For all conditioning trials, females were primed with EB and P as described in Experiment 1. Conditioning trials occurred as in Experiment 1.

Partner preference test: Four days following the last conditioning trial, each male was placed in an open field arena and allowed to habituate for 5 min. At the end of this

period, two receptive females, one almond-scented and the other unscented, were placed simultaneously into two diagonal corners of the open field at approximately equal distance from the male. All animals were able to interact freely with one another for 30 min. These tests were recorded on DVDs and scored.

Behavioural Measures and Statistical Analyses

Male copulatory preference was determined by latency and frequency measures for male mounts, intromissions, and ejaculations during the partner preference test. Female solicitations (defined as a headwise orientation to the male followed by a runaway, encouraging the male to chase the female), hops and darts, lordosis magnitude were all recorded as well. One-way ANOVAs were used to determine differences in the frequencies and latencies of copulatory behaviors among the two conditioning groups. Non-parametric chi-square analysis was used to determine the difference in the proportion of females selected for first three and last three mounts and intromissions and first three ejaculations during the copulatory preference test. The level of significance for all comparisons was set to $p < .05$.

Results

Figure 5 illustrates the average frequencies of ejaculation for all males with females associated with 1-hole and 4-hole pacing conditions. Males displayed significantly more ejaculations with females associated with the 4-hole pacing condition than with those associated with 1-hole condition ($F(1, 56) = 10.781$, $p < 0.05$). Figure 6 shows the proportions of intromissions for all males with females associated with 1-hole and 4-hole pacing condition before the first ejaculation. For their last intromission, males chose females associated with 4-hole paced copulation ($X^2 = 4.17$, $p < 0.05$).

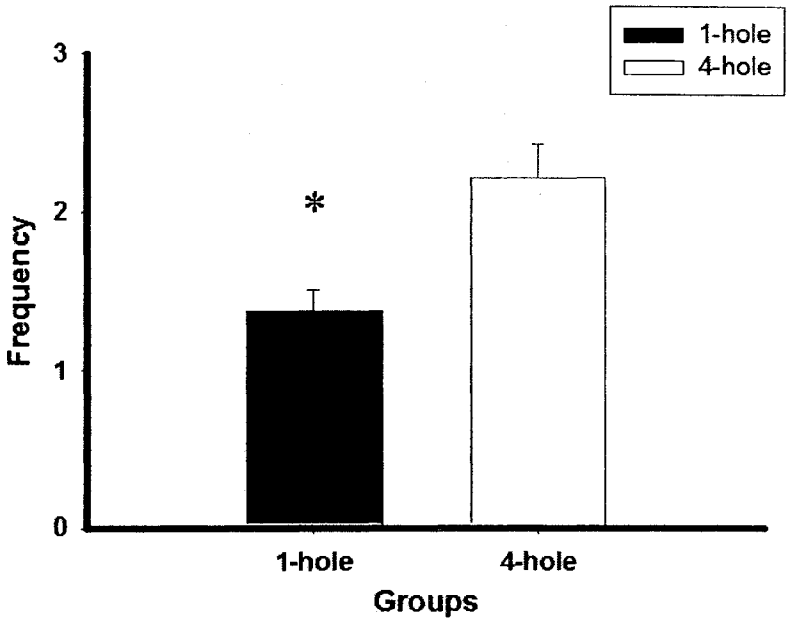

Figure 5: Average frequencies of ejaculation for all males with females associated with 1-hole and 4-hole pacing condition. (1-hole: n= 29 males, 4-hole: n= 29 males).

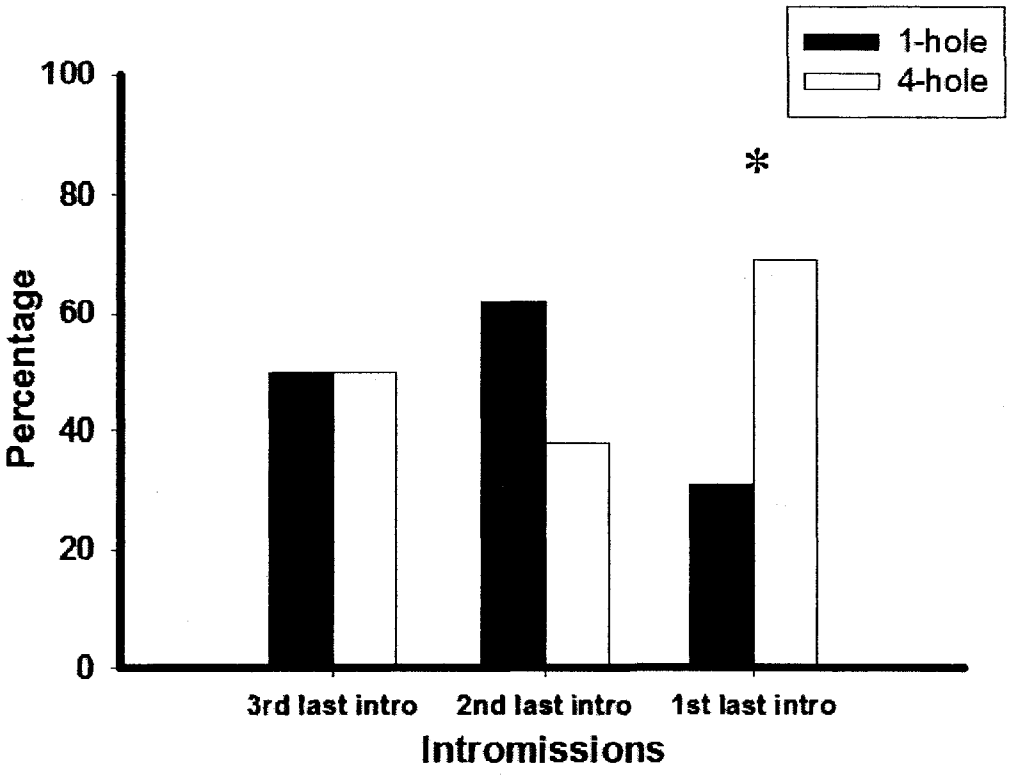

Figure 6: Distribution of the last three intromissions between females associated with 1-hole and 4-hole paced copulation during the first ejaculation. (1-hole: n= 29 males, 4-hole: n= 29 males).

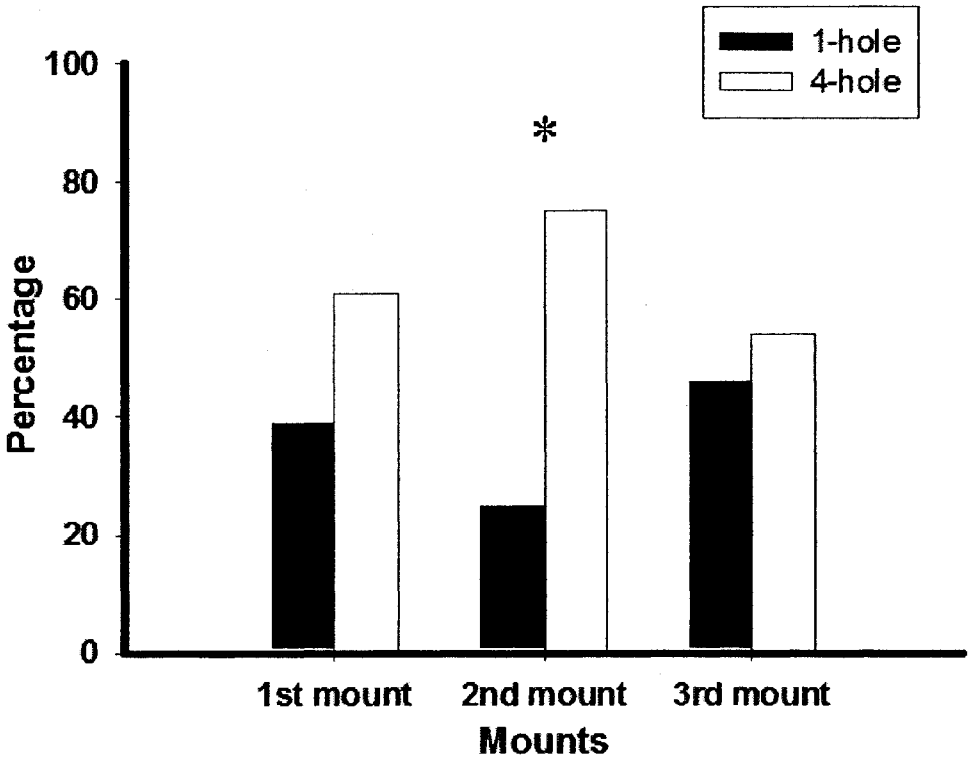

Figure 7: Distribution of the first three ejaculations between females associated with 1-hole and 4-hole paced copulation. (1-hole: n= around 28 males, 4-hole: n= around 28 males).

General Discussion

The findings of the first experiment revealed that males trained to copulate in the 4-hole pacing chamber had significantly shorter ejaculation latencies and ejaculated with significantly fewer intromissions than did males trained in the 1-hole pacing chamber. On the last trial, males trained in 1-hole pacing chambers were switched to 4-hole pacing chambers and vice-versa. Consistent with our first hypothesis about the stability of copulatory behavior once it is acquired, the males maintained their pattern of intromissions to ejaculation when the environments were switched, despite the difference in external contingency that induced the differences in the first place. In the second experiment, males displayed a conditioned ejaculatory preference for females associated with 4-hole paced copulation relative to females associated with 1-hole paced copulation, indicating that the males preferred the experience of copulating in the 4-hole relative to 1-hole condition. This is consistent with our second hypothesis that the increased availability of the female in the 4-hole condition would allow males to copulate at their preferred rate relative to the more restricted access to females in the 1-hole condition.

It is possible that the different contexts induced different amounts of sexual arousal in the males. Sexual arousal is coordinated by the combined actions of the sympathetic and parasympathetic nervous systems, both of which are activated in different organs during sexual arousal. For example, in males activation and maintenance of erection is mediated by the parasympathetic division, whereas the concurrent increases in heart and breathing rate are mediated by the sympathetic division (Hull & Dominguez, 2007; McKenna, 1999; Rowland, 2005). Activation of sympathetic outflow to the penis facilitates ejaculation, after which erection is inhibited. We note that males in the 1-hole

condition displayed longer ejaculation latencies, suggesting that they experienced more parasympathetic arousal that maintained erection but delayed ejaculation. In contrast, males in the 4-hole condition may have experienced more sympathetic arousal which could promote faster ejaculation. The fact that ejaculation patterns were maintained despite the switch in environment indicates that patterns of sexual arousal and behavior are shaped by the environment in which sexual experience is acquired, and that once acquired those patterns become relatively "fixed".

Female availability has profound effects on the development of copulatory patterns and conditioning in male rats. For example, Larsson (1956) shaped the number of intromissions male rats required for ejaculation using an "enforced interval" paradigm. Males were allowed to copulate for 8 trials with a receptive female but the female was removed between each intromission for either short (i.e. 30 sec to 3 min), long (i.e. more than 7 min), or normal (spontaneous, without interruption) intervals. Data from the final test day were used to assess the effect of the training procedure. Males that received short enforced intervals between intromissions ejaculated more rapidly and with fewer intromissions than males that had free access to the female. However, when longer intervals were enforced, a reduced number of males reached ejaculation in the set time frame. These findings suggest that there is an optimal timing of penile stimulation for males to reach ejaculation, and that stimulation delays that are slightly longer than the uninterrupted interintromission interval results in more rapid ejaculation whereas stimulation delays that are too protracted in time makes it more difficult for males to achieve ejaculation. More rapid ejaculation is likely induced by an overactivation of sympathetic outflow whereas the delayed ejaculation may stem from either overactivation

of parasympathetic outflow or a deficit in stimulation of sympathetic outflow. Similarly, Silberberg and Adler (1974) allowed males to copulate with a receptive female until ejaculation or 7 intromissions, whichever happened first. After 20 trials, males in the intromission-alone group learned to ejaculate after 6 or fewer intromissions, suggesting a facilitation of ejaculation that may be mediated by increased sympathetic outflow. Finally, McClintock (1984) reported that wild male rats have shorter interintromission intervals and ejaculate after fewer intromissions than do domesticated males despite the fact that they are copulating in the same large seminatural environment with the same females. Although this could be due to genetic differences between wild and domesticated rats, differences in the sexual behavior between wild and domesticated females suggest that it could also be due to differences in female availability in the wild and in the laboratory settings which could shape the pattern of copulation in males. Indeed, in her analysis McClintock noted that longer intervals between intromissions were more likely to occur when wild females were enforcing or pacing the copulatory contact. Taken together with the data of the present study, we suggest that the 4-hole condition produces a small delay in the availability of the female, which increases sympathetic outflow during copulation and leads to a faster ejaculation threshold compared to the 1-hole condition. This condition also increases solicitations and hops and darts significantly in the females, which may also lead to an increase in sympathetic sexual arousal. In the 1-hole condition, males must wait longer for females to return to their side, which may lead to a slower activation of sympathetic outflow for ejaculation and thus a longer ejaculation latency. Females in this condition also display significantly

fewer solicitations and hops and darts, which may also contribute to a decrease in sympathetic sexual arousal in the males.

Ejaculation is necessary for the development of both conditioned place and partner preferences (Ågmo & Berenfeld, 1990; Mehrara & Baum, 1990; Miller & Baum, 1987; Kippin and Pfaus, 2001a,b). However, results from Martinez and Paredes (2001) suggested that ejaculation is necessary but not sufficient to induce conditioning. Similarly, in the present study, males displayed partner preference for females associated with the 4-hole relative to 1-hole condition. Given that males also ejaculated in both conditions, but displayed a partner preference for females in the 4-hole condition, our data support the findings of Martinez and Paredes in that ejaculation is necessary, but not sufficient, to induce sexual reward. The arousal level experienced during copulation may also affect the intensity of sexual reward. Males may prefer 4-hole over 1-hole paced copulation because they experience more sympathetic arousal in the 4-hole condition. Moreover, the 4-hole condition allows males to have greater access to females and optimize their preferred rate of copulation.

Another factor that may increase arousal of the male is the degree of appetitive or precopulatory behavior of the female. In the original studies of conditioned ejaculatory preference by Kippin and colleagues (Kippin et al., 1998; Kippin & Pfaus 2001a,b; Kippin, Samaha, Sotiropoulos, & Pfaus, 2001) males were trained to associate an almond odor on sexually receptive females that they copulated with to ejaculation in bilevel pacing chambers. Those chambers have two levels and a set of ramps that allow females to solicit and pace the copulatory contact by running from level to level, forcing the males to chase them. On a final test conducted in a large open field, males were presented

with two sexually receptive females, one scented with almond and the other unscented. A significant preference to ejaculate with the scented female occurred simply because the odor was present on the female during the postejaculatory interval. Although intromissions or ejaculation alone were not sufficient to induce conditioning (i.e., in males for which the scented female was removed immediately after 5 intromissions or after the first ejaculation), the state induced by ejaculation was. However, it is noteworthy that males had to chase the females in order to copulate. Evidence suggests that the strength of female precopulatory behavior (e.g., solicitations, hops and darts, pacing, etc.) can maintain male copulatory behavior following castration (Madlafousek, Hliňák, & Beran, 1976) or subthreshold 6-OHDA lesions of mesolimbic dopamine terminals in the nucleus accumbens (Everitt, 1990). This suggests that waiting for, or chasing, a sexually receptive female at optimal intervals between intromissions may sum with ejaculation to induce a sexual reward state sufficient to condition partner preferences. The same may hold true for sexually conditioned place preferences, given that ejaculation and its aftermath are a necessary unconditioned stimulus.

In summary, the findings of the present study show that ejaculation is necessary but not sufficient for the development of conditioning. A high or optimal level of arousal during copulation may also be a necessary component of sexual reward that makes it of sufficient intensity to condition partner preferences. The availability of the female is a critical feature of the context in which copulatory behaviors are developed, and can permanently shape the pattern of copulation perhaps due to differences in optimal levels of arousal.

CHAPTER TWO

The experiments of Chapter 1 showed that female availability differs during copulation in 1-hole and 4-hole pacing chambers, which may lead to differences in the development of baseline copulatory patterns in males, and the establishment of a preference for females associated with easier access relative to more restricted access . For example, females spend more time away from the males in 1-hole relative to 4-hole pacing chambers. Males trained in 4-hole pacing chambers displayed shorter ejaculation latencies compared to males trained in 1-hole conditions, perhaps because greater female availability in 4-hole chambers increased the level of arousal experienced during copulation. Furthermore, the results in Chapter 1 also showed that males preferred to copulate with females associated with 4-hole over 1-hole chambers, possibly because males better control the rate of copulatory contact in 4-hole chambers and achieve their optimal rates of copulation. Could males develop partner preference for a familiar female following repeated copulation in 4-hole pacing chambers? The experiments in Chapter 2 examined whether males can develop a conditioned ejaculation preference for a familiar female following repeated copulation in 4-hole pacing chambers, and also the conditions necessary for the development of this preference.

Pacing conditions contribute to the conditioned ejaculatory preference for a familiar female in the male rat

Nafissa Ismail, Hélène Gelez, Ivonne Lachapelle, James G. Pfaus*

Center for Studies in Behavioral Neurobiology, Department of Psychology
Concordia University, Montréal, QC H4B 1R6, Canada

RUNNING HEAD: Paced copulation and partner preference

* Corresponding author. Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, 7141 Sherbrooke W., Montréal, QC, H4B 1R6 Canada.

E-mail address: Jim.Pfaus@concordia.ca.

Citation: *Physiology & Behavior, in press*, (2008).

Abstract

Male rats show greater unconditioned sexual arousal and mating preference for a novel female compared to a familiar one. However, they also display a conditioned preference to ejaculate with a female bearing an odor paired previously with copulation to ejaculation, suggesting that their copulatory strategies are not fixed. The aim of the present study was to examine if males might prefer a familiar or a novel female after repeated copulation with the same female in a pacing chamber bisected by a 1-hole or a 4-hole divider. Sexually naïve male Long-Evans rats were assigned to copulate with the same almond-scented or unscented female in a 1-hole or 4-hole pacing chamber for 10 conditioning trials at 4-day intervals. Four days following the last trial, each male was given a partner preference test during which they had the choice to copulate with either the familiar or a novel scented or unscented female. Results showed that males trained to copulate in 1-hole pacing chambers developed a conditioned ejaculatory preference for their familiar almond-scented female. However, if the familiar female was not scented with almond odor or if a novel female was bearing the almond odor, 1-hole trained males failed to display conditioned ejaculatory preference. Males trained in the 4-hole condition did not display a conditioned ejaculatory preference. These findings indicate that pacing conditions in which males have restricted access to the female contribute to the conditioned ejaculatory preference for familiar females bearing a neutral odor.

Keywords: Sexual arousal, Partner preference, Olfactory discrimination, Paced copulation, Pavlovian conditioning

Introduction

Male rats are generally thought to be polygamous (Calhoun, 1962). In the wild, male rats are often found chasing estrous females as a pack and competing to mate with the female (Robitaille & Bouvet, 1976). Indeed, the so-called "Coolidge effect" describes a phenomenon whereby sexual behavior can be either maintained longer in sexually active males, or reinitiated in sexually sated males, by presenting them with a novel female (e.g. Beach & Jordan, 1956). The ultimate advantage of the Coolidge effect on reproductive fitness is that it enables the male to inseminate more than one female (Daly & Wilson, 1978; Barash, 1979). For example, replacing a familiar female with a novel estrous female following sexual exhaustion in a male rat results in a weak resumption of copulatory activity in that male (Beach & Jordan, 1956). Second, McClintock and colleagues (McClintock, Aniski & Adler, 1982a) noted that during group mating, when many females are present, males ejaculate twice as much than during pair mating, when only one female is present. Replacing a stimulus female by a novel one also results in increased dopamine release in the nucleus accumbens of sexually sated male rats (Fiorino, Coury & Phillips, 1997). These findings suggest that males are more sexually aroused when presented with a novel female after copulating to several ejaculations with a familiar female, and this increased arousal may direct their mate choice.

Despite evidence for polygamous mating strategies in rats, there is emerging evidence that male rats can learn to associate cues with sexual reward and display preferences for partners that bear those familiar cues. Kippin and colleagues (Kippin & Pfaus, 2001; Kippin & Pfaus, 2001a; Kippin, Samaha, Sotiropoulous & Pfaus, 2001b) trained males to associate a neutral odor (almond extract) painted on the neck and

anogenital region of a sexually receptive female with copulation to ejaculation. Two control conditions were used, a non-paired condition in which males copulated with unscented receptive females and were given access to the almond odor on gauze in their home cages between tests, and a random-paired group in which the odor was presented on the female every other trial (so that males had an equal number of trials with and without the odor). After the conditioning phase, males were tested for their partner preference in an open field where they were given the choice to copulate with either an almond-scented or an unscented receptive female. Males in the paired condition chose to ejaculate with the scented female first, and significantly more often, throughout the 30-min test compared with control males in non-paired or random-paired groups. However, males in the non-paired group ejaculated more frequently with the unscented female, suggesting a similar preference for familiarity. These findings indicated that male rats display a conditioned ejaculatory preference (CEP) for a female bearing a familiar odor when this odor was paired with copulation. Subsequent studies (Kippin & Pfaus, 2001a) showed that the post-ejaculatory state was the necessary unconditioned stimulus because males had to be in the presence of the scented female during this period to make the association. This was reminiscent of the sexually-conditioned place preference (CPP) shown by males that requires ejaculation as a necessary unconditioned stimulus or reward.

Whether a male will display preferences towards "familiarity" or "novelty" may depend on female availability and accessibility. If males have easy access to females, then they gain an obvious advantage to ejaculate with a different one every time to increase their reproductive fitness. However, if access to females is restricted, then it may

be advantageous for the males to remain with the female they have already ejaculated with and mate with her again at a later point and display preferences for this familiar female or these familiar cues. These differences in mating strategies have been reported in other rodents, for example prairie and meadow voles (Winslow, Shapiro, Carter & Insel, 1993) that are found in either sparsely or densely populated groups, respectively. Field studies have demonstrated that prairie voles share their nests with their partner throughout the mating season (Getz & Hofman, 1986) and are actively involved in parental care; they are thus termed monogamous (Insel, Young & Wang, 1997). In contrast, meadow voles are deemed polygamous because the males do not form pair bonds and display minimal parental care (Insel, Young, Wang, 1997).

Rats appear to have the ability to learn both polygamous-like and monogamous-like mating strategies depending on their initial sexual experiences, and in particular, the circumstances in which their initial experiences with sexual reward occur. Although opioid transmission is an important component of the postejaculatory reward state that supports CPP (Miller & Baum, 1987), it is not clear that this is the only determinant of the sexual reward state or its intensity; the level of arousal that the male experiences during copulation may also play an important role during conditioning (Pfaus, Kippin & Centeno, 2001). The arousal level can be modulated by a number of factors, one being the environment in which males copulate (Borg, Esbenshade, Johnson, Lunstra & Ford, 1992). For instance, environments in which males have restricted access to the female increase the level of arousal as males anticipate future copulatory contact with the female (Amstislavskaya & Popova, 2004).

The present study sought to determine whether ejaculation alone is sufficient to induce a sexual reward state in male rats, or whether the arousal level that males experience prior to ejaculation also plays a role. This was accomplished using the pacing chambers described previously for the study of female sexual behavior (Erskine, Kornberg & Cherry, 1989; Paredes & Vasquez, 1999; Coria-Avila, Ouimet, Pacheco, Manzo & Pfaus, 2005). These chambers are bisected by a Plexiglas partition with either one or several holes cut out of the bottom that are big enough for the female to pass through, but too small for a male. This restricts the male to one side and allows the female to control the initiation and rate of copulation by moving freely from the unoccupied side to the male's side and back again. In a previous study (Ismail, Zhao & Pfaus, 2008), we showed that in 1-hole pacing chambers females spend more time away from males, forcing the males to wait for the females for longer periods of time than pacing chambers bisected with a 4-hole divider. Thus, copulation in 1-hole pacing chambers may be more arousing to males since access to the female is more restricted. Consequently, it is possible that males trained to copulate in the 1-hole condition may be more likely to display preferences for familiar cues than males trained in the 4-hole condition. Accordingly, we first examined whether males can develop preferences for familiar cues when trained exclusively in 1-hole or 4-hole pacing chambers. We then investigated whether the preferences displayed by males trained in 1-hole pacing chambers were for the familiarity of the female or the odor cue.

Materials and methods

Subjects

Male Long Evans rats, weighing approximately 300 g at the start of the experiment, were obtained from Charles River Laboratories (St-Constant, Quebec). Rats were housed in groups of four, in plastic solid floor cages, with free access to food (Purina Rat Chow) and water, and maintained on a 12-hour dark/light cycle (lights on at 8 pm) in a temperature-controlled room (21 ± 2°C). Behavioral testing was conducted in the dark phase of the cycle. Males were sexually naïve at the start of the experiment.

Female Long Evans rats, weighing approximately 200 g at the start of the experiment, were obtained from the same supplier and were housed under the same conditions as the males. Females were anaesthetized with a mixture of ketamine (50 mg/ml) and xylazine hydrochloride (4 mg/ml), at a ratio of 4:3, respectively, administered intraperitoneally in a volume of 1 ml/kg of body weight. Anaesthetized females were then bilaterally ovariectomized via a lumbar incision. All females were given a week of postsurgical recovery prior to sexual training. Following recovery, all females were placed in a pacing chamber with intact and sexually vigorous males during four sexual training trials. For all behavioral tests, sexual receptivity was induced by priming the females with an injection of 10 μg (s.c. in 0.1 ml of sesame oil) of estradiol benzoate (EB) 48 hrs and 500 μg (s.c. in 0.1 ml of sesame oil) of progesterone (P) 4 hrs prior to behavioral testing.

All animal procedures conformed to the guidelines of the Canadian Council on Animal Care, and were approved by the Concordia University Animal Research Ethics Committee.

Apparatus

Copulatory conditioning trials took place in pacing chambers (38 cm x 60 cm x 38 cm) with bedded floors and bisected by a clear Plexiglas divider with either one hole or four holes cut into the bottom of the divider. These holes were large enough to allow the female to cross through but small enough to prevent the males from doing the same. All conditioning sessions were recorded on DVD and scored using a PC-based program (Cabilio, 1996).

Procedure

2.1. CEP for a familiar almond-scented female following 1-hole paced copulation.

Forty sexually naïve males were randomly assigned to one of two groups and given 10 copulatory trials with the same almond-scented female; however, one group was placed in 1-hole pacing chambers, while the other group was in 4-hole pacing chambers at every trial. For all conditioning sessions, males were given a five-minute habituation period into a pacing chamber, after which, a female was placed into the chamber for 20 min. Females were primed with EB and P as described above. Conditioning trials occurred at 4-day intervals during the middle third of the rat's dark cycle following hormone priming. Four days after the last conditioning trial, males were placed in an open field and allowed to habituate for 5 min. At the end of this period, the familiar scented female and a novel unscented female were placed simultaneously into two diagonal corners of the open field at approximately equal distance from the male. All animals were able to interact freely with one another for 30 min.

2.2. CEP for a familiar unscented female following 1-hole paced copulation.

Forty sexually naïve males were randomly assigned to one of two groups. One group was given ten copulatory trials in 1-hole pacing chambers with the same unscented female and the other group was given ten copulatory trials in 4-hole pacing chambers with the same unscented female. Four days following the last conditioning trial, males were placed in an open field and allowed to habituate for 5 min. At the end of this period, the familiar and novel unscented females were placed simultaneously into two diagonal corners of the open field at approximately equal distance from the male. All animals were able to interact freely with one another for 30 min.

2.3. CEP for novel females bearing a familiar almond odor following 1-hole paced copulation.

Forty sexually naïve males were randomly assigned to one of two groups. One group was given ten copulatory trials in 1-hole pacing chambers and the other group was given ten copulatory trials in 4-hole pacing chambers with the same scented female on every trial. Males were placed individually into a pacing chamber for 5 min, after which the scented female was placed into the chamber for 20 min. Four days after the last conditioning trial, males were placed in an open field and allowed to habituate for 5 min. At the end of this period, a novel scented female and a novel unscented female were placed simultaneously into two diagonal corners of the open field at approximately equal distance from the male. All animals were able to interact for 30 min.

2.4. Behavioral measures and statistical analyses

Latency and frequency measures were recorded for all mounts, intromissions, and ejaculations. Criteria for sexual behaviors were those described by Sachs and Barfield

[23]. Female solicitations (defined as a headwise orientation to the male followed by a runaway forcing the male to chase the female), hops and darts and the frequency of lordosis magnitude (on a scale from 1 to 3, with 1 representing low magnitude, 2 representing moderate magnitude and 3 representing high magnitude, as in Hardy and Debold [24], and as we have used previously [20]) were recorded. To examine copulatory preference, the frequency of ejaculation with each female, and the female chosen for the first three ejaculations were also recorded. One-way analyses of variance (ANOVAs) were used to analyze all frequency and latency data. Chi-square analyses were used to determine the difference in the proportion of females selected for the first two ejaculations during the copulatory preference test. The criterion for statistical reliability for all comparisons was set to $p < 0.05$.

3. Results

3.1. Development of a CEP for a familiar almond-scented female following 1-hole paced copulation.

Figure 8 illustrates the average frequencies of copulatory behaviors for 1-hole males with the familiar and the novel females, as well as the choice of female for the first two ejaculations. Overall, 1-hole males ejaculated significantly more with the familiar scented female over the novel unscented female ($F(1, 34) = 6.641$, $p < 0.05$) (Figure 8a). One-hole males also tended to chose more their familiar female for the first ejaculation ($\chi^2 = 2.58$, $p = 0.1$) (Figure 8b). No differences were found in the latencies of male sexual behaviors. There were also no differences in the frequency of ejaculation in 4-hole males between the familiar and novel females (Figure 9).

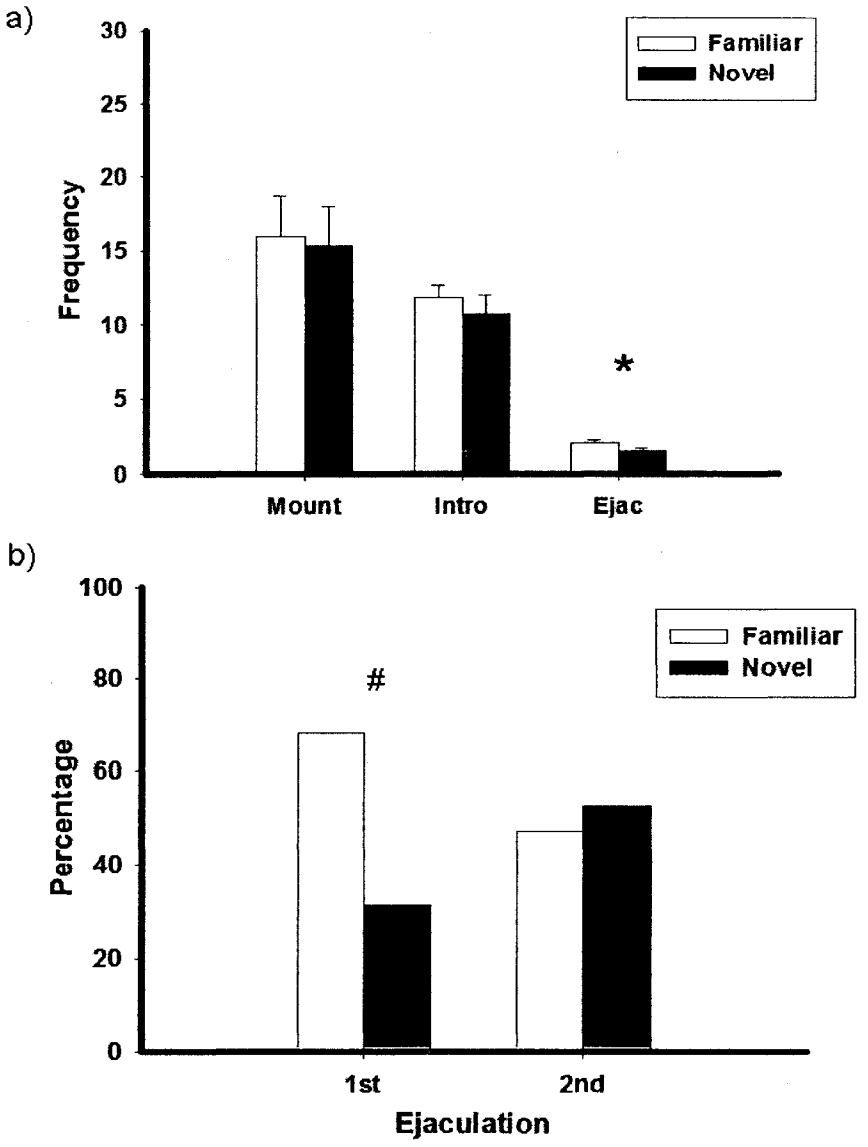

Figure 8: a) Frequencies of copulatory behaviors (mounts, intromissions, and ejaculations) displayed by 1-hole males towards the familiar scented and novel unscented females. b) Distribution of first two ejaculations between familiar almond-scented and novel unscented females in 1-hole males. Data are means ± SEMs. * $p < 0.05$, familiar vs. novel. # $p < 0.1$, familiar vs. novel.

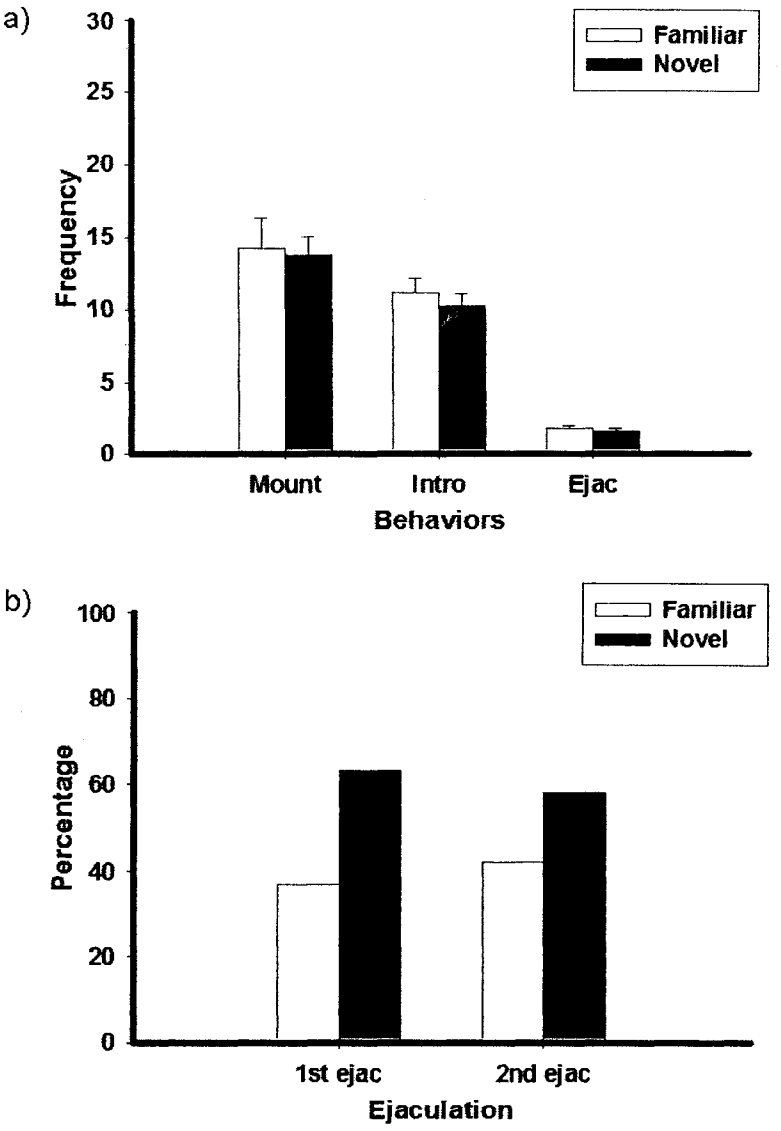

Figure 9: a) Frequencies of copulatory behaviors displayed by 4-hole males towards the familiar scented and novel unscented females. b) Distribution of first two ejaculations between familiar almond-scented and novel unscented females in 4-hole males. Data are means ± SEMs.

Figure 10 illustrates the average frequencies of sexual behaviors in the familiar and novel females trained in 1-hole pacing chambers. 1-hole familiar females displayed significantly more solicitations ($F(1, 34) = 4.29, p < 0.05$) and more hops and darts ($F(1, 34) = 4.892, p < 0.05$) than novel females. There was no difference in the frequency of behaviors of 4-hole familiar and novel females (Figure 11).

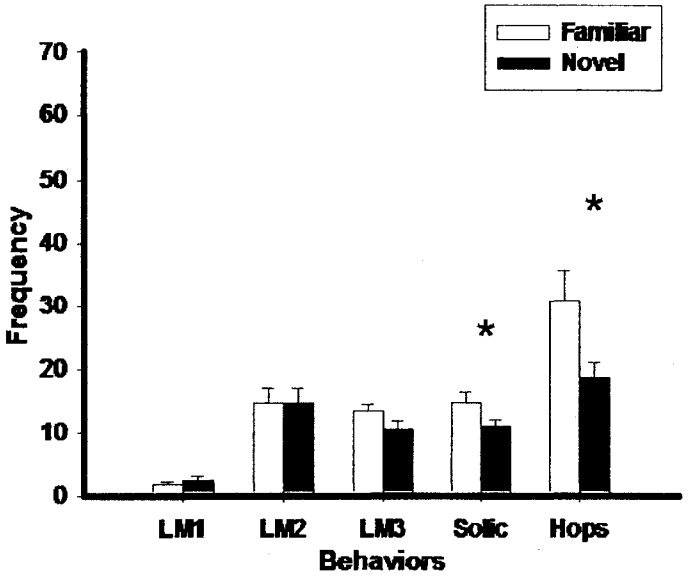

Figure 10: Frequency of sexual behaviors displayed by 1-hole familiar scented and novel unscented females. (LM1 = lordosis magnitude 1; LM2 = lordosis magnitude 2; LM3 = lordosis magnitude 3; Solic = solicitations; Hops = hops and darts). Data are means ± SEMs. * $p < 0.05$, familiar vs. novel.

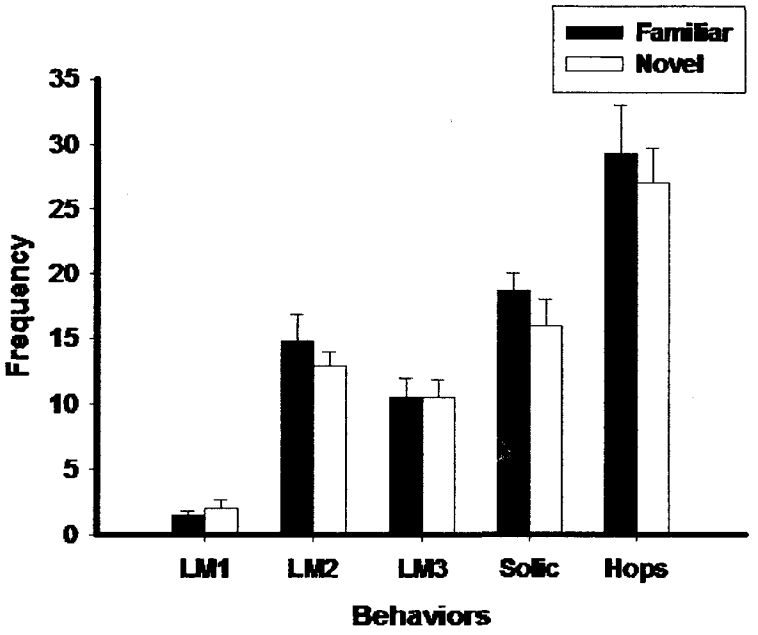

Figure 11: Frequency of sexual behaviors displayed by 4-hole familiar scented and novel unscented females. Data are means ± SEMs.

3.2. Failure to develop CEP for a familiar unscented female following 1-hole paced copulation.

As can be seen in Figs. 12a and 13a, 1-hole and 4-hole males did not ejaculate more with their familiar female as opposed to the novel one. Moreover, 1-hole males did not ejaculate first with their familiar female (Figure 12b). Interestingly, as in the previous experiment, 1-hole familiar females displayed significantly more solicitations ($F(1, 34) = 3.8, p = 0.05$) and more hops and darts ($F(1, 34) = 22.11, p < 0.05$) than novel females (Fig. 14). Four-hole familiar females also solicited the males more than did novel females ($F(1, 26) = 5.57, p < 0.05$) (Fig. 15).

3.3. Failure to develop CEP for novel females bearing a familiar almond odor following 1-hole paced copulation.

Fig. 16a shows that 1-hole males tended to ejaculate more with the unscented female as opposed to the scented one ($F(1, 27) = 3.23, p < 0.1$). However, males trained in 1-hole pacing chambers chose significantly more the scented female for their first ejaculation ($\chi^2 = 3.27, p < 0.1$) (Figure 16b). There were no differences in the frequency of ejaculations made by the 4-hole males with the scented and unscented female (Figs. 17a and 17b). Finally, there were no differences in female sexual behaviors between the scented and unscented females trained in 1-hole or 4-hole pacing chambers (Figs. 18 and 19).

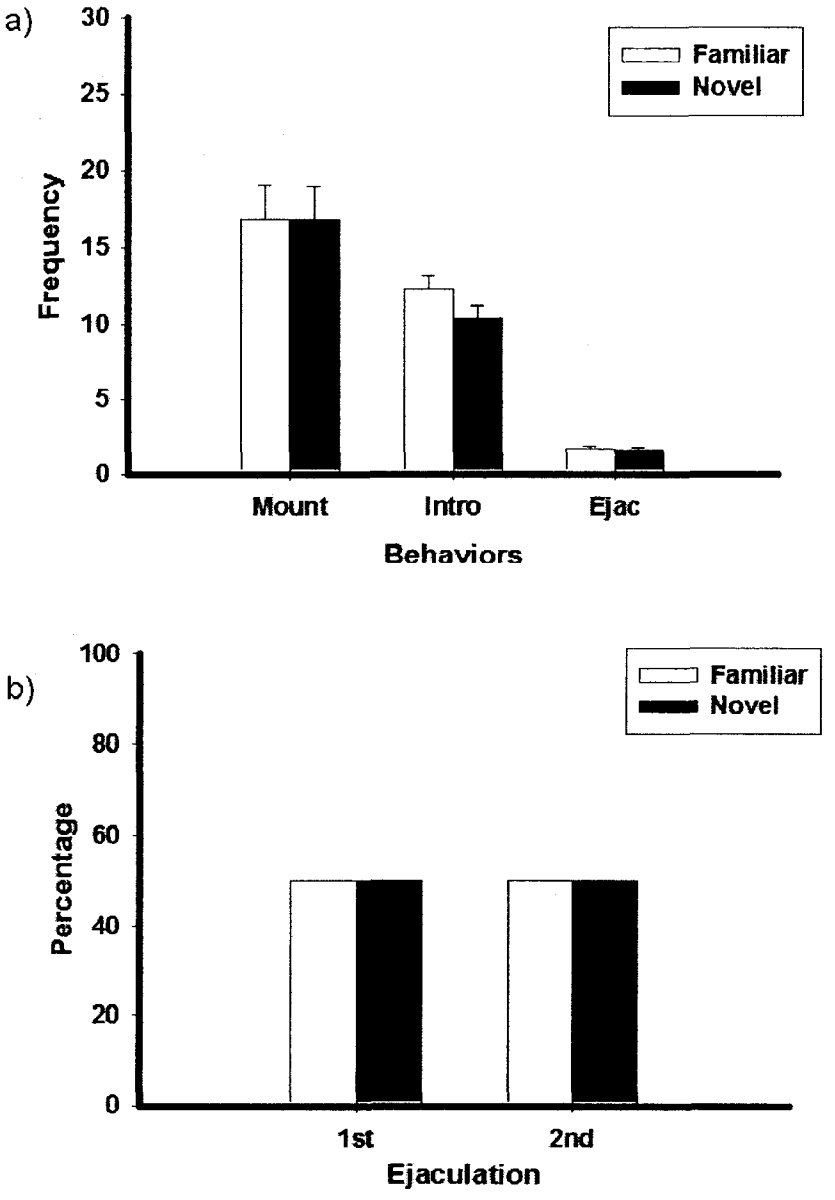

Figure 12: a) Frequencies of copulatory behaviors displayed by 1-hole males towards the familiar and novel unscented females. b) Distribution of first two ejaculations between familiar and novel unscented females in 1-hole males. Data are means ± SEMs.

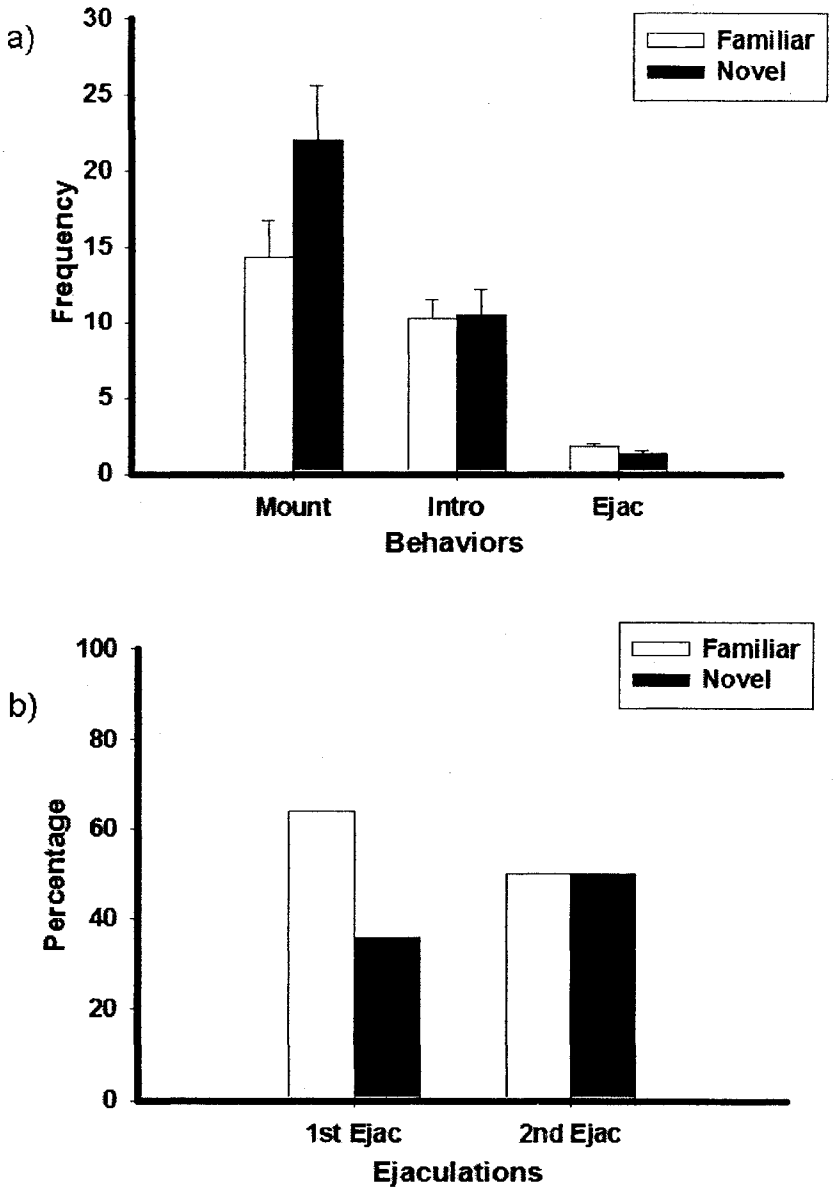

Figure 13: a) Frequencies of copulatory behaviors displayed by 4-hole males towards the familiar and novel unscented females. b) Distribution of first two ejaculations between familiar and novel unscented females in 4-hole males. Data are means ± SEMs.

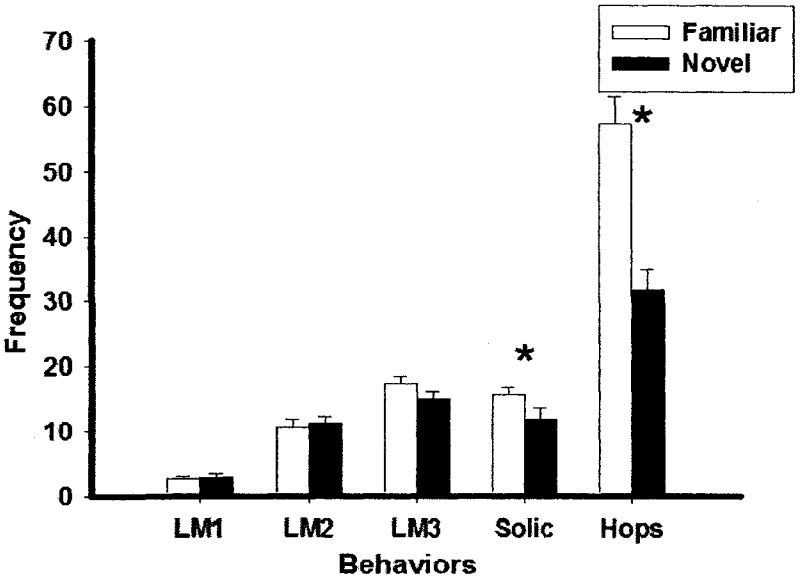

Figure 14: Frequency of sexual behaviors displayed by 1-hole familiar and novel unscented females. Data are means ± SEMs. * $p < 0.05$, familiar vs. novel.

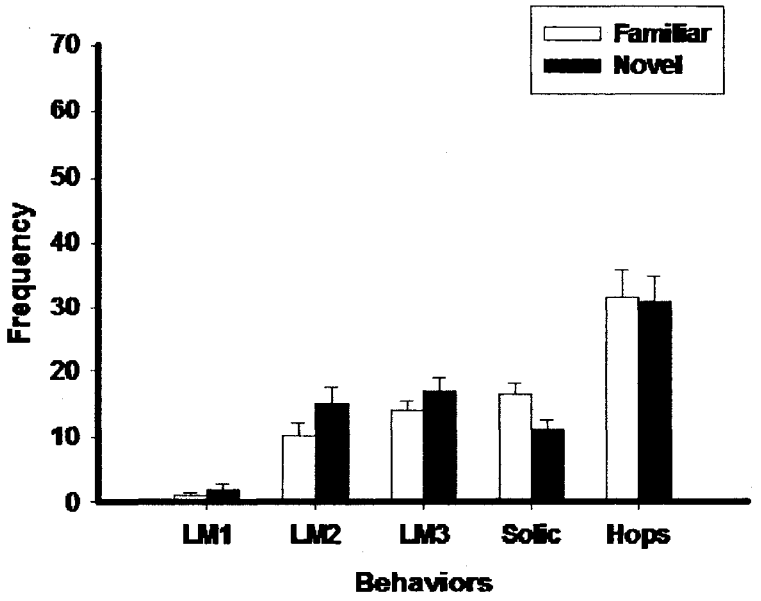

Figure 15: Frequency of sexual behaviors displayed by 4-hole familiar and novel unscented females. Data are means ± SEMs.

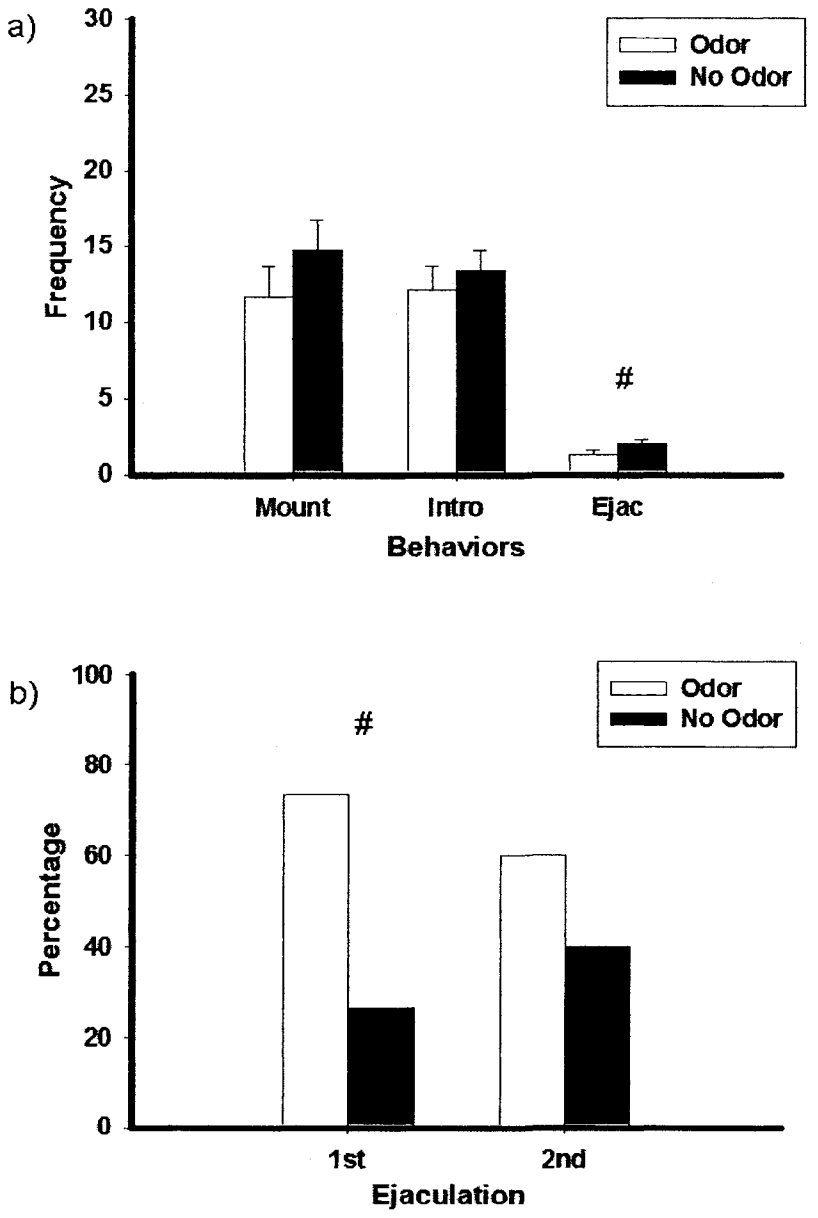

Figure 16: a) Frequencies of copulatory behaviors displayed by 1-hole males towards the novel scented and unscented females. b) Distribution of first two ejaculations between novel scented and unscented females in 1-hole males. Data are means ± SEMs. # $p < 0.1$, familiar vs. novel.

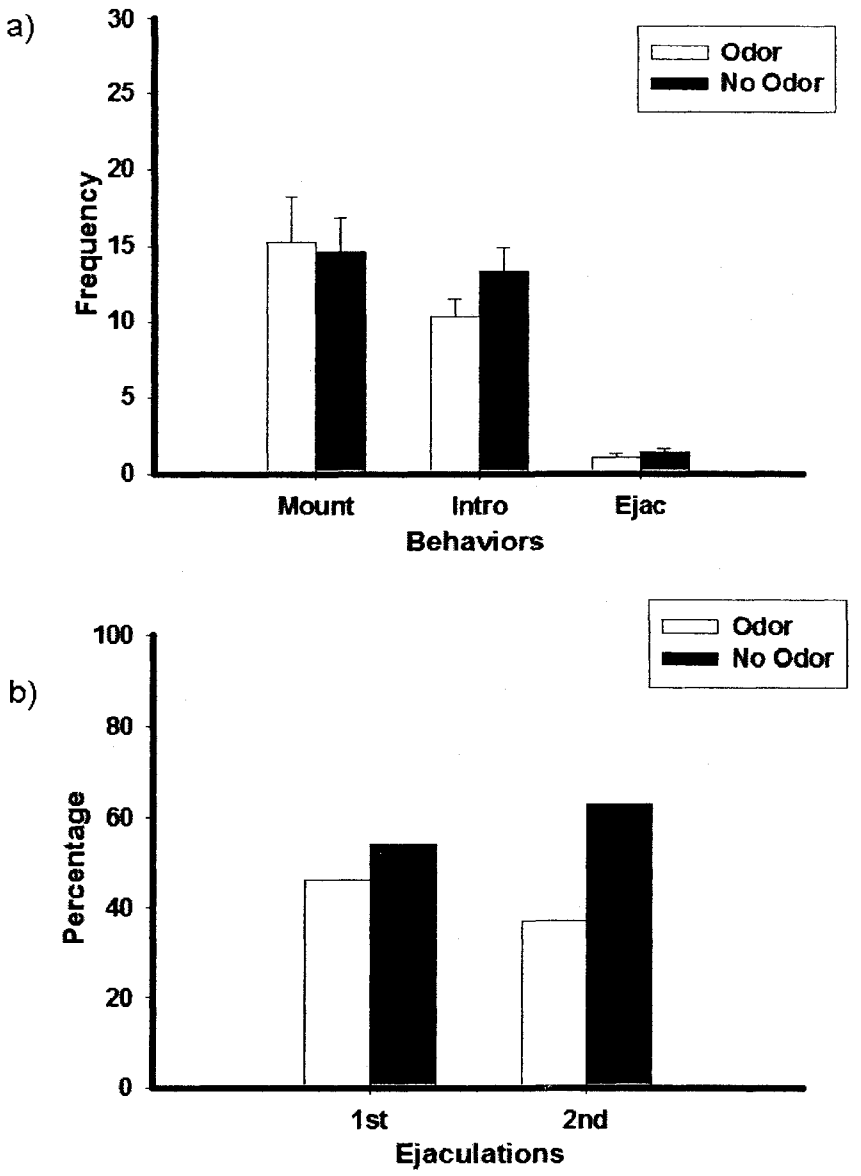

Figure 17: a) Frequencies of copulatory behaviors displayed by 4-hole males towards the novel scented and unscented females. b) Distribution of first two ejaculations between novel scented and unscented females in 4-hole males. Data are means ± SEMs.

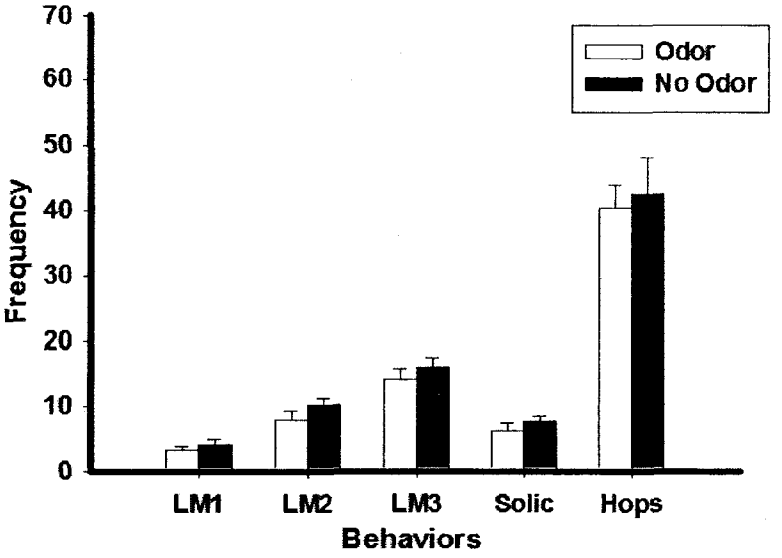

Figure 18: Frequency of sexual behaviors displayed by 1-hole novel scented and unscented females. Data are means ± SEMs.

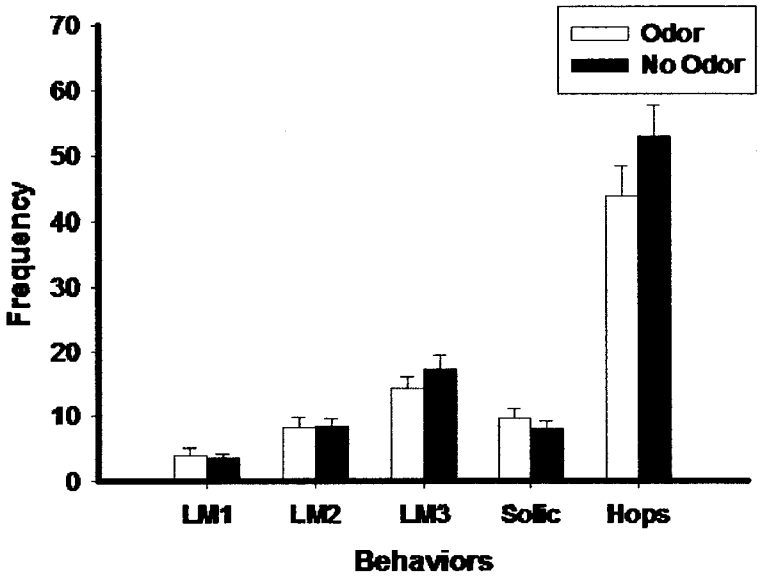

Figure 19: Frequency of sexual behaviors displayed by 4-hole novel scented and unscented females. Data are means ± SEMs.

4. Discussion

Males trained to copulate in 1-hole pacing chambers ejaculated significantly more with their familiar almond-scented female compared to the novel unscented female. They also tended to choose their familiar scented female for their first ejaculation. Thus, males trained to copulate in 1-hole pacing chambers developed a significant CEP for their familiar scented female. In contrast, no differences were found in ejaculation frequency or in the choice of the female for the first ejaculation in 4-hole males. Females trained in the 1-hole condition hopped more and solicited more than novel females or females in the 4-hole condition. Familiarity of an unscented female did not elicit a significant CEP, nor did a familiar odor placed onto a novel female. Thus, both components are necessary for males trained in a 1-hole pacing chamber to display CEP for their familiar scented female. However, these components failed to facilitate the development of CEP in the 4-hole pacing condition.

Opioid transmission during or following ejaculation is necessary for the association of cues to sexual reward and for the development of sexually conditioned place and partner preference (Kippin & Pfaus, 2001; Miller & Baum, 1987). In the present study, however, rats ejaculated in the 1-hole and 4-hole conditions, yet only rats in the 1-hole condition developed a significant CEP. This strongly indicates that ejaculation alone is not sufficient for male rats to develop a partner preference. Rather, contextual variables that comprise the availability or accessibility of the female may be important for the development of partner preference. We have shown previously that the female's return latencies are significantly shorter in the 4-hole relative to the 1-hole condition (Ismail, Zhao & Pfaus, 2008). This is likely due to the ease with which females

could cross the 4-hole versus 1-hole divider, given that males often obstruct the entry of the 1-hole divider with their heads. It is possible that the different contexts activate different sexual arousal mechanisms in males. We note that males ejaculated faster and with fewer intromissions in the 4-hole condition relative to the 1-hole condition. Males may thus experience more parasympathetic genital activation in the 1-hole condition and more sympathetic genital activation in the 4-hole condition. In the same study, we also found that males given copulatory experience in both 1-hole and 4-hole conditions displayed a CEP for females associated with the 4-hole condition relative to females associated with the 1-hole condition. At first glance, this appears inconsistent with the results of the present study in which males developed significant CEP in the 1-hole condition but not in the 4-hole condition. However, it is possible that males prefer to copulate in environments in which they have easier access to females and can thus copulate at their preferred temporal rate. This is consistent with the findings of Martinez and Paredes (Martinez & Paredes, 2001) showing that males develop a sexual CPP for their preferred rate of copulation (using a pacing chamber with no divider) relative to a rate that the females control (in the same pacing chamber bisected by a 1-hole divider). Taken together, these results suggest that when males are exposed to both 1-hole and 4-hole environments, they display preferences for the 4-hole environment and for females associated with that environment. However, when males are exposed only to the 1-hole or 4-hole pacing conditions, they develop CEP only in the 1-hole condition, and only if they are presented with their familiar almond-scented female relative to a novel unscented female.

The 1-hole familiar females solicited the males more than the novel females during the copulation preference test, suggesting that the CEP observed in 1-hole males in the first experiment might have been dependent on the number of female solicitations. However, 1-hole familiar females also solicited the males in Experiment 2 more than the novel females, but those males failed to display CEP for their familiar female, indicating that the display of CEP in males is independent of female solicitation. Those results are interesting in their own right, as they indicate that females recognized the males they copulated with during conditioning trials. Those females also displayed aggressive behaviors toward the novel females, and more so if the familiar female bore the conditioned odor (data not shown). Mate guarding is used as an index of partner preference or mate choice in monogamous species (Getz & Hofman, 1986; Ismail, Zhao & Pfaus, 2008). The fact that it can come under Pavlovian control in putatively polygamous female rats adds to a growing body of evidence that sexual strategies are flexible and based on experience with sexual reward, as we have shown elsewhere for appetitive measures like solicitations and the choice of male for ejaculation (Coria-Avila, Ouimet, Pacheco, Manzo & Pfaus, 2005).

In previous conditioning studies by Kippin and colleagues (Kippin & Pfaus, 2001; Kippin & Pfaus, 2001a; Kippin, Samaha, Sotiropoulous & Pfaus, 2001b) male rats developed significant CEP in bilevel chambers or in unilevel racetrack chambers. In both conditions, males chase females that solicit and run away, forcing the males to copulate at a rate imposed by the female. It appears that the wait and the anticipation of the females' return may increase arousal levels in males in the 1-hole condition and facilitate the development of CEP for females associated with those contextual variables. Lipp (2002)

showed that the arousal value of an anticipated event can modulate the development of conditioning; therefore, it is possible that higher levels of parasympathetic arousal may have facilitated the development of CEP. Kippin and Pfaus (2001) found that placing the conditioned odor on a nonreceptive female induced paired-trained, but not unpaired-trained, males to mount the female. Moreover, presenting a paired-trained male with an unscented female increased both the mount and intromission latency, and decreased the intromission ratio, suggesting that the odor carried conditional signals for parasympathetic arousal, as indicated by erection and intromission. It is thus possible that chasing a pacing female in bilevel chambers or unilevel racetrack chambers increases arousal in a manner similar to that observed in the 1-hole condition in the present study.

The data of the present experiments are strikingly similar to those found in the literature on voles. Prairie voles, found in sparsely populated environments, are monogamous and display copulatory preferences for their familiar female (Winslow, Shapiro, Carter & Insel, 1993). In contrast, meadow voles, found in more densely populated environments, fail to display such preferences (Insel, Young & Wang, 1997). These vole species display endogenous differences in several neurochemical markers, most notably the distribution of vasopressin and corticotrophin releasing factor receptors in the brain (Lim, Hammock & Young, 2004; Lim, Liu, Ryabinin, Bai, Wang & Young, 2007). However, over-expression of the vasopressin 1a receptor gene in the ventral pallidum of male Meadow voles induced by viral vector transfection dramatically increased partner preference for a familiar female. This indicates that mating strategies can be flexible depending on the hormonal and neurochemical milieu present, which

may, in turn, be altered by the social constraints imposed by female availability in different environments.

Although these results are consistent with reports on voles, they are contrary to the assumption of the Coolidge effect. According to the Coolidge effect, males have a natural tendency to prefer to copulate and ejaculate with a novel female rather than one with which they have already ejaculated (Beach & Jordan, 1956). Although male rats may have such tendency, the current data suggest that following the association of an odor cue with sexual reward, this natural tendency can be overridden. This flexibility in mating strategy appears to be based on early experience with sexual reward (Pfaus, Kippin & Centeno, 2001), which generates a preference for cues associated with the reward. A similar phenomenon occurs in humans during the early phase of sexual experience that enhances the development of a preference or "love map" for features and activities associated with sexual reward (Krafft-Ebing, 1929; Money, 1997).

In summary, copulation to ejaculation in a situation with restricted access to a female (1-hole pacing situation) potentiates the development of a CEP in males for a familiar scented female. In contrast, copulation to ejaculation in a situation with easy or unrestricted access to a female (4-hole pacing situation) does not result in the development of a CEP. Thus, although the reward state induced by ejaculation is necessary for CEP (Kippin & Pfaus, 2001), it is not sufficient. The present results suggest that the level of arousal that underlies the reward state is a critical factor that determines whether the sexual reward state leads to the development of a CEP. This arousal state depends on the context in which copulation occurs, and is sensitive to

factors such as the availability or accessibility to the female, her level of familiarity to the male, and the presence of cues that signal sexual reward.

Acknowledgements

This rsearch was supported by a grant from the Canadian Institutes of Health Research (MOP-74563) to JGP. The authors would like to thank Drs. Michael Baum, Genaro Coria-Avila, Tod Kippin, and Larry Young, for useful discussions.

CHAPTER THREE

The results reported in Chapter 1 suggest that males prefer to copulate in environments that provide them with easy access to females perhaps because they can achieve their optimal rates of copulation. However, the results in Chapter 2 showed that only males trained to copulated in 1-hole pacing chambers, which is associated with restricted access to the female partner, develop CEP for their familiar almond-scented female. Males trained in 4-hole pacing chambers failed to develop such preferences. The next experiment assessed the preference in males for females that are more or less accessible during copulation while keeping the environment of copulation constant.

Sexual responses and partner preferences of male rats paired with haloperidol-treated female rats.

Nafissa Ismail, Clemence Laroche, Fabienne Girard-Bériault, Shann Ménard, Jonathan A. Greggain, James G. Pfaus*

Center for Studies in Behavioral Neurobiology
Department of Psychology, Concordia University
Montréal, QC H4B 1R6 Canada

Running Head: Copulation and partner preference with haloperidol-treated females

*Corresponding author: Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, 7141 Sherbrooke W., Montréal, QC H4B 1R6 Canada.

E-mail: Jim.Pfaus@concordia.ca.

For submission to *Physiology & Behavior.*

Abstract

We have recently shown that only males trained to copulate with the same almond-scented female, in environments that restrict access to the female during copulation, develop a conditioned ejaculation preference (CEP) for their familiar female over a novel one. These findings suggest that copulation in environments in which the female spends more time away from the male and in which the male anticipates female copulatory contact facilitates the development of CEP. However, conditioned place and partner preference studies have shown that males prefer easy over obstructed access to the female partners during copulation. The objective of this experiment was to attempt to understand the discrepancy between the findings by examining the importance of female proceptive behavior and genital stimulation in the development of partner preference while keeping the environment constant. Experiment 1 investigated the effect of administering a range of doses of haloperidol, a dopamine D2-receptor antagonist, to females, on female and male sexual behavior. Results showed that haloperidol treatment significantly reduced female proceptive behaviors in a dose-dependent manner. Experiment 2 examined the importance of female proceptive behaviors in the development of partner preference by inhibiting these behaviors with a haloperidol treatment. Following alternating copulatory trials with haloperidol- or saline-treated females, males were subjected to a partner preference test. Males displayed a preference for copulating with haloperidol-treated females, suggesting that males do not require female proceptive behaviors for the development of partner preference and that penile stimulation is sufficient for the development of partner preference in males.

Keywords: Conditioning, Pacing, Sexual behavior, Sexual reward, Dopamine antagonist

1. Introduction

Male rats develop preferences for environments that predict an optimal rate of sexual stimulation and sexual reward. Using conditioned place preference, Martinez and Paredes (2001) demonstrated that male rats prefer to spend time in environments associated with unobstructed access to the female as it allowed the males to achieve their preferred rate of copulation. Males were given sequential copulatory experience in a pacing chamber with either a 1-hole divider or no divider. Following each copulatory trial, males were placed into one of two distinctive sides of a conditioned place preference (CPP) apparatus. On the final test, males were placed into the CPP apparatus and allowed to move freely between the two sides. Males spent more time on the side associated with copulation without the divider, suggesting that unobstructed access to the female was more rewarding than obstructed access with the divider.

We recently showed that male rats develop a conditioned ejaculatory preference (CEP) for females bearing an olfactory cue associated with unrestricted access during copulatory training (Ismail, Zhao and Pfaus, 2008). Males were given 10 alternating copulatory trials at 4-day intervals in pacing chambers divided by a 1-hole or 4-hole partition. The partition created conditions in which males had relatively restricted (1-hole) or unrestricted (4-hole) access to females. Females scented with almond extract were placed in one condition and unscented females were presented in the other condition and scenting was counterbalanced between the conditions. Following conditioning trials, males underwent a partner preference test in an open field where they had unrestricted access to two females, one scented and the other unscented, which had been associated with copulation in each type of pacing chamber. Regardless of odor, the males displayed

a significant CEP for females associated with copulation in 4-hole pacing chambers. Those findings were in agreement with Martinez and Paredes (2001) conclusion that male rats prefer conditions in which an optimal rate of copulatory stimulation can occur.

Conversely, when males are given copulatory trials with almond-scented females in either a 1-hole or a 4-hole pacing chamber, only those exposed to the female in the 1-hole chamber develop CEP for the almond-scented female (Ismail, Gelez, Lachapelle, and Pfaus, 2008). Those findings suggest that when males are exposed to both 1-hole and 4-hole conditions, they prefer the 4-hole condition perhaps because they recognize the difference between restricted and unrestricted access. When males are given exclusive access to females in either 1- or 4-hole conditions, they require a greater level of arousal or reward to induce significant CEP. In our previous studies of CEP (Kippin et al., 1998; Kippin & Pfaus, 2001a.b) males were given access to scented or unscented females in bilevel chambers. Such chambers allow females to pace the copulatory contact by running from level to level, forcing the males to chase them. The development of significant CEP in those chambers may occur because males must chase the females, increasing their level of arousal. Together, the findings of those studies suggest that female sexual behavior, along with female availability and accessibility, play an important role in the regulation of sexual arousal, copulatory responses, and partner preferences of male rats.

Indeed, Madlafousek, Hliňák, and Beran (1976) reported that castrated males can maintain copulatory responding longer after castration if they receive unobstructed access to ovariectomized (OVX) females treated fully with estrogen (E) and progesterone (P) so that they display high rates of solicitation and lordosis. Castrated males that receive

either obstructed access to primed females, or that receive access to females treated with E alone (and that do not display appetitive behaviors) show a faster decline in copulatory responding. Males given subthreshold 6-hydroxydopamine lesions to the nucleus accumbens will copulate normally with saline-treated females primed fully with E and P, but less with females primed fully but injected with the non-selective dopamine (DA) antagonist flupenthixol (Everitt & Stacey, 1987). Flupenthixol treatment disrupts appetitive solicitations and hops and darts in female rats, but augments the lordosis reflex intensity and duration that females hold the posture. This suggests that sexually hypo-arousable males, like castrated males, may require female appetitive behaviors to experience adequate levels of arousal during copulation.

The present study asked whether the development of CEP depends upon female precopulatory behavior or an optimal rate of penile stimulation. To investigate this we inhibited appetitive precopulatory behaviors in females by giving them systemic injections of the D2 DA receptor antagonist haloperidol. The first experiment examined the effect of various doses of haloperidol treatement to females on female and male sexual behavior in bi-level chambers. Since the greatest effect on sexual behavior was observed with the highest dose of haloperidol, in the second experiment, males were given alternating copulatory trials with females injected with the high dose of haloperidol and females injected with saline. Then, males underwent a partner preference test to examine their preference for either female.

2. Materials and methods

All animal procedures conformed to the guidelines of the Canadian Council on Animal Care, and were approved by the Concordia University Animal Research Ethics Committee.

2.1. Dose-response effects of haloperidol on female sexual behavior

2.1.1. Subjects and surgery

Males. 30 sexually naïve male Long Evans rats, weighing approximately 300 g at the beginning of the experiment, were purchased from Charles River Laboratories (St-Constant, Quebec). Rats were housed in groups of four, in plastic solid floor cages, with free access to food (Purina Rat Chow) and water, and maintained on a 12-hour dark/light cycle (lights on at 8 pm) in a temperature-controlled room (21 ± 2°C). Behavioral testing was conducted in the dark phase of the cycle.

Females. 30 Long Evans female rats, weighing approximately 200 g at the start of the experiment, were purchased from the same supplier and were housed under the same conditions as the males. Females were anaesthetized with a mixture of ketamine (50 mg/ml) and xylazine hydrochloride (4 mg/ml), at a ratio of 4:3, respectively, administered intraperitoneally in a volume of 1 ml/kg of body weight. Anaesthetized females were then bilaterally ovariectomized via a lumbar incision. All females were given one week of postsurgical recovery prior to sexual training. Following recovery, all females were placed in a pacing chamber with intact and sexually vigorous males during four sexual training trials. For all behavioral tests, sexual receptivity was induced by priming the females with an injection of 10 μg (sc in 0.1 ml of sesame oil) of estradiol benzoate (EB) 48 hrs and 500 μg (sc in 0.1 ml of sesame oil) of progesterone (P) 4 hrs

prior to behavioral testing. One hour prior to conditioning, females were injected subcutaneously with saline, 0.1mg/kg haloperidol, or 0.2mg/kg haloperidol.

2.1.2. Apparatus

Copulatory conditioning trials took place in rectangular chambers (46 cm x 39 cm x 37 cm) covered with a grid floor and bedding. All conditioning sessions were recorded on a DVD and subsequently scored using a PC-based program (Cabilio, 1996).

2.1.3. Procedure

The females were primed with EB and P as described above. The 30 females were randomly assigned to receive saline, a low dose of haloperidol (0.1mg/kg), or a high dose of haloperidol (0.2mg/kg) one hour before the test. At the time of testing, males were placed individually into the bi-level chambers and the females were introduced for 20 min.

2.1.4. Behavioral measures and statistical analyses

Frequency data were recorded for all mounts, intromissions, and ejaculations during the copulatory preference test. Criteria for sexual behaviors were those described by Sachs and Barfield (1970). Female solicitations (defined as headwise orientation to the male followed by a runaway, forcing the male to chase the female), hops and darts, lordosis magnitude (on a scale from 1 to 3, with 1 representing low magnitude, 2 representing moderate magnitude, and 3 representing high magnitude, as in Hardy & DeBold, 1972). One-way analyses of variance (ANOVAs) were used to determine differences in the frequencies and latencies of copulatory behaviors among the two conditioning groups. The criterion for statistical reliability for all comparisons was set to $p < 0.05$.

2.2. Development of CEP for haloperidol-treated females.

2.2.1. Subjects and surgery

Males. 40 sexually naïve male Long Evans rats, weighing approximately 300 g at the start of the experiment, were purchased from the same supplier and were housed under the same conditions as in Experiment 1. Behavioral testing was conducted in the dark phase of the cycle.

Females. 40 Long Evans female rats, weighing approximately 200 g at the start of the experiment, were also purchased from the same supplier and housed under the same conditions as in Experiment 1. Females were ovariectomized the same way as in Experiment 1. For all behavioral tests, sexual receptivity was induced by priming the females with an injection of 10 μg (sc in 0.1 ml of sesame oil) of EB 48 hrs and 500 μg (sc in 0.1 ml of sesame oil) of P 4 hrs prior to behavioral testing. On alternate trials, females were subcutaneously injected with haloperidol (0.2 mg/kg) one hour before conditioning.

2.2.2. Apparatus

Copulatory conditioning trials took place in rectangular chambers (46 cm x 39 cm x 37 cm) covered with a grid floor and bedding. All conditioning sessions were recorded on a DVD and subsequently scored using a PC-based program (Cabilio, 1996).

2.2.3. Procedure

Conditioning phase. The 40 naïve males were randomly assigned to one of three groups. One group was given ten copulatory trials with an almond-scented haloperidol injected female and with an unscented saline injected female on alternate trials. The other group was the counterbalance of the first group. This group was given ten copulatory

trials with an unscented haloperidol-injected female and with an almond-scented saline injected female on alternate trials. For all conditioning trials, males were placed individually into the chamber for 5 min, after which either an almond-scented or unscented, haloperidol or saline injected female was placed into the chamber for 20 min. Females were primed with EB and P as described above. Conditioning trials occurred at 4-day intervals during the middle third of the rat's dark cycle.

Copulatory Preference Test. Four days following the last conditioning trial, males were placed in an open field and allowed to habituate for 5 min. At the end of this period, an almond-scented female and an unscented female were placed simultaneously into two diagonal corners of the open field at approximately equal distance from the male. Both females were drug free at this stage. All animals were able to interact freely with one another for 30 min.

2.2.4. Behavioral measures and statistical analyses

Latency and frequency data were recorded for all mounts, intromissions, and ejaculations during the copulatory preference test. Criteria for sexual behaviors were those described by Sachs and Barfield (1970). Female solicitations (defined as a headwise orientation to the male followed by a runaway, forcing the male to chase the female), hops and darts, lordosis magnitude (on a scale from 1 to 3, with 1 representing low magnitude, 2 representing moderate magnitude, and 3 representing high magnitude, as in Hardy & DeBold, 1972), and the time spent away from the male were also recorded. One-way analyses of variance (ANOVAs) were used to determine differences in the frequencies and latencies of copulatory behaviors among the two conditioning groups. The criterion for statistical reliability for all comparisons was set to $p < 0.05$.

3. Results

3.1. Dose-response effects of haloperidol on female sexual behavior

Figure 20 illustrates the frequency and latency of female sexual behaviors in females treated with saline, a low dose of haloperidol or a high dose of haloperidol. One-way ANOVAs revealed a significant difference in solicitation between the three groups ($F(2,27) = 3.74$, $p < 0.05$). Post-hoc Tukey pairwise comparisons revealed that the high dose induced a significant decrease in solicitation rate when compared to the low dose (Mean difference = -7.17, $p < 0.05$) and compared to saline (Mean difference = -13.8, $p < 0.05$) (Figure 20a). One-way ANOVAs also revealed a significant difference in lordosis duration between the three groups ($F(2, 27) = 12.74$, $p < 0.05$). Tukey pairwise comparisons showed that the high dose of haloperidol increased lordosis durations significantly compared to the low dose (Mean difference = 10.39, $p < 0.05$) and saline (Mean difference = 13.46, $p < 0.05$). No difference was found in hops and darts, defensive/rejection responses and overall lordosis magnitude (data not shown here).

Figure 21 illustrates the frequency of ejaculation. One-way ANOVAs showed a significant difference in ejaculation frequency between the three groups ($F(2,27) = 10.84$, $p < 0.05$). Post-hoc analyses revealed that males paired with females that received the highest dose of haloperidol ejaculated significantly less than males paired with females that received the lowest dose of haloperidol (Mean difference = 1.7, $p < 0.05$) or saline (Mean difference = 1.4, $p < 0.05$). No difference was found in intromission and mount frequencies (data not shown here).

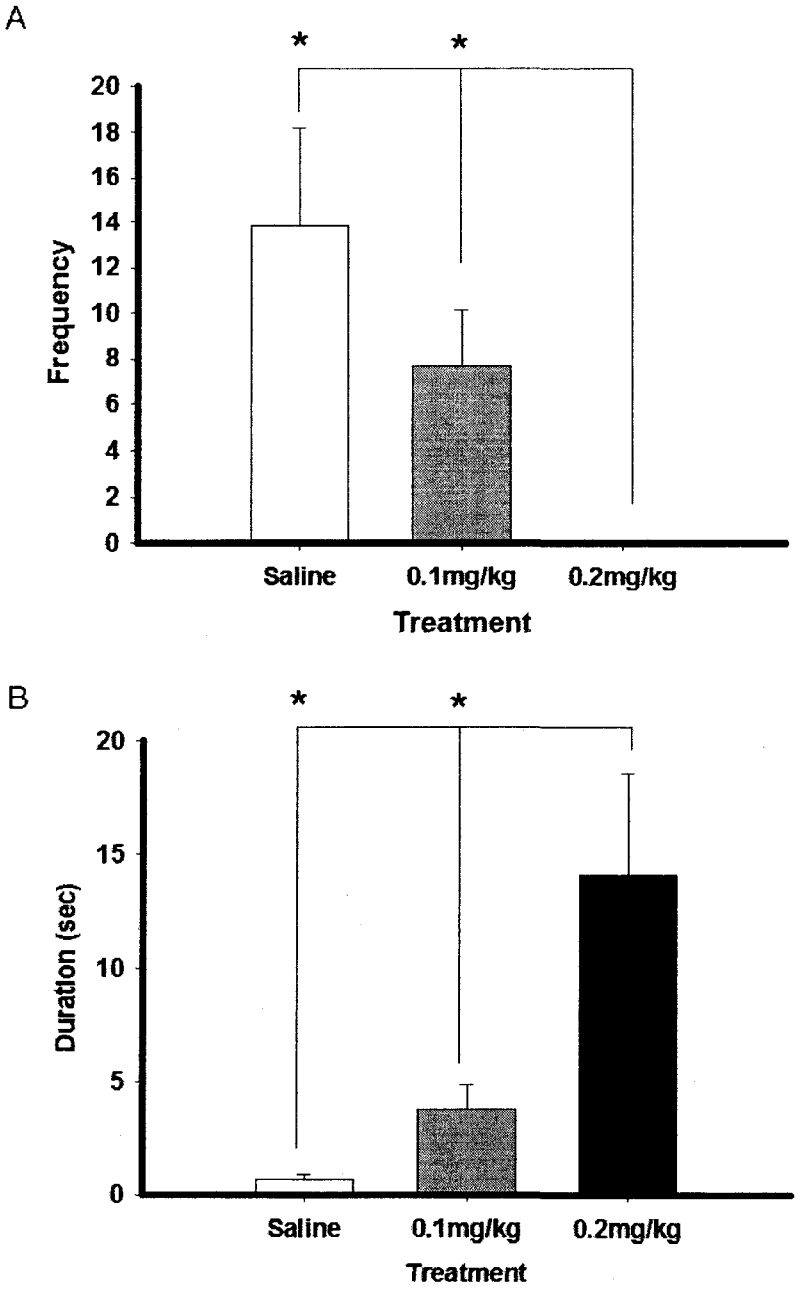

Figure 20: a) Average frequencies of solicitation and b) durations of the lordosis posture treated with saline or a low dose of haloperidol (0.1 mg/kg) or a high dose of haloperidol (0.2 mg/kg). * $p < 0.05$.

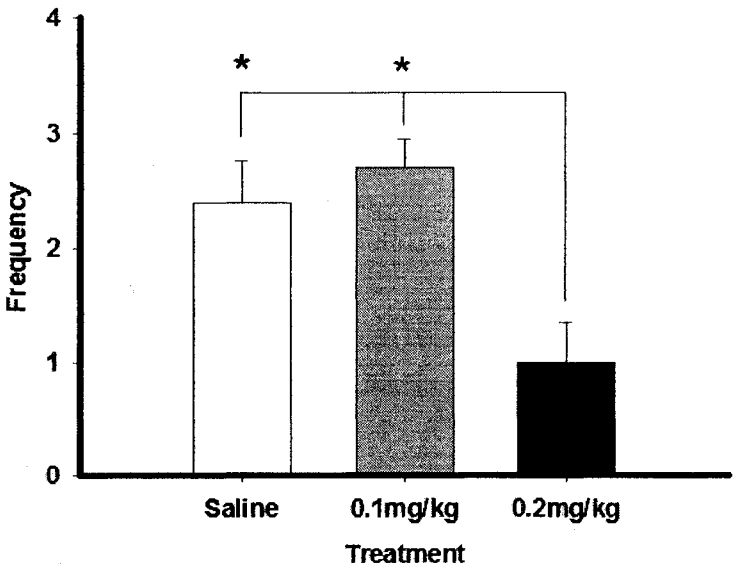

Figure 21: Average frequencies of ejaculation in males paired to copulate with females treated with saline or a low dose (0.1 mg/kg) or a high dose of haloperidol (0.2 mg/kg). * $p < 0.05$.

3.2. Development of CEP for haloperidol-treated females.

Figure 22 illustrates the frequency of male sexual behaviors with females associated with haloperidol or saline injected females. No differences in mounts, intromissions or anogenital investigations were observed (Table 1). However, males ejaculated significantly more times with scented females associated previously with haloperidol- injected females ($F(1, 66) = 4.48, p < 0.05$) (Figure 22). No differences were found in the latency to mount, intromit or ejaculate with either haloperidol or saline associated females. Moreover, there were no differences in the choice of female for the first ejaculation (Table 1).

Differences in female behaviors were also observed. For instance, females associated to haloperidol injection display significantly more lordosis magnitude 3 than females associated to saline injection ($F(1, 66) = 5.02, p < 0.05$) (Figure 23a). Moreover, females injected with haloperidol displayed significantly longer lordosis duration than saline treated females ($F(1, 25) = 199, p < 0.05$) (Figure 23b).

Table 1. Average frequencies and latencies of male sexual behavior

Behaviors	Saline	Haloperidol
Frequency		
Mount	14.47±1.84	15.91±2.21
Intromissions	9.44±1.03	12.38±1.19
Anogenital invest.	7.50±0.88	8.74±1.14
Latency		
Mount	45.23±7.25	51.20±11.84
Intromission	91.27±35.03	57.27±12.99
Ejaculation	688.53±74.99	611.99±92.72

Table 1: Average frequencies and latencies of male sexual behaviour towards females associated to copulation with saline-treated or haloperidol-treated females. The data are expressed as means±SEM.

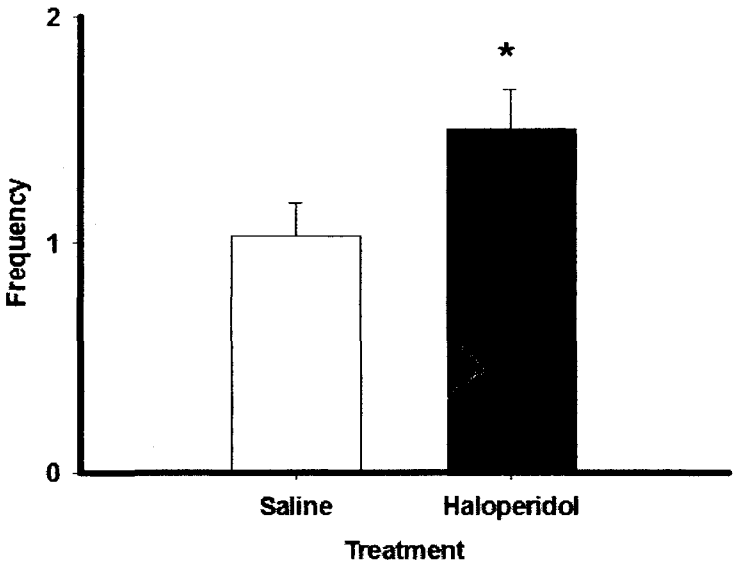

Figure 22: Average frequencies of ejaculation with females associated with haloperidol or saline treated females during the copulatory preference test. * $p < 0.05$.

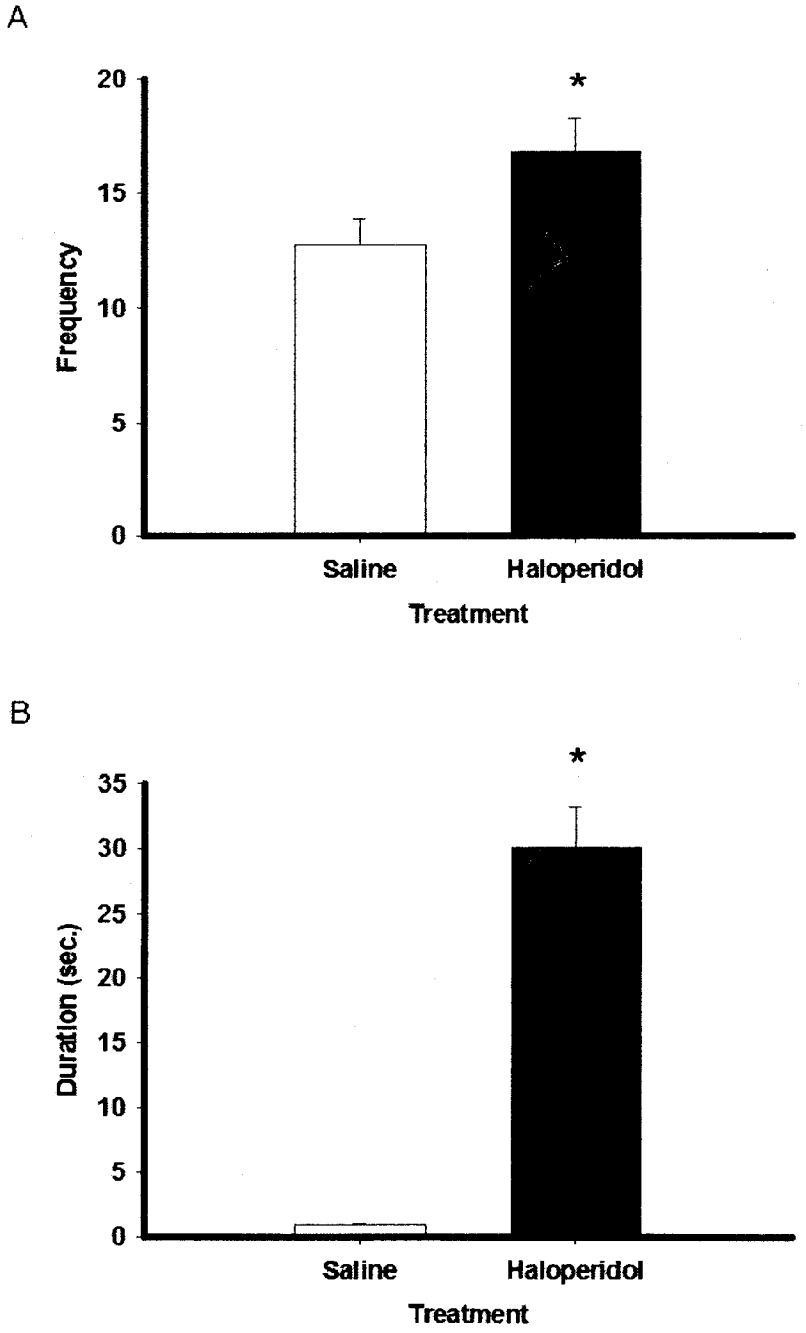

Figure 23: a) Average frequencies of lordosis magnitude 3 and durations of the lordosis posture in females treated with saline and with haloperidol. * $p < 0.05$.

4. Discussion

The present study shows that treating females with haloperidol affected both female and male sexual behavior in a dose-dependent manner. Females injected with the high dose of haloperidol (0.2 mg/kg) displayed significantly fewer solicitations relative to saline-treated females, and significantly longer lordosis durations. Males that copulated with those females displayed significantly fewer ejaculations, perhaps due to lower sexual arousal because of a lack of appetitive sexual behaviors displayed by the females. However, somewhat paradoxically, on the final partner preference test, males displayed a significant CEP for females bearing the odor previously paired with copulation with haloperidol-treated females. This indicates that males prefer to copulate with females that are more accessible during their initial copulatory experiences. Those results are consistent with our previous findings (Ismail, Zhao and Pfaus, 2008) and with those of Martinez and Paredes (2001) in that males prefer to copulate in settings that provide them with easy access to females perhaps because they can better control the rate of copulation and achieve an optimal amount of stimulation.

Although the present findings suggest that the rate of copulatory stimulation is related to the degree of sexual reward experienced by male rats, the males in the present study were able to differentiate between haloperidol-treated and untreated females. This is reminiscent of the method used in our previous study (Chapter 1) in which male rats were able to differentiate between pacing conditions of unrestricted versus restricted access to females (Ismail, Zhao, & Pfaus, 2008). In that study, males displayed a CEP for females bearing an odor that predicted unrestricted access. It is important to note that the present findings do not seem in agreement with those of Madlafousek, Hliňák, and

Beran (1976) or with Everitt and Stacey (1987). In those experiments, hyporeactive males (made so by castration or subthreshold mesolimbic dopamine depletion) maintained copulation only if females displayed appetitive behaviors in addition to lordosis. It may be that basal levels of dopamine within mesolimbic or other terminal regions have a critical threshold above which the appetitive behavior of the female becomes less critical in the stimulation of copulatory behaviors. This is reminiscent of the difference between sexually naïve versus experienced males. Treatments that disrupt copulation in sexually naïve males, such as olfactory ablation, novelty stress, castration, or penile desensitization, are far less effective in sexually experienced males (Pfaus & Wilkins, 1995). Indeed, dopamine release in mesolimbic terminals sensitizes with sexual experience (Mitchell & Gratton, 1991), and may well play a role in the "automatization" of sexual responding.

Mehrara and Baum (1990) and Kippin and Pfaus (2001) showed that ejaculation is necessary for the induction of CPP and CEP, respectively. However, in our present study, males copulated to ejaculation with both saline-treated and haloperidol-treated females, but displayed CEP for the olfactory cue associated with the reward state induced by copulating with haloperidol-treated females. This suggests that the arousal level induced by an optimal rate of copulatory stimulation prior to ejaculation also plays an important role in the development of conditioning. Thus, we argue that ejaculation is necessary but not sufficient for the induction of conditioning. Contextual factors that contribute to sexual arousal, including the availability of the female and the copulatory rate, appear to sum with ejaculation to induce the reward state. This is similar to the results of a previous study that examined the inhibition of infantacide in male rats by

prior sexual experience (Pfaus, Kippin, & Centeno, 2001). In that study, males that ejaculated with a female were significantly less likely to display infanticidal behaviors toward a rat pup placed in their home cages 21 days later, relative to males that copulated to 5 intromissions but did not ejaculate. However, when the ejaculation group was further subdivided by median split into males that achieved ejaculation following more than 9 intromissions versus those that achieved it with fewer than 9 intromissions, a striking result emerged: None of the males that displayed more than 9 intromissions prior to ejaculation displayed infanticidal behaviors whereas no significant differences existed between males that achieved ejaculation with fewer than 9 intromissions compared to those that experienced only 5 intromissions. Thus, the rate of copulation, the degree of penile stimulation, and perhaps the experience of sexual arousal appear to augment the level of sexual reward experienced by male rats.

In summary, the findings of the present study showed that the highest dose of haloperidol administered to females resulted in the greatest change in male and female sexual behavior. The findings of this current study also demonstrated that males prefer to copulate with haloperidol-treated over saline-treated females suggesting that males prefer to copulate under conditions that provide them with easier access to females. These results emphasize the importance of the context of copulation, which consists of the arousal level and the availability and accessibility of the female, on the development of CEP in rats.

Acknowledgements

This research was supported by a grant from the Canadian Institutes of Health Research (MOP-74563) to JGP.

CHAPTER FOUR

Chapter 2 demonstrated that males trained to copulate in 1-hole pacing chambers with the same almond-scented female at every trial develop a conditioned ejaculatory preference for their familiar female as opposed to a novel one. The study of Chapter 4 examined whether the development of CEP could be modulated by the strain of the female partner, as can be demonstrated in female rats for the strain of the male (Coria-Avila et al., 2006).

The effect of paced copulation on the development of partner preference for strain of female in the male rat.

Nafissa Ismail, Sherri Lee Jones, M. Dean Graham, Sarah Sylvester, James G. Pfaus*

Center for Studies in Behavioral Neurobiology
Department of Psychology, Concordia University
Montréal, QC, H4B 1R6 Canada

Running Head: Strain conditioning and partner preference

*Corresponding author: Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, 7141 Sherbrooke W., Montréal, QC H4B 1R6 Canada.

E-mail: Jim.Pfaus@concordia.ca.

For submission to *Physiology & Behavior.*

Abstract

Although male rats are reported to show greater sexual arousal and mating preference for a novel female compared to a familiar one, we have shown that after repeated copulation to ejaculation with a female bearing a neutral odor in pacing chambers bisected by a 1-hole divider, male rats display a conditioned ejaculatory preference for a female bearing the odor relative to a female not bearing the odor. The aim of the present study was to examine whether the strain of the female plays a role in the development of conditioned ejaculatory preference in male rats after repeated copulation with the same female in a pacing chamber bisected by either a 1-hole or 4-hole divider. In this experiment, male Long-Evans rats were given 10 copulatory trials with the same Long Evans or Wistar female in either the 1-hole or 4-hole condition. Copulatory preferences were then examined in an open field where males had the choice to copulate with either the familiar female or a novel one of a different strain from the familiar female. Results indicated that Long-Evans males trained in the 1-hole condition with the same Long Evans female displayed a conditioned ejaculatory preference for the familiar vs. novel female. However, males trained in the 1-hole condition with the same Wistar female at every trial copulated indiscriminately with the familiar and novel females. No preference was detected in males trained in the 4-hole condition. These findings suggest that, following training in a 1-hole pacing chamber, males displayed an ejaculatory preference only if the familiar female is of their own strain.

Keywords: assortative mating, ejaculatory preference, paced copulation

1. Introduction

Male rats develop a conditioned ejaculatory preference for females bearing a neutral odor (e.g., almond) paired with the sexual reward state induced by ejaculation (Kippin et al., 1998; Kippin & Pfaus, 2001). Similar findings have been reported in female rats. A study by Coria-Avila and colleagues (2005) showed that females can develop conditioned partner preference for males bearing an almond odor associated with copulation in pacing chambers. Pacing chambers are uni-level chambers bisected with a divider and are often used to study female sexual behavior and preference because they provide females with control over the rate of copulation (Erskine, Kornberg & Cherry, 1989; Paredes & Vazquez, 1999; Coria-Avila, Ouimet, Pacheco, Manzo & Pfaus, 2005). Together, these findings suggest that simple Pavlovian procedures can direct the mate choice of male and female rats. Although compelling from a learning perspective, one can ask from a more ethological question how relevant are those data given that rats are not presented with different neutral odors on partners in the wild.

Erskine (2005) has argued that the conditioned partner preference paradigm provides us with important tools to examine sexual motivation and mate choice in laboratory settings but it is unclear whether this information applies to rats in their natural environment. Rats are known to be polygamous (Calhoun, 1962). In the wild, males have been reported to chase estrous females as a pack and compete to mate with the female (Robitaille & Bouvet, 1976). Similarly, in semi-natural settings, females have been found to shift partners throughout the mating sequence, although some females take ejaculations exclusively from a single male (McClintock, 1985; McClintock, Anisko & Adler, 1982).

The mating strategies used by males and females during group mating thus seem highly variable, and may therefore be highly modifiable by experience.

Different strains of rats (such as albino and pigmented) have been used to study sexual competition and females seem to interact more with partners of their own strain relative to those of different strains (Austin & Dewsbury, 1986; Coria-Avila, Pfaus, Hernandez, Manzo & Pacheco, 2004). These findings suggest that females are able to identify individuals of their own strain. Although the exact cues the females attend to have not been identified (e.g., olfactory, visual, and/or auditory), these cues may ultimately influence their partner choice. Coria-Avila and colleagues (2006) examined whether the development of partner preference in females for males associated with paced copulation was dependent on the strain of the male. Indeed, they showed that the strain of the partner plays an important role in the development of partner preference. Long Evans and Wistar females experienced either paced copulation with a Long Evans male and non-paced copulation with a Wistar male or paced copulation with a Wistar male and non-paced copulation with a Long Evans male. Females solicited males associated with paced copulation selectively, regardless of strain. However, females chose to receive their first ejaculation from males of their own strain, and to receive more ejaculations from those males if they were paired with paced copulation. This suggests that females have a natural preference for males of the same strain, although appetitive aspects of sexual behavior can be modulated away from this preference if the other strain is paired with sexual reward.

Do male rats develop a preference for the strain of female paired with the reward state induced by ejaculation? The present study addressed this question as in Coria-Avila

and colleagues (2006) using the strain of the partner as the conditioned cue. Based on the results reported by Coria-Avila and colleagues (2006), we hypothesize that Long Evans males given copulatory trials with Long Evans females will be more likely to develop partner preference than Long Evans males given copulatory trials with Wistar females.

2. Methods

All animal procedures conformed to the guidelines of the Canadian Council on Animal Care, and were approved by the Concordia University Animal Research Ethics Committee.

2.1. Subjects and surgery

58 sexually naïve male Long Evans rats, weighing approximately 300 g at the start of the experiment, were purchased from Charles River Laboratories (St-Constant, Quebec). Rats were housed in groups of four, in plastic solid floor cages, with *ad lib* access to food (Purina Rat Chow) and water, and maintained on a 12-hour dark/light cycle (lights on at 8 pm) in a temperature-controlled room (21 ± 2°C). Behavioral testing was conducted in the dark phase of the cycle.

29 Long Evans and 29 Wistar female rats, weighing approximately 200 g at the start of the experiment, were purchased from the same supplier and were housed under the same conditions as the males. After a week of habituation, females were anaesthetized with a mixture of ketamine (50 mg/ml) and xylazine hydrochloride (4 mg/ml), at a ratio of 4:3, respectively, administered intraperitoneally in a volume of 1 ml/kg of body weight. Anaesthetized females were then bilaterally ovariectomized via a lumbar incision. All females were given one week of postsurgical recovery prior to

sexual training. Following recovery, all females were placed in a pacing chamber with intact and sexually vigorous males during four sexual training trials. For all behavioral tests, sexual receptivity was induced by priming the females with an injection of 10 μg (sc in 0.1 ml of sesame oil) of estradiol benzoate (EB) 48 hrs and 500 μg (sc in 0.1 ml of sesame oil) of progesterone (P) 4 hrs prior to behavioral testing.

2.2. Apparatus

Copulatory conditioning trials took place in rectangular pacing chambers (46 cm x 39 cm x 37 cm) covered with a grid floor and bedding and bisected with a clear Plexiglas divider with either one hole or four holes cut into the bottom of the divider. These holes were small enough to only allow the female to cross and not the male. We have previously shown that copulation in these chambers induces CEP (Ismail, Gelez, Lachapelle & Pfaus, 2008). All conditioning sessions were recorded on a DVD and subsequently scored using a PC-based program (Cabilio, 1996).

2.3. Procedure

2.3.1. Conditioning phase. The 58 naïve Long Evans males were randomly assigned to one of two groups. One group was given ten copulatory trials in 1-hole pacing chambers and the other group was given ten copulatory trials in 4-hole pacing chambers at every trial. Half of the males assigned to the 1-hole condition were paired to copulate with the same Long Evans female at every trial. The other half was given access to the same Wistar female during conditioning. Similarly, the males assigned to the 4-hole condition were also given access to either the same Long Evans or the same Wistar female during copulation. For all conditioning trials, males were placed individually into a pacing chamber for 5 min, after which either a Long Evans or Wistar female primed with EB

and P as described above was placed into the chamber for 20 min. Conditioning trials occurred at 4-day intervals during the middle third of the rat's dark cycle following hormone priming.

2.3.2. Copulatory preference test. Four days following the last conditioning trial, males were placed in an open field and allowed to habituate for 5 min. At the end of this period, the familiar female and a novel female of a different strain (Long Evans or Wistar) than the familiar one were placed simultaneously into two diagonal corners of the open field at approximately equal distance from the male. All animals were able to interact freely with one another for 30 min.

2.4. Behavioral measures and statistical analyses

Latency and frequency data were recorded for all mounts, intromissions, and ejaculations. Criteria for sexual behaviors were those described by Sachs and Barfield (1970). Female solicitations (defined as a headwise orientation to the male followed by a runaway, forcing the male to chase the female), hops and darts, lordosis magnitude (on a scale from 1 to 3, with 1 representing low magnitude, 2 representing moderate magnitude, and 3 representing high magnitude, as in Hardy & DeBold, 1972), and the time spent away from the male were also recorded. One-way analyses of variance (ANOVAs) were used to determine differences in the frequencies and latencies of copulatory behaviors among the two conditioning groups. The criterion for statistical reliability for all comparisons was set to $p < 0.05$.

3. Results

Figure 24 illustrates the frequency of ejaculation in 1-hole-trained males with Long Evans and Wistar females during the copulatory preference test. Males trained in 1-

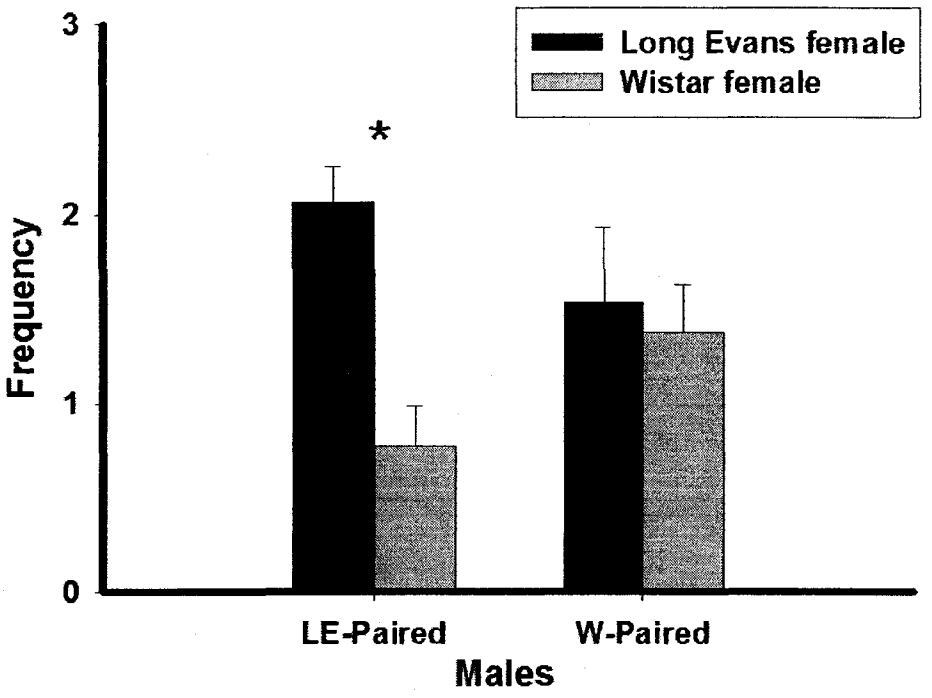

Figure 24: Average frequencies of ejaculations during the copulatory preference test in males trained in 1-hole pacing chambers with either a Long Evans (LE-paired) or a Wistar female (W-paired). * $p < 0.05$.

hole pacing chambers and paired with a Long Evans female ejaculated significantly more with their familiar Long Evans female than with the novel Wistar one ($F(1,31) = 19.57$, $p < 0.05$). In contrast, males trained in 1-hole pacing chambers and paired with a Wistar female ejaculated indiscriminately with the novel Long Evans and the familiar Wistar females ($F(1, 31) = 0.009$, $p > 0.05$). Moreover, Figure 25a shows that 1-hole-trained males paired with a Long Evans female tended to choose more often their familiar Long Evans as opposed to the novel Wistar female for their first ($\chi^2 = 2.25$, $p = 0.1$) and second ejaculations ($\chi^2 = 6.25$, $p < 0.05$). One-hole-trained males paired with a Wistar female did not display such preferences for their first three ejaculations (Figure 25b). Contrary to 1-hole trained males, 4-hole-trained males ejaculated indiscriminately with both Long Evans or Wistar females (Figures 26 and 27).

Differences in female behaviors were also observed. One-hole Long Evans females solicited the Long Evans-paired males significantly more frequently than did the Wistar females ($F(1,31) = 6.67$, $p < 0.05$) (Figure 28). Four-hole Wistar females solicited the Wistar-paired males significantly more often than did the Long Evans females ($F(1,20) = 8.58$, $p < 0.05$) (Figure 29).

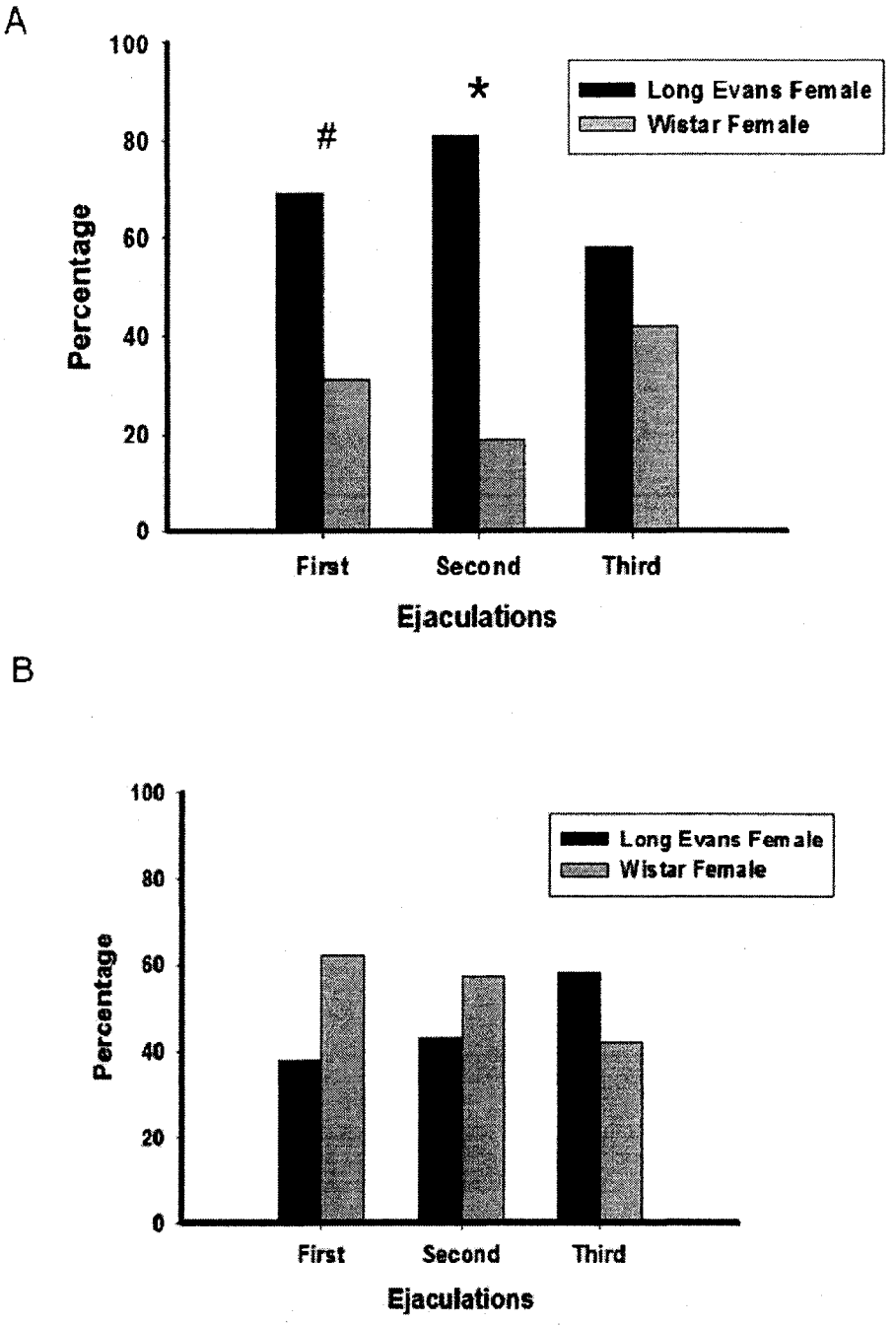

Figure 25: Choice of female for the first three ejaculations in a) LE-paired or b) W-paired 1-hole males. * $p < 0.05$, # $p < 0.1$.

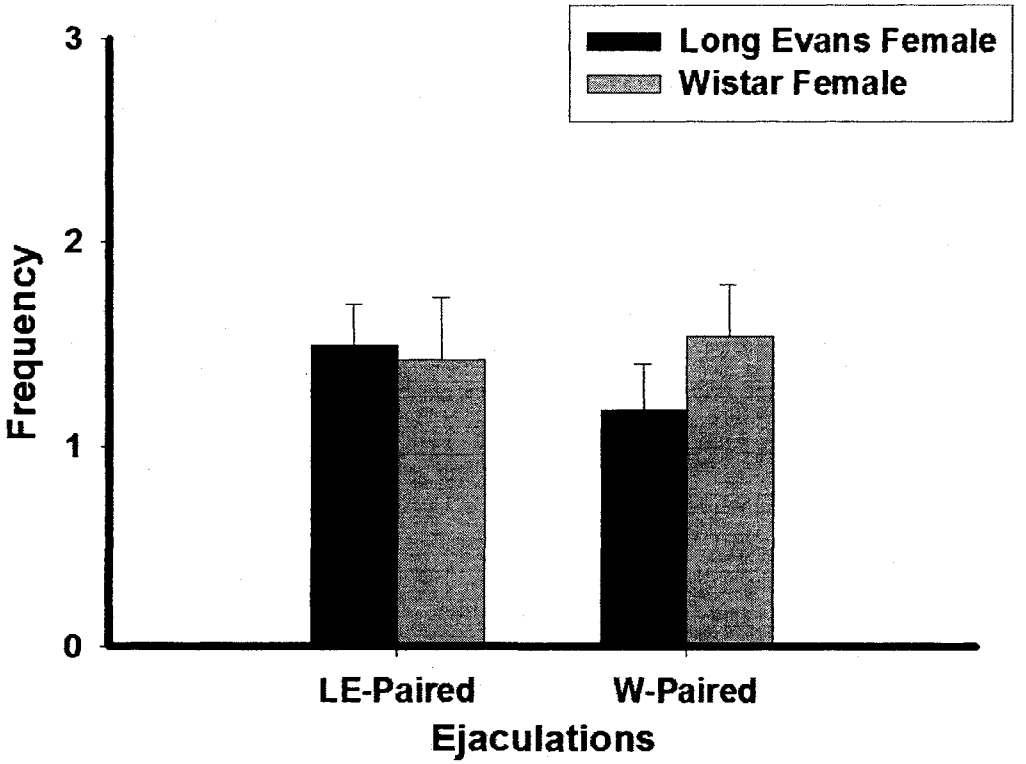

Figure 26: Average frequencies of ejaculations during the copulatory preference test in males trained in 4-hole pacing chambers with either a Long Evans (LE-paired) or a Wistar female (W-paired).

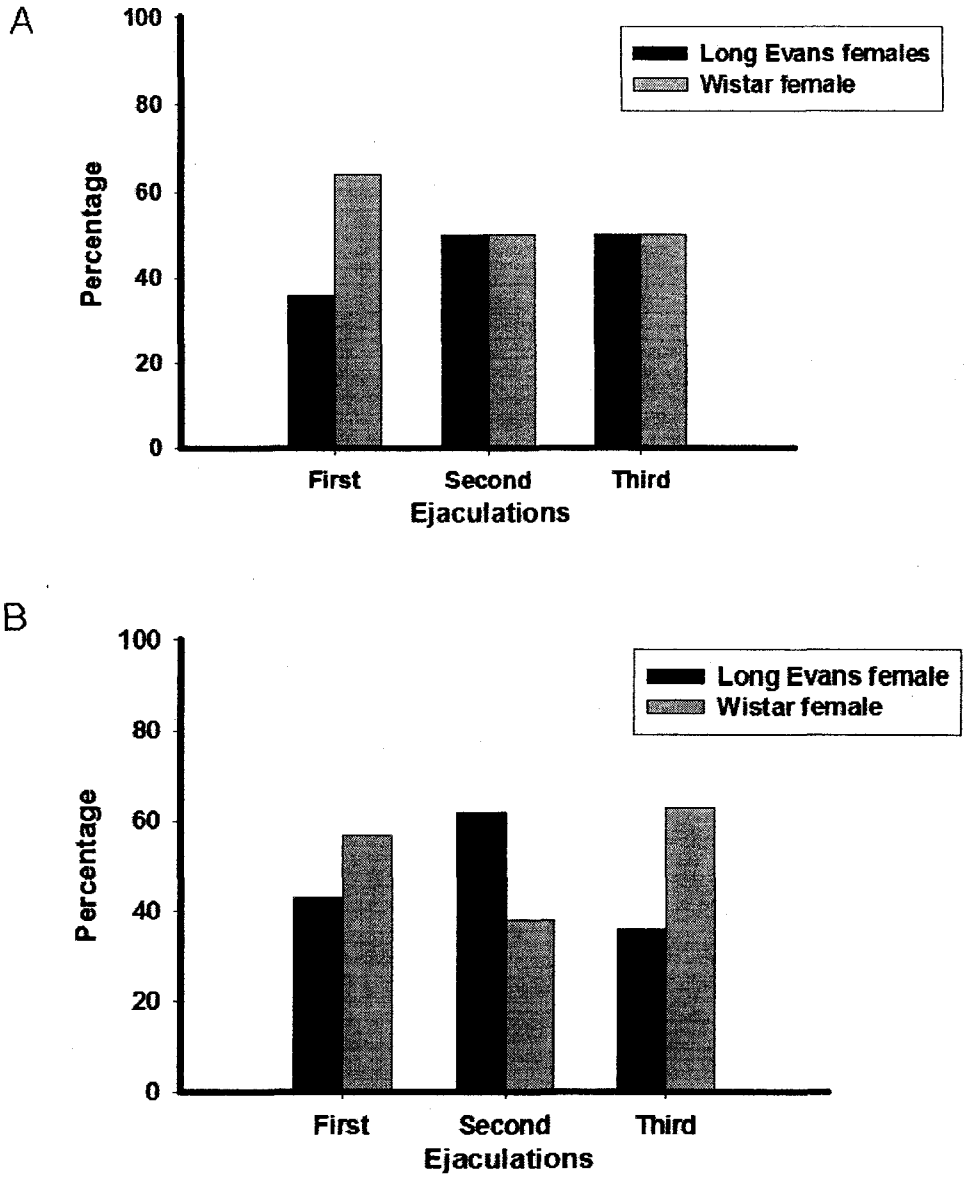

Figure 27: Choice of female for the first three ejaculations in a) LE-paired or b) W-paired 4-hole males.

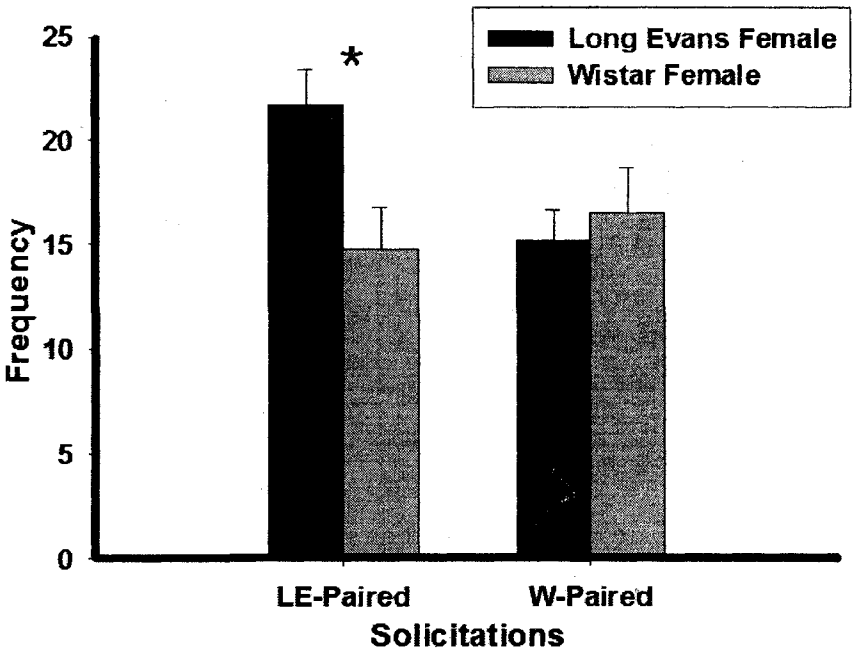

Figure 28: Average solicitations frequency in 1-hole Long Evans and Wistar females with LE-paired and W-paired males. * $p < 0.05$.

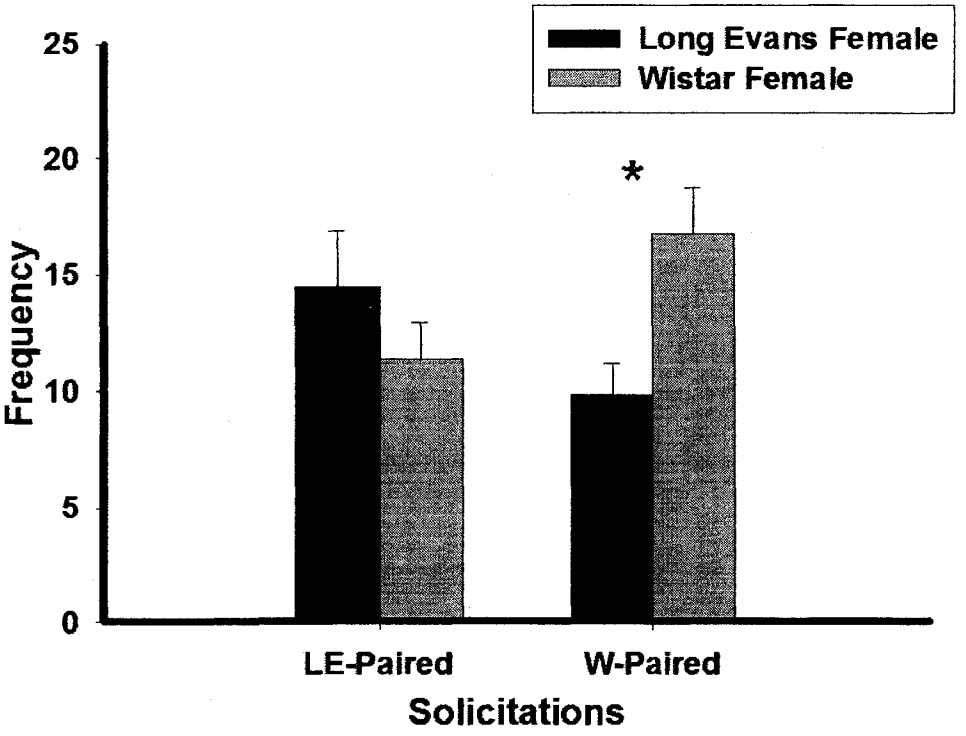

Figure 29: Average solicitations frequency in 4-hole Long Evans and Wistar females with LE-paired and W-paired males. * $p < 0.05$.

4. Discussion

The present study demonstrates that male rats display a significant CEP for the strain of partner, but only if the familiar female is of the same strain as the male. This preference was demonstrated by a higher ejaculation frequency and the choice of the familiar Long Evans female for the first two ejaculations in Long Evans males trained in 1-hole pacing chambers.

Partner preference is a well-established paradigm to study sexual motivation (Adkins-Regan, Mansukhani, Thompson & Yang, 1997; Ballard & Wood, 2007). In males, it requires them to identify, interpret, and respond to cues such as odors, appearance, and vocalizations from the stimulus female (Everitt, 1990; Hughes, Everitt & Herbert, 1990; Matusczyk & Larsson, 1994; Kippin & Pfaus, 2001; Ballard & Wood, 2007). In the present study, it was observed that 1-hole-trained males paired with Long Evans females developed CEP for their familiar female while males paired with Wistar females did not. This suggests that conditioning is stronger when males are paired with females of their own strain during copulatory trials, perhaps because they have a natural preference for females of their own strain. Early life experiences may shape animals to recognize individuals of their own strain and selectively mate with them. In fact, D'Udine & Alleva (1983) showed that early postnatal experiences with parents and siblings induce sexual preference in animals for their own strain. Early postnatal exposure of male rats to a neutral odor smeared on their mother's teats and anogenital regions results in a preference to ejaculate with estrous females bearing this neutral odor. Moreover, mice raised by rat mothers attempt to copulate more with rats than with mice (Lagerspetz & Heino, 1970). In agreement with the findings of Coria-Avila and colleagues (2006), our

results suggest that most animals engage in assortative mating, a strategy that maintains homozygosity by preventing strains from outbreeding positive characteristics. However, this phenomenon appears more plastic in females than in males.

Coria-Avila and colleagues (2006) showed that Long Evans and Wistar females can develop a conditioned partner preference for males associated with paced copulation, regardless of the strain of males but the development of this preference is facilitated when females are given access to paced copulation with males of their own strain, indicating that females have a natural preference for males of the same strain. This suggests that females' initial sexual experiences can modify the effects of early postnatal experience on partner preference. However, this does not appear to be the case for males. In our study, 1-hole-trained males displayed CEP for their familiar female only if she was of their own strain. This suggests that in males, experience with sexual reward superimposes a mate preference only for traits (e.g. a neutral odor) associated with an already-established preference for a familiar strain. It is also possible that females are more sensitive to certain strain cues (e.g., pheromones) that males cannot detect. This is one possible explanation of why females in the studies by Coria-Avila and colleagues were able to differentiate males on the basis of strain alone, whereas males in the present study could not do so.

The choice of female for the first three ejaculations shows that males trained in 4-hole pacing chambers were more influenced by the Coolidge effect, a natural phenomenon whereby males display increased sexual arousal for novel females (Beach & Jordan, 1956), than males trained in 1-hole pacing chambers. This suggests that males trained in 1-hole pacing chambers expressed higher levels of arousal during conditioning

and that this increased sexual arousal overcame the natural desire to copulate with a novel female when given the choice. More specifically, 1-hole-trained Long Evans paired males consistently chose their familiar female for their first two ejaculations more often than the novel female. This indicates that in 1-hole-trained males, the strength of the conditioning had overcome the males' natural tendency to prefer novel females. In contrast, 4-hole-trained males alternated between the Long Evans and Wistar females, indicating that their choice was influenced by the Coolidge effect.

It could be argued that the CEP observed in 1-hole-trained Long Evans-paired males for their familiar Long Evans female was influenced by the increased solicitations from their familiar female during the copulatory preference test. However, this does not appear to be the case as the Wistar females also tended to solicit more often toward the 4-hole Wistar-paired males and this did not result in the display of CEP in these males for Wistar female. Moreover, in our recent publication (Ismail, Gelez, Lachapelle & Pfaus, 2008), we showed that female solicitation does not influence the display of CEP in male rats.

The results of our present study show that males trained in 4-hole pacing chambers failed to display a CEP for their familiar female regardless of the strain of the female. As we have shown previously (Ismail, Zhao and Pfaus, 2008; Ismail, Gelez, Lachapelle and Pfaus, 2008), the development of conditioning for place or partner cues is not solely dependant on ejaculation, as suggested previously by Miller and Baum (1987) and Kippin and Pfaus (2001; 2001a, b), but rather seems additionally modulated by the level of sexual arousal during copulation. In our recent study (Ismail, Zhao & Pfaus, 2008, Chapter 1), we showed that males trained to copulate in 1-hole and 4-hole pacing

chambers display different patterns of copulation which result from different levels of arousal experienced during copulation in these chambers. For instance, males trained in 1-hole pacing chambers display significantly longer ejaculation latencies than males trained in 4-hole pacing chambers. Moreover, females spent more time away from the males in the 1-hole condition, compelling the males to wait for the females for longer periods of time than males in the 4-hole condition (Ismail, Gelez, Lachapelle & Pfaus, 2007). Thus, the anticipated access to the female in 1-hole pacing chambers may result in higher levels of arousal in males and this may facilitate conditioning and the development of partner preference. This idea is consistent with the findings of Lipp (2002) that the arousal level of an anticipated event can modulate the development of conditioning. Indeed, Richardson and Gratton (2008) recently showed that dopamine transmission in the nucleus accumbens was increased when a reward was presented at variable, rather than at fixed intervals. Those findings suggest that rats are more aroused and excited in situations in which the occurrence of the reward was anticipated and unpredictable than in situations in which the occurrence of the reward was expected and predictable. Similarly, in our study, males trained in the 1-hole paced condition may have experienced high levels of arousal during copulation because the contact with the female partner was unpredictable in this environment. The anticipation of future contact with the female may have increased sexual desire and facilitated the development of partner preference for the familiar female. In contrast, males trained in 4-hole pacing chambers may have experienced lower levels of sexual arousal because the contact with the female partner was much more regular and predictable and this may have contributed to the failure to develop partner preference for the familiar female.

In summary, the results of the present study suggest that males trained to copulate in 1-hole pacing chambers develop CEP for strain cues only if the familiar female is of their own strain. Thus, although cues associated with sexual reward come to direct appetitive sexual responses in both male and female rats, mate choice (in this case the choice to give or receive ejaculations to or from a particular partner) may be more "hardwired" by assortative pressures in which animals prefer their own strain over a novel one.

Acknowledgements

This research was supported by a grant from the Canadian Institutes of Health Research (MOP-74563) to JGP. The authors would like to thank Ladan Mohebbian, Caroline Frank and Crystal McDubbin for technical assistance.

CHAPTER FIVE

The findings reported in the previous chapters showed that males trained to copulate in 1-hole pacing chambers with the same almond-scented female develop a CEP for the familiar female relative to a novel female. A similar CEP develops for strain cues, but only if the familiar female is of their own strain. A nearly identical, but stronger, pattern of preferences develop in female rats (Coria-Avila et al. 2006). Because the development of conditioned partner preference for odor rats can be disrupted in female by treatment with opioid or dopamine receptor antagonists during training (Coria-Avila et al., 2008a, 2008b), the experiments in Chapter 5 examined whether these two neurochemical systems are involved similarly in the development of CEP in male rats.

Naloxone, but not Flupenthixol, Disrupts the Development of Conditioned Ejaculatory Preference in the Male rat.

Nafissa Ismail, Fabienne Girard-Bériault, Satoru Nakanishi, & James G. Pfaus

Concordia University

RUNNING HEAD: NALOXONE, FLUPENTHIXOL AND PARTNER PREFERENCE

Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, Montréal, QC.

This research was supported by a grant from the Canadian Institutes of Health Research (MOP-74563) to JGP. The authors would like to thank Jonathan Greggain for technical assistance.

Correspondence concerning this article should be addressed to James G. Pfaus, Ph.D., Center for Studies in Behavioral Neurobiology, department of Psychology, Concordia University, Montréal, QC H4B 1R6 Canada. E-mail: Jim.Pfaus@concordia.ca.

For submission to *Behavioral Neuroscience.*

Abstract

Male rats display a conditioned preference to ejaculate with a female bearing an odor paired previously with copulation to ejaculation. The present study examined the role of endogenous opioid and dopamine systems in this preference. Male rats received saline, the opioid antagoinst naloxone, or the dopamine antagonist flupenthixol prior to 10 conditioning trials in a pacing chamber with an almond-scented female. On the final test, all males were injected with saline and given access to two females, one scented and the other unscented, in an open field. Only males injected with naloxone during training failed to manifest a conditioned ejaculatory preference. Activation of opioid, but not dopamine, systems during sexual interaction are necessary for conditioned ejaculatory preference in male rats.

Keywords: Opioids, Dopamine, Naloxone, Flupenthixol, Partner preference

It has been shown that polygamous male rats can develop a conditioned ejaculatory preference (CEP) for females bearing a familiar odor cue paired previously with the reward state induced by ejaculation (Kippin et al., 1998). In a subsequent study, Kippin and Pfaus (2001a) demonstrated that ejaculation is necessary for conditioning to occur and that the post-ejaculatory interval is the stage of copulation during which males associate a conditioned stimulus with sexual reward. These findings are consistent with those of conditioned place preference (CPP) in which ejaculation is also necessary for conditioning to occur (Miller & Baum, 1987; Mehrara & Baum, 1990). Recently, we showed that males can develop CEP for a familiar female after repeated copulation with this female in pacing chambers bisected by a partition containing 1 hole, but not 4 holes (Ismail, Gelez, Lachapelle and Pfaus, 2008; Chapter 2). Pacing chambers are often used to study female sexual behavior (Erskine, Kornberg & Cherry, 1989; Paredes & Vazquez, 1999; Coria-Avila, Ouimet, Pacheco, Manzo & Pfaus, 2005). Though males ejaculated in the 1-hole and in the 4-hole conditions, only males trained in the 1-hole condition developed CEP. These findings suggest that ejaculation alone is not sufficient for conditioning to occur. This makes it possible that the arousal level experienced during copulation also plays a role in the development of conditioning.

The pharmacology underlying the development of CEP for a familiar female remains to be investigated. However, previous studies have identified both endogenous opioids and dopamine as possible candidates. For example, the opioid receptor antagonist naloxone disrupts the display of a sexually conditioned place preference (CPP) in male rats that develops when a place is paired with the postejaculatory refractory period (Ågmo and Berenfeld, 1990; Miller & Baum, 1987; Mehrara & Baum, 1990).

Naloxone also disrupts the acquisition of a conditioned partner preference in female rats for males bearing odor or strain cues associated with paced copulation (Coria-Avila et al., 2008). Another neurotransmitter possibly involved in the development of CEP is dopamine (DA). DA has long been known to be important for male sexual motivation and behavior (Blackburn, Pfaus & Phillips, 1992; Pfaus & Phillips, 1991; Sachs & Meisel, 1988) and for directing attention to a rewarding stimulus (Mirenowicz & Schultz, 1994). DA release increases phasically in hypothalamic and mesolimbic terminals, such as the medial preoptic area (mPOA) and nucleus accumbens (NAc), in response to cues associated with copulation such as estrous vaginal secretions or neutral odors associated with the ejaculatory reward state (Blackburn, Pfaus & Phillips, 1992; Damsma et al., 1992; Gelez et al., submitted; Mitchell & Gratton, 1991; Pfaus et al., 1990; Richardson & Gratton, 2008). DA is released in a relatively continuous or tonic manner during copulation in the mPOA, NAc, and striatum of male rats, but declines precipitously in the mPOA and NAc after ejaculation (Blackburn, Pfaus, & Phillips, 1992; Lorrain, Riolo, Matuszewich, & Hull, 1999). Given that DA plays an important role in the prediction of the occurrence of a rewarding event (Schultz, 1998), it is possible that activation of DA release is part of the mechanism that directs male rats toward odors or other cues associated with sexual reward. Interestingly, in females, administration of the DA receptor antagonist flupenthixol disrupts the acquisition of sexually conditioned partner preference for an odor cue associated with paced copulation, but not for strain cues associated with paced copulation (Coria-Avila et al., 2008b). Moreover, administration of DA antagonists do not disrupt the expression of sexually conditioned CPP (Ågmo & Berenfeld, 1990; Garcia Horsman & Paredes, 2004; Paredes & Ågmo, 2004).

Given that opioid, but not DA, transmission is important for the expression of sexual CPP in male and female rats, but that both play a role in sexually conditioned partner preference in female rats, the present study examined whether endogenous opioid or DA transmission plays a role in the development of CEP in males.

Experiment 1

Effect of the Opioid Receptor Antagonist Naloxone

Methods

Subjects and Surgery

Males. Forty male Long Evans rats, weighing approximately 300 g at the start of the experiment, were obtained from Charles River Laboratories (St-Constant, Quebec). Rats were housed in groups of four, in plastic solid floor cages, with *ad libitum* access to food (Purina Rat Chow) and water, and they were maintained on a 12-hour dark/light cycle (lights on at 8 pm) in a temperature-controlled room (21 ± 2°C). Behavioral testing was conducted in the dark phase of the cycle. Males were sexually naïve at the start of the experiment.

Females. Forty female Long Evans rats, weighing approximately 200 g at the start of the experiment, were obtained from the same supplier and were housed under the same conditions as the males. Females were anaesthetized with a mixture of ketamine (50 mg/ml) and xylazine hydrochloride (4 mg/ml), at a ratio of 4:3, respectively, administered intraperitoneally in a volume of 1 ml/kg of body weight. Anaesthetized females were then bilaterally ovariectomized via a lumbar incision. All females were given a week of postsurgical recovery prior to sexual training. Following recovery, all

females were placed in a pacing chamber with intact and sexually vigorous males during four sexual training trials. For all behavioral tests, sexual receptivity was induced by priming the females with an injection of 10 μg (s.c. in 0.1 ml of sesame oil) of estradiol benzoate (EB) 48 hrs and 500 μg (s.c. in 0.1 ml of sesame oil) of progesterone (P) 4 hrs prior to behavioral testing.

Drugs

Naloxone hydrochloride (Sigma; St-Louis, MO), a non-specific opioid receptor antagonist, was dissolved in 0.9% physiological saline, and was injected into the males subcutaneously at a dose of 5 mg/kg in a volume of 1 ml/kg (as in Miller & Baum, 1987), five minutes before each trial. Males in the control group were injected subcutaneously with 0.9% physiological saline in a volume of 1ml/kg 5 min before each trial.

Apparatus

Copulatory conditioning trials took place in rectangular pacing chambers (46 cm x 39 cm x 37 cm) covered with a grid floor and bedding and bisected with a clear Plexiglas divider with a hole cut into the bottom of the divider. This hole was small enough to allow only the female to cross, but not the male. All conditioning sessions were recorded on a DVD and subsequently scored using a PC-based program (Cabilio, 1996).

Procedure

Conditioning phase. The 40 naïve males were randomly assigned to one of two groups. Both groups were subjected to ten copulatory trials in a pacing chamber with the same almond-scented female; however, one group was injected subcutaneously with saline 5 minutes before each trial, while the other group was injected with naloxone, a

non-specific opioid receptor antagonist (s.c. 5mg/kg), five minutes before each trial. For all conditioning sessions, males were given a five-minute habituation period to a pacing chamber, after which, a female was placed into the chamber for 20 min. Females were primed with EB and P as described above. Conditioning trials occurred at 4-day intervals during the middle third of the rat's dark cycle following hormone priming.

Copulatory Preference Test. Four days following the last conditioning trial, males were placed in an open field and allowed to habituate for 5 min. At the end of this period, the familiar scented female and a novel unscented female were placed simultaneously into two diagonal corners of the open field at approximately equal distance from the male. All animals were able to interact freely with one another for 30 min. All males were drug-free during this test.

Behavioral Measures and Statistical Analyses

Latency and frequency measures were recorded for all mounts, intromissions, and ejaculations. Criteria for sexual behaviors were those described by Sachs and Barfield (1970). Female solicitations (defined as a headwise orientation to the male followed by a runaway forcing the male to chase the female), hops and darts and lordosis magnitude (on a scale from 1 to 3, with 1 representing low magnitudes, 2 representing moderate magnitudes and 3 representing high magnitudes, as in Hardy and Debold, 1972) were recorded. To examine copulatory preference, the frequency of ejaculation with each female, and the female chosen for the first three ejaculations were also recorded. One-way analyses of variance (ANOVAs) were used to analyze all frequency and latency data. Chi-square analyses were used to determine the difference in the proportion of

females selected for the first two ejaculations during the copulatory preference test. The criterion for statistical reliability for all comparisons was set to $p < 0.05$.

Results

Figure 30 illustrates the mean frequencies of copulatory behaviors for previously saline- and naloxone-treated males with the familiar and the novel females. Saline-treated males ejaculated significantly more often with their familiar female as opposed to the novel one ($F(1, 22) = 9.54, p < 0.05$) (Figure 30a). In contrast, males that were injected with naloxone during conditioning ejaculated as often with the familiar as with the novel females. Interestingly, these males tended to mount ($F(1, 24) = 3.70, p < 0.1$) the novel female more frequently and to intromit significantly ($F(1, 24) = 18.27, p < 0.05$) more frequently the novel female (Figure 30b) .

Males previously injected with saline also seemed to choose their familiar female more often than the novel one for the first three ejaculations, but this difference did not reach statistical significance. Naloxone-treated males seemed to choose more often the novel female for the first and third ejaculation, but this did not reach statistical reliability (Figure 31).

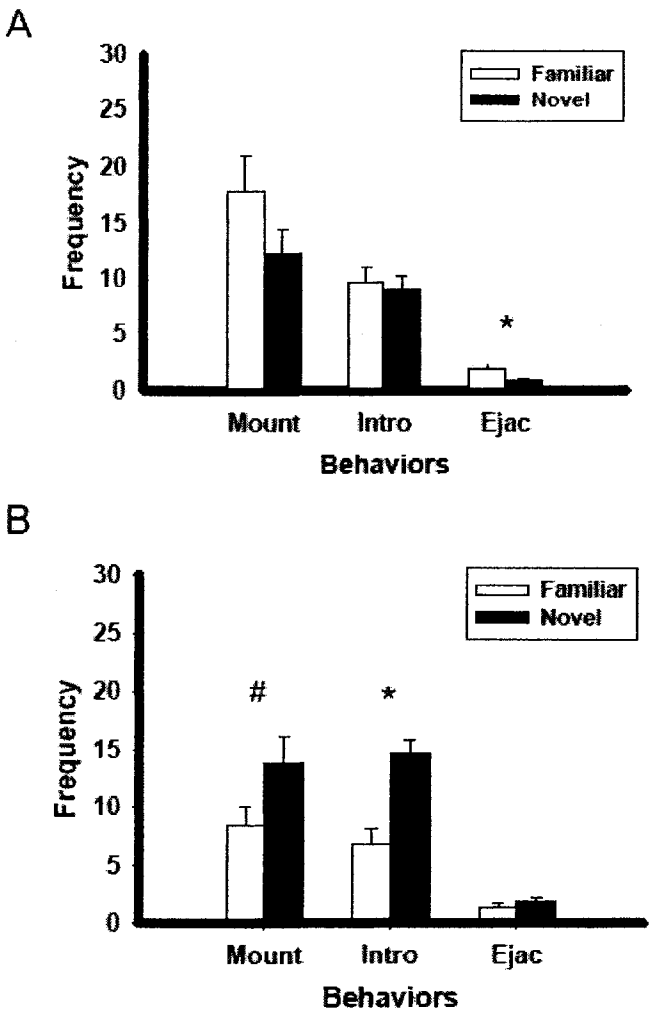

Figure 30: Mean frequency of mounts, intromissions and ejaculations in a) saline- and b) naloxone-treated males with the familiar scented and novel unscented females.
* $p < 0.05$; # $p < 0.1$.

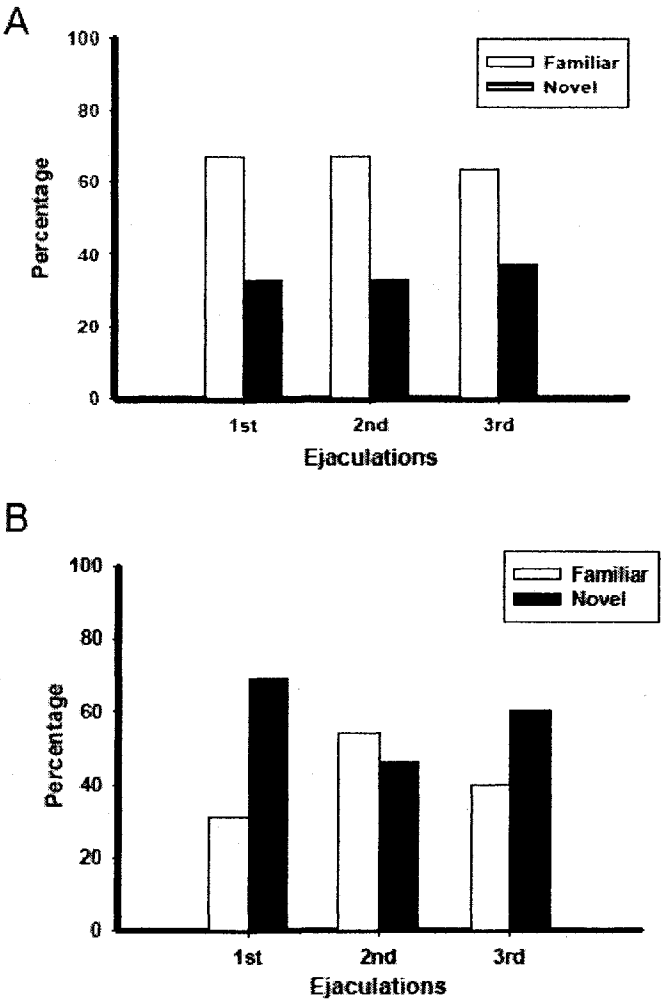

Figure 31: Distribution of first three ejaculations between familiar almond-scented and novel unscented females in a) saline- and b) naloxone-treated males.

Experiment 2

Effect of the Dopamine Receptor Antagonist Flupenthixol

Methods

Subjects

Males and Females. Forty male and 40 female Long Evans rats served as subjects in this experiment. Subjects were acquired from the same supplier and housed under the same conditions as subjects in Experiment 1. For all conditioning trials and the copulatory preference test, females were primed with EB and P.

Drugs

Cis(Z)- Flupenthixol (Sigma; St-Louis, MO), a D1/D2 receptor antagonist was dissolved in 0.9% physiological saline and was injected intraperitoneally at a dose of 0.5 mg/kg in a volume of 1 ml/kg 30 min before every trial (as in Agmo, 2003) Males in the control group were injected intraperitoneally with 0.9% physiological saline with a volume of 1ml/kg, 30 min before each trial.

Apparatus

All conditioning sessions and copulatory preference testes took place in the same pacing chambers and open fields used in Experiment 1.

Procedure

Conditioning Phase. The 40 naïve males were randomly assigned to one of two groups. One group was given ten copulatory trials in a pacing chamber with the same scented female and was injected with saline before each trial. The other group was given ten copulatory trials in pacing chambers with the same scented female and was injected

with flupenthixol (i. p. 0.1mg/kg), a non-specific dopamine antagonist before every trial. Conditioning trials occurred in the same way as in Experiment 1.

Copulatory Preference Test. Copulatory preference test also took place in the same way as in Experiment 1. The males were drug-free during this test.

Behavioral Measures and Statistical analyses

Latency and frequency data of male and female sexual behaviors during the open field test were recorded and analyzed using 1-way ANOVAs as in Experiment 1. Chi-square analyses were used to determine the differences in the proportion of females selected for the first two ejaculations. Copulatory preference was determined the same way as in Experiment 1. The criterion for statistical reliability for all comparisons was set to $p < 0.05$.

Results

Figure 32 illustrates the frequency of sexual behavior in males injected with saline or with flupenthixol during conditioning. It can be seen in this figure that saline-treated males displayed significantly more ejaculations with their familiar female compared to the novel one ($F(1,29) = 4.12, p = 0.05$) (Figure 32a). Interestingly, males injected with flupenthixol during conditioning also ejaculated significantly more with the familiar female as opposed to the novel one ($F(1,36) = 4.01, p = 0.05$) (Figure 32b).

Saline-treated males also seemed to choose more frequently their familiar female for the first and third ejaculation, but this did not reach statistical significance. Similarly, flupenthixol-treated males also seemed to choose more frequently their familiar female for the first two ejaculations, but this failed to reach statistical criterion for reliability (Figure 33).

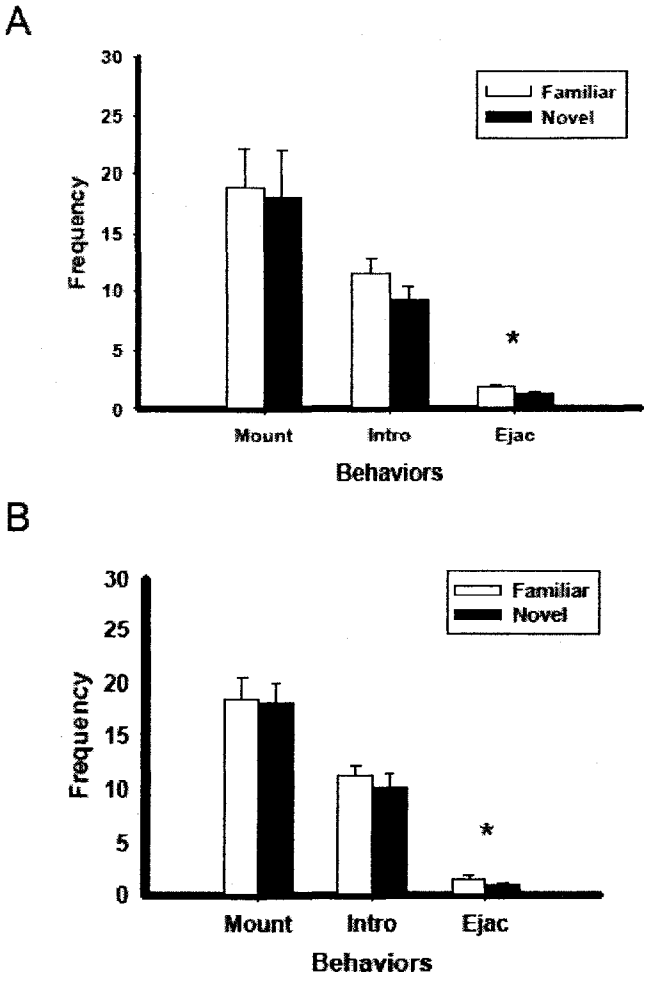

Figure 32: Average frequency of mounts, intromissions and ejaculations in a) saline- and b) flupenthixol-treated males towards the familiar scented and novel unscented females on the partner preference test when no drugs were given.
* $p < 0.05$.

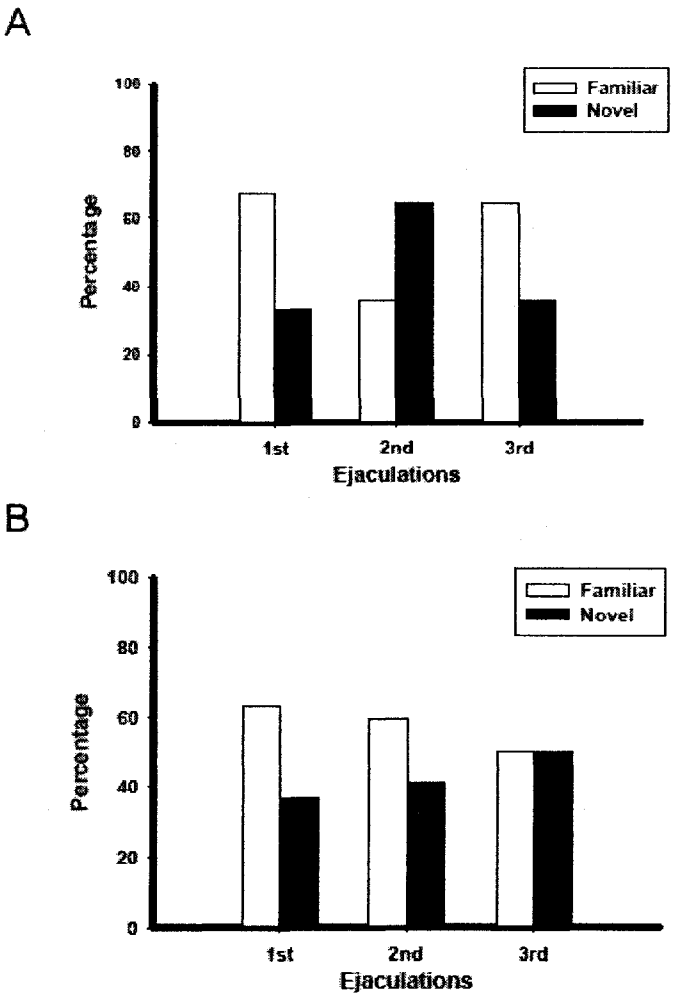

Figure 33: Distribution of first three ejaculations between familiar almond-scented and novel unscented females in a) saline- and b) naloxone-treated males.

Discussion

The present study shows that the development of CEP is disrupted by the administration of naloxone, but not flupenthixol, during conditioning trials. This suggests that activation of opiods receptors, but not DA receptors, is necessary for the development of CEP for a familiar female following copulatory training in 1-hole pacing chambers. These findings are similar to those of Ågmo and Berenfeld (1990), in which naloxone, but not the DA receptor antagonist pimozide, disrupted the expression of sexually-conditioned CPP in male rats. Thus, similar neurochemical mechanisms appear to underlie the development of CPP and CEP.

Endogenous opioids figure prominently in the control of pleasure and reward. There are three major types of opioid receptors: mu, delta and kappa. These receptors are found predominantly in hypothalamic, limbic, and cortical areas such as the amygdala, the MPOA, the PVN, the NST and the cingulate cortex (Clarke, Zimmer, Zimmer, Hill & Kitchen, 2003; Reinoso-Barbero & de Andres, 1995). The effect of opioids on sexual behavior was first reported in clinical accounts of long-term opioid users that experienced a deterioration of their sexual function. In men, long-term opioid use results in delayed ejaculation, decreased ejaculate volume, anorgasmia, elimination of sexual dreams and even infertility in certain cases. Similarly, in women, long-term opioid use has been associated with anorgasmia, absence of menstrual periods, elimination of sexual dreams and infertility in some cases (Pfaus & Gorzalka, 1987). Interestingly, effects of acute opioid administration on sexual behavior are very different from long-term opioid use. For example, opioid users describe the acute administration of heroin as producing an instantaneous, orgasm-like "rush" of euphoria (Pfaus & Gorzalka, 1987). Furthermore,

Whipple & Komisaruk (1985) have shown that vaginal stimulation significantly increases pain tolerance and pain detection thresholds. Similarly, Gonzalez-Mariscal and colleagues (1994) have reported in male rats that during copulation, analgesic effects reach peak levels during ejaculation. Based on these findings, endogenous opioids appear to be released during genital stimulation and ejaculation, and may play an important role in the perception of the intensity of sexual reward by binding to opioid receptors in the amygdala, the MPOA, the PVN, the NST and/or cingulate cortex and by mediating the activation of other areas that receive projections from these areas.

The results of the present study are only partially in agreement with those of Coria-Avila and colleagues (2008b) who found that both opioid- and DA-receptor antagonists block the development of conditioned partner preference in female rats following paced copulation. One obvious possible explanation is that different neuronal mechanisms underlie the development of partner preference in males and in females. However, another potential explanation is that DA does play a role in the development of CEP in males but that the effect of flupenthixol was not noticeable with our current experimental design. Although both dopamine and opioid antagonists bind competitively with their respective neurotransmitters, the binding kinetics and release patterns of opioids and dopamine during sexual behavior are not equivalent. Endogenous opioids are released as a "bolus" in different brain regions at the time of ejaculation (Szechtman, Hershkowitz, & Simantov, 1981), whereas DA transmission is increased continuously throughout copulation but decreases precipitously after ejaculation (Pfaus et al., 1990). Given that conditioning occurs at the the post-ejaculatory interval (Kippin, Samaha, Sotiropoulos & Pfaus, 2001b) and that opioid levels are high but that DA levels are low

during this stage of copulation, this suggests that opioid release is necessary for conditioning to occur. Although microinjections of flupenthixol to the mPOA or VTA disrupt male copulatory behavior and appetitive maze running to obtain a receptive female (Pehek et al., 1988; Warner et al., 1991), and although the dose of flupenthixol used in the present study blocked the acquisition of conditioned partner preference in female rats (Coria-Avila et al., 2008b), it is possible that this dose did not compete efficiently with the increased DA release during copulation. Indeed, the increase in NAc DA in females during paced copulation is far lower than the increase observed in males (Jenkins & Becker, 2003), so it is possible that a higher dose of flupenthixol would be needed to block DA receptors sufficiently. In contrast, the dose of naloxone used in the present study has consistently prevented CPP in male and female rats, and conditioned partner preference in female rats. This suggests that naloxone pretreatment competes with opioid released as a bolus during the experience of sexual reward.

Paredes and Ågmo (2004) have argued against a role of DA in sexual behavior, but their argument is predicated on the fact that DA antagonists do not alter sexual CPP or an unconditioned olfactory preference for an estrous versus nonreceptive female. In their studies, a single administration of the same dose of flupenthixol was given before training, which, as suggested above, may not be sufficient to compete with endogenous DA release during copulation. Numerous other evidences suggest that DA plays an important role in male sexual behavior. The VTA is part of the midbrain and is rich in dopaminergic neurons. This area is considered to be a part of the reward system as activities that produce pleasure tend to activate this area (McBride, Murphy & Ikemoto, 1999). One area to which the VTA projects to is the NAcc. The latter is known to play a

key role in limbic circuits that are responsible for motivated and goal-directed behaviors (Groenewegen & Uylings, 2000; Kelley, 1999), such as sexual behavior. Most of the neuronal innervations to the NAcc are dopaminergic innervations from the VTA. Overall, the neurotransmitter DA enhances sexual motivation and copulatory ability in rodents, humans and nonhuman primates. For example, data from several laboratories have indicated that extracellular DA in the NAcc increases in the presence of sexually relevant stimuli (Pfaus, Damsma, Nomikos, Wenkstern, Blaha, et al., 1990; Mas, Gonzalez-Mora, Louilot, Sole & Guadalupe, 1990; Damsma, Pfaus, Wenkstern, Phillips & Fibiger, 1992; Fiorino, Coury & Phillips, 1997; Fiorino & Phillips, 1999). Moreover, subcutaneous injection of apomorphine, a DA agonist, elicited penile erection whereas this effect was blocked by the subcutaneous injection of the DA antagonist haloperidol (Rampin, Jerome & Suaudeau, 2003). Also, Lopez and Ettenberg (2000) demonstrated that males injected with haloperidol before their first copulatory experience exhibited diminished motivation to approach estrous females on subsequent drug-free trials, even though all males ejaculated on their first copulatory experience. Dopamine is also widely believed to be associated with the anticipatory phase of reward-seeking behaviors (Schultz & Dickinson, 2000). Moreover, studies comparing monogamous prairie voles to non-monogamous meadow voles reported that blockade of DA D2 receptors in the NAc disrupts copulation-induced partner preference (Liu & Wang, 2003; Young, Lim, Gingrich & Insel, 2001). Thus, although in the present study, disruption of the dopaminergic system failed to block the development of CEP for a familiar female, DA may still play an important role in the pattern of male copulatory behavior.

Future experiments could examine the role of oxytocin in the development of CEP as oxytocin may also play an important role during conditioning. Arletti and colleagues (1985) examined the effects of oxytocin on male copulatory behavior. In their study, animals injected with oxytocin displayed a significantly shorter ejaculation latency and postejaculatory interval than saline-treated animals, suggesting that oxytocin plays a facilitatory role in ejaculatory behavior. Oxytocin also modulates the development of pair bonding in monogamous prairie voles (Young et al., 2004). For instance, female prairie voles injected with oxytocin develop pair bonds even in the absence of copulation (Young et al., 2001). Moreover, injection of an oxytocin antagonist directly in the NAc disrupts the development of pair bond following mating. Interestingly, systemic injection of oxytocin to male rats prior to their first sexual experience with an almond scented female increases the number of males that display conditioned ejaculatory preference (Gelez et al., unpublished observation). Together, these findings suggest that oxytocin may play an important role in the development of CEP for a familiar female.

In summary, the results of the present study suggest that the release of endogenous opioids but not DA is necessary for the development of partner preference in males for a familiar female. However, the role of DA in the development of CEP requires further investigation. These findings shed light on the neurochemical mechanisms underlying the development of CEP for a familiar female following 1-hole paced copulation in male rats.

CHAPTER SIX

The final chapter of the thesis examined potential brain regions involved in CEP. Males that had been trained to associate an almond odor associated with sexual reward received access to scented or unscented females behind a screen, or access to a cotton gauze pad saturated with the almond odor. The induction of Fos protein by the sensory stimulation was counted in different brain regions and compared statistically among the groups.

NEURONAL ACTIVATION BY CONDITIONED ODORS AND NATURAL CUES IN MALE RATS

N. Ismail, J. Spape, F. Girard-Bériault, A. Knezevic, Z. El-Jabary, J. G. Pfaus*

Center for Studies in Behavioral Neurobiology, Department of Psychology
Concordia University, Montréal, QC H4B 1R6, Canada

---

* Corresponding author: Tel: +1-514-848-2424x2189; fax: +1-514-848-2817.
E-mail address: Jim.Pfaus@concordia.ca (J. G. Pfaus).

Abbreviations: accessory olfactory bulb (AOB), arcuate nucleus of the hypothalamus (Arc), basolateral amygdala (BLA), caudate putamen (CPu), lateral habenula (LHb), lateral septum (LS), medial habenula (MHb), main olfactory bulb (MOB), medial preoptic area (MPOA), nucleus accumbens core (NAccore), nucleus accumbens shell (NAccsh), paraventricular nucleus of the hypothalamus (PVN), piriform cortex (Pircx), posteromedial dorsal amygdala (PDMA), suprachiasmatic nucleus (SCN), supraoptic nucleus (SON), ventral pallidum (VP), ventral tegmenatal area (VTA), ventromedial hypothalamus (VMH), and olfactory tubercle (Tu).

For submission to *Neuroscience*.

Abstract

Male rats display a preference to ejaculate with females bearing olfactory cues paired previously with the reward state induced by ejaculation. However, we have recently found that ejaculation alone is not sufficient to induce the development of a conditioned ejaculatory preference (CEP); the level of sexual arousal experienced during copulation also plays an important role. The present study examined regions of the brain activated by sexually conditioned cues using immunocytochemical detection of Fos protein in the brains of conditioned males following exposure to a familiar almond-scented female or to the almond odor alone. In the first two experiments, males were randomly assigned to copulate in a 1-hole or 4-hole pacing chamber in a paired (same female at every trial) or random (different female at every trial) condition. The results showed that, following exposure to the familiar almond-scented female, 1-hole paired males displayed significantly more Fos-IR in the ventral tegmental area (VTA), arcuate nucleus (Arc), paraventricular nucleus of the hypothalamus (PVN), posterodorsal medial amygdala (PDMA) and a tendency towards more Fos-IR in the piriform cortex (PIR) and the lateral septum (LS) compared to males in the other groups. The results also showed that following exposure to the almond odor alone, 1-hole paired males displayed significantly more Fos-IR in the VTA and the ventromedial nucleus of the hypothalamus (VMH). In the third experiment, 1-hole paired males were either pretreated with saline or with flupenthixol before each conditioning trial. Although flupenthixol did not interfere with the development of partner preference, differences in the pattern of Fos activation were observed between saline- and flupenthixol-treated males. Following exposure to the almond odor alone, 1-hole saline-treated males displayed significantly less Fos-IR in

VTA and a tendency towards less Fos-IR in the Arc and the nucleus accumbens core (NAccore). In the final experiment, 1-hole paired males were either pretreated with saline or naloxone before each conditioning trial. Naloxone-treated males failed to develop partner preference for their familiar female and examination of Fos activation revealed that, following exposure to the almond odor alone, naloxone-treated males displayed significantly less Fos-IR in Arc and a tendency towards less Fos-IR in the VTA. Together, these findings suggest that males that developed CEP for a familiar female displayed more Fos-IR in a number of areas, but more consistently in the VTA and the Arc.

Keywords: Fos, Olfactory, Sexual behavior, Partner preference

Introduction

Olfactory stimuli modulate sexual behavior in a variety of species (Cain & Paxinos, 1974; Edwards, Griffis &Tardivel, 1990; Kippin, Cain & Pfaus, 2003; Larsson, 1971; McClintock, 1978, 1984; McClintock and Adler, 1978; Schank and McClintock, 1997), including humans (McClintock, 1971, Stern and McClintock, 1998). For example, rats are able to differentiate between individuals of their own strain and those of a different strain based on vocal, visual and olfactory cues (Austin & Dewsbury, 1976). Moreover, estrous odors emitted from sexually receptive females increase testosterone and luteinizing hormone levels in the blood (Graham and Desjardins, 1980) and produce sexual arousal (Sachs, 1997).

Brain areas mediating the effects of estrous odors have been examined using Fos immunoreactivity (IR) as a marker of neuronal activation. Exposure to estrous odors increased Fos-IR in the accessory olfactory bulb (AOB)/vomeronasal organ, medial amygdala (MEA), medial bed nucleus of the stria terminalis (mBNST), nucleus accumbens core and shell (NAcc core and NAcc sh), medial preoptic area (mPOA), and the ventral tegmental area (VTA) in rats, hamsters, gerbils and ferrets (Bialy & Kaczmarek, 1996; Newman, Parfitt & Kollack-Walker, 1997; Pfaus & Heeb, 1997; Swann & Fiber, 1997; Veening & Coolen, 1998).

Neutral odors associated with sexual reward can also modulate neuroendocrine function, sexual behavior and partner preference. For example, Graham and Desjardins (1980) reported that exposure to a neutral odor (methyl salicylate or wintergreen) increased plasma levels of testosterone and luteinizing hormone. Kippin and Pfaus (2001) demonstrated that male rats can associate cues with sexual reward and display

preferences for partners bearing those familiar cues. Males were exposed to a random almond-scented female at every trial for nine conditioning trials in bilevel chambers. Following these conditioning trials, males underwent a partner preference test where they were placed in an open field arena with two females, one scented and one unscented female. Results showed that, when given the choice, males ejaculated significantly more with the almond-scented female over the unscented one. These findings suggest that males had associated the almond-odor to sexual reward and developed preferences for partners bearing this scent. Subsequent examination of the brain areas involved in the development of this preference using Fos immunocytochemistry revealed that, following exposure to the conditioned almond-odor in the bedding, males had increased Fos induction in the piriform cortex (Pircx), nucleus accumbens core (NAccore), anterior portion of the lateral hypothalamic area, and basolateral amygdala (BLA), relative to males that had never experienced the odor paired with copulation to ejaculation (Kippin, Cain & Pfaus, 2003).

Recently, we showed that copulation in chambers in which access to the female is restricted, facilitates the development of partner preference in males for a familiar almond-scented female over a novel one (Ismail, Gelez, Lachapelle & Pfaus, 2008). These chambers, also known as pacing chambers, are commonly used to study female sexual behavior (Coria-Avila, Ouimet, Pacheco, Manzo & Pfaus, 2005; Coria-Avila et al., 2006; 2008 a,b; Erskine, Kornberg & Cherry, 1989; Paredes & Vasquez, 1999). The chambers are bisected with a divider that has one or more holes cut into the bottom of the divider and these holes are small enough to only allow the female to cross, giving females control over the rate of copulation and restricting males from easily accessing the females

during mating. In our study, we assigned males to one of two groups. One group copulated in a 1-hole pacing chamber with the same almond-scented female at every trial. The second group copulated in a 4-hole pacing chamber with the same almond-scented female at every trial. After ten conditioning trials, males underwent a partner preference test where they had the option to copulation with their familiar almond-scented female or a novel unscented female. Results showed that only males previously trained to copulate in the 1-hole pacing chamber developed a conditioned ejaculatory preference (CEP) for their familiar female over a novel one. We have previously reported that, females spend more time away from males in the 1-hole than in the 4-hole pacing chambers (Ismail, Zhao & Pfaus, 2008), suggesting that access to the female is more restricted in the 1-hole than in the 4-hole condition. Together, these findings suggest that copulation in environments in which access to the female is restricted facilitates the development of partner preference in males for a familiar female. We also noted that the development of this preference only occurs when the familiar female is of the same strain as the male (Ismail, Jones, Graham, Sylvester, & Pfaus, in preparation, Chapter 4). In an attempt to identify the neural circuits involved in the development of partner preference, we examined the effects of flupenthixol, a non-specific dopamine (DA) receptor antagonist and the effects of naloxone, a non-specific opioid receptor antagonist, administered before each conditioning trial. We observed that, contrary to flupenthixol treated males, those pretreated with naloxone failed to develop conditioned partner preference for their familiar female. These findings suggest that intact opioid systems but not dopaminergic systems are necessary for the development of partner preference for their familiar female (Ismail, Girard-Bériault, Nakanishi & Pfaus, in preparation, Chapter 5).

Although the brain areas important for the conditioning of a neutral odor in males have been identified (see Kippin, Cain & Pfaus, 2003), the brain areas involved in the development of partner preference for a familiar female remain to be investigated. The objective of the present study was to examine the brain areas that are activated when males are exposed to the almond-odor alone or to their familiar almond- scented female behind a mesh screen following copulatory training in 1-hole or 4-hole pacing chambers. We also investigated the pattern of Fos induction following exposure to a conditioned odor in males treated with naloxone or flupenthixol during conditioning.

EXPERIMENTAL PROCEDURES

Animals and Surgery

Sexually naïve Long Evans male rats (about 300 grams) and Long Evans and Wistar female rats, were purchased from Charles River Laboratories (St-Constant, Quebec). Rats were housed in groups of four, in plastic solid floor cages, with free access to food (Purina Rat Chow) and water, and maintained on a 12-hour dark/light cycle (lights on at 8 pm) in a temperature-controlled room (21 ± 2°C). Behavioral testing was conducted in the dark phase of the cycle. All animal procedures were approved by the Concordia University Animal Research Ethics Committee in compliance with the guidelines of the Canadian Council on Animal Care. All possible efforts were made to reduce the number of animals used and to minimize their suffering.

Females were anaesthetized with a mixture of ketamine (50 mg/ml) and xylazine hydrochloride (4 mg/ml), at a ratio of 4:3, respectively, administered intraperitoneally in a volume of 1 ml/kg of body weight. Anaesthetized females were then bilaterally

ovariectomized via a lumbar incision. All females were given one week of postsurgical recovery prior to sexual training. Following recovery, all females were placed in a pacing chamber with intact and sexually vigorous males during 4 sexual training trials. For all behavioral tests, sexual receptivity was induced by priming the females with an injection of 10 μg (sc in 0.1 ml of sesame oil) of estradiol benzoate (EB) 48 hrs and 500 μg (sc in 0.1 ml of sesame oil) of progesterone (P) 4 hrs prior to behavioral testing. On alternate trials, females were subcutaneously injected with haloperidol (0.2 mg/ml) one hour before conditioning.

Odor Conditioning

Copulatory conditioning trials took place in rectangular chambers (46 cm x 39 cm x 37 cm) covered with a grid floor and bedding. Sexually naïve males were randomly assigned to one of four groups. One group was given ten copulatory trials in a pacing chamber bisected by a 1-hole divider with the same almond-scented female at each trial (1-hole paired). The second group was given ten copulatory trials in a 1-hole pacing chamber with a random almond scented female at every trial (1-hole random). The third group was placed with the same almond-scented female at every trial for ten trials in a 4-hole pacing chamber (4-hole paired). Lastly, the fourth group was placed with a random almond-scented female at every trial in a 4-hole pacing chamber (4-hole random). For all conditioning trials, males were placed individually into the chamber for 5 min, after which the same or a random almond-scented female was placed into the pacing chamber for 20 min. Females were primed with EB and P as described above. Conditioning trials occurred at 4-day intervals during the middle third of the rat's dark cycle following hormone priming.

Odor Conditioning under Flupenthixol or Naloxone pretreatment

Conditioning procedure was similar to that of the odor conditioning without pretreatment, in that sexually naïve males were randomly assigned to one of three groups. The first group of males was pretreated with Flupenthixol and was assigned to copulate in a 1-hole pacing chamber with the same almond-scented female for ten trials. The second group was pretreated with naloxone and was assigned to copulate with the same almond-scented female in a 1-hole pacing chamber. The third group was pretreated with saline as a control and was assigned to copulate with the same almond-scented female in a 1-hole pacing chamber for 10 conditioning trials. Conditioning trials again occurred at 4-day intervals, during which males were placed individually for 5 minutes. Then, the females were placed into the cage for 20 minutes.

Activation of Fos-IR by odor cue alone or by an almond-scented female

Each odor conditioned male was placed in a chamber where half of the males were exposed to the almond-odor alone on a cotton gauze and the other half to an almond-scented EB and P-primed female behind a wire mesh screen to avoid all physical contact with the cotton gauze or the female for 60 minutes undisturbed. The flupenthixol- and naloxone-pretreated males were exposed to the almond-odor alone on a cotton gauze behind a mesh screen for 60 min undisturbed. This allowed for auditory, visual and olfactory cues to be detected but prevented physical contact. At the termination of the 60 min period, males were injected with an overdose of sodium pentobarbital (120 mg/kg, i.p.) and intracardially perfused with 250 ml of phosphate buffered saline followed by 250 ml of 4% paraformaldehyde. Brains were carefully extracted and post-fixed in 4%

paraformaldehyde for 4 hours and stored overnight in 30% sucrose solution. Then, brains were then stored at -80°C until sectioning.

Fos immunocytochemistry

Each brain was sliced on a coronal plane at 30 μm from the accessory olfactory bulb to the VTA, corresponding to plates 4-44 of Paxinos and Watson (1998), using a cryostat. The sliced sections were incubated first in a mixture of 30% hydrogen peroxide (H_2O_2) in Tris-buffered saline (TBS) for 30 min at room temperature. Then, the sections were incubated in a solution of 3% normal goat serum (NGS) in 0.2% Triton TBS for two hours at room temperature. Subsequently, the sections were incubated in a mixture of 3% NGS and in 0.05% Triton TBS and rabbit polyclonal anti-Fos antibody (Calbiochem, Gibbstown, New Jersey, USA) diluted in a concentration of 1:10,000 for 72 hours at 4°C. At the end of the 72-hour period, the sections were incubated in a mixture of 3% NGS and biotinylated goat anti-rabbit IgG (Vector laboratories, Burlingame, CA, USA; 1:200) in 0.05% Triton TBS for one hour at 4°C after which the sections were incubated in a solution of 3% NGS and avidin-biotinylate-peroxidase complex (Vectastain Elite ABC Kit, Vector Laboratories; diluted 1:55) for 2 hours at 4°C in 0.2% Triton TBS. Sections were washed in TBS three times for 5 min each between each incubation. To stain the immunoreactions, the sections were incubated at room temperature in 50-mM Tris buffer for 10 min, 3,3'-diaminobenzidine (DAB) in 50-mM Tris for 10 min and then in a mixture of DAB and 3% H_2O_2 and 8% nickel chloride for 10 min. Then, all the sections were mounted on gel-coated slides and allowed to dry for at least 24 hours. The slides were dehydrated in increasing concentrations of alcohol 70%, 90% and 100% and cleared in Xylenes for 2 hours, cover-slipped and examined under a Zeiss light microscope.

Histological and Statistical Analyses

For quantification of the numbers of Fos-IR neurons following the different behavioral situations, Fos-IR were counted the accessory olfactory bulb (AOB), main olfactory bulb (MOB), nucleus accumbens core (NAcc), nucleus accumbens shell (NAcsh), lateral septum (LS), piriform cortex (Pircx), olfactory tubercle (Tu), caudate putamen (CPu), ventral pallidum (VP), suprachiasmatic nucleus (SCN), supraoptic nucleus (SON), paraventricular nucleus (PVN), medial preoptic area (MPOA), arcuate nucleus (Arc), ventral medial hypothalamus (VMH), basolateral amygdala (BLA), posterodorsal medial amygdala (MeApd), medial habenula (MHb), lateral habenula (LHb) and ventral tegmental area (VTA) in a standard area of 400 μm^2, in five adjacent sections representative for each brain, using Scion Image. The number of Fos positive cells were counted compared between all four groups using ANOVA followed by a Tukey post-hoc test. The criterion for statistical reliability for all comparisons was set to $p < 0.05$.

RESULTS

Brain activation by an almond-scented female

One-way ANOVAs revealed significantly more Fos-positive neurons in the VTA ($F(3,16) = 5.49, p < 0.05$), the Arc ($F(3,14) = 5.42, p < 0.05$), the PVN ($F(3,12) = 10.65, p < 0.05$) and the PDMA ($F(3,15) = 3.51, p < 0.05$) of males conditioned to copulate in a 1-hole pacing chamber with the same almond-scented female. Moreover, in these males, there was a tendency towards more Fos in the Pircx ($F(3, 14) = 3.0, p < 0.1$) and in the LS ($F(3, 15) = 2.89, p < 0.1$). Post-hoc Tukey pairwise comparisons revealed a significant difference in Fos-positive cells in the VTA between the 1-hole paired group and the 4-

hole paired group (Mean difference = 10.12, $p < 0.05$) and between the 1-hole paired group and the 4-hole random group (Mean difference = 9.86, $p < 0.05$). Pairwise comparisons also revealed significantly more Fos-positive neurons in the 1-hole paired group than in the 4-hole random group in the Arc (Mean difference = 26.62, $p < 0.05$). In the PVN, Tukey pairwise comparisons revealed significantly more Fos-positive cells in the 1-hole paired group than in the 1-hole random group (Mean difference = 9.94, $p < 0.05$), 4-hole paired group (Mean difference = 10.23, $p < 0.05$) and 4-hole random group (Mean difference = 17.18, $p < 0.05$). In the PDMA, the pairwise comparisons revealed that the 1-hole paired group displayed significantly more Fos-positive cells than the 4-hole random group (Mean difference = 21.19, $p < 0.05$). Tukey pairwise comparisons also revealed that 1-hole paired group tended to display more Fos-positive neurons than the 4-hole random group in the Pircx (Mean difference = 31.38, $p < 0.1$) and in the LS (Mean difference = 15.29, $p < 0.05$) (Figures 34 and 35). There were no significant differences in the MHb, LHb, SCN, MOB, AOB and NAcccore, NAccsh, MPOA, BLA, VP, CPu, VMH, SON, Tu (Table 2).

Brain activation by the almond odor alone following conditioning

One-way ANOVAs revealed a significant difference in the number of Fos-positive cells in the VTA ($F(3,11) = 12.03$, $p < 0.05$). The ANOVAs also revealed a tendency towards a significant difference in the Pircx ($F(3,14) = 2.56$, $p < 0.1$) and in the MPOA ($F(3,12) = 2.63$, $p < 0.1$). Tukey pairwise comparisons showed that the 1-hole

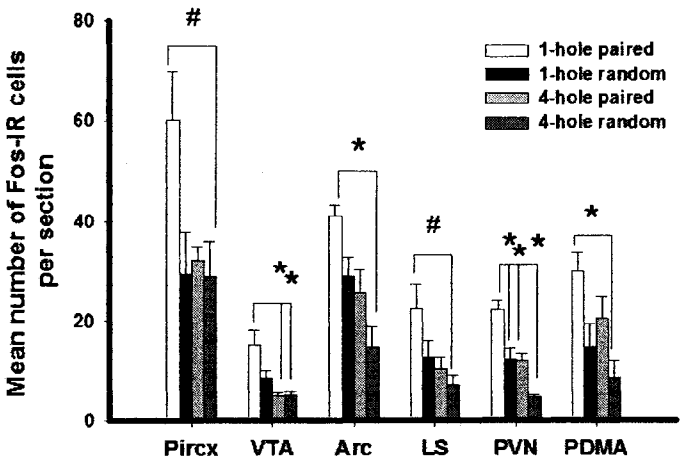

Figure 34. Brain areas of male rats that expressed difference in Fos-IR following exposure to an almond-scented female. The bars represent the mean ± SEM number of Fos-IR cells within a sample area of the same dimensions for all regions (Pircx: piriform cortex, VTA: ventral tegmental area, Arc: arcuate nucleus of the hypothalamus, LS: lateral septum, PVN: paraventricular nucleus of the hypothalamus, PDMA: posterodorsal medial amygdala).
* $p < 0.05$; # $p < 0.1$, between 1-hole and 4-hole paired and random groups.

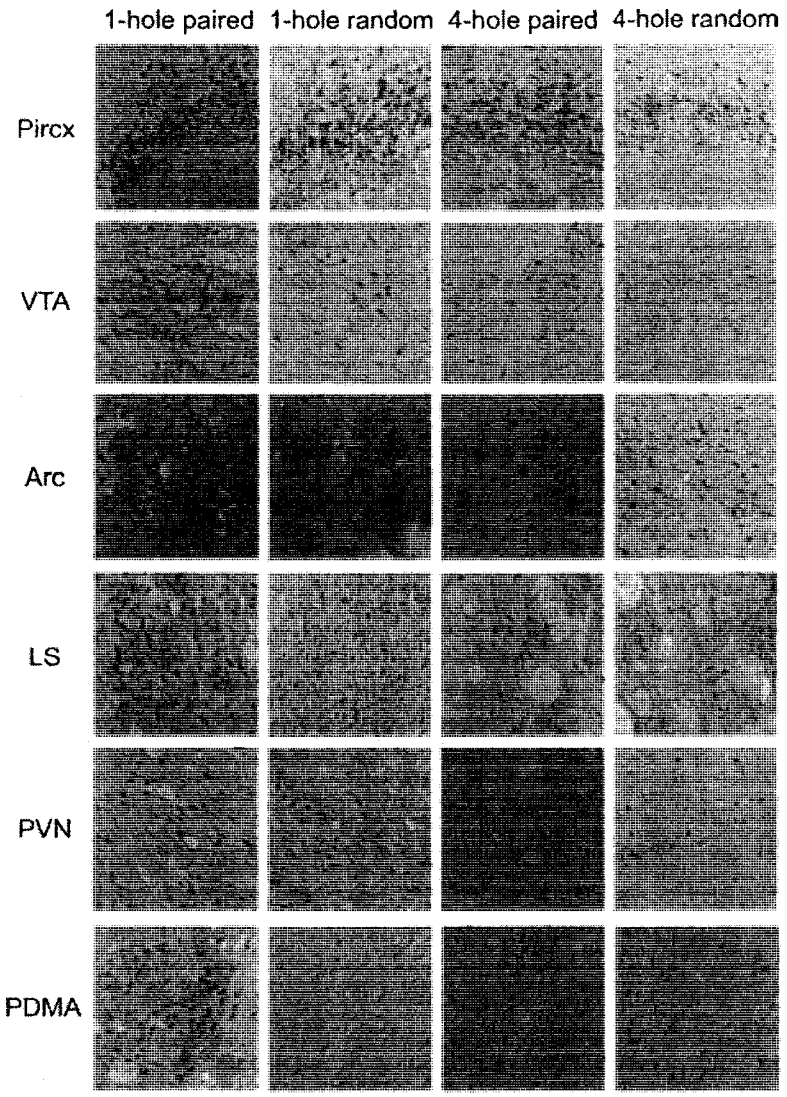

Figure 35. Brain areas that express significantly more Fos-IR in male rats in the 1-hole paired group relative to the 1-hole random and 4-hole paired and random groups following exposure to an almond-scented female behind a screen. (Pircx: piriform cortex, VTA: ventral tegmental area, Arc: arcuate nucleus of the hypothalamus, LS: lateral septum, PVN: paraventricular nucleus of the hypothalamus, PDMA: posterodorsal medial amygdala) (all images were captured at 400 x 400 μm).

Table 2. Brain areas of male rats that expressed Fos-IR following exposure to an almond-scented female

Area	1-hole paired	1-hole random	4-hole paired	4-hole random
MHb	11.45±2.30	11.80±2.69	9.75±3.42	5.46±1.22
LHb	11.55±2.21	9.13±3.59	5.45±1.06	7.28±2.54
SCN	56.57±14.89	34.72±7.47	21.75±5.67	19.75±2.53
MOB	66.45±12.55	73.57±19.71	55.27±11.32	60.12±12.45
AOB	68.7±13.90	42.33±9.48	48.75±22.66	26.03±7.90
NAcccore	22.23±3.53	19.48±5.21	17.42±5.60	10.74±3.37
Naccsh	29.95±6.20	13.88±4.50	13.52±2.32	13.15±4.30
MPOA	16.23±3.62	16.80±4.83	22.18±4.27	12.65±4.07
BLA	14.68±3.74	13.06±4.05	6.80±1.20	5.80±1.84
VP	20.33±2.15	21.32±3.26	9.98±2.84	20.15±4.75
Cpu	24.45±7.66	11.08±5.54	7.87±2.64	5.65±2.46
VMH	18.55±5.18	12.22±5.39	7.80±1.70	10.52±3.38
SON	14.43±6.03	11.58±1.09	15.08±6.82	3.90±0.61
Tu	51.06±7.12	40.18±10.80	35.33±10.01	30.54±7.16

Table 2. The mean number of Fos-IR cells in all the regions that did not display significant differences between the groups. The data are expressed as means±SEM.

paired group had significantly more Fos-positive neurons than the 1-hole random group (Mean difference = 19.25, p <0.05), 4-hole paired (Mean difference = 18.84, $p < 0.05$) and 4-hole random groups (Mean difference = 21.18, $p < 0.05$) in the VTA (Figures 36 and 37). There were no significant differences in the Arc, MHb, LHb, SCN, MOB, AOB, NAccore, Naccsh, LS, BLA, PVN, VP, CPu, VMH, PDMA, SON and Tu (Table 3).

Brain activation by the almond odor alone following conditioning with flupenthixol or saline pretreatment.

ANOVAs revealed a significant difference in the number of Fos-positive neurons between the males pretreated with flupenthixol and those pretreated with saline in the VTA ($F(1, 8) = 6.24$, $p < 0.05$) and a tendency towards a significant difference in the Arc ($F(1,8) = 3.59$, $p < 0.1$) and in the NAcccore ($F(1, 8) = 3.46$, $p < 0.1$) (Figures 38 and 39). No differences were found in the Pircx, MHb, LHb, SCN, MOB, AOB, NAccsh, MPOA, LS, BLA, PVN, VP, CPu, VMH, PDMA, SON and Tu (Table 4).

Brain activation by the almond odor alone following conditioning with naloxone or saline pretreatment.

One-way ANOVAs revealed a significant difference in the number of Fos-positive cells between males pretreated with naloxone and those pretreated with saline in Arc ($F(1, 7) = 7.56$, $p < 0.05$) and a tendency towards a significant difference in the VTA ($F(1, 5) = 6.12$, $p < 0.1$) (Figures 40 and 41). No differences were found in the Pircx, MHb, LHb, SCN, MOB, AOB, NAcccore, NAccsh, MPOA, LS, BLA, PVN, VP, CPu, VMH, PDMA, SON, and Tu (Table 5).

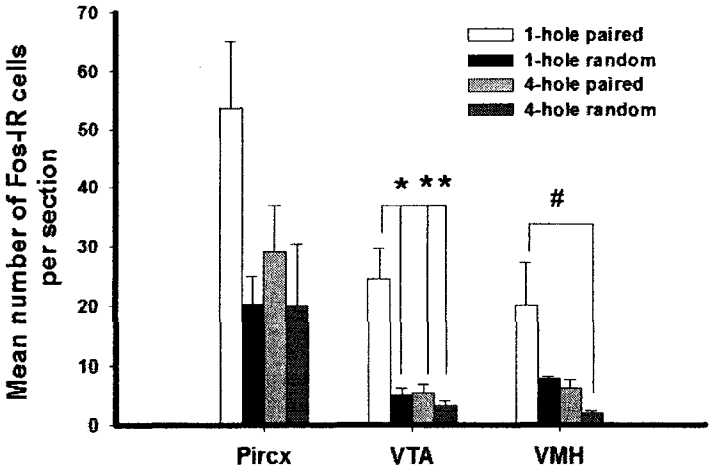

Figure 36. Brain areas of male rats that expressed difference in Fos-IR following exposure to the almond odor. The bars represent the mean ±SEM number of Fos-IR cells within a sample area of the same dimensions for all regions (Pircx: piriform cortex, VTA: ventral tegmental area, VMH: ventromedial nucleus of the hypothalamus). * $p < 0.05$; # $p < 0.1$, between 1-hole and 4-hole paired and random groups.

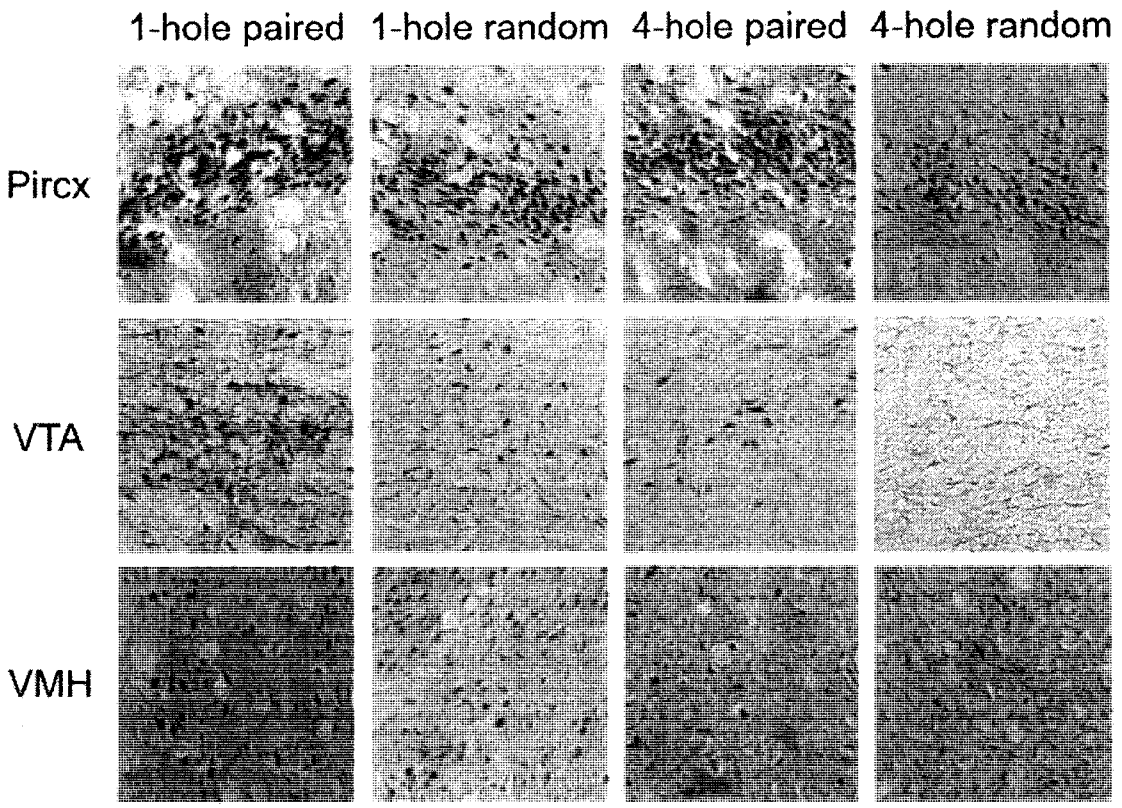

Figure 37. Brain areas that express significantly more Fos-IR in male rats in the 1-hole paired group relative to the 1-hole random and 4-hole paired and random groups following exposure to the conditioned almond odor behind a screen. (Pircx: piriform cortex, VTA: ventral tegmental area, VMH: ventromedial nucleus of the hypothalamus) (all images were captured at 400 x 400 μm).

Table 3. Brain areas of male rats that expressed Fos-IR following exposure to the almond odor

Area	1-hole paired	1-hole random	4-hole paired	4-hole random
Arc	29.90±7.49	29.46±10.56	33.15±5.95	20.25±4.84
MHb	15.60±9.63	6.43±1.54	13.08±4.54	6.06±2.16
LHb	16.35±4.52	9.73±2.19	8.54±2.06	4.80±1.82
SCN	33.80±5.12	20.65±1.36	34.10±10.03	23.58±7.74
MOB	60.87±15.24	58.13±9.39	61.08±20.00	31.70±8.34
AOB	27.07±9.08	12.00	33.20±7.14	5.30
Nacccore	22.80±5.03	18.68±2.37	16.86±4.56	14.28±4.76
Naccsh	19.06±3.52	14.90±4.86	14.82±3.85	12.20±5.35
LS	33.15±15.48	9.93±1.22	16.88±3.07	13.50±4.98
BLA	9.58±1.88	7.85±3.06	4.48±1.43	6.12±2.69
PVN	27.70±12.67	7.30±1.08	8.46±2.51	3.93±1.36
VP	13.54±4.35	9.90±0.82	15.86±6.80	8.50±3.56
Cpu	19.00±6.45	13.50±2.10	7.68±4.83	11.75±4.87
VMH	20.03±7.20	7.87±0.51	6.26±1.55	2.03±0.43
PDMA	20.50±7.44	9.00±0.48	10.46±2.50	8.00±4.36
SON	13.67±3.72	8.10±0.47	10.40±2.64	5.00±1.41
Tu	42.74±13.96	33.57±7.25	36.12±6.27	21.43±7.25

Table 3. The mean number of Fos-IR cells in all the regions that did not display significant differences between the groups. The data are expressed as means±SEM.

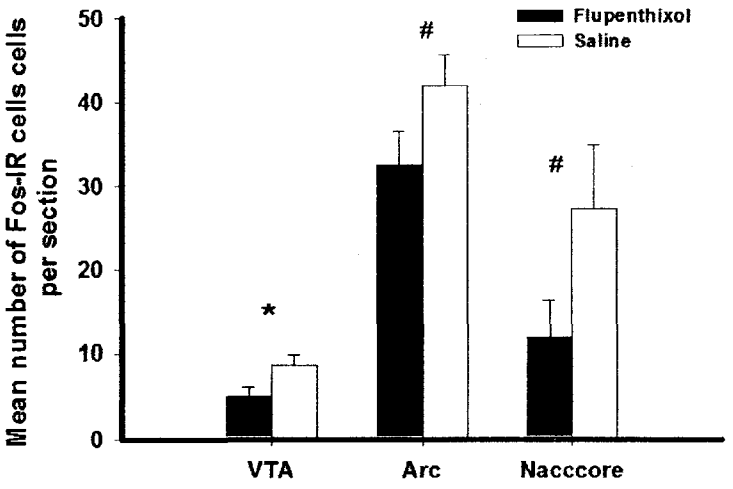

Figure 38. Brain areas of male rats that expressed difference in Fos-IR following exposure to the almond odor. The bars represent the mean ±SEM number of Fos-IR cells within a sample area of the same dimensions for all regions (Pircx: piriform cortex, VTA: ventral tegmental area, NAcccore: nucleus accumbens core). *$p < 0.05$; # $p < 0.1$, between flupenthixol and saline groups.

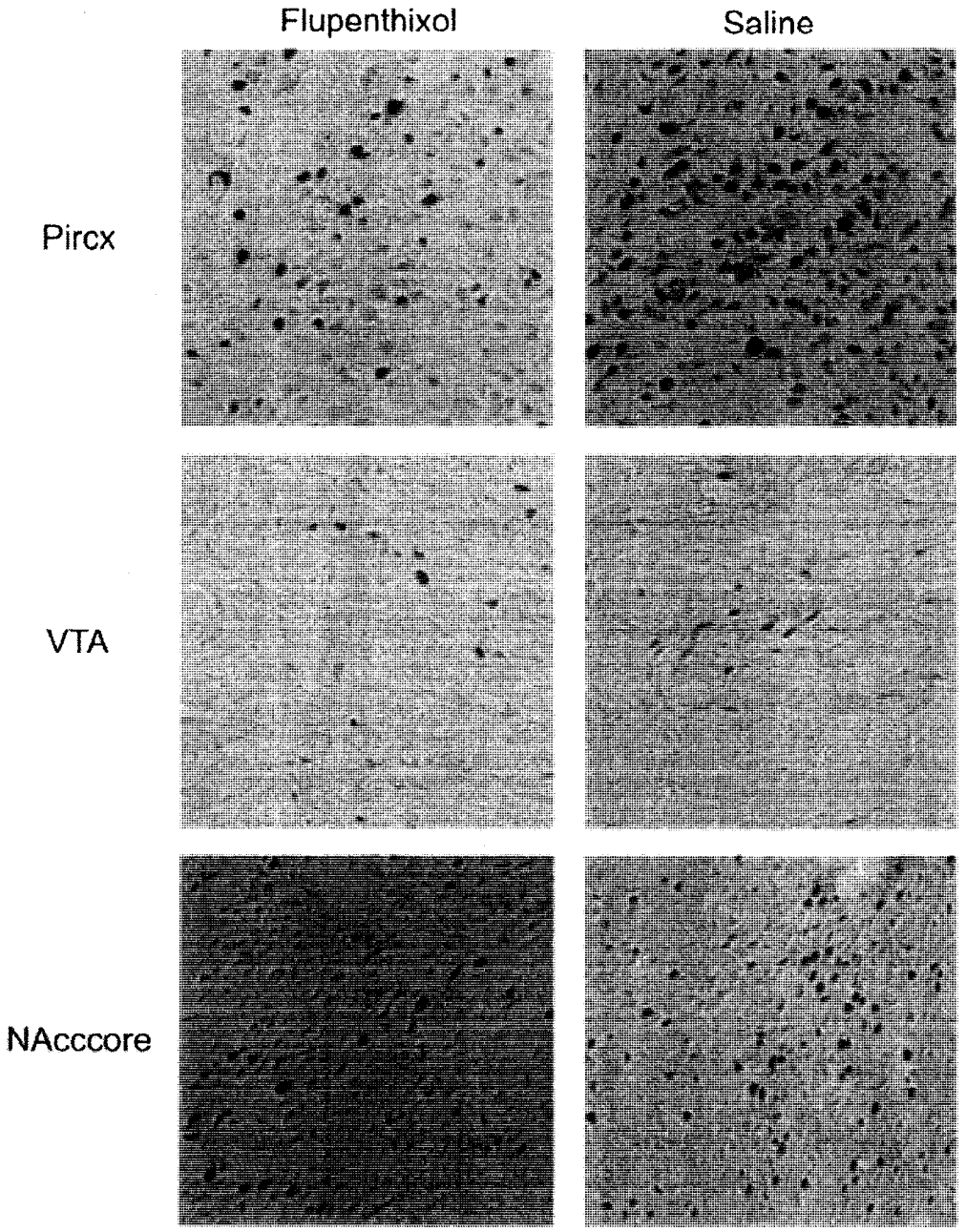

Figure 39. Brain areas that express significantly more Fos-IR in male rats previously treated with saline relative to those treated with flupenthixol following exposure to the conditioned almond odor behind a screen. (Pircx: piriform cortex, VTA: ventral tegmental area, NAcccore: nucleus accumbens core) (all images were captured at 400 x 400 μm).

Table 4. Brain areas of male rats that expressed Fos-IR following exposure to the almond odor

Area	Flupenthixol	Saline
Pir	49.08±10.20	64.18±8.31
MHb	12.86±5.88	5.32±0.93
LHb	22.14±4.53	18.60±2.53
SCN	16.18±2.19	38.34±13.45
MOB	66.06±19.57	71.90±10.93
AOB	69.23±7.29	30.50±
Naccsh	9.48±1.90	18.20±6.92
MPOA	10.60±2.53	16.86±3.69
LS	10.92±3.46	18.20±4.12
BLA	7.78±3.02	11.88±3.97
PVN	19.34±3.96	17.74±4.53
VP	26.06±8.24	24.28±4.99
Cpu	10.40±1.36	25.28±10.28
VMH	8.78±1.76	13.20±2.60
PDMA	19.94±3.66	24.26±4.54
SON	6.04±1.25	7.54±1.48
Tu	52.54±10.94	37.68±8.57

Table 4. The mean number of Fos-IR cells in all the regions that did not display significant differences between the groups. The data are expressed as means±SEM.

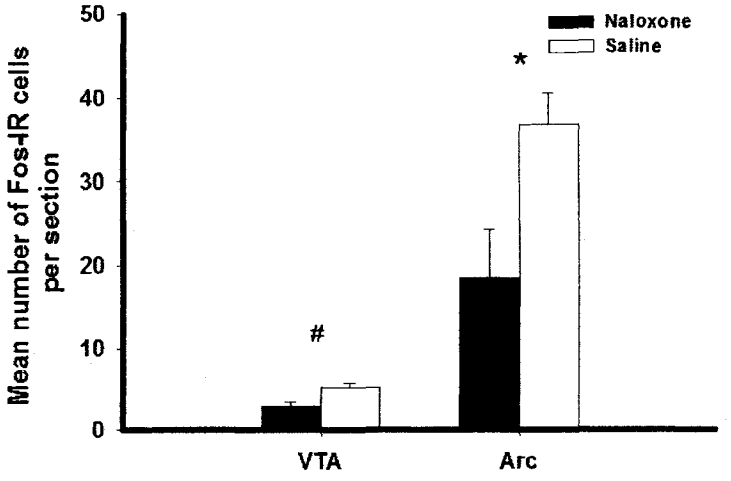

Figure 40. Brain areas of male rats that expressed difference in Fos-IR following exposure to the almond odor. The bars represent the mean ±SEM number of Fos-IR cells within a sample area of the same dimensions for all regions (VTA: ventral tegmental area, Arc: arcuate nucleus of the hypothalamus). * $p < 0.05$; # $p < 0.1$, between flupenthixol and saline groups.

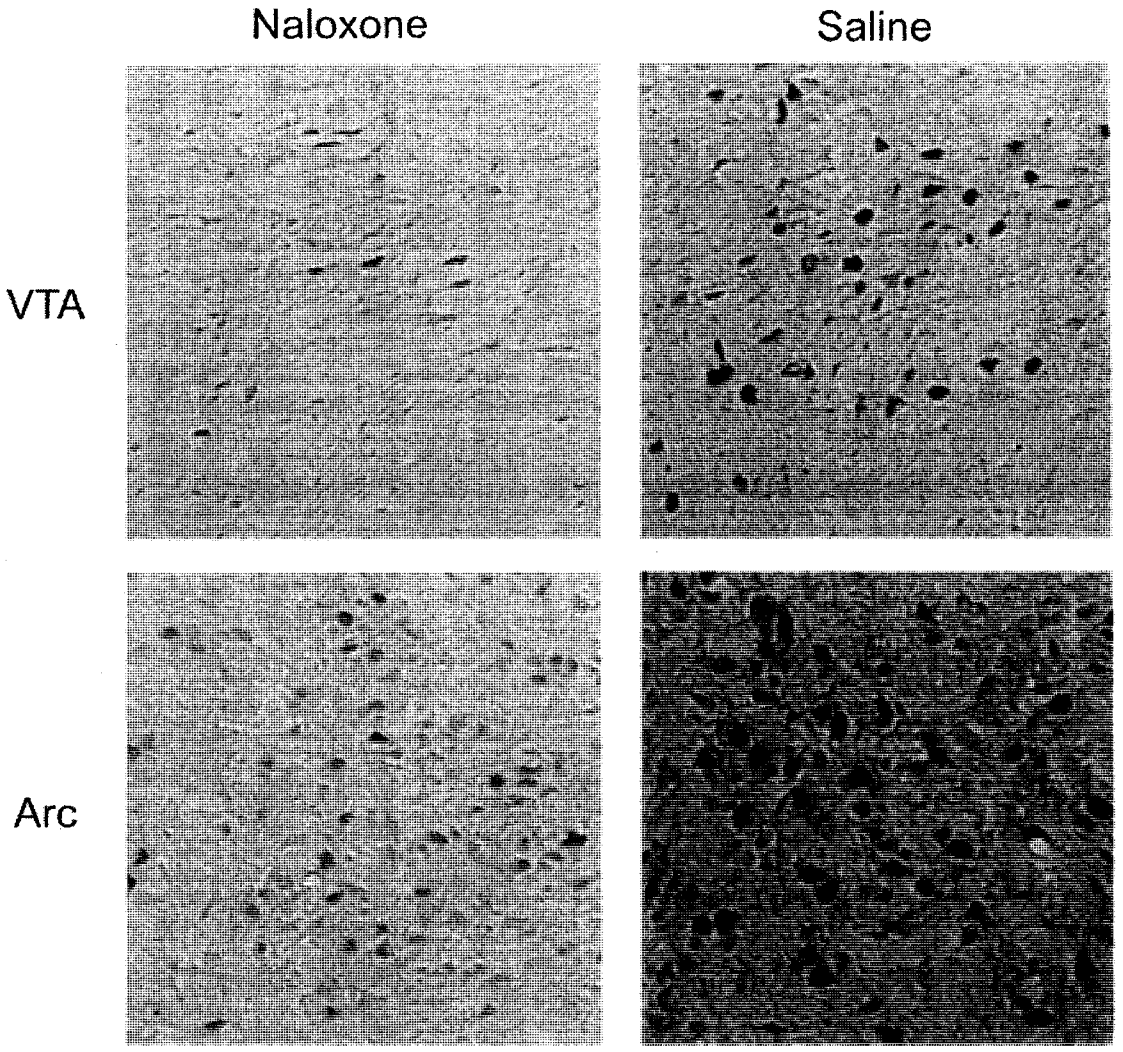

Figure 41. Brain areas that express significantly more Fos-IR in male rats previously treated with saline relative to those treated with naloxone, following exposure to the conditioned almond odor behind a screen. (VTA: ventral tegmental area, Arc: arcuate nucleus of the hypothalamus) (all images were taken at 400 x 400 μm).

Table 5. Brain areas of male rats that expressed Fos-IR following exposure to the almond odor

Area	Naloxone	Saline
Pir	42.85±11.64	51.98±7.35
MHb	6.23±3.38	5.78±0.97
LHb	16.50±6.34	11.35±2.94
SCN	30.57±10.87	34.00±2.37
MOB	61.10±24.33	70.15±4.52
AOB	10.00	37.00
Nacccore	27.60±7.69	26.80±1.72
Naccsh	17.35±4.96	16.70±2.13
MPOA	19.80±6.79	25.77±0.58
LS	24.15±8.86	34.82±6.28
BLA	10.33±4.37	17.16±3.28
PVN	12.43±2.50	18.67±0.91
VP	18.00±5.12	15.84±1.95
Cpu	15.65±3.46	25.14±3.75
VMH	7.48±4.23	5.93±1.44
PDMA	16.57±6.21	31.12±6.58
SON	7.67±4.13	12.87±3.16
Tu	25.83±5.69	34.50±6.51

Table 5. The mean number of Fos-IR cells was taken from a sample area of the same dimensions for all the regions that did not display significant differences between the groups. The data are expressed as means±SEM.

DISCUSSION

The present study was designed to identify brain areas activated by olfactory and partner cues that direct the expression of a CEP. We first examined neural activation following exposure to an almond-scented female behind a mesh screen in males conditioned to copulation with the same almond-scented female in a 1-hole pacing chamber (1-hole paired group), in males conditioned to copulate with a random almond-scented female in a 1-hole pacing chamber (1-hole random group), in males conditioned to copulation with the same almond-scented female in a 4-hole pacing chamber (4-hole paired group), and in males conditioned to copulate with a random almond-scented female in a 4-hole pacing chamber (4-hole random group). The results showed that there was more Fos-immunoreactivity (Fos-IR) in the 1-hole paired group in the VTA, Arc, PVN and PDMA and there was a trend for significance in the Pircx and LS. These results suggest that these areas are involved in the development and expression of CEP for a familiar female. Then, the pattern of Fos-IR following exposure to the conditioned almond odor alone on a cotton gauze behind a mesh screen was also examined. The results showed that males in the 1-hole paired group displayed significantly more Fos-positive cells in the VTA and a trend for significance in the Pircx and the MPOA. Interestingly, when males are pretreated with flupenthixol before each conditioning trial, no differences were observed in their ability to develop CEP for their familiar female, but they displayed significantly less Fos-IR in the VTA and a trend towards significantly less Fos-IR in the Arc and the NAcccore than saline-treated animals following exposure to the almond odor alone on a gauze pad behind a screen. In comparison, males pretreated with naloxone before each conditioning trial, failed to develop CEP for their familiar females

and displayed significantly fewer Fos-positive cells in the Arc and a trend towards fewer Fos-positive cells in the VTA following exposure to the almond odor on a gauze pad. Taken together, these findings suggest that multiple areas show Fos-IR following conditioning with an almond-scented familiar female in a 1-hole pacing chamber. However, the VTA and the Arc showed the most consistent Fos-IR across the different studies, suggesting that these two areas play a critical role in the development of partner preference for a familiar female.

The VTA contains the cell bodies of dopamine (DA) neurons that project throughout the limbic system (Fallon & Moore, 1978; Phillipson, 1979). The mesolimbic DA system plays an important role in directing attention towards reward-related stimuli (Robinson & Berridge, 1993). For instance, DA transmission is increased during exposure to estrous odors in sexually naïve and experienced male rats (Mitchell & Gratton, 1991, 1992; Wenkstern, Pfaus & Fibiger, 1993), suggesting that DA release sensitizes with experience. These findings suggest that dopaminergic neurons in the VTA play an important role in male sexual behavior. Given that in the present study males that developed CEP for their familiar female displayed more Fos-IR in the VTA than males in the other groups, it is possible that this increase in Fos-IR be due to the stimulation of DA neurons in the VTA making these conditioned males more attentive to their familiar female or to the odor associated with copulation with their familiar female.

The dopaminergic projections in the VTA are under tonic inhibition by local GABAergic interneurons (Balfour, Yu and Coolen, 2004). Electrophysiological and pharmacological studies have shown that stimulation of the G-coupled μ-opioid receptor inhibits these GABAergic interneurons which in turn disinhibits dopaminergic

projections in the VTA (Matthews & German, 1984; Johnson & North, 1992; Klitenick, DeWitte & Kalivas, 1992; Ikemoto, Kohl and McBride, 1997). Moreover, injection of opioid receptor agonist into the VTA facilitates sexual behaviors and sexual arousal in hypo-arousal castrated male rats (Mitchell & Stewart, 1990). Thus, increased Fos-IR in the VTA following exposure to estrous or conditioned odors of males that developed CEP could also be due to the possibility that exposure to these odors stimulates opioid neurons in the VTA. This is consistent with our previous finding (Ismail, Girard-Bériault, Nakanishi & Pfaus, in preparation) that administration of an opioid-receptor antagonist before each conditioning trial blocks the development of CEP for a familiar female, suggesting that intact opioid system, perhaps in the VTA, is necessary for the development of partner preference for a familiar female.

Similar to the VTA, the Arc was also found to display increased Fos-IR in conditioned animals in our present study. The Arc nucleus contains most of the neural circuitry required for the regulation of metabolism and reproduction (Chen, Tsai, Yeh, Tai & Tsai, 2007). The Arc has been reported to contain DA (Hoffman & Sladek, 1980) and noradrenergic terminals (Kizer, Muth & Jacovowitz, 1976). Although neurons in the Arc are known to project to the MPOA (Leranth, MacLusky, Shanabrough & Naftolin, 1988), an area known to play a crucial role in male sexual behavior (Hull, Meisel & Sachs, 2002), the actual function of the Arc in male sexual behavior remains to be investigated. At the moment, it is known that there are age-related changes in the activity and number of DA-containing neurons in the Arc (Hoffman & Sladek, 1980), which may partially account for the age-related decline in copulatory behavior in males. In fact, Chen and colleagues (2007) found that adequate levels of DA tissue are necessary in the

Arc and the MPOA for males to display sexual behavior. Furthermore, like the VTA, the Arc also contains μ-opioid-receptors. Prenatal activation of μ-opioid-receptors in the Arc alters male sexual behavior (Bonelli & Vannelli, 1983; Siddique, Haq & Shah, 1997). Together, these findings suggest that opioid and dopamine systems in the Arc may also play an important role in the development of partner preference in males.

The results of our present study are not consistent with those of Kippin and colleagues (2003) who failed to find increase Fos-IR in the VTA and the Arc following exposure to a conditioned odor. These differences could perhaps be due to methodological differences between the two studies. While in the present study males were conditioned to copulate in pacing chambers, Kippin and colleagues conditioned their males in bi-level chambers. In bi-level chambers, males can chase the female to mate with her but in pacing chambers, males are required to wait for the female to return to their side to copulate. This results in different patterns of copulation and different levels of arousal experienced during sexual behavior in these chambers (see Ismail, Zhao & Pfaus, 2008). Moreover, Kippin and colleagues applied the conditioned odor in the bedding while in the current study the odor was presented on a cotton gauze behind a mesh screen. This may explain the broader neural activation in Kippin and colleagues' study as males were not prevented from interacting with the scented bedding. However, the results of our present study are consistent with those of Kippin and colleagues when they examined Fos-IR following exposure to estrous odors. They reported increased Fos-IR in a number of areas, namely in the VTA. These findings suggest that the VTA plays an important role in attending to natural odors, such as estrous odors in conditioned males.

The findings of our present experiment are also consistent with those of Coria-Avila and Pfaus (2007) in female rats. As in the present study, Coria-Avila and Pfaus also used paced copulation to condition their subjects and examined the brain areas involved in the development of partner preference by exposing the females to the conditioned odor behind a mesh screen. They reported increased Fos-IR in a number of areas such as Pircx, MPOA and VTA. The relationship between those regions that subseve the perception of the reward state (e.g., mPOA, VTA), the perception of the conditioned olfactory stimuli (mPOA, PIR), and an integrator of the unconditioned and conditioned stimuli (mPOA, NAc) was presented as a neuronal model of the development of sexually conditioned place and partner preference following paced copulation. This model may well apply to male rats, although it may include several other regions along with some sex-related differences in the way unconditioned cues (estrous odors, strain cues) are processed.

In summary, the findings of our present study suggest that among other areas, the VTA and the Arc seem to play an important role in the development of partner preference for a familiar almond-scented female following 1-hole paced copulation.

GENERAL DISCUSSION

The experiments in this dissertation are among the first to demonstrate that context, level of sexual arousal, female availability, and intensity of sexual reward, play a role in the development of male copulatory behavior and conditioned partner preference The results of Chapter 1 showed that males trained in 1-hole pacing chambers displayed longer ejaculation latencies than males trained to copulate in 4-hole pacing chamber. When the animals' environments were switched, they maintained the differences in ejaculation latency, suggesting that the pattern of copulation had become conditioned by the initial environment of copulation in males. Moreover, the partner preference test demonstrated that males prefer to copulate with females associated with 4-hole paced copulation over those associated with 1-hole paced copulation. These findings suggest that males prefer to copulate in environments in which the female is more accessible. The results of Chapter 2 revealed that only males trained to copulate in a 1-hole pacing chamber with the same almond scented female developed CEP for their familiar almond-scented female. Males trained in 4-hole pacing chambers failed to develop such preference, suggesting that environments that restrict access to the female facilitate the development of partner preference in males. In Chapter 3, the results showed that males exposed to both saline-treated and haloperidol-treated females develop CEP for females associated with copulation with haloperidol-treated females, suggesting that female proceptive behaviors do not play an important role in the development of CEP in males. In Chapter 4, the findings demonstrated that males only develop CEP for their familiar female following 1-hole paced copulation if the female is of their own strain. These results suggest that conditioning did not override the innate preference of males for

assortative mating. The results of Chapter 5 showed that naloxone but not flupenthixol blocked the development of CEP for a familiar female, suggesting that intact opioid but not dopamine system is necessary for the development of partner preference in males. In Chapter 6, examination of Fos-IR following exposure to an almond-scented female or to the almond-odor alone revealed that the VTA and the Arc play an important role in the development of CEP for a familiar female. Taken together, the results collected in this dissertation suggest that the context of copulation, which consists of sexual arousal, the intensity of sexual reward, and female availability or accessibility during copulation, plays an important role in directing the acquisition of male sexual behavior and partner preferences.

First and foremost, context influences the acquired pattern of copulation. For instance, males trained by Pfaus and Phillips (1991) to copulate in bi-level chambers ejaculated more rapidly (250 sec) than males trained by Vega Matuszcyk, Larsson, and Eriksson (1998) in a circular open field (600 sec). Similarly, Fadem and Barfield observed that males trained in chambers with a 1-hole divider displayed longer intervals between each intromission, longer ejaculation latencies, and longer post-ejaculatory intervals, compared to males tested in the chambers without the divider. In our study (Ismail, Zhao & Pfaus, 2008), we also noted differences in the pattern of copulation acquired in different environments in that males trained in 4-hole pacing chambers ejaculate faster than males trained in 1-hole pacing chambers. These findings suggest that environments in which males have easier access to the females, namely 4-hole pacing chamber, facilitate ejaculation while environments in which access to the female is restricted delay ejaculation, like 1-hole pacing chambers. This could either be due to

more general arousal during copulation in 4-hole pacing chambers or it could be due to the activation of different arousal mechanisms during copulation in 1-hole and 4-hole pacing chambers. Sexual arousal is coordinated by the combined actions of the sympathetic and parasympathetic nervous systems, both of which are activated in different organs during sexual arousal. For example, in males, activation and maintenance of erection is mediated by the parasympathetic division, whereas the concurrent increases in heart and breathing rate are mediated by the sympathetic division. Activation of sympathetic outflow to the penis facilitates ejaculation after which erection is inhibited. Thus, longer ejaculation latency during copulation in 1-hole pacing chambers suggests that males experienced more parasympathetic arousal that maintained erection but delayed ejaculation. In contrast, males in the 4-hole condition may have experienced more sympathetic arousal which could promote faster ejaculation. The fact that ejaculation patterns were maintained despite the switch in environment indicates that the patterns of sexual arousal and behavior are shaped by the environment in which sexual experience is acquired, and that once acquired those patterns become relatively "fixed".

The context of copulation also influences the preference of males for different copulation environments. For instance, males that acquire sexual experience in both 1-hole and 4-hole pacing chambers, display preferences for females paired with copulation in 4-hole pacing chambers possibly because it provides them with easier access to the females (Ismail, Zhao & Pfaus, 2008, Chapter 1). These findings are consistent with those of Martinez and Paredes (2001) that showed using CPP that males prefer to copulate in environments in which they have easier access to females because they can better control the rate of copulation and achieve optimal rates. However, optimality

between access to the female and the environment of copulation differs in different contexts. For example, male rats that are rendered hypo-arousal either by castration (Madlafousek, Hliňák, & Beran, 1976) or mPOA 6-OHDA lesions (Everitt & Stacey, 1987) require being able to chase female partners that display high rates of solicitation and lordosis in order to copulate. These results suggest that, contrary to normal males, sexually hypo-arousal males necessitate female precopulatory behaviors to experience adequate levels of arousal during copulation. Males with "normal" levels of arousal do not appear to require female precopulatory behavior for conditioning to occur. Rather they seem to respond to penile stimulation, although even that may not be required to induce ejaculation once animals have had a sufficient amount of sexual experience (Pfaus, Kippin, & Centeno, 2001).

Because male rats in our first study preferred to ejaculate with females associated with copulation in environments that provided the males with unrestricted access to the female (e.g., the 4-hole condition in the pacing chambers used in the present thesis), it was expected that males would develop partner preference for a familiar female following copulation in those chambers in the second study. However, only males trained in 1-hole condition displayed significant CEP for their familiar almond-scented female over the novel unscented one. This suggested that copulation in environments in which access to the female is restricted facilitates the development of partner preference for a familiar female. One important discrepancy between the two studies is that in the first, males were given experience in both 1-hole and 4-hole paced copulation conditions, whereas in the second males were given exclusive experience in one of the two conditions. It may be the case that when males are allowed to choose between partners

that represent two distinct copulatory experiences, their choice is driven by the experience that is less restrictive. On the other hand, when males have only one reference point for copulatory experience, the role of sexual arousal during copulation may be accorded more weight as a predictor of the reward state. Hints of this exist in the literature. For example, male rats learn to ejaculate with fewer intromissions if the female is removed by the experimenter for a period longer than the male's normal inter-intromission interval, a phenomenon known as the "enforced interval effect" (Larsson, 1956). A similar phenomenon was reported by Silberberg and Adler (1974) in which male rats learned to ejaculate on the 7th intromission when the female was consistently removed for good in previous tests after the 7th intromission. The hyperstimulation of sympathetic arousal that induces ejaculation, however, does not in itself support sexually-conditioned place preference. Camacho and colleagues (2007) found that the facilitation of ejaculation induced by injections of the 5-HT1A receptor agonist 8-OH-DPAT prior to CPP training did not result in CPP.

As noted above, males in the first study in Chapter 1 maintained longer ejaculation latencies in the 1-hole versus 4-hole condition. There may well be an optimal timing of ejaculation that results in a reward state of sufficient intensity to allow conditioning to occur. This state may be driven by the balance between parasympathetic arousal that maintains penile erection and inhibits ejaculation, and sympathetic arousal that brings about ejaculation. It may also be the case that males with more than one reference point for copulatory experience are more naturally aroused by the differences between the experiences relative to males that have only one reference point. This is reminiscent of the differential conditioning described by Pavlov (1927) in which dogs

maintained baseline rates of conditioned responding longer if the conditioning procedures and stimuli they were exposed to changed from trial to trial. Indeed, giving males access to different females associated with each copulatory context may have imposed a "Coolidge effect"; arousal may have increased sufficiently to reveal a reward state associated with unrestricted access to females in which males could copulate at their desired rate. It could be argued then that males given one copulatory reference point may be more dependent on female precopulatory behaviors, and in particular, the ability of females to pace the copulatory contact in a more restrictive way, as in the 1-hole condition in the present studies or in bilevel chambers in the previous studies of Kippin and colleagues. Indeed, pacing conditions affect the timing of ejaculation in pacing chambers (Chapter 1) and bilevel chambers (Pfaus, Smith, & Coopersmith, 1999). Thus it is likely that an optimal level of arousal helps to induce an optimal level of reward.

The development of CEP for a familiar female following 1-hole paced copulation is also restricted to females of the same strain as the males (Ismail, Jones, Graham, Sylvester, & Pfaus, 2008; Chapter 4), perhaps because they have a natural preference for females of their own strain. Early life experiences seem to shape animals to recognize individuals of their own strain and engage in assortative mating. D'Udine & Alleva (1983) showed that early postnatal experiences with parents and siblings induce sexual preference in animals for their own strain. Early postnatal exposure of male rats to a neutral odor smeared on their mother's teats and anogenital regions results in a preference to ejaculation with estrous females bearing this neutral odor. Moreover, mice raised by rat mothers attempt to copulate more with rats than with mice (Lagerspetz & Heino, 1970). These findings are somewhat consistent with those of Coria-Avila and

colleagues (2006) who showed that the development of partner preference in female rats is facilitated when the male partner is of the same strain as the female. However, Coria-Avila and colleagues also showed that female solicitations are most sensitive to the reward context, which for solicitations overrides early experiences that contribute to assortative copulation.

The strength of conditioning is dependent on the intensity of the sexual reward and is manifested in the attention an animal pays to stimuli that predict sexual reward. Two neurochemicals known to be important for the experience of sexual reward are opioids and DA. For example, DA release is increased phasically in both the mPOA and NAc upon presentation of a female or cues associated with sexual reward. During copulation, DA release in these regions occurs continuously until ejaculation, when it declines precipitously back to baseline during the absolute refractory phase, but climbs back up during the relative refractory phase (Blackburn, Pfaus, & Phillips 1992; Pfaus et al., 1990). The dopaminergic projections in the VTA are under tonic inhibition by local GABAergic interneurons (Balfour, Yu and Coolen, 2004). Van Furth and van Ree (1996) showed that endogenous opioids in the VTA contribute to the stimulation of sexual motivation presumably by stimulating DA neurons in the VTA. Electrophysiological and pharmacological studies have shown that stimulation of the G-coupled μ-opioid receptor inhibits these GABAergic interneurons which, in turn, disinhibits dopaminergic projections in the VTA (Matthews & German, 1984; Johnson & North, 1992; Klitenick, DeWitte & Kalivas, 1992; Ikemoto, Kohl and McBride, 1997). Given that opioids and DA are important for the experience of sexual reward and that the development of

conditioning is dependent on sexual reward, it is possible that both DA and opioids play important roles in the development of place and partner preference.

Ågmo and Berenfeld (1990) reported that naloxone blocked the development of place preference; whereas pimozide, a non-specific DA-receptor antagonist, did not affect the development of this preference. These findings suggest that opioid but not DA play an important role in the development of place preference. Similar to the results of Ågmo and Berenfeld, peripheral injections of naloxone during copulatory training in 1-hole pacing chambers in Chapter 5 disrupted the development of partner preference in males, while peripheral injections of flupenthixol failed to disrupt the development of CEP for a familiar female (Ismail, Girard-Bériault, Nakanishi & Pfaus, 2008; Chapter 5). These findings suggest that, like the development of CPP, intact opioid systems but not DA systems are necessary for the development of CEP for a familiar almond-scented female. These findings add to evidence from Hughes and colleagues (1990) that there is a common reward system for CPP and CEP in males.

At first glance, our findings appear inconsistent with those on female partner preference. Coria-Avila and colleagues (2008a,b) showed that both dopamine and opioid systems play an important role in the development of partner preference. Although, these results suggest that the mechanism involved in the development of partner preference differs between males and females, the role of DA in the development of partner preference in males cannot be completely dismissed. It is possible that the dose of the DA receptor antagonist used was not sufficient to compete with the sustained increase in DA during copulation. Future experiments should examine the effect of different doses of flupenthixol or multiple flupenthixol injections of the same dose at different time points

on male copulatory behavior and on the development of partner preference for a familiar female following 1-hole-paced copulation.

Examination of the neural structures important for the development of CEP for a familiar female revealed that the VTA and the Arc are two areas that display increased Fos-IR following exposure to the familiar almond-scented female or the conditioned almond odor alone (Ismail, Spape, Girard-Bériault, Knezevic, El-Jabary & Pfaus, in preparation). The VTA, an area rich in dopamine (DA) cell bodies and opioid receptors, is known to play an important role in mediating attention to reward-related cues (Robinson & Berridge, 1993) and also plays a role in the prediction of the occurrence of a reward (Schultz, 1998). The Arc nucleus contains most of the neural circuitry required for the regulation of metabolism and reproduction (Chen, Tsai, Yeh, Tai & Tsai, 2007). The Arc has been reported to contain DA (Hoffman & Sladek, 1980) and noradrenergic terminals (Kizer, Muth & Jacovowitz, 1976). Although neurons in the Arc are known to project to the mPOA (Leranth, MacLusky, Shanabrough & Naftolin, 1988), an area known to play a crucial role in male sexual behavior (Hull, Meisel & Sachs, 2002), the actual function of the Arc in male sexual behavior remains to be investigated. Like the VTA, the Arc also contains μ-opioid-receptors and neurons in the Arc give rise to both opioid and melanocortin systems that project to hypothalamic and limbic structures (O'Donohue & Dorsa, 1982) that play an important role in male sexual behavior and reward. For example, prenatal activation of μ-opioid-receptors in the Arc alters male sexual behavior (Bonelli & Vannelli, 1983; Siddique, Haq & Shah, 1997). Moreover, the Arc is the source of ascending endorphin projections that are likely activated by ejaculation and may be conditionally activated during 1-hole paced copulation (Cheung,

Salinas, & Hammer, 1995). Together, these findings suggest that copulation in 1-hole pacing chambers facilitates the development of partner preference for a familiar female through activation of opioid and/or DA systems in the VTA and in the Arc.

Although Fos activation was not observed consistently in brain areas reported to be activated in studies by Kippin, Cain, and Pfaus (2003) and Coria-Avila and Pfaus (2007), it is still possible that some of these areas may contribute to the development of CEP for a familiar female. The counting protocol employed in Chapter 6 used a more conservative "stereological" approach, in which small patches of Fos within a region were counted randomly among sections from each animal. This is similar to the protocol used in studies of Fos induction by ejaculation in gerbils (Heeb & Yahr, 2001), but differs considerably from the method used by Kippin, Coria-Avila, and others (e.g., Coolen, Peters, & Veening, 1997), in which an entire region is counted in multiple samples per rat that are picked after a qualitative assessment has been made of regions that have the most Fos induced by a particular treatment. Fos counts, like the results of other brain activation techniques, should not be used exclusively to determine which regions of the brain are important for a behavior. For example, the NAc did not show a significant activation in the present study when rats were presented with stimuli associated with sexual reward. However, it may well play an important role in male sexual behavior and in the development of CEP. The NAc receives dopaminergic inputs from the VTA (Phillipson, 1979). Pharmacological studies that measured DA release in the NAc have reported that DA is increasingly released in the NAc during copulation with a peak around the time of ejaculation (Pfaus et al., 1990). Kippin and colleagues (2004) showed that lesions to the NAc inhibit intomission behavior and impair non-

contact erections. Studies examining the neurochemistry underlying monogamy in prairie voles reported that DA D2-receptor subtype within the NAc appears to be critical for the formation of pair bond in prairie voles (Aragona, Liu, Curtis, Stephan, & Wang, 2003; Gingrich, Liu, Cascio, Wang, & Insel, 2000). For example, blockage of DA D2-receptors in the NAc disrupted copulation-induced partner preference (Liu & Wang, 2003; Young, Lim, Gingrich & Insel, 2001). Thus, the NAc may also play an important role in the development of CEP for a familiar female, despite the lack of significant Fos induction in response to cues associated with sexual reward in the present study.

In addition to DA, oxytocin, a hormone that also acts as a neurotransmitter in the NAc and elsewhere also seems to be important in the development of partner preference. For instance, female prairie voles injected with oxytocin in the NAc develop pair bonds even in the absence of copulation (Young et al., 2001). Moreover, injection of an oxytocin antagonist directly in the NAc disrupts the development of pair bond following mating. These findings suggest that oxytocin plays an important role in the formation of pair bonds in prairie voles. Interestingly, systemic injection of oxytocin to male rats prior to their first sexual experience with an almond-scented female increases the number of males that display conditioned ejaculatory preference (Gelez et al., unpublished observation). These findings suggest that oxytocin may play an important role in the development of CEP for a familiar female in male rats. Similar to oxytocin, vasopressin, a peptide hormone, also seems to facilitate the development of pair-bonding. For instance, systemic administration of vasopressin to males facilitates pair-bonding (Insel et al., 1995). Given that DA, oxytocin and vasopressin have been shown to be important for the development of pair-bonding (Young et al., 2001; Insel et al., 1995), future

experiments should examine the role of DA and oxytocin in the NAc (and elsewhere) and arginine vasopressin on the development of CEP for a familiar female in male rats.

In conclusion, the findings of the present dissertation suggest strongly that the context of copulation, which comprises sexual arousal, the intensity of sexual reward, and female availability/accessibility, plays a critical role in the development of male sexual behavior and partner preferences. Contrary to hypotheses that male sexual behavior is driven by an "ultimate" genetic mandate to spread the gene pool far and wide (e.g., Buss, 2003), the findings in this thesis suggest that male sexual behavior is driven by a more "proximate" desire to find a mate that fits a particular template of features associated with early experience of sexual reward and to copulate with her under the right conditions. Linking sexual behavior to mechanisms of reward assures that many members of a species will actually copulate, even if it also opens up the door to mate choice based on Pavlovian associations with particular cues or characteristics predictive of sexual reward, a rudiment of the supposedly "hard-wired" sexual strategy of monogamy. The end result of this in evolutionary terms is a counterweight – perhaps THE counterweight – that prevents uncontrolled outbreeding. It also allows for a large degree of variance in what becomes preferred, down to the idea that a somewhat different template of cues associated with sexual reward becomes preferred in each individuals experience (see Money, 1997). With reference to the sexual desire machine mentioned in the introduction, males are not mere on-off switches, but rather they also have dials that have to be set just right, like in females. These dials correspond to the level of sexual arousal experienced, availability and accessibility of the female, and cues that make a particular sex partner and/or type of sexual behavior predictive of sexual reward.

Like in male rats, sexual behavior in men is also influenced by context, which comprises of the availability of partners, level of arousal, and intensity of sexual reward experienced or expected (Colson, Lemaire, Pinton, Hamidi, & Klein, 2006). In men, the context of sexual behavior influences ejaculation latency (Waldinger et al., 2005) and the intensity of sexual reward experienced during orgasm (Masters, Johnson, & Kolodny, 1983). Money (1997) argued that the context of early sexual experience creates a template in which particular features of a partner and particular types of sexual activity become preferred. This is reminiscent of the suggestion made by Krafft-Ebing (1929) that early sexual experiences create "fetishes" that can include socially-acceptable features of a partner to more "exotic" features of forbidden partners or actions, to inanimate objects that must be present for sexual arousal to occur at all. The fact that male rats display similar phenomena as a result of their early sexual experiences is a strong indication that the brains of rats and men have evolved to learn about and experience sex in similar yet very individual ways.

REFERENCES

Adkins-Regan, E., Mansukhani, V., Thompson, R., & Yang, S. (1997). Organizational actions of sex hormones on sexual partner preference. *Brain Research Bulletin,* 44(4), 497-502.

Ågmo, A., & Berenfeld, R. (1990). Reinforcing properties of ejaculation in the male rat: role of opioids and dopamine. *Behavioral Neuroscience*, 104, 177-182.

Amstislavskaya, T. G., & Popova, N. K. (2004) Female-induced sexual arousal in male mice and rats: behavioral and testosterone response. Hormones and Behavior, 46(5): 544-550.

Althof, S. (2006). The psychology of premature ejaculation: therapies and consequences. *Journal of Sexual Medecine*, 3 (Suppl. 4), 324-331.

Aragona, B. J., Liu, Y., Curtis, J. T., & Wang, Z. X. (2003). A critical role for dopamine in pair bonding in male Prairie voles. *Journal of Neuroscience*, 23, 3483-3490.

Austin, D., & Dewsbury, D. A. (1976). Possible influence of strain differences on pregnancy initiation in laboratory rats. *Physiology & Behavior*, 37, 621-625.

Austin, D., & Dewsbury, D. A. (1986) Possible influence of strain differences on pregnancy initiation in laboratory rats. *Physiology & Behavior*, 37: 621-625.

Balfour, M. E., Yu, L., Coolen, L. M. (2004). Sexual behavior and sex-associated environmental cues activate the mesolimbic system in male rats. *Neuropsychopharmacology*, 29(4): 718-730.

Ballard, C. L., & Wood, R. I. (2007). Partner preference in male hamsters: steroids, sexual experience and chemosensory cues. *Physiology & Behavior*, 91, 1-8.

Barash, D. "The Whisperings Within". Harper, New York. 1979.

Barfield, R. J., & Sachs, B. D. (1968). Sexual behavior: stimulation by painful electrical shock to skin in male rats. *Science*, 161(839), 392-393.

Basson, R., & Brotto, L. A. (2003). Sexual psychophysiology and effects of sildenafil citrate in oestrogenised women with acquired genital arousal disorder and impaired orgasm: a randomized controlled trial. *BJOG*, 110 (11), 1014-1024.

Baum, M. J., & Everitt, B. J. (1992). Increased expression of c-fos in the medial preoptic area after mating in male rats: role of afferent inputs from the medial amygdala and midbrain central tegmental field. *Neuroscience*, 50 (3), 627-646.

Beach, F. A. (1942). Analysis of the stimuli adequate to elicit mating behavior in the sexually inexperienced male rat. *Journal of Comparative Physiology and Psychology*, 33, 163-207.

Beach, F. A. (1956). Characteristics of masculine "sex drive". *Nebraska Symposium on Motivation*, 4, 1-32.

Beach, F. A., & Jordan, E. (1956). Sexual exhaustion and recovery in the male rat. *Quart. J. Psychol.*, 8, 121-133.

Bialy M, & Kaczmarek L (1996) c-Fos expression as a tool to search for the neurobiological base of the sexual behaviour of males. *Acta Neurobiol Exp (Warsz)*, 56, 567-577.

Bodenmann, G., Ledermann, T., Blattner, D., & Galluzzo, C. (2006). Associations among everyday stress, critical life events, and sexual problems, *The Journal of nervous and mental disease*, 194(7), 494-501.

Bonelli, A., & Vannelli, G. (1983). Effects of prolonged administration of morphine on the estrus cycle of the rat. *Boll Soc Ital Biol Sper*, 59(8), 1210-1216.

Borg, K. E., Esbenshade, K. L., Johnson, B. H, Lunstra, D. D., & Ford, J. J. (1992). Effects of sexual experience, season, and mating stimuli on endocrine concentrations in the adult ram. *Hormones & Behavior*, 26(1): 87-109.

Buss, D. M. (2003). The evolution of desire: Strategies of human mating, revised edition. Basic Books, New York.

Buvat, J., Lemaire, A., Buvat-Herbaut, M., Fourlinnie, J. C., Racadot, A., & Fossati, P. (1985). Hyperprolactinemia and sexual function in men. *Hormone Research*, 22(3), 196-203.

Cabilio, S. (1996). Behavioral observation program. [unpublished computer software]. Concordia University.

Caggiula, A. R., & Eibergen, R. (1969). Copulation of virgin male rats evoked by painful peripheral stimulation. *Journal of Comparative and Physiological Psychology*, 69, 414-419.

Cain, D. P., & Paxinos, G. (1974). Olfactory bulbectomy and mucosal damage : Effects on copulation, irritability, and interspecific aggression in male rats. *Journal of Comparative and Physiological Psychology*, 86(2), 202-212.

Calhoun, J. B. (1962). The ecology and sociology of the norway rat. Bethesda, MD: U.S. Department of Health, Education and Welfare.

Camacho, F. J., Castro, M., Hernández, V., & Paredes, R. G. (2007). Facilitation of ejaculation induced by 8-OH-DPAT does not produce conditioned place preference in male rats. *Behavioral Neuroscience*, 121(3), 579-585.

Carr, W. J., Loeb, L. S., & Dissinger, M. L. (1965). Responses of rats to sex odors. *Journal of Comparative and Physiological Psychology*, 59, 370-377.

Carr, W. J., Loeb, L. S., & Wylie (1966). Responses to feminine odors in normal and castrated male rats. *Journal of Comparative and Physiological Psychology*, 62(2), 336-338.

Centeno, S., Coopersmith, C. B., & Pfaus, J. G. (2001). Sexual experience diminishes the inhibitory effect of penile anesthesia, castration, and 8-OH-DPAT on sexual behavior in the male rat. *Physiology & Behavior* (submitted).

Cerny, J. (1978). Biofeedback and the voluntary control of sexual arousal in women. *Behav Ther*, 9, 847-855.

Chen, J. C., Tsai, H-W., Yeh, K-Y., Tai, M-Y., & Tsai, Y-F. (2007). Male sexual behavior and catecholamine levels in the medial preoptic area and arcuate nucleus in middle-aged rats. *Brain Research*, 1184:186-192.

Colson, M. H., Lemaire, A., Pinton, P., Hamidi, K., & Klein, P. (2006). Sexual behaviors and mental perception, satisfaction and expectations of sex life in men and women in France. *Journal of Sexual Medecine*, 3(1), 121-131.

Coolen, L. M., Peters, H. J. P. W., & Veening, J. G. (1996). Fos immunoreactivity in the rat brain following consummatory elements of sexual behavior: A sex comparison. *Brain Research*, 738, 67-82.

Coria-Avila, G. A., & Pfaus, J. G. (2007). Neuronal activation by stimuli that predict sexaul reward in female rats. *Neuroscience*, 148(3):623-632.

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

Coria-Avila, G. A., Solomon, C. E., Barbosa Vargas, E., Lemme, I., Ryan, R., Ménard, S., Gavrila, A. M., & Pfaus, J. G. (2008a). Neurochemical basis of conditioned partner preference in the female rat : I. Disruption by naloxone. *Behavioral Neuroscience*, 122(2), 385-395.

Coria-Avila, G. A., Gavrila, A. M., Boulard, B., Charron, N., Stanley, G., & Pfaus, J. G. (2008b). Neurochemical basis of conditioned partner preference in the female rat : II. Disruption by flupenthixol. *Behavioral Neuroscience*, 122(2), 396-406.

Coria-Avila, G. A., Jones, S. L., Solomon, C. E., Gavrila, A. M., Jordan, G. J., & Pfaus, J. G. (2006). Conditioned partner preference in female rats for strain of male. *Physiology & Behavior*, 88(4-5), 529-537.

Coria-Avila, G. A., Ouimet, A. J., Pacheco, P., Manzo, J., & Pfaus, J. G. (2005). Olfactory conditioned partner preference in the female rats. *Behavioural Neuroscience,* 119(3), 716-25.

Crowley, W. R., Popolow, H. B., & Ward, O. B. (1973). From dud to stud: copulatory behavior elicited through conditioned arousal in sexually inactive male rats. *Physiology & Behavior*, 10, 391-394.

Curtis, J. T., Stowe, J. R., & Wang, Z. (2003). Differential effects of intraspecific interactions on the strial dopamine system in social and non-social voles. *Neuroscience*, 118(4), 1165-1173.

D'Udine, B., & Alleva, E. (1983). Early experience and sexual preferences in rodents. In: Batenson, P. editor. Mate choice. Cambridge, UK: Cambridge University Press.

Daly, M., & Wilson, M. Sex, evolution and behavior. Duxbury, North Scituate, MA, 1978.

Damsma, G., Pfaus, J. G., Wenkstern, D., Phillips, A. G., & Fibiger, H. C. (1992). Sexual behavior increases dopamine transmission in the nucleus accumbens and striatum of male rats: comparison with novelty and locomotion, *Behavioral Neuroscience*, 106(1), 181-191.

Dewsbury, D. A. (1969). Copulatory behavior of rats (Rattus Norvegicus) as a function of prior copulatory experience. *Animal Behavior*, 17, 217-223.

Domjan, M., O' Vary, D., & Greene, P. (1988). Conditioning of appetitive and consummatory sexual behavior in male Japanese quail. *Journal of Experimental Analysis of Behavior*, 50, 505-519.

Drori, D., & Folman, Y. (1964). Effects of Cohabitation on the reproductive system, kidneys, and body composition of male rats. *Journal of Reproduction and Fertility*, 8, 351-359.

Duvauchelle, C. L., Fleming, S. M., & Kornetsky, C. (1996). Involvement of σ- and μ-opioid receptors in the potential of brain-stimulation reward. *European Journal of Pharmacology*, 316(2-3), 137-143.

Edwards, D. A., Griffis, K. T., Tardive, l. C. (1990). Olfactory bulb removal: effects on sexual behavior and partner-preference in male rats. *Physiology & Behavior,* 48(3): 447-450.

Emmerick, J. J., & Snowdon, C. T. (1976). Failure to show modification of male golden hamster mating behavior through taste/odor aversion learning, *Journal of Comparative and Physiological Psychology*, 90(9), 857-869.

Erskine, M. S. (1989). Solicitation behavior in the estrous female rat: a review. *Hormones & Behavior*, 23(4), 473-502.

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

Erskine, M. S., Kornberg, E., & Cherry, J. A. (1989). Paced copulation in rats: effects of intromission frequency and duration on luteal activation and estrus length. *Physiology & Behavior*, 45(1), 33-39.

Everitt, B. J. (1990). Sexual motivation: a neural and behavioral analysis of the mechanisms underlying appetitive and copulatory responses of male rats. *Neuroscience and Biobehavioral Reviews*, 14(2), 217-232.

Everitt, B. J., & Stacey, P. (1990) Studies of instrumental behavior with sexual reinforcement in male rats (Rattus norvegicus: II. Effects of preoptic area lesions, castration, and testosterone. *Journal of Comparative Psychology*, 101(4), 407-419.

Fadem, B. H., & Barfield, R. J. (1982). Differences between male and female rats in the temporal pattern of copulation. *Behavioral and Neural Biology*, 36, 411-415.

Fallon, J. H., Moore, R. Y. (1978). Catecholamine innervation of the basal forebrain. IV. Topography of the dopamine projection to the basal forebrain and neostriatum. *J Comp Neurol,* 180, 545-580.

Fillion, T. J., & Blass, E. M. (1986). Infantile experience with suckling odors determines adult sexual behavior in male rats. *Science*, 231 (4739), 729-731.

Fiorino, D. F., Coury, A., & Phillips, A. G. Dynamic changes in nucleus accumbens dopamine efflux during the Coolidge effect in male rats. J Neurosci. 1997,

17(12): 4849-4855.

Fiorino, D. F., & Phillips, A. G. (1999). Facilitation of sexual behavior and enhanced dopamine efflux in the nucleus accumbens of male rats after d-amphetamine-induced behavioral sensitization, *Psychopharmacology,* 19(1), 456-463.

Gelez, H., Coria-Avila, G.A., Woehrling, D., Rajabi, H., & Pfaus, J.G. (2005). A neutral odor previously paired with copulation enhances dopamine release in the nucleus accumbens of male rats. *Ninth Annual Meeting of the Society for Behavioral Neuroendocrinology* (Austin, TX).

Getz, L.L, & Hofman, J. E. (1986). Social organization in free living prairie voles. *Microtus ochrogaster.*

Ghiselin, M. T. (1974). The economy of nature and the evolution of sex. Berkeley, Los Angeles: University of California press.

Gingrich, B., Liu, Y., Cascio, C., Wang, Z., & Insel, T. R. (2000). Dopamine D2-receptors in the nucleus accumbens are important for social attachment in female Prairie voles (Microtus Ochrogaster). *Behavioral Neuroscience*, 114(1), 173-183.

González-Mariscal, G., Gómora, P, & Bayer, C. (1994). Participation of opiatergic, GABAergic, and serotonergic systems in the expression of copulatory analgesia in male rats, *Pharmacology, Biochemistry & Behavior*, 49(2), 303-307.

Graham, J. M., & Desjardins, C. (1980). Classical conditioning: Induction of luteinizing hormone and testosterone secretion in aniticipation of sexual activity, *Science*, 210: 1039-1041.

Gray, G. D., Smith, E. R., Dorsa, D. M., & Davidson, J. M. (1981). Sexual Behavior and Testosterone in Middle-Aged male rats. *Endocrinology*, 109, 1597-1604.

Groenewegen, H. J.,& Uylings, H. B. (2000). The prefrontal cortex and the integration of sensory, limbic and autonomic information. *Progress in Brain Research*, 126, 3-28.

Gutiérrez, G., & Domjan, M. (1997). Differences in the sexual conditioned behavior of male and female Japanese quail (Coturny Japonica). *Journal of Comparative Psychology*, 111, 135-142.

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

Hayashi, S., & Kimura, T. (1976). Sexual behavior of the native male mouse as affected by the presence of a male and a female performing mating behavior. *Physiology & Behavior*, 17, 807-810.

Herz, Z. Y. Folman, Y., & Drori, D. (1969). The testosterone content of the testes of mated and unmated rats. *Journal of Endocrinology*, 44, 127-128.

Hoffman, G. E., & Sladek, Jr J. R. (1980). Age-related changes in dopamine, LHRH and somatostatin in the rat hypothalamus. *Neurobiol. Aging* 1:27-37.

Hollis, K. L., Cadieux, E. L., & Colbert, M. M. (1989). The biological function of Pavlovian conditioning : a mechanism for mating success in the Blue Gourami (Trichogacter Trichopterus). *Jouranl of Comparative Psychology*, 103(2), 115-121.

Hughes, A. M., Everitt, B. J., & Herbert, J. (1990). Comparative effects of preoptic area infusions of opioid peptides, lesions and castration on sexual behavior of male rats: Studies of instrumental behavior, conditioned place preference and partner preference. *Psychopharmacology* 102, 243–256.

Hull, E. M. & Dominguez, J. M. (2007). Sexual behavior in male rodents. *Hormones and Behavior, 52,* 45-55.

Hull, E. M., Meisel, R. L., Sachs, B. D. (2002). Male sexual behavior. In: Pfaff DW, Arnold AP, Etgen AM, Fahrbach SE, Rubin RT (Eds), Hormones, Brain and Behavior, vol. 1. Academic Press, New York, pp. 3-137.

Ikemoto, S., Kohl, R. R., McBride, W. J. (1997). GABA(A) receptor blockade in the anterior ventral tegmental area increases extracellular levels of dopamine in the nucleus accumbens of rats. *J Neurochem* 69: 137-143.

Insel, T. R., Winslow, J. T., Wang, Z. X., Young, L., & Hulihan, T. J. (1995). Oxytocin and the molecular basis of monogamy. *Advances in Experimental Medecin and Biology*, 395, 227-234.

Insel, T. R., Young, L., & Wang, Z. (1997). Molecular aspects of monogamy. *Ann N Y Acad Sci*, 807: 302-316.

Ismail, N., Gelez, H., Lachapelle, I., & Pfaus, J. G. (2008). Pacing conditions contribute to the conditioned ejaculatory preference for a familiar female in the male rat. *Physiology & Behavior.* (in press).

Ismail N, Girard-Bériault F, Nakanishi S, Pfaus JG. (2008). Naloxone but not flupenthixol, disrupts the development of conditioned ejaculatory preference in the male rat. (in preparation).

Ismail, N., Jones, S. L., Graham, M. D., Sylvester, S. & Pfaus, J. G. (2008). The effect of paced copulation and strain conditioning on the development of partner preference. (in preparation).

Ismail, N., Laroche, C., Girard-Bériault, F., Ménard, S., Greggain, J. A., Pfaus, J. G. (2008). Sexual responses and partner preferences of male rats paired with haloperidol-treated female rats. (in preparation).

Ismail, N., Spape, J., Girard-Bériault, F., Knezevic, A., El-Jabary, Z., & Pfaus. J. G. (2008). Neuronal activation by conditioned odors and natural cues in male rats. (in preparation).

Ismail N, Zhao Y, Pfaus JG (2008) Context-dependent acquisition of copulatory behavior in the male rat: Role of female availability. Behav Neurosci. (in press).

Jenkins, W. J., & Becker, J. B. (2003). Dynamic increases in dopamine during paced copulation in the female rat. *The European Journal of Neuroscience*, 18(3), 1997-2001.

Johnson, S. W., North, R. A. (1992). Opioids excite dopamine neurons by hyperpolarization of local interneurons. *J Neurosci,* 12: 483-488.

Johnston, R. E., Zahorik, D. M., Immler, K., & Zakon, H. (1978). Alterations of male sexual behavior by learned aversions to hamster vaginal secretion. *Journal of Comparative and Physiological Psychology*, 92, 85-93.

Jowaisas, D., Taylor, J., Dewsbury, D. A., & Malagodi, E. F. (1971). Copulatory behavior of male rats under an imposed operant requirement. *Psychonom Sci*, 25, 287-290.

Kantorowitz, D. A. (1978). An experimental investigation of preorgasmic reconditioning and postorgasmic deconditining. *J. Appl. Behav. Anal*, 11, 23-24.

Kelley, A. E. (1999). Functional specificity of ventral striatal compartments in appetitive behaviors. *Ann N Y Acad Sci*, 877, 71-90.

Kippin, T. E., Cain, S. W., & Pfaus, J. G. (2003). Estrous odors and sexually conditioned neutral odors activate separate neural pathways in the male rat. *Neuroscience* 117: 971-979.

Kippin, T. E. & Pfaus, J. G. (2001b). The nature of the conditioned response mediating olfactory conditioned ejaculation preference in the male rat. *Behavioural Brain Research,* 122(1): 11-24.

Kippin, T. E. & Pfaus, J. G. (2001a). The development of olfactory conditioned ejaculatory preferences in the male rat. I. Nature of the unconditioned stimulus. *Physiol Behav., 73,* 457-469.

Kippin, T. E., Samaha, A. N., Sotinopoulos, V., & Pfaus J. G. (2001). The development of olfactory conditioned ejaculatory preferences in the male rat. II. Parametric manipulation of conditioning session number and duration. *Physiology & Behavior*, 73(4), 471-485.

Kippin, T. E., Sotiropoulos, V., Badith, J., & Pfaus, J. G. (2004). Opposing roles of the nucleus accumbens and anterior lateral hypothalamic area in the control of sexual behavior in the male rat. *The European Journal of Neuroscience*, 19(3), 698-704.

Kippin, T. E., Talianakis, S., & Pfaus, J. G. (1997). Olfactory conditioning of sexual behavior in the male rat: Odor habituation, explicitly unpaired, and random controls. *Society for Neuroscience Abstracts*, 23, 1356. (New Orleans, LA).

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

Kizer JS, Muth E, Jacobowitz DM (1976) The effect of bilateral lesions of the ventral noradrenergic bundle on endocrine-induced changes of tyrosine hydroxylase in the rat median eminence. *Endocrinology,* 98: 886-893.

Klitenick, M. A., DeWitte, P., Kalivas, P. W. (1992). Regulation of somatodendritic dopamine release in the ventral tegmental area by opioids and GABA: and *in vivo* microdialysis study. *J Neurosci* 12: 2623-2632.

Koch, P. G., & Peters, R. H. (1987). Suppression of adult copulatory behaviors following LiCl-induced aversive contingencies in juvenile male rats. *Developmental Psychobiology*, 20(6), 603-611.

Kovas, Y., & Plomin, R. (2006). Generalist genes: implications for the cognitive sciences. *Trends in Cognitive Sciences*, 10(5), 198-203.

Krafft-Ebing, R. von. Psychopathia sexualis. New York: Physicians and Surgeons Book Co. 1929.

Lagerspetz, K., & Heino, T. (1970) Changes in social reactions resulting in early experience with another species. *Psychol. Rep.*, 27, 255-262.

Larsson, K. (1956). Conditioning and sexual behavior in the male albino rat. Sockholm: Almqvist & Wiksell.

Larsson K (1971) Impaired mating performances in male rats after anosmia induced peripherally or centrally. Brain Behav Evol 4(6): 463-471.

Lawrence, G. J., & Kiefer, S. W. (1987). Cessation of male rat copulatory behavior using illness as punishment: facilitation with a novel odor. *Behavioral Neuroscience*, 101(2), 289-291.

Leranth, C., MacLusky, N. J., Shanabrough, M., & Naftolin, F. (1988). Catecholaminergic innervation of luteinizing hormone-releasing hormone and glutamic acid decarboxylase immunopositive neurons in the rat medial preoptic area. An electron-microscopic double immunostaining and degeneration study. *Neuroendocrinology* 48(6): 591-602.

Letourneau, E. J., & O'Donohue, W. (1997). Classical conditioning of female sexual arousal. *Arch Sex Behav*, 26, 63-78.

Lim, M. M., Hammock, E. A., & Young, L. J. The role of vasopressin in the genetic and neural regulation of monogamy. *J Neuroendocrinol.* 2004, 16(4): 325-332.

Lim, M. M., Liu, Y., Ryabinin, A. E. Bai, Y., Wang, Z., & Young, L. J. (2007). CRF receptors in the nucleus accumbens modulate partner preference in prairie voles. *Hormones & Behavior*, 51(4): 508-515.

Lipp, O. V. (2002). Anticipation of a non-aversive reaction time task facilitates the blink startle reflex. Biol Psychol. 2002, 59(2): 147-162.

Lisk, R. D., & Heiman, J. (1980). The effects of sexual experience and frequency of testing on retention of copulatory behavior following castration in the male hamster. *Behavioral and Neural Biology*, 28, 156-171.

Liu, Y., & Wang, Z. X. (2003). Nucleus accumbens oxytocin and dopamine interact to regulate pair bond formation in female Prairie voles. *Neuroscience*, 121, 537-544.

Lodder, J. (1975). Penile deafferention and the effect of mating experience on sexual motivation in adult male rats. *Physiology & Behavior*, 17, 571-573.

Lopez, H. H. & Ettenberg, A. (2000). Haloperidol challenge during copulation prevents subsequent increase in male sexual motivation, *Pharmacology, Biochemistry & Behavior*, 67(2), 387-393.

Madlafousek, J. & Hliňák, Z. (1982). Importance of female's precopulatory behaviour for the primary initiation of male's copulatory behaviour in the laboratory rat. *Behaviour, 86,* 237–249.

Madlafousek, J., Hliňák, Z., & Beran, J. (1976). Decline of sexual behavior in castrated male rats: effects of female precopulatory behavior. *Hormones and Behavior, 7,* 245–252.

Marr, J. N., & Gardner, L. E. Jr. (1965). Early olfactory experience and later social behavior in the rat: preference, sexual responsiveness, and care of young. *The Journal of Genetic Psychology*, 107 (1st Half), 167-174.

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

Mas, M., Gonzalez-Mora, J. L., Louilot, A., Solé, C., Guadalupe, T. (1990). Increased dopamine release in the nucleus accumbens of copulating male rats as evidenced by in vivo voltammetry, *Neuroscience Letters*, 110(3), 303-308.

Masters, W. H. Johnson, V. E. (1966). Human Sexual Response. Toronto, New York: Bantam Books.

Masters, W. H. Johnson, V. E. (1970). Human Sexual Inadequacy. Toronto: New York: Bantam Books.

Masters, W. H. Johnson, V. E., & Koldny, R. C. (1983). Sex and human loving. Boston: Little Brown.

Matthews, R. T., German, D. C. (1984). Electrophysiological evidence for excitation of rat ventral tegmental area dopamine neurons by morphine. *Neuroscience* 11(3): 617-625.

Matusczyk, J. V., & Larsson, K. (1994). Experience modulates the influence of gonadal hormones on sexual orientation of male rats. *Physiology & Behavior*, 55(3), 527-531.

McBride, W. J., Murphy, J. M., & Ikemoto, S. (1999). Localization of brain reinforcement mechanisms: intracranial self-administration and intracranial place-conditioning study, *Behavioral Brain Research*, 101(2), 129-152.

McClintock, M. K. (1971). Menstrual synchrony and suppression. *Nature*, 229(5282), 244-245.

McClintock, M. K. (1978) Estrous synchrony and its mediation by airborne chemical communication (Rattus norvegicus). Horm Behav 10(3): 264-275.

McClintock, M. K. (1984). Group mating in the domestic rat as a context for sexual selection: consequences for the analysis of sexual behavior and neuroendocrine responses. *Advances in the Study of Behavior*, 14, 1-50.

McClintock, M. K. (1984) Estrous synchrony: modulation of ovarian cycle length by female pheromones. Physiol Behav 32(5): 701-705.

McClintock, M. K., Adler NT (1978) Induction of persistent estrus by airborne chemical communication among female rats. Horm Behav 11(3): 414-418.

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

McConaghy, N. (1970). Subjective and penile plethysomograph responses to aversion therapy for homosexuality: A follow-up study. *British Journal of Psychiatry*, 17, 555-560.

McConaghy, N. (1974). Penile volume responses to moving and still pictures of male and female nudes. *Arch Sex Behav*, 3, 565-570.

McGill, T. E. (1962a). Reduction in "head-mounts" in the sexual behavior of the mouse as a function of experience. *Psychol Rep*, 10, 284

McGill, T. E. (1962b). Sexual behavior in three inbred strains of mice. *Behavior*, 19, 341-350.

McKenna, K. E. (1999). Central nervous system pathways involved in the control of penile erection. *Annual Review of Sex Research, 10,* 157-183.

Mehrara, B. J., & Baum, M. J. (1990). Naloxone disrupts the expression but not the acquisition by male rats of a conditioned place preference response for an oestrous female. *Psychoparmacology,* 110(1), 118-125.

Ménard, S., Gelez, H., Coria-Avila, G.A., Jacubovich, M., & Pfaus, J.G. (2006). Impact of neonatal olfactory conditioning of later sexual preference in the male rat. Tenth Annual Meeting of the Society for Behavioral Neuroendocrinology (Pittsburgh, PA)

Michael, R. P. (1961). Observations upon the sexual behavior of the domestic cat (Felis catus 1) under laboratory conditions. *Behavior*, 18, 1-24.

Miller, R. L., & Baum, M. J. (1987). Naloxone inhibits mating and conditioned place preference for an estrous female in male rats soon after castration. *Pharmacology, Biochemistry and Behavior*, 26(4), 781-789.

Mirenowicz, J. & Schultz, W. (1994). Importance of unpredictability for reward responses in primate dopamine. *Journal of Neurophysiology*, 72(2), 1024-1027.

Mitchell, J. B., & Gratton, A. (1991). Opioid modulation and sensitization of dopamine release elicited by sexually relevant stimuli: a high speed chronoamperometric study in freely behaving rats. *Brain Research*, 551, 20-27.

Mitchell, J, B., & Gratton, A. (1992). Mesolimbic dopamine release elicited by activation of the accessory olfactory system: a high speed chronoamperometric study. *Neurosci Lett,* 140:81-84.

Mitchell, J. B., & Stewart, J. (1990). Facilitation of sexual behaviors in the male rat associated with intra-VTA injections of opiates. *Pharmacol Biochem Behav* 35: 643-650.

Money, J. (1997). Evolutionary sexology: the hypothesis of song and sex. Med Hypotheses, 48: 399-402.

Nadler, R. D., & Bartlett, E. S. (1997). Penile erection: a reflection of sexual arousal and arousability inmale chimpanzees. *Physiology & Behavior*, 61(3), 425-432.

Newman SW, Parfitt DB, Kollack-Walker S (1997) Mating-induced c-fos expression patterns complement and supplement observations after lesions in the male Syrian hamster brain. *Ann NY Acad Sci* 807:239-259.

Paredes, R. G. & Agmo, A. (2004). Has dopamine a physiological role in the control of sexual behavior? A critical review of the evidence. *Progress in Neurobiology*, 73(3), 179-226.

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., & Vasquez, B. (1999). What do female rats like about sex? Paced mating. *Behavioral Brain Research,* 105, 117–127.

Pattij, T., de Jong, T. R., Uitterdijk, A., Waldinger, M. D., Veening, J. G., Cools, A. R., Van der Graaf, P. H., & Olivier, B. (2005). Individual differences in male rat ejaculatory behavior: searching for models to study ejaculation disorders. *The European Journal of Neuroscience*, 22(3), 724-734.

Paxinos, G., & Watson, C. (1998). The rat brain in stereotaxic corrdinates. San Diego: Academic Press.

Peters, R. H. (1983). Learned aversions to copulatory behaviors in male rats. *Behavioral Neuroscience*, 97, 140-145.

Pfaus, J. G. (1999). Revisiting the concept of sexual motivation. *Annual Review of Sex Research*, 10, 120-156.

Pfaus, J. G., Damsma, G., Nomikos, G. G., Wenkstern, D. G., Blaha, C. D., Phillips, A. G., & Fibiger, H. C. (1990). Sexual behavior enhances central dopamine transmission in the male rat. *Brain Reseach*, 530(2), 345-348.

Pfaus, J. G., Damsma, G., Wenkstern, D., Fibiger, H. C. (1995) Sexual activity increases dopamine transmission in the nucleus accumbens and striatum of female rats. *Brain Research*, 693, 21-30.

Pfaus, J. G., & Gorzalka, B. B. (1987). Selective activation of opioid receptors differentiation affects lordosis behavior in female rats, Peptides, 8(2), 309-317.

Pfaus, J. G., Heeb, M. (1997) Implications of immediate-early gene induction in the brain following sexual stimulation of female and male rodents. *Brain Res Bull,* 44, 397-407.

Pfaus, J. G., Kippin, T. E., & Centeno, S. Conditioning and sexual behavior: a review. Horm Behav. 2001, 40(2): 291-321.

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

Pfaus, J. G., Mendelson, S. D., Phillips, A. G. (1990) A correlation and factor analysis of anticipatory and consummatory measures of sexual behavior in the male rat. *Psychoneuroendocrinology*, 15, 329-340.

Pfaus, J. G., & Phillips, A. G. (1991). Role of dopamine in anticipatory and consummatory aspects of sexual behavior in the male rat. *Behavioral Neuroscience*, 105, 727-743.

Pfaus, J. G., Smith, W. J., Byrne, N., & Stephens, G. (2000). Appetitive and consummatory sexual behaviors of female rats in bilevel chambers. II. Patterns of estrus termination following vaginocervical stimulation. *Hormones and Behavior*, 37(1), 96-107.

Pfaus, J. G., Smith, W. J., Coopersmith, C. B. (1999). Appetitive and consummatory sexual behaviors of female rats in bilevel chambers. I. A correlational and factor analysis and the effects of ovarian homones. *Hormones and Behavior*, 35(3), 224-240.

Pfaus, J. G, & Wilkins, M. F. (1995). A novel environment disrupts copulation in sexually naive but not experienced male rats: reversal with naloxone. *Physiology and Behavior*, 57, 1045-1049.

Phillipson, O. T. (1979). The cytoarchitecture of the interfascicular nucleus and ventral tegmental area of Tsai in the rat. J Comp Neurol 187:85-98.

Pomerantz, S. M. (1990). Apomorphine facilitates male sexual behavior of rhesus monkeys. *Pharmacology, Biochemistry & Behavior*, 35(3), 659-664.

Rachman, S. (1966). Sexual fetishism : An experimental analogue. *Psychol Res,* 16, 293-296.

Rachman, S., Hodgson, F. J. (1968). Experimentally-induced "sexual fetishism": Replication and development. *Psychol Res,* 18, 25-27.

Rampin, O., Jérôme, N., & Suaudeau, C. (2003). Proerectile effects of apomorphine in mice, *Life Sciences*, 72(21), 2329-2336.

Reinoso-Barbero, F., & de Andrés, I. (1995). Effects of opioid microinjection in the nucleus of the solitary tract on the sleep-wakefulness cycle states in cats. *Anesthesiology*, 82(1), 144-152.

Reynolds, B. S. (1980). Biofeedback and facilitation of erection in men with erectile dysfunction. *Arch Sex Behav*, 9, 101-113.

Richardson, N. R., & Gratton, A. (2008). Changes in nucleus accumbens dopamine transmission associated with fixed- and variable-time schedule-induced feeding. *European Journal of Neuroscience*, 27, 2714-2723.

Robertson, G. S., Pfaus, J. G., Atkinson, L. J., Matsumura, H., Phillips, A. G., & Fibiger, H. C. (1991), Sexual behavior increases c-fos expression in the forebrain of male rat. *Brain Research*, 564(2), 352-357.

Robinson, T. E., & Berridge, K. C. (1993). The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res Rev*, 18, 247-291.

Robitaille, J. A., & Bouvet, J. (1976). Field observations on the social behaviour of the Norway, Rattus Norvericus (Berkenhout). Biol Behav, 1, 289-308.

Rodríguez-Manzo, G., & Fernández-Guasti, A. (1995). Opioid antagonists and the sexual satiation phenomenon. *Psychopharmacology (Berl)*, 122(2), 131-136.

Rosen, R. C. (1973). Suppression of penile tumescence by instrumental conditioning. *Psychosom Med*, 35, 509-514.

Rosenbaum, J. F., & Pollack, M. H. (1998). Anhedonic ejaculation with desipramine. *International Journal of Psychiatry in Medecin*, 18(1), 85-88.

Rowland, D. L. (2005). Psychophysiology of ejaculatory function and dysfunction. *World Journal of Urology, 23,* 82-88.

Sachs, B. D. (1978). Conceptual and neural mechanisms of masculine copulatory behavior. In: McGill, T. E.; Dewsbury, D. A.; Sachs, B. D. eds. Sex and behavior. New York: plenum Press; 267-295.

Sachs, B. D. (1995). Placing erection in context: The reflexogenic-psychogenic dichotomy reconsidered. *Neuroscience and Biobehavioral Reviews*, 19, 211-224.

Sachs, B. D. (1997). Erection evoked in male rats by airborne scent from estrous females. *Physiology & Behavior*, 62, 921-924.

Sachs, B. D., Akasofu, K., Citron, J. H., Daniels, S. B., Natoli, J. H. (1994). Non-contact stimulation from estrous females evokes penile erection in rats. *Physiology & Behavior*, 55(6), 1073-1079.

Sachs, B. D., & Barfield, R. J. (1970). Temporal patterning of sexual behavior in the male rat. *The Journal of Comparative Physiological Psychology,* 73, 359-364.

Sachs, B. D., & Garinello, L . D. (1978). Interaction between penile reflexes and copulation in male rats. *Journal of Comparative and Physiological Psychology*, 92(4), 759-767.

Sachs, B. D., & Meisel, R. L. (1988). The Physiology of male sexual behavior. In: Knobil E, Neill J (Eds) *The Physiology of Reproduction.* Raven, New York, 1393-1485.

Schank JC, McClintock MK (1997) Ovulatory pheromone shortens ovarian cycles of female rats living in olfactory isolation. *Physiology & Behavior* 62(4): 899-904.

Schultz, W. (1998). Predictive reward signal of dopamine neurons. *Journal of Neurophysiology*, 80(1), 1-27.

Schultz, W., & Dickinson, A. (2000). Neuronal coding of prediction errors, *Annu Rev Neurosci*, 23, 473-500.

Sheffield, F. D., Wulff, J. J., & Backer, R. (1951). Reward value of copulation without sex drive reduction. *Journal of Comparative Physiological Psychology*, 44(1), 3-8.

Siddiqui A, Haq S, Shah BH (1997) Perinatal exposure to morphine disrupts brain norepinephrine, ovarian cyclicity, and sexual receptivity in rats. *Pharmacol Biochem Behav*, 58(1), 243-248.

Siegel, L. I., Nunez A. A., & Wade, G. N. (1981). Copulation affects body weight but not food intake or dietary self-selection in male rats. *Physiology and Behavior*, 27, 943-946.

Silberberg, A., & Adler, N. (1974). Modulation of the copulatory sequence of the male rat by a schedule of reinforcement. *Science*, 185(4148), 374-376.

Stern, K., & McClintock, M. K. (1998). Regulation of ovulation by human pheromones, *Nature*, 392(6672), 177-179.

Stone, C. P. (1922). The congenital sexual behavior of the young male albino rat. *Journal of Comparative Psychology*, 2, 95-153.

Swann, J., & Fiber, J. M. (1997). Sex differences in function of a pheromonally stimulated pathway: role of steroids and the main olfactory system. *Brain Res Bull,* 44:409-413.

Thor, D, & Flannelly, K. J. (1977). Social-Olfactory experience and initiation of copulation in the virgin male rat. *Physiology and Behavior*, 19, 411-417.

Toates, F. M. (1986). Motivational Systems. Cambridge University Press, Cambridge.

Valenstein, E. S., & Goy, R. W. (1957). Further studies of the organization and display of sexual behavior in male guinea pigs. *Journal of Comparative and Physiological Psychology*, 50, 115-119.

Van Furth, W. R., & van Ree, J. M. (1996). Sexual motivation: involvement of endogenous opioids in the ventral tegmental area, *Brain Research*, 729(1), 20-28.

Veening, J. G., & Coolen, L. M. (1998). Neural activation following sexual behavior in the male and female rat brain. *Behav Brain Res,* 92, 181-193.

Vega Matuszcyk, J., Larsson, K., & Eriksson, E. (1998). The selective serotonin reuptake inhibitor fluoxetine reduces sexual motivation in male rats. *Pharmacology, Biochemistry and Behavior*, 60(2), 527-532.

Waldinger, M . D., Zwinderman, A. H., Olivier, B., & Schweitzer, D. H. (2005). Proposal for a definition of lifelong premature ejaculation based on epidemiological stopwatch data. *Journal of Sexual Medecin*, 2(4), 498-507.

Wenkstern, D., Pfaus, J. G., & Fibiger, H. C. (1993). Dopamine transmission increases in the nucleus accumbens of male rats during their first exposure to sexually receptive female rats. *Brain Research,* 618, 41-46.

Wersinger, S. R., Baum, M. J., & Erskine, M. S. (1993). Mating-induced FOS-like immunoreactivity in the rat forebrain: A sex comparison and a dimorphic effect of pelvic nerve transaction. *Journal of Neuroendocrinology*, 5, 557-568.

Whalen, R.E. (1961). Effects of mounting without intromission and intromission without ejaculation on sexual behavior and maze learning. *Journal of Comparative and Physiological Psychology,* 54, 409-415.

Whipple, B., & Komisaruk, B. R. (1985). Elevation of pain threshold by vaginal stimulation in women. *Pain*, 21(4), 357-367.

Will, M. J., Franzblau, E. B., & Kelley, A . E. (2003). Nucleus accumbens μ-opioids regulate intake of a high-fat diet via activation of a distributed brain network, *Journal of Neuroscience*, 23(7), 2882-2888.

Winslow, J. T., Shapiro, L., Carter, C. S., & Insel, T. R. (2001). Oxytocin and complex social behavior: species comparisons. Psychopharmacol Bull, 29(3), 409-414.

Young, L. J., Lim, M. M., Gingrich, B., & Insel, T. R. (2001). Cellular mechanisms of social attachment. *Hormones and Behavior*, 40(2), 133-138.

Zamble, E., Hadad, G. M., & Mitchell, J. B. (1985a). Pavlovian conditioning of sexual arousal: Unsuccessful attempts with an ejaculatory US. *Bull Psychonom Soc*, 23, 149-152.

Zamble, E., Mitchell, B., & Findlay, H. (1986). Pavlovian conditioning of sexual arousal: Parametric and background manipulations. *J Exp Psychol Anim Behav Proc*, 12, 403-412.

Zingheim, P. K., & Sandman, C. A. (1978). Discriminative control of the vaginal vasomotor response. *Biofeedback Self-Regulation*, 3, 29-41.