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Olfactory Conditioned Ejaculatory Preference in the Male Rat: Implications for the Role of Learning in Sexual Partner Preferences.

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A Thesis

in

The Department

of

Psychology

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

March, 2000

ABSTRACT

Olfactory Conditioned Ejaculatory Preference in the Male Rat:
Implications for the Role of Learning in Sexual Partner Preferences.

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Concordia University, 1999

The development, expression, and extinction of a novel conditioned sexual behavior, conditioned ejaculatory preference (CEP), were studied. Male rats allowed to copulate with sexually-receptive females bearing an artificial odor (almond or lemon) displayed a subsequent preference for a female bearing that odor over a female that did not. Males receiving explicitly-unpaired or randomly-paired training failed to display this preference, implicating classical conditioning mechanisms in the development of this behavior. Examination of the time course of the development of CEP found that it develops rapidly, demonstrating the importance of early sexual experience in the determination of sexual partner preferences. Extinction occurred during copulation tests with one scented and one unscented female. Further, the rate of extinction was faster following massed training than distributed training. Analysis of the components of copulation required to support the development of CEP revealed that ejaculation was necessary, but not sufficient to support CEP. Rather CEP development is critically dependent upon the presence of a scented female during the postejaculatory period. Finally, the nature of the conditioned response mediating CEP was shown to be a bias of copulatory responses toward the scented female near the point of ejaculation, not facilitated ejaculation *per se*. The present findings are interpreted in Pavlovian and incentive motivational models to provide a framework for understanding the role of learning in sexual partner preference.

ACKNOWLEDGEMENTS

I wish to express my gratitude to Jim Pfaus for his supervision, tireless enthusiasm, and friendship. He has been a revitalizing force as well as a tremendous source of resources and support during the time I have been in his lab. I like to thank all my lab mates, especially Mark Wilkins, Soraya Centeno, Wendy Smith, Marlene Taube, and Myriam Lavoie for their camaraderie in and out of the lab. I also wish to extend my gratitude to my lab technicians and assistants, especially Colleen Manitt, Stella Talianakis, Veneta Sotiropoulos, Anna Samaha, and Sean Wright for their faithful help in maintaining the lab and carrying out portions of the experiments. I also thank Drs. Jane Stewart and Dave Mumby for their time, insight, and reading less-polished versions of this thesis. For keeping me from working too hard, I would like to thank, among many others, Alfonzo Abazaid, the Players, and all of the members of Club Carnivore. I would like to extend special thanks to Tanya Bergevin for being a constant friend and partner in crime and to Devin Mueller for always being available for (occasional) workouts , latenight outings, and the best conference roommate. I would like to thank Karen Szumlinski for her help during the crunch time. I also wish to thank my parents for all their love and support throughout my studies.

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General Introduction

The development of competent and successful sexual behavior in mammals involves not only the myriad biological and physiological changes present at puberty, but importantly psychological and social influences that can occur before and after puberty. The influence of the early social environment on the development and expression of sexual behavior has been the subject of empirical and theoretical analyses. The importance of the contribution of experience to the expression of sexual behavior has long been recognized (Freud, 1905; Pavlov, 1927; Watson, 1925) and is largely taken for granted;

> "...common to most [theories of sexual behavior] is the claim that to some extent sexual behavior, including sexual arousal, is learned. ...theories which are not explicitly conditioning-based accounts also rely on the notion that to some extent sexual behavior and arousal are learned" (O'Donohue and Plaud, 1994, pp. 321).

In spite of this, however, there are many aspects of the issue that have yet to be understood.

The general goal of the present thesis is to examine how learning influences the selection of sexual partners. First, I review the literature regarding the roles that learning plays in the development of sexual behaviors. In this review, I attempt to define what types of learning shape behavior to bring about, facilitate, or direct copulatory behavior.

In the second, empirical part of the thesis, I report on a series of studies carried out in the male rat to determine the role of associative learning in copulatory partner preferences. In these studies, males are allowed to copulate with a female scented with a neutral odor and subsequently are allowed to copulate with two females, one scented and one not. In Chapter 1, the nature of the influence of the odor stimulus on sexual interactions with the female bearing the odor are examined. In Chapter 2, the course of development and extinction of the conditioned preference is examined. In Chapter 3, the copulatory components of the unconditioned stimulus that support the development of the conditioned preference are identified. Finally, in Chapter 4, the behavioral components that comprise the conditioned response underlying the preference are identified. In the general discussion, an attempt is made to tie the results of the present experiments to general motivational theories and provide a framework for understanding the role of learning in sexual partner preference.

Roles of Learning in Sexual Behavior

Despite the widespread acceptance of the general idea that learning plays a role in sexual behavior, the exact form of this influence and the specific learning process involved are often undefined. The present review represents an attempt to see exactly what is known about the issues and, therefore, includes all aspects of behavioral change that are typically termed learning. Defining learning is problematic as it must be differentiated from superficially similar processes such as receptor adaptation, muscle fatigue, and

maturation (see e.g. Flaherty, 1987). I will use a basic definition derived from others (Flaherty, 1987; Kimble, 1961; Pearce, 1997) which is that learning processes are relatively permanent changes in behavior or behavior potentiality which occur as a result of experience. Three aspects of this definition are critical. First, relatively permanent distinguishes learning processes from transient receptor adaptation and muscle fatigue as well as from permanent maturational changes. "Relative permanency" may be operationalized as a change that remains in effect as long as it is appropriate to the situation, but can be changed relatively quickly in new circumstances. Second, "a change in behavior or behavior potentiality" implies that the response to a situation or set of stimuli is altered in some way, but that the change is not necessarily apparent at the time of learning. Third, "learning processes occur as a result of experience" indicates that the appropriate environmental stimuli must be encountered to produce the change in behavior.

The focus of the present review will be on how learning guides sexual and other reproductive behaviors and how it interacts with motivational factors. I review the impact of environmental factors on the ability of animals to identify external stimuli that signal sexual encounters, to predict where sexual partners can be found, to actively seek out or work to obtain sex partners, to distinguish pheromonal stimuli or behavioral patterns of potential sex partners from those of animals not sexually receptive, and to pursue desired sex partners once contact has been made. Further, where possible, I will examine the critical conditions that lead to the learning of such relationships.

Learning Processes

Although it is not my central goal to distinguish between different types of learning processes, it is necessary to briefly describe some of the forms of learning that have been implicated in learned aspects of sexual behavior. Three types of learning appear to be crucial in the determination of successful adult sexual behavior; these are sexual imprinting, instrumental learning, and Pavlovian conditioning.

Experience early in life that has a latent effect on subsequent sexual behavior has been termed sexual imprinting (Bateson, 1978a; b). As with other forms of imprinting (see Lorenz, 1970), the exact nature of associations and reinforcement contingencies involved in this type of learning are not well understood. However, it has been argued that imprinting follows contingency rules similar to those important for classical conditioning (Hollis, ten Cate, & Bateson, 1991).

The contingencies involved in instrumental (operant) learning have been more clearly defined. Learning of this form has been thoroughly described by several authors, most notably Skinner (1953; 1966). Instrumental learning is said to occur when there is a change in the frequency or effectiveness of a behavioral response as a result of contingent reinforcement or punishment. Response contingent reinforcement increases the frequency of behavioral responses and response contingent punishment decreases the frequency of behavioral responses. Traditionally, it has been assumed that operant learning is the result of an association between a behavioral response and its consequences, i.e. response-outcome associations are formed (Thorndike, 1911). Several variants of instrumental

conditioning are of interest to the study of sexual behavior. For instance, successful mounting and intromitting appears to be reinforced by sensory feedback from copulatory experience, performance of arbitrary responses can be reinforced by mate presentation, and behavioral responses may be diminished by the removal of sexual partners or reward .

When an association is formed between two stimuli the type of learning is termed classical or Pavlovian conditioning. As described by Pavlov (1927), when an initially neutral stimulus (one that does not elicit the specific behavioral response) is paired with a second stimulus that unconditionally elicits the specific behavioral response, the neutral stimulus will gain the ability to elicit a response by itself. That is, the stimulus comes to be able to elicit a conditioned response (CR). The CR is not necessarily the same as the unconditioned response (UCR). Rather, the CR may serve to prepare the organism for the performance of the UCR (see for instance, Hollis, 1984). There are at least three ways in which classical conditioning may have a role in sexual behavior. In the first, a mate is conceived as an array of stimuli, some of which will unconditionally elicit sexually relevant responses and others of which will not. With sexual experience, initially ineffective stimuli become associated with behaviorally significant ones, and thereby, come to elicit sexually relevant responses. Second, initially neutral, elemental stimuli that are arbitrary and separated physically from an unconditional stimulus can, through contiguous pairings, come to elicit sexually relevant responses. Third, configural features of an environment or context can become associated with a sexually relevant unconditioned stimulus and thereby come to elicit sexually relevant responses.

I will adopt a neural perspective in my review of the role of learning in sexual behavior (e.g. Pavlov, 1927). In such a perspective, it is the neural representations of stimuli and events that are paired. To illustrate, take the example of a male rat exposed to a sexually receptive female paired with a neutral odor. The representation of the conditioned stimulus (CS) is relatively easy to define as the neural activity generated by the neutral odor. The representation of the unconditioned stimulus (UCS) is the pattern or patterns of neural stimulation generated by some features of the female, as well as those generated by copulating behavior in the male. In a relatively simple conditioning trial in which the male is allowed to copulate with the female, there are multiple UCSs that evoke separate aspects of behavior and that are paired with the CS. Further, the context in which the encounter occurs may also gain control over behavior in as much as its neural representation is paired with the neural representation of the UCSs. Because learning mechanisms occur in the central nervous system, it is the neural representation of the neutral stimuli that enters into association with the neural representation of the unconditional stimuli. Such a perspective benefits from the large literature on neural plasticity to understand how conditioning occurs at the neural level and then uses this understanding to explain the generation of conditioned responses.

Sexual Behaviors

Sexual behavior in mammals is composed of a cascade or sequence of behavioral events which include, but are not limited to, copulation. For these behavioral events to

occur, animals must respond to a variety of internal and external stimuli that trigger their own sexual desire and signal that of a potential mate. Many of these stimuli are present in the absence of a mate, and are important for finding a mate as well as eliciting sexual arousal (defined as increased genital blood flow) and sexual excitement (defined as heightened locomotor activity). Other stimuli are derived from a potential mate and may lead to sexual arousal, elicit courting behaviors and the initiation of sexual interactions. An animal's ability to respond appropriately to such stimuli requires not only innate mechanisms, but also a great deal of flexibility in order to learn what stimuli are useful predictors of copulatory success or failure. Although copulation is an unambiguously sexual behavior, it is important to emphasize that it is clearly not the only behavior that is sexual.

Differentiating sexual behavior from non-sexual behavior can be difficult. The goal here is not to provide a definitive list of behaviors that may be classified as sexual or otherwise, but rather to offer definitional criteria that have an appropriate scope in order to be meaningful. For the purpose of the present review, sexual behaviors are those that are motivated by the desire for, and are reinforced or punished by copulatory responses. Accordingly, I will review the empirical evidence regarding the influence of learning on the elicitation of sexual excitement, behaviors that bring about the opportunity to mate, courtship displays, copulatory parameters, and sexual partner preferences.

Influence of Learning on Sexual Excitement

Before an actual sexual encounter, anticipatory responses are often performed with great excitement (Pfaus, 1996; 1999). Stimuli that are predictive of such encounters induce sexual excitement and are critical to the performance of these anticipatory behaviors. Contextual and discrete stimuli paired with sexual stimulation come to elicit sexual excitement as measured operationally by general locomotor activity.

Mendelson and Pfaus (1989) found that male Long-Evans rats will increase the number of level changes made in a bilevel chamber in anticipation of a conspecific if they have previously received access to a sexually receptive female in the bilevel chamber. Males given access to unreceptive females in bilevel chambers (with intervening access to receptive females in unilevel chambers) failed to develop this behavior. Following the establishment of increased anticipatory level changing in trials with sexually receptive females, this response was extinguished if males received access to no conspecific during the subsequent trials in bilevel chambers, but not if males received access to a nonreceptive female (Mendelson & Pfaus, 1989). The reason for this apparent contradiction in the development and maintenance of conditioned sexual excitement is unclear.

Conversely, Van Furth and Van Ree (1996b) found that male Wistar rats exposed to bilevel chambers with either receptive females, nonreceptive females, or no female displayed increased anticipatory level changing. However, in this study, the development of anticipatory level changing was disrupted by anosmia produced by zinc sulphate

infusion into the nasal cavity. Based on these results, Van Furth and Van Ree concluded that olfactory stimuli from animals copulating previously in the bilevel chambers are a critical determinant in the development of anticipatory level changing in male Wistar rats. Consistent with this hypothesis is the finding that neurochemical responses to estrous odors in the male rat sensitize with repeated exposure (Mitchell & Gratton, 1991).

The reasons for the discrepancies between these studies is unclear. In both studies, sexually experienced males were examined and the chambers were not cleaned between trials. One possibility is that strain differences contributed to the differences in results; Wistar rats appear to display more robust responses to sex-related odors than do Long-Evans rats (Carr, Loeb, & Dissinger, 1965; Lydell & Doty, 1972; Merkx, 1983). Alternatively, in the Mendelson and Pfaus (1989) experiment, males trained with nonreceptive females were allowed to copulate with receptive females between each trial in unilevel chambers; this procedure was not followed in the Van Furth and Van Ree (1996b) study. Thus, the subjects in Mendelson and Pfaus (1989) study may have been able to discriminate between the value of the two types of chambers for predicting copulation and to respond subsequently to the dirty bilevel chambers as predictive of the lack of opportunity to copulate.

An analogous finding of increased anticipatory locomotor behavior in the rat has been demonstrated in the Japanese quail. Akins, Domjan, and Gutierrez (1994) found that general activity of the male quail was increased in response to a CS (red light) if the interval between the CS and the UCS (presentation of a receptive female) was 20 minutes

or longer. In contrast, for intervals shorter than 5 minutes, the male quail approaches and remains near the CS, a behavior similar to that observed with a visible female. These results, along with those of Mendelson and his collaborators, suggest that stimuli present before copulation elicit sexual excitement which is evidenced by increased locomotor activity. One benefit of increased locomotor activity may be to increase the chance of encountering a mate.

The effect of opiates on conditioned sexual excitement employing conditioned level changing in bilevel chambers has been examined. Van Furth, Wolterink-Donselaar, and Van Ree (1994) and Van Furth and Van Ree (1996b) found that naloxone (an opiate receptor antagonist) injected systemically prior to each training trial attenuated the development of conditioned level changing. They also found that in males showing high levels of conditioned level changing, naloxone given before each test produces a gradual decrease in conditioned level changing across subsequent trials. Unfortunately, it is not clear from those data whether the expression of conditioned level changing was blocked or if the behavior was extinguished because a final vehicle test was not reported. Van Furth and Van Ree (1996c) also examined the effects of infusions of opioid drugs into the ventral tegmental area (VTA) on the expression of conditioned level changing. In agreement with systemic delivery, VTA infusions of naloxone given prior to each test session blocked the development of conditioned level changing. In contrast, VTA infusion of β-endorphin given prior to each test session did not block the development of conditioned level changing but instead lowered the frequency of level changes, compared

to controls. The effects of these manipulations on the expression of previously acquired conditioned level changing was not examined.

The role of dopamine in the expression of conditioned sexual excitement has been examined by Pfaus and Phillips (1991), employing conditioned level changing in bilevel chambers. Systemic injections of either SCH 23390, a D1 receptor antagonist, or sulpiride, a D2 receptor antagonist, both produced a decrease in the frequency of conditioned level changing in male rats, these effects do not appear to be due to general locomotor impairments as the latencies to level change were not affected. They also examined the neuroanatomical basis of the dopaminergic influence on conditioned level changing by infusing haloperidol (a nonselective D2 and D1 receptor antagonist) into the nucleus accumbens, anteriodorsal striatum, and the medial preoptic area (mPOA). Infusions of haloperidol into the nucleus accumbens or the mPOA, but not into the striatum, decreased conditioned level changing. Interestingly, haloperidol infusions into the mPOA also reduced the amount of pursuit of a receptive female, suggesting that this area is involved in responses to both conditioned and unconditioned sexual stimuli. The effects of manipulations of dopamine systems on the development of conditioned level changing has not been examined.

A number of issues remain to be clarified regarding conditioned sexual excitement. First, more attention needs to be given to elucidating what factors influence the development and expression of conditioned sexual excitement. Second, more attention needs to be given to controlling for general performance effects that may be independent

of conditioned effects. For instance, decreased level changing may be due to motoric effects of a drug independent of stimulus-elicited sexual excitement. Third, disruptions of conditioned sexual excitement are generally interpreted as disruptions of sexual motivation. However, such a conclusion is seldom warranted. Diminished sexual excitement may be produced by disruptions of mnemonic or associative processes independently of purely motivational processes. Rats may fail to make associations or forget the predictive value of stimuli, rather than exhibiting decreased responsiveness to sexually relevant stimuli. This criticism is relevant to other research attempting to use conditioned behaviors as indices of sexual motivation. One way to examine memory processes independently from motivational influences is the post-trial manipulation during training method developed by McGaugh and colleagues to examine fear conditioning (for reviews see McGaugh, Cahill, & Roosezdall, 1996; McGaugh, 1989). In this method, subjects are given a conditioning trial or session of trials, receive a manipulation following the training, and then are tested at a later time. For instance, a tone (CS) may be paired with a shock (UCS), followed by drug administration, and tested once the drug is no longer present. In such a case, the drug can not influence the performance of responses necessary for the development or expression of conditioning, but rather it specifically influences memory retention or consolidation processes. Conversely, manipulations that precede a training or test session may influence motivational, performance, and mnemonic processes.

Influence of Learning on Locating a Mate

One initial problem in mating is locating a mate. This might be accomplished simply by relying upon chance encounters; however, unless the individual resides in an area that has a very high density of potential partners, it is unlikely to be a very successful strategy. Accordingly, animals could increase their chances for reproductive success by using past experience in the search for a mate. Studies using both contextual and discrete stimuli demonstrated that animals tend to approach and remain in the vicinity of stimuli that have been paired with copulation.

Animals display a preference to remain in a context that has been paired consistently with access to a mate over a context that has not; this is commonly referred to as a conditioned place preference (CPP). CPP is typically demonstrated using an apparatus with two connected distinctive compartments. First, the compartments are paired differentially with unconditional stimuli (e.g. one side is paired with a sex partner, food, or a rewarding drug and the other side is paired with either nothing or a control manipulation). Then, on a test session, the subject is allowed to move freely between the compartments. A CPP is said to have developed when the subject spends more time in the reward paired compartment than in the other one. UCSs that are capable of supporting CPP are referred to as rewards, as opposed to reinforcers, because the subject has never been required to move into the paired compartment to experience the UCS. Thus CPP behavior is not reinforced as it is being displayed spontaneously.

In male rats, CPPs have been established with sexual reward using two different conditioning procedures. In one procedure, copulation to ejaculation is allowed to occur within one distinctive environment and this environment is subsequently preferred over the other environment in which copulation was not allowed (e.g. Everitt, 1990)--CPP developed by this procedure can be referred to as copulatory CPP. Such a copulatory CPP can be maintained by intromissive stimulation alone, whereas prevention of intromission disrupts a previously established CPP (Hughes, Everitt, & Herbert, 1990).

In a second procedure, male rats are allowed to copulate to ejaculation in a separate arena and then transferred immediately to one distinctive compartment of the CPP apparatus; following such training this compartment will be preferred over the other compartment (e.g. Agmo & Berenfiled, 1988)--CPP produced by this procedure can be referred to as post-ejaculatory CPP. Demonstrations of post-ejaculatory CPP might appear puzzling from the perspective of Pavlovian conditioning. It would appear, at least superficially, that the CS was presented following the UCS or reward (copulation), a situation involving a "backward" pairing of the stimuli that is not supposed to yield conditional responding to the CS. However, if the neural state induced by ejaculation is considered to be the UCS, then the pairing of CS and UCS is simultaneous and post-ejaculatory CPP can be accounted for by the rules of Pavlovian conditioning. Be that as it may, both CPP procedures produce effects of similar magnitude. However, there do appear to be differences in the underlying neurobiology as demonstrated by differential effects of drugs (see below).

Conditioned place preferences have also been demonstrated in female rats and hamsters. However, in contrast to the robust preferences seen in males, some reports demonstrate only weak effects in females. Oldenburger et al. (1992) found that when copulation occurred within one of the distinctive compartments of a typical CPP apparatus female rats showed only a weak CPP. Conversely, Paredes and Alonso (1997) demonstrated a robust CPP in female rats which depended on whether or not the females were able to pace the rate of copulation without employing defensive behaviors (referred to as "paced" copulation). Females acquired a strong preference for a context if they were placed into the context immediately following paced copulation. In contrast, no preference was found if the copulation was unpaced. Thus, for a female rat CPP develops only when she is able to control the temporal aspects of copulation without defensive behavior. Paredes and Alonso have interpreted these results as a postcopulatory reward state in the female rat. However, these results may also reflect the presence of aversive properties during unpaced mating conditions given that the female must resort to defensive behavior in an attempt to pace the male's sexual behavior. Examining CPP in female rats produced by paced mating within a distinctive environment would evaluate if paced copulation itself is rewarding.

CPP for the environment in which the copulation has occurred previously has also been demonstrated in female hamsters (Meisel & Joppa, 1994). However, it is important to note that the latter study in hamsters found that females displayed a CPP for compartments paired with aggressive encounters. Thus, there appears to be a species

difference between female hamsters and female rats regarding the rewarding properties of aggressive encounters. In hamsters, aggressive encounters may serve to strengthen a copulatory CPP. Conversely, in rats, aggressive encounters (produced by unpaced mating) may serve to weaken a copulatory CPP. Nevertheless, the body of evidence on sexually rewarded CPP demonstrates that both males and females approach and remain in a context that has previously been paired with sexual stimulation or reward. However, it is not yet clear whether the nature of sexual stimuli capable of supporting CPP are the same or different for males and females.

Discreet stimuli within an environment that are consistently paired with sexual interactions can also elicit conditioned approach behavior. Domjan, Lyons, North, and Bruell (1986) found that when a visual stimulus consistently preceded the arrival of a female, male quail would approach and remain near the stimulus. Similar results have been produced in female quail (Gutierrez & Domjan, 1997). One study in the male gerbil (Villarreal & Domjan, 1998) is of particular interest because it suggests that even when a CS is paired inconsistently with sexual reward it is still able to elicit conditioned approach behavior. In this study, male gerbils were exposed to a neutral odor that was paired with access to their impregnated mates. On some of the training trials, copulation took place whereas on other trails, no copulation occurred. Despite this, the males displayed conditioned approach behavior to the neutral odor in the absence of their mates.

Recently, Pfaus, Theberge, and Kippin (in preparation) have found that an initially aversive stimulus paired with copulation can acquire attractive properties. In

this study, groups of male rats were allowed to copulate either with cadaverine-scented females or with unscented females for 9 sessions. Cadaverine, a component of decaying corpses, is innately aversive to rats (Pinel, Gorzalka, & Ladak, 1981). Following the conditioning procedure, all males were tested in a novel environment into which a wooden dowel wrapped in cotton containing cadaverine was placed. Males that had previously copulated with unscented females avoided the dowel. Few of these males made contact with it, many tried to escape the test chamber, and all spent more time on the distal side of the chamber. Conversely, males that had previously copulated with cadaverine-scented females readily approached and showed appetitive responses toward the scented dowel. All of these males made contact with and remained in close proximity to it, most picked it up, and gnawed on it, and none tried to escape the test chamber. Following subsequent cadaverine habituation sessions, the control group still displayed aversive responses to cadaverine. Thus, pairing the aversive cadaverine odor with copulation not only diminished the aversive properties of the odor but made it an attractive conditional stimulus. This finding may have important implications for the formation of sexual paraphilias in humans involving erotic responses to stimulation that may otherwise be considered aversive.

Demonstrations of conditioned place preference and conditioned approach behavior are consistent with the much larger literature documenting that animals will approach conditioned stimuli that have been paired with rewarding stimuli (for reviews see Nader, Bechara, & van der Kooy, 1997; Tzschentke, 1998; Wise, 1989). Presumably

this reflects a cognitive search strategy based upon past experience which increases the probability of coming into contact with mates and other rewards. The findings of post-ejaculatory CPP and that drugs of abuse induce CPP suggest it may be the rewarding aspects of mating, not the discovery of a mate, that elicit stimulus approach behaviors. However, it is not clear whether copulatory behavior *per se* is capable of supporting the development of CPP.

The neurobiological substrates of conditioned approach behaviors have been examined primarily using the conditioned place preference model in the male rat. Both copulatory and post-ejaculatory CPPs have been studied using opioid agents, yielding somewhat inconsistent results. Naloxone appears to disrupt both types of CPP, but in different ways. Agmo and Berenfield (1988) found that the development of post-ejaculatory CPP is blocked by naloxone injections prior to each training session. Conversely, the development of copulatory CPP was unaffected by naloxone prior to each training session (Meharra & Baum, 1990). However, once a copulatory CPP had developed, its expression was blocked by an injection of naloxone prior to the test session (Meharra & Baum, 1990; Hughes et al., 1990). There is also evidence that the site of action of naloxone is different for these effects. Agmo and Gomez (1993) found that naloxone disruption of the development of post-ejaculatory CPP can be achieved with infusions into the mPOA, whereas naloxone into this brain region prior to a test session did not disrupt the expression of copulatory CPP (Hughes et al., 1990). Interestingly, lesions of this area caused a time-dependent disruption of a copulatory CPP (Hughes, et

al., 1990). The differential effects of opioids on the two types CPP suggest that post-ejaculatory CPP may be less robust than copulatory CPP or that multiple opioid systems are involved in CPPs produced by sexual reward. One way to resolve this issue is to test the influence of a naloxone injection prior to the test on the expression of post-ejaculatory CPP.

The effect of dopaminergic drugs on conditioned approach behavior has also been examined. Agmo & Berenfield (1988) found that the development of post-ejaculatory CPP in male rats was blocked by injections of pimozide (a D2/D3 receptor antagonist) prior to each training session. Similarly, Meisel, Joppa, and Rowe (1996) found that the development of copulatory CPP in female hamsters was blocked by injections of the D2 receptor antagonists, sulpiride and raclopride, prior to each training session. No studies have reported on the effects of dopaminergic drugs on the expression of CPP. However, other results suggest involvement of the mesolimbic dopamine systems in responding to conditioned stimuli. For example, West, Clancy, and Michael (1992) examined the responsiveness of the nucleus accumbens in male rats to odors. They found that odors paired previously with a receptive female produced more single unit activity in medium spiny neurons than odors paired with either a nonreceptive female or with no conspecific. Further, the responsiveness was higher for males that ejaculated during training than in those who did not. Although, conditioned approach behaviors would likely have been elicited following this training procedure, no behavioral measures were reported to

confirm this and the relation of these conditioned physiological changes to conditioned approach behavior is unclear.

Limited attention has been given to assessing the role of the endocrine system in conditioned approach behavior. Castration disrupts the expression of copulatory CPP on the first post-operative test (Miller & Baum, 1987; Hughes et al., 1990). As well, acquisition of copulatory CPP was blocked by naloxone in castrated, but not intact, males (Meharra & Baum, 1990). It is interesting to note that endocrine responses have been detected following exposure to either contextual or discrete stimuli paired previously with copulation. Kamel, Mock, Wright, & Frankel (1975) found that testosterone, luteinizing hormone, and prolactin levels were elevated following exposure to an arena in which prior copulation occurred. Similarly, Graham and Desjardin (1980) found that testosterone and luteinizing hormone were increased following exposure to an odor (methyl salicylate or wintergreen) paired previously with copulation. However, it is again important to note that no behavioral measures were reported, thus the relation of these conditioned physiological changes to conditioned approach behavior is unclear

A number of issues remain to be clarified with sexually rewarded CPP. First, the differences between copulatory CPP (which includes ejaculation) and post-ejaculatory CPP need to be examined further. Specifically, more work needs to be done to determine whether these types of CPP are mediated by the same or different physiological mechanisms. Also, as with conditioned sexual excitement, more attention needs to be paid to assessing the way factors influence development and expression of CPP.

Similarly, studies need to determine if disruptions of CPP can be attributed to disruptions of sexual motivation or independent disruptions of mnemonic processes.

Influence of Learning on Overcoming Obstacles to Mating

In order to gain access to a mate, it may not be sufficient to merely approach a location that has previously been the site of copulation. Sometimes there are obstacles to overcome. Males and females of many species have demonstrated a strong willingness to work for many rewards, including access to a sex partner. The propensity to perform arbitrary behaviors that result in the presentation of a mate has been demonstrated in several species. Anecdotal evidence from human experience tells us that both men and women will perform various operants to attract or gain favor with potential mates, however, the empirical data are restricted to studies involving animals.

Numerous empirical reports have demonstrated that male rats easily learn to bar press in order to receive access to a receptive female (Beck, 1971; 1978; Beck & Chmielewska, 1976; Jowaises, Taylor, Dewsbury, & Malagodi, 1971; Larsson, 1956; Schwartz, 1956). Other studies have demonstrated that female rats learn to bar press for a sexually active male (Beck, 1971; 1974; 1978; Bermant, 1961; Bermant & Westbrook, 1966; French, Fitzpatrick, & Law, 1972). Typically, these studies involve the subject learning to bar press in a modified Skinner box in order to cause a mate to be delivered into the box allowing copulation to commence. Bar pressing for mates has also been demonstrated in both male (Micheal & Keverne, 1968) and female (Keverne, 1972) rhesus monkeys. Similar studies demonstrate that male pigeons will key peck (Gilbertson, 1975)

and male stickleback fish will swim through a ring (Sevenster, 1973) in order to gain access to a receptive female. Correct performance of a T-maze in order to locate a mate is also performed by male rats (Drewett, 1973; Hetta & Meyerson, 1978; Kagan, 1955; Whalen, 1961) and female rats (Drewett, 1973; Meyerson & Lindstrom, 1973; Eliasson & Meyerson, 1975). Other studies found that male rats (Beach & Jordan, 1956a; Ware, 1968) and guinea pigs (Seward & Seward, 1940) will run an alley and male rats will climb over a hurdle (Sheffield, Wulff, & Backer, 1951) to gain access to a receptive female. To gain access to a receptive female, male rats can also be trained to dig through sand (Anderson, 1938), cross shock grids or perform other aversive tasks (Anderson, 1938; Meyerson & Lindstrom, 1973; Warner, 1927), turn a wheel (Denniston, 1954) and master obstruction boxes (Moss, 1924; Warner, 1927; Jenkins, 1928; Stone, Barker, & Tomlin, 1935). These results demonstrate that males and females of many species have the ability to learn to overcome many obstacles, as well as to endure painful stimulation in order to gain access to a mate.

The effect of allowing only incomplete copulation (i.e. intromission without ejaculation) on several operants has been examined. Males allowed to copulate to ejaculation rather than incomplete copulations made more consistent choices (Kagan,1955) and developed faster running speeds (Whalen, 1961) in T-mazes and hurdle climbing (Sheffield et al., 1951). Bermant & Westbrook (1966) examined lever press latencies following intromission alone or with ejaculation in male and female rats. They found that for both sexes the longer response latencies were obtained with the completion

of the entire sequence of sexual behavior suggesting transient sexual satiety is produced by ejaculation. These results support the notion that copulation and ejaculation have differential effects on behavior, however, they do not indicate whether such differences are quantitative or qualitative in nature.

Everitt and colleagues have used a modified version of lever pressing for a receptive female to examine the neurobiology of sexually-reinforced operant behavior in male rats. Everitt, Fray, Kostarcyzk, Taylor, and Stacey (1987) demonstrated that rats trained to bar press for a receptive female can subsequently be trained to bar press for a light or tone that is paired with copulation on a second-order (FI: FR10) schedule of reinforcement. Using this procedure, they were able to obtain high and consistent rates of lever pressing allowing an examination of the neurochemical basis of the expression of this conditioned responding. Dopamine antagonism by intraperitoneal injection of α-flupentixol, dose dependently decreased, whereas infusion of amphetamine into the nucleus accumbens increased, instrumental responding under this second-order reinforcement schedule (Everitt, 1990). Lesions of the basolateral amygdala selectively disrupted conditioned lever pressing for a secondary reinforcer, and this effect was reversed by infusion of amphetamine into the nucleus accumbens. Such lesions did not affect copulation (Everitt, Cador, & Robbins, 1989). These results implicate a projection from basolateral amygdala to nucleus accumbens in the control of operant responding for sexual incentives. However, it appears that this circuit is not specialized for sexual incentives, as similar disruptions are seen in responding for ingestive incentives (Everitt,

1990). In contrast, lesions of the mPOA disrupted copulation, but had a small, indirect influence on conditioned lever pressing, in initial postlesion tests, responding was high and decreased with subsequent testing. This suggests that lever pressing was extinguished due to an inability to obtain reinforcement through copulation (Everitt & Stacey, 1987). The effects of castration on lever pressing were similar to those of mPOA lesions--initially normal rates followed by extinction. Additionally, systemic injections, but not intra-mPOA infusions, of naloxone reduced conditioned responding for second-order reinforcement (Hughes, et al., 1990).

Influence of Learning on Mate Recognition

Once a conspecific has been encountered, mating does not necessarily follow. First, an individual must be able to recognize a sexually receptive mate from a nonreceptive conspecific and respond appropriately. Efficient mate recognition is a crucial task for the reproductive success of individuals. Failure to recognize a mate and respond with the appropriate sexual vigour will result in a missed opportunity to mate. In nature, where such opportunities are typically rare, this could prove deleterious to the individual's contribution to the gene pool. Equally, failure to recognize conspecifics that are not sexually-receptive and withhold sexual advances can result not only in an inappropriate use of vital energy and time, but also lead to potentially severe social repercussions and risks of injury.

The ability of animals to discriminate potential mates from other conspecifics has been studied largely through preference tests. During choice tests, a male often spends more time in the proximity of an estrous female than a diestrous one and in the proximity of estrous stimuli (e.g. soiled bedding) than diestrous stimuli. Similarly, females often display proximity preferences for a male over a female conspecific. Substantial evidence implicates learning processes in both preferences for potential mates over non-mates, and in the generation of appropriate responses toward each.

Preferences for conspecifics of varying sexual status has been studied extensively in a few species; however, the generality of the findings is unclear. Male rats appear to have a readily demonstrable preference for an estrous female over a male or a diestrous female. Male rats prefer to spend more time in the proximity of a caged estrous female than either a caged diestrous female or a caged male (Carr, Loeb, & Dissinger, 1965; Stern, 1970). They spend more time investigating an anaesthetized estrous female than an anaesthetized diestrous female (Landauer, Wiesse, & Carr, 1977; Stern, 1970). And they spend more time investigating soiled bedding (Carr et al., 1965; Landauer et al., 1977), or preputial gland extract (Thody & Dijstra, 1978) from estrous than diestrous females. However, Brown (1977) found that male rats do not display a preference for urine of estrous over diestrous females. When female rats are in estrous, they prefer to spend time in the proximity of a caged male over a female conspecific (Carr, Wylie, & Loeb, 1970) with a sexually-active male being preferred over a castrated one (Drewett, 1973).

Evidence for estrous preferences in other species is equally convincing. Male mice prefer vaginal secretions (Hayashi & Kimura, 1974) and urine (Rose & Drickamer, 1975) from estrous females over those from diestrous females. Female mice also prefer male mice over female mice (Nimomiya & Kimura, 1988; Brown, 1985). Similar preferences for female conspecifics have been demonstrated in male desert wood rats (Fleming, Che, & Vaccarino, 1981), brown and collared lemmings (Huck & Banks, 1984), Mongolian gerbils (Block, Volpe, & Hayes, 1981), beagles (Doty & Dunbar, 1974), monkeys (Goldfoot, 1981), and Japanese quail (Domjan & Hall, 1986a; b; Domjan, Akins, & Vandergriff, 1992).

The evidence for estrous preferences in some species is more equivocal. Male hamsters, for example, display estrous preferences for soiled bedding (Johnston, 1980; 1983) and female-primed cages (Carmichael, 1980). However, they display no preference when the stimulus females are actually present (Landauer, Banks, & Carter, 1978). Male prairie voles, but not male montane voles, display an estrous preference (Taylor & Dewsbury, 1988). Kangaroo rats of the *Dipodomys merriami* strain display an estrous preference, whereas, those of the *D. spectabilis* strain do not (Randall, 1985; 1986). Deer mice do not appear to have an estrous preference for soiled bedding from anesthetized or caged females (Dewsbury, Ferguson, Hodges, & Taylor, 1986). Thus, the belief that all animals display preferences for receptive and vigorous mates seems to be an overgeneralization (for a review see Taylor & Dewsbury, 1990). The failure to display robust preferences for mates or mate-related stimuli in some species does not necessarily

indicate the lack of discrimination of mates from non-mates, rather that it is not manifested as a preference in these species. For instance, in some species high levels of aggression exhibited by females of that species (e.g. hamsters) may deter the male from spending time in the female's presence. In such cases, the potential mates may possess both positive and negative attributes which compete during a conspecific preference test.

In several species in which robust preferences for particular conspecifics exist, learning has been demonstrated to play a role in the development of this preferential responding. Sexual experience has been shown to have a profound effect on estrous preferences in male rats. Males given either discrete encounters with only estrous females (Lydell & Doty, 1972; Landauer et al., 1977), or given experience with diestrous as well as estrous females (Carr et al., 1965), showed a stronger estrous preference than sexually naive males. Similar results have been produced with lemmings (Huck & Banks, 1984), mice (Rose & Drickamer, 1975; Hayashi & Kimura, 1974), hamsters (Johnston, 1980), and dogs (Doty & Dunbar, 1974). In contrast, sexually-naive, male prairie voles display an estrous preference that is largely unaltered by sexual experience (Taylor & Dewsbury, 1988). A particularly interesting study by Hayshi & Kimura (1976) found that male mice did not need direct interaction with a receptive female to develop an estrous preference. They found that exposure to conspecifics engaged in copulation resulted in expression of an estrous preference in sexually-naive male mice, demonstrating that, on some measures, observational experience is sufficient for sexual learning in rodents. However, it should be noted that the possible influence of a pheromone produced only during copulation was

not ruled out. Nevertheless, this study suggests that no copulatory experience is explicitly necessary for the development of an estrous preference.

One explanation for the inconsistency in the comparison of naive- and sexually-experienced males in different species may be the that estrous odors have innate effects in the brain in some, but perhaps not all, species. Estrous odors on first contact produce increased dopamine release in the nucleus accumbens of male rats (Wenkstern, Pfaus, & Fibiger, 1993). Further exposure to estrous odors (without sexual experience) produces sensitization of dopamine release in the nucleus accumbens in male rats (Mitchell & Gratton, 1991). Thus, in the rat, sexual experience or exposure to estrous odors may increase an innate response via sensitization of this dopamine system, which may produce approach preferences to females in estrous. Accordingly, it is necessary to examine innate and experienced responses in species in which males do not display estrous preferences (e.g. montane voles; Taylor & Dewsbury, 1988) as well as in species in which sexual experience does not appear to increase estrous female preferences (e.g. prairie voles; Taylor & Dewsbury, 1988).

A study by Pfaus & Pinel (1989) demonstrated a functional outcome of estrous preferences in male rats. They found that in order for male rats to learn to direct mounting behavior towards females in estrous and suppress mounting towards diestrous females, the males had to have experience with females in both reproductive states. Almost all sexually-experienced males which had not been exposed to nonreceptive females attempted to copulate with these females, despite their nonestrous reproductive

status and high level of defensive behaviors. However, after several trials with both estrous and diestrous females, these same rats only attempted to mount estrous females.

Experience is also an important variable in the development of female conspecific preferences. Sexually-experienced, but not sexually-naive female rats and mice display preferences for male conspecifics over female conspecifics. Perhaps the most convincing demonstration of the influence of learning on preferences for conspecifics in females comes from two experiments by de Jonge, Burger, Van Haaren, Overdijk, and Van de Poll, (1987). In one experiment, they replicated the findings of others that esterous females that had received heterosexual experience with males, but not sexually-naive females, spent more time near a sexually-active male rat than near a female rat. Conversely, in a second experiment, they found that ovariectomized females that had been treated with testosterone propionate to induce mounting of conspecific females subsequently displayed a preference for an estrous female over either a sexually-active male or a diestrous females. Thus, after engaging in sexual or social experience with other females, female rats display a male-like preference.

In contrast to the apparent dependence of conspecific preferences on odor in rodents, studies by Domjan and his colleagues in the Japanese quail have demonstrated that, in this avian species, vision plays an important role in determining status of conspecifics. Further, they claim that preferences for females by males is under instrumental control. Their claim is based upon studies that have measured the preference of male Japanese quail to approach and remain near a female or a female-like model. Like

the measures of estrous preference with male rodents, this approach behavior of the male quail occurs in sexually-experienced, but not sexually-naive, individuals (Domjan & Hall, 1986a; b). Once males learn to approach and remain near a visible female, they will also approach and remain near a visible male. However, this male-male approach behavior declines if the exposure is not reinforced by sexual access to a female (Domjan & Revert, 1991). Further studies demonstrated that male quail appear to discriminate the sex of the other quail through sexual reinforcement (Nash, Domjan, & Akins, 1989). It appears that approach behavior to stimulus animals of either sex can be induced and maintained as long as it is reinforced by an opportunity to engage in sexual behavior. Additionally, Domjan and Nash (1988) found that male quail would also approach a taxidermic model of a female in a similar manner to that of an actual female. By altering the shape of the model they were able to determine that only certain configurations of the component stimuli of the model would elicit approach behavior. Specifically, they found that proper orientation of the head of the model was necessary for animals to elicit approach and that a hooded model failed to elicit approach.

Influence of Learning on Courtship Behavior or Solicitation

In many species, once animals come into the proximity of a conspecific that is sexually-receptive, copulation is preceded by one (usually the male) or both potential partners engaging in behaviors which entice the other partner to mate. A number of studies have reported that certain components of courting behavior can be elicited by

stimuli associated with a mate. Studies in rodents have found that learning plays an important role in the production of vocalizations associated with copulation. It has been demonstrated that ultrasonic vocalizations in response to olfactory stimuli in male mice were dependent on prior sexual experience for their expression (Maggio, Maggio, & Whitney, 1983; Dizinno, Whitney, & Nyby, 1978).

Classical conditioning has been implicated in the elicitation of courtship behaviors by several studies demonstrating that previously neutral stimuli paired with mating opportunities are also capable of eliciting elements of courtship behavior. Nyby and his colleagues (Nyby, Bigelow, Kerchner, & Barbehenn, 1983; Nyby, Whitney, Schmitz, & Dizinno, 1978) have demonstrated that artificial odors paired with access to receptive female mice become capable of eliciting ultrasonic vocalizations from male mice. Hollis and colleagues (Hollis, Cadieux, & Colbert, 1989; Hollis, Pharr, Dumas, Brittion & Field, 1997) demonstrated that repeatedly pairing a light with non-contact exposure to a receptive female resulted in conditioning of the sexual behavior in male gouramies. Following training, males responded to the light alone with fin displays that are normally associated only with courtship. Sevenster (1973) reported that male stickleback fish made courtship displays towards a floating ring that they had been trained to swim through in order to gain access to a female. Similar results have been obtained in avian species. Gilbertson (1975) reported that courtship displays were elicited in male pigeons during operant key pecking for a female, and Farris (1967) found that male Japanese quail made courtship displays following a tone that had reliably predicted presentation of a

female. Interestingly, in several studies using a visual CS, Domjan and colleagues (for a review see Domjan, 1994) have failed to replicate the conditioned courtship displays reported by Farris. This inconsistency may indicate the differential effects of conditioning produced by stimuli of different sensory modalities and may reflect constraints on learning regarding the conditioned elicitation of courtship behaviors.

Conditioning can also attenuate preparatory and courting behaviors. Peters, Koch, Blythe, and Sufka (1988) found that ultrasonic vocalizations preceding copulation were inhibited in male rats that had previously received injections of lithium chloride (LiCl) paired with access to a receptive female. Hamsters not only learn to prefer the odors of estrous females but they also readily lick and consume vaginal secretions when presented on a slide (Johnston, 1972; 1974). Johnston and his colleagues have produced a conditioned taste aversion to this normally highly attractive stimulus through a punishment procedure that pairs vaginal secretions with an injection of LiCl. Male hamsters treated in such a manner took longer to initiate licking, spent less time licking, consumed less vaginal secretions presented on a slide than did control animals (Johnston & Zahorik, 1975; Zahorik & Johnston, 1976; Johnston, Zahorik, Immler, & Zakon, 1978), and consumed less of a dilute solution of vaginal secretions than control males (Zahorik & Johnston, 1976). Further, when given the opportunity to interact with an estrous female, conditioned males had increased latencies for orogenital contact with a receptive female (Johnston et al., 1978).

Influence of Learning on Copulatory Parameters

Once two potential mates have come in contact with each other, engaged in courtship behavior, and both are mutually receptive, copulation may begin. For all mammals, copulation involves the insertion of the male's penis into the female's vagina to allow for sperm delivery to produce fertilization as well as vaginocervical stimulation to facilitate pregnancy. For males of most mammalian species, this involves mounting a receptive female, pelvic thrusting with the subsequent achievement of penile intromissions that eventually culminates in ejaculation, followed by a period of quiescence. For females of most species, copulation involves the act of assuming appropriate positioning to facilitate intromission by the male, solicitation and pacing of the male's copulatory behavior, and a period of quiescence following completion of copulation. The behavioral cascade of copulatory behavior necessitates a high level of similarity between species, however, substantial diversity does exist. For instance, in a comparative analysis, Dewsbury (1972; 1973; 1975) has found that rodent copulatory behavior differed qualitatively between species based on the presence of a copulatory lock, thrusting, multiple intromission, or multiple ejaculations. From this analysis, he found that rodents display a wide range of copulatory patterns. Across a wider range of species, there is no doubt that a greater amount of diversity would be found.

It is generally well accepted that sexual experience aids in the coordination of appropriate copulatory responses and studies reporting plasticity in these behaviors have confirmed this notion in a number of species. Of the species studied, the role of sexual

experience has been best described in the rat. Larsson (1956) and Dewsbury (1969) have found that the number of sexual experiences increases the percentage of successful attempts to intromit by male rats (i.e. the intromission ratio increases). Further, the number of intromissions to achieve ejaculation decreases and the time between intromissions decreases with sexual experience. Similar improvements in copulatory efficiency have been noted in cats (Micheal, 1961; Rosenblatt, 1965; Whalen, 1963), hamsters (Bunnell & Kimmel, 1965), and guinea pigs (Valenstein & Goy, 1957). Additionally, McGill (1962a) reported that, in mice, the number of mounts inappropriately directed towards the female's head decreased with sexual experience. These effects of experience on sexual behavior are likely due to instrumental leaning and appear to bring male copulatory responses to a homogenous form. However, no studies have reported the effect of sexual experience on the sexual efficiency of female animals or humans of either gender.

Other learning effects on copulatory behaviors have also been reported. Silberberg and Adler (1974) reported that rats can learn to control their intromission frequency under a negative punishment schedule of responding. They found that rats decreased the number of intromissions required to ejaculate if they were limited to seven per copulation session, whereas control rats showed no alteration in intromission frequency. Jowaisas, Taylor, Dewsbury, and Malagodi (1971) found that rats allowed to copulate under an imposed operant requirement produced altered intromission patterns similar to that produced by an "enforced interval effect" (see Larsson, 1956) in which males ejaculated

with fewer intromissions. Female quail display increased squatting (a measure of sexual receptivity) frequency and duration if the appearance of a male quail is signalled (Gutierrez & Domjan, 1998).

Perhaps the most widely studied dependent measure of conditioning of sexual behavior has been the study of sexual arousal. Sexual arousal is one of the few components of sexual behavior to which there are both substantial human and animal literatures. Assessment of sexual arousal in human studies is strictly defined as the measurement of blood flow to the genitalia--penile erection in men and vaginal pulse in women. Penile erections elicited by nonaccessible female are also measured in primates (e.g. Nadler & Bartlett, 1997; Pomerantz, 1990) and rats (Sachs, Akasofu, Citron, Daniels, & Natoli, 1994; Sachs, 1995a). Additionally, penile erection produced by manual stimulation by the experimenter is also widely studied in rodents (see Meisel & Sachs, 1994). Unfortunately, the more common approach in animal models has been to use the latencies to intromit and ejaculate as indices of sexual arousal. Thus, the measures of sexual arousal used in humans and animals studies have often not been the same. This is especially true for the case of studies of conditioned sexual arousal.

Studies using human subjects have demonstrated that sexual arousal can be altered through the use of a number of manipulations, including habituation, classical conditioning, and instrumental learning. Habituation of erectile responses in men has been demonstrated with repeated exposure to the same erotic slides (O'Donohue & Geer, 1985) or audiotapes (O'Donohue & Plaud, 1991). Using women subjects, Meuwissen and Over

(1990) found that vaginal pulse habituated with repeated presentations of the same erotic film segment, and then dishabituated with novel film segments.

A number of studies have demonstrated that classical conditioning can produce sexual arousal. Rachman (1966) and Rachman and Hodgson (1968) found that following pairing with erotic slides, a pair of women's boots was able to elicit erections in men. Similarly, McConaghy (1970; 1974) demonstrated conditioned erection elicited by colored circles or squares paired previously with erotic videotapes or still pictures in heterosexual and homosexual men. A particularly informative study by Kantorowitz (1978) further examined the nature of association between the unconditioned stimuli and conditioned arousal induced by still pictures. For each subject, he paired three different slides with the plateau, refractory, and resolution stages of masturbation. During subsequent testing, stimuli paired with the plateau phase produced an increase in penile erection, stimuli paired with the refractory phase produced a decrease in erection, and stimuli paired with the resolution phase had no effect. Remarkably these responses were still present after 3 months. Only one study has examined the classical conditioning of sexual arousal in women. Letourneau and O'Donahue (1997) failed to find significant effects of conditioning on sexual arousal in women. However, the authors note that the UCSs (erotic films) produced only moderate levels of arousal whereas in studies with male subjects such stimuli produced high levels of arousal. Thus, this failure to demonstrate conditioned arousal in women may have been due to an ineffective UCS.

Several studies have attempted to demonstrate instrumental control of sexual arousal in men and women. Rosen, Shapiro, and Schwartz (1975) found that given feedback and contingent monetary reinforcement, men learned to become sexually aroused in the absence of erotic stimuli. Other studies have found that men, as instructed, can suppress (Rosen & Kopel, 1977; Rosen, 1973) or increase (Reynolds, 1980) penile erection with feedback; however, these studies failed to demonstrate learning effects across trials. Similarly, as instructed, women can increase 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 occurred. In summary, the evidence regarding instrumental control of sexual arousal is limited to the one report in which monetary reinforcement and feedback were provided.

Evidence from animal studies has demonstrated a clear influence of previous sexual experience in the speed of copulation. Larsson (1956), and subsequently Dewsbury (1969), reported the effect of sexual experience on the development of sexual behavior. They both found that ejaculation latency was reduced as a function of prior copulation and Dewsbury found that mount and intromission latencies were also reduced. Similar results have been obtained with mice (McGill, 1962b), cats (Michael, 1961), and guinea pigs (Valenstein & Goy, 1957). Kippin, Talianakis, and Pfaus (1997) have recently examined the influence of ejaculation on the development of sexual behavior. Male rats were allowed to obtain multiple intromissions without ejaculation, one ejaculation, or two ejaculations on each of 9 training sessions, then all males were allowed to copulate for a

30 min test. There were no differences between groups on a range of copulatory parameters, including intromission latency, ejaculation latency, intromission frequency, interintromission interval, and post-ejaculatory interval. These results suggest that intromissions are sufficient for the development of copulatory efficiency. Additionally, 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 mating behavior.

Classically conditioned stimuli are also capable of increasing sexual arousal as reflected by copulatory rate measures. Zamble and his colleagues (Zamble, Hadad, Mitchell, & Cutmore, 1985; Zamble, Mitchell, & Findlay, 1986) used placement of male rats in a holding cage as a conditioned stimulus to signal non-copulatory exposure to a receptive female on several training trials. On test trials, they found that placing the males into the holding cage prior to copulation resulted in significantly shorter latencies to intromit and ejaculate than if the conditioned stimulus was omitted. Subsequent studies found that second-order conditioned stimuli were effective at eliciting arousal (Zamble et al., 1985). Hollis, Cadieux, and Colbert (1989) demonstrated that repeatedly pairing a light with non-contact exposure to a receptive female resulted in conditioning of sexual behavior in male gouramies. They found that males receiving the conditioning treatment displayed significantly lower latencies to initiate copulation and lower levels of aggression towards females when the conditioned stimulus was presented before access to a female. Similar results have been demonstrated in Japanese quail. Males that had previously

received repeated exposure to females following the presentation of a conditioned stimulus displayed significantly shorter latencies to initiate copulation when the stimulus was present compared to when it was absent (Domjan, O'Vary, & Greene, 1988). Pfaus, Talianakis, and Kippin (in preparation) have recently found evidence that somatosensory stimuli can be used to condition sexual arousal. Male rats that had received prior sexual experience with receptive females while wearing an unattached harness jacket displayed faster intromission and ejaculation latencies if tested with the jacket than without it.

Aversive conditioning can also influence copulatory latencies. Male hamsters and rats injected with LiCl following copulation subsequently displayed significantly longer intromission latencies than controls (hamsters: Johnston et al., 1978; rats: Peters, 1983). However, Emmerick and Snowdon (1976) failed to find inhibition following a similar treatment. In rats, the addition of a neutral stimulus (almond odor: Lawrence & Kiefer, 1987) or a component of scent marking (phenylacetic acid: Emmerick & Snowdon, 1976) facilitated the conditioned aversion to females. Similarly, juvenile rats injected with LiCl following exposure to estrous females, displayed longer latencies to intromit during copulation in adulthood (Koch & Peters, 1987). Finally, Sachs (1995b) reported that in male rats erections elicited by non-contact exposure to a female were attenuated by prior pairings of such exposure with injections of LiCl.

Interestingly, copulatory parameters are affected by CSs that have been paired with either aversive or rewarding stimuli of a nonsexual nature. Fillion and Blass (1986) found that adult male rats displayed shorter ejaculation latencies with receptive female

rats bearing an odor paired with nursing during infancy compared with receptive females not bearing the odor (see also Marr & Gardner, 1965). It has been demonstrated that moderately painful stimuli have a facilitatory effect on copulation in male rats. For example, administration of painful skin shock decreased intromission latency and postejaculatory refractory period (Barfield & Sachs, 1968) and adminstration of painful tailshock can induce previously noncopulating rats to copulate (Caggiula & Elbergen, 1969). Moreover, the presentation of a CS previously paired with shock can induce noncopulating male rats to copulate (Crowley, Popolow, & Ward, 1973). Contextual stimuli paired with drug administration also have effects on copulation. Mitchell & Stewart (1990) found that a context previously paired with morphine increased the amount of female-directed behaviors in intact male rats and decreased the intromission latencies in castrated male rats. The influence on sexual behavior of stimuli paired with other drugs or aversive stimuli in males, or any drugs or aversive stimuli in females have not been reported; such studies would be of great importance to understanding how sexual arousal and motivation interacts with motivational and arousal mechanisms for nonsexual incentives.

Influence of Learning on Sexual Partner Preferences

Individuals exhibit preferences, not only for sexually-receptive conspecifics over nonreceptive ones, but also for specific receptive potential mates. One can prefer the features of one potential mate over those of another. There has been much theoretical

speculation and some empirical evidence that learning plays a role in the development of these preferences, and mate preferences appear to be influenced by experiences both early in life and in adulthood.

Preferences for specific mates is determined, at least in part, by sexual imprinting. Several studies have demonstrated that adult males preferentially mate with females that have attributes similar to those of the female that nursed them early in life. Yamazaki, Beauchamp, Kupniewski, Bard, Thomas, and Boyse (1988) found that male mice nursed by foster mothers choose to mate with females that resembled their foster mother rather than their biological mother. Similarly, Cooke and colleagues have determined that the coloration of the nursing lesser snow goose is preferred by adult ganders both in laboratory experiments (Cooke & McNally, 1975) and field studies (Cooke, Finney, & Rockwell, 1976; Cooke, Mirsky, & Seiger, 1972). Bateson (1978a) claims that sexual imprinting allows adult males to mate with an optimal outbreeding strategy in order to avoid inbreeding. He provided evidence for this hypothesis from a study with Japanese quail using three distinctively colored strains. In a series of mate-choice tests, he reported that males showed the highest preference to approach and to copulate with females whose coloration differed slightly from that of their foster mothers as compared to females with the exact same coloration or drastically different coloration. Perhaps the most provocative report of sexual imprinting is that of Kendrick, Hinton, and Atkins (1998), demonstrating that sexual partner preferences can be achieved between goats and sheep using crossfostering to manipulate the imprinting process. In both males and

females of both species, sexual partner preferences were toward members of the opposite sex of the species of the foster, rather than the biological, mother.

Another approach to studying sexual imprinting has been to examine the influence of artificial stimuli attached to nursing mothers. Two such studies have examined the influence of pairing novel odors with nursing dams on subsequent conspecific preferences in male rats. Marr and Gardener (1965) found that subjects that had a novel odor paired with nursing until weaning displayed an approach preference for conspecifics bearing that odor. Similarly, subjects with normal scented dams showed a preference for unscented females. Recently, Moore, Jordan, and Wong (1996) failed to replicate these findings. However, during preference tests in the latter study, the subjects were allowed to contact conspecifics, whereas in the former study no contact could occur. As well, different odors were used. Why these methodological differences would produce different results is unclear and this contradiction needs to be clarified. Moore et al. (1996) also found no differences during contact with anaesthetized conspecifics or during a simultaneous sexual test with a scented and unscented female. These studies have been performed only in rats using odors as stimuli; it would be interesting to examine the generalizablity of such findings to other sensory modalities and to other species.

The importance of adult sexual experience on partner preferences has been clearly demonstrated in two different lines of evidence. First, studies of social interaction of pairbonded prairie voles, a socially-monogamous species, show preferential responding to partners that are sexually familiar over partners that are novel. Second, studies of social

interaction in the seasonally socially-monogamous, Japanese quail, have shown that pairing neutral stimuli with copulation produces subsequent preferential responding toward individuals bearing the familiar stimuli.

The behavioral consequences of past sexual interactions on sexual preferences have been studied extensively in voles. Such studies employ a comparative framework in which socially-monogamous prairie voles (*Microtus orchrogaster*) are compared to polygamous montane voles (*M. montanus*). The results of research from several laboratories demonstrates that prairie voles, but not montane voles, prefer an opposite-sex conspecific with whom they have previously copulated and/or cohabited over an opposite-sex conspecific that is unfamiliar. Although well-established breeding pairs show sexual preferences for each other (Getz, Carter, & Gavish, 1981), sexual preferences are rarely studied in this model (Carter, DeVries, & Getz, 1995). Typically, non-copulatory social interactions are studied because they are displayed by newly formed pairs. The primary measure used in these studies has been the amount of side-to-side contact exhibited by female prairie voles during the opportunity to interact with one familiar and one unfamiliar male. In such tests, females will mate indiscriminately with both males, but will show more contact with the familiar male (Williams, Catania, & Carter, 1992)--I refer to this behavior as a social proximity preference. This preference is independent of behavioral responses of the males as females still display preferences when the males are anaesthetized.

Classical conditioning of stimuli associated with sexual behavior plays a major role in the development of mate-choice preferences. Studies by Domjan and colleagues show that male Japanese quail respond differentially to females based on the presence of stimuli paired previously with copulation. Nash & Domjan (1991) allowed male quail to copulate with females of two strains of quail that have different plumage coloration (brown or blond). Subsequently, males choose to spend more time in the proximity of females whose coloration was the same as the coloration of females with whom they had copulated previously (Nash & Domjan, 1991). Similarly, males allowed to copulate with females that were adorned with bright orange feathers subsequently spent more time near, and engaged in more sexual activity with, females similarly adorned than unadorned females. Moreover, males trained with adorned females engaged in mating behavior with a taxidermic model of a female quail only if it was adorned with the feathers (Domjan, O'Vary, & Greene, 1988). It is important to note that several studies have claimed to assess conditioned sexual preferences by examining differential responding during copulatory diad tests. However, these studies do not truly provide evidence of preference behavior as the subjects are never allowed to choose between partners.

The neurobiology of partner preference in voles has been examined using pharmacologic and neuroendocrine manipulations in prairie voles and by comparative analysis of physiological parameters between prairie and montane voles. Extensive research has determined that pituitary hormones control social proximity preferences in both male and female prairie voles. However, the hormone responsible is different in

males and females (for recent reviews, see Carter, DeVries, & Getz, 1995; Insel, Winslow, Wang, & Young, 1998; Young, Wang, & Insel, 1998). Female sexual partner preferences appear to be under the control of oxytocin. In female prairie voles, oxytocin in the absence of mating is sufficient for social proximity preference formation and oxytocin antagonists disrupt social proximity preference formation (Wang, Smith, Major, & DeVries, 1994). Conversely, male social proximity preferences are influenced by vasopressin. In male prairie voles, vasopressin in the absence of mating is sufficient for social proximity preference formation and vasopressin antagonists disrupt social proximity preference formation (Winslow, Hastings, Carter, Harbaugh, & Insel, 1993). In socially nonmonogamous species of voles, oxytocin and vasopressin do not induce social proximity preferences. Further, the adrenal hormone, corticosterone, inhibits social proximity preference in females, but facilitates it in males (Carter, DeVries, Taymans, Roberts, Williams, & Chrousos, 1995; DeVries, DeVries, Taymans, & Carter, 1995; DeVries, Taymans, & Carter, 1997). Additionally, social proximity preference in female prairie voles is not influenced by manipulations of the gonadal hormones or gonadectomy (Carter, Witt, Thompson, & Carlstead, 1988; Williams et al., 1992; Williams, Insel, Harbaugh, & Carter, 1994). This latter finding is of particular interest because it demonstrates that not all sexual or sexually-reinforced behaviors are dependent on the gonads.

Comparative investigations of oxytocin and vasopressin neural systems have revealed differences in the brain organization that may underlie species differences in

partner preferences. The distribution of oxytocin and vasopressin cells appears to be similar between socially monogamous and nonmonogamous species (Wang, Zhou, Hulihan, & Insel, 1996). Thus, it appears that species difference in partner preferences are mediated by the cellular response to, rather than the release of, these hormones. Accordingly, there appear to be striking differences in the distribution of oxytocin and vasopressin receptors between prairie and montane voles.

Autoradiography studies demonstrated the different distributions of vasopressin receptors in prairie and montane voles (Insel, Wang, & Ferris, 1994). Despite no sex differences nor any overall species differences, the regions of highest receptor density were different between species; in the prairie vole, vasopressin receptor densities were highest in accessory olfactory bulb, laterodorsal thalamus, superior colliculus, and diagonal band of Broca; in contract, vasopressin receptor densities were highest in accessory olfactory bulb, superior colliculus, and lateral septum in the montane vole.

Similarly, autoradiographic analysis of oxytocin receptors revealed no sex differences or overall species differences in binding densities between prairie and montane voles (Insel & Shapiro, 1992). Again, the regions of highest receptor densities were different between species. In the prairie vole, the highest binding densities were found in the prelimbic cortex, bed nucleus of the stria terminalis, nucleus accumbens, midline nuclei of the thalamus, and the lateral aspects of the amygdala, whereas in the montane vole, the highest binding densities were found in the lateral septum, ventromedial nucleus of the

hypothalamus, and cortical nucleus of the amygdala. Thus, there was almost no overlap in brain regions of highest oxytocin receptors between the species.

It is unclear how to interpret the receptor density differences for the understanding of sexual partner preferences. The underlying cause of these species differences in receptor densities is not yet known. However, the finding that there are few differences in gene sequence and promoters (Young, Huot, Nilsen, Wang, & Insel, 1996) suggests that the answers lie in events upstream that influence the expression of these genes, rather than the genes themselves. Further, species differences in receptor densities do not provide a clear mechanistic explanation of species differences in sexual partner preferences. However, these differences implicate specific brain regions as starting points for future studies to elucidate the neural pathways for sexual partner preferences.

Rationale of the Development of a Rat Model of Conditioned Sexual Partner Preferences

The present thesis investigates the role of learning in sexual partner preferences in the male rat. Past sexual experience has been shown to alter preferences in sexual partners. Male and female prairie voles exhibit copulatory and approach preferences for sexually-familiar conspecifics over novel ones. Male Japanese quail display approach preferences for females of coloration or adornment resembling that of past sexual partners.

Although these studies have demonstrated that learning is involved in the selection of mates, the generalization of these findings is limited for at least two reasons. First, although studies in Japanese quail have clearly implicated classical conditioning as the mechanism responsible for the influence of learning on sexual partner preference, no studies in voles or any other mammalian species has identified the learning mechanism or mechanisms that can influence sexual preferences. The present thesis employs a mammalian species, the rat, in order to address this issue. Further, several studies have revealed effects of conditioning on sexual behavior in the rat, however, none have employed a testing situation in which the subject has an opportunity to select between mates. Thus, although classical conditioning and sexual imprinting are known to influence male copulatory behavior (e.g., sexual arousal), it is not known if learning can alter sexual partner preferences in this species.

Second, studies that specifically test for learned preferences have only been conducted in socially monogamous species; in the wild, Prairie voles are socially monogamous (Carter et al. 1995) and Japanese quail are serially socially monogamous with a single mate selected each breeding season (Mills, Crawford, Domjan, & Faure, 1997). Thus, it is not clear whether the results from these species would generalize to polygamous species. As polygamy is a far more common mating strategy than social monogamy, it is important to determine whether past sexual experience alters subsequent selection of sexual partners and the direction of such alterations in a polygamous species.

Accordingly, the present series of studies employs the polygamous rat to examine the influence of prior sexual experience on sexual preferences.

Additionally, a rat model of conditioned sexual partner preferences would be highly amenable to revealing underlying neurobiological mechanisms for a number of reasons. First, this species has been the species of choice for neurobiological studies since the inception of the field. More is known about the neurobiology of this species than perhaps any other. Second, the reproductive physiology and neurobiology of the rat is well understood. Moreover, there are striking similarities between human and rat reproductive neurobiology especially in males. For instance, male rat and human sexual behavior show similar alterations following administration of a wide spectrum of drugs (see Meisel & Sachs, 1994; Pfaus & Everitt, 1996). Third, the neurobiology that underlies associative learning has been examined extensively in the rat. Much of this work has been directed toward the study of conditioning with aversive unconditional stimuli. Thus, a model of conditioned sexual preferences would be ideal for comparisons between the neurobiology underlying aversively conditioned and appetitively conditioned responses.

Accordingly, the present thesis develops a model of conditioned sexual partner preferences in the rat. This model uses initially neutral odors as CSs that are physically attached to a potential sexual partner. During training, male rats have access to females bearing the odor CS. Then, during a test session, the male has access to two females, one bearing the CS and one not.

The present thesis comprises four distinct chapters each describing crucial elements of conditioned sexual partner preferences in the rat. Chapter 1 examines the elementary aspects of the model. In three experiments neutral odors (either almond or lemon) are paired with the opportunity to copulate, then males are tested with one scented and one unscented female in an unobstructed fashion; I refer to this test situation as a copulatory preference test (CPT). This test situation allows interaction between all three subjects, thus mimicking the reproductive ethology of the group mating rat (see McClintock, 1984). In the first chapter the necessary explicitly-unpaired and randomly-paired control groups are examined to rule out nonassociative forms of learning. The results of Chapter 1 demonstrate that, under the conditions described, male rats ejaculate more frequently with females bearing an odor that was paired previously with copulation--I refer to this phenomenon as a conditioned ejaculatory preference (CEP).

Having established the associative nature of CEP, Chapter 2 investigates the course of development and extinction of CEP. The course of CEP development is examined by varying both the number of conditioning sessions (each of fixed length) and by varying the length of a single conditioning. Then males were give a CPT to assess CEP. The course of extinction was examined in males displaying CEP by employing multiple CPTs.

Chapter 3 determines the components of copulation that comprise the unconditioned stimulus that is capable of supporting the development of CEP. Although, studies in the Japanese quail by Domjan and colleagues have demonstrated that

conditioned approach behavior requires copulation with a female, they did not demonstrate this in a preference test. Further, due to the copulatory pattern of the quail, they were unable to analyze the role of various components of copulation in supporting the development of conditioning. Conversely, copulation in rats follows a multiple intromission pattern which is ideal for such an analysis. In chapter 3, male rats were allowed to copulate with scented females, with session termination at various stages of copulation. Males were allowed to copulate without ejaculation, with ejaculation, or with multiple ejaculations on each conditioning session. Then all males were given a CPT.

Chapter 4 investigates the nature of the conditioned response that mediates CEP. Essentially, the chapter focuses on two basic explanations for CEP: Facilitated ejaculation with the scented female during the CPT or preferential ejaculation with the scented female during the CPT. To this end, the CR elicited by the CS was examined in the absence of a female or with a sexually-nonreceptive female and copulatory behavior in the absence of the CS was assessed. Finally, I re-examined the distribution of mounts at different points during CPT from all of the experiments in the first three chapters in which males displayed CEP in order to ascertain whether males show distinct copulatory strategies at different points of copulation.

The present results are interpreted in both a Pavlovian and incentive motivational framework. The generalizablity of these findings to the development of sexual partner preferences are examined. Finally, future studies of CEP are suggested.

CHAPTER 1: OLFACTORY CONDITIONING OF SEXUAL BEHAVIOR IN THE MALE RAT

The influence of conditioning in differential copulatory responses to sexual partners has been examined. Domjan, O'Vary, and Greene (1988) found that male Japanese quail exhibited shorter latencies to engage and complete copulation with a female bearing a CS (coloured feathers) paired previously with copulation compared with the latencies of males that copulated with a female not bearing the CS. Fillion and Blass (1986) found that adult male rats displayed shorter ejaculation latencies with receptive female rats bearing an odor paired with nursing during infancy compared with receptive females not bearing the odor (see also Marr & Gardner, 1965). In these studies, the conditioned effect was demonstrated by differences in responding to females either bearing or not bearing the CS.

Although such differential responding is indicative of conditioned increases in sexual arousal, it should not be taken as evidence of a conditioned preference for a particular mate for one important reason: There was no opportunity for the male to select between potential mates. Investigation of the relation between conditioned stimuli and mate preferences would require that (1) the conditioned stimulus be attached to a potential mate (as in the Domjan et al., 1988 and Fillion and Blass, 1986 studies) and that (2) males would have unrestricted access to at least two potential mates.

In a preliminary study (Pfaus, Jacobs, & Wong, 1986), a neutral odor (almond extract) used as an olfactory CS paired with copulation produced an ejaculatory preference. Male rats were allowed access to sexually receptive females with either the

CS or distilled water applied to their neck and anogenital region during copulatory training trials. The influence of this conditioning procedure on copulatory preference was tested subsequently by allowing males to copulate to ejaculation with simultaneous, unrestricted access to two receptive females, one bearing the CS and one not. More males in each group ejaculated with females similar to those they had previously copulated with. The present study replicates the results of this pilot study and provides the explicitly unpaired and randomly paired control conditions necessary to establish classical conditioning as the mechanism underlying the development of the ejaculatory preference.

Experiment 1

In Experiment 1, we examined the effect of repeated pairing of an olfactory stimulus (almond extract) with access to a receptive female rat on the acquisition of sexual behavior and on the subsequent copulatory preferences between potential mates in the male rat. The explicitly unpaired and the randomly paired control conditions necessary to determine whether or not classical conditioning has been established were also examined (Rescorla, 1967). This was accomplished by giving three groups of male rats alternating training trials with both receptive and nonreceptive female rats. One group always had exposure to almond odor paired with access to receptive females (Receptive-Paired-Trained condition) and trials with nonreceptive females that were unscented. A second group always received exposure to almond odor paired with nonreceptive females (Nonreceptive-Paired-Trained condition) and trials with receptive females that were

unscented. A third group had exposure to almond odor paired with receptive or nonreceptive females in a random fashion (Random-Trained condition).

Methods

Subjects

Males. The 54 Long-Evans rats that served as subjects in this experiment were obtained from Charles River Canada, (St. Constant, Québec). The males weighed approximately 300 g and were sexually naive at the start of the experiment. They were housed in pairs in Plexiglas cages (36 cm x 26 cm x 19 cm) with ad lib access to food (Purina Rat Chow) and water. All rats were kept in a 12:12 hour reversed light-dark cycle colony room maintained at 21°C.

Females. Female Long-Evans rats from the same supplier as above were ovariectomized via bilateral lumbar incisions under ketamine/xylazine anaesthesia at least two months prior to the start of the experiment and were sexually experienced. Sexual receptivity was induced by subcutaneous administration of estradiol (10 μg) 48 hr prior and progesterone (500 μg) 4-6 hr prior to each test trial. Females were housed under the same conditions as males. Stimulus females were selected at random for use during Conditioning Trials and Copulatory Preference Tests. Female rats were scented with approximately 1 ml of either almond extract (Blue Ribbon, Etobicoke, Ontario, Canada) or distilled water applied to both the back of the neck and the anogenital area using a cotton swab.

Apparatus

Conditioning Trials took place in bilevel chambers constructed of Plexiglas (outside dimensions of 18 cm x 25 cm x 65 cm) with a platform (40 cm in length) elevated by a set of ramps at each end dividing the chamber into two levels (see Pfaus, Mendelson, & Phillips, 1990 for further details). The bilevel chambers were cleaned with water and Coverage 256 (Conva Tec, St. Louis, MO) and soiled bedding was replaced with clean bedding prior to each Conditioning Trial. All Conditioning Trials were recorded on video and scored subsequently using a PC-based program (Cabillio, 1996). Copulatory Preference Tests took place in a large open field (123 cm x 123 cm x 46 cm); Copulatory Preference Tests were scored at the time of testing.

Procedure

Conditioning Phase. Male rats were pre-exposed to the bilevel chambers once a day for 15 min each day in order to habituate them to the training environment. This habituation procedure lasted 7 days and has been shown previously to increase the proportion of males that become vigorous copulators (Pfaus & Wilkins, 1995). Then all males received a total of 18 Conditioning Trials at two day intervals during the middle third of the dark phase of the light:dark cycle. Access to sexually-receptive and sexually-nonreceptive females occurred on alternating trials; the first trial was counterbalanced with respect to female status for each group. For males in the Receptive-Paired-Trained condition, all trials with sexually-receptive females were with females that had almond extract applied to each area (A+E females) and all trials with sexually-nonreceptive

females were with females that had distilled water applied to each area (N-Alone females). For males in the Nonreceptive-Paired-Trained condition, all trials with sexually-receptive females were with females that had distilled water applied to each area (E-Alone females) and all trials with sexually-nonreceptive females were with females that had almond extract applied to each area (A+N females). And for the males in the Random-Training condition, half the trials with sexually-receptive females were with A+E females and half with E-Alone females and half the trials with sexually-nonreceptive females were with A+N females and half with N-Alone females: The scent of the females on all these trials was counterbalanced and followed a pseudo-random scheduled determined prior to the start of the experiment.

For all Conditioning Trials, males were placed individually into a bilevel chamber for 5 min, after which a female of the appropriate sexual status and appropriate scent was placed into the chamber for a 30 min test of copulation. Latency and frequency data for all mounts, intromissions, and ejaculations were recorded during each Conditioning Trial. Criteria for sexual behaviors were those described by Sachs and Barfield (1976) and Meisel and Sachs (1994).

Copulatory Preference Test. Four days after the final Conditioning Trial, each male was placed in the large open field and allowed to habituate for 5 min. At the end of this period, one A+E female and one E-Alone female were placed simultaneously into two diagonal corners of the open field at approximately equal distances from the male. All

copulatory behaviors and the females to which they were directed were recorded during each male's test. Tests were terminated 30 min after the females were introduced.

Statistical Analysis.

Mixed-design between-within ANOVAs were used to analyze the level changing data from the receptive and nonreceptive Conditioning Trials with significant values being followed by post hoc analysis of individual means using the Tukey method. Chi square analysis was used for analysis of proportions of female selected for first mounts, first intromissions, and first ejaculations on the Copulatory Preference Test. Mixed ANOVAs were used for analysis of the distributions of mounts, intromissions, and ejaculations throughout the 30 min Copulatory Preference Test. T-tests were used for analysis of proportions of mounts and intromissions directed toward the female selected for ejaculation in a series. The level of significance for all comparisons was 0.05.

Results

Conditioning Phase. No substantial between group differences were detected during the Conditioning Phase. Mean level change latency decreased and mean level change frequency increased across trials for all groups (data not shown); levels were similar to those found in previous studies using the bilevel chambers (see Pfaus et al., 1990). The proportion of males that ejaculated with receptive females increased during the Conditioning Phase from 67% on the first Trial to 95% on the final Trial; there were no between group differences. The proportion of males that mounted nonreceptive

females decreased during the Conditioning Phase from 30% on the first trial to 5% on the final trial; again there were no between group differences.

Copulatory Preference Test. Of the 54 males, all but 11 copulated to ejaculation on the Copulatory Preference Test. Of the males that failed to ejaculate, more were in the Nonreceptive-Paired-Trained group (n = 7) than in the Receptive-Paired-Trained group (n = 3) and the Random-Trained group (n = 1). The proportion of Nonreceptive-Paired-Trained males that failed to copulate on the Copulatory Preference Test was significantly greater than on the Conditioning Trial 9 ($\chi 2 = 8.69$, $p < 0.05$) and was significantly greater than the proportion of Random-Trained males ($\chi 2 = 5.79$, $p < 0.05$).

Figure 1 displays the selection of female for first mount, first intromission, and first ejaculation for each group. More Receptive-Paired-Trained males ejaculated first with A+E females than did males in the other two groups and more Nonreceptive-Paired-Trained males ejaculated first with E-Alone females than did males in the paired group. Chi square analyses confirmed the statistical significance of these observations: Receptive-Paired-Trained versus Nonreceptive-Paired-Trained $\chi 2 = 5.42$, $p < 0.05$; Receptive-Paired-Trained versus Random-Trained $\chi 2 = 3.35$, $p < 0.05$; Nonreceptive-Paired-Trained versus Random-Trained $\chi 2 = 0.56$, $p > 0.05$. No significant differences were found between the groups for selection of female for first mount or first intromission.

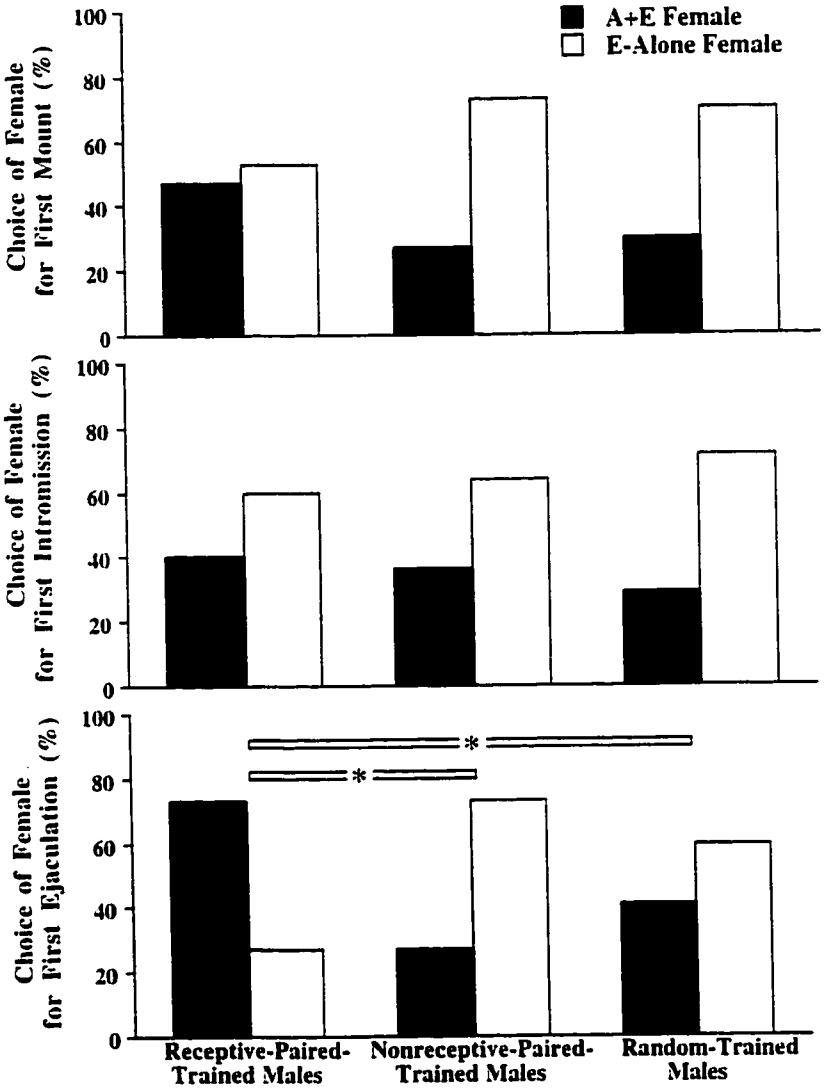

Figure 1. Proportional distribution by female type of first mount (top panel), first intromission (middle panel), and first ejaculation (bottom panel) during the Copulatory Preference Test in Experiment 1. * denotes $p < 0.05$ for between groups comparison.

The mean mounts per female per series, intromissions per female per series, and mean ejaculations per female over the 30 min test are displayed in Figure 2. Receptive-Paired-Trained males mounted more frequently than did the males in the other two groups and the E-Alone females received more mounts than did the A+E females, there was not a significant interaction between Group and Female Type. The significance of these observations were confirmed by a 3 x 2 mixed ANOVA (for Group: $F(2, 80) = 6.86$, $p < 0.05$, post hoc comparisons revealed that Receptive-Paired-Trained males differed significantly from Nonreceptive-Paired-Trained and Random-Trained males, but that Nonreceptive-Paired-Trained and Random-Trained did not differ significantly; for Female Type: $F(1,80) = 10.56$, $p < 0.05$; for Group x Female Type: $F(2,80) = 0.37$, $p > 0.05$). Also, E-Alone females received more intromissions than did A+E females, but there were no other significant differences in the distribution of intromissions; the significance of these observations were confirmed by a 3 x 2 mixed ANOVA (for Group: $F(2, 80) = 1.03$, $p < 0.05$; for Female Type: $F(1,80) = 6.10$, $p < 0.05$; for Group x Female Type: $F(2,80) = 1.45$, $p < 0.05$). Moreover, for ejaculation distribution, Receptive-Paired-Trained males ejaculated more frequently with A+E females than with E-Alone females, Nonreceptive-Paired-Trained males ejaculated more frequently with E-Alone females than with A+E female, and Random-Trained males ejaculated with both females with equal frequency. A 3 x 2 mixed ANOVA revealed no significant main effects (for Group: $F(2,80) = 0.40$, $p > 0.05$; for Female Type: $F(1,80) = 0.50$, $p > 0.05$). However, there was a significant interaction between Group and Female Type ($F(2,80) = 11.01$,

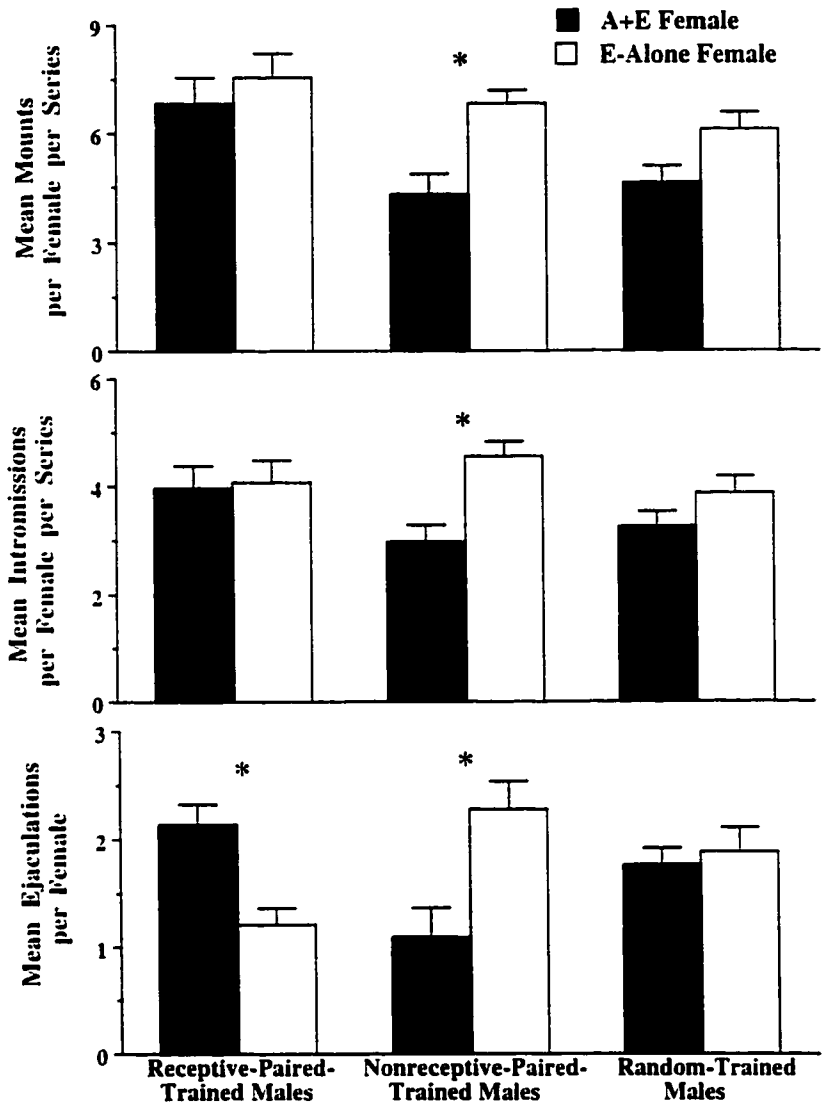

Figure 2. Distribution of mean (+SE) mounts per female per series (top panel), mean (+SE) intromissions per female per series (middle panel), and mean (+SE) ejaculations per female (bottom panel) during the Copulatory Preference Test in Experiment 1. * denotes $p < 0.05$ for between female types comparison.

$p < 0.05$). Post hoc comparisons revealed that Receptive-Paired-Trained males ejaculated more times with A+E females than they did with E-Alone females; that Receptive-Paired-Trained males ejaculated with A+E females more times than the males in the other two groups; that Receptive-Paired-Trained males ejaculated with E-Alone females less times than did the males in the other two groups; that Nonreceptive-Paired-Trained males ejaculated more times with E-Alone females than they did with A+E females; that Nonreceptive-Paired-Trained males ejaculated more times with E-Alone females than did Random-Trained males; and that Nonreceptive-Paired-Trained males ejaculated with A+E females less times than did Random-Trained males.

The proportion of males that switched females for consecutive ejaculations is displayed in Figure 3. Analysis of choice of females for consecutive ejaculations revealed that there were differences between groups. On the second series more than half of the Random-Trained males (59%) switched females, whereas about half of the Receptive-Paired-Trained males (47%) and less than half of the Nonreceptive-Paired-Trained males (27%) switched females. A chi squared analyses revealed that the statistical significance of the difference between the Random-Trained and Nonreceptive-Paired-Trained groups ($\chi 2 = 5.04$, $p < 0.05$), but no other differences were significant. Analysis of subsequent ejaculatory series failed to reveal significant differences in the proportions of males switching on the third or fourth series.

No other measures of copulatory behavior differed significantly between the two groups. However, it is noteworthy that the selection of female for ejaculation was not

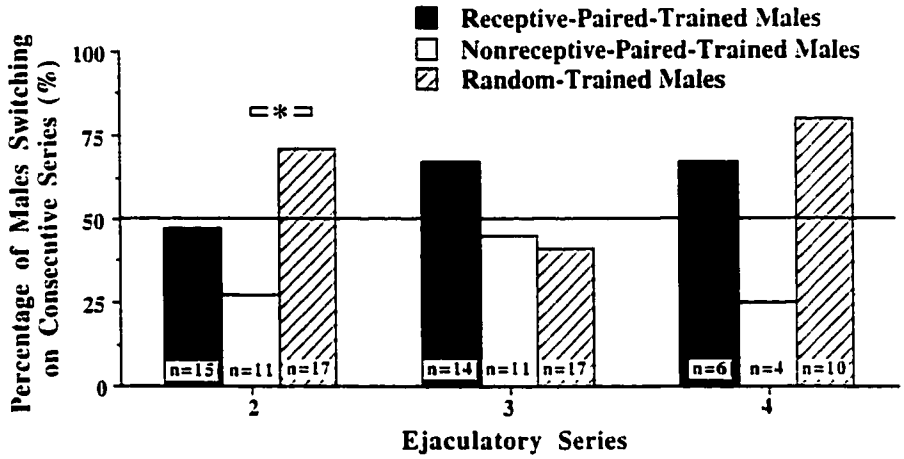

Figure 3. Proportion of males in each group switching females for ejaculation on consecutive ejaculatory series during the Copulatory Preference Test in Experiment 1. * denotes $p < 0.05$ for between groups comparison.

related to either the distribution of mounts or the distribution of intromissions. The same female was selected for first ejaculation and for first mount by about half of the Receptive-Paired-Trained (60%), Nonreceptive-Paired-Trained (43%), and the Random-Trained (53%) males, and for first intromission by about half of the Receptive-Paired-Trained (53%), Nonreceptive-Paired-Trained (36%), and Random-Trained (59%) males. For all three groups of males, the mean proportion of mounts across all ejaculatory series directed toward the female selected for ejaculation on the same series was not substantially different from chance (50%) probability (for Receptive-Paired-Trained males: $t(14) = 0.33$, $p > 0.05$; for Nonreceptive-Paired-Trained males: $t(10) = 1.9$, $p > 0.05$; for Random-Trained males: $t(16) = 0.21$, $p > 0.05$). Similarly, the mean proportion of intromissions across all ejaculatory series directed toward the female selected for ejaculation on the same series was not substantially different from chance probability in either group (for Receptive-Paired-Trained males: $t(14) = 0.87$, $p > 0.05$; for Nonreceptive-Paired-Trained males: $t(10) = 0.41$, $p > 0.05$; for Random-Trained males: $t(16) = 0.31$, $p > 0.05$.).

Discussion

The finding that the Receptive-Paired-Trained and Nonreceptive-Paired-Trained males ejaculated preferentially with females during the Copulatory Preference Test refines the generally-accepted notion that male rats mate indiscriminately with groups of females (see Dewsbury, 1982; McClintock, 1984; Symons, 1979) or that they have a preference for novel females (Dewsbury, 1981). Males were allowed to copulate with two females

that had been rendered highly distinguishable by addition of the CS (almond) odor to one of the females. Under these conditions, males displayed discriminative mating. On the Copulatory Preference Test, more males in Receptive-Paired-Trained and Nonreceptive-Paired-Trained groups ejaculated with the female that was similar to females that they had previously copulated with than ejaculated with the other female; the Receptive-Paired-Trained males displayed a preference for A+E females and Nonreceptive-Paired-Trained males displayed a preference for E-Alone females. These findings support previous work demonstrating that under certain conditions a male can discriminate between, and prefer to mate with, certain females (Dewsbury, 1982; McClintock, Anisko, & Adler, 1982; see also McClintock, 1984), and further suggest that when there are distinguishable differences between females, males will use stimuli that have previously been paired with female reproductive status in directing their ejaculations.

It is important to note that in the present experiment, the expression of the male preference occurred in the presence of interactions between the two females. McClintock, Anisko, and Adler (1982) and Tiefer (1969) have shown during group mating, female rats compete actively while mating with a male and that such competition is highest when a male is about to ejaculate, especially for early ejaculations. This competition between females (e.g., interception of the male as he chases another female) was observed during the Copulatory Preference Test. Thus, the current demonstration of male sexual preference for a particular female for his first ejaculation in the presence of

two females suggests that conditioned ejaculatory preference in the male rat is a particularly robust phenomenon.

An unexpected and potentially interesting finding in this experiment was the significant reduction in the proportion of Nonreceptive-Paired-Trained males copulating on the Copulatory Preference Test. The reason for this is not clear. One possible explanation is that the almond odor may have produced inhibition of sexual behavior on the test. During the Conditioning Trials, the Nonreceptive-Paired-Trained males received repeated trials with A+N females. Male rats in all of the groups learned to inhibit their sexual behavior toward the nonreceptive females, but for the Nonreceptive-Paired-Trained males, almond odor was always a reliable indicator of the necessity of this inhibition. Thus, through classical conditioning almond odor alone may have become able to inhibit sexual behavior. During the Copulatory Preference Test, almond odor was likely present from two sources: first, from the A+E female; and second, the open field was not cleaned between tests which likely resulted in residual almond odor being present. This intriguing pattern of results is of potential importance because this effect may provide a useful model for studying the effects of inhibitory conditioning on sexual behavior. Previous studies of conditioned inhibition of sexual behavior used nausea produced by lithium chloride injections paired with copulation to reduce sexual arousal (e.g. Johnston & Zahorik, 1975). The present findings suggest that conditioned sexual inhibition may be produced by pairing a CS (a neutral odor) with a naturally inhibiting UCS (a nonreceptive female).

Another unexpected finding was that significantly more Random-Trained males switched their choice of female for second ejaculation ("switching") compared with the other groups, suggesting an alteration in the general mating strategies of male rats in the presence of an arbitrary odor that has been paired previously with female reproductive status. The significance of switching lies in its resemblance to the Coolidge Effect (Beach & Jordan, 1956b; see Dewsbury, 1981). As is the case with the Coolidge Effect, switching involves a relative increase in the incentive value of a second (or novel) female following copulation with a first (or familiar) female. In the original study by Beach and Jordan (1956b), the higher incentive value of a novel female was apparent by her ability to stimulate copulatory behavior from a male rat that had become sexually exhausted through repeated ejaculations with a familiar female. In the present study, the higher incentive value of an unejaculated female over an ejaculated one was apparent in Random-Trained males, most of which switched their choice of female for the second ejaculation. The fact that the other groups of males were less likely to display switching suggests that in addition to a conditioned ejaculation preference, the conditioning procedure used here can influence the general mating strategy employed by male rats. The nature and consequences of these differences in copulatory strategy are unclear, and to our knowledge similar conditioning effects have not been reported previously. However, the finding that Random-Trained and Nonreceptive-Paired-Trained males differed in terms of their selection of female for the second ejaculation under equivalent conditions further

suggests that male selection controlled the distribution of ejaculations during the Copulatory Preference Test.

Finally, it is of interest to note that the addition of training trials with nonreceptive females does not appear to influence the development of conditioned level changing. Mendelson and Pfaus (1989) found that sexually-naive males receiving access to receptive females developed conditioned level changing, whereas those receiving access to nonreceptive females did not. They further found that once males had developed conditioning level changing, substitution of a nonreceptive female for the receptive one did not disrupt the behavior. Thus, the present results are consistent with the notion that the development of conditioned level changing is dependent upon receiving access to receptive females in the bilevel chamber. Moreover, as the chambers were cleaned prior to each trial, these findings are also support the notion that sex odors from previous copulations are not necessary for the development of conditioned level changing in Long-Evans rats.

Experiment 2

The purpose of Experiment 2 was to replicate and extend the results of Experiment 1. In Experiment 1, we found that when males were given training trials with both receptive and unreceptive females which always involved a consistent odor pairing that males would develop a preference for the receptive females of the odor type that they had previously encountered as sexually-receptive. The purpose of Experiment 2

was to determine if mate preferences could be generated with only the trials with receptive females. Accordingly, Paired-Trained males received 9 trials with A+E females, Nonpaired-Trained males received 9 trials with E-Alone females, and Unpaired-Trained males received 9 trials with E-Alone females and exposure to almond odor in isolation. Then each male received a Copulatory Preference Test with one A+E and one E-Alone female.

Methods

Subjects

Forty-five male rats (n = 15 per group) of the same strain from the same supplier were housed in the same conditions as those of Experiment 1 served as subjects. Female rats were housed and receptivity was induced in the same way as in the first experiment.

Apparatus

All Conditioning Trials were conducted in the same bilevel chambers (cleaned prior to each Trial) and the Copulatory Preference Tests were conducted in the same open field as used in Experiment 2. All conditioning Trials were recorded on video and scored subsequently using a PC-based program (Cabillio, 1996).

Procedure

Conditioning Phase. As in Experiment 1, male rats received 7 daily 15-min preexposure sessions to the bilevel chambers. The subsequent 9 Conditioning Trials were identical to the trials with receptive females in the first experiment. Both Paired-, Nonpaired-, and Unpaired-Trained males were placed individually into a bilevel chamber

for 5 min, after which a receptive female bearing the appropriate scent (almond extract or distilled water) was placed into the chamber for a 30 min test of copulation. Unpaired-Trained males received exposure to almond odor in isolation by applying approximately 1 ml of almond extract to a cotton roll which was placed in their home cage for 30 min--males in the Unpaired-Trained group were housed in a separate colony from the males in the other two groups.

Copulatory Preference Test. Four days following the final Conditioning Trial, each of the males was placed in the open field and allowed to habituate for a period of 5 min. As in Experiment 1, one A+E female and one E-Alone female were placed simultaneously into two diagonal corners of the open field at approximately equal distances from the male. All copulatory activity and the female to which they were directed was recorded during each male's test. The test was terminated after 30 min.

Statistical Analysis

Mixed-design between-within ANOVAs were used to analyze all data from the Conditioning Trials with significant values being followed by post hoc analysis of individual means using the Tukey method. Chi square analysis was used for analysis of proportions of female selected for first mount, first intromission, and first ejaculation on the Copulatory Preference Test. Mixed ANOVAs were used for analysis of the distributions of mounts, intromissions, and ejaculations throughout the 30 min test period. T-tests were used for analysis of proportions of mounts and intromissions

directed toward the female selected for ejaculation in a series. The level of significance for all comparisons was 0.05.

Results

Conditioning Phase. Of the 45 males used as subjects in this experiment, 4 failed to ejaculate on the first Conditioning Trial but all males mounted, intromitted, and ejaculated on subsequent Trials. No significant differences between groups were observed during the Conditioning Phase. The mean latency to level change decreased and the mean number of level changes increased across Conditioning Trials, however, there were no between group differences on these measures (data not shown). Similarly, the mean latencies to intromit and ejaculate of rats in each group decreased across trials during the Conditioning Phase; again, no between group differences were detected (data not shown). Data were consistent with other studies of copulation in bilevel chambers (see Pfaus et al., 1990),

Copulatory Preference Test. All 45 males mounted, intromitted, and ejaculated at least twice during the Copulatory Preference Test. Figure 4 displays the choice of female for first mount, first intromission, and first ejaculation for each group. Consistent with the findings of Experiment 1, more Paired-Trained males ejaculated with A+E females, whereas approximately equal numbers of Nonpaired-Trained and Unpaired-Trained males ejaculated with E-Alone and A+E females, a chi square confirmed the statistical significance of these observations: Paired-Trained versus Nonpaired Trained, $\chi 2 = 3.60$, $p < 0.05$; Paired-Trained versus Unpaired-Trained, $\chi 2 = 5.00$, $p < 0.05$; Nonpaired-

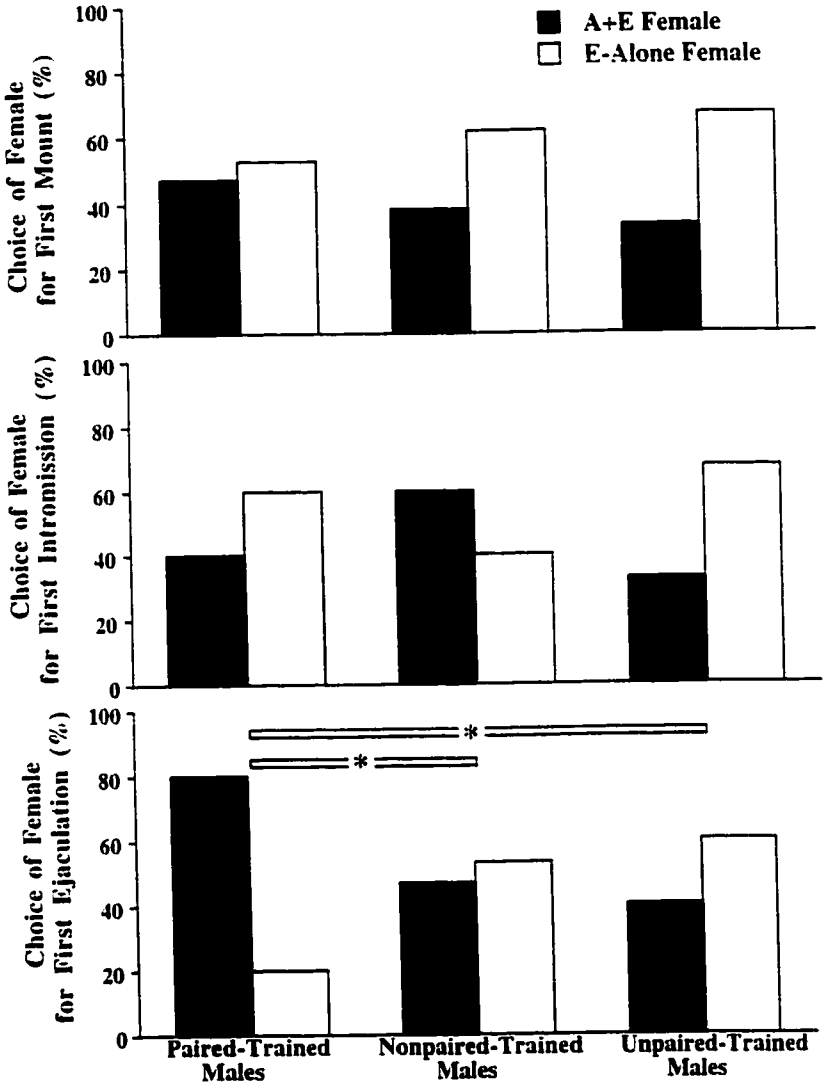

Figure 4. Proportional distribution by female type of first mount (top panel), first intromission (middle panel), and first ejaculation (bottom panel) during the Copulatory Preference Test in Experiment 2. * denotes $p < 0.05$ for between groups comparison.

Trained versus Unpaired-Trained, $\chi 2 = 0.14$, $p > 0.05$. No significant differences were found between the groups for choice of female for first mount or first intromission.

The mean mounts per female per series, intromissions per female per series, and mean ejaculations per female over the 30 min test are displayed in Figure 5. For mount distribution there were no significant effects; this was confirmed by a 2 x 2 mixed ANOVA (for Group: $F(2, 84) = 0.49$, $p > 0.05$; for Female Type: $F(1, 84) = 0.93$, $p < 0.05$; for Group X Female Type: $F(2, 84) = 2.86$, $p > 0.05$). For intromission distribution, there were also no significant effects (for Group: $F(2, 84) = 2.67$, $p > 0.05$; for Female Type: $F(1, 84) = 0.18$, $p < 0.05$; for Group X Female Type: $F(2, 84) = 1.39$, $p > 0.05$). Moreover, for ejaculation distribution, a 2 x 2 mixed ANOVA revealed no significant main effects (for Group: $F(2, 84) = 0.29$, $p > 0.05$; for Female Type: $F(1, 84) = 0.76$, $p > 0.05$). There was, however, a significant interaction between Group and Female Type ($F(2, 84) = 7.81$, $p < 0.05$). Post hoc comparisons revealed that Paired-Trained males ejaculated significantly more times with A+E females than E-Alone females and that Nonpaired-Trained males ejaculated significantly more times with E-Alone females than A+E females. And Unpaired-Trained males did not ejaculate with either female significantly more.

Figure 6 displays the proportion of males in each group that switched female for choice of ejaculation on consecutive series. Analysis of the second ejaculatory series revealed that the three groups differed in the choice of female for ejaculation with respect to the female with which they ejaculated on the first series. A large proportion of the

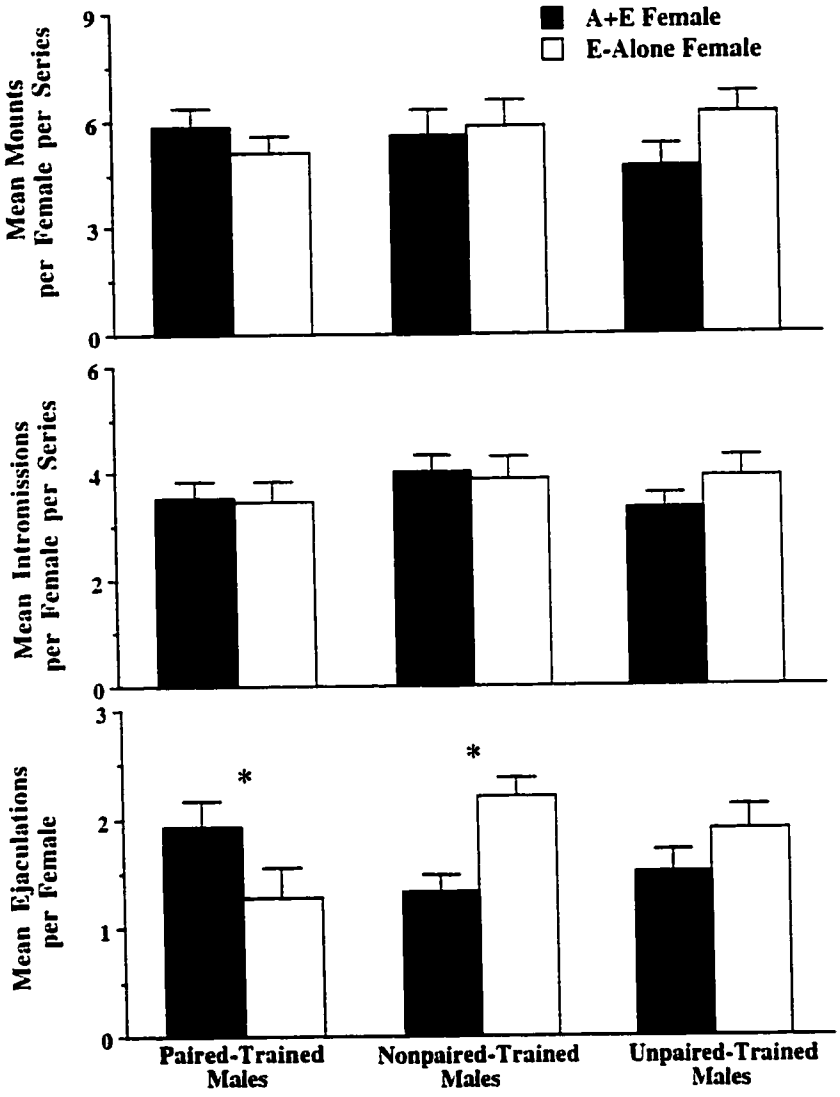

Figure 5. Distribution of mean (+SE) mounts per female per series (top panel), mean (+SE) intromissions per female per series (middle panel), and mean (+SE) ejaculations per female (bottom panel) during the Copulatory Preference Test in Experiment 2. * denotes $p < 0.05$ for between female types comparison.

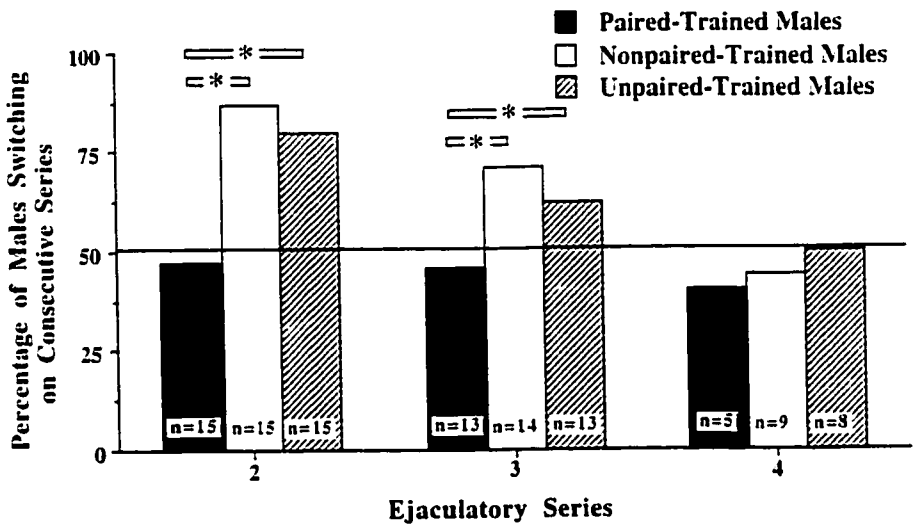

Figure 6. Proportion of males in each group switching females for ejaculation on consecutive ejaculatory series during the Copulatory Preference Test in Experiment 2. * denotes $p < 0.05$ for between groups comparison.

Nonpaired-Trained males (87%) and the Unpaired-Trained males (80%) switched their choice of female for ejaculation from the first to the second series, whereas less than half of the Paired-Trained males switched (47%). A chi-squared analysis confirmed the statistical significance of these observations: Paired-Trained versus Nonpaired-Trained, $\chi 2 = 5.40$, $p < 0.05$; Paired-Trained versus Unpaired-Trained, $\chi 2 = 3.60$, $p < 0.05$; Nonpaired-Trained versus Unpaired-Trained, $\chi 2 = 0.24$, $p > 0.05$. Analysis of subsequent ejaculatory series failed to reveal significant differences in the proportion of males switching females on consecutive ejaculatory series. Further, just over half (60%) of the Paired-Trained males ejaculated with both females during the 30 min test compared with all (100%) of the Nonpaired-Trained and Unpaired-Trained males. A chi squared analysis confirmed the statistical significance of these observations: Paired-Trained versus Nonpaired-Trained, $\chi 2 = 7.50$, $p < 0.05$; Paired-Trained versus Unpaired-Trained, $\chi 2 = 7.50$, $p < 0.05$; Nonpaired-Trained versus Unpaired-Trained, $\chi 2 = 0.00$, $p > 0.05$.

No other measures of copulatory behavior differed significantly between the three groups. Again, it is noteworthy that the selection of female for ejaculation was not related to either the distribution of mounts or the distribution of intromissions. The same female was selected for first ejaculation and for first mount by about half of the Paired-Trained (53%), Nonpaired-Trained (60%), and Unpaired-Trained (47%) males, and for first intromission by about half of the Paired-Trained (40%), Non-Paired-Trained (53%), and Unpaired-Trained (53%) males. For all groups of males, the mean proportion of mounts across all ejaculatory series directed toward the female selected for ejaculation on

the same series was not substantially different from chance (50%) probability (for Paired-Trained males: $t(14) = 0.63$, $p > 0.05$; for Nonpaired-Trained males: $t(14) = 0.68$, $p > 0.05$; for Unpaired-Trained males: $t(14) = 0.58$, $p > 0.05$). Similarly, the mean proportion of intromissions across all ejaculatory series directed toward the female selected for ejaculation on the same series was not substantially different from chance probability in either group (for Paired-Trained males: $t(14) = 1.67$, $p > 0.05$; for Nonpaired-Trained males: $t(14) = 0.28$, $p > 0.05$; for Unpaired-Trained males: $t(14) = 1.01$, $p > 0.05$.).

Discussion

The results of Experiment 2 confirmed the general findings of Experiment 1 on the Copulatory Preference Test. Paired-Trained males directed their ejaculations preferentially toward A+E females as indicated by the selection of female for their first ejaculation and the distribution of ejaculations. In contrast, Nonpaired-trained males directed their ejaculations preferentially toward E-Alone females as indicated by the distribution of ejaculations, but not on their selection of female for their first ejaculation. Unpaired-Trained males did not preferentially direct their ejaculations toward either female (Figures 4 and 5). Together the results of these experiments demonstrate that the pairing of a neutral odor with access to a receptive female produces a preference for a female bearing that odor over a female that does not. The fact that differential distribution of copulatory behaviors among the two female types were only observed with ejaculatory measures indicates that such conditioned preferences influence only the distribution of ejaculations and not the distribution of mounts or intromissions.

Experiment 3

The purpose of Experiment 3 was to replicate and extend the results of the preceding experiments. In Experiment 1 and 2, we found that males receiving training trials with almond-scented receptive females developed a preference to ejaculate with an almond-scented female over an unscented one. Experiment 3 examines the development of conditioned mate preferences using a different neutral odor, lemon. Accordingly, Paired-Trained males received 9 trials with lemon-scented receptive females (L+E), Nonpaired-Trained males received 9 trials with E-Alone females, and Unpaired-Trained males received 9 trials with E-Alone females and exposure to lemon odor in isolation. Then each male received a Copulatory Preference Test with one L+E and one E-Alone female.

Methods

Subjects

Seventy-two male rats (n = 24 per group) of the same strain from the same supplier were housed in the same conditions as those of Experiment 1 and 2 served as subjects. Female rats were housed and receptivity was induced in the same way as in the first two experiments. Female rats were scented with approximately 1 ml of either lemon extract (Blue Ribbon, Etobicoke, Ontario, Canada) or distilled water applied to both the back of the neck and the anogenital area using a cotton swab.

Apparatus

All Conditioning Trials were conducted in the same bilevel chambers (cleaned prior to each Trial) and the Copulatory Preference Tests were conducted in the same open field as used in Experiments 1 and 2. All conditioning Trials were recorded on video and scored subsequently using a PC-based program (Cabillio, 1996).

Procedure

Conditioning Phase. As in Experiment 2, male rats received 7 daily 15-min preexposure sessions to the bilevel chambers. The subsequent 9 Conditioning Trials were identical to the trials with receptive females in Experiment 2 with the substitution of lemon odor for almond odor. Paired-, Nonpaired-, and Unpaired-Trained males were placed individually into a bilevel chamber for 5 min, after which a receptive female bearing the appropriate scent (lemon extract or distilled water) was placed into the chamber for a 30 min test of copulation. For Paired-Trained males access was always to lemon-scented females (L+E); for Nonpaired-Trained and Unpaired-Trained males access was always to E-Alone females. Unpaired-Trained males received exposure to lemon odor in isolation by applying approximately 1 ml of lemon extract to a cotton roll which was placed in their home cage for 30 min--males in the Unpaired-Trained group were housed in a separate colony from the males in the other two groups.

Copulatory Preference Test. Four days following the final Conditioning Trial, each of the males was placed in the open field and allowed to habituate for a period of 5 min. As in Experiments 1 and 2, one L+E female and one E-Alone female were placed

simultaneously into two diagonal corners of the open field at approximately equal distances from the male. All copulatory activity and the female to which they were directed was recorded during each male's test. The test was terminated after 30 min.

Statistical Analysis

Mixed-design between-within ANOVAs were used to analyze all data from the Conditioning Trials with significant values being followed by post hoc analysis of individual means using the Tukey method. Chi square analysis was used for analysis of proportions of female selected for first mount, first intromission, and first ejaculation on the Copulatory Preference Test. Mixed ANOVAs were used for analysis of the distributions of mounts, intromissions, and ejaculations throughout the 30 min test period. T-tests were used for analysis of proportions of mounts and intromissions directed toward the female selected for ejaculation in a series. The level of significance for all comparisons was 0.05.

Results

Conditioning Phase. Of the 72 males used as subjects in this experiment, 9 failed to ejaculate on the first Conditioning Trial but all males mounted, intromitted, and ejaculated on subsequent Trials. No significant differences between groups were observed during the Conditioning Phase. The mean latency to level change decreased and the mean number of level changes increased across Conditioning Trials, however, there were no between group differences on these measures (data not shown). Similarly, the mean latencies to intromit and ejaculate decreased across Conditioning Trials, again, no between

group differences were detected (data not shown). Data were consistent with other studies of copulation in bilevel chambers (see Pfaus et al., 1990),

Copulatory Preference Test. Of the 72 males, 9 failed to copulate to ejaculation during the copulatory preference test (3 in the Paired-Trained group, 2 in the Nonpaired-Trained group, and 4 in the Unpaired-Trained group), all other males mounted, intromitted, and ejaculated at least twice during the Copulatory Preference Test. Figure 7 displays the choice of female for first mount, first intromission, and first ejaculation for each group. Consistent with the findings of Experiment 1, more Paired-Trained males ejaculated with L+E females, whereas approximately equal numbers of Nonpaired-Trained and Unpaired-Trained males ejaculated with E-Alone and L+E females, a chi square confirmed the statistical significance of these observations: Paired-Trained versus Nonpaired Trained, $\chi 2 = 4.54$, $p < 0.05$; Paired-Trained versus Unpaired-Trained, $\chi 2 = 4.58$, $p < 0.05$; Nonpaired-Trained versus Unpaired-Trained, $\chi 2 = 0.07$, $p > 0.05$. No significant differences were found between the groups for choice of female for first mount or first intromission.

The mean mounts per female per series, mean intromissions per female per series, and mean ejaculations per female over the 30 min test are displayed in Figure 8. For mount distribution there were no significant effects; this was confirmed by a 2 x 2 mixed ANOVA (for Group: $F(2, 120) = 1.21$, $p > 0.05$; for Female Type: $F(1, 120) = 0.43$, $p < 0.05$; for Group X Female Type: $F(2, 120) = 2.19$, $p > 0.05$). For intromission distribution, there were also no significant effects (for Group: $F(2, 120) = 1.07$, $p > 0.05$;

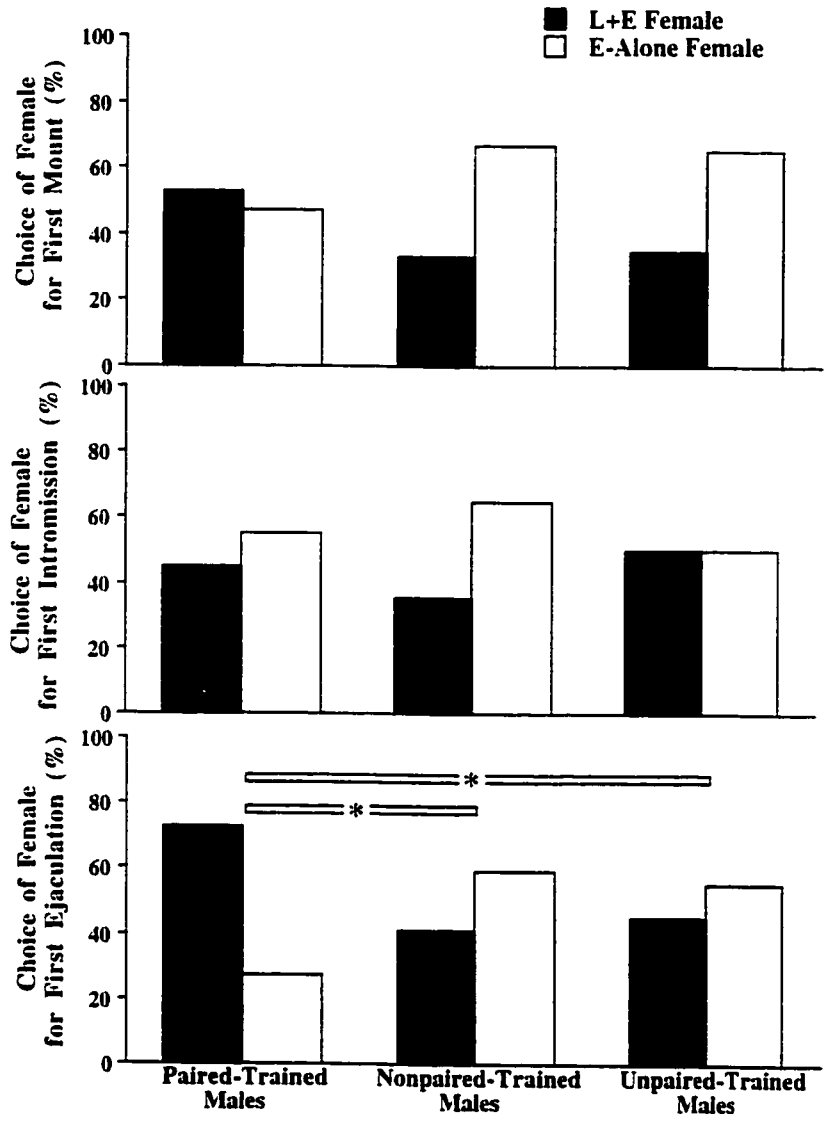

Figure 7. Proportional distribution by female type of first mount (top panel), first intromission (middle panel), and first ejaculation (bottom panel) during the Copulatory Preference Test in Experiment 3. * denotes $p < 0.05$ for between groups comparison.

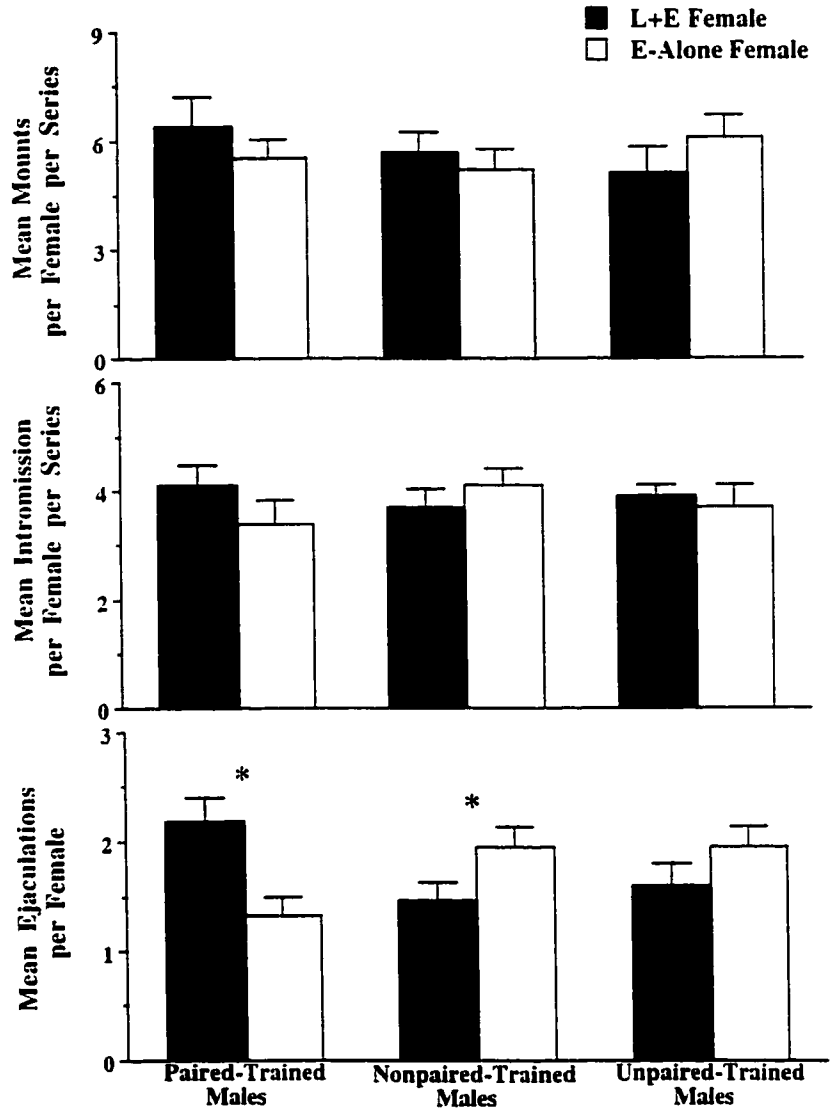

Figure 8. Distribution of mean (+SE) mounts per female per series (top panel), mean (+SE) intromissions per female per series (middle panel), and mean (+SE) ejaculations per female (bottom panel) during the Copulatory Preference Test in Experiment 3. * denotes $p < 0.05$ for between female types comparison

for Female Type: $F(1, 120) = 0.78$, $p < 0.05$; for Group X Female Type: $F(2, 120) = 0.89$, $p > 0.05$). Moreover, for ejaculation distribution, a 2 x 2 mixed ANOVA revealed no significant main effects (for Group: $F(2, 120) = 0.07$, $p > 0.05$; for Female Type: $F(1, 120) = 0.58$, $p > 0.05$). There was, however, a significant interaction between Group and Female Type ($F(2, 120) = 5.57$, $p < 0.05$). Post hoc comparisons revealed that Paired-Trained males ejaculated significantly more times with L+E females than E-Alone females. Nonpaired-Trained males ejaculated significantly more times with E-Alone females than L+E females. Finally, Unpaired-Trained males did not ejaculate significantly more with either type of female.

Figure 9 displays the proportion of males in each group that switched female for choice of ejaculation on consecutive series. Analysis of the second ejaculatory series revealed that the two groups differed in the choice of female for ejaculation with respect to the female with which they ejaculated on the first series. A large proportion of the Nonpaired-Trained males (68%) and the Unpaired-Trained males (70%) switched their choice of female for ejaculation from the first to the second series, whereas less than half of the Paired-Trained males switched (41%). A chi-squared analysis confirmed the statistical significance of these observations: Paired-Trained versus Nonpaired-Trained, $\chi 2 = 3.30$, $p < 0.05$; Paired-Trained versus Unpaired-Trained, $\chi 2 = 3.58$, $p < 0.05$; Nonpaired-Trained versus Unpaired-Trained, $\chi 2 = 0.02$, $p > 0.05$. Analysis of subsequent ejaculatory series failed to reveal significant differences in the proportion of males switching females on consecutive ejaculatory series.

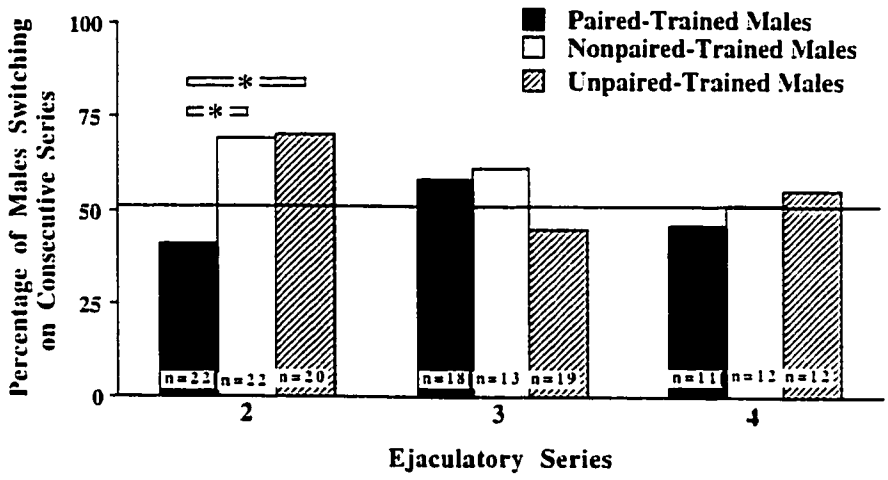

Figure 9. Proportion of males in each group switching females for ejaculation on consecutive ejaculatory series during the Copulatory Preference Test in Experiment 3. * denotes $p < 0.05$ for between groups comparison.

No other measures of copulatory behavior differed significantly between the groups. Again, it is noteworthy that the selection of female for ejaculation was not related to either the distribution of mounts or the distribution of intromissions. The same female was selected for first ejaculation and for first mount by about half of the Paired-Trained (55%), Nonpaired-Trained (32%), and Unpaired-Trained (59%) males, and for first intromission by about half of the Paired-Trained (50%), Non-Paired-Trained (43%), and Unpaired-Trained (57%) males. For all groups of males, the mean proportion of mounts across all ejaculatory series directed toward the female selected for ejaculation on the same series was not substantially different from chance (50%) probability (for Paired-Trained males: $t(21) = 1.10$, $p > 0.05$; for Nonpaired-Trained males: $t(21) = 0.97$, $p > 0.05$; for Unpaired-Trained males: $t(19) = 0.59$, $p > 0.05$). Similarly, the mean proportion of intromissions across all ejaculatory series directed toward the female selected for ejaculation on the same series was not substantially different from chance probability in either group (for Paired-Trained males: $t(21) = 1.42$, $p > 0.05$; for Nonpaired-Trained males: $t(21) = 0.92$, $p > 0.05$; for Unpaired-Trained males: $t(19) = 1.38$, $p > 0.05$.).

Discussion

The results of Experiment 3 confirmed the general findings of Experiments 1 and 2 on the Copulatory Preference Test. Paired-Trained males directed their ejaculations preferentially toward L+E females as indicated by the selection of female for their first ejaculation and the distribution of ejaculations. Nonpaired-Trained males directed their ejaculations preferentially toward E-Alone females as indicated by the distribution of

ejaculations but not selection of female for their first ejaculation. Unpaired-Trained males did not ejaculate preferentially with either female for first ejaculation or the distribution of ejaculations (Figures 7 and 8). Together the results of present experiments demonstrate that the pairing of a neutral odor with access to a receptive female produces a conditioned ejaculatory preference for a female bearing that odor over a female that does not.

General Discussion

The present series of experiments demonstrate that the pairing of a neutral odor with a receptive female alters consummatory measures of sexual behavior and mate selection in the male rat. Males trained with scented receptive females ejaculate with scented females significantly more than with unscented females. Males trained with unscented females showed the opposite, albeit weaker pattern, tending to ejaculate more often with unscented females, however, this effect was strengthened with the addition of training with scented-nonreceptive females. Males trained with random pairings of almond odor and female receptivity showed no preference for one female over the other. Furthermore, for the second ejaculation during the copulatory preference test, Paired-Trained males displayed a significant reduction in switching behavior, whereas almost all of the males trained with unscented females or random pairings tended to switched females. Together, these results suggest that olfactory conditioning produces an ejaculatory preference in male rats by making them more likely to direct their ejaculation toward stimuli previously paired with sexually-receptive females.

The present findings that male rat sexual behavior can be conditioned by pairing a CS with a copulatory unconditioned stimulus (UCS) also stand in contrast to those of Zamble and colleagues (Zamble, Hadad, & Mitchell, 1985a), who failed to find any evidence of conditioning using a copulatory UCS. Probably the most important reason for these differences in results is the manner in which each study assessed conditioning. The present study assessed the influence of conditioning on a qualitative measure, selection of a mate in a triad mating situation, whereas Zamble et al. assessed the influence of conditioning on a quantitative measure, ejaculation latency in a dyad mating situation. Thus, the type of conditioned effects reported here could not have been detected in the Zamble et al. study. It is not known whether the present conditioning procedure might have produced differential response latencies similar to that reported by Zamble et al. because diad testing conditions were not used, although there were no differences between the groups on copulatory latencies during the Conditioning Phase.

Second, the CS-UCS interval was qualitatively different between studies. In the current experiments, the CS was presented throughout the period of exposure to the receptive female, whereas in the Zamble et al. (1985a) study, it was presented only prior to access to the receptive female on training trials. Akins, Domjan, and Gutierrez (1994) have demonstrated that in Japanese Quail, the topography of the conditioned responses is dependent on the CS-UCS interval: With a long interval, males displayed a general increase in locomotion, whereas with a short interval males displayed behavior directed toward the CS (i.e. conditioned approach). If the same principles apply to the rat then it

seems likely that the relatively long interval employed by Zamble et al. facilitated sexual behavior by increasing arousal in general, whereas the shorter CS-UCS interval in the current experiments produced conditioned effects on sexual behavior directed at the CS (e.g., selection of female with CS odor).

The present findings demonstrate not only the ability of a conditioned odor to produce an ejaculatory preference in the male rat, but also the utility of testing male sexual behavior in the presence of multiple receptive females. The conditioned effects observed on the pattern of copulatory preferences could not have been detected if a more standard diad test had been employed because simply, there would be no opportunity for the males to display a preference. An alternative test strategy could have been one similar to those employed in studies of conspecific preference (e.g. Gilman & Westbrook, 1978; de Jonge, Burger, Van Haaren, Overdijk, & Van de Pol, 1987). In those studies, subjects are allowed to investigate confined conspecifics or bedding from conspecifics. Although such experiments would be of interest, they do not allow direct assessment of copulatory preferences per se, but rather are useful in assessing preferential approach behavior. Furthermore, rats mate naturally in groups (see McClintock, 1984), and the triad mating test might therefore represent a more ethologically-relevant method of assessing mate preferences in this species.

Comparison of the results from distribution of mounts and intromissions with the distribution of ejaculations during the Copulatory Preference Test reveals another unexpected finding. Both groups of males preferentially distributed their ejaculations, but

the same was not true for other measures of copulatory activity. Although, Paired-Trained males displayed preferences for scented females for first ejaculation, and both groups of males displayed a preference for one type of female over the other for total number of ejaculations. They did not show any consistent preference for either female for first mount, first intromission, nor for the mean number of mounts or intromissions in any ejaculatory series or across series. A male's choice of female for the first mount or intromission on a given ejaculatory series thus could not be used to predict which female that male would ejaculate with. This suggests that in group mating situations there are differences in how males distribute mounts and intromissions compared to how they distribute ejaculations. Although our results are consistent with McClintock's view (1984), that "[u]sually, during group mating, a male rat simply mates with the female that is closest and soliciting him" (pp. 26), this does not appear to be true for ejaculation. At the point of ejaculation or immediately prior to it, males may become discriminating and choose between potential mates. Thus, in the male rat, as in the male human, selection of a partner for copulation per se and selection of a partner for mating may be differentiated (Buss & Schmitt, 1993; Kenrick, Sadalla, Groth, & Trost, 1990; Townsend & Levy, 1990; Townsend & Roberts, 1993).

In conclusion, the present results extend the findings of several previous studies that report the conditioning of different aspects of sexual behavior in the male rat. These studies demonstrate that conditioned stimuli can increase sexual excitement (e.g. Pfaus, Mendelson & Phillips, 1990), arousal (e.g. Zamble et al., 1985), neuroendocrine function

(Graham & Desjardins, 1980), and that subjects can be trained to perform arbitrary operants to obtain access to a mate or a second-order stimulus associated with a mate (e.g. Everitt et al., 1987). The present study demonstrates that conditioned stimuli can also direct ejaculation in a situation in which a male rat has the opportunity to copulate with multiple partners.

CHAPTER 2: THE DEVELOPMENT AND EXTINCTION OF CEP

In Chapter 1, it was demonstrated that pairing a neutral odor (almond extract) with copulation produced a conditioned ejaculatory preference (CEP). In that study, male rats were allowed access to sexually receptive females that had the odor CS applied to their necks and anogenital regions during copulatory conditioning sessions. The influence of this conditioning procedure on copulatory preference was tested subsequently by allowing males to copulate simultaneously with two receptive females, one bearing the CS and one not. On the copulatory preference test, most males trained with the neutral odor paired with access to receptive females, ejaculated first and more often with the scented female than with the unscented one. In contrast, males with no prior experience with the neutral odor or trained with the neutral odor that was either unpaired or paired randomly with sexually-receptive females did not display a preference for scented females.

Early sexual experiences are thought to be particularly powerful influences on the development of sexual preferences in humans. Storms (1981) has provided a theoretical framework that posits sex drive development and ensuing sexual experiences as the critical elements in the formation of sexual preferences. Correlative support for this notion has been derived from retrospective self-reports in which a correlation between early sexual activity and later sexual preferences in both men and women was found (Bell, Weinberg, & Hammersmith, 1981; Van Wyk & Geist, 1984). Moreover, similar to my finding (see Chapter 3) that ejaculation is critical in the development of CEP in rats, adult sexual

preferences were highly correlated with early satisfying sexual experiences in these studies. This lead to the development of the hypothesis that adult sexual preferences are strongly related to the incidence of rewarding sexual activities. The CEP phenomenon in rats provides an experimental method to examine this hypothesis.

Accordingly, the present study examined the development and extinction of CEP. In Chapter 1, conditioning consisted of 9 copulatory sessions with almond-scented females, each 30 min in duration. In Experiment 4, I manipulated the number of sessions and examined the subsequent copulatory preferences. In Experiment 5, I employed a single conditioning session and manipulated either the duration of the session or the amount of copulatory stimulation, and examined the subsequent copulatory preferences. In Experiment 6, I compared the extinction rates of CEP produced by either 9 conditioning sessions each of 30 min duration or a single conditioning session of 4 hr duration. Extinction was produced by a series of copulatory preference tests in which males were given access to both a scented and an unscented female.

Experiment 4

In Chapter 1, I found CEP was produced following 9 conditioning sessions, each 30 min in duration. In the present experiment, Long-Evans male rats were given either 1, 5, or 9 sessions with almond-scented receptive females, each 30 minutes in duration. Then, their copulatory preferences were assessed with simultaneous access to two females, one scented and one not.

Methods

Subjects

Males. The 58 Long-Evans rats that served as subjects in Experiment 4 were obtained from Charles River Canada, (St. Constant, Québec, Canada). The males weighed approximately 300 g and were sexually naive at the start of the experiment. They were housed in pairs in Plexiglas cages (36 cm x 26 cm x 19 cm) with ad lib access to food (Purina Rat Chow) and water. All rats were kept in a 12:12 hour reversed light-dark cycle colony room maintained at 21°C.

Females. Female Long-Evans rats from the same supplier as above were ovariectomized via bilateral lumbar incisions under ketamine/xylazine anaesthesia at least two months prior to the start of the experiment and were sexually experienced. Sexual receptivity was induced by subcutaneous administration of estradiol (10 μg) 48 hr prior and progesterone (500 μg) 4-6 hr prior to each test trial. Females were housed under the same conditions as males. Stimulus females were selected at random for use during conditioning sessions and copulatory preference tests. Female rats were scented with approximately 1 ml of either almond extract (Blue Ribbon, Etobicoke, Ontario, Canada) or distilled water applied to both the back of the neck and the anogenital area using a cotton swab.

Apparatus

Conditioning sessions took place in unilevel pacing chambers constructed with standard laboratory Plexiglas cages (36 cm x 26 cm x 19 cm) with a Plexiglas insert. The

insert was made by attaching a Plexiglas divider (30 cm x 20 cm x 0.5 cm) length wise to the center of a Plexiglas base (35 cm x 18 cm x 0.5 cm). The insert was then placed into the Plexiglas cage, covered with bedding material, and a piece of wire mesh (0.25 cm grid, 35 cm x 18 cm) with a groove cut into the center to fit over the divider. A cover constructed of wire mesh (0.5 cm grid, 36 cm x 20 cm) was placed over the chamber. All conditioning sessions were recorded on video and scored subsequently using a PC-based program. Copulatory preference tests took place in a large open field (123 cm x 123 cm x 46 cm) with a thin layer of Beta Chip bedding material over the floor. Copulatory preference tests were scored at the time of testing.

Procedure

Conditioning Phase. Conditioning sessions were conducted in unilevel pacing chambers in the same manner as in Chapter 1. Male rats were preexposed to the chambers once a day for 15 min each day in order to habituate them to the training environment. This habituation procedure lasted 7 days and has been shown previously to increase the proportion of males that become vigorous copulators (Pfaus & Wilkins, 1995). Then males received either 1 (n = 22), 5 (n = 18), or 9 (n = 18) conditioning sessions at four day intervals during the middle third of the dark phase of the light:dark cycle. In each conditioning session, males were placed in the chamber for 5 min then a sexually-receptive female which had almond extract applied to the back of the neck and anogenital area (A+E female) was placed into the chamber. Rats were allowed to copulate for a period of 30 min after which the session was terminated. Latency and

frequency data for all mounts, intromissions, and ejaculations were recorded during each conditioning session. Criteria for sexual behaviors were those described by Sachs and Barfield (1976) and Meisel and Sachs (1994).

Copulatory Preference Test. Four days after the final conditioning session, each male was placed in the large open field and allowed to habituate for 5 min. At the end of this period, one A+E female and one unscented female (E-Alone) were placed simultaneously into two diagonal corners of the open field at approximately equal distances from the male. All copulatory behaviors and the females to which they were directed were recorded during each male's test. Tests were terminated 30 min after the females were introduced.

Statistical Analysis.

Chi square analyses were used for the proportion of females selected for first mount, first intromission, and first ejaculation on the copulatory preference test. The previous experiments in Chapter 1 found consistently that these olfactory conditioning procedures produce preferences for the distribution of ejaculations, but not for distribution of mounts or for distribution of intromissions. Accordingly, the distribution of ejaculations between females on the copulatory preference tests were analyzed using planned orthogonal comparisons (Glass & Hopkins, 1984) with comparisons made only between the A+E and E-Alone female for each group of males and effect size estimates (Glass & Hopkins, 1984) were calculated from the distribution of ejaculations between females for each group. The distribution of mounts and intromissions between the two

females during the copulatory preference tests were analyzed using mixed ANOVAs with followup comparisons using the Tukey method. The level of significance for all comparisons was 0.05.

Results

Conditioning Phase. Of the 58 males used as subjects in this experiment, 21 failed to ejaculate on the first conditioning session; 8 in the 1-Session group, 7 in the 5-Session group, and 6 in the 9-Session group. On the fifth trial all but 4 males in the 5-Session group and 2 in the 9-Session group ejaculated. On the ninth trial all males in the 9-Session group ejaculated. No significant differences between groups were observed during the conditioning phase. The mean latencies of rats in each group to mount, intromit, and ejaculate decreased across trials during the conditioning phase; no between group differences were detected (data not shown).

Copulatory Preference Test. Of the 58 males tested, 9 failed to copulate to ejaculation (2 in the 1-Session group, 5 in the 5-Session group, and 2 in the 9-Session group) which were excluded from further analyses. The mean number of ejaculations ($\pm$ SEM) per group were: 1-Session, 3.25 $\pm$ 0.86; 5-Sessions, 3.69 $\pm$ 0.73, 9-Sessions, 3.75 $\pm$ 0.74.

Figure 10 displays the choice of female for first mount, first intromission, and first ejaculation. More males in the 5-Session and the 9-Session groups ejaculated first with A+E females than did males in the 1-Session group. Chi square analyses confirmed the statistical significance of these observations: 5-Session versus 1-Session $\chi 2$ (n = 33) =

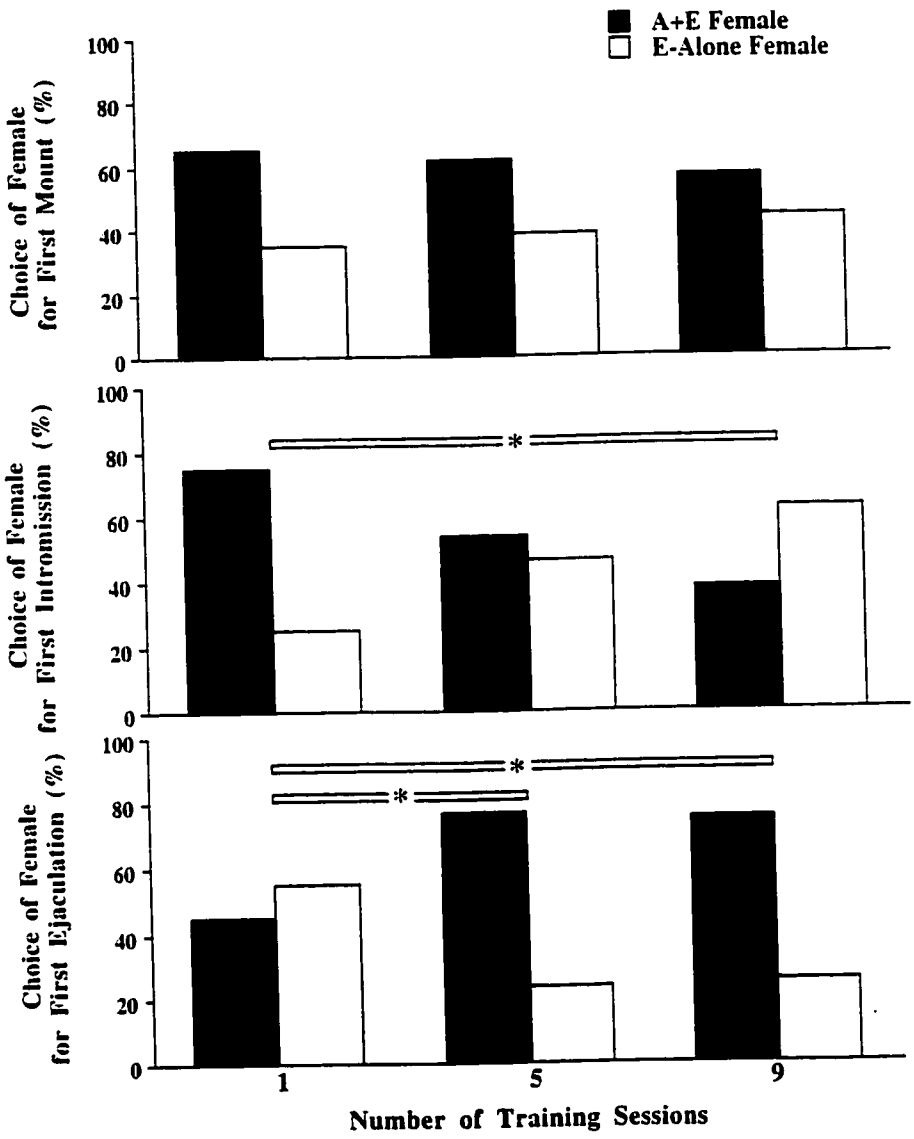

Figure 10. Proportional distribution by female type of first mount (top panel), first intromission (middle panel), and first ejaculation (bottom panel) during the Copulatory Preference Test in Experiment 4. * denotes $p < 0.05$ for between groups comparison.

3.29, $p < 0.05$; 9-Session versus 1-Session $\chi 2$ (n = 36) = 3.30, $p < 0.05$; 5-Session versus 9-Session $\chi 2$ (n = 29) = 0.56, $p > 0.05$. Additionally, the 9-Session and 1-Session group differed on the choice of female for first intromission, $\chi 2$ (n = 33) = 5.60, $p < 0.05$; no other significant differences were found between the groups for selection of female for first mount or first intromission.

Figure 11 displays the mean mounts per female per series, mean intromissions per female per series, and mean ejaculations per female over the 30 min test for each group. Males in the 9-Session group mounted less frequently than did the males in the other two groups, but total mounts were distributed equally between females and there was no interaction between groups and females. The significance of these observations were confirmed by a 3 x 2 mixed ANOVA (for Group: F (2, 92) = 7.23, $p < 0.05$, post hoc comparisons revealed that 9-Session males differed significantly from 5-Session and 1-Session males, but 5-Session and 1-Session males did not differ significantly; for Female Type: F (1,92) = 1.87, $p > 0.05$; for Group x Female Type: F(2,92) = 0.42, $p >0.05$). Similarly, males in the 9-Session group intromitted fewer times than those in the other two groups, but total intromissions were distributed equally between females and there was no interaction between groups and females. The significance of these observations were confirmed by a 3 x 2 mixed ANOVA (for Group: F (2, 92) = 3.23, $p < 0.05$, post hoc comparisons revealed that 9-Session males differed significantly from 5-Session, but 9-Session and 1-Session males and 5-Session and 1-Session males did not differ significantly; for Female Type: F (1,92) = 0.06, $p > 0.05$; for Group x Female Type: F

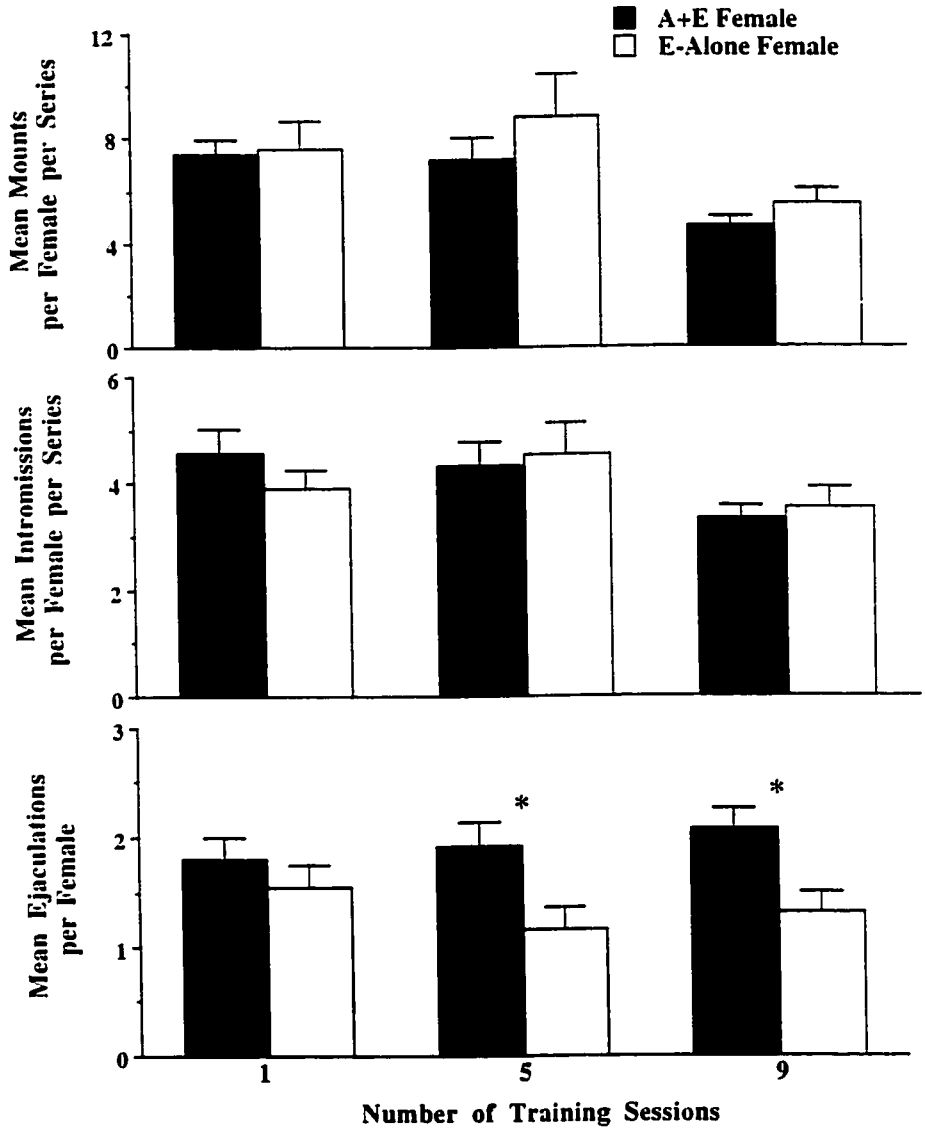

Figure 11. Distribution of mean (+SE) mounts per female per series (top panel), mean (+SE) intromissions per female per series (middle panel), and mean (+SE) ejaculations per female (bottom panel) during the Copulatory Preference Test in Experiment 4. * denotes $p < 0.05$ between female types comparison.

(2,92) = 0.88, p >0.05). Moreover, males in the 9-Session and the 5-Session groups ejaculated more frequently with the A+E female, whereas the males in the 1-Session group ejaculated with both females with approximately the frequency. Planned orthogonal contrasts confirmed the significance of these observations: 9-Session group t (92) = 3.09, $p < 0.005$; 5-Session group t (92) = 2.49, $p < 0.05$; 1-Session group t (92) = 1.00, $p > 0.05$. Effect size estimates were 1.23 for 9-Session group, 0.98 for 5-Session group, and 0.29 for 1-Session group.

Exploratory analysis of the data for the 1-Session males revealed a subset of these males did in fact display a preference. Males were classified based on the number of ejaculations they achieved during the conditioning session and a series of t-tests were performed on the data from the males achieving 1 or more (n = 14), 2 or more (n = 11), 3 or more (n = 10), and 4 or more ejaculations (n = 8; only 2 males had more than 4 ejaculations). Statistically significant preferences were detected for males achieving 2 or more and 3 or more, but not 1 or more and 4 or more, ejaculations during training; 1 or more t (26) = 0.881, $p > 0.05$; 2 or more t (20) = 2.56, $p < 0.05$; 3 or more t (14) = 2.29, $p < 0.05$; 4 or more t (14) = 1.78, $p > 0.05$.

Discussion

The finding that the 9-Session group in the present experiment displayed CEP replicates the main finding of Chapter 1, and the 5-Session group extends this effect to a shorter conditioning procedure. Moreover, in agreement with my previous studies, the influence of conditioning on copulatory preferences were only apparent on the choice of

female for first ejaculation and the distribution of ejaculations; mounts and intromissions were not preferentially distributed toward either of the females.

Additionally, the results of the analyses of the distribution of ejaculations in the 1-Session group suggest that CEP can develop within a single conditioning session providing that a certain level of copulation occurs; a hypothesis that was investigated in the next experiment of the present study. Further, the present finding that the development of CEP required multiple ejaculation on a single conditioning session is consistent with the findings of other experiments in which development of CEP was critically dependent on ejaculation over the course of multiple conditioning sessions (Chapter 3).

Experiment 5

The purpose of Experiment 5 was to further examine the effect of a single conditioning session on subsequent copulatory preferences. In Experiment 4, males that received a single conditioning session and ejaculated several times displayed significant CEP, whereas males that ejaculated only once did not. Increasing the amount of copulatory experience in a single Session with a scented female might increase the strength of the preference. Accordingly, males received a single conditioning session with either a duration or copulatory criterion imposed. In Experiment 5A, males received a single session in which they were allowed to copulate with multiple almond-scented females in sequence for either 60 min with 2 females, 120 min with 4 females, 180 min with 6

females or 240 min with 8 females. In Experiment 5B, males received a single session in which they were allowed to copulate with multiple almond-scented females until they obtained 2, 4, or 6 ejaculations.

Methods

Subjects

Male rats of the same strain from the same supplier were housed in the same conditions as those of Experiment 4 served as subjects in both Experiment 5A (n = 73) and Experiment 5B (n = 36). Female rats were housed and treated in the same manner as in the first experiment.

Apparatus

All conditioning sessions were conducted in the same chambers (cleaned prior to each session) and the copulatory preference tests were conducted in the same open field as used in Experiment 4. All conditioning sessions were recorded on video and scored subsequently using a PC-based program (Cabillio, 1996).

Procedure

Conditioning Phase. As in Experiment 4, male rats received 7 daily 15-min preexposure sessions to the bilevel chambers. The subsequent conditioning sessions were identical to those in the first experiment. Males in each group were placed individually into a chamber for 5 min, after which a receptive female bearing almond extract was placed into the chamber for 30 min, then another almond-scented, receptive female was substituted for the previous one and the pair was allowed to copulate for 30 min; this

procedure was repeated until the criterion was achieved. In Experiment 5A, conditioning sessions were either 60 min (n = 24), 120 min (n = 18), 180 min (n = 15), or 240 min (n = 16) in duration. In Experiment 5B, conditioning sessions were terminated upon completion of 2 ejaculatory series (n = 12), 4-ejaculatory series (n = 12), or 6 ejaculatory series (n = 12).

Copulatory Preference Test. Four days following the final conditioning session, each of the males was placed in the open field and allowed to habituate for a period of 5 min. As in Experiment 4, one A+E female and one E-Alone female were placed simultaneously into two diagonal corners of the open field at approximately equal distances from the male. All copulatory activity and the female to which they were directed was recorded during each male's test. The test was terminated after 30 min.

Statistical Analysis

The statistics used in Experiment 4 were used in Experiment 5. Chi square analyses were used for the proportion of females selected for first mount, first intromission, and first ejaculation on the copulatory preference test. The distribution of ejaculations between females on the copulatory preference tests were analyzed using planned orthogonal comparisons of the ejaculations received by the A+E and E-Alone females for each group of males. The distribution of mounts and intromissions between the two females during the copulatory preference tests were analyzed using mixed ANOVAs. The level of significance for all comparisons was 0.05. Additionally, effect size estimates were calculated for males that received a single conditioning session using

the data from both Experiments 4 and 5 in order to compare the effect of different amounts of copulation (defined by session duration or number of ejaculations) in producing CEP.

Results

Conditioning sessions.

Of the 73 males used in Experiment 5A, 22 males failed to ejaculate during the conditioning session; 11 in the 60-min-Session group, 8 in the 120-min-Session group, and 3 in the180-min-Session group. The mean number of ejaculations ($\pm$ SEM) during the conditioning sessions for each group were: 60-min-Session group, 2.35 ± 0.68; 120-min-Session group, 3.83 ± 0.93; 180-min-Session group, 5.50 ± 0.57; 240-min-Session group, 6.31 ± 0.36

Of the 36 males used in Experiment 5B, 8 failed to achieved the copulatory criterion during the conditioning session and were excluded from further analyses; 2 in the 2-ejaculation group, 3 in the 4-ejaculation group, and 3 in the 6-ejaculation group. The mean number of number of females ($\pm$ SEM) required to achieve the copulatory criterion during the conditioning sessions for each group were: 2-ejaculation group, 1.1 ± 0.10; 4-ejaculation group, 2.0 ± 0.17; 6-ejaculation group, 3.89 ± 0.45.

Copulatory Preference Test.

Duration Criteria. Of the 73 males used in Experiment 5A, 20 failed to copulate to ejaculation during the copulatory preference test; 11 in the 60-min-Session group, 6 in the 120-min-Session group, and 3 in the 180-min-Session group--most were the same as

those that failed to copulate during the conditioning session. The mean number of ejaculations (± SEM) for each group were: 60-min, 2.85 ± 0.87; 120-min, 3.33 ± 0.88, 180-min, 2.58 ± 0.59; 240-min, 3.06 ± 0.92. Figure 12 displays the choice of female for first mount, first intromission, and first ejaculation. More males in each group ejaculated first with the A+E female than with the E-Alone female. No significant differences were found between groups for first ejaculation, for first intromission, or first mount.

Figure 13 displays the mean mounts per female per series, mean intromission copulatory preference test for each group in Experiment 5A. The 60-min group displayed more mounts than the 240-min group and no other significant differences were found for distribution of mounts (results of 3 X 2 mixed ANOVA: for Group: F (2, 98) = 4.86, $p < 0.05$ with 60-min group mounting significantly more than the 240-min group; for Female Type: F (1, 98) = 1.46, $p > 0.05$; for Group x Female Type: F (2, 98) = 1.73, $p > 0.05$) No significant differences were found for distribution of intromissions (results of 3 X 2 mixed ANOVA: for Group: F (2, 98) = 1.54, $p > 0.05$; for Female Type: F (1, 98) = 1.04, $p > 0.05$; for Group x Female Type: F (2, 98) = 0.82, $p > 0.05$). In contrast, males in the 120-min Session, the180-min Session, and the 240-min Session groups ejaculated more frequently with the A+E female, whereas the males in the 60-min Session group ejaculated with both females with approximately equal frequency. Planned orthogonal contrasts confirmed the significance of these observations: 120-min-Session group, $t(98) = 3.42$, $p < 0.05$; 180-min-Session group, $t(98) = 2.68$, $p < 0.05$; 240-min Session group, $t(98) = 1.90$, $p < 0.05$; 60-min-Session group, $t(98) = -0.70$, $p > 0.05$.

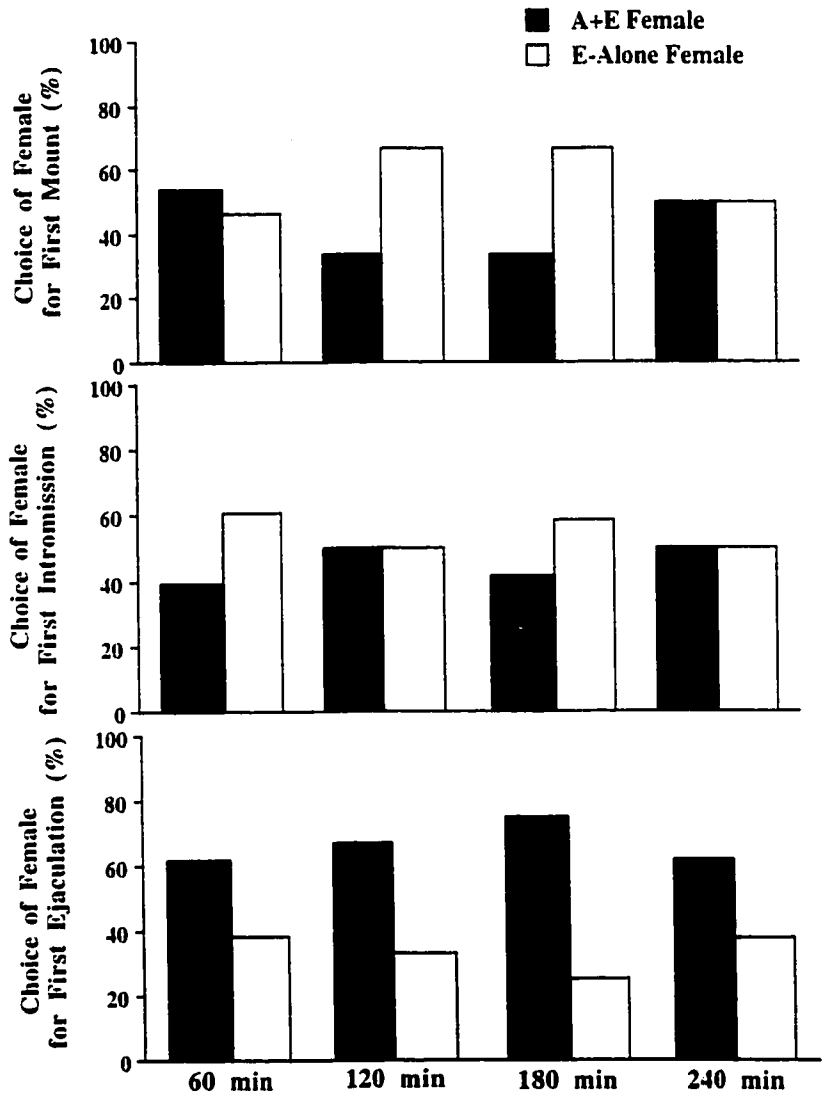

Figure 12. Proportional distribution by female type of first mount (top panel), first intromission (middle panel), and first ejaculation (bottom panel) during the Copulatory Preference Test in Experiment 5A. * denotes $p < 0.05$ for between groups comparison.

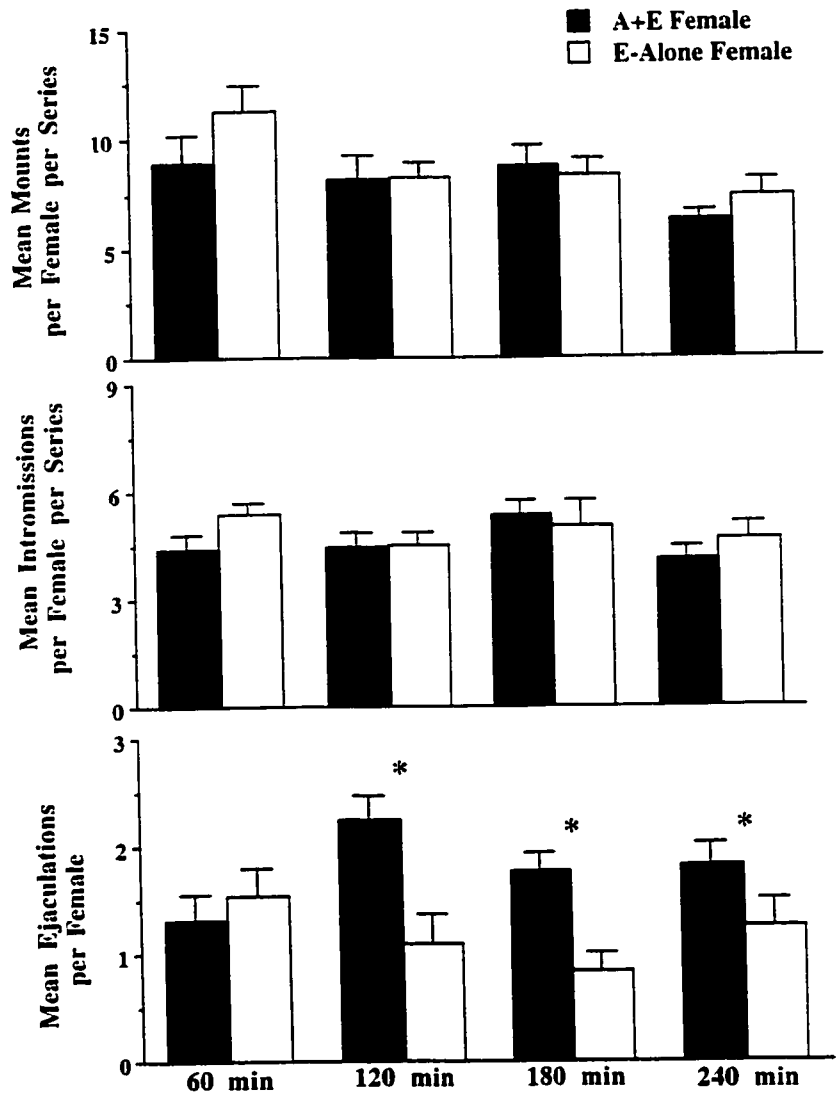

Figure 13. Distribution of mean (+SE) mounts per female per series (top panel), mean (+SE) intromissions per female per series (middle panel), and mean (+SE) ejaculations per female (bottom panel) during the Copulatory Preference Test in Experiment 5A.
* denotes $p < 0.05$ for between female types comparison.

Copulatory Criteria. All of the males that received a copulatory preference test in Experiment 5B copulated to ejaculation. The mean number of ejaculations (± SEM) for each group were: 2-ejaculations, 3.30 ± 1.03; 4-ejaculations, 3.00 ± 0.69, 6-ejaculations, 3.11 ± 1.12. Figure 14 displays the choice of female for first mount, first intromission, and first ejaculation. More males in each group ejaculated first with the A+E female than with the E-Alone female. No significant differences were found between groups for first ejaculation, for first intromission, or first mount.

Figure 15 displays the mean mounts per female per series, mean intromissions per female per series, and mean ejaculations per female during the copulatory preference test for each group in Experiment 5B. No significant differences were found for distribution of mounts (results of 3 X 2 mixed ANOVA: for Group: $F(2, 50) = 0.55$, $p > 0.05$; for Female Type: $F(1, 50) = 0.03$, $p > 0.05$; for Group x Female Type: $F(2, 50) = 0.44$, $p > 0.05$). Similarly, no significant differences were found for distribution of intromissions (results of 3 X 2 mixed ANOVA: for Group: $F(2, 50) = 1.25$, $p > 0.05$; for Female Type: $F(1, 50) = 1.47$, $p > 0.05$; for Group x Female Type: $F(2, 50) = 0.92$, $p > 0.05$). Although, males in the all three groups ejaculated more frequently with the A+E female, only the 4-ejaculation group displayed a substantial preference. Planned orthogonal contrasts confirmed the significance of these observations: 2-ejaculation group, $t(50) = 1.15$, $p > 0.005$; 4-ejaculation group, $t(50) = 3.72$, $p < 0.05$; 6-ejaculation group, $t(50) = 1.45$, $p > 0.05$--males in the 2-ejaculation and 6-ejaculation group displayed a trend toward CEP with $p < 0.15$ and $p < 0.10$, respectively.

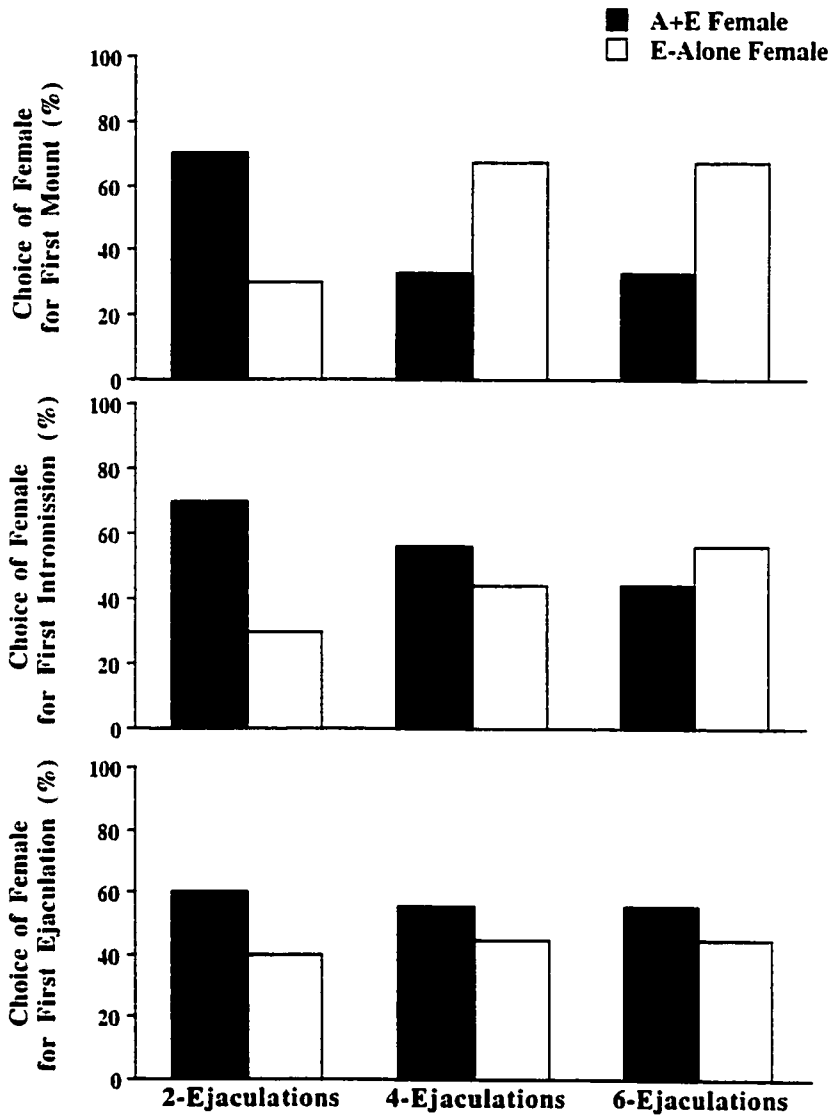

Figure 14. Proportional distribution by female type of first mount (top panel), first intromission (middle panel), and first ejaculation (bottom panel) during the Copulatory Preference Test in Experiment 5B. * denotes $p < 0.05$ for between groups comparison.

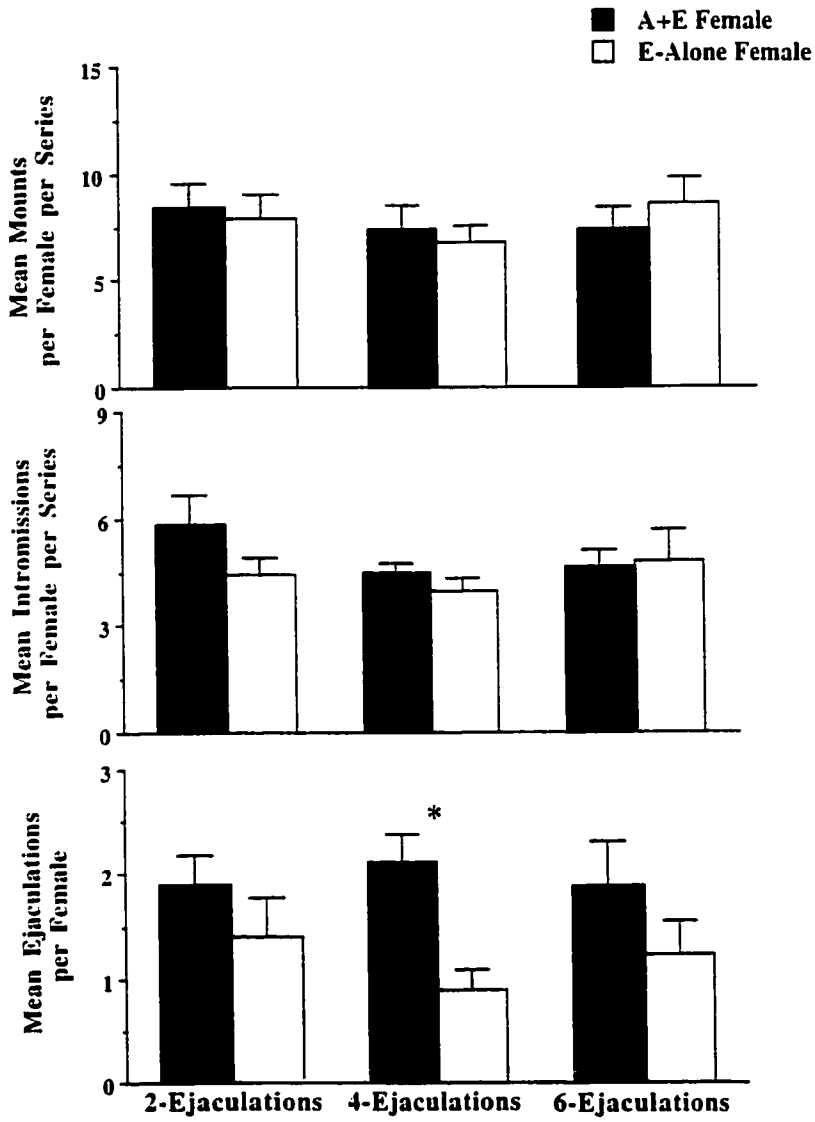

Figure 15. Distribution of mean (+SE) mounts per female per series (top panel), mean (+SE) intromissions per female per series (middle panel), and mean (+SE) ejaculations per female (bottom panel) during the Copulatory Preference Test in Experiment 5B.
* denotes $p < 0.05$ for between female types comparison.

The data from Experiments 4, 5A, and 5B, were all included in effect size estimate analyses to compare the strength of CEP produced by different durations of a single conditioning session or different numbers of ejaculations during a single conditioning session. Males were grouped for session duration based on number of females that they copulated with (approximately 30 min each), in Experiments 4 and 5A, this was defined by criterion and in Experiment 5B, this was defined by the number of females required to achieved the copulatory criterion. Males were grouped for number of ejaculations, this was defined by criterion in Experiment 5B and defined by number achieved in Experiments 4 and 5A. Figure 16 displays the effect size estimates for each of these conditions. Effect size estimates did not vary as a linear function of either number of females copulated with or number of ejaculations. Rather, maximal levels of conditioning were obtained with 3 or 4 females and 4 ejaculations in a single conditioning session; further increases on either variable during the conditioning session produced smaller effect sizes estimates.

Discussion

The results of Experiment 5 show that CEP can be produced with a single conditioning session. In Experiment 5A, males given a conditioning session of 120 min with 4 almond-scented females in succession, males given a conditioning session of 180 min with 6 almond-scented females in succession, and males given a conditioning session of 240 min with 8 almond-scented females in succession subsequently displayed a

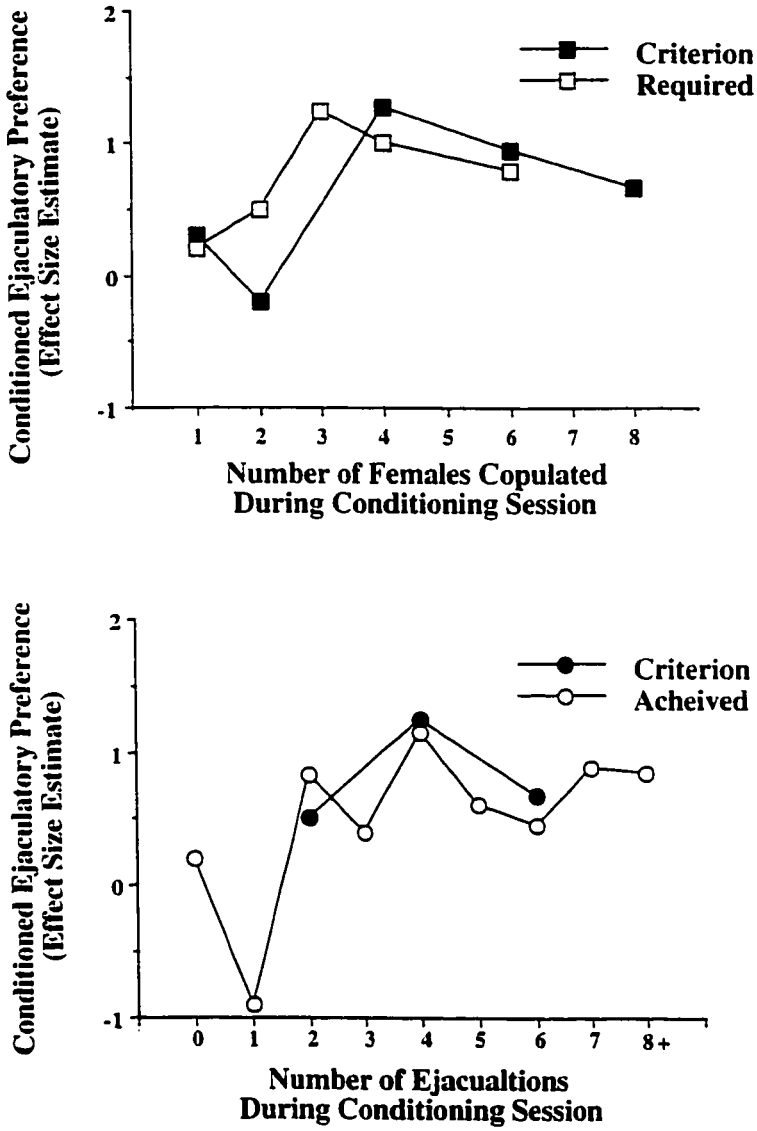

Figure 16. Effect size estimates (based on distribution of ejaculations between female types) of CEP strength produced during a single conditioning session for different durations of a single conditioning session (top panel) or different numbers of ejaculations (bottom panel) in Experiments 4, 5A, and 5B.

preference to ejaculate with an almond-scented female over an unscented female for both first ejaculation and distribution of ejaculations. Similarly, in Experiment 5B, males allowed 4 ejaculations with almond-scented females during a conditioning session displayed a preference to ejaculate with an almond-scented female over an unscented female for both first ejaculation and distribution of ejaculations. Males given either 2 or 6 ejaculations during a conditioning session displayed trends in the same direction, but this failed to meet statistical significance.

The strength of the CEP produced by a single conditioning session appears to be of similar magnitude to that produced by 5- or 9-Sessions each 30 min in duration. Effect size estimates for 5-Session and 9-Session groups in Experiment 4 were approximately equivalent to the peak effect size estimates produced during single conditioning session in Experiment 5. Moreover, the strength of CEP produced during single conditioning session appears to be optimal at intermediate levels of copulation; peak effect size estimates coincided with conditioning sessions consisting of 120 min of copulation or 4 ejaculations with scented females.

Experiment 6

In previous experiments, CEP was assessed during a single copulatory preference test and the endurance of CEP was not examined. In the present experiment, males received repeated copulatory preference tests to assess the resiliency of CEP. Males received either 9 training session with A+E females each of 30 min duration or a single

training session with A+E females of 240 min duration. Then, all males received a series of three copulatory preference tests.

Methods

Subjects

Thirty-one male rats of the same strain from the same supplier were housed in the same conditions as those of previous experiments served as subjects in Experiment 6. Female rats were housed and treated in the same manner as in the first experiment.

Apparatus

All conditioning sessions were conducted in the same chambers (cleaned prior to each session) and the copulatory preference tests were conducted in the same open field as used in Experiments 4 and 5. All conditioning sessions were recorded on video and scored subsequently using a PC-based program (Cabillio, 1996).

Procedure

Conditioning Phase. As in Experiments 4 and 5, male rats received 7 daily 15-min preexposure sessions to the bilevel chambers. Then, males in the 9-Session group (n = 15) received 9 training sessions with a single A+E female each of 30 min duration and males in the 240-min Session group (n = 16) received a single training session with 8 A+E females for 30 min per female with total duration of 240 min. At the start of each conditioning session, each male was placed individually into a chamber for 5 min, after which an A+E female was placed into the chamber. For the 9-Session group, the training session was terminated following 30 min of access to the A+E female and the next training

session took place 4 days later. For the 240-min Session group, following 30 min of access to the A+E female, another A+E female was substituted for the previous one and the pair was allowed to copulate for 30 min this process was repeated until each male had copulated with 8 females.

Copulatory Preference Tests. Four to six day following the final conditioning session, each of the males was placed in the open field and allowed to habituate for a period of 5 min. As in Experiments 4 and 5, one A+E female and one E-Alone female were placed simultaneously into two diagonal corners of the open field at approximately equal distances from the male. All copulatory activity and the female to which they were directed was recorded during each male's test. The test was terminated after 30 min. Each male received two more copulatory preference tests at 4-7 day intervals.

Statistical Analysis

The statistics used in Experiment 4 and 5 were used in Experiment 6. Chi square analyses were used for the proportion of females selected for first mount, first intromission, and first ejaculation on the copulatory preference test. The distribution of ejaculations between females on the copulatory preference tests were analyzed using planned orthogonal comparisons of the ejaculations received by the A+E and E-Alone females for each group of males. The distribution of mounts and intromissions between the two females during the copulatory preference tests were analyzed using mixed ANOVAs. The level of significance for all comparisons was 0.05.

Results

Conditioning Phase. Of the 31 males used as subjects in this experiment, 4 failed to ejaculate on the first conditioning session; all 4 in the 9-Session group. On the second trial all males in the 9-Session group ejaculated. No significant differences between groups were observed during the conditioning phase.

Copulatory Preference Tests. All of the 31 males tested copulated to at least 2 ejaculations on each copulatory preference test. For the 9-Session group, the mean number of ejaculations ($\pm$ SEM) per tests were 3.20 ± 0.99, 3.53 ± 0.95, 3.53 ± 1.19 and for the 240 min-Session group, the mean number of ejaculations ($\pm$ SEM) per test were 3.06 ± 0.92, 3.69 ± 1.03, 3.38 ± 0.91.

Figure 17 displays the choice of female for first ejaculation on each of the copulatory preference tests. Most of the males in the 9-Session groups ejaculated first with A+E females than E-Alone females on the first and second, but not the third, copulatory preference tests. Most of the males in the 240 min-Session group ejaculated first with the A+E female than the E-Alone female on the first, but not the second and third, copulatory preference test. There were no significant differences between groups on any of the tests: test 1 $\chi 2$ ($n = 31$) = 1.16, $p > 0.05$; test 2 $\chi 2$ ($n = 31$) = 2.64, $p > 0.05$; test 3 $\chi 2$ ($n = 31$) = 0.03, $p > 0.05$. For the 9-Session group only test 1 differed from test 3: test 1 versus test 2 $\chi 2$ ($n = 30$) = 0.68, $p > 0.05$; test 1 versus test 3 $\chi 2$ ($n = 30$) = 3.59, $p < 0.05$; test 2 versus test 3 $\chi 2$ ($n = 30$) = 1.22, $p > 0.05$. For the 240 min-

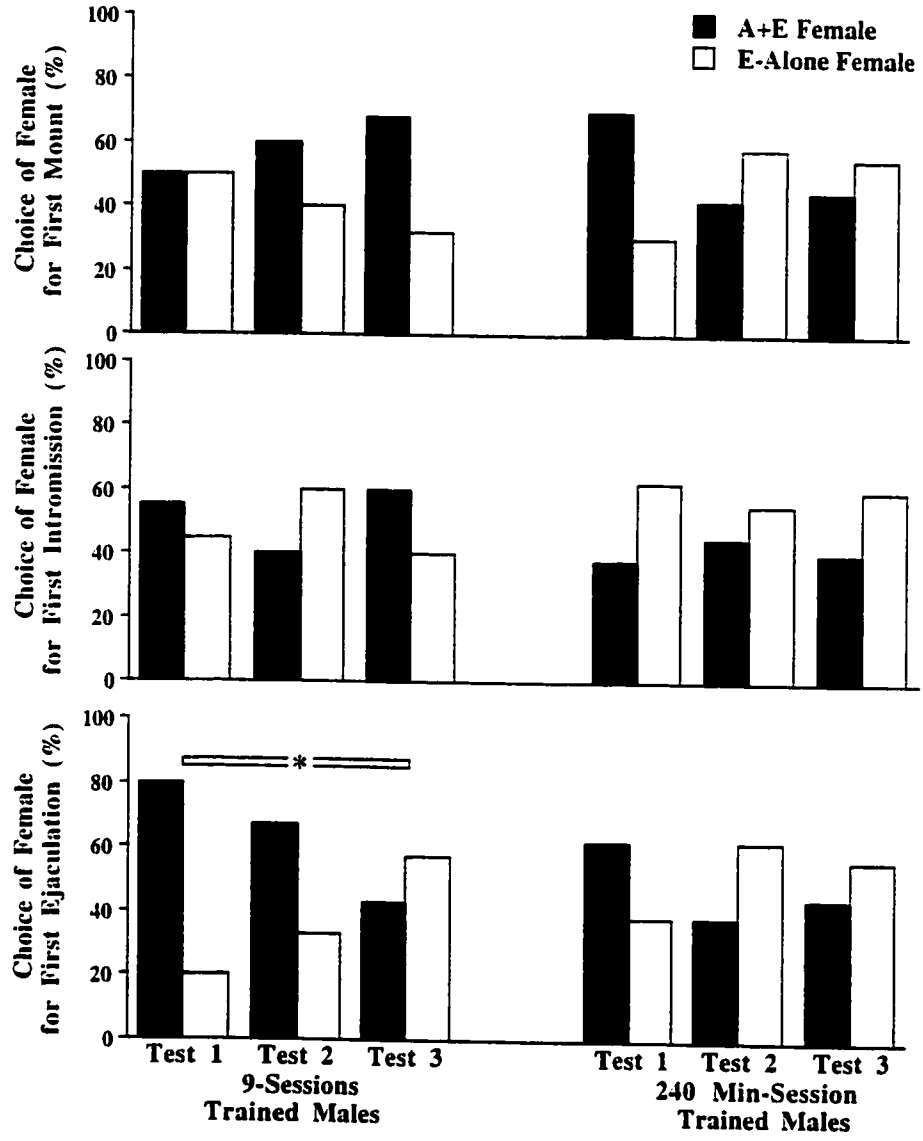

Figure 17. Proportional distribution by female type of first mount (top panel), first intromission (middle panel), and first ejaculation (bottom panel) during the three successive Copulatory Preference Tests in Experiment 6. * denotes $p < 0.05$ for between tests comparison.

Session group no tests differed: test 1 versus test 2 $\chi 2$ (n = 32) = 2.00, $p > 0.05$; test 1 versus test 3 $\chi 2$ (n = 32) = 1.13 $p > 0.05$; test 2 versus test 3 $\chi 2$ (n = 32) = 0.13, $p > 0.05$. There were no significant differences between groups on any of the tests or between tests for either of the groups on the choice of female for first mount or for first intromission.

Figure 18 displays the mean mounts per female per series, the mean intromissions per female per series, and the mean ejaculations per female during each test for each group. Males in the 9-Session group ejaculated more frequently with the A+E female than the E-Alone female on the first and second, but not the third, copulatory preference tests. Planned orthogonal contrasts confirmed the significance of these observations: test 1 t (58) = 2.21, $p < 0.05$; test 2 t (58) = 2.02, $p < 0.05$; test 3 t (58) = -0.17, $p > 0.05$. Males in the 240 min-Session group ejaculated more frequently with the A+E female than the E-Alone female on the first, but not the second and third, copulatory preference tests. Planned orthogonal contrasts confirmed the significance of these observations: test 1 t (58) = 1.73, $p < 0.05$; test 2 t (58) = -0.53, $p > 0.05$; test 3 t (58) = 0.34, $p > 0.05$. There were no significant effects of group, female type or interactions on any of the tests or across tests for the distribution of mounts or distribution of intromissions.

Discussion

The results of the present experiment demonstrate that CEP can be disrupted during copulatory preference tests. Males in both the 9-Session and 240 min-Session groups displayed CEP on the first copulatory preference test, whereas on the third test

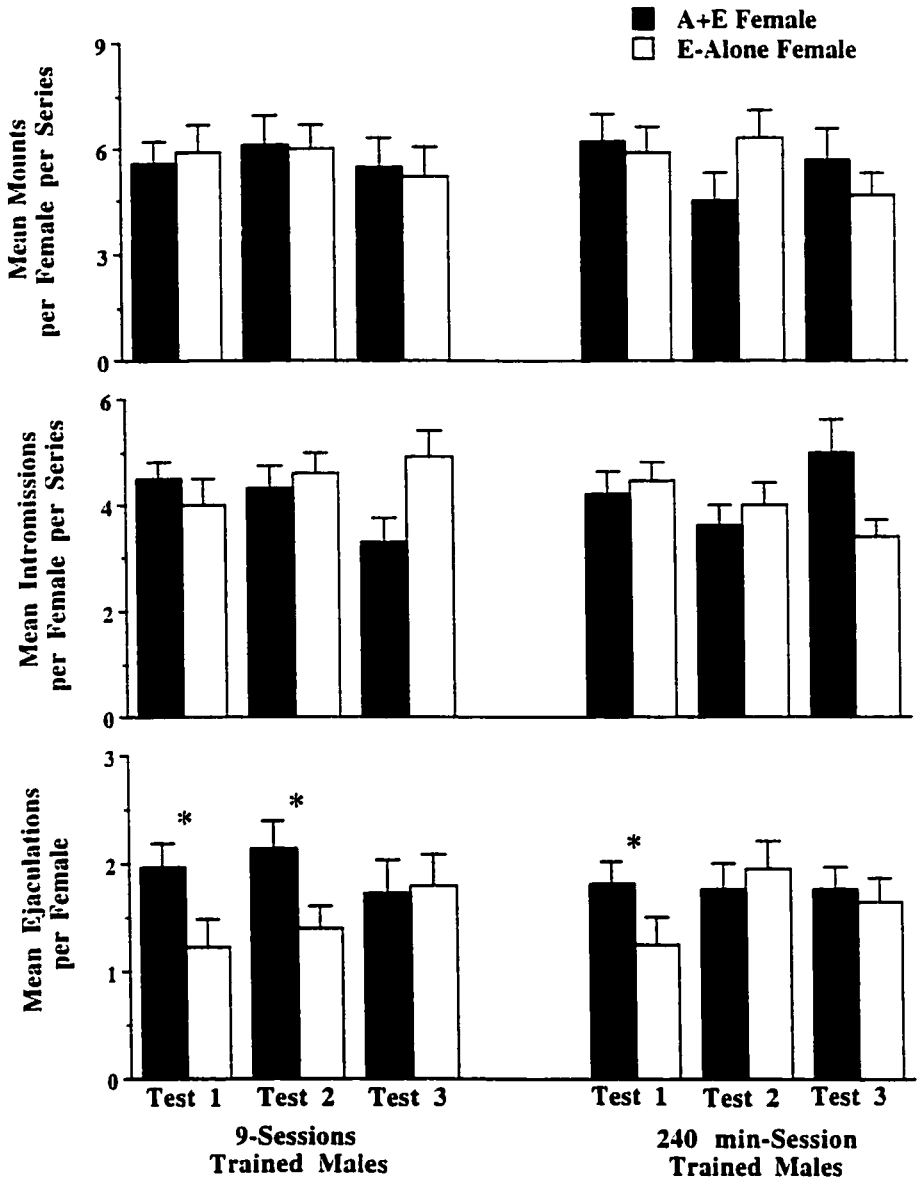

Figure 18. Distribution of mean (+SE) mounts per female per series (top panel), mean (+SE) intromissions per female per series (middle panel), and mean (+SE) ejaculations per female (bottom panel) during the three successive Copulatory Preference Tests in Experiment 6. * denotes $p < 0.05$ between female types comparison.

neither group displayed CEP. Because CEP is produced by copulation with a single scented female, these results suggest that copulation with an unscented female is sufficient to disrupt CEP. However, it may be that copulation with multiple females (scented or not) produces alterations in copulatory preference. Accordingly, a more controlled analysis of the disruption of CEP using extinction procedures would be informative. Although the present procedures likely mimic such procedures, extinction procedures examine the effect of repeated presentation of the CS alone on the subsequent expression of the conditioned response (Pavlov, 1927; Rescorla, 1988). Additionally, determining what components of copulation that are sufficient to disrupt CEP would be insightful. For instance, the effect of mounts without intromission, intromissions without ejaculation, or complete copulatory series with E-Alone female on previously established CEP could be assessed.

Comparison of the diminution of CEP between the 9-Session and 240 min-Session groups revealed that CEP appears to be more enduring following multiple conditioning session training than following a single conditioning session training. Males in the 9-Session group displayed CEP on both the first and second copulatory preference test. These males tended to ejaculate first and more often with the A+E female than the E-Alone female during test 1 and test 2. Conversely, males in the 240 min-Session group displayed CEP only on the first copulatory preference test. These males tended to ejaculate first and more often with the A+E female than the E-Alone female only during the first test. This finding is in agreement with the general principle that massed learning

is more easily disrupted than distributed learning (see for e.g. Carron, 1969; Fishman, Keller, & Atkinson, 1968; Nunnally, Duchnowski, & Knott, 1967; Williams, Frame, & LoLordo, 1991; Yin, Barnet, & Miller, 1994). This implies that each conditioning session produces an additive effect that enhances not only the magnitude of CEP but also its resiliency.

The present finding that CEP diminishes during repeated opportunities to copulate with one scented and one unscented female bears a resemblance to the findings of Kendrick et al. (1998) regarding the disruption of sexually imprinted partner preferences in male sheep and goats. They found that in males the preference for a female similar to their foster mother diminished as a result of the opportunity to copulate with dissimilar females. Conversely, sexually imprinted partner preferences in females were highly resistant to disruption by the same procedures. Accordingly, it would be interesting to examine if classically conditioned copulatory preference is also sexually-dimorphic in regards to its resiliency.

General Discussion

The findings of the present study extend the results of the experiments in Chapter 1 which show that pairing a neutral odor with access to a sexually-receptive female produces a subsequent preference to ejaculate with a female bearing the odor over one that does not. In Experiment 4, males given either 5 or 9 conditioning sessions each 30 min in duration with a single almond-scented female displayed a subsequent CEP for

almond-scented females. In Experiment 5, males given only a single conditioning session of either 120 min or 180 min in duration or in which they were allowed 4 ejaculations with almond-scented females displayed a subsequent CEP. The present experiments also support previous observations that the preferences produced by pairing an odor with sexual stimulation is specific to ejaculation; no significant preferences to mount or intromit with either female were observed on the copulatory preference tests.

Comparison of the strengths of CEP across different conditioning regimens yielded several interesting results. First, the strength of CEP, as measured by choice of female for first ejaculation, effect size estimates calculated from distribution of ejaculations, and proportion of males switching, did not differ for 5-Session and 9-Session groups in Experiment 4. This suggests that the CEP is maximal following five 30-min conditioning sessions. Further, a single conditioning session produced effect size estimates of nearly equal magnitude as both the 5- and 9-Sessions suggesting that the strength of CEP is similar across these treatments. However, there were a number of subtle differences between these treatments that effect size estimates do not take into account. First, total numbers of ejaculations were somewhat higher in the 5- and 9-Session groups (average 3.70) than in the single-Session groups (average 3.10). The relevance of this difference to assessment of CEP strength is unclear, but changes in number of ejaculations on the copulatory preference test could alter the appearance of CEP. For instance, preferences may be less obvious in males ejaculating an even number of times than males ejaculating an uneven number of times--in such cases, order of females

selected to ejaculate with may provide more insight than mean number of ejaculations per female. Second, proportion of males that choose the almond-scented female for first ejaculation were slightly higher in the 5- (78%) and the 9-Session (82%) groups than in the 120-min group (67%), 180-min group (75%), 240-min group (62%), and the 4-ejaculation group (67%). Third, the proportion of males that switched choice of female for the second ejaculation were slightly lower in the 5- (46%) and the 9-Session (48%) groups than in the 120-min group (65%), 180-min group (64%), 240-min group (56%), and the 4-ejaculation group (56%). These latter two differences are directly related to measures previously shown to reflect CEP and therefore suggest stronger CEP with multiple conditioning sessions. Moreover, the findings of Experiment 6 demonstrate that the CEP produced by multiple training sessions is more enduring than CEP produced in a single training session. This finding is in agreement with the general principle of massed versus distributed learning. Accordingly, it appears that the strength of CEP is increased by multiple conditioning sessions.

An interesting and unexpected finding in the single conditioning session results was that the effect of increased session duration or increased number of ejaculations during conditioning on the strength of CEP (as reflected by effect size estimates) appeared to vary in a nonlinear manner. CEP increased with lower levels of both variables, peaked at moderate levels, and showed a modest decline with further increases. This finding likely reflects a complex interaction between CS-UCS pairing and sexual motivation or satiety. Other experiments have identified ejaculation as a critical

component of the UCS for CEP during multiple conditioning session treatments (see Chapter 3). It is likely that the same UCS is involved in single conditioning session treatments. Accordingly, we predicted that increases in either number of ejaculations or duration of sessions (which indirectly increases the number of ejaculations) during conditioning would produce a linear increase in CEP. This was not the case. Rather, it appears that increases beyond a certain level actually produce a weaker CEP. An explanation for this may be that as conditioning sessions are lengthened, increases in sexual satiety may weaken the conditioning. This could happen in at least two ways. First, more sexual satiety could decrease the explicitness of the pairing between CS and UCS, i.e., more exposure to CS between ejaculations as the refractory periods increases with more ejaculations. Second, pairing the CS with sexual satiety may alter the strength of CEP more directly. CS exposure paired with high levels of sexual satiety may act to compete with or extinguish CEP and therefore override any benefits derived from additional CS-UCS pairings. Interestingly, decreased sexual motivation has been found to exert similar effects on the expression of conditioned appetitive sexual behaviors following multiple ejaculations (Van Furth & Van Ree, 1996a) or castration (Everitt et al., 1987). Further study of these effects may provide novel insights into the interaction of motivational variables with the conditioning of sexual behavior.

The finding that a CS paired with an increase in sexual satiety might weaken CEP along with findings regarding the neurobiology of sexual satiety may provide clues concerning the underlying neurobiology of CEP. Rodriguez-Manzo and Fernandez-

Guasti (1994; 1995a; 1995b) have employed a behavioral model of sexual exhaustion to assess the ability of psychopharmacological agents to restore copulatory behavior. They found that adrenergic and opioid antagonists and the 5HT1A-selective agonist, 8-OH-DPAT, increase levels of copulation relative to controls during a test 24 hr following a 2 hr sexual exhaustion trial. In a similar study, apomorphine was also found to induce copulation in sexually sated rats (Mas, Fumero, & Perez-Rodriguez, 1995). However, it is important to note that the reversal of sexual exhaustion in these studies was only partial despite using a wide range of doses of the drugs. Full reversal may require site specific administration, polypharmacology, or both. Evidence regarding neurochemical correlates of sexual exhaustion have also been studied. Mesolimbic dopamine release in response to sexual incentives and during copulation declines with repeated ejaculations (Fiorino, Coury, & Phillips, 1998). Increased activation of opioid peptide systems has been detected following 120 min, but not 30 min, of copulation (Szechtman, Hershokowitz, Simatov, 1981). However opioid release has been implicated following a single ejaculation by the ability of antagonists to reverse analgesia (Szechtman et al., 1981) and conditioned place preferences (Agmo & Berenfeld, 1990). Accordingly, it may be inferred that the disruption of CEP by prolonged mating may be due to the increased release of serotonin or certain opioid peptides or decreased dopaminergic or noradrenergic tone. Given that an optimal level of CEP develops during prolonged mating, it is likely the effects of these neurochemicals on CEP would also have an optimal range.

The present findings that CEP develops early in the course of sexual experience has important theoretical implications. Some theories have implicated early sexual experience in the development of sexual preferences (e.g. Storms, 1981), yet little work has been done to examine this notion empirically. Our finding that CEP can develop during the first sexual experience provides support in the context of an animal model. Further, the fact that on some measures CEP produced by a single conditioning session is as strong as those developed over a longer period gives direct evidence that initial experiences have particularly powerful influences on sexual preference. Relatively few ejaculations (i.e. 4) within a single session have similar effects as many (i.e. over 20 ejaculations in 9 sessions) distributed across several sessions. However, it should be noted that the effect of prior or subsequent copulation in the absence of the CS (i.e. with an unscented female) on the development of CEP produced by copulation in the presence of the CS (i.e. with a scented female) has yet to be examined.

Storms (1981) suggested that the onset of puberty and the ensuing development of sexual motivation are key factors in determining the effect of conditioning: Stimuli present before puberty (when motivation is low) do not result in conditioning, whereas those after puberty (when motivation is high) become associated with sexual stimulation to produce preferences. In our study, we have found indirect evidence that when sexual motivation is low (i.e. after several ejaculations or during the onset of sexual satiety) conditioning appears to be impaired; thus beyond an optimal point CEP strength declines.

Our findings and Storms' hypothesis share the common notion that neutral stimuli become capable of influencing sexual preferences when sexual motivation is high.

In summary, the present study replicates and further explores the influence of pairing a neutral olfactory stimulus with copulation and its subsequent effects of sexual partner preferences. As in previous experiments, males displayed conditioned preferences on ejaculatory measures when allowed to copulate with more than one female. The present finding that such conditioning can be produced during a single session, demonstrates the relative importance of early sexual experience in determining sexual preferences. That an optimal level of conditioning can be produced in one session demonstrates that the development of CEP involves a complex interaction between CS-UCS pairings and motivational factors.

CHAPTER 3: NATURE OF THE UNCONDITIONED STIMULUS.

The present study examines the nature of the UCS for the conditioning of copulatory preferences. In previous chapters, a neutral odor (almond extract) used as an olfactory CS paired with copulation produced a conditioned ejaculatory preference (CEP). In these studies, male rats were allowed access to sexually receptive females with a neutral odor applied to their neck and anogenital region during copulatory training sessions. The influence of this conditioning procedure on copulatory preferences was tested subsequently by allowing males to copulate simultaneously with unrestricted access to two receptive females, one bearing the CS and one not. On the copulatory preference test, most males trained with the almond scented females ejaculated first and more often with the almond scented female than the unscented one. Males with no experience with almond odor, or trained with almond odor in an unpaired or randomly paired manner, did not display a preference for almond scented female. Thus demonstrating that the learning was of a Pavlovian nature.

The UCS for CEP was determined by examining the effect of different amounts of copulation with scented females on the development of CEP. This was accomplished by allowing males either multiple intromissions without ejaculation, ejaculation, or multiple ejaculations with almond-scented females, and then assessing their copulatory preferences for an almond-scented female or an unscented female.

Experiment 7

Our previous demonstrations of conditioned ejaculatory preferences involved training schedules of 9 sessions each 30 min in duration. In Experiment 7, Long-Evans male rats were given 9 sessions with almond-scented receptive females which were terminated following either 2 ejaculations, 1 ejaculation plus the first intromission after the postejaculatory interval (PEI), 1 ejaculation without a PEI, or 5 intromissions without ejaculation. Then subsequent copulatory preferences were assessed during a 30 min test with two females, one almond-scented and one not.

Methods

Subjects

Males. The 66 Long-Evans rats that served as subjects in Experiment 7 were obtained from Charles River Canada, (St. Constant, Québec, Canada). The males weighed approximately 300 g and were sexually naive at the start of the experiment. They were housed in pairs in Plexiglas cages (36 cm x 26 cm x 19 cm) with ad lib access to food (Purina Rat Chow) and water. All rats were kept in a 12:12 hr reversed light-dark cycle colony room maintained at 21°C.

Females. Female Long-Evans rats from the same supplier as above were ovariectomized via bilateral lumbar incisions under ketamine/xylazine anaesthesia at least two months prior to the start of the experiment and were sexually experienced. Sexual receptivity was induced by subcutaneous administration of estradiol (10 μg) 48 hr prior and progesterone (500 μg) 4-6 hr prior to each training or test session. Females were

housed under the same conditions as males. Stimulus females were selected at random for use during conditioning sessions and copulatory preference tests. Female rats were scented with either approximately 1 ml of almond extract (Blue Ribbon, Etobicoke, Ontario, Canada) or distilled water applied to both the back of the neck and the anogenital area using a cotton swab.

Apparatus

Conditioning sessions took place in bilevel chambers constructed of Plexiglas (outside dimensions of 18 cm x 25 cm x 65 cm) with a platform (40 cm in length) elevated by a set of ramps at each end dividing the chamber into two levels (for further details see Pfaus, Mendelson, & Phillips, 1990). The bilevel chambers were cleaned with water and Coverage 256 (Conva Tec, St. Louis, MO) and the soiled bedding was replaced with clean bedding prior to each conditioning session. All conditioning sessions were recorded on video and scored subsequently using a PC-based program. Copulatory preference tests took place in a large open field (123 cm x 123 cm x 46 cm) with a thin layer of bedding covering the floor. Copulatory preference tests were scored at the time of testing.

Procedure

Conditioning Phase. Conditioning sessions were conducted in the same manner as in Chapter 1 with the exception that trials were terminated once a criterion of copulation was achieved. Male rats were preexposed to the chambers once a day for 15 min each day in order to habituate them to the training environment. This habituation procedure lasted 7 days and has been shown previously to increase the proportion of males that

become vigorous copulators (Pfaus & Wilkinson, 1995). Then males were allowed to achieve either 2 ejaculations (2EJ; n = 14), 1 ejaculation plus the first intromission after the PEI (PEI; n = 14), 1 ejaculation (1EJ; n = 14), or 5 intromissions (INT; n = 24); conditioning sessions were conducted at four day intervals during the middle third of the dark phase of the light:dark cycle. In each of 9 conditioning sessions, males were placed in the chamber for 5 min then a sexually-receptive scented female (A+E female) was placed into the chamber. Rats were then allowed to copulate until they reached their copulatory criterion then they were returned to their home cages; conditioning sessions were terminated by removing the female. Latency and frequency data for all mounts, intromissions, and ejaculations were recorded during each conditioning session. Criteria for sexual behaviors were those described by Sachs and Barfield (1976) and Meisel and Sachs (1994).

Copulatory Preference Test. Four days after the final conditioning session, each male was placed in the large open field and allowed to habituate for 5 min. At the end of this period, one A+E female and one female scented with distilled water (E-Alone female) were placed simultaneously into two diagonal corners of the open field at approximately equal distances from the male. All copulatory behaviors and the females to which they were directed were recorded during each male's test. Tests were terminated 30 min after the females were introduced.

Statistical Analysis.

Mixed-design between-within ANOVAs were used to analyze the level changing data from the receptive and nonreceptive conditioning sessions with significant values being followed by post hoc analysis of individual means using the Tukey method. Chi square analysis was used for the proportion of female selected for first mount, first intromission, and first ejaculation on the copulatory preference test. Previous experiments have consistently found that olfactory conditioning procedures similar to those used in the present experiment produce preferences on ejaculation, but not on mount or intromission, measures. Accordingly, the distribution of ejaculations between females on the copulatory preference tests were analyzed using planned orthogonal comparisons (Glass & Hopkins, 1984); comparisons were made only between the A+E and E-Alone female for each group of males. Effect size estimates (Glass & Hopkins, 1984) were calculated from the distribution of ejaculations between females for each group. The distribution of mounts and intromissions between the two females during the copulatory preference tests were analyzed using mixed ANOVAs with significant values being followed by post hoc analysis of individual means using the Tukey method. The level of significance for all comparisons was 0.05.

Results

Conditioning Phase. Of the 66 males used as subjects in this experiment, 7 failed to achieve the copulatory criterion on the first conditioning session and were excluded from the experiment; 1 in the 2EJ group, 3 in the PEI group, and 3 in the INT group. An

additional 4 males in the INT group were excluded from analysis because they ejaculated on one or more conditioning sessions.

The mean latency to level change decreased and the mean number of level changes increased across conditioning sessions for all groups. Interestingly, males in the INT group reached an asymptotic latency to level change higher than that of the other three groups and an asymptotic level change frequency lower than that of the 2EJ and 1EJ groups (data not shown). The statistical significance of these differences were confirmed by mixed-design between-within ANOVAs. For level change latency, there was a significant effect of group [$F(3, 48) = 4.04$, $p < 0.05$; INT group differed significantly from the other three groups and no other groups differed significantly], a significant effect of session [$F(8, 384) = 35.47$, $p < 0.05$; session 1 differed from all other sessions, but no other sessions differed significantly], but the interaction between group and sessions failed to reach significance [$F(8, 384) =$, $p > 0.05$]. For level change frequency, there was a significant effect of group [$F(8, 48) = 5.90$, $p < 0.05$; INT group differed significantly from the 2EJ and 1EJ groups, no other groups differed significantly], a significant effect of sessions [$F(8, 384) = 35.47$, $p < 0.05$; session 1 differed from all other sessions, session 2 differed from sessions 4 to 9, and session 3 differed from session 7, no other sessions differed significantly], and a significant interaction between group and session [$F(24, 384) = 1.60$, $p < 0.05$; for session 1, 2EJ group differed from the 1EJ and PEI groups, for session 3, 2EJ group differed from the other three groups and 1EJ group

differed from INT group, for sessions 4, 5, and 7, INT group differed from the other three groups, for sessions 6, 8, and 9, INT group differed from 2EJ and 1EJ groups].

Conversely, males in all groups displayed similar copulatory behaviors during training; latency to mount, latency to intromit, and inter-intromission intervals decreased to asymptotic levels that did not differ between groups, intromission ratio (number of successful intromission to intromission attempts, i.e. mounts) increased to asymptotic levels that did not differ between groups, and pursuit of female (number of level changes per mount) increased to asymptotic levels that did not differ between the four groups. Similarly, ejaculation latency decreased to asymptotic levels that did not differ between the 2EJ, PEI, and 1EJ groups, and postejaculatory refractory period decreased to asymptotic levels that did not differ between the 2EJ and PEI. groups (data not shown).

Copulatory Preference Test. Of the 55 males tested, 7 failed to copulate to ejaculation on the copulatory preference test (1 in the 2EJ group, 3 in the PEI group, and 3 in the INT group). All copulatory parameters were similar between all groups; no significant differences were detected between groups for intromission latency, intromission frequency, inter-intromission interval, ejaculation latency, and postejaculatory interval.

The choice of female for first mount, first intromission, and first ejaculation for each group in Experiment 7 are displayed in Figure 19. Males in the 2EJ and PEI, but not the 1EJ, groups tended to ejaculate first with A+E females more than did males in the INT group. Chi square analyses confirmed the statistical significance of these

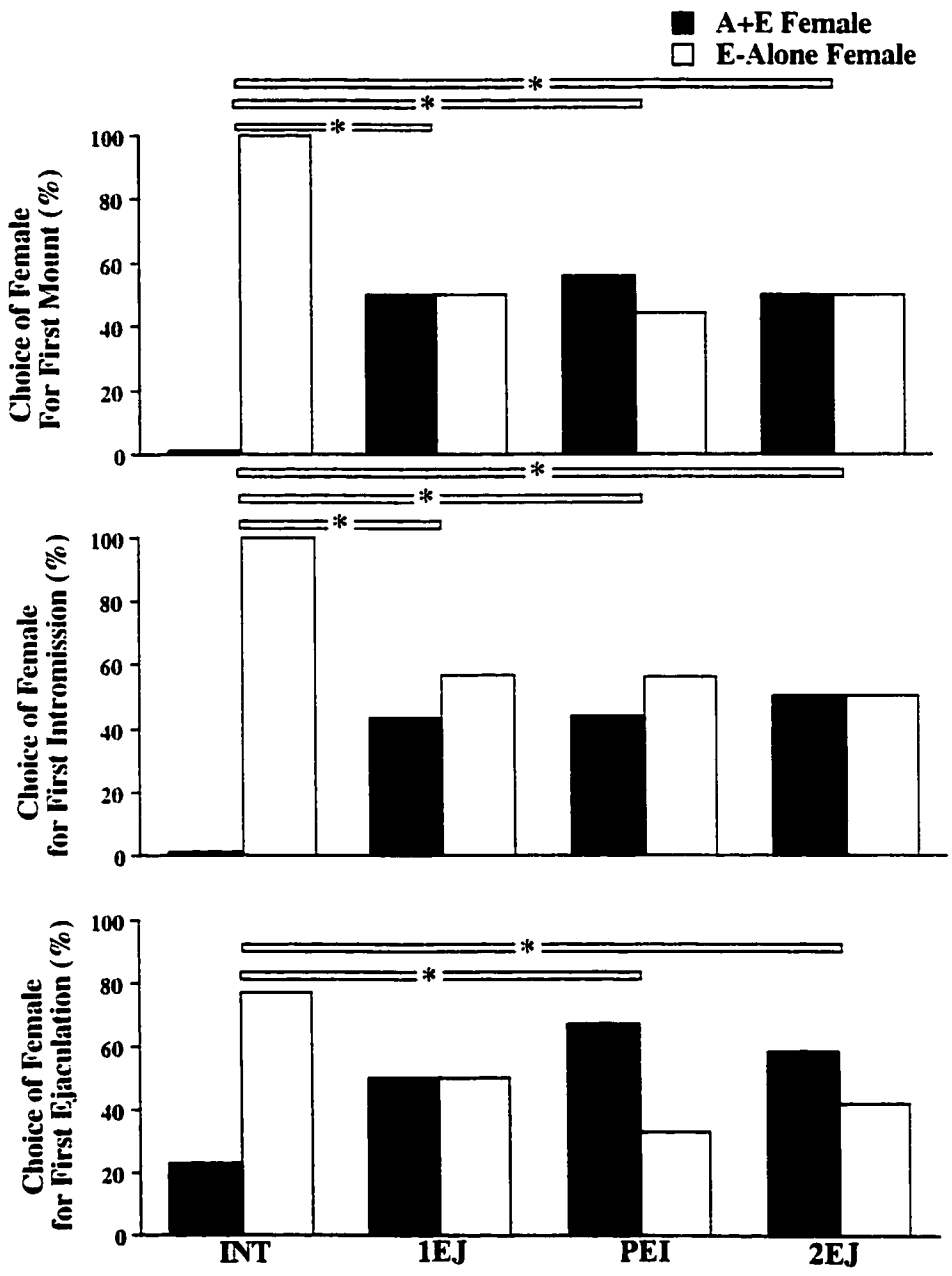

Figure 19. Proportional distribution by female type of first mount (top panel), first intromission (middle panel), and first ejaculation (bottom panel) during the Copulatory Preference Test in Experiment 7. * denotes $p < 0.05$ for between groups comparison.

observations: 2EJ versus PEI $\chi 2$ (n = 21) = 0, $p > 0.05$; 2EJ versus 1EJ $\chi 2$ (n = 26) = 1.47, $p > 0.05$; 2EJ versus INT $\chi 2$ (n = 25) = 4.81, $p < 0.05$; PEI versus 1EJ $\chi 2$ (n = 23) = 1.24, $p > 0.05$; PEI versus INT $\chi 2$ (n = 22) = 4.18, $p < 0.05$; 1EJ versus INT $\chi 2$ (n = 27) = 1.19, $p > 0.05$. More males in the 2EJ, PEI, and 1EJ group mounted and intromitted first with the almond-scented female than males in the INT group; none of the males in the INT group mounted or intromitted first with the almond-scented female. Chi square analyses confirmed the statistical significance of these observations. For mounts: 2EJ versus PEI $\chi 2$ (n = 21) = 0.06, $p > 0.05$; 2EJ versus 1EJ $\chi 2$ (n = 26) = 0, $p > 0.05$; 2EJ versus INT $\chi 2$ (n = 25) = 8.55, $p < 0.05$; PEI versus 1EJ $\chi 2$ (n = 23) = 0.07, $p > 0.05$; PEI versus INT $\chi 2$ (n = 22) = 9.35, $p < 0.05$; 1EJ versus INT $\chi 2$ (n = 27) = 8.77, $p < 0.05$. For intromissions: 2EJ versus PEI $\chi 2$ (n = 21) = 0.06, $p > 0.05$; 2EJ versus 1EJ $\chi 2$ (n = 26) = 0.13, $p > 0.05$; 2EJ versus INT $\chi 2$ (n = 25) = 8.55, $p < 0.05$; PEI versus 1EJ $\chi 2$ (n = 23) = 0.01, $p > 0.05$; PEI versus INT $\chi 2$ (n = 22) = 7.06, $p < 0.05$; 1EJ versus INT $\chi 2$ (n = 27) = 7.16, $p > 0.05$.

The mean mounts per female per series, mean intromissions per female per series, and mean ejaculations per female over the 30 min test for each group in Experiment 7 are displayed in Figure 20. There were no significant differences between groups in the distribution of mounts or intromissions toward either female. However, males in the 2EJ and PEI groups ejaculated more frequently with the A+E female, whereas the males in the

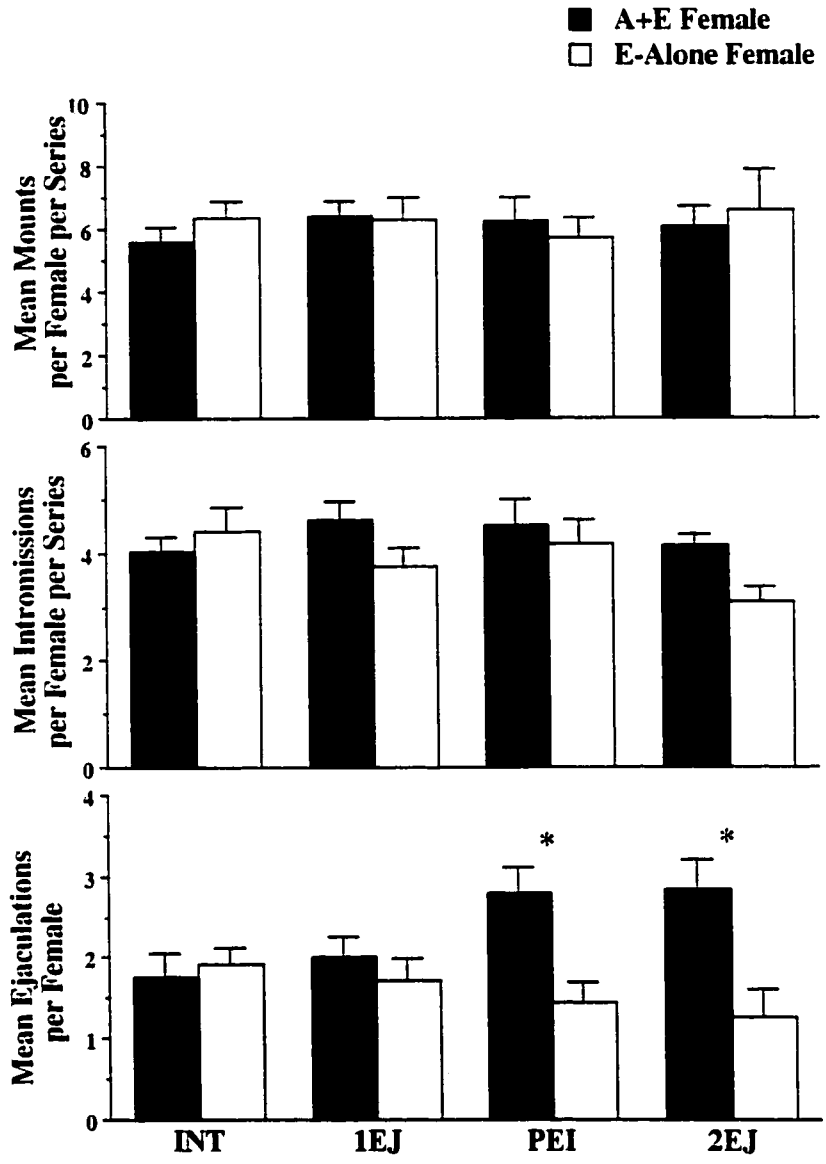

Figure 20. Distribution of mean (+SE) mounts per female per series (top panel), mean (+SE) intromissions per female per series (middle panel), and mean (+SE) ejaculations per female (bottom panel) during the Copulatory Preference Test in Experiment 7. * denotes $p < 0.05$ for between female types comparison.

1EJ and INT groups ejaculated with both females with approximately the same frequency. Planned orthogonal contrasts confirmed the statistical significance of these observations: 2EJ group $t(88) = 3.88$, $p < 0.005$; PEI group $t(88) = 2.83$, $p < 0.05$; 1EJ group $t(88) = 0.80$, $p > 0.05$; INT group $t(88) = -0.39$, $p > 0.05$. Effect size estimates for the differences in distribution of ejaculations were for the 2EJ group, 1.31, for the PEI group, 1.58, for the 1EJ group, 0.30, for the INT group, -0.17.

Discussion

The findings of the present experiment demonstrate that the development of CEPs are dependent upon ejaculation with a scented female during the conditioning sessions. Males not allowed to ejaculate during training, as in the INT group, failed to demonstrate a preference. Moreover, it appears that the male must be exposed to the scented female following ejaculation for the preference to develop. When the female was removed immediately following ejaculation, as in the 1EJ group, no preference was displayed. These results suggest that the UCS for CEP is an event produced by ejaculation, not the ejaculation per se. However, the present experiment failed to control for duration of exposure to the almond-scented female, thus the differences between groups may simply be due to the length of exposure resulting from copulatory criteria that require more time to achieve. This confound was addressed subsequently in Experiment 8.

Interestingly, in the INT group, which did not display CEP for the scented female, it appears that the males in this group displayed an initial copulatory preference for the unscented female. All males in this group choose the unscented female for first mount

and first intromission and the majority of them choose the unscented female for first ejaculation. This finding is interesting for two reasons. First, it is the first demonstration that conditioned copulatory preferences can be displayed in choice of female for mounts or intromissions. Our previous studies found differences only for distribution of ejaculations. Second, the finding that the direction of the preference displayed by the INT group was toward the unscented female, whereas all their previous copulatory experience was with scented females, suggests that interruption of copulation prior to ejaculation may produce an aversive state sufficient to act as a UCS for a CEP in the opposite direction of the CEP observed when males are allowed to copulate to ejaculation.

The difference between groups in the development of conditioned level changing in anticipation of the arrival of the female also reveals a number of interesting points regarding the nature of sexual conditioning. First, consistent with the findings of previous experiments, all groups in the present experiment displayed levels of anticipatory level changing that increased with copulatory experience in chambers that were cleaned suggesting that conditioned level changing is not elicited solely by sex odors in Long-Evans males. Non-olfactory stimuli or perhaps even the scent of the clean chambers must have served as the CS to facilitate anticipatory level changing. This finding clarifies conclusions of Van Furth and Van Ree (1996b) that conditioned level changing is driven by sex odors based on their finding that olfactory bulb lesions disrupt the development of this conditioned behavior. Our results do not stand in contrast to

theirs because the chambers in our experiment, although cleaned, undoubtably contained distinctive odors (e.g. that of Coverage 256 detergent). However, it is not known whether conditioned level changing could be elicited by strictly non-olfactory stimuli. This idea could be tested by changing the odors in the chambers between training and testing sessions. Second, although all groups displayed a decrease in latency to level change and an increase in frequency of level changes, the INT group reached asymptotic levels with a longer latency and lower frequency than the other groups. This suggests that the type of copulation obtained can affect the magnitude of the conditioned response. Stimuli associated with ejaculation appear to produce more sexual excitement than stimuli associated with interrupted copulation. Third, the finding that INT group displayed increased anticipatory level changing during training, but that the scented female appeared to be devalued during the copulatory preference test, demonstrates not only that there are different UCSs for different conditioned behaviors but also that a single UCS can have various influences, and in apparently opposing directions, depending on what behavior is measured (i.e. increase sexual excitement and decrease in ejaculatory preference).

The present study also reveals novel information regarding the acquisition of copulatory behavior. Although previous studies (e.g. Whalen, 1961) have examined the maintenance of copulation following intromissive experience alone, the subjects in those experiments were all sexually-experienced. In the present experiment, all subjects were initially sexually-naive and received various amounts of copulation during training. Consistent with findings in sexually-experienced animals, intromissive experience alone

was sufficient to maintain copulatory behavior in sexually-naive males. Moreover, parameters of copulatory behavior did not differ between groups either during training or on the copulatory preference test demonstrating that intromissive experience alone is sufficient for the acquisition of the copulatory efficiency typical of sexually-experienced males.

Experiment 8

The results of Experiment 7 demonstrate that either 2EJ or PEI with a scented female present during conditioning sessions is sufficient for the development of CEP. However, it is not clear why males in the 1EJ and the INT conditions failed to develop the preferences. It may be argued that the critical reason that PEI group, but not the 1EJ group, displayed CEP was due to the length of exposure to the scented female and not due to achieving a specified amount of copulation (i.e. exposure to female for PEI group was approximately 15-20 min on session 1 and 8-10 min on session 9 versus for 1EJ group was approximately 10-15 min on session 1 and 3-5 min on session 9). Accordingly, the goal of Experiment 8 was to determine whether length of exposure to the scented female, or copulation during exposure, is the critical factor in the UCS for CEP. This was achieved by allowing males to copulate to different criteria followed by exposure without access to the scented female for a total 30 min period.

Methods

Subjects

Male rats of the same strain from the same supplier were housed in the same conditions as those of Experiment 7 served as subjects in Experiment 8 (n = 78). Female rats were housed and treated in the same way as in the first experiment.

Apparatus

All conditioning sessions were conducted in the semicircular chambers fitted with dividers. The chambers were 65 cm long at the front and 40 cm at the widest point and were 40 cm high. The chambers had a plexiglass front, a wood floor covered with a thin layer of bedding, a rounded metal rear portion, and a metal mesh lid (grid of 1.2 cm). The front and back had plexiglass grooves so that a divider of plastic coated metal mesh (grid 2.5 cm) could be easily inserted to form two separate compartments. Subjects on either side of the divider could see, hear, smell, and have limited contact with each other, but could not copulate. Copulatory preference tests were conducted in the same open field as used in Experiment 7. All conditioning sessions were recorded on video and scored subsequently using a PC-based program and copulatory preference tests were scored at the time of testing.

Procedure

Conditioning Phase. As in Experiment 7, male rats received 7 daily 15-min preexposure sessions to the training chambers. Each male received 9 conditioning sessions consisting of two phases: First, male subjects and stimulus females were

allowed to copulate to criterion and then the male was exposed without access to the female. Males were allowed to copulate with an A+E female to either 2 ejaculations (2EJ+; n = 26), 1 ejaculation (1EJ+; n = 26), or 5 intromissions (INT+; n = 26) before the divider was inserted. On each conditioning session, males in each group were placed individually into a chamber for 5 min, after which an A+E female was placed into the chamber and the pair was allowed to copulate until the criterion was achieved. Then the divider was quickly inserted and, if appropriate, the female was placed on the opposite side of the divider of the male. Then, the pair was left undisturbed until 30 min had elapsed from the time that the female was first placed into the chamber. This ensured that all males in different groups received the same duration of exposure to the A+E females.

Copulatory Preference Test. Four days following the final conditioning session, each male was placed in the open field and allowed to habituate for a period of 5 min. As in Experiment 7, one A+E female and one E-Alone female were placed simultaneously into two diagonal corners of the open field at approximately equal distances from the male. All copulatory behaviors, and the female to which they were directed, were recorded during each male's test. The test was terminated after 30 min.

Statistical Analysis

The statistics used in Experiment 7 were used in Experiment 8. Chi square analysis was used for the proportion of female selected for first mounts, first intromissions, and first ejaculations on the copulatory preference test. The distribution

of ejaculations between females on the copulatory preference tests was analyzed using planned orthogonal comparisons of the ejaculations received by the A+E and E-Alone females for each group of males. Effect size estimates were calculated from the distribution of ejaculations between females for each group. The distribution of mounts and intromissions between the two females during the copulatory preference tests was analyzed using mixed ANOVAs. The level of significance for all comparisons was 0.05.

Results

Conditioning Sessions.

Of the 78 males used in Experiment 8, 23 males failed to ejaculate during the first conditioning session and were not included in any analyses; 7 in the 2EJ+ group, 8 in the 1EJ+ group, and 8 in the INT+ group. Males in all groups displayed similar copulatory behaviors during training; latency to mount, latency to intromit, and inter-intromission intervals decreased to asymptotic levels that did not differ between groups and intromission ratio increased to asymptotic levels that did not differ between groups. Similarly, ejaculation latency decreased to asymptotic levels that did not differ between the 2EJ+ and 1EJ+ groups (data not shown).

Copulatory Preference Test.

Of the 55 males tested in Experiment 8, 5 failed to copulate to ejaculation during the copulatory preference test; 2 in the 1EJ+ group and 3 in the INT+ group. Copulatory behaviors were similar between all groups. No significant differences were detected between groups for intromission latency, inter-intromission interval, ejaculation latency,

or ejaculation frequency. However, a significant difference was found between groups for the postejaculatory interval [$F(2, 47) = 3.98$, $p < 0.05$; follow up analysis revealed the INT+ group had longer postejaculatory intervals than the other two groups].

The choice of female for first mount, first intromission, and first ejaculation for each group of males in Experiment 8 are displayed in Figure 21. More males in the 2EJ+ and 1EJ+ group ejaculated first with the A+E female than males in the INT+ group. The statistical significance of these observations were confirmed with chi square analyses: 2EJ+ versus 1EJ+ $\chi 2$ ($n = 35$) = 0.57, $p > 0.05$; 2EJ+ versus INT+ $\chi 2$ ($n = 34$) = 2.98, $p < 0.05$; 1EJ+ versus INT+ $\chi 2$ ($n = 31$) = 5.43, $p < 0.05$. No significant differences were observed between groups for first intromission or first mount.

The mean mounts per female per series, mean intromissions per female per series, and mean ejaculations per female during the copulatory preference test for each group in Experiment 8 are displayed in Figure 22. No significant differences were found for distribution of mounts (results of 3 X 2 mixed ANOVA: for Group: $F(2, 94) = 0.54$, $p > 0.05$; for Female Type: $F(1, 94) = 0.06$, $p > 0.05$; for Group x Female Type: $F(2, 94) = 1.16$, $p > 0.05$). Similarly, no significant differences were found for distribution of intromissions (results of 3 X 2 mixed ANOVA: for Group: $F(2, 94) = 0.29$, $p > 0.05$; for Female Type: $F(1, 94) = 1.37$, $p > 0.05$; for Group x Female Type: $F(2, 94) = 1.86$, $p > 0.05$). Moreover, males in the 2EJ+ and 1EJ+ groups ejaculated more frequently with the A+E female, whereas the males in the INT+ group ejaculated more frequently with the unscented female. Planned orthogonal contrasts confirmed the statistical significance

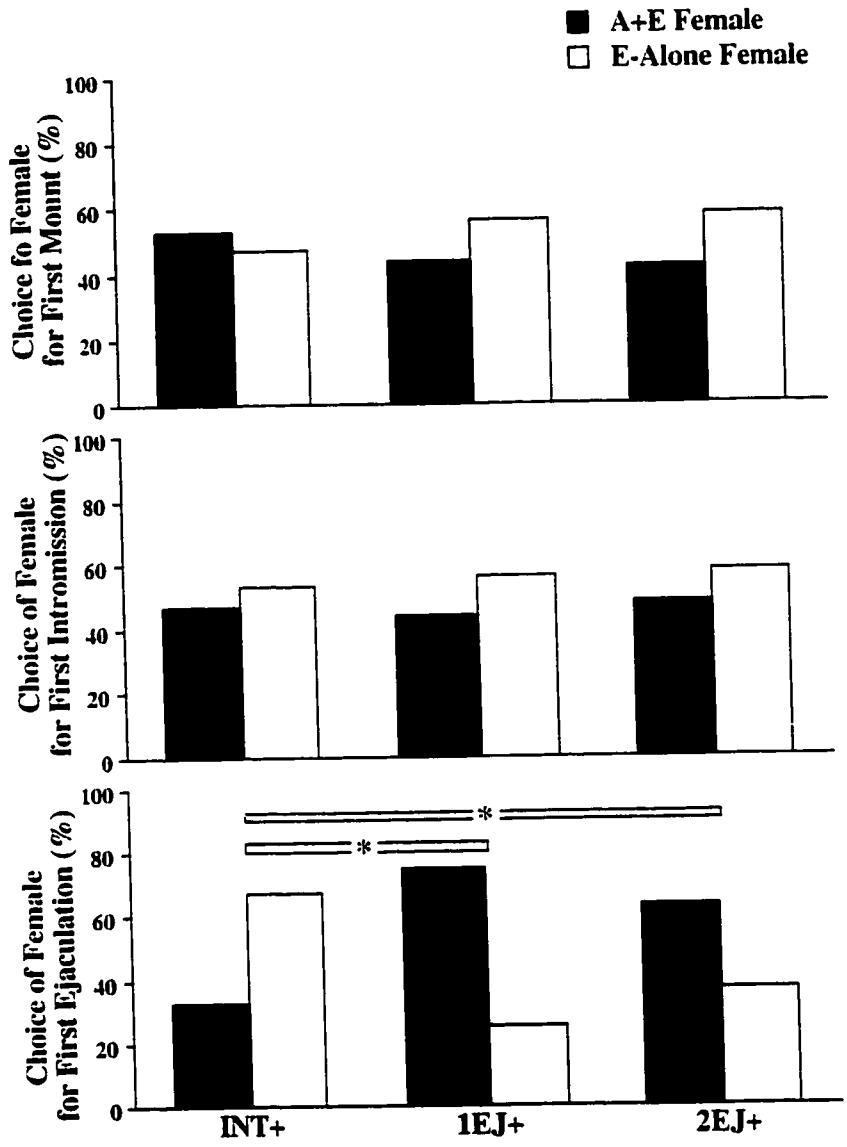

Figure 21. Proportional distribution by female type of first mount (top panel), first intromission (middle panel), and first ejaculation (bottom panel) during the Copulatory Preference Test in Experiment 8. * denotes $p < 0.05$ for between groups comparison.

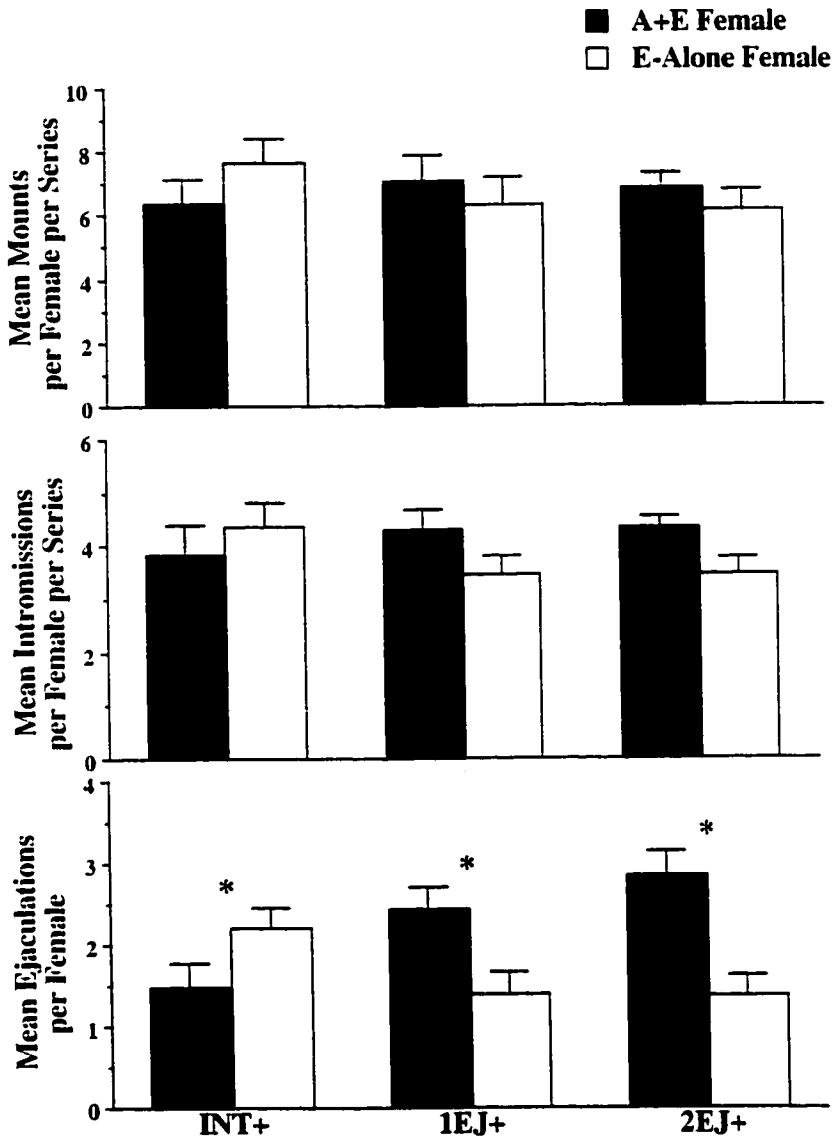

Figure 22. Distribution of mean (+SE) mounts per female per series (top panel), mean (+SE) intromissions per female per series (middle panel), and mean (+SE) ejaculations per female (bottom panel) during the Copulatory Preference Test in Experiment 8. * denotes $p < 0.05$ for between female types comparison.

of these observations: 2EJ+ group, $t(94) = 4.01$, $p < 0.05$; 1EJ+ group, $t(94) = 2.65$, $p < 0.05$; INT+ group, $t(94) = -1.77$, $p > 0.05$. Effect size estimates for the differences in distribution of ejaculations were for the 2 ejaculations group, 1.24; for the 1EJ+ group, 1.10; for the INT+ group, -0.66.

Discussion

The results of Experiment 8 confirm and extend the findings of Experiment 7. The finding that the INT+ group did not display a preference for the scented female even when time of exposure during training sessions was 30 min, demonstrates that length of exposure to the scented female is not in itself sufficient for the development of CEP. Additionally, the finding that the 2EJ+ and 1EJ+ groups displayed a preference for the scented female demonstrates that ejaculation is critical for the development of CEP. Moreover, these results show that the presence of the scented female following ejaculation is critical to the development of CEP. When the female was removed following ejaculation (1EJ group in Experiment 7) no CEP developed; however, when the female was present even without access (1EJ+ group in Experiment 8) a significant CEP developed.

The males in the INT+ group in the present experiment displayed an ejaculatory preference for the unscented female as demonstrated by choice of female for first ejaculation and distribution of ejaculations. This finding is in partial agreement with the finding for the INT group in Experiment 7. In Experiment 7, the INT group displayed only an initial preference for the unscented female, as demonstrated by choice for first mount, first intromission, and first ejaculation, but did not show a preference for

distribution of ejaculations for the rest of the test session. Given the discrepancies between the nature of the preference displayed by each group, it is not clear if the incomplete copulation treatment results in a preference that is altered by removal or presence of the female. In other studies, males trained with unscented females displayed an ejaculatory preference for unscented females when given the opportunity to copulate with a scented and unscented female. It is not clear whether this represents a default condition or if there is a conditioned preference displayed by males trained with unscented females. In such a case the addition of an odor would produce a deviation from the conditioned preference. Thus, the preference for unscented females displayed by males trained with unscented females and males trained with incomplete copulation with scented females may be interpreted as an absence of any conditioned preferences (i.e. a default) or that one or both of these treatments resulted in conditioning that is the same in expression but not in underlying nature. The resolution of these issues will require further investigation.

Experiment 9

The findings of Experiments 7 and 8 suggest that the critical aspect of copulation that comprises the UCS for development of CEP was the presence of the scented female following ejaculation. The purpose of Experiment 9 was to determine if exposure to an A+E female only following ejaculation is sufficient to support CEP. Accordingly, male rats were allowed to copulate to ejaculation with an unscented female immediately after which they were exposed (without access) to the same female bearing almond odor for a period of 30 min.

Methods

Subjects

Male rats of the same strain from the same supplier were housed in the same conditions as those of Experiments 7 and 8 served as subjects (n = 30). Female rats were housed and treated in the same way as in the first two experiments.

Apparatus

All conditioning sessions were conducted in the semicircular chambers fitted with dividers that were used in Experiment 8. Copulatory preference tests were conducted in the same open field as used in Experiments 7 and 8. All conditioning sessions were recorded on video and scored subsequently using a PC-based program and copulatory preferences tests were scored at the time of testing.

Procedure

Conditioning Phase. As in Experiments 7 and 8, male rats received 7 daily 15-min preexposure sessions to the testing chambers. Each male then received 9 conditioning sessions consisting of two phases: First, male subjects and stimulus females were allowed to copulate until the male ejaculated, after which the male was exposed to the female without access. Males were allowed to copulate to 1 ejaculation with either an A+E (1EJ+; n = 12) or unscented female (PEI-only; n = 18), after which both groups were exposed without access to an A+E female behind the divider. At the start of each conditioning session, males in each group were placed individually into a chamber for 5 min, after which a receptive female bearing the appropriate scent (almond extract or

distilled water) was placed into the chamber until the male ejaculated. Then, the female was immediately removed and scented with almond odor, the divider was inserted, and the female placed on the opposite side of the divider as the male for 30 min of exposure.

Copulatory Preference Test. Four days following the final conditioning session, each male was placed in the open field and allowed to habituate for a period of 5 min. As in Experiments 7 and 8, one A+E female and one E-Alone female were placed simultaneously into two diagonal corners of the open field at approximately equal distances from the male. All copulatory behaviors, and the female to which they were directed, were recorded during each male's test. The test was terminated after 30 min.

Statistical Analysis

The statistics used in Experiments 7 and 8 were used in Experiment 9. Chi square analysis was used the proportion of females selected for first mounts, first intromissions, and first ejaculations on the copulatory preference test. The distribution of ejaculations between females on the copulatory preference tests were analyzed using planned orthogonal comparisons of the ejaculations received by the A+E and E-Alone females for each group of males. Effect size estimates were calculated from the distribution of ejaculations between females for each group. The distribution of mounts and intromissions between the two females during the copulatory preference tests were analyzed using mixed ANOVAs. The level of significance for all comparisons was 0.05.

Results

Conditioning Sessions.

Of the 30 males used in Experiment 9, 9 failed to copulate to ejaculation on the first conditioning session and were not included in any analyses; 6 in the PEI-only group and 3 in the 1EJ+ group. No significant differences on any copulatory behaviors were detected between the groups.

Copulatory Preference Test.

All of the 21 the males that completed training copulated to ejaculation and no copulatory behaviors differed between groups. The choice of female for first mount, first intromission, and first ejaculation for each group of males in Experiment 9 are displayed in Figure 23. More males in the 1EJ+ group ejaculated first with the A+E female than males in the PEI-only group; however, this difference failed to reach statistical significance $\chi 2$ ($n = 20$) = 1.25, $p > 0.05$. No significant differences were found between groups for first mount or first intromission.

The mean mounts per female per series, mean intromissions per female per series, and mean ejaculations per female during the copulatory preference test for both groups in Experiment 9 are displayed in Figure 24. Males in the 1EJ+ group mounted more frequently than males in the PEI-only group (F (1, 36) = 10.46, $p < 0.05$), but there was no significant difference for distribution of mounts between females (F (1, 36) = 0.04, $p > 0.05$) nor was there a significant interaction between group and female type (F (2, 50) = 0.44, $p > 0.05$). No significant differences were found for distribution of intromissions

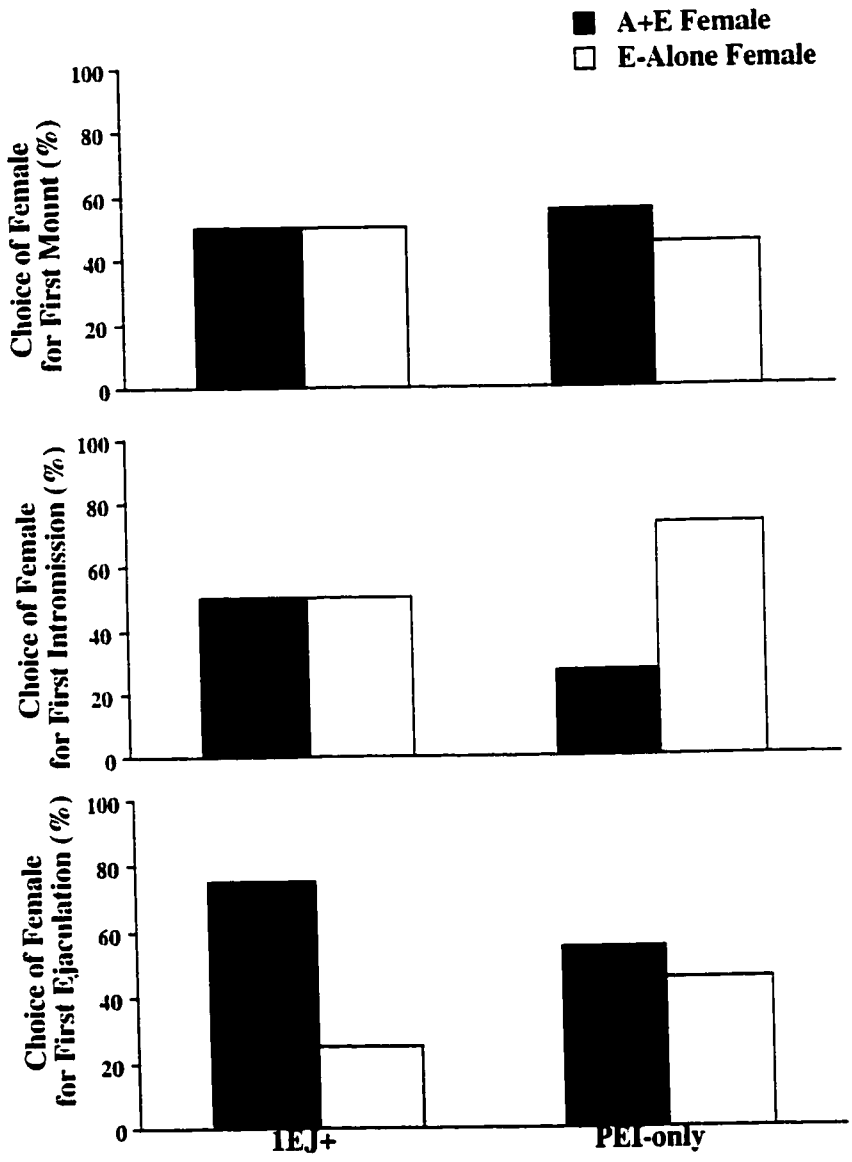

Figure 23. Proportional distribution by female type of first mount (top panel), first intromission (middle panel), and first ejaculation (bottom panel) during the Copulatory Preference Test in Experiment 9. * denotes $p < 0.05$ for between groups comparison.

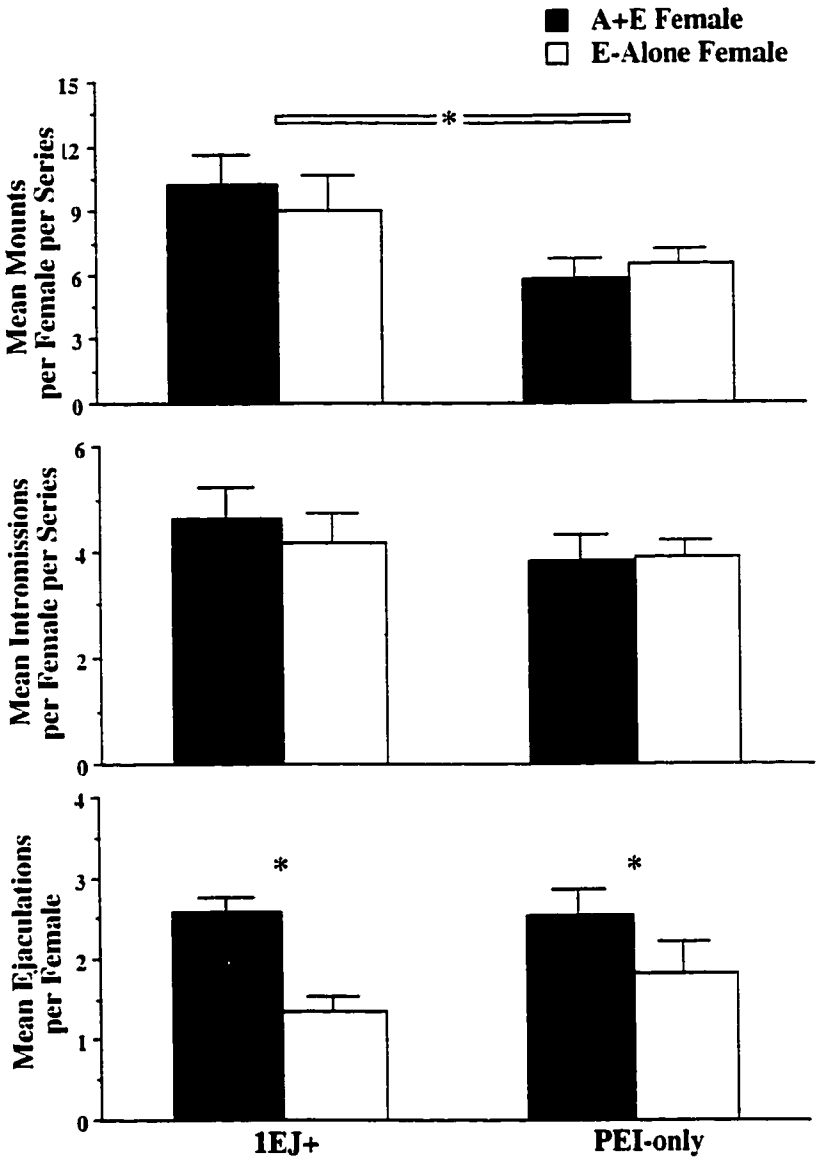

Figure 24. Distribution of mean (+SE) mounts per female per series (top panel), mean (+SE) intromissions per female per series (middle panel), and mean (+SE) ejaculations per female (bottom panel) during the Copulatory Preference Test in Experiment 9. * denotes $p < 0.05$ for between female types comparison.

(results of 3 X 2 mixed ANOVA: for Group: F (1, 36) = 2.75, p > 0.05; for Female Type: F (1, 36) = 0.01, p > 0.05; for Group x Female Type: F (1, 36) = 0.10, p >0.05). However, males in the both groups ejaculated more frequently with the A+E female than the E-Alone female. Planned orthogonal contrasts confirmed the significance of these observations: 1EJ+ group, t (36) = 2.28, p < 0.05; PEI-only group, t (36) = 2.00, p < 0.05. Effect size estimates for the differences in distribution of ejaculations for the 1EJ+ group and the PEI-only group are 1.92 and 0.69, respectively.

Discussion

The results of Experiment 9 confirm the findings of the first two experiments that one ejaculation per training session followed by exposure to the scented female is sufficient for the development of a significant CEP. This finding supports the notion that the critical component of the UCS for CEP is the presence of scented female during the PEI, and does not require continuous copulation with a scented female.

Interestingly, the CEP displayed by the PEI-only males was weaker than that displayed by the 1EJ+ males. This pattern of results is consistent with the interpretation that events during the PEI constitute the critical UCS. The difference in magnitude of the CEP between the two groups is also in agreement with the general principles of Pavlovian conditioning which is typically stronger when the CS (i.e. almond scented female) is present prior to the UCS (i.e. postejaculatory events) than when the CS is present at the same time as the UCS (Rescorla, 1988). This pattern of results may also be interpreted as suggesting that the CEP produced by pairing an odor with the UCSs present during the

postejaculatory period is augmented by pairing the odor with other UCSs present during copulation. However, the finding in Experiment 7, that removal of the scented female immediately following ejaculation does not produce CEP demonstrates that pairing the CS with copulatory UCSs is, by itself, insufficient for the development of CEP. Further, it is unlikely that the disturbance of removing the female blocks CEP development because both groups in the present experiment experienced the removal of the female (and subsequent return) but still displayed CEP.

General Discussion

The results of the present study reveal that the development of CEP is dependent upon ejaculation followed by the presence of a scented female. CEPs can be displayed when the male has access to a scented female throughout the PEI or when he is exposed without access to a scented female for an extended interval following ejaculation (presumably throughout the PEI). Perhaps the most remarkable finding is that males do not actually have to copulate with a scented female in order to subsequently display CEP; the female must only be scented following ejaculation. Together these findings implicate processes during the postejaculatory interval as the critical components of sexual behavior which constitute the UCS that supports the development of CEP.

The findings of the present study that the UCS for CEP and the UCS for conditioned sexual excitement are different highlights the fact that not all conditioning effects on sexual behavior involve the same UCS. Conditioned sexual excitement (Van

Furth & Van Ree, 1996b), conditioned sexual arousal (McConaghy, 1967; Rachman, 1966; Rachman & Hodgson, 1968; Zamble, Hadad, & Mitchell, 1985; Zamble, Hadad, Mitchell, & Cutmore, 1986), and instrumental behavior (Kagen, 1955; Sheffield et al., 1951; Whalen, 1961), all appear to be supported by UCSs which do not involve ejaculation. Conversely, CEP and conditioned place preferences (Agmo & Benefeld, 1990; Parades & Alonso, 1997) both require ejaculation. Further, they both are supported by pairing stimuli with the postejaculatory period. These findings suggest that highly complex interactions occur between various aspects of the neural stimulation produced by components of sexual behavior (i.e. UCSs) and environmental stimuli (i.e. CSs) which result in a multifaceted impact of learning on subsequent sexual behaviors.

The PEI is defined as the period from an ejaculation to the next mount with intromission (Sachs & Barfield, 1976; Meisel & Sachs, 1994). Accordingly, there are at least two sequential processes that occur during the PEI. Immediately following ejaculation there is an inhibitory phase, often referred to as a refractory period, which is accompanied by a characteristic vocalization produced by the male (Brown, 1979). The inhibition diminishes progressively until the male can be rearoused and copulates. From the present studies, it is unclear whether the inhibitory processes, the rearousal processes, or both, constitute the UCS for CEP. Given that the UCS for CEP are events following ejaculation, it would be useful to identify what these events are. However, it is inherently difficult to isolate the underlying neurobiological events of individual components of copulation. For example, the details regarding the processes of genital

stimulation that trigger ejaculation and that produce and remove the transient inhibition of copulatory behavior following ejaculation are not completely understood at the neural level.

Several studies aimed at correlating neural activation with sexual behavior have elucidated certain neural events related to ejaculation. This has been done by measuring electrophysiological activity and using induction of the immediate-early gene, *c-fos*, as a marker of neuronal activation. In the hippocampus, electrophysiological recordings have revealed that theta rhythm predominates prior to intromissions followed by transient desynchronized, large-amplitude irregular activity. This irregular activity is markedly prolonged following ejaculation (Kurtz & Adler, 1973). Similarly, activity in the mPOA increases prior to intromission, and is followed by a sharp drop in firing rate. This drop is firing rate is also prolonged following ejaculation (Horio, Shimura, Hanada, & Shimokochi, 1986). Fos protein appears to be induced selectively by ejaculation in a number of brain regions in several species (for a review see Pfaus & Heeb, 1997). Ejaculation-related Fos has been reported in the posterodorsal preoptic nucleus (Baum, & Everitt, 1992; Coolen, Peters, & Veening, 1996; Heeb & Yahr, 1996), caudal portion of the medial bed nucleus of the stria terminalis in rats (Coolen, Peters, & Veening, 1996), but not in gerbils (Heeb & Yahr, 1996), posterodorsal portion of the medial amygdala (rat: Baum, & Everitt, 1992; Coolen, Peters, & Veening, 1996; Wersinger, Baum, & Erskine, 1993; hamsters: Fernandez-Fewell, & Meredith, 1994; Kollack & Newman, 1992; Wood & Newman, 1992; gerbils: Heeb & Yahr, 1996), and lateral tegmentum (rats: Baum, &

Everitt, 1992; Wersinger, Baum, & Erskine, 1993; gerbils: Heeb & Yahr, 1996). There are also greater amounts of Fos found in the parvocellular regions of the hypothalamic paraventricular nuclei in rats allowed to ejaculate than in those not allowed to ejaculate (Witt & Insel, 1994). These findings are highly consistent across species despite differences in the behaviors that induce them, suggesting that the individual sensory afferents or their convergence may be critical components for brain activation by sexual stimulation (Pfaus & Heeb, 1997). However, it must be noted that ejaculation versus intromission comparisons involve one group receiving more stimulation (e.g. more intromissions, longer exposure) than the other. Subtractive differences between groups may be qualitative, quantitative, or both in these studies. Accordingly, interpreting the relevance of such effects to the neural basis of the UCS for CEP must be viewed cautiously.

Other studies have examined the correlation between sexual behavior and neurochemistry. Microdialysis has shown that levels of dopamine (DA) and its metabolites rise with the initiation of mating in the nucleus accumbens (Damsma, Pfaus, Wenkstern, Phillips, & Fibiger, 1992; Fiorino, Coury, & Phillips, 1997; Pfaus, Dasma, Nomikos, Wenkstern, Blaha, Phillips, & Fibiger, 1990; Pleim, Matochik, Barfield, R & Auerback, 1990) and the medial preoptic area (Hull, Du, Lorrain, & Matuszewich, 1995; Sato, Wada, Horita, Suzuki, Shibuya, Adachi, Kato, Tsukamoto, & Kumamoto, 1995). These levels appear to remain elevated throughout copulation. In studies employing voltammetry, DA oxidation signals in the nucleus accumbens and medial preoptic area,

levels rose and fell with the onset and termination of each ejaculatory series (Blackburn, Pfaus, & Phillips, 1992). This drop in DA during the PEI was confirmed subsequently with microdialysis (Nakamura, Yells, Jacques, & Hendricks, 1994). Similarly, lutenizing hormone secretion increases during initiation of copulation (Bronson, & Desjardins, 1982; Kamel, Mock, Wright, & Frankel, 1975; Oaknin, Rodriguez del Castillo, Guerra, Battaner, & Mas, 1989). Conversely, brain levels of serotonin (5-HT) in the lateral hypothalamic area (Lorrain, Matuszewich, Freidman, & Hull, 1997), and 5-HT metabolite levels in brain homogenates (Mas, Rodriguez del Castillo, Guerra, Davidson, & Battaner, 1987), are elevated only following ejaculation. Similarly, ejaculation is associated with cerebrospinal fluid increases in oxytocin (Hughes, Everitt, Lightman, & Todd, 1987), prolactin (Bronson, & Desjardins, 1982; Oaknin, Rodriguez del Castillo, Guerra, Battaner, & Mas, 1989), and gamma-amino butyric acid (GABA) (Qureshi & Sodersten, 1986). Further, endogenous opioids have been implicated in ejaculatory mechanisms because opioid antagonists disrupt ejaculation-induced analgesia (Szechtman, Hershokowitz, & Simatov, 1981), antianxiety (Fernandez-Guasti, Roldan-Roldan, & Saldivar, 1989; Saldivar-Gonzalez & Fernandez-Guasti, 1994), and conditioned place preferences (Agmo & Benefeld, 1990). Although oxytocin likely plays a role in triggering ejaculation, opioids, 5-HT, prolactin, and GABA, which are inhibitory to copulation, may be involved specifically in the

inhibitory phase of the PEI. Determining the exact neurochemistry and neuroanatomy of CEP will require further investigation.

In summary, the present experiments demonstrate that the UCS for CEP are postejaculatory events that do not require copulation with a scented female per se. This stands in sharp contrast to the development of copulation or conditioned sexual excitement which requires intromissions but not ejaculation as the necessary UCS. Understanding the specific components of sexual behavior that comprise UCSs for conditioned behaviors will guide subsequent investigations into the neural substrates of conditioned influences on sexual behavior.

CHAPTER 4: THE NATURE OF THE CONDITIONED RESPONSE.

The present study investigates the nature of the conditioned response (CR) that underlies CEP. Given equal distribution of mounts and intromission and the unequal distribution of ejaculation, there are at least two ways in which CEP could be mediated. First, males trained with scented females may have a lower threshold to ejaculate with a scented female than an unscented one. During the mate choice test, the male would be expected to copulate indiscriminately with both females but ejaculation would be facilitated when mounting the scented female perhaps due to higher sexual arousal or excitement (i.e. the CR is autonomic in nature). Alternatively, the male may select one female preferentially over the other for ejaculation but not for mounts or intromissions. Thus, during the mate choice test, the male would be expected to copulate indiscriminately with both females until he is about to ejaculate, then select the scented female for to receive his ejaculation.

The present study examined the nature of the CR and the final experiment examined the alternative explanations for the mechanisms of CEP. In Experiment 10, the response to the conditioned odor in the absence of a conspecific was assessed. Experiment 11 examined the effect of omitting the olfactory CS on copulatory behavior. Experiment 12 examined the response of males to sexually nonreceptive females bearing the olfactory CS. Finally, I re-examined data from past studies in order to determine whether mounts are preferentially distributed during different phases of an ejaculatory series.

Experiment 10

The purpose of Experiment 10 was to assess how males trained with almond-scented sexually-receptive females, a procedure previously shown to produce CEP, respond to almond odor alone. Males were allowed to copulate with either scented or unscented females for a duration known to produce reliable CEPs. Next, males were placed in an empty chamber that had either almond odor or no odor in the bedding material and their responses were recorded. This experiment thus determined the form of the CR without the presence of competing unconditioned copulatory stimulus (UCS).

Methods

Subjects

Males. The 26 Long-Evans rats that served as subjects in Experiment 10 were obtained from Charles River Canada, (St. Constant, Québec). The males weighed approximately 300-350 g and were sexually naive at the start of the experiment. They were housed in pairs in Plexiglas cages (36 cm x 26 cm x 19 cm) with ad lib access to food (Purina Rat Chow) and water. All rats were kept in a 12:12 hour reversed light-dark cycle colony room maintained at 21°C.

Females. Female Long-Evans rats from the same supplier as above were ovariectomized via bilateral lumbar incisions under ketamine/xylazine anaesthesia at least two months prior to the start of the experiment and were sexually experienced. Sexual receptivity was induced by subcutaneous administration of estradiol (10 μg) 48 hr prior and progesterone (500 μg) 4-6 hr prior to each test trial. Females were housed under the

same conditions as males. Stimulus females were selected at random for use during each session. Female rats were scented with approximately 1 ml of either almond extract (Blue Ribbon, Etobicoke, Ontario, Canada) or distilled water applied to both the back of the neck and the anogenital area using a cotton swab.

Apparatus

Conditioning sessions took place in unilevel pacing chambers constructed with standard laboratory Plexiglas cages (36 cm x 26 cm x 19 cm) with a Plexiglas insert. The insert was made by attaching a Plexiglas divider-piece (30 cm x 20 cm x 0.5 cm) length wise to the center of a Plexiglas base (35 cm x 18 cm x 0.5 cm). The insert was then placed into the chamber, the base was covered with bedding material, and a piece of wire mesh (0.25 cm grid, 35 cm x 18 cm) with a groove cut into the center was placed over the divider insert. A cover constructed of wire mesh (0.5 cm grid, 36 cm x 20 cm) was placed over the chamber. All conditioning sessions were recorded on video and scored subsequently using a PC-based program (Cabilio, 1996). Olfactory stimulus tests were carried out in the same Plexiglas cages lined with bedding material without the divider-insert.

Procedure

Conditioning Phase. Conditioning sessions were conducted in unilevel pacing chambers in the same manner as in previous experiments. Male rats were preexposed to the chambers once a day for 15 min each day in order to habituate them to the training environment. This habituation procedure lasted 7 days and has been shown previously to

increase the proportion of males that become vigorous copulators (Pfaus & Wilkins, 1995). Then males received 9 conditioning sessions at four day intervals during the middle third of the dark phase of the light:dark cycle. In each conditioning session, males were placed in the chamber for 5 min after which a sexually-receptive female was placed into the chamber and the animals were allowed to copulate for 30 min. For A+E-Trained males (n = 13), access was always to estrous females that had almond extract applied to the neck and anogenital regions (A+E female) and for E-Trained males (n = 13), access was always to estrous females that had distilled water applied to these same regions (E-Alone female). The chambers were cleaned prior to each trial in order to reduce extraneous olfactory stimuli. Latency and frequency data for all mounts, intromissions, and ejaculations were recorded during each conditioning session. Criteria for sexual behaviors were those defined by Sachs and Barfield (1976) and Meisel and Sachs (1994).

Olfactory Stimulus Test. Four days after the final conditioning session, each male was placed in the test chamber which had either 5 ml of either almond extract (A+E-Trained, n = 7; E-Trained, n= 7) or 5 ml of distilled water (A+E-Trained, n = 6; E-Trained, n = 6) mixed into the bedding. Each tests lasted 40 min. The frequency of the following behaviors was recorded: (1) side changes (locomotion); (2) rearing; (3) digging/rooting; (4) gnawing on bedding; (5) body/head grooming; and (6) genital grooming. Behaviors were scored for frequency of bouts not duration of bouts.

Statistical Analyses

Mixed-design between-within ANOVAs were used to analyze all data from the conditioning sessions with significant values being followed by post hoc analysis of individual means using the Tukey method. Two factor between subjects ANOVAs were used to analyze the frequency data from the olfactory stimulus tests with significant values being followed by post hoc analysis of individual means using the Tukey method. Chi square analyses were used where appropriate for analysis of proportions of males displaying a behavioral sequence. The level of significance for all comparisons was 0.05.

Results

Conditioning Phase. Of the 26 males used in Experiment 10, 4 males failed to ejaculate during the first conditioning session; 2 in the A+E-Trained group and 2 in the E-Trained group. Males in all groups displayed similar copulatory parameters during training: Latency to mount, latency to intromit, inter-intromission intervals, ejaculation latency, and post-ejaculatory interval decreased to assymtopic levels that did not differ between groups. The intromission ratio (number of successful intromissions / number of mounts with or without intromissions) and ejaculation frequency increased to asymtopic levels that did not differ between groups (data not shown).

Olfactory Stimulus Test. The mean frequency of each behavioral bout for each training condition under each testing condition is displayed in Table 1.

All subjects engaged in digging and rooting, but males exposed to almond odor displayed more bouts of digging and rooting than the males exposed to distilled water and

Table 1. **Response to Almond odor or no odor in bedding material.**
Mean ± S.E. Numbers in parentheses represent subjects displaying the behavior.

	A+E-/Alm. Odor	A+E-/No Odor	E-/Alm. Odor	E-/No Odor
digging/rooting	39.3 + 4.3 *** (7/7)	15.7 + 2.9 (6/6)	27.2 + 3.5 * (7/7)	25.2 + 1.7 (6/6)
gnawing	3.14 + 1.16 *** *** (5/7)	0.16 + 0.41 (1/6)	0.14 + 0.38 (1/7)	0.00 + 0.00 (0/6)
body/head grooming	11.4 + 1.4 (7/7)	8.5 + 1.1 (6/6)	8.6 + 1.6 (7/7)	5.7 + 1.4 (6/6)
genital grooming	2.14 + 0.70 *** ** (5/7)	0.33 + 0.21 (2/6)	0.14 + 0.14 (1/7)	0.17 + 0.41 (1/6)
side changes	46.8 + 4.2 (7/7)	38.5 + 4.8 (6/6)	54.7 + 9.7 (7/7)	39.5 + 5.5 (6/6)
rearing	77.6 + 8.3 (7/7)	69.3 + 7.4 (6/6)	80.9 + 11.2 (7/7)	79.2 + 7.8 (6/6)

*** A+E-trained / Almond odor versus all other groups, $p < 0.05$
** A+E-trained / Almond odor versus E-Trained / Amond odor and E-Trained / No odor, $p < 0.05$
* E-Trained / Almond odor versus A+E-Trained / No odor, $p < 0.05$

the A+E-Trained males displaying more bouts than the E-Trained males when exposed to almond odor. The statistical significance of these findings were confirmed with ANOVAs. Although there was no significant effect of training [$F(1, 22) = 0.02$, $p > 0.05$], there was a significant effect of test odor [$F(1, 22) = 11.8$, $p < 0.05$], there was also a significant interaction between training and test odor [$F(1, 22) = 7.97$, $p < 0.05$; A+E-Trained males exposed to almond odor displayed more digging and rooting than all other groups and E-Trained males exposed to almond odor displayed more digging and rooting than the A+E-Trained exposed to no odor]. Further, several A+E-Trained males engaged in digging that was much more intense than that observed in the other groups. The intensity of which was similar to that observed in defensive burying (Pinel & Treit, 1978) with bedding material being air-borne at times. However, in this case there was no object to bury and the digging was not directed in any particular direction as the rat moved about the cage during the digging bout.

Of the 26 subjects, only 7 gnawed on bedding during the test, most of which were in the A+E-Trained and almond exposed condition: 5 A+E-Trained males exposed to almond odor; 1 A+E-Trained male exposed to distilled water; and 1 E-Trained males exposed to almond odor. Chi square analyses confirmed the statistical significance of these observations: A+E-Trained exposed to almond versus A+E-Trained exposed to distilled water $\chi 2$ ($n = 13$) $= 3.91$, $p < 0.05$; A+E-Trained exposed to almond versus E-Trained exposed to almond $\chi 2$ ($n = 14$) $= 4.67$, $p < 0.05$. A+E-Trained males exposed to almond also displayed more frequent gnawing than the other groups. The statistical

significance of these findings were confirmed with ANOVAs. There was a significant effect of training [$F(1, 22) = 5.54$, $p < 0.05$ with A+E-Trained gnawing more frequently]. There was a significant effect of test condition [$F(1, 22) = 11.8$, $p < 0.05$ with almond exposed males eating more frequently]. Finally, there was a significant interaction [$F(1, 22) = 7.97$, $p < 0.05$; A+E-Trained males exposed to almond odor gnawed more than all other groups and no other groups differed significantly].

All males engaged in head and body grooming with A+E-Trained males exposed to almond displaying the most bouts and E-Trained males exposed to distilled water displaying the fewest. There was a significant effect of training [$F(1, 22) = 4.83$, $p < 0.05$ with A+E-Trained grooming more frequently]. There was a significant effect of test condition [$F(1, 22) = 4.90$, $p < 0.05$ with almond exposed males grooming more frequently]. No significant interaction was detected [$F(1, 22) < 1.0$, $p < 0.05$].

Of the 26 subjects, only 9 engaged in genital grooming during the test, most of which were in the A+E-Trained exposed to almond odor condition: 5 A+E-Trained males exposed to almond odor, 2 A+E-Trained male exposed to distilled water, 1 E-Trained males exposed to almond odor, and 1 E-Trained male exposed to distilled water. Chi square analyses confirmed the statistical significance of these observations: A+E-Trained exposed to almond versus A+E-Trained exposed to distilled water $\chi 2$ ($n = 13$) $= 1.89$, $p > 0.05$; A+E-Trained exposed to almond versus E-Trained exposed to almond $\chi 2$ ($n = 14$) $= 4.77$, $p < 0.05$. A+E-Trained males exposed to almond also displayed more frequent genital grooming than the other groups. The statistical significance of these

findings were confirmed with Anovas. There was a significant effect of training [$F(1, 22) = 6.98$, $p < 0.05$ with A+E-Trained males genital grooming more frequently]. There was a significant effect of test condition [$F(1, 22) = 4.74$, $p < 0.05$ with almond exposed males genital grooming more frequently]. And there was a significant interaction [$F(1, 22) = 5.00$, $p < 0.05$; A+E-Trained males exposed to almond odor differed from all other groups and no other groups differed significantly]. Additionally, two A+E-Trained males exposed to almond odor were observed to have erections accompanying genital grooming, however, it is unclear if other males had erections as the chambers used for the odor exposure tests did not permit ventral viewing which is necessary for accurate detection of erections in rats (see Sachs et al., 1994).

Discussion

The results of the present study demonstrate that pairing a neutral olfactory stimulus with copulation results in the elicitation of a CR when the CS is presented in the absence of a conspecific. Males that had received conditioning sessions with almond scented females displayed increased amounts of digging and rooting, gnawing, and genital grooming when exposed to almond odor alone than males in the other training and testing conditions. These findings also demonstrate that the CR is a complex one involving both self-directed behaviors (genital grooming) and CS-directed behaviors (gnawing, digging, and rooting).

The finding that exposure to the CS elicits genital grooming is particularly interesting in light of recent work by Sachs and colleagues showing that genital grooming

is usually associated with erections. In their studies, they have found that noncontact exposure to an estrous female (Sachs, 1996; Sachs, Akasofu, Citron, Daniels, & Natoli,, 1994) or to her air-borne odors (Sachs, 1997) will induce both erection and genital grooming. The present finding that an initially neutral odor paired with copulation can subsequently elicit genital grooming suggests that sexual arousal and erections can be produced by exposure to the CS. Conditioned sexual arousal has been reported for visual stimuli as measured by erection volume and frequency in humans (Barr & McConaghy, 1971; Kantorowitz, 1978; McConaghy, 1974; Rachman, 1966; Rachman & Hodgson, 1968) and as measured by intromission latency in rats (Zamble, Hadad, Mitchell, & Cutmore, 1985; Zamble, Mitchell, & Findlay, 1986). Thus, the present result provides indirect evidence to extend conditioned sexual arousal to an olfactory CS paired with copulation.

The present indirect demonstration of conditioned sexual arousal elicited by the olfactory CS may provide a clue to the mechanism underlying CEP. Stimuli that increase sexual arousal (typically measured by a decrease in intromission latency during copulation) also decrease ejaculatory threshold (as measured by ejaculation latency). For instance, many pharmacologic manipulations that influence intromission latency have similar effects on ejaculation latency (for reviews see Meisel & Sachs, 1994; Pfaus & Everitt, 1995). Similarly, exposure to stimuli paired previously with noncontact exposure to estrous females also reduces both intromission and ejaculation latencies (Zamble et al., 1985; Zamble et al., 1986). If the olfactory CS used in the present CEP experiments is

capable of increasing sexual arousal then it may also be capable of facilitating ejaculation and thus CEP may increase the likelihood of ejaculation with an almond-scented female relative to an unscented one during indiscriminate mating in the copulatory preference test. This hypothesis is explored further in Experiment 11.

Whereas the increased genital grooming is likely to reflect an autonomic component of the CR, the increased digging, rooting, and gnawing of bedding are mediated by the skeletal systems and are directed at the CS. This finding is interesting for two reasons. First, the expression of a non-sexual CR in response to stimuli paired previously with copulation demonstrates that a CR is influenced by not only the nature of the CS-UCS pairing during conditioning, but also the presentation of the CS once conditioning has developed.

The present finding that a CS paired with copulation elicits CS-directed responses may also provide insight to the mechanism of CEP. In my observations of CEP, males ejaculate more frequently with the CS-bearing females than a female without the CS. In light of a CS-directed CR, the bias to ejaculate with CS-bearing females may be produced by males voluntarily directing their ejaculation toward one female over another. If males display CS-directed copulatory responses in the absence of a UCS eliciting copulatory responses, then this would suggest that CEP is mediated by a voluntary CR. Experiment 12 further investigates this hypothesis by assessing responses of A+E-Trained males toward a nonreceptive female bearing the CS.

Experiment 11

The purpose of Experiment 11 was to assess the influence of the CS (almond odor) on copulatory behavior in males receiving copulatory training with scented females which has previously been shown to produce CEP. As in Experiment 10, males were allowed to copulate with either almond-scented or unscented females. Then, they received a copulatory odor-reversal test in which males trained with almond-scented females were allowed access to an unscented female and males trained with unscented females were allowed access to an almond-scented one. This procedure allowed us to assess the effect of omitting the olfactory CS in the A+E-Trained males and the effect of a novel odor in the E-Trained males on copulatory behavior. If CEP is mediated by facilitated ejaculation with the scented female during a copulatory preference test, then an increased ejaculatory threshold should be evident when the olfactory CS is absent during copulation.

Methods

Males and Females. The 24 male Long-Evans rats that served as subjects and the female Long-Evens rats that served as stimuli in Experiment 11 were from the same supplier and housed in the same manner as the subjects in Experiment 10.

Apparatus

Conditioning sessions and the copulatory odor-reversal test took place in bilevel chambers constructed of Plexiglas (outside dimensions of 18 cm x 25 cm x 65 cm) with a platform (40 cm in length) elevated by a set of ramps at each end dividing the chamber

into two levels (see Pfaus et al., 1990 for further details). The bilevel chambers were cleaned with water and Coverage 256 (Conva Tec, St. Louis, MO) and soiled bedding was replaced with clean bedding prior to each conditioning session and test. All copulatory sessions were recorded on video and scored subsequently using a PC-based program (Cabilio, 1996).

Procedure

Conditioning Phase. Conditioning sessions were conducted in the bilevel chambers in the same manner as in Experiment 10. Male rats were preexposed to the chambers once a day for 15 min each day. Then males received 9 conditioning sessions at four day intervals during the middle third of the dark phase of the light:dark cycle. In each conditioning session, males were placed in the chamber for 5 min, after which a sexually-receptive female was placed into the chamber and the animals were allowed to copulate for 30 min. For A+E-Trained males (n = 12), access was always to an A+E female and for E-Trained males (n = 12), access was always to an E-Alone female. The chambers were cleaned with a water and coverage mixture prior to each trial and copulatory data were collected as described in Experiment 10. The final conditioning session served as a baseline for comparison to the copulatory odor-reversal test.

Copulatory Odor-Reversal Test. Four days after the final conditioning session, each male was placed in a bilevel chamber for 5 min. Then, as in conditioning sessions, a sexually-receptive female was placed in the chamber and the pair was allowed to interact for 30 min. However, during the copulatory odor-reversal test, males previously trained

with A+E females now received access to an E-Alone female and males previously trained with E-Alone females now received access to an A+E female. The chambers were cleaned prior to each test and copulatory data were collected as described above.

Statistical Analyses

Mixed-design between-within ANOVAs were used to analyze all data from the conditioning sessions. Mixed-design between-within ANOVAs were used to analyze the raw data from the final conditioning session and the copulatory odor-reversal test. Significant F values were followed by post hoc analysis of individual means using the Tukey method. Additionally, the difference scores between the final conditioning session and the copulatory odor-reversal test were computed and analyzed using the Walsh statistic for nonparametric data (Siegel, 1956). The level of significance for all comparisons was 0.05.

Results

Conditioning Phase. Of the 24 males used in Experiment 11, 3 males failed to ejaculate during the first conditioning session; 2 in the A+E-Trained group and 1 in the E-Trained group. Males in all groups displayed similar copulatory parameters during training; latency to mount, latency to intromit, inter-intromission intervals, ejaculation latency, and post-ejaculatory interval decreased to assymtopic levels that did not differ between groups, and intromission ratio (number of intromissions / number of mounts with or without intromission) and ejaculation frequency increased to assymtopic levels that did not differ between groups (data not shown). There were no significant

differences on of any these measures between the final conditioning session and the three conditioning sessions that preceded; thus, the final conditioning session can serve as a stable baseline.

Copulatory Odor-Reversal Test. All males included in this experiment copulated and ejaculated at least 3 times on both the final conditioning session and the copulatory odor-reversal test. The copulatory parameters for the final conditioning session, for the copulatory odor-reversal test, and for the mean differences between tests are presented in Table 2. There were no significant differences between groups, female type, or significant interactions between group and female type for most measures. All ANOVAs and Walsh tests for ejaculation latency, mount frequency, intromission frequency, inter-intromission interval, ejaculation frequency, and level changes per mount (pursuit) failed to reach statistical significance (all p's > 0.05).

In contrast, there were significant effects for mount latency, intromission latency, and intromission ratio. Mount latency was significantly increased for A+E-Trained males when they copulated with unscented females and for E-Alone-Trained males when they copulated with scented females. The statistical significance of the former observation was confirmed by ANOVAs [group effect: $F(1, 22) = 0.27$, $p > 0.05$; female type: [$F(1, 22) = 0.023$, $p > 0.05$; interaction between group and female type: $F(1, 22) = 4.39$, $p < 0.05$; post-hoc comparisons revealed A+E-Trained males with A+E females had lower intromission latencies than A+E-Trained males with E-Alone females]. Further, Walsh statistical analysis revealed that the distribution of mount latency difference scores was

Table 2. Influence of omitting the CS or addition of a novel odor on copulation.
Mean ± SE. Numbers in parentheses are lowest andhighest socres.

	A+E-Trained Males			E-Trained Males		
	Baseline (A+E Female)	Reversal (E-Alone Female)	Mean Difference (Reversal - Baseline)	Baseline (E-alone Female)	Reversal (A+E Female)	Mean Difference (Reversal - Baseline)
Mount Latency (s)	7.14±1.4 (4) - (19)	7.85±0.74 (6) - (11)	3.5±3.4 (-12) - (23)	8.8±1.3 (4) - 11)	16.4±7.6 (5) - (52)	7.6±8.4 ** (-5) - (41)
Intromission Latency (s)	7.14±1.4 (5) - (19)	13.0±2.9 * (6) - (29)	8.49±3.51 ** (-12) - (25)	8.8±1.3 (4) - (11)	17.8±8.7 (5) - (52)	9.0±8.26 ** (-5) - (41)
Ejaculation Latency (s)	82.5±7.4 (51) - (136)	118.9±33.1 (57) - (442)	42.9 ± 31.3 (-62) - (306)	76.6 ± 19.3 (25) - (141)	127.4 ± 41.7 (26) - (245)	36.8 ± 24.4 (-27) - (147)
Mount Frequency	6.9 ± 0.7 (5) - (9)	8.7 ± 1.8 (4) - (18)	3.1 ± 2.2 (-3) - (5)	7.4 ± 1.4 (5) -(11)	6.8 ± 1.4 (5) - (11)	-0.8 ± 1.5 (-5) - (3)
Iintromission Frequency	5.6 ± 0.6 (3) - (12)	6.0 ± 1.1 (4) - (11)	1.1 ± 1.7 (-5) - (4)	7.4 ± 1.4 (5) - (11)	6.6 ± 1.2 (5) - (10)	-0.6 ± 1.6 (-5) - (4)
Ejaculation Frequency	3.9 ± 0.1 (3) - (4)	3.7 ± 0.2 (3) - (4)	-0.1 ± 0.1 (-1) - (1)	3.8 ± 0.2 (3) - (4)	3.9 ± 0.2 (3) - (4)	0.1 ± 0.3 (-1) - (1)
Post-Ejaculatory Interval	311.1 ± 25.0 (228) - (412)	330.6 ± 35.3 (259) - (537)	28.3 ± 20.8 (-64) - (125)	329.2 ± 47.6 (270) - (407)	341.8 ± 41.9 (294) - (447)	22.7 ± 17.8 (-56) - (113)
Intromission Ratio	0.81 ± 0.06 (.66) - (1.00)	0.70 ± 0.04 * (0.50) - (0.83)	-0.18 ± 0.07 ** (-0.25) - (0.14)	1.00 ± 0.00 (1.00) - (1.00)	0.98 ± 0.02 (0.90) - (1.00)	-0.02 ± 0.02 (-0.10) - (0.00)
Inter-Intromission Interval	15.8 ± 1.6 (12.8) - (22.7)	20.67 ± 5.9 (10.5) - (38.3)	7.2 ± 4.9 (-5.6) - (38.3)	9.84 ± 2.9 (6.5) - 18.4)	19.6 ± 7.7 (8.3) - (49.0)	9.7 ± 7.6 (-2.3) - (39.7)
Level Changes per Mount	1.06 ± 0.22 (0.40) - (2.00)	0.91 ± 0.18 (0.28) - (1.53)	0.23 ± 0.41 (-1.30) - (0.90)	0.89 ± 0.34 (0.33) - (1.8)	1.17 ± 0.32 (0.75) - (1.85)	-0.42 ± 0.50 (-0.65) - (1.17)

* A+E-Trained Males with A+E female versus A+E-Trained Males with E-alone female, $p < 0.05$.
** Mean difference scores, $p < 0.05$.

statistically significant for the A+E-Trained males {min[d5, 1/2(d1+d8) > 0, $p < 0.05$]} and for the E-Trained males {min[d5, 1/2(d1+d8) > 0, $p < 0.05$]}. Intromission latency was significantly increased for A+E-Trained males when they copulated with unscented females and for E-Alone-Trained males when they copulated with scented females. The statistical significance of the former observation was confirmed by ANOVAs [group effect: $F(1, 22) = 0.27$, $p > 0.05$; female type: $f(1, 22) = 0.023$, $p > 0.05$; interaction between group and female type: $F(1, 22) = 4.39$, $p < 0.05$; post-hoc comparisons revealed A+E-Trained males with A+E females had lower intromission latencies than A+ETrained males with E-Alone females]. Further, Walsh statistic analysis revealed that the distribution of intromission latency difference scores was statistically significant for the A+E-Trained males {min[d5, 1/2(d1+d8) > 0, $p < 0.05$]} and for the E-Trained males {min[d5, 1/2(d1+d8) > 0, $p < 0.05$]}. Intromission ratios were significantly lower in A+E-Trained males than E-Trained males, moreover, intromission ratios were significantly reduced for the A+E-Trained males when they copulated with unscented females. The statistical significance of this observations was confirmed by ANOVAs [group: $F(1, 22) = 27.01$, $p < 0.05$; female type: $F(1, 22) = 1.56$, $p > 0.05$; interaction between group and female type interaction: $F(1, 22) = 4.72$, $p < 0.05$; post-hoc comparisons revealed A+E-Trained males with A+E females displayed higher intromission ratios than A+E-Trained males with E-Alone females]. Similarly, Walsh statistical analysis revealed that the distribution of intromission ratio difference scores

was statistically significant for the A+E-Trained males {max[d8, 1/2(d5+d12) > 0, $p < 0.05$]}, but not the E-Trained males {max[d8, 1/2(d5+d12) < 0, $p > 0.05$]}.

Discussion

The results of Experiment 11 indicate that there are significant differences in copulatory parameters when an olfactory CS previously paired with copulation is omitted but not when a neutral odor is added. When A+E-Trained males were allowed access to E-Alone females they displayed significantly longer mount and intromission latencies and significantly lower intromission ratios. Similarly, when E-Trained males were allowed access to A+E females, they displayed significantly longer mount and intromission latencies, but there was no change in intromission ratio.

The present results are consistent with those of Experiment 10 suggesting that the olfactory CS used in CEP experiments increases sexual arousal. A+E-Trained males copulating with E-Alone females displayed longer intromission latencies and lower intromission ratios, both measures indicate that the absence of the olfactory CS resulted in delayed and lower incidence of penile erection. In Experiment 10, A+E-Trained males exposed to the olfactory CS in isolation exhibited substantially increased genital grooming which might be an indicator of penile erection. Thus, the presence or absence of the CS appears to influence sexual arousal as measured by genital grooming or intromission latency.

However, the present experiment failed to provide evidence that the olfactory CS used in CEP experiments facilitates ejaculation. Although the A+E-Trained males

displayed a trend for longer latencies to ejaculate with E-Alone females than A+E ones (with ejaculation latency in two rats being 2 and 5 min longer with E-Alone females than A+E ones), the data suggest that ejaculatory threshold is not generally altered by the omission of the CS. To achieve ejaculation, A+E-Trained males required a similar numbers of intromissions, similar amounts of time, and achieved similar numbers of ejaculation regardless of the female that they were copulating with. Accordingly, the present findings are not consistent with the hypothesis that CEP is mediated by facilitated ejaculation during completely indiscriminate mating in the copulatory preference test.

Experiment 12

The purpose of Experiment 12 was to assess the effect of an olfactory stimulus paired previously with copulation on behavior directed to towards a sexually-nonreceptive female bearing that odor. Males received either almond odor consistently paired with or randomly paired with sexually-receptive females. Further, all males were given experience with sexually-nonreceptive females, a procedure that reduces the amount of inappropriate sexual behavior directed toward nonreceptive females (Pfaus & Pinel, 1989) and does not interfere with the development of CEP (see Experiment 1). Then all males were given a receptivity-reversal test, in which they were allowed access to a sexually-nonreceptive female bearing almond odor.

Methods

Males and Females. The 36 male Long-Evans rats that served as subjects and the female Long-Evens rats that served as stimuli in Experiment 12 were from the same supplier and housed in the same manner as the subjects in previous experiments.

Apparatus

All conditioning sessions and the receptivity-reversal test took place in the same bilevel chambers and followed the same cleaning and video recording procedures as used in Experiment 11.

Procedure

Conditioning Phase. As in Experiments 10 and 11, male rats received 7 daily-15 min preexposure sessions to the bilevel chambers. Then all males received a total of 18 conditioning sessions at two day intervals. Access to receptive and nonreceptive females occurred on alternating trials; the first trial was counterbalanced with respect to female status for each group. For males in the A+E-Trained group, all sessions with sexually-receptive females were with A+E females and all sessions with sexually-nonreceptive females were with unscented (N-Alone) females. For the males in the Control group, half the sessions with sexually-receptive females were with A+E females and half with E-Alone females and half the sessions with sexually-nonreceptive females were with almond-scented (A+N) females and half with N-Alone females--the scent of the females on these sessions was counterbalanced and followed a pseudo-random schedule determined prior to the start of the experiment. For all conditioning sessions, males were

placed individually into a bilevel chamber for 5 min, after which a female of the appropriate sexual status and appropriate scent was placed into the chamber for 30 min. The bilevel chambers were cleaned prior to each session and copulatory data were collected as described in previous experiments.

Receptivity-Reversal Test. The receptivity-reversal tests took place 4 days following the final conditioning session and were conducted in a similar manner. Each male was placed in a bilevel chamber for 5 min, after which an A+N female was placed in the chamber and the pair was allowed to interact for 30 min. In addition to the sexual behaviors recorded in the previous experiment, the number of contacts between the male's forepaw and the female's rear was also recorded because this behavior appeared to be an attempt by the male to mount the female who would prevent an actual mount by moving away or engaging in defensive behavior.

Statistical Analysis

The proportions of males in each group mounting the nonreceptive females were analyzed using Chi square tests where appropriate. The mean mounts and contacts were compared between groups using independent sample t-tests. The level of significance for all comparisons was 0.05.

Results

Conditioning Phase. No substantial between group differences were detected during the conditioning sessions. The proportion of males that ejaculated with receptive females increased during the conditioning phase from 67% on the first session to 95% on

the final session; there were no between-group differences. The proportion of males that mounted nonreceptive females decreased during the conditioning phase from 35% on the first session to 6% on the final session; again there were no between-group differences.

Receptivity-Reversal Test. The proportion of males that mounted and the mean ($\pm$ S.E.M.) mounts, contacts, and level changes for each group of males is displayed in Figure 25. All males level changed in the bilevel chambers and made forepaw to rear contacts with the A+N female at least once. Additionally, 67% of A+E-Trained males and only 11% of Control males actually mounted the A+N females; the statistical significance of this latter finding was confirmed with chi square analysis ($\chi 2 = 11.69$, $p < 0.05$). The two groups did not differ in mean number of level changes ($p > 0.05$). However, A+E-Trained males made significantly more contacts with the A+N female than did the males in the control group [$t(34) = 3.05$, $p < 0.05$]. And A+E-Trained males mounted the A+N female significantly more than males in the control group [$t(34) = 4.14$, $p < 0.05$].

Discussion

The results of the present experiment demonstrate that pairing a neutral odor with copulation enables that odor to elicit CS-directed copulatory behavior in the absence of a copulation-eliciting UCS. Consistent with the findings of Pfaus and Pinel (1989), males that receive repeated exposure to receptive and nonreceptive females learned to inhibit their sexual behavior toward nonreceptive females, but not receptive females. Moreover, A+E-Trained males that had learned to inhibit their copulatory behavior toward

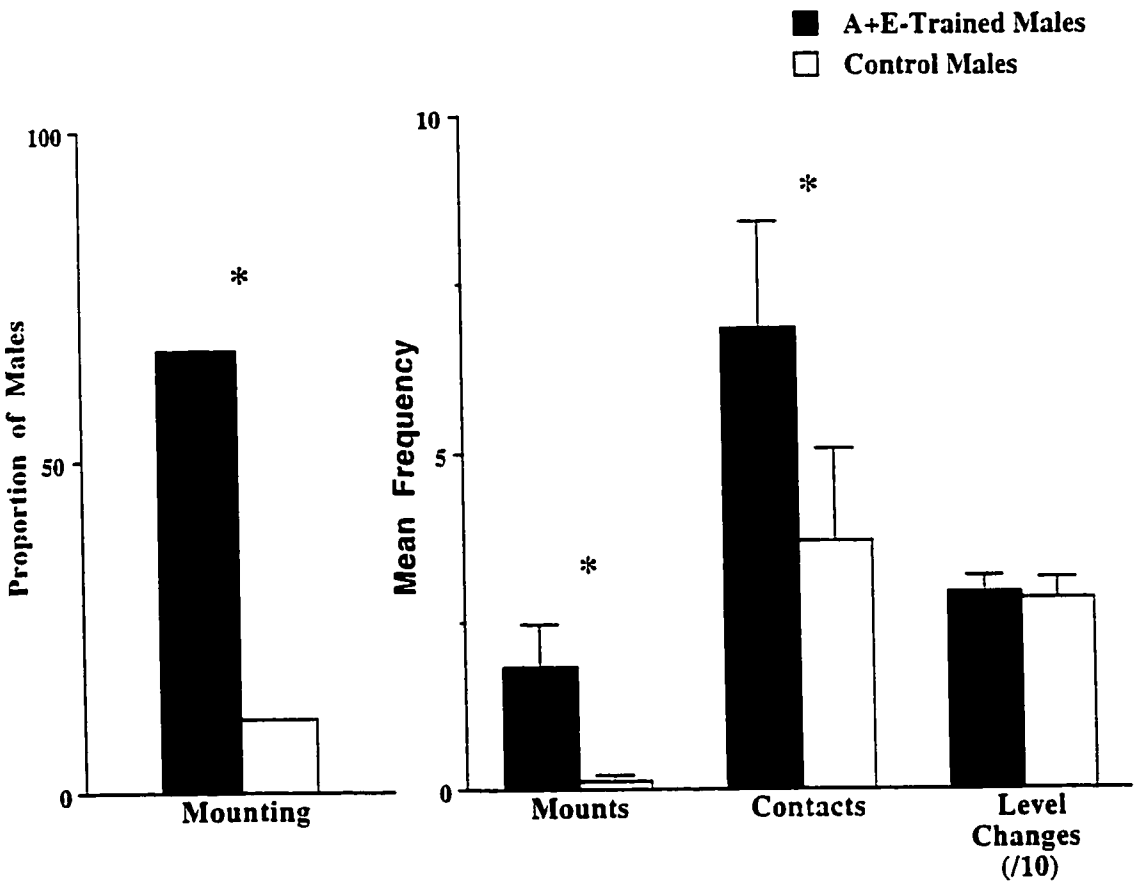

Figure 25. Proportion of males that mounted the nonreceptive female (left panel) and the mean (+SE) mounts, mean (+SE) contacts, and mean (+SE) level changes (right panel) during the Receptivity-Reversal Test in Experiment 12. * denotes $p < 0.05$ for between groups comparison.

unscented nonreceptive females displayed more contacts toward and more mounts with A+N females; males receiving equal experience with the almond odor paired randomly with receptive and nonreceptive females displayed significantly fewer contacts and mounts.

The present finding that olfactory sexual conditioning produces CS-directed copulatory behavior is important for two reasons. First, it extends the findings of CS-directed behavior of Experiment 10. In Experiment 10, CS-directed behavior was expressed as digging and gnawing of bedding that was scented with the olfactory CS. In the present experiment, the same CS-UCS pairing produce CS-directed behavior that is sexual in nature. Together these findings emphasize that the presentation of the CS both during conditioning and during elicitation of a CR determines the nature of the CR.

Moreover, the finding that the same conditioning procedure that produces a CEP also produces CS-directed copulatory behaviors in the absence of a sexual UCS provides insight into the underlying mechanism of CEP. On one hand, the present finding is consistent with the hypothesis that CEP results from selective mating on the part of the conditioned male, in which the male selects the CS-bearing female to ejaculate with. However, this can not explain why no preference is seen in the distribution of mounts and intromissions. One way to reconcile these two findings is the interpretation that at the point of ejaculation or immediately prior to it, males may become discriminating and make selections between potential mates. Experiment 13 further investigates this hypothesis

by reexamining data from previous experiments in Chapters 1, 2, and 3 for the distribution of mounts at different time points during ejaculatory series.

Experiment 13

In previous chapters, I have consistently found that if males are allowed to copulate with a female bearing a neutral odor and are subsequently allowed to copulate with two females, one bearing the odor and one not, then they will ejaculate with the scented female more frequently despite mounting and intromitting equally with both females. In light of the results of the proceeding experiments in this study, that the same training procedures produce CS-directed copulatory behavior but not CS facilitated ejaculation, a reanalysis of the distribution of copulatory behavior during ejaculatory series seems appropriate. Accordingly, I analyzed the distribution of the first 3, first 5, last 3, and last 5 mounts of the first and all ejaculatory series for groups of males that displayed CEP in previous studies.

Methods

Subjects and Procedures

The subjects and procedures used to generate the data for the present analysis have been described in detail in previous chapters. In all of these studies, males were trained with sexually receptive female bearing a neutral odor (either almond or lemon), however the details of the training varied from experiment to experiment. Briefly, data from a total of 186 males was included in the analysis. Males were all in groups that

displayed a CEP during a copulatory preference test with one scented and one unscented female. Forty-eight males received nine 30-min sessions with scented females and no other training (Experiment 2, almond: n = 15; Experiment 4, almond n = 12; Experiment 3, lemon n = 21). Twelve males received five 30-min sessions with almond-scented females and no other training (Experiment 4). Thirty-three males received a single session with almond-scented females which lasted either 3 hr (n = 11), 2 hr (n = 13), or 4 ejaculatory series (n = 9) and no other training (Experiment 5). Fifteen males received nine 30-min sessions with almond-scented female with concurrent training with unscented nonreceptive females (Experiment 1). Seventy-eight males received 9 sessions with almond-scented females terminating following the postejaculatory interval (Experiments 7, 8 and 9).

The data from the 186 rats were analyzed for the distribution of mounts at different points during an ejaculatory series. The mean number of mounts directed at the scented female was calculated for: (1) the first 3 mounts of the first series; (2) the first 5 mounts of the first series; (3) the last 3 mounts of the first series; (4) the last 5 mounts of the first series; (5) the mean first 3 mounts of all series; (6) the mean first 5 mounts of all series; (7) the mean last 3 mounts of all series; (8) the mean last 5 mounts of all series. All males mounted at least 5 times during the first series and at least 3 times, but not always 5 times, during each subsequent series. If a male failed to mount at least 5 times during a given series, then that series was not included in the analysis for first and last 5 mounts and a mean mounts score was calculated from the remaining series.

Statistical Analysis.

One sample t-tests were used to analyze the mean mounts for each of the above describe measures. The level of significance was 0.05 for all comparisons.

Results

The mean number of mounts for the first 3, first 5, last 3, and last 5 mounts for the first and for all series is displayed in Figure 26. As revealed in Figure 26 the deviations from expected random values were small, ranging from -5.0% to + 7.3%. The means for the first 3 mounts of the first series and across series, for the first 5 mounts across series, and for the last 5 mounts of the first series and across series failed to reached statistical significance (all $p > 0.05$). Conversely, the mean for the first 5 mounts of the first series was significantly lower than the expected value and the means for the last 3 mounts of the first series and for the last 3 mounts across all series were significantly higher than the expected values. T-tests confirmed the statistical significance of these observations: For the first 5 mounts of the first series $t(185) = -2.39$, $P < 0.05$; for last 3 mounts of the first series $t(185) = 1.98$, $p < 0.05$; and for last 3 mounts across all series $t(185) = 4.36$, $p < 0.05$.

Discussion

The results of the present analysis demonstrate that males do direct their mounts preferentially towards specific females during a copulatory preference test. Although the previous experiments found that males do not display an overall preference to mount with one female over another may be correct, it does not necessarily indicate

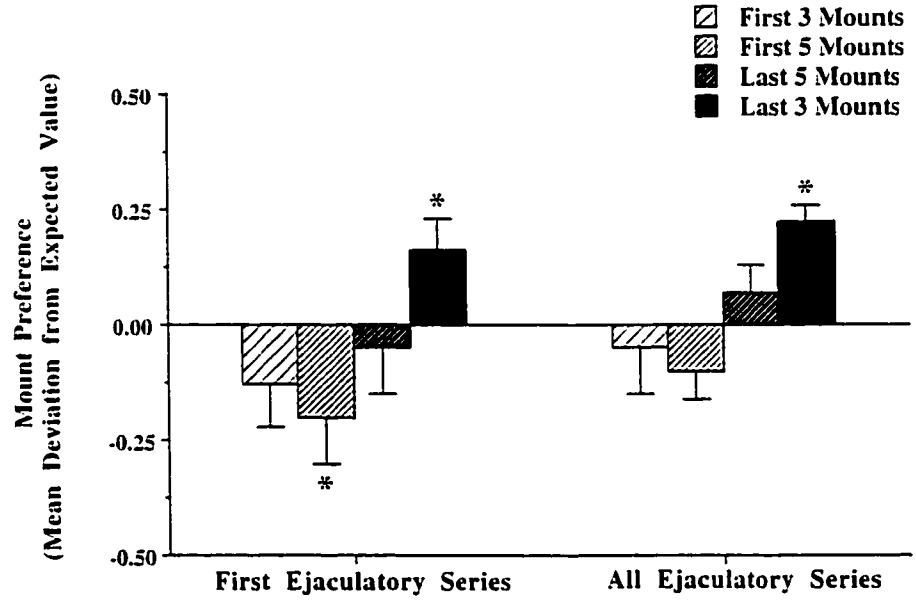

Figure 26. The mean (± SE) deviations from expected values of the first 3, first 5, last 3, and last 5 mounts of the first ejaculatory series (left) and all ejaculatory series (right) during a Copulatory Preference Test for all males analyzed in Experiment 13. * denotes $p < 0.05$ for deviation from expected value comparison.

indiscriminate copulating. The present analysis found that males mount more frequently with the E-Alone during the early, but not necessarily the initial, portion of the first ejaculatory series and then mount more frequently with the A+E female during the final portion of all ejaculatory series. It is important to note that the present results are small biasses in the distribution of copulatory behavior towards a female, nonetheless, given the large sample used in the analysis significant effects were detected.

The present findings have important implications for understanding the nature of the CR that underlies CEP. They support the hypothesis that during a copulatory preference test, males are selecting the female with which to ejaculate in a manner different from the female with which they copulate: Males trained with scented-females mounted more often with the scented female than the unscented one only during the 3 last mounts of each series. These data along with the findings of Experiment 12, that A+E-Trained males display CS-directed copulatory responses, suggest that during the copulatory preference test, CEP is mediated by selective mounting near the point of ejaculation.

General Discussion

The present experiments demonstrate that the CR to an olfactory CS paired with copulation can be elicited in the absence of a sexual UCS. Males trained with almond-scented sexually receptive females displayed CRs in response to the almond odor in bedding and to almond odor on sexually nonreceptive females. These findings

demonstrate that the CS has taken on significance independent of the context in which it was presented. Moreover, the finding that the CR to almond-scented bedding involved gnawing the bedding (Experiment 10), whereas the CR to an almond-scented nonreceptive female elicited mounting (Experiment 12) demonstrates that the form of the CS presentation determines in part the form of the CR. According to a functional perspective of CRs (see Hollis, 1984; Pavlov, 1927), one would expect that a stimulus consistently paired with copulation should elicit CRs appropriate to copulation; i.e. the CR should be similar to the UCR. Evidence that the nature of CS-directed CRs and the nature of the UCR are congruent has been provided by Jenkins and Moore (1973). Pigeons exposed to a food-signalling key light made sharp, vigorous pecks at the light as if it were food, whereas pigeons exposed to a water-signalling key light made water-pecks, involving slower movements with more contact with the key light. Conversely, in the present study, a CS paired with copulation did not elicit CS-directed copulatory behavior, rather it elicited CS-directed investigation and gnawing. This likely occurred because the rats could not engage in copulation with bedding. Similarly, Fillion and Blass (1986) found that when a nursing-paired CS is subsequently encountered on a sexually-receptive female, male rats will copulate with her more vigorously than in the absence of the CS, but they do not nurse because there is no opportunity to do so. Further, Mitchell and Stewart (1990) found that copulation is enhanced in an environment that has been paired previously with morphine but not saline. These findings demonstrate that conditioned *signal-centered action patterns* (Hollis, 1984) are not narrowly constrained by the UCR

but are flexible to the situation in which the CS is encountered. Further, it appears that "pre-organized behavior patterns" (Jenkins, Barrera, Ireland, & Woodside, 1978) may underlie signal-centered action patterns but the particular behavior pattern elicited is sensitive to the nature of the CS presentation.

The present findings also illustrate that pairing a stimulus with copulation enables the stimulus to elicit a complex CR. A+E-Trained males responded to the almond odor with both CS-directed behaviors and increased sexual arousal. Presentation of the CS in the absence of a sexual UCS resulted in CS-directed behaviors whether the CS was in bedding or on a conspecific. Conditioned sexual arousal may also have been present in A+E-Trained males by the increased incidence of genital grooming in response to the CS in bedding (Experiment 10) and by increased latencies to intromit and lower intromission ratios when confronted with an unscented sexually-receptive female (Experiment 11). Thus, the CR has components that involve both the skeletal and autonomic responses.

The findings of the present Experiments provide insight into the nature of CEP. In previous chapters, I have used the term CEP to describe the finding that males having copulated only with females scented with an initially-neutral odor will ejaculate more frequently with a female bearing that odor over one that does not. At the start of this chapter, I outlined two basic mechanisms for this effect: (1) facilitated ejaculation with the scented female; and (2) selection of the scented female to receive an ejaculation. The present findings do not support the first mechanism. No evidence for CS-facilitated ejaculation was found. Although A+E-Trained males copulating with unscented females

displayed decreased sexual arousal, as evidenced by longer intromission latencies and more mounts without intromission, there was no evidence for a significant change in the ejaculatory threshold. A+E-Trained males did not take longer to ejaculate, did not require more intromissions to ejaculate, or achieve fewer ejaculations with unscented females as compared to A+E ones. It is noteworthy that in Experiment 11, two A+E-Trained males (17%) did have substantially longer ejaculation latencies (2 and 5 min) with unscented females. It is tempting to speculate that such a response could occur in a small number of males and manifest itself as a CEP during a copulatory preference test. Although, this cannot account for CEP displayed by the group, it does suggest that there could be multiple mechanisms to produce a biased distribution of ejaculations between two females.

The present experiment provides evidence to support the notion that CEP is mediated by a CS-directed selection of the scented female over the unscented one at the time of ejaculation. Both Experiments 10 and 12 demonstrate CS-directed behavior, including CS-directed copulatory behavior despite a UCS (the nonreceptive female) that is usually capable of inhibiting copulatory behavior. Further, the reanalysis of previous data demonstrated an increased proportion of mounts directed toward the CS-bearing female specific to the period immediately proceeding ejaculation. Together these findings suggest that CEP is mediated by CS-directed copulatory behavior that is elevated immediately prior to ejaculation. Thus, males appear to change their selection strategy at

different points during copulation, and CEP results from a bias toward mounting the scented female near ejaculation.

The hypothesized selective ejaculation mechanism of CEP depends on the idea that male rats may be aware of, and behave differently near, their impending ejaculation. There is some evidence for this in the literature. Male rats emit a 22 kHz ultrasonic vocalization that is specific to the period immediately prior to ejaculation (Brown, 1979). Further, female rats appear to be able to detect the point during copulation in which a male is about to ejaculate as evidenced by increased female-female competition (McClintock, 1984; McClintock et al., 1982). Accordingly, given that females can detect it and males sing about it, the sexually-experienced male rat is likely able to predict his own ejaculation.

Regardless of whether a male rat can detect his impending ejaculation, the question remains as to why CS-directed behavior should be limited to the point of ejaculation during tests of copulatory behavior with receptive females. One explanation for this can be derived from the concept of incentive salience (Bindra, 1974; Bindra, 1978; Toates, 1986). According to this principle the amount of impact that a stimulus has on behavior depends on its ability to predict reinforcement (incentive value) and the importance that the specific reinforcement is now prescribed (saliency). To illustrate, food and food-predictive stimuli do not impact significantly on behavior unless a desire for food is also present. Further, as there are usually multiple incentives in an environment, saliency

must be considered as a relative quality. Even when not hungry, food can be desirable in the absence of other incentives.

Incentive salience can be applied to the proposed selective ejaculation mechanism underlying CEP. During the initial phases of copulation, the olfactory CS may have little salience because sexual UCSs predominate. As copulation proceeds, the male's own internal state of heightened sexual stimulation approaches the ejaculation threshold. With ejaculation impending, the salience of a CS paired previously with sexual reward is increased. Thus, when a female bearing an olfactory stimulus previously paired with the postejaculatory interval (chapter 3) is present she is a more attractive mate than a female without that odor and more CS-directed copulatory behavior is produced. This relative selection of a CS-bearing female near ejaculation explains why these females receive most, but not all, ejaculations from a male, despite not receiving more mounts overall.

But why would a selective ejaculation exist in the male rat? A selective ejaculation mechanism versus a selective copulation mechanisms may have been produced in rats because in the wild they tend to mate in groups (McClintock, 1984). Although each female requires a certain rate of vaginocervical stimulation to help induce pregnancy, it is the female that a male ejaculates with that he will actually impregnate, as vaginocervical stimulation can be provided sequentially by other copulating males. Accordingly, a male need only ejaculate selectively to proliferate his genes with the most desirable mate.

The present findings suggest that CS-directed copulatory behavior mediates CEP, but they do not distinguish clearly whether the development of the CR follows Pavlovian

or instrumental learning. A number of dichotomies have been proposed for Pavlovian and instrumental learning. For example, these two forms of learning were once thought to be distinguished by skeletal versus autonomic responses, however the demonstration of reinforcement of autonomic responses with biofeedback (Schwartz, 1972; 1975) and the phenomenon of autoshaping (Brown & Jenkins, 1968; Jenkins & Moore, 1973) weighed against this interpretation. Each type of learning has been argued to be the basis for the other (see Hollis, 1984) and it is currently viewed that the dichotomy between Pavlovian and instrumental learning lies within procedural differences in laboratory experiments (see e.g. Flaherty, 1987; Wasserman & Miller, 1997). Pavlovian contingencies involve stimulus-reinforcer associations whereas instrumental contingencies involve response-reinforcer associations. However, the rules that govern these forms of learning are very similar (see Dickinson, 1980) and most instances of learning likely involve properties of both contingencies. Currently, the best way to distinguish between the two types of associative contingencies is the employment of an omission procedure (Lajoie & Bindra, 1978) in which an animal must refrain from making a response to obtain a reward. This procedure is thought to distinguish between Pavlovian autoshaping behavior and operant responding by decreasing the latter but not the former. Unfortunately, the CEP model does not appear to be readily amenable to such an analysis because the conditioning procedure involves the subject's own copulatory responses to induce a UCS (postejaculation reward state) that when paired with the olfactory CS produces the CS-directed copulatory behavior near the ejaculation threshold. Omitting the UCS

(postejaculatory interval) when the UCR (copulation) occurred would mean that the CS would always be presented alone. However, if a postejaculatory state could be induced in the absence of copulation, for instance by brain stimulation or pharmacological treatment, then the omission procedure could be used to ascertain if the development of CEP is Pavlovian or instrumental in nature.

General Discussion

The major finding of the present thesis is that associative learning plays a role in determining the selection of sexual partners in male rats. The experiments in this thesis have shown repeatedly that allowing a male rat to copulate with a female bearing a neutral odor produces a subsequent preference to ejaculate with a female bearing that neutral odor over a female that does not. No ejaculatory preference was observed if the odor was presented in isolation or if it was paired randomly with the opportunity to copulate. Accordingly, I have referred to this behavior as a conditioned ejaculatory preference (CEP). CEP appears to develop early during sexual experience and further conditioning increases the strength of the CEP. The development of CEP does not appear to be critically dependent upon copulation with a scented female per se, but rather it appears that the presence of a scented female following ejaculation is sufficient for minimal CEP development. Interestingly, preferences are not observed for copulation in general, as shown by the fact that mounts and intromissions are not distributed preferentially across the ejaculatory series. Rather, copulatory behavior is directed preferentially toward the scented female only immediately prior to ejaculation. Thus, the expression of CEP appears to be the result of a switch from indiscriminant copulating early in an ejaculatory series to discriminant copulating near ejaculation. This results in the male directing his ejaculations to a female bearing a stimulus paired previously with sexual reward.

CEP in Pavlovian and Incentive Motivational Frameworks

Pavlovian Conditioning Account of CEP. The present findings can be interpreted within a Pavlovian conditioning framework. In this case the CS is readily identifiable as the initially-neutral odor applied to the female rat. After it has been paired with copulation, the CS is able to elicit copulatory behavior directed toward a female bearing it, as demonstrated by increased mounting of a scented, sexually-nonreceptive females by the Paired-Trained males (Experiment 12). The ejaculation-specific nature of CEP stems from the relative strength of the CS to direct behavior at different points in copulation (Experiment 13): This finding is discussed below. Further, the CR also appears to involve components of sexual arousal as evidenced by genital grooming in the presence of the CS in isolation (Experiment 10) and the delay of intromission during copulation with a female not bearing the CS (Experiment 11). However, ejaculation, *per se,* does not appear to be influenced by the conditioning in as much as ejaculation latency and intromission frequency are not altered significantly by the omission of the CS during copulation (Experiment 11). Thus, the CR produced by the conditioning procedures used in the present thesis is complex and appears to involve both autonomic and skeletal elements.

Conversely, elements of the unconditional stimulus upon which the CEP is based appear to fit into a Pavlovian framework only if the neural, rather than the behavioral, aspects are considered. In Chapter 3, it was demonstrated that the scented female must be present during the postejaculatory interval in order for CEP to develop. Superficially, this might suggest that the UCS is ejaculation and the UCR is post-ejaculatory inactivity.

From this perspective, the CEP might appear to develop under the backward conditioning contingency of ejaculation followed by the odor. Given the wide body of evidence that backward conditioning is extremely rare (e.g. Flaherty, 1987; Rescorla, 1988), it is unlikely that this is the case. Conversely, if one considers the neural activity set up by ejaculation to be the UCS, the conditioning appears to follow established principles. I have argued that the neural activity triggered by ejaculation comprises the UCS for CEP. For example, following ejaculation hippocampal activity is desynchronized, mesolimbic dopamine transmission is decreased, serotonin, oxytocin, prolactin, and opioid transmission are increased, and Fos protein is produced in specific areas of the hypothalamus, amygdala, and tegmentum. The behavioral response that accompanies these neurochemical changes is clear, the male rat becomes transiently inactive following ejaculation. However, it is the neural activity set up by ejaculation that enters into association with the neural representation of the CS. The neurochemical events triggered by ejaculation likely produce local circuit activity suppression in regions controlling copulatory reflexes (i.e., in the mPOA), but may also promote memory formation in other neural circuits. Thus, the post-ejaculatory brain may be highly amenable to long-lasting alterations in functional activation and responsiveness. In other words, ejaculation not only inhibits copulation, but also facilitates learning. Accordingly, one component of the UCR to ejaculation-triggered events is a state of neural plasticity that is responsive to sensory stimuli (CSs) in such a way as to make these sensory stimuli attractive,

especially during subsequent states of high sexual stimulation (i.e., near the ejaculatory threshold).

Incentive Motivational Account of CEP. The present findings can also be interpreted within a general incentive motivational framework, emphasizing the role of incentive salience. Accordingly, I have formulated a model to describe the development and expression of CEP based on the incentive motivational theories of Toates (1986; 1998). Figure 27 depicts the model's account of the events that occur before, during, and after copulation at the time of an initial olfactory conditioning session. At the time of a conditioning session, a male is placed in a chamber (CS2) and soon after he is joined by a scented, sexually-receptive female. The features of the female are comprised of her unconditional stimulus properties (UCS1) and the conditional odor (CS1). In reality, the female provides a complex of multiple UCSs. At least two aspects of the sexually-receptive female elicit responses unconditionally from males. Estrous odors, by themselves, are capable of eliciting approach (e.g. Bressler & Baum, 1992) and of inducing sexual arousal (e.g. Sachs, 1997). Additionally, the female's movements have been shown to provide important stimulus elements as demonstrated by lower incentive value of a female immobilized by haloperidol (see Edwards & Maillard, 1988; Everitt, 1990).

Figure 27A depicts the primary features of the model. Initially, CS1 and CS2 are neutral stimuli that do not elicit any responses, with the potential to enter into any number of associations and elicit conditional responses. Each stimulus could be paired with other UCSs or CSs (as in second order conditioning), hence the notation of CR1x and

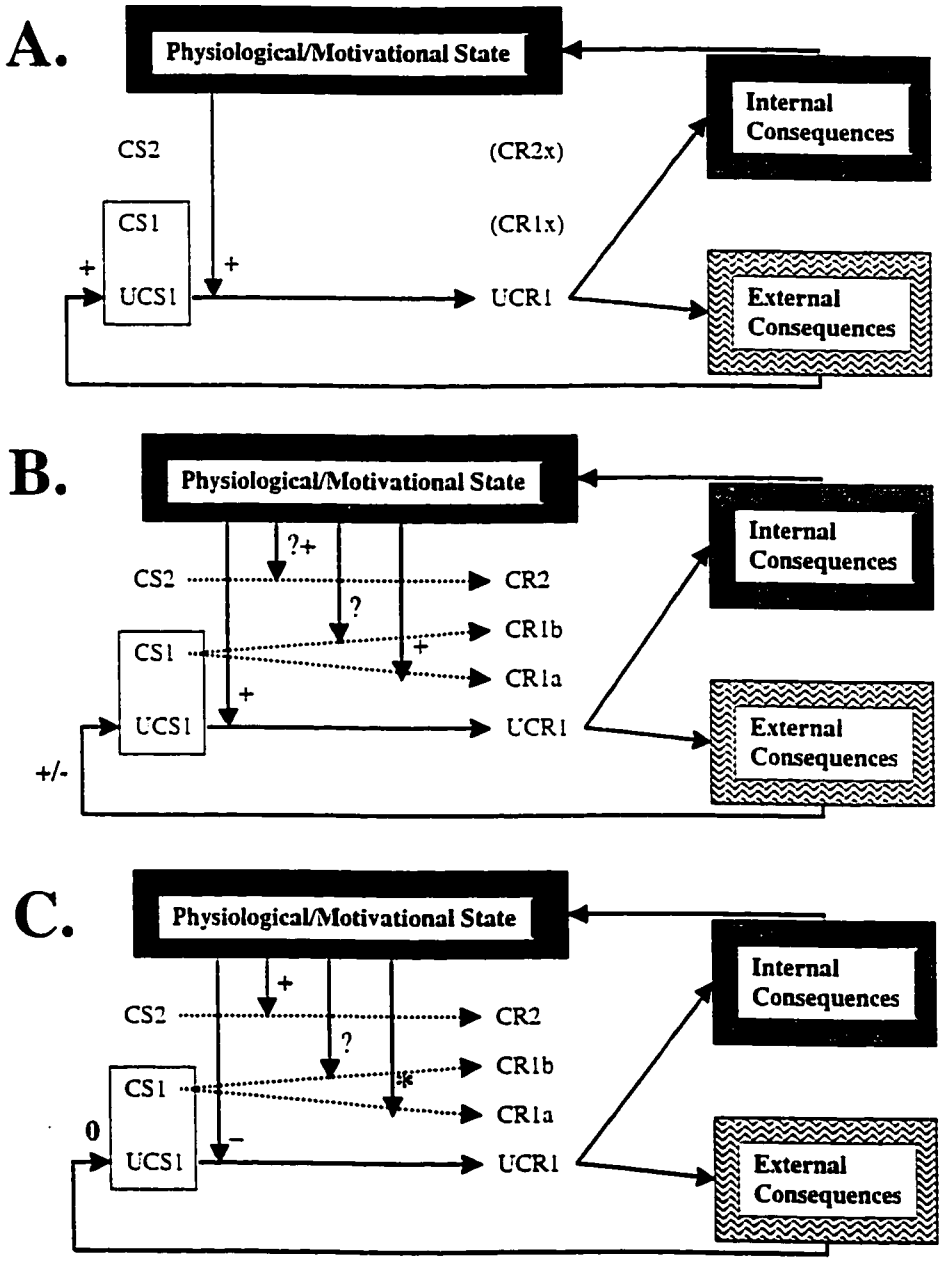

Figure 27. Incentive Motivational model of the development of CEP, conditioned sexual excitement, and conditioned sexual arousal during copulation with a scented receptive female.

CR2x; where x notes an as yet undetermined CR. Conversely, UCS1 is initially capable of eliciting a limited set of behavioral responses. The unconditional responses are likely complex, but for simplicity, I will describe UCR1 only for the ability of UCS1 to elicit copulatory responses.

Once UCR1 is performed (Figure 27B), a complex chain of events is set into motion. UCR1 has external consequences that will feedback to alter UCS1 and perhaps its ability to elicit responses. For instance, copulation will alter the proceptivity and receptivity of the female that will influence the male's response to her. Additionally, UCR1 has internal consequences that alter the physiological/motivational state of the male subject. In turn, these physiological/motivational changes feedback to influence the incentive salience of UCS1 itself. For instance, copulation may increase the male's motivation to interact with UCS1. In addition, as a result of the heightened physiological/motivational arousal elicited by interacting with the UCS1 from the female, the neutral stimuli are imparted importance though their association with the state. CS1 and CS2 become meaningful stimuli, predictive of the internal consequences of copulation. Accordingly, through the neural changes induced by their association with the UCS they gain the ability to elicit CRs. CR1a, CR1b, and CR2 represent the conditional responses studied in the present thesis.

CR1a is the stimulus directed behavior elicited by CS1 (odor). In the case of CEP, CR1a is copulatory behavior directed at the CS-bearing female. CS-directed behavior was demonstrated in Chapter 4 in the absence of a sexually-receptive female, including

copulatory responses directed at a scented nonreceptive female. CR1a appears to be strengthened by copulation with a scented female; CEP is stronger when a male is allowed to copulate to ejaculation with a scented female followed by exposure to a scented female than when a male is allowed to copulate to ejaculation with an unscented female followed by exposure to a scented female (Experiment 9). Conversely, it appears that if copulation is disrupted prior to ejaculation, then CS1 takes on incentive value of an entirely different nature. Males allowed to copulate to five intromissions without ejaculation with a scented female display a subsequent preference for an unscented female over a scented one (Experiments 7 & 8). Thus, pairing a neutral stimulus with copulation, *per se*, does not appear to determine the qualitative nature of a CS-directed response. Rather, the qualitative nature of a CR appears to be directed by subsequent events. Sexual reward (i.e., ejaculation) results in subsequent behavior being directed toward the CS. Whereas, sexual frustration (i.e., disrupted copulation) results in subsequent behavior being directed away from the CS.

CR1b is the sexual arousal elicited by CS1. This was demonstrated in the present studies by increased genital grooming elicited by the CS in the absence of a female (Experiment 10) and increased intromission latencies during copulation with an unscented female (Experiment 12). The parameters surrounding the development of CR1b were not examined explicitly in the present thesis. Conditioned sexual arousal has been demonstrated in the absence of copulation in rats (Zamble et al., 1985) and with masturbation in men (Kantorowitz, 1978). However, no study has compared levels of

conditioned sexual arousal directly under these situations. Thus, it is with some speculation that I suggest that conditioned sexual arousal is facilitated by copulation.

CR2 is the sexual excitement (as demonstrated by increased locomotor activity in anticipation of the arrival of a female on subsequent trials) elicited by CS2 (chamber). CR2 develops following copulation without ejaculation, however, the minimum parameters for the development of conditioned sexual excitement were not determined in the present thesis and are not clear from the present literature. Van Furth and Van Ree (1996b) have suggested that sex odors are critical determinants of conditioned sexual excitement in the male rat based upon the ability of olfactory bulbectomy to disrupt conditioned level changing. Conversely, Mendelson and Pfaus (1989) found that conditioned level changing developed when males have access to receptive females in the bilevel chambers, but not when males have access to nonreceptive females. In the latter study, sex odors were likely present during all conditioning sessions, accordingly, their results are in apparent conflict with those of Van Furth and Van Ree. Moreover, in the present experiments, conditioned level changing was found to develop in bilevel chambers that were cleaned thoroughly prior to each conditioning session. However, it is important to note that Van Furth and Van Ree used Wistar rats for their experiments, whereas Mendelson and Pfaus and the present studies employed Long-Evans rats. Relative to Long-Evans, Wistar rats have poorer vision and rely more heavily on olfactory stimuli (e.g. Boyes & Dyer, 1983; Creel, Dustman, & Beck, 1970; Dyer & Swartzwelder, 1978). Hence, I have speculated that conditioned sexual excitement is enhanced by copulation.

Repeated performance of UCR1 culminates in ejaculation which triggers the dramatic alteration of the physiological/motivational state resulting in both short- and long-term consequences (Figure 27C). These physiological/motivational changes have a powerful inhibitory influence on the performance of UCR1 as postejaculatory inhibition of copulation is strong enough to completely devalue the receptive female, an otherwise highly arousing UCS. Moreover, they have powerful influences on the development of CRs elicited by the scent of the female and the chamber. The development of CR1a is critically dependent upon the presence of the CS (presence of scented female) during the postejaculatory period. If the scented female is removed, no CEP develops whatsoever. However, the present thesis did not examine if the presence of the odor by itself during the postejaculatory interval would support CEP. This could be tested by allowing a male to copulate to ejaculation followed by exposure to the CS on a cotton roll. Conversely, the development of CS2 is not critically dependent upon ejaculation, however, it is enhanced. Subsequent copulation and ejaculations with a scented female or females in a single session or across sessions serve to strengthen the CR-eliciting ability of the CS1 and CS2. It is important to point out that a CS paired with copulation may not be elicit only the CRs examined in the present thesis and should receive attention in the future.

Expression of CEP is also best interpreted within an incentive motivational framework. Figure 28 depicts the events during a copulatory preference test following conditioning procedures capable of producing CEP. Here UCS2 represents the stimulus properties of the unscented female and UCR2 is copulatory behavior directed at her.

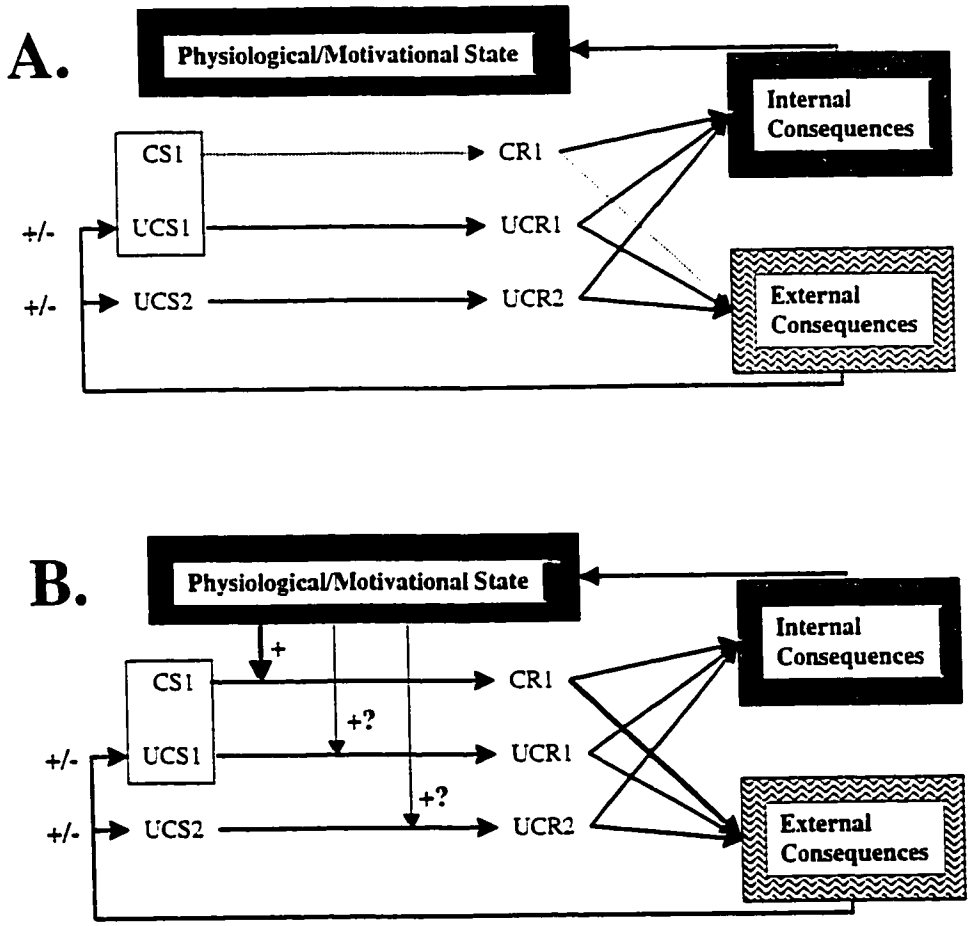

Figure 28. Incentive Motivational model of the expression of CEP during the Copulatory Preference Test with one scented (CS1-UCS1) and one unscented female (UCS2).

UCS1 represents the stimulus properties of the scented female, independent of the CS1 (that is her incentive value without the scent) and UCR1 is the copulatory response directed at her, independent of the CR1. CS1 is the odor paired previously with copulation and CR1 is the copulatory behavior that it can elicit. UCS1 and CS1 are physically connected as the odor is applied directly to the female.

Figure 28A depicts the circumstances in the copulatory preference test (CPT) at the initiation of an ejaculatory series. Initially, the ability of UCS1 and UCS2 to elicit UCR1 and UCR2, respectively, are equal and very strong relative to the ability of CS to elicit CR1. The incentive value of the two females is approximately the same, thus, the amount of copulatory behavior directed toward the scented and unscented female is equal. The feedback systems are similar to those described in Figure 27 with the exception that the external consequences occur from two females.

As copulation during the CPT continues, the internal consequences of sexual stimulation become apparent (Figure 28B). The external consequences of copulating with each female are represented in a connected manner and have not been examined in the present thesis. For instance, the amount of copulation one of the female receives relative to the other probably influences their relative proceptivity and receptivity, but are always interconnected through intrasexual competition (see McClintock, 1984). Such effects have been studied by conducting group mating tests using females that had received various amounts of copulation from other males. These effects were generally

unsystematic and require further study before their impact on copulatory preferences can be assessed (see Dewsbury, 1981).

Conversely, the effect of sexual stimulation on the internal consequences and subsequent changes in physiological/motivational states clearly influences the manner in which copulatory responses are directed. As ejaculation approaches, the ability of the CS to elicit copulatory responses increases. Males trained previously with scented females bias their copulatory responses toward the scented female near ejaculation. This is evident in the number of mounts just prior to ejaculation and the number of ejaculations received by the scented female relative to the unscented one. Thus, CEP is the result of the increased incentive salience of a CS during heightened sexual stimulation.

Roles of Learning in Sexual Partner Preferences

Non-human Animal Studies. Sexual partner preferences involve complex patterns of social behavior that include, but are not limited to, copulatory preferences. Animal studies have implicated several measures related to selective mating. These include: male and female cohabitation, selective aggression, biparental care, social preferences (Carter et al., 1995), as well as, affiliative and alliance formation (see Fairbanks et al., 1977; Vasey, 1995). Accordingly, the copulatory preferences revealed in the present thesis are but one component in the larger phenomenon of sexual partner preferences.

The relations between different components of sexual partner preferences have been studied in various contexts. In socially monogamous and serially socially

monogamous species in which mating pairs form extended and limited partnerships, many of the components are correlated. For instance, in the socially monogamous prairie vole, male and female pairs cohabitate, display reduced aggression to mates, engage in parental care, and display preferences to remain in the proximity of their mate over an unfamiliar conspecific (I refer to this latter behavior as a social proximity preference). Carter et al. (1995) have suggested that the amalgamation of these preferential behaviors determine a pairbond.

The behavioral patterns that make up the pairbond do not appear to be restricted to purely socially monogamous species. Japanese quail form seasonal pairbonds that last the duration of a breeding season, then new mates are sought for the following season (Mills et al., 1997). Even the notoriously polygamous male rat has been observed to display elements of pairbonding. Rats typically live in burrow systems, thus, cohabitation is more common in this species than most rodents. Within rat colonies, there are social controls that suppress aggression directed toward members of the colony, but not toward intruders (see for e.g. Blanchard & Blanchard, 1990). Further, male rats have been observed to display extensive parental behavior (Brown & Moger, 1983). The only pairbond correlate which rats have not been found to display is social proximity preference for familiar sex partners.

Social proximity preferences are robust in socially monogamous prairie voles, but have been observed in a polygamous species. Social proximity preferences have been observed in the polygamous meadow vole (Phillips, Parker, & Lee, 1999; Salo &

Dewsbury, 1995), but not the polygamous montane vole (see Carter et al., 1995). Although both meadow and montane voles are polygamous, they do not share entirely the same social patterns. Meadow voles appear to be far more social than montane voles as measured by huddling behavior (Salo, Shapiro, & Dewsbury, 1993; Shapiro, Meyer, & Dewsbury, 1989). As such, the presence or absence of social proximity preferences for familiar sex partners may relate, in part, to the social or nonsocial nature of the species under study. If this is the case, then it would follow that social proximity preferences are part of a general behavioral response system that mediates approach of stimuli paired with sexual stimulation. In conditioned place preferences (CPPs) based on copulation in rats, the stimuli are arbitrary and contextual; in the conditioned approach behavior based on copulation in the quail, the stimuli are arbitrary and discrete; and in CEP in male rats and in social proximity preferences in the vole, the stimuli arise from the sex partner and are arbitrary or naturally-occurring, respectively.

Accordingly, it would be interesting to examine whether social proximity preferences could be demonstrated with discrete, arbitrary stimuli manipulated by the experimenter. For instance, the procedures used in the present thesis to produce CEP could be followed by a different test to address this question. For example, male rats trained to copulate with scented females could be tested with two females, one scented and one not, placed at different ends of a runway. A number of variants of this test would be of interest. Males may or may not be given access to the female. The females could be sexually-receptive or nonreceptive.

Conversely, it would also be of interest to further examine the responses to stimuli paired with sexual behavior and sexual UCSs in Pine voles. Pine voles do not demonstrate social proximity preferences for familiar sex partners and they do not exhibit preferences for estrous females over diestrous ones. Thus, it may be that this species do not show strong approach to sexual stimuli, in general. Accordingly, it would be interesting to examine how this species responds to arbitrary predictors of copulation, for example, assessing CPPs produced by copulation. Perhaps various species of voles possess novel nervous system organizations that differ around sexual reward mechanisms and perhaps nonsexual reward mechanisms. Understanding these differences could provide insight into behavior through a comparative approach that extends beyond the social monogamy-polygamy distinction. Such a comprehensive approach could serve to bring together the findings from disparate literatures into a single framework.

Currently, it is not clear to what degree copulatory preferences are related to the other factors described in pairbonding. Despite the long-standing belief that pairbonded socially monogamous species do not engage in extrapair copulation, recent paternity tests in field studies have revealed that mixed paternity offspring are common in such situations. Field and laboratory studies revealed that pairbonded female prairie voles carry mixed paternity litter (Carter, Williams, & Witt, 1990). Even when females display clear social proximity preferences, they do not display copulatory preferences. Mixed paternity litters are also found in a variety of other species (e.g. Bercovitch & Nurnberg, 1996; d'Orgeix & Turner, 1995; Shimmin, Sofronidis, Bowden, & Temple-Smith, 1995;

Stockley, 1997; Tegelstrom, Searle, Brookfield, & Mercer, 1991). Accordingly, copulatory preferences appear to be a distinct form of sexual partner preference that is independent of social proximity or other types of preferences.

It is widely believed that sexual novelty is preferred by males, and perhaps females, of most, if not all, species. This idea is embodied empirically in the Coolidge effect (Beach & Jordan, 1956b). Laboratory findings of the Coolidge effect are generally limited to the ability of a novel copulatory partner to rearouse sexually sated males or females (for a review, see Dewsbury, 1981). However, the notion has also been extended to explain the apparent preference to mate with novel partners. The present thesis finds another measure of this form of preference in switching behavior. I use the term switching to refer to the propensity of a male to direct his second ejaculation toward the female who did not receive his first ejaculation during the course of a copulatory preference test. Males that displayed either weak or no ejaculatory preferences tend to display a high degree of switching. Conversely, males that display robust ejaculatory preferences appear to wait until later ejaculatory series to switch females. Males trained with scented females tend to direct their first and second ejaculations toward a scented female then distribute subsequent ejaculation equally between females. Thus, it appears that the expression of copulatory preference reflects the interaction of multiple mating strategies: Directing ejaculations toward a familiar scented female and distributing them between two females. Preferences for novel sex partners would also be expected to be evident in social proximity preferences. Evidence for this is provided by the finding that

female prairie voles often mate with both the familiar and unfamiliar males during social proximity tests. Accordingly, the confinement of the males allow both the copulatory preference for novelty and social preference for familiarity to be displayed.

In summary, animal studies of sexual partner preferences have revealed an enormous complexity of social and sexual motivations. Species differences may better be understood by analysis of the competing motives rather than dichotomous classification into social monogamy and polygamy. Rats appear to be social and highly sexual; male rats and female rats in heat appear to copulate readily. Conversely, prairie voles appear to be social but less sexual as many members of this species fail to display sexual behavior during their lifetime despite constant mixed-sex social structures. And Montane voles appear to be relatively sexual but exhibit little social behavior. The expression of social or sexual preferences is controlled by these often competing goals. As well, situational factors, both past and present, play a role in directing behavior towards a specific conspecific. Accordingly, a more global approach to the study of social interaction needs to examine motivational, experiential, and temperamental factors to understand how preferential behavior towards conspecifics is mediated. Moreover, the present finding that male rats direct their ejaculation toward specific females during the context of group mating demonstrates that male mate choice does occur and is an important determinant of the sexual selection process. Accordingly, the present model provides a basis to re-evaluate sexual selection theories as a complex interaction of choices by both males and females, rather than the prevailing bias to focus on female mate choice.

Only when there are meaningful differences between potential mates is mate choice necessary and it appears that past experience has a powerful role in determining the meaning of these differences.

Generalizations to Humans. The preceding analysis of partner preferences has been limited to nonhuman studies, however, it appears that similar factors operate in humans. Indeed, a host of factors have been found to be related to the attractiveness of a mate in humans. These include physical, personality, and social features (see Buss & Schimdt, 1993). Humans also form pairbonds of variable length. Buss and colleagues have described long- and short-term mating strategies with their primary focus and evidence stemming from studies of sex differences. From their studies, it is clear that different features are preferred and different criteria are used for different types of relationships. Like the prairie vole, people tend to enter into relationships that are of a long-term nature in which selective aggression, biparental care, cohabitation, and perhaps social preferences are displayed. Moreover, despite implicit expectations of sexual exclusivity, pairbonded humans also engage in extrapair copulation. Accordingly, it appears that human mating patterns bear much in common with mating patterns seen in other species. Preferences are not global, but are comprised of several competing factors.

People exhibit preferences for copulatory partners. Buss and colleagues have used the term short-term mating to describe transient relationships that are unrelated to childrearing and characterized by partner preferences that are most heavily reliant upon physical characteristics for both men and women. These are essentially copulatory

preferences. During short-term mating both men and women display preferences for certain characteristics of sex partners. Although Buss and colleagues posit that evolved psychological mechanisms underlie these preferences, they do not offer any form of proximal mechanisms for the establishment of these preferences. Evidence of learned sexual preferences may fill in this void.

CEP may occur during actual copulation or during masturbation with real and fantasized partners becoming preferred. The development of preferences during actual copulation would explain the anecdotal evidence that individuals often continually pursue partners with features similar to previous partners. Even following past abuse, similar partners are often sought despite the negative consequences. Additionally, preferences developed during masturbation may also contribute to the adherence to cultural values. The features of fantasized partners could be comprised of culturally-valued characteristics. When these are paired with sexual reward, preferences would be established or strengthened. Thus, cultural values may also determine what features will be preferred in a mate. This can explain not only the status quo of physical preferences within a culture, but also those of past eras.

Accordingly, Buss' evolved psychological mechanism guiding copulatory preferences may in fact be learning. Learning provides an efficient mechanism to guide behavior toward the stimuli that are predictive of fertility and reproductive success. Sexually imprinted maternal stimuli may be excellent predictors of fertility. Classically conditioned stimuli paired with sexual reward are likely to be excellent predictors of

receptivity. Moreover, the relative impact of imprinted and conditioned stimuli in sexual preferences in humans may be magnified in comparison to the rat because sexual status (i.e., menstrual cycle) is masked in women (Alexander & Noonan, 1979). According to the incentive motivational model that I used to explain the expression of CEP (Figure 28), sexually imprinted and conditioned stimuli would be expected to have more powerful influences in the absence of sexual USs. This would indeed be the case in men for whom the reproductive status of women can not be determined directly. Accordingly, copulatory attempts may be appropriately or inappropriately directed toward or away from women based largely upon learned stimuli rather than actual reproductive status.

Implications for Understanding Sexual Orientation. The above discussion has followed the implicit assumption that sexual preferences are for members of the opposite sex. This is not always the case. The present findings of CEP may have implications for both homosexual partner preferences and the development of sexual orientation.

Sexual partner preference in homosexual individuals appears to be similar to that of heterosexuals as highly valued attributes are similar in straight and gay men and women. For example, Howard, Blumstein, & Schwartz (1987) examined ratings for the importance of the qualities of expressive, attractive, athletic, aggressive, and ambitious in both gay and straight men and women. Similar to the finding of Buss and Schmidt (1993), men put more emphasis on attractiveness than did women with gay and straight men not differing; however, gay women desired attractiveness more than did straight women. Women preferred aggressive and ambitious mates more than men, with straight women

scoring higher than gay women and gay men scoring higher than straight men. Similarly, Bailey, Gaulin, Agyei, and Gladue (1994) found that both homosexual and heterosexual men placed a high value on visual sexual stimuli and physical attractiveness, but not on partner socioeconomic status, whereas homosexual and heterosexual women valued a partner's status and sociosexuality. Thus, it appears that similar mechanisms are involved in the development of sexual partner preferences in gay and straight individuals.

Although, traditional learning theories attempting to explain the development of sexual orientation have usually posited homosexuality as a result of pathological learning (e.g. Freud, 1905; James, 1967), a number of recent theories have tried to account for the development of both homosexual and heterosexual orientations as part of the normal sexual maturational process. Storms (1981) suggested that the timing of early sexual experience is the critical determinant of sexual orientation. He proposed that individuals who mature sexually at an early age when their social groups are composed of same-sex members are predisposed to be homosexual through sexual interactions with other members of the social group. Same- or opposite-sex features that are associated with early sexual experience (either through masturbation or intercourse) set the course for the development of one's orientation. The present findings that CEP can be produced by a single conditioning session (see Chapter 2) gives direct evidence that initial experiences have particularly powerful influences on sexual preference. Further, the present findings also demonstrate the critical role of sexual reward in determining sexual preferences (see Chapter 3). Thus, the phenomenon of CEP provides evidence in the context of an animal

model for Storms' hypothesis regarding the critical role of early experiences that are sexually rewarding in the determination of what becomes attractive.

Conversely, Bem (1996) has proposed that the development of sexual orientation is secondary to the development of temperaments. According to this hypothesis, genetically-determined temperaments produce sex-typical or sex-atypical behavior. Behavioral variables result in secondary feelings of similarity for like-behaving individuals and feelings of dissimilarity (exotic) for differently-behaving individuals. The exotic feelings are made erotic via heightened autonomic arousal during subsequent antagonistic interactions with these individuals. The findings of CEP do not bear directly on this mechanism but certainly suggest that other mechanisms are at work. Moreover, the finding that stimuli paired with disrupted copulations (e.g., five intromissions without ejaculation) are subsequently devalued during preference tests appear to contradict or at least limit Bem's hypothesis. Disrupted copulation undoubtably produces a state of heightened autonomic arousal, yet the incentive value of associated stimuli is devalued. Consequently, Bem's hypothesis that stimuli paired with increased autonomic arousal become sexually-preferred does not hold for all patterns of arousal. Perhaps only certain patterns of autonomic arousal would increase incentive value of associated stimuli, but to date these have not been specified. The exact nature of autonomic arousal that would produce sexual inclinations and disinclinations must be examined before this hypothesis can be fully evaluated.

It is important to note that both Bem (1996) and Storms (1981) have inappropriately treated sexual orientation as an unitary construct that has only two outcomes, homosexual or heterosexual. Future theories of sexual orientation development should take the multidimensional nature of orientation into account (see Stein, 1997). A comprehensive theory of sexual orientation should account for the development of the various dimensions of sexuality, how these dimensions interact, how they relate to the attractiveness of specific stimuli, and how all these factors vary across different contexts (e.g. short- versus long-term mating) and across age (e.g., Money, 1987).

Summary. There are multiple dimensions that comprise mate preferences, but the exact way in which these dimensions coexist needs to be further examined. In well-established pairs of prairie voles and perhaps some human couples, it appears that all the aforementioned measures of pairbonding are displayed together. Such pairs cohabitate, raise offspring together, as well as exhibit social and copulatory preferences. Conversely, other pairs appear to exhibit only part of this pattern or the full pattern but only for short periods of time. Further, copulatory preferences appear to be separate from all the other sexual partner preference behaviors. This discrepancy makes sense in terms of what is gained from each of the behaviors. When biparental care increases the survival of offspring, the cluster of social measures of pairbonding is required. Biparental care suffers in situations in which cohabitation or selective aggression are absent. However, covert extrapair mating would not affect the quality of parental care and would benefit the philanderer by increasing genetic combinations that may prove to be of higher adaptive

value. Placing the dimensions of partner preferences into a framework that emphasize proximal mechanisms (e.g. incentive salience) will likely provide valuable insight into the underlying factors that determine what and when mates are attractive.

Future Directions

The present thesis examined the role of conditioning in sexual partner preferences by pairing a neutral odor with copulation, then allowing the male the opportunity to copulate with two receptive females that differed with respect to the presence or absence of an arbitrary, olfactory CS. This procedure revealed an ejaculatory preference for the scented female. Further studies should examine the relation between copulatory preferences and other forms of sexual partner preferences. One way to investigate this issue would be to assess other forms of preferences produced by conditioning procedures that are able to produce CEP. For example, would males trained with scented, receptive females display proximity preferences for a scented, nonreceptive female over an unscented, nonreceptive female?

One of the reasons for carrying out these experiments on conditioning of sexual partner preferences was to be able to investigate the neural mechanisms underlying the learning in sexual partner preferences. In a preliminary study using Fos immunoreactivity to assess activation of neural pathways (Kippin & Pfaus, in preparation), I demonstrated the utility of CEP in this regard. By using an initially neutral, arbitrary stimulus as the CS, it was possible to present a CS (which was meaningful in mating preferences) to the

male without the confounds of having the female present. Future research could use the findings of this brain activation study as a starting point to investigate the role of various structures in the mediation of CEP. Brain inactivation during different phases of the conditioning paradigm would reveal valuable information regarding the coordinated action of the limbic loop and mPOA circuit in producing long-term behavioral consequences. Similarly, neurochemical and neuropharmacological studies employing similar strategies could reveal which neurotransmitters and hormones mediate CEP. Additionally, psychopharmacological studies could provide valuable information regarding the effect of drugs on conditioned responses important to sexual behavior.

Although this thesis focussed on CEP produced by pairing a CS with sexual reward, two additional effects of conditioning on sexual partner preferences were revealed. When a neutral odor was paired with access to a nonreceptive female subsequent conditioned inhibition of sexual behavior and stimulus devaluation were displayed (Experiment 1). Several male rats trained with scented nonreceptive females failed to copulate in the presence of the odor despite the presence of two sexually-receptive females, one of which was unscented. This finding suggests that conditioned inhibition of sexual behavior may be particularly powerful; able to suppress the effects of highly arousing UCSs. Of the males trained with scented nonreceptive females that did copulate during the CPT, an ejaculatory preference was revealed for the unscented female. This latter finding demonstrates that sexual partner preference is a relative quality in which past experience can decrease, as well as increase, the incentive value of stimuli.

Similarly, the pairing of almond odor with intromission without ejaculation produced a preference for an unscented female over a scented one. This finding suggests that stimuli paired with sexual frustration become devalued. These findings also highlight the relative nature of preference behavior. Comparisons of these devaluation procedures and elucidation of neurobiology that underlies them would cast our understanding of sexual partner preferences in a new direction. Investigations of the interaction of increased and decreased incentive stimuli is necessary for the understanding of preferences at both a behavioral and neural level.

The present findings have important implications for the treatment of deviant sexual preferences and behaviors. Deviant sexual preferences are thought to develop through conditioning processes (e.g. Abel & Blanchard, 1974; Laws & Marshall, 1990; McGuire, Carlisle & Young, 1965) and conditioning techniques are often employed in an attempt to reduce or eliminate these preferences. Common techniques include: directed masturbation, in which subjects masturbate to nondeviant themes; satiation, in which subjects masturbate well past the first orgasm to deviant themes; and masturbatory reconditioning, in which subjects masturbate to nondeviant themes followed by fantasizing to deviant themes (Brownell, Hayes, & Barlow, 1977; Marquis, 1970; Marshall, 1979). These techniques are often employed despite limited evidence of their effectiveness or in spite of evidence of their ineffectiveness (Laws & Marshall, 1991; Johnston, Hudson, & Marshall, 1992). The finding that pairing a stimulus with sexual reward (i.e. postejaculatory period) increases the incentive value of sex partners bearing

that stimulus suggest that directed masturbation should be followed by exposure (either real or fantasized) to nondeviant stimuli and may explain the weak effects of satiation and masturbatory reconditioning. Additionally, the findings that pairing a stimulus with sexual frustration (sexual stimulation that does not accompany sexual reward) or with lack of sexual stimulation in a sexual context decreases the incentive value of sex partners bearing that stimulus suggest new venues for conditioning of sexual preferences in clinical treatment. Pairing deviant stimuli with sexual frustration or with the lack of opportunity for sexual stimulation may enhance the effectiveness of directed masturbation to alter sexual preferences.

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