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The Biopsychology of Sexual Motivation in the Male Rat: Effects of Primary and Secondary Incentives

A Dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy in Psychology

by

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ABSTRACT

The Biopsychology of Sexual Motivation in the Male Rat:

Effects of Primary and Secondary Incentives

by

Hassan Habib López

A series of experiments exploring the motivational impact of primary and secondary sexual incentives was conducted using male laboratory rats. In order to measure appetitive processes independent of consummatory ability, an operant paradigm was adopted in which subjects traversed a straight-arm runway in order to approach a variety of stimulus targets. Subjects never experienced sexual reinforcement within the apparatus, thus precluding the establishment of S-R, instrumental associations. The first pair of experiments demonstrated that sexually-naïve adult male rats are inherently attracted to estrous female cues, and that systemic administration of the dopamine-receptor antagonist, haloperidol, attenuates the unconditional incentive value of estrous cues. The second pair of experiments further noted that when male subjects are provided with one copulatory episode of sexual experience, culminating in ejaculation, they are subsequently more motivated to approach an estrous female in

the runway apparatus. However, this incentive enhancement does not occur if subjects are merely given one intromission of sexual experience, or if haloperidol pre-treatment is administered prior to copulatory activity. In a third pair of experiments, subjects were conditioned to associate two previously neutral olfactory scents with either social isolation or copulation with a receptive female. The sex-paired scent (CS+) was subsequently capable of eliciting approach behavior within the runway, an effect that was attenuated by haloperidol pre-treatment. Lastly, in order to identify neural regions that are activated by the perception of sexual incentives, an experiment comparing incentive-induced *c-fos* expression in naïve and experienced subjects was conducted. Results indicated that estrous cues cause enhanced *c-fos* expression in the nucleus accumbens of experienced but not naïve males. Taken together, these results suggest that both primary female and secondary, conditioned incentives activate sexual motivational systems via dopaminergic release, and that reward-mediated incentive-enhancement is also dependent upon dopaminergic activity.

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Chapter I: Introduction

Motivational Theory

Motivational states function to choose and initiate behavioral sequences that increase the probability of achieving a particular goal-state, given current environmental circumstances. Representations of goals may become activated either by internal homeostatic mechanisms sensitive to a particular threshold (e.g., nutrient deprivation) or by the perception of external stimuli associated with a goal (e.g., estrous pheromones). Once a desired goal-state is activated, the organism must determine a course of action that will bring it closer to acquisition of that goal. Motivational systems co-opt suites of decision-making algorithms that continually compare "present-state" with "desired-state", select behaviors that might efficiently diminish the existing discrepancy, and only become quiescent when the two are equivalent. These algorithms may activate representations of sub-goals that are necessary prerequisites, and in turn, recruit the aid of other motivational systems (e.g., hunger stimulating predatory aggression). Motivation, under this formulation, is composed of a set of hierarchically organized information-processing modules that function to increase the survival probability and reproductive success of an individual organism.

Homeostatic regulation of motivation has historically been linked to the notion of an internal "drive" mediating behavior (Hull, 1943). These drives presumably exist to ensure the organism engages in specific, goal-directed behaviors on a periodic basis in order to fulfill physiological needs essential for survival. In contrast, "incentive-motivation" theory stresses behavioral responsiveness to goal-

related, environmental stimuli (Bindra, 1976; Bolles, 1975; Toates, 1986; Tolman, 1932). The perception of reward-predictive cues tends to stimulate approach behavior. Incentive-mediated processes function to bring an organism into consummatory contact with a desirable goal once it is in relative close spatial proximity, and perhaps to encourage an organism to take advantage of opportune circumstances, even if physiological requirements are not dire. Modern motivational theorists tend to synthesize external (incentive) and internal (homeostatic)-driven mediators of behavior, in an attempt to portray a comprehensive explanation of goal-directed activity (Bolles, 1975; Dickinson & Balleine, 1994; Wilson et al., 1995).

A possible exception to this may be sexual motivation, as current theoretical speculations within this domain tend to be driven by incentive-motivational hypotheses (Agmo, 1999; Pfaus, 1996; Stewart, 1995). The reason for this bias is most likely rooted in the fact that copulation is not required for individual survival, and consequently, no obvious homeostatic mechanism that increasingly encourages sexual activity after periods of deprivation has been discovered. In addition, copulation often requires a complex behavioral interaction between two organisms, such that individual "drive" is considered an inadequate determinant. The existence of more observable "sexual signals", emitted by male and female members of many species, also lends credence to the notion that the perception of external incentives is fundamental in the generation of sexual motivation.

Sexual Motivation: Theoretical Considerations

The ultimate goal of a sexually-motivated organism is copulation with a designated sex-object. What constitutes "sex-object" differs between species, sexes, and often even individuals within a species. This dissertation specifically focuses on heterosexual motivation within the adult, male laboratory rat. Use of a non-human species allows for examination of neurobiological substrates, but introduces the complication of finding an accurate, observable measure of a predominantly "inner" process. Motivation is generally viewed as an intervening variable between a homeostatic trigger and/or sensory perception of relevant incentives, and the activation of appropriate goal-seeking behavioral programs. Therefore, the state of being sexually motivated presumably exists throughout the process of seeking a receptive mate and the copulatory act itself. If researchers are interested in studying those processes or neural correlates that underlie male sexual motivation, independent of those that control the actual sexual act, it is in their best interest to examine behaviors that immediately precede copulation.

The theoretical dichotomy between sexual desire and performance mimics the classic ethological distinction between appetitive and consummatory behaviors (Everitt, 1990; Krebs & Davies, 1993). Appetitive behaviors are reflective of a motivational state insofar as they are correlated with goal-seeking behavior. They tend to be more labile, dependent upon environmental input, and subject to variation between individuals (Pfaus, 1996). Consummatory responses are initiated once an organism comes into contact with a desired goal, and often consist of stereotyped

behavioral patterns reflecting activation of a fixed motor program. Precopulatory courtship behavior would be considered appetitive, while the mammalian male pattern of mounting a female followed by repeated penile intromission is clearly consummatory.

This distinction between appetitive and consummatory processes spawned Frank Beach's proposal (1956) that male sexual behavior is determined by the operation of two relatively independent mechanisms: a sexual arousal mechanism (SAM) and an intromission and ejaculatory mechanism (IEM). The function of SAM is to bring sexual excitement to a critical threshold at which point the IEM is activated, coordinating a sequence of mounts and intromissions culminating in ejaculation. This formulation has more or less endured to the present, although a number of reappraisals and revisions have been proposed. Most notably, Sachs and Barfield (1976) conducted a detailed factor analysis of ten measures of male copulatory behavior, and concluded that four factors, representing four relatively independent processes, underlie the entire sexual response: initiation, copulatory rate, hit rate, and intromission count. The initiation factor was virtually identical to Beach's SAM, while the IEM was now divided into three interactive mechanisms that regulate male consummatory sexual behavior.

Beach's basic theoretical formulation has shaped many neuroscientists' approaches to studying male sexual motivation. It is generally believed that neurological systems underlying appetitive processes are distinct from those that coordinate copulation itself (Everitt, 1990). Both neuroanatomical and

pharmacological manipulations can alter motivation independent of performance, and vice-versa. To explore this dichotomy, researchers have adopted a number of behavioral variables that they believe reflect motivational intensity independent of copulatory ability.

Methodological Issues

Male rats do not engage in a stereotyped courtship pattern prior to copulation that can be quantified and interpreted as an index of motivational intensity. Although males often display a certain amount of female-investigation (especially anogenital) and pursuit prior to their first mount, these behaviors vary widely between individuals and are highly dependent upon the responsiveness of the female. For these reasons, researchers studying sexual motivation in the male rat have applied one of three methodological strategies.

The first targets particular aspects of male copulatory behavior as indices of preceding motivation. Many studies have analyzed mount, intromission and ejaculation latencies, as well as post-ejaculatory intervals (Hull et al., 1986; Melis & Argiolas, 1995; Pfaus & Phillips, 1989). The underlying assumption is that highly motivated males will take less time to initiate copulation and achieve ejaculation. However, not only are these variables easily influenced by female motivation and receptivity, but they also confound appetitive processes with performance and arousal aspects of copulation (Everitt, 1990). In response to these critiques, many laboratories have developed methodologies that minimize the role of female

responsiveness and equate male sexual motivation with a well-defined behavioral output. The two most widely used approaches are partner preference and operant response paradigms.

Preference studies typically involve simultaneously presenting subjects with the stimulus properties of two target animals within a preference apparatus. The targets are located in different areas, and are often harnessed behind mesh barriers so as to prevent physical interaction between subject and target. Choice of one partner, as reflected by a greater amount of time spent in the vicinity of that target, indicates a higher motivation to associate with that partner. Thus, if a male rat spends more time around an estrous female than a nonestrous female, he is expressing an increase in sexual motivation generated by perception of estrous cues. However, preference scenarios present a difficult interpretive problem (Everitt, 1990). Choice of an estrous female over a nonestrous female may indicate either that the stimulus cues of the estrous target are positively motivating, or that they are merely less aversive than those of the nonestrous target. This criticism is particularly applicable in scenarios where subject males are given the choice between an estrous female and another male (e.g., Eliasson & Meyerson, 1981; Vega Matuszczyk, Appa, & Larsson, 1994; Vega Matuszczyk & Larsson, 1993).

Operant procedures generally eliminate this drawback by providing only one target for motivational arousal. A number of procedures and apparatuses have been utilized in the study of sexual motivation, including obstruction-grids (Moss, 1924; Warner, 1927), lever-pressing chambers (Everitt et al., 1987; Everitt & Stacey, 1987;

Jowaisas et al., 1971; Schwartz, 1956), X-mazes (which combine aspects of partner preference and operant emission; Hull et al., 1991; Warner et al., 1991), bi-level chambers (Mendelson & Gorzalka, 1987; Mendelson & Pfaus, 1989; Van Furth & Van Ree, 1996ab), and straight-arm runways (Beach & Jordan, 1956; Sheffield, Wulff, & Backer, 1951; Ware, 1968). Each procedure requires that subjects emit an arbitrary behavioral response in order to obtain access to a receptive female. The reinforcing properties of copulation and ejaculation increase the subsequent probability of the instrumental response under test conditions. Following training, the rate/intensity/strength of the operant behavior becomes a measure of the animal's motivation to copulate with the female target. These procedures possess the advantage of measuring subjects' motivation outside of a choice-scenario. However, because copulatory experience is used as a reinforcer, they suffer from two essential flaws: 1) they do not allow for the analysis of sexual motivation in sexually-naive males, and 2) they introduce a critical confound into the interpretation of data. Specifically, subjects' motivation to gain access to a female on a given trial may be dependent on the incentive value of perceived estrous cues (present within the apparatus), conditioned incentives, and the past history of reinforcement within that environment. Dissociating these multiple factors is crucial if one hopes to understand the effect that individual motivational variables have on male sexual biopsychology.

To circumvent this array of difficulties in our research program, we have developed an operant runway task that does not require extensive training or the introduction of copulatory reinforcement. In addition, the task incorporates aspects of

place preference methodology (i.e., approaching a place associated with incentive cues) but does not involve a choice between targets. For each of the experiments discussed in Chapers II and III, subjects are presented with comparable procedural scenarios. Prior to each trial, they are pre-exposed to a target animal located within the runway goalbox. A Plexiglas partition within the goalbox allows subjects to perceive visual, olfactory and auditory cues emitted by the target, but prevents them from engaging in physical contact. Subjects are removed after a set period of time and placed in the runway's startbox. Their motivation to return to the goalbox and approach the target within is objectively assessed by recording their "run time" down the alley. Using this methodology, it becomes possible to isolate the behavioral impact of numerous motivational factors, including primary female incentives, sexual experience, and with some modifications, conditioned incentives (as discussed in Chapter IV).

Factors that Influence Male Sexual Motivation

Numerous specific factors influence the activation of male sexual motivation, such that reproductive behavior only occurs under certain conditions. Some factors are internal, in the sense that they are rooted in the physiological state of the organism, and may further be categorized as static (e.g., male-typical sexually-dimorphic brain regions) or dynamic (e.g., changing levels of steroid hormones). These factors often bias the interpretation of perceived external stimuli within the local environment of an organism that signal the possibility of goal-attainment

(Stewart, 1995). This dissertation focuses primarily on the motivational impact of perceived sexual incentives, and the effect that one internal factor (degree of sexual experience) has on the value of such cues. However, it is important to note that many other factors influence male sexual motivation; while these factors are not explicitly studied within the following experiments, their effect is consistently present and the patterns of results found may reflect their influence alongside that of the variables under examination. For this reason, a brief discussion of these alternate factors, and how they were controlled for, follows.

Internal Factors

a. Other motivational states

Motivational states are continuously interacting in the generation of behavior. As such, numerous other motivational processes may affect sexual desire, positively and negatively. Perhaps the most common examples of this in the experimental setting are the inhibitory effects of fear and stress. Male rats are less likely to initiate copulation in novel environments, where they may experience anxiety over potential danger (Pfaus & Wilkins, 1995). If potential predators (e.g., human researchers) or unfamiliar male rats are in close proximity, caution may negate any desire for copulation until conditions are deemed safer. Extreme hunger, thirst, or fatigue may also reduce sexual motivation. To control for these effects in the following experiments, all animals were given free access to food and water, were handled for several days by all researchers upon arrival, were acclimated to all experimental-

environments and apparatuses prior to testing, and were always transported between the vivarium and the laboratory in individual holding containers.

b. Degree of sexual experience

Male rats with sexual experience tend to be more motivated to engage in copulatory behavior with female rats, when compared to sexually-naïve males. The evidence and meaning of this effect is discussed in greater detail in a later section of this chapter.

c. Degree of sexual satiation/deprivation

Male rats experience a significant reduction in sexual motivation immediately following ejaculation with a female partner (Pfaus, 1996). During this "refractory period," males are generally unresponsive to female solicitation and are, to some degree, physiologically incapable of continuing copulation. In addition, male rats will achieve "sexual exhaustion" if given unrestricted access to a receptive female, following 10 to 15 ejaculations. Alternately, if a male rat is allowed to intromit with a receptive female but is prevented from ejaculating, he will experience an increase in sexual motivation until he is allowed to complete the copulatory sequence (Sheffield, Wulff, & Backer, 1951).

Most motivational states increase in intensity from the last occurrence of goal-satisfaction. For example, an organism feels hungrier the longer it has been since last feeding, and a greater desire to rest since the last occurrence of sleep (Finan & Taylor, 1940). Sexual deprivation has also been shown to impact sexual motivation under certain experimental circumstances, which suggests the existence of a hidden

"sexual drive" (Lorenz & Leyhausen, 1973; Warner, 1927). In order to control for this, when subjects in the following experiments (particularly within Chapter III) were given copulatory experience, motivational testing occurred after an equivalent amount of time had passed for all subjects.

d. Age of male

Sexual motivation varies across the life-span in male rats (Eliasson & Meyerson, 1981; Spruijt, Meyerson, & Hoglund, 1989; Vega Matuszczyk, Appa, Larsson, 1994). Prior to puberty, males express little interest in sexual activity or estrous females, in comparison to their interest in parental figures or other male conspecifics. Motivation reaches its peak during adolescence, when testosterone secretion is highest. As old age approaches, sexual motivation declines steadily; senescent mammals express less interest in sexual activity and suffer more difficulties in sexual performance. For these reasons, all subject males in the following experiments were within the late adolescent, early adulthood age-range (70-150 days old).

e. Individual variation

Mating tests reveal that male rats of similar age and health nonetheless vary in their sexual motivation, as measured by mount latency, run times in an alley, etc. In addition, roughly 20-40% of male laboratory rats persistently fail to mate despite numerous opportunities with a receptive female (Whalen, 1964). The proximate causes of these variations in motivation between individuals are unknown, although numerous theories have been proposed, including differences in basal testosterone,

social status, and emotionality (Pottier & Baran, 1973). In studies of male sexual motivation, such "non-copulators" are often dropped from the experiment following failure in an initial mating pre-test. This tradition was abandoned in these dissertation experiments for two reasons: 1) if one wishes to study sexual motivation in sexually-naïve males, copulatory pre-tests are precluded, and 2) this tradition potentially excludes low-motivation subjects, creating an interpretive bias.

External Factors

a. Primary Incentives

Primary incentives are defined as those stimulus features of a goal that have become tagged with either positive or negative valence depending upon whether they are predictive of either a rewarding or aversive experience. From a cognitive-evolutionary perspective, it seems reasonable to assume that if particular behavioral interactions with specific objects reliably grant fitness benefits to an organism, then that organism should possess information-filtering mechanisms that grant certain classes of goal-related stimuli privileged access to domain-specific motivational centers. Thus, incentives are defined by their adaptive significance, and provide a link between the ecological history of a species and the design features of an individual mind. In reference to the heterosexual motivation of male rats, certain stimuli associated with the presence of female rats are considered primary sexual incentives. These include both general feminine cues (Beach, 1942), and those specifically emitted by females in "heat." Spontaneous ovulators, such as rats, engage in a periodic hormonal cycle that is directly tied with their sexual and

reproductive status. Increasing levels of the ovarian hormone estradiol cause a surge in lutenizing hormone every 4-5 days within the female rat, initiating the process of ovulation (Feder, 1981). It is at this point that the female enters the estrus state. She experiences an increase in sexual motivation (Erskine, 1989; Meyerson & Lindstrom, 1973; Warner, 1927) and in sexual receptivity – i.e., her willingness to engage in copulation with a male rat (Beach, 1976; Boling and Blandau, 1939; Whalen, 1974). She also begins to emit specific cues across a variety of sensory domains, including vaginal pheromones and behavioral proceptive displays (hop-darts and ear-wiggles; Beach, 1976).

Adult male rats display a consistent and replicable preference for estrous females over nonestrous females, presumably due to the positive incentive value of those behavioral and physiological cues associated with estrus (Beach, 1956, 1976). Males spend more time in the vicinity of an estrous female, even if direct physical contact with the female is prevented (Hetta & Meyerson, 1978; Landauer, Wiese, & Carr, 1977; Merkx, 1983), and prefer the odor of an estrous female over that of a nonestrous female (Carr, Loeb, & Dissinger, 1965; LeMagnen, 1952; Lydell & Doty, 1972; Stern, 1970). Sexually-experienced, sexually-naive, and castrated males are all more likely to initiate copulatory sequences with females displaying proceptive behaviors than with nondisplaying females (Hlinak & Madlafousek, 1972; Landau & Madden, 1983; Madlafousek, Hlinak, & Beran, 1976; Tiefer, 1969). However, there is debate over whether such cues are inherently attractive to adult males or acquire

incentive value following sexual experience; Chapter II focuses on this central issue and provides fairly compelling evidence in favor of the former hypothesis.

b. Attractiveness of female

Individual females of any species differ in their genotype and phenotype, resulting in variations in mate-value. Female mate-value, or "attractiveness", in large part is dependent on the perceived reproductive value and/or fertility of a particular female (Symons, 1979). Thus, cues of post-pubescent age, health, and reproductive viability (such as previous successful pregnancies) may sexually motivate males of many mammalian species, including rats. Unfortunately, while a great deal of intriguing psychological work has been conducted on the nature of human female attractiveness (Buss, 1994; Symons, 1979), we have no idea what role this factor plays in the motivation of male rats. A number of preference experiments (e.g., comparing a subject's desire to approach old vs. young females, or healthy vs. sick females) could be conducted to identify those physical characteristics that determine relative attractiveness. In the following experiments, all females fell within the post-pubescent, fertile age-range (70-180 days), and noticeably sick females were immediately excluded from further experimentation.

c. Novelty of female

Male rats given unlimited exposure to a receptive female will achieve sexual satiation following 10 to 15 ejaculations. At that point, the male will not initiate any further copulatory behavior with that female, but if a novel female is introduced he will rapidly begin a new mounting sequence (Fisher, 1962; Tiefer, 1969; Wilson,

Kuehn, & Beach, 1963). This is the commonly known "Coolidge effect," and is strongest in species where females within the community come into estrus together and male parental investment is relatively low (Daly & Wilson, 1983). Presumably it evolved because males that were sexually-aroused by female novelty and were physiologically capable of copulating with a variety of different females during this time period would have accrued significant reproductive benefits compared to males that limited their copulatory advances to a small sub-set of the available females. The Coolidge effect requires that males be able to distinguish between individual females, recognize females with whom they have mated, and be attracted to females that possess novel characteristics. However, it is not clear to what extent female novelty plays a role in the sexual motivation of a non-satiated male. The experiments detailed in Chapter IV utilize a methodology in which male subjects were given multiple sexual episodes over a period of several days. To maximize the probability of inducing copulation, a different female was paired with the male for each session; each subject experienced five sexual episodes with five different females.

d. Secondary incentives

The definition and role of secondary incentives are discussed in detail in the next section, "The Role of Sexual Experience."

e. General environmental conditions

Rats are nocturnal creatures, and generally engage in copulatory behavior at night, when light cues are absent (Wallen & Turek, 1981). Thus, sexual behavior and, presumably, motivation follow circadian rhythms (Stefanick, 1983). Within the

laboratory, the presence of white light often disrupts the initiation of copulation, such that mating tests are typically scheduled during the dark portion of the rats' photoperiod and conducted under red light (so that the experimenters can observe the behavior). This protocol was adhered to throughout the following experiments.

The Role of Sexual Experience

It has been well-documented that sexually-experienced male rats initiate copulation sooner than inexperienced males (Dewsbury, 1969; Rabedeau & Whalen, 1959). They are also less sensitive to losses of sensory information (Beach, 1942). For example, sexually-experienced males that have been rendered anosmic will continue to approach, mount, and complete copulatory sequences with receptive females, while anosmic sexually-naive males will not (Larsson, 1975; Saito & Moltz, 1986; Thor & Flannelly, 1977). In addition, sexually-experienced males are less sensitive to the anxiety-provoking effects of novel environments (Pfaus & Wilkins, 1995), to the loss of testosterone following castration (Mitchell & Stewart, 1989), and to lesions of the medial preoptic area of the hypothalamus (Allendash & Gorski, 1983; DeJonge et al., 1989).

These observations suggest that sexual experience impacts motivation by either increasing the incentive value of female cues or reducing a hypothetical "copulatory threshold" (Beach, 1942) through sexual reward-mediated processes. It is certainly true that copulation acts as an effective reinforcer within a variety of operant paradigms (Beach & Jordan, 1956; Everitt et al., 1987; Everitt & Stacey,

1987; Hull et al., 1991; Jowaisas et al., 1971; Moss, 1924; Schwartz, 1956; Sheffield, Wulff, & Backer, 1951; Ware, 1968; Warner et al., 1991; Warner, 1927). In addition, ejaculation can establish a reliable place preference in a conditioned place preference apparatus (Agmo & Berenfeld, 1990; Mehrara & Baum, 1990).

This dissertation asserts that sexual experience influences motivational systems through two pathways: the value-enhancement of currently existing incentives and the establishment of new, conditioned incentives. Chapter III presents two experiments that demonstrate the former process. Conditioned (or secondary) incentives are environmental stimuli that become associated with copulation through Pavlovian learning, and thus acquire positive value. The perception of conditioned incentives on subsequent occasions is capable of increasing sexual motivation. For example, Everitt (1990) demonstrated that male rats trained under second-order instrumental conditions learn to bar-press for presentation of a light previously associated with copulation. In addition, male rats will spend a majority of their time on a sex-paired side of a conditioned place-preference apparatus, even if conditioning consists of only a single ejaculation, suggesting that the local environmental cues of the preference apparatus acquire positive value (Agmo & Berenfeld, 1990). Chapter IV presents two experiments that explore the process by which sexual experience establishes a conditioned incentive, and how conditioned incentives stimulate approach behavior.

It should be noted that many researchers within this field make the implicit assumption that all sexual incentives, including estrous cues, are conditioned

incentives. Thus, the distinction between primary and secondary incentives is merely one of proximity to the goal: primary incentives are those stimuli directly tied to the goal-object (such as the smell given off by a food, or the ultrasonic call of an estrous female rat), while secondary incentives are more distal environmental stimuli physically separate from the goal. As I shall discuss in Chapter VI, this distinction is not only trivial, but incorrect.

Dopamine: Incentive Salience, Reinforcement, or Both?

The link between midbrain dopaminergic pathways and motivational processes has been under debate for several decades. While it has been commonly observed that the administration of dopamine-receptor antagonist drugs to laboratory animals tends to inhibit the display of goal-directed, motivated behavior (see Blackburn, Pfaus, & Phillips, 1992; Salamone, 1994 for reviews), there are several possible interpretations of this effect. First, as it is well-established that dopaminergic activity (particularly within the nigrostriatal pathway) plays an essential role in the initiation of voluntary movement, non-specific dopamine-receptor blockade may cause motor impairment not dissimilar to that exhibited by Parkonsonian patients (Everitt, 1990; Melis & Argiolas, 1995). This motor impairment may be interpreted incorrectly as a motivational deficit, since motivation is usually measured by behavioral output.

Second, dopamine-receptor antagonists may disrupt the rewarding or reinforcing effects associated with the goal (e.g., copulation, feeding), and thus

indirectly decrease the motivation of the subject to seek that goal in the future. Indeed, midbrain dopamine systems have been implicated in the reinforcing consequences of numerous natural rewards and drugs of abuse, independent of their role in generating motor responses (for reviews, see Ettenberg, 1989; Koob, 1992; Koob & Goeders, 1989; Phillips, 1984; Wise, 1982; Wise & Rompre, 1989). These conclusions are based in part upon the finding that dopamine-receptor antagonists reduce operant responding for food and water (e.g., Ettenberg & Horvitz, 1990; Gerber, Sing, & Wise, 1981; Mason et al., 1980; Tombaugh, Tombaugh, & Anisman, 1979; Wise et al., 1978; Wise, Spindler, & Legault, 1978), electrical brain stimulation (Fouriezos, Hansson, & Wise, 1978; Fouriezos & Wise, 1976; Gallistel et al., 1982; Stellar & Corbett, 1989; Stellar, Kelley, & Corbett, 1983), amphetamine (Yokel & Wise, 1976), cocaine (de Wit & Wise, 1977; Roberts, Corcoram & Fibiger, 1977), and morphine (Bozarth & Wise, 1981). The pattern of decline in operant responding observed under such conditions mimics that which occurs following removal of a reinforcer, during extinction curves (e.g., Franklin, 1978; Franklin & McCoy, 1979; Gallistel et al., 1982; Wise et al., 1978), suggesting that dopaminergic blockade attenuates the positive reinforcing qualities of both natural and artificial rewards. Based upon these observations, it has been proposed that the euphoric properties of drugs of abuse stem from their ability to activate central dopamine pathways whose primary function is to reinforce adaptive behaviors, such as feeding and copulation (Di Chiara et al., 1993; Nesse & Berridge, 1997).

A third alternative interpretation is that dopamine-receptor blockade directly inhibits motivational centers, possibly by reducing the incentive value of goal-related stimuli. This view, common among contemporary motivational theorists, proposes that mesolimbic dopamine acts as a response signal triggered by the perception of potentially rewarding and/or aversive stimuli, thus leading to activation of systems that mediate approach and avoidance behavior (Berridge & Robinson, 1998; Blackburn, Pfaus, & Phillips, 1992; Blackburn, Phillips, & Fibiger, 1987; Blackburn et al., 1989; Horvitz, 2000; Ikemoto & Panksepp, 1999; Kiyatkin, 1995; Mogenson, Jones, Yim, 1980; Phillips, Pfaus, & Blaha, 1991; Robbins & Everitt, 1996; Salamone, 1994, 1996; Schultz, 1998; Schultz, Dayan, & Montague, 1997; Schultz et al., 1992).

As an example of this process, Shultz and colleagues have conducted a series of experiments in which they record the activity of dopaminergic neurons within the ventral striatum of primates in response to the presentation of a primary reward (food) and a conditioned stimulus predictive of the presence of food (Schultz, 1998; Schultz, Dayan, & Montague, 1997; Schultz et al., 1992). Prior to conditioning, it was noted that dopaminergic activity increased during food consumption. Over the course of training, the dopaminergic signal shifted from the consummatory phase to presentation of the predictive stimulus. Eventually, the signal completely dissipated, presumably reflecting the reduced role of attentional and incentive processes in the performance of a S-R "habit." The authors concluded from this pattern of results that dopamine acts specifically as a signal of unexpected reward. This view has been

expounded by a number of other researchers (Berridge & Robinson, 1998; Horvitz, 2000), as it seems to provide a plausible synthesis of dopamine's role in both reinforcement and incentive-motivation.

Dopamine and Male Sexual Behavior

It certainly seems to be the case that dopamine plays a significant role in the generation of male copulatory behavior and in the mediation of sexual reward (for reviews, see Bitran & Hull, 1987; Everitt, 1990, 1995; Hull et al., 1999; Melis & Argiolis, 1995; Pfaus & Everitt, 1995; Wilson, 1993). Conclusions regarding the dopaminergic regulation of sexual activity have been primarily founded upon the effects of systemic and central administration of dopamine-receptor agonists and antagonists; these treatments tend to stimulate and inhibit male sexuality, respectively. However, behavioral changes following dopaminergic manipulation may reflect alterations in sexual motivation, the capacity to perform, and/or the experience of sexual reward. Indeed, the lack of specific methodologies able to dissociate these variables (as discussed above), and the consequent failure to resolve the exact role of dopamine was a primary justification for the series of experiments detailed within this dissertation. Having said this, prior research does suggest that dopamine may play a specific role in mediating the behavioral-activating effects of sexual incentives and the generation of male sexual motivation.

Dopamine-receptor agonists, such as apomorphine, and the dopamine precursor, l-DOPA, are capable of stimulating copulatory behavior in both sexually

inactive and castrated male rats (Malmnas, 1976, 1977; Scaletta & Hull, 1990; Tagliamonte et al., 1974). Systemic administration of apomorphine also reduces mount latencies and the number of intromissions necessary for ejaculation (Bitran & Hull, 1987). Selective dopamine D2-receptor agonists, such as LY 171555 and LY 163502, stimulate sexual behavior in a similar fashion to apomorphine (Melis & Argiolas, 1995).

In contrast, systemic administration of dopamine-receptor antagonists, such as chlorpromazine, haloperidol, and pimozide, delay or inhibit the initiation of copulation, reduce frequency of intromissions, decrease the total number of ejaculations achieved in a test-situation, and in some cases, increase the post-ejaculatory interval (reviewed in Pfaus & Phillips, 1989). Interestingly, at moderate doses, both haloperidol and pimozide also reduce anticipatory level-changing in a bilevel chamber without having a significant effect on the initiation of copulation (Pfaus & Phillips, 1991). At high doses however, these drugs completely abolish both the initiation of copulation and level-changing behavior (Pfaus & Phillips, 1991). Clozapine and the D2-receptor antagonist, sulpiride, cause dose-dependent delays in the initiation of copulation in male rats, but have no effect on copulation once it has been initiated (Pfaus & Phillips, 1989). These drugs also attenuate level-changing behavior (Pfaus & Phillips, 1991). The selective D2-receptor antagonist, raclopride, abolishes copulatory behavior in male rats, while the selective D1-receptor antagonist, SCH 23390, increases mount and intromission latencies but does not eliminate sexual behavior, even at high doses (Pfaus & Phillips, 1991). Finally, the

mixed D1/D2-receptor antagonist, alpha-flupenthixol, dose-dependently decreases responding for access to a receptive female under a second-order schedule, and prolongs mount and intromission latencies at doses that have no effect on copulatory performance (Everitt, 1990). The different behavioral effects of these various antagonists have been interpreted as indicating that drugs selectively blocking dorsal striatal dopamine receptors predominantly affect copulatory performance, while those blocking ventral-striatal dopamine receptors, such as the atypical neuroleptics, specifically attenuate motivation (Everitt, 1990).

Clearly, what is objectively defined as "motivation" differs across many of these studies, and is often a reflection of more than one component of the male sexual response, or the impact of multiple external and internal factors. For example, many studies interpret facets of sexual performance (such as mount latency) as motivational indicators, potentially confounding the influence of appetitive and consummatory processes. This is particularly problematic, considering the aforementioned importance of dopamine in mediating voluntary movement. For those studies utilizing operant methodologies, the typical decrease in operant responding for access to receptive females observed in male rats following dopamine-receptor blockade (Everitt, 1990; Pfaus & Phillips, 1991) may be due to a direct attenuation of sexual motivation (based on the incentive devaluation of either the test-female or conditioned cues), or may reflect a reduction in the reinforcing properties of copulation that, over trials, extinguishes the instrumental response. The resolution of this issue lies in the need to experimentally isolate the motivational impact of primary

incentives, secondary incentives, and sexual reward, and individually test the effects of dopamine-receptor antagonists on each of these factors. A large portion of this dissertation was geared towards that end, as will be discussed in Chapters IIB, IIIB, and IV.

Neuroanatomy of Sexual Motivation

The neuroanatomical circuitry underlying the male sexual response has been slowly delineated over the past 40 years through application of numerous techniques including neural ablation, electrical stimulation, electrophysiology, localized injection of pharmacological substrates, and immunocytochemical staining of immediate early gene products. The shared goal of these various procedures is assignment of a functional role to distinct populations of neurons, to link the activation of a particular cluster with some aspect of male copulation. Unfortunately, the methodological failings mentioned earlier abound within this literature as well, often making it difficult to determine whether a chosen neural region is primarily tied to appetitive or consummatory processes. Nonetheless, a number of potential brain regions responsible for the initiation of male copulatory behavior have been proposed. These include the central and basolateral nuclei of the amygdala, the medial preoptic area of the hypothalamus (MPOA), the bed nucleus of the stria terminalis (BNST), and the nucleus accumbens (NA).

Central Amygdala

Within rats, olfactory information from both the accessory and main olfactory bulbs is passed onto the central nuclei of the amygdala (Kostarczyk, 1986). This immediately suggests that this region may be an initial processing station for certain sexual incentives, in particular pheromonal odors emitted by estrous females. In support of this hypothesis, it has been found that lesions to the central nucleus of the amygdala lead to reliable deficits in copulatory performance within male rats. Lesioned males decrease their courtship activities and avoid females more than nonlesioned controls, suggesting a specific decrease in sexual motivation (Michal, 1972). In accord with this hypothesis, Rasmusen et al. (1960) noted that male rats with amygdala lesions are less likely to cross an electrified grid in order to reach an estrous female. Lesions to the central amygdala completely suppress copulatory behavior in sexually inexperienced males (Kondo, 1992). Furthermore, lesioned rats do not express reduced ejaculation latencies when allowed to copulate following pre-exposure to an estrous female (presented behind a wire mesh screen so as to prevent physical contact), as non-lesioned controls do (De Jonge et al., 1992). Presumably, the pre-exposure period increases the sexual motivation and arousal of the subjects, and the integrity of the central amygdala is necessary for this "priming" effect to occur.

Electrophysiological recordings have revealed that central amygdala neurons fire selectively when a male subject is presented with a receptive female, but not in non-sexual social circumstances (e.g., for a nonreceptive female or another male) or

in non-social circumstances (e.g., eating, sleeping, exploring; Minerbo et al., 1994). These discharges occur considerably before copulation actually begins, implying that this region is primarily involved in appetitive, motivational processes.

Basolateral Amygdala

In striking contrast to the findings above, lesions to the basolateral nuclei of the amygdala do not affect the normal copulatory behavior of male rats (Harris & Sachs, 1975; Kondo, 1992). Males continue to investigate receptive females, mount and intromit, and achieve ejaculation. However, Everitt and colleagues have proposed that the basolateral amygdala is an essential link in a neural circuit designed to build stimulus-reward associations and establish secondary incentives (Everitt, Cador, & Robbins, 1989; Everitt et al., 1991). In a series of studies, male rats were trained to lever-press within an operant chamber for access to a receptive female. Subsequently, a light was paired with the delivery of sexual reinforcement. Subjects then lever-pressed for presentation of the light under a second-order reinforcement schedule. Bilateral lesions of the basolateral amygdala resulted in a marked decrease in instrumental responses maintained by the light, even though they had no impact upon the unconditioned sexual behavior of the males (Everitt, Cador, & Robbins, 1989). Thus, the basolateral amygdala may contribute to sexual motivation by processing information regarding relevant secondary incentives (i.e., stimuli that have predicted copulation in the past), while the central amygdala processes primary incentives (particularly olfactory cues).

Bed Nucleus of Stria Terminalis and Medial Preoptic Area

Both the BNST and MPOA receive a major afferent input, via the stria terminalis, from the central amygdala, placing them squarely in the center of the neuroanatomical circuit underlying male sexual behavior (Kondo & Yamanouchi, 1995; Simerly & Swanson, 1986). Lesions to the BNST cause partial deficits in the initiation of copulation, as well as more severe deficits in the achievement of ejaculation (Emery & Sachs, 1976). More recently, it was found that lesions to the BNST severely impair the ability of males to demonstrate non-contact erections in response to the presentation of an estrous female, with only moderate effects on copulation (Liu, Salamone, & Sachs, 1997). These studies suggest that the BNST integrates information regarding the presence of sexual incentives, and communicates with the male genitalia in coordination of the erectile response.

Electrical stimulation of the MPOA accelerates copulation by reducing the number of mounts and intromissions preceding ejaculation, as well as reducing the ejaculation latency and post-ejaculatory interval (Malsbury, 1971). In contrast, electrolytic and neurotoxic lesions of the MPOA permanently eliminate copulatory behavior in most male subjects, including mounts, intromissions, and ejaculations (Giantonio, Lund, & Gerall, 1970; Hansen, 1982; Hansen et al., 1982; Heimer & Larsson, 1966; Larsson & Heimer, 1964). Unilateral lesions of the MPOA, when combined with contralateral but not unilateral lesions of the corticomedial amygdala, abolish copulatory behavior in male rats (Kondo & Arai, 1995). Interestingly, bilateral transections of the stria terminalis do not disrupt copulatory behavior as

much as lesions of the medial amygdala, suggesting the existence of a nonstrial pathway flowing from the medial amygdala to the MPOA (Kondo & Yamanouchi, 1995). MPOA lesions also reduce self-grooming and female-pursuit in some male subjects (Paredes, Highland, & Karam, 1993). These observations, along with the fact that neurons within the MPOA fire during both precopulatory pursuit and actual copulation (Shimura, Yamamoto, & Shimokochi, 1994), suggest that the MPOA is critically involved in both motivational and performance dimensions of male sexual behavior.

However, the role of the MPOA has been an area of intense debate, with some experimenters claiming that the region primarily regulates copulatory performance independent of motivation. Several researchers have reported that MPOA-lesioned males continue to vigorously pursue and investigate estrous females, even attempting incomplete mounts without the stereotyped pelvic thrust pattern necessary for intromission (Giantonio, Lund, & Gerall, 1970; Hansen, 1982; Hansen et al., 1982). Similarly, rhesus monkeys with MPOA lesions show increased autosexual behavior when presented with receptive females, but are incapable of successfully mounting them (Slimp, Hart, & Goy, 1978). Lesioned male rats continue to show a preference for estrous females over nonestrous females, although the magnitude of the preference is significantly lower compared to that of nonlesioned controls (Edwards & Einhorn, 1986; Edwards, Walter, & Liang, 1996; Hughes, Everitt, & Herbert, 1990). Lastly, MPOA lesions disrupt neither instrumental responding for presentation of a conditioned cue associated with copulation nor investigation of the

receptive female once she is introduced, while such lesions do abolish mounts, intromissions, and ejaculation (Everitt & Stacey, 1987).

Experiments involving central pharmacological manipulation have further complicated this debate. Central infusions of haloperidol into the MPOA increase both mount and intromission latencies, and decrease anticipatory level-changing in a bilevel chamber, suggesting a strong motivational effect (Pfaus & Phillips, 1991). Central administration of cis-flupenthixol leads to fewer copulatory initiations, a slower copulatory rate, a decreased number of ejaculations, reduced penile erection, and a longer PEI (Pehek et al., 1988; Warner et al., 1991). In addition, males display reduced sexual motivation in an X-maze, as reflected by a reduction in percentage of trials on which they choose a goalbox containing a receptive female over an empty goalbox (Warner et al., 1991). Administration of both the D1-receptor antagonist, SCH-23390, and the D2-receptor antagonist, raclopride, also decreases sexual motivation under these conditions (Moses et al., 1995). Both dopamine agonists and antagonists administered to the MPOA produce only minor alterations in noncopulatory behaviors, such as eating, drinking, and global activity (Pehek et al., 1988). These effects suggest that dopamine-receptor antagonism within the MPOA leads to an overall inhibition of sexual motivation, performance, *and* arousal.

In vivo microdialysis and voltammetry have revealed that extracellular dopamine increases within the MPOA both prior to copulation, when a male is presented with an estrous female behind a mesh barrier, and during copulation (Hull et al., 1995). No increase in dopamine was found when male subjects were presented

with other males, ate a highly palatable food, or exercised in running wheels. Most interestingly, all males that showed increases in dopamine during precopulatory exposure to the female subsequently copulated; this included all intact animals, all testosterone-treated castrates, and 9 of 14 one-week castrates. Dopamine levels did not increase in any male that subsequently failed to copulate, including the remaining one-week, and all two-week castrates. These results provide strong evidence that the MPOA is involved both in copulatory performance and in the generation of sexual motivation, that dopamine released within the MPOA in response to primary incentives (estrous cues) is an integral part of this process, and that testosterone may promote copulation through a permissive action on dopamine release (Hull et al., 1995).

Nucleus Accumbens

The NA is the critical terminal site along the mesolimbic dopamine pathway, and has been implicated in mediating the reinforcing effects of numerous natural rewards and drugs of abuse (Bozarth, 1991). In addition, it seems to play a role in sexual behavior, although whether this role lies in mediating sexual reward and/or motivation is unclear. Electrolytic lesions of the NA cause only minor impairments in copulatory behavior (Barr & Leipheimer, 1993) while neurotoxic lesions induced by 6-hydroxy-dopamine markedly delay the initiation of copulation, an effect enhanced when males are paired with non-proceptive females (Everitt, 1990; Liu, Sachs, and Salamone, 1998). Infusions of amphetamine and apomorphine into the NA dose-dependently reduce mount and intromission latencies, but have no effect on

consummatory measures, such as copulatory rate or number of intromissions (Everitt, 1990). These same treatments also dose-dependently increase the rate of instrumental responding for a receptive female presented under a second-order schedule of sexual reinforcement (Everitt, 1990). In addition, dopamine-receptor agonist infusions to the NA are capable of restoring instrumental responding to baseline levels following basolateral amygdala lesions that radically reduce responding (Everitt, Cador & Robbins, 1989). This has led Everitt and colleagues to propose that the basolateral amygdala and nucleus accumbens comprise an integrated neural circuit responsible for the behaviorally-activating effects of conditioned incentives.

Central infusions of dopamine antagonists into the NA tend to inhibit sexual motivation. Bilateral infusion of haloperidol into the NA reduces anticipatory level-changing in a bilevel chamber without affecting consummatory measures of sexual behavior (Pfaus & Phillips, 1991). Cis-flupenthixol dose-dependently reduces responding under a second-order schedule and increases mount and intromission latencies, while having no further effect on copulatory behavior (Everitt, 1990). The specific D2-receptor antagonist raclopride produces slightly longer mount and intromission latencies when males are paired with receptive and proceptive females; however, when tested with females treated with alpha-flupenthixol, which abolishes proceptive behavior, the majority of male subjects never initiate a copulatory sequence (Everitt, 1990). These results suggest that NA dopaminergic activity may also be involved in regulating the value of primary incentives, such as estrous odor and proceptive displays.

Initial *in vivo* neurochemical tests indicated that dopamine concentrations within the NA rose during male copulatory activity (Pfaus et al., 1990; Pleim et al., 1990). It was later specified that dopamine levels increase significantly when males simply perceive a receptive female (even if she is located behind a mesh barrier preventing physical interaction), and increase further during actual copulation (Damsma et al., 1992; Fiorino, Coury, & Phillips, 1997). Intriguingly, dopamine concentration within the NA falls to baseline levels during periods of sexual satiety, but is augmented following exposure to, and copulation with, a novel female (but not one previously copulated with). These data suggest that dopaminergic fluctuations within the NA directly parallel changes in sexual motivation, and that these changes may account for the well-known Coolidge Effect (Fiorino, Coury, & Phillips, 1997).

Perhaps most interestingly, NA dopamine concentration increases during a male rat's very first exposure to a sexually receptive female (Wenkstern, Pfaus, & Fibiger, 1993). Estrous female odors alone, but not odors of other males or nonestrous females, also induce selective increases in dopamine release within the NA of both sexually naive and experienced males (Louilot et al., 1991; Mitchell & Gratton, 1991, 1992). Repeated exposure to estrous female bedding leads to an increased, or sensitized, dopaminergic response within the NA (Mitchell & Gratton, 1991). Such sensitization to primary female incentives may explain the progressive decrease in mount and intromission latencies typically observed in male rats following repeated sexual experience (Wenkstern, Pfaus, & Fibiger, 1993).

The pattern of accumbal dopamine release observed in microdialysis and voltammetry studies during male sexual behavior suggests the existence of two distinct phases: a small increase in response to the perception of primary and secondary sexual incentives, and a larger, more sustained increase during active copulation (Mitchell & Gratton, 1994). The increased mesolimbic dopamine transmission during the preparatory and consummatory phases of sexual behavior in male rats could serve both to reward ongoing sexual activity and to facilitate preparatory sexual behaviors that are elicited by primary or secondary incentives.

Primary Questions

This survey of the biopsychological literature on the sexual motivation of male rats reveals a number of central questions that remain unanswered. First and foremost, while the perception of female incentives and in particular, estrous cues, seems to be pivotal in the stimulation of male sexual motivation, it remains unclear whether male rats are endowed with the inherent capacity to attribute positive salience to such stimuli, or must learn through copulatory experience that certain feminine cues tend to predict positive sexual outcomes. The field as a whole appears to be biased in the latter direction, as can be seen within the following quotations from relatively recent reviews:

> "Presumably, [wild] rats learn to inhibit their sexual advances toward inappropriate stimuli during adolescence... and learn to associate certain olfactory and pheromonal cues, behavioral responses, and ultrasonic vocalizations exclusively with estrus. Male rats in the laboratory almost never learn to distinguish sexually receptive from nonreceptive females." (Pfaus, 1998)

> "Whether copulation is to be initiated or not, in the inexperienced animal, is determined by the random occurrence of appropriate tactile stimulation. In the experienced animal, the execution of copulatory acts may have been conditioned." (Agmo, 1999)

Thus, it is assumed that a successful copulatory encounter with an estrous female is an essential ingredient in the ontogeny of male sexual motivation. The delicate issue of how the male is supposed to know where/who to turn to in order to achieve that initial goal is carefully avoided through evocation of "random" exploratory behavior. The organism is allowed to possess inherent mechanisms that classify sexual activity as rewarding, as well as a small set of general-purpose learning systems that allows for the establishment of stimulus-response, CS-US, or action-outcome associations. This theoretical stance is not only popular in sexual biopsychology, but throughout the motivational literature:

> "An individual's initial contact with a hedonic reward may occur incidentally, triggering 'liking' as the individual explores its world, sampling food, etc. Such natural rewards have hedonic value presumably because of their survival value in evolutionary history." (Berridge, 1998)

An adaptationist perspective on male sexual motivation finds such a hypothesis untenable due to the vast computational improbability of matching an appropriate (utilitarian) behavioral response to a particular environmental scenario without the aid of motivational predispositions acquired through natural selection. The primary hypothesis of this dissertation was therefore that sexually-naïve male rats would be capable of discriminating between nonestrous and estrous females, and would be

more motivated to approach and engage the latter because of their greater incentive value.

However, it is also clear that sexual experience does have a profound impact on the subsequent copulatory behavior of male rats. It was a further goal of this dissertation to determine whether sexual experience affects motivation, independent of its effects on copulatory efficiency (i.e., a practice effect). Intuitively, it seems as if a change in subsequent sexual motivation could be mediated by two distinct processes: first, the enhancement of primary female incentives (or those cues associated with the female herself). A memory of the rewarding experience might then be layered upon motivational predispositions that already exist. Second, experience could allow for the establishment of conditioned incentives, or in other words, teach the male that certain recurring environmental stimuli are reliable predictors of copulation and should therefore be approached. The experiments within Chapters III and IV were dedicated to the dissociation of these processes.

The third primary question this dissertation attempted to resolve concerned the role of dopamine in regulating male sexual motivation. The brief review included above indicates that there is a significant degree of debate within the field over whether dopamine is involved in sexual reward (and reinforcement-mediated learning) or incentive-motivation. As discussed, the methodological structures of the vast majority of experiments reviewed do not allow one to dissociate these possibilities. Therefore, we developed a protocol in which sexual motivation can be measured without introducing the confounding effects of copulatory reinforcement.

Peripheral administration of haloperidol, a dopamine-receptor antagonist, under a variety of experimental conditions allowed us to detail dopamine's role in these multiple processes. Specifically, subjects were given haloperidol prior to engaging in copulation to test whether dopaminergic release during sexual activity is involved in mediating the potential motivational changes that transpire. In other experiments, subjects were treated with haloperidol prior to being exposed to either primary female incentives or secondary, conditioned incentives, to determine dopamine's role in mediating behavioral responsiveness to motivationally-salient cues. Systemic administration of a drug unfortunately tells one nothing about which neural locations are involved in the processes under consideration. In lieu of performing a subsequent series of experiments involving the central administration of haloperidol, a study examining *c-fos* induction in male rats following exposure to female incentives was conducted, and is discussed in Chapter VI.

Chapter II: Sexual Motivation in Naïve Male Rats

A. The Inherent Attractiveness of Sexual Incentives

Adult male rats display a consistent and replicable preference for estrous females over nonestrous females, presumably due to the motivating properties of those behavioral and physiological female cues (e.g., pheromones, hop-darts) emitted specifically during estrus (Beach, 1956, 1976). Males spend more time in the vicinity of an estrous female, even if direct physical contact with the female is prevented (Hetta & Meyerson, 1978; Landauer, Wiese, & Carr, 1977; Merkx, 1983), and prefer the odor of an estrous female over that of a nonestrous female (Carr, Loeb, & Dissinger, 1965; LeMagnen, 1952; Lydell & Doty, 1972; Stern, 1970). Sexually-experienced, sexually-naive, and castrated males are all more likely to initiate copulatory sequences with females displaying proceptive behaviors than with nondisplaying females (Hlinak & Madlafousek, 1972; Landau & Madden, 1983; Madlafousek, Hlinak, & Beran, 1976; Tiefer, 1969).

The process by which male rats come to find these female precopulatory cues sexually attractive has remained largely obscure. One possibility is that males come pre-equipped with specialized neuronal mechanisms designed to respond to sexually-relevant sensory information. Beach (1942) reported that sexually-naive males were unconditionally motivated to engage estrous females but not nonestrous females, and that even elimination of a single sensory modality within these males did not eradicate their preference for estrous females. Later studies demonstrated that sexually-naive males do express preferences for estrous over nonestrous females (Landauer, Wiese, & Carr, 1977; Merkx, 1983), estrous female odor over nonestrous female odor (LeMagnen, 1952), and increased sexual motivation in response to

female proceptive displays (Hlinak & Madlafousek, 1972). Recent work by Sachs (1997) has shown that sexually-naive Long-Evans males display noncontact erections in response to inaccessible, estrous females located upwind, even when the females are not visible, indicating that male rats are unconditionally aroused by female precopulatory cues, in particular olfactory pheromonal signals. Perhaps most interestingly, Van Furth and Van Ree (1996a) have demonstrated that sexually-naive Wistar Albino males increase their number of anticipatory level changes in a bi-level chamber over time, if they perceive the presence of estrous odors within the chamber. If "level-changing" behavior is an accurate measure of sexual motivation, as the authors argue, then this suggests that olfactory precopulatory cues unconditionally activate appetitive systems within this strain.

An alternative view is that males learn with repeated sexual experience that estrous cues are associated with positive sexual outcomes, and are punished by failed copulatory attempts with nonreceptive females (e.g., Pfaus, 1996). In support of this proposition, many experiments have documented that sexually inexperienced males do not express estrous odor preferences (Carr at al., 1965; Carr, Loeb, & Wylie, 1966; Landauer, Wiese, & Carr, 1977; Lydell & Doty, 1972; Stern, 1970).

As discussed in Chapter I, the majority of experiments within this field have adopted either partner preference paradigms that intrinsically suffer from interpretive problems, or operant response procedures that preclude the examination of sexual motivation in naïve males. In order to circumvent these difficulties, and potentially resolve some of the discrepancies within this literature, we tested sexually-naïve male

subjects within an operant runway for their motivation to approach one of five potential goalbox targets: an empty goalbox, a conspecific male, a nonestrous female, a receptive but non-proceptive female, and a fully estrous female. We hypothesized that subjects would demonstrate faster run times for estrous females over nonestrous females, for females over males, and for males over an empty goalbox.

Method

Animals

A total of 12 male and 6 female Long-Evans rats were obtained from Charles Rivers Laboratories (Wilmington, MA). The males were 70-90 days old and the females were 90-120 days old at the start of testing. All animals were housed individually in hanging wire cages within a 22°C vivarium environment maintained under a reverse 14:10 light-dark schedule (lights on 2300 h-1300 h). Food (Purina Rat Chow) and water were provided on an *ad libitum* basis.

Prior to arrival at our vivarium, the males were group housed with other males (typically, 10-15 individuals) but did not have access to females. Therefore, they were sexually-naive insofar as they lacked heterosexual (but possibly not homosexual) copulatory experience. In addition, it should be noted that in the Charles Rivers facilities, males and females are group housed within the same room, such that it is possible that males receive exposure to cues of estrous (e.g., pheromones) prior to use in the following experiments.

Surgery

Females were ovariectomized (OVX) through a single lower abdominal incision two months prior to testing using standard aseptic surgical techniques and under deep anesthesia. Atropine (0.3 mg/kg; Pittman-Moore, Washington Crossing, NJ) was administered intraperitoneally (IP) before anesthesia to reduce potential respiratory problems. Each animal received 30 mg/kg IP sodium pentobarbital (Nembutal: Pittman-Moore) 15 minutes after atropine treatment. Supplementary doses of Nembutal and methoxyflurane (Metofane: Pittman-Moore) were administered during surgery as needed.

Apparatus

The test apparatus was a straight-arm runway consisting of a startbox (25 cm x 25 cm x 20 cm), an alley (160 cm x 10 cm x 20 cm), and a cylindrical Plexiglas goalbox (45 cm diameter, 40 cm height). Removable doors were located between the startbox and alley, and between the alley and goalbox. Infrared photocell emitter-detector pairs were located within the alley just outside the startbox and just inside the goalbox. Interruption of the photobeam outside the startbox initiated a timer that stopped when the subject entered the goalbox. This apparatus is comparable to that used successfully by our laboratory for studying other motivating goalbox events including food (Chausmer & Ettenberg, 1997; Ettenberg & Camp, 1986a; Horvitz & Ettenberg, 1989; McFarland & Ettenberg, 1998), water (Ettenberg & Camp, 1986b; Ettenberg & Horvitz, 1990), and drugs of abuse (Ettenberg & Geist, 1993; Ettenberg, MacConnell, & Geist, 1996; McFarland & Ettenberg, 1995, 1997). Within the

goalbox, a removable Plexiglas partition divided the arena into two semi-circular halves. Sixteen 1.2 cm diameter holes drilled into the partition and spaced 8 cm apart from one another allowed air to pass between the two sides. Thus, the partition prevented even minimal tactile contact between subject and target, although visual, auditory and olfactory cues were accessible.

Procedure

On two separate days, all 12 subjects were allowed to individually explore the entire apparatus for 10 minutes. The targets (three females and one male) were each given two 10 minute exposures to the goalbox. This was done to acclimate the animals to the novel runway environment. All testing took place under red light conditions during the dark portion of the rats' photoperiod.

On any given test day, all 12 subjects ran for the same target in the goalbox; only one trial per day per subject was conducted. Before a day's trials, the designated target animal was placed into the goalbox for several minutes. The partition was then introduced into the goalbox, with the target animal placed on the side farthest from the goalbox entrance. At this point, the trials began: first, a subject male was placed into the goalbox on the opposite side of the partition from the target for five minutes. The subject was then removed and immediately placed into the startbox. After 10 seconds, the goal-door and start-door were lifted, and the time required for the subject to traverse the alley was recorded. Once the subject had re-entered the goalbox, the door was closed and the animal was left for one minute before being removed and returned to his home cage. The next subject's trial was then initiated. Testing

continued, one animal at a time, until all 12 subject males were run. The order of subjects run was held constant throughout the experiment.

Over the course of five days, subjects ran for five different targets: this constituted one cycle. Every five days a new cycle was begun with the order of target conditions counterbalanced. A total of seven cycles (35 days) was completed with the first cycle regarded as practice and not included in statistical analysis. The five target conditions were as follows: 1) empty goalbox; 2) male; 3) OVX female treated with vehicle (0.1 ml sesame oil injected subcutaneously (SC) 48 and 24 hours before testing, and an additional SC injection of 0.1 ml propylene glycol 4-5 hours before testing); 4) OVX female treated with 15 μg of estradiol benzoate (dissolved in sesame oil and administered SC 48 and 24 hours before testing, with an additional SC injection of 0.1 ml propylene glycol 4-5 hours before testing); and 5) OVX female treated with both 15 μg estradiol benzoate and 500 μg progesterone (estradiol prepared in sesame oil and injected SC 48 and 24 hours before testing, followed by a SC progesterone injection prepared in a vehicle of propylene glycol given 4-5 hours before testing). Steroid hormones were purchased from Sigma Chemical Company, St. Louis, MO. Each of the three target females was rotated through each of the three female conditions twice.

On every test day that entailed the use of a female target, a brief one minute pretest was conducted in another room to assess the female's responsivity to a Long-Evans stimulus male (taken from another experiment). These tests confirmed that non-hormonally treated females (nonreceptive condition) never displayed lordosis or

any proceptive behaviors, females given only estradiol injections (receptive condition) always displayed lordosis upon physical contact with the male but few, if any, proceptive behaviors (always less than five hop-darts and ear-wiggles total), and females given both estradiol and progesterone (receptive+proceptive condition) displayed both lordosis and numerous proceptive behaviors in the space of a minute (always over ten hop-darts and ear-wiggles total).

Dependent Measures

The primary dependent measure in this experiment was run time - i.e., the time elapsed between when the subject left the startbox and entered the goalbox. Lower run times reflect a greater motivation to approach the goalbox target. Past experiments utilizing the runway apparatus have shown that subjects will often stop at some point along the alley, turn around, and return to the startbox rather than continue directly to the goalbox. These "retreats" (Ettenberg & Geist, 1993) are presumed to reflect the subject's motivational state insofar as they have been shown to increase in number as the valence of the goalbox experience becomes less positive (Geist & Ettenberg, 1997). The total number of retreats produced by each subject on each trial was also recorded.

In addition, when the target female had received both estradiol and progesterone injections, the number of proceptive behaviors (hop-darts and ear-wiggles) displayed to the male subject during the five-minute exposure period prior to running was recorded. No female in either of the other two conditions (no hormones or estradiol-treated) ever displayed proceptivity behind the Plexiglas partition.

Results

Run Time

Each subject male ran for every goalbox target six separate times. To assess whether operant running for the same target changed over the course of the experiment, a one-way repeated measures ANOVA was computed on the mean run times of the single group of subjects across the six replications (i.e., assessing the effects of repeated testing independent of goalbox condition). This ANOVA revealed no reliable effect of repeated testing, $F(5,20)=0.747$, $p=0.598$. Note that this stable performance produced by the one-trial per day protocol is in marked contrast to most operant conditioning studies in which subjects increase the rate/intensity of behavioral responding over the course of the experiment. The data for each of the six replications were therefore averaged for each target condition.

Figure 1, panel A displays the subjects' mean run time as a function of the target condition. A one-way repeated measures ANOVA comparing these means yielded a highly significant main effect of target condition, $F(4, 44) = 7.432$, $p<0.005$. A limited set of planned comparisons (paired-sample, one-tailed, student t-tests) were conducted, comparing subjects's run times for 1) an empty goalbox vs. a male, 2) a male vs. a nonreceptive female, 3) a nonreceptive female vs. a receptive/proceptive female, and 4) a receptive female vs. a receptive/proceptive female. These analyses revealed a significant difference in run times between the male and nonreceptive female condition, $t(11)=1.938$, $p=0.04$, and between the nonreceptive female and receptive/proceptive female, $t(11)=2.391$, $p=0.018$.

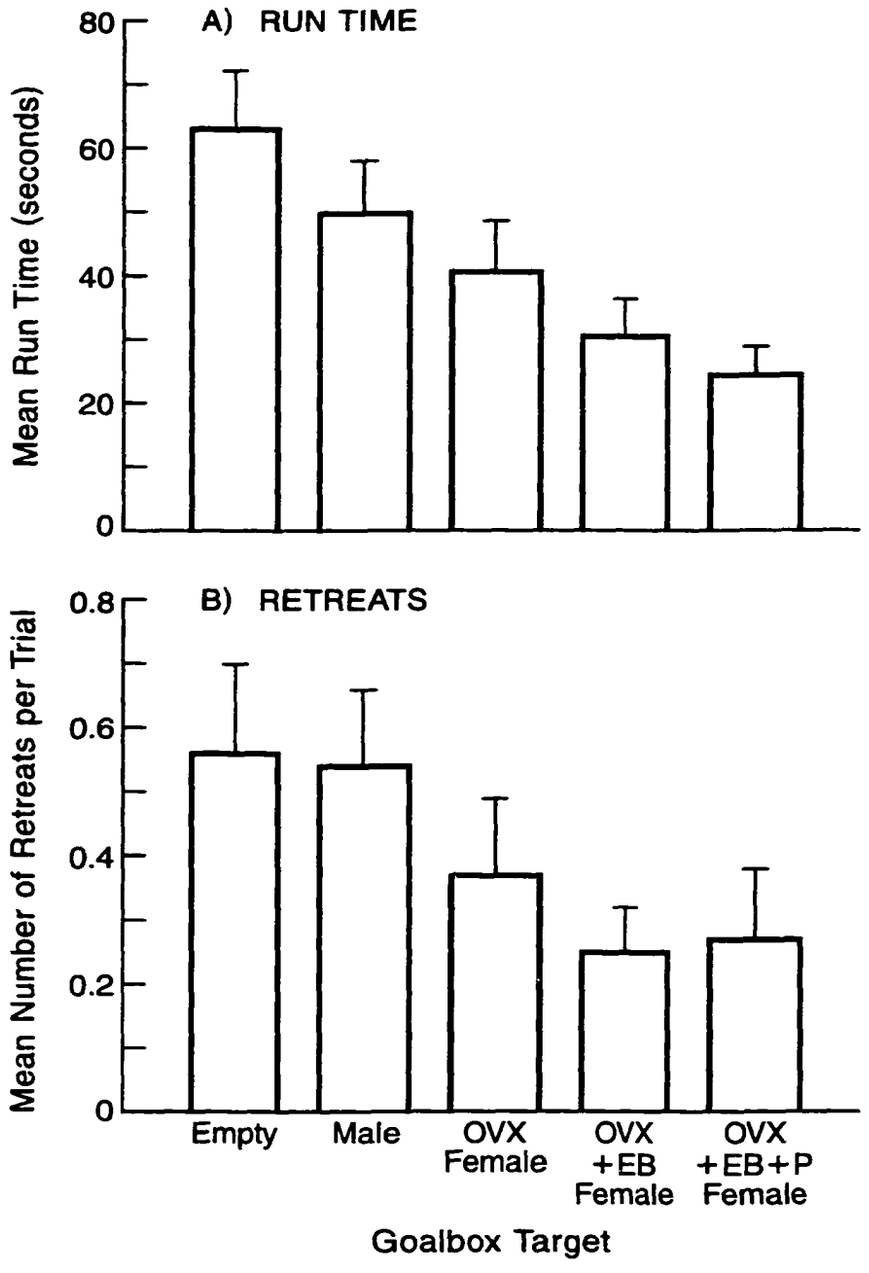

Figure 1. Mean (+SEM) performance of 12 subject males for each of the five goalbox targets (averaged across six trials). Panel A (top) depicts mean run times while panel B (bottom) depicts the mean number of retreats per trial.

Retreats

Figure 1, panel B illustrates the mean number of runway "retreats" exhibited by the subjects within the alley for each condition. A one-way repeated measures ANOVA on these data revealed a significant main effect of target condition, $F(4, 44) = 3.150$, $p=0.023$. Further analysis revealed that subjects committed significantly more retreats when running for a male target than when running for a nonreceptive female target, $t(11)=2.366$, $p=0.018$.

Proceptive Displays

Only one of the three female targets, when given the estradiol+progesterone hormone treatment, emitted proceptive displays in the presence of a male subject on the opposite side of the Plexiglas barrier. Specifically, the target female displayed to seven of the subject males: the mean (±SEM) run time of this subgroup was 11.8 (±1.48) seconds. The five remaining subjects were not exposed to any proceptive behaviors over the course of these two trials: the mean (±SEM) run time of this subgroup was 29.7 (±9.94) seconds. An independent samples, one-tailed *t*-test comparing the means of these two subgroups was computed and yielded a significant difference in run times, $t(10)=2.125$, $p=0.030$.

Discussion

The present data confirm that running behavior within an alley can be a useful index of a subject's motivation to seek a goal stimulus, even when the subject is not allowed to engage in consummatory behavior within the goalbox. The present results

also confirm previous findings that copulatory experience is not necessary for males to prefer the proximity of estrous over nonestrous females (Hetta & Meyerson, 1978; Landauer, Wiese, & Carr, 1977; Merkx, 1983), or estrous females over other males (Eliasson & Meyerson, 1981; Vega Matuszczyk, Appa, & Larsson, 1994; Vega Matuszczyk & Larsson, 1993). However, the experiments conducted here were able to address the interpretive difficulties of the preference literature in two important ways. Analysis of the retreat data (see Fig 1, panel B), which are reflective of the subjects' ambivalence about entering the goalbox (Ettenberg & Geist, 1993; Geist & Ettenberg, 1997), suggests that faster running for female targets compared to male targets might indeed be due to a negative motivational state associated with returning to a place in which a male target is located. In contrast, the faster running of males for a receptive+proceptive female over a nonreceptive female cannot easily be accounted for by differences in the negative valence of the targets since there was not a significant difference in the mean numbers of retreats subjects' exhibited when running for these targets. Procedurally, the current operant runway approach has the additional advantage over preference methods in that run times expressed for each of the target conditions can be compared to an appropriate control condition (i.e., an empty goal-box).

Van Furth and Van Ree (1996a) have argued that many of the conflicting results in this literature can be explained by strain differences in the regulation of masculine sexual arousal. Specifically, sexually-naive Sprague-Dawley and Long-Evans males fail to demonstrate a preference for estrous females, whereas sexually-

naive Wistar males do. Data from the present experiment were inconsistent with this distinction, in that sexually-naive Long-Evans males did demonstrate a greater motivation to return to a goalbox containing an estrous female versus a nonestrous female. However, there may be strain differences in pheromonal sensitivity. Sexually-naive Long-Evans males do not show preferences for the mere odor of estrous females (Carr at al., 1965; Carr, Loeb, & Wylie, 1966; Landauer, Wiese, & Carr, 1977; Lydell & Doty, 1972), while naive Wistar males do (LeMagnen, 1952). It is possible that Long-Evans males require more than olfactory sensory information (i.e., visual, auditory, etc.) in order to be sexually motivated by estrous females. However, as stated in the introduction, Sachs (1997) recently demonstrated that olfactory cues are sufficient to provoke noncontact erections in sexually-naive Long-Evans males. These disparate findings suggest the nonintuitive hypothesis that Long-Evans males respond with unconditional arousal (i.e., erections) to pheromones, but are not sexually *motivated* by them (i.e., as reflected by approach behavior).

In Experiment 1, only one female target treated with estradiol and progesterone displayed proceptive behaviors in the proximity of male subjects. This is in stark contrast to the high number of hop-darts and ear-wiggles that females in estrus will normally display when allowed to physically interact with a male (Fadem, Barfield, & Whalen, 1979; Tennent, Smith, & Davidson, 1980; Whalen, 1974), an observation that was confirmed when the estradiol and progesterone-treated female targets were paired with a stimulus male in one-minute pretests. It would seem then that actual physical contact between a male and female is necessary to stimulate the

full expression of these behaviors. Nevertheless, when proceptive displays did occur, subject run times achieved their lowest mean: 11.8 seconds. One interpretation of this finding is that sexually-naive males are unconditionally motivated by female proceptive displays, as others have concluded (Hlinak & Madlafousek, 1972). However, the correlational nature of this analysis does not allow for a firm conclusion regarding a potential causal link between female proceptivity and male sexual motivation.

In contrast to what some researchers have suggested, this experiment successfully demonstrated that sexually-naïve male rats are not only capable of distinguishing between nonestrous and estrous females, but are more motivated to approach the latter (Pfaus, 1996; Agmo, 1999). The next goal of our research program was to test whether dopaminergic activity plays a causal role in mediating subjects' approach behavior towards sexual incentives.

Chapter II: Sexual Motivation in Naïve Male Rats

B. Haloperidol Attenuates Sexual Motivation

Numerous excellent reviews have discussed the importance of dopaminergic transmission for the generation of male sexual motivation (Bitran & Hull, 1987; Everitt, 1990, 1995; Hull et al., 1999; Melis & Argiolis, 1995; Pfaus & Everitt, 1995; Wilson, 1993). In general, researchers note that the administration of dopamine-receptor agonists stimulates male sexual motivation, while antagonists inhibit it. Perhaps most revealing have been the results of recent *in vivo* neurochemical analyses that have correlated central dopaminegic release with several aspects of male sexual behavior, including precopulatory perception of estrous female cues (for reviews, see Mitchell & Gratton, 1994; Phillips, Pfaus, & Blaha, 1991).

However, it remains to be determined exactly how dopamine modulates sexual motivation. Numerous internal factors (hormonal condition, degree of sexual experience, etc.) and external inputs (female pheromones, proceptive displays, sexually-conditioned contextual cues, etc.) influence a male's desire for copulation. While dopamine may indeed regulate the processing of some or all of these factors, research methods employed to study male sexual motivation have often been unable to delineate and isolate their relative impact. The use of copulatory performance variables or sexually-reinforced operant behaviors as indices of motivation have made conclusions regarding dopamine's role ambiguous. It is often unclear as to whether dopamine receptor blockade is directly affecting sexual motivation by reducing the incentive value of primary female cues and/or sexually-conditioned cues, or indirectly reducing sexual motivation by attenuating the rewarding consequences of sexual reinforcement, leading to eventual extinction of the measured operant response. The

use of sexually-experienced subjects is also potentially problematic in that it can confound the motivational impact of primary incentives with the effects of prior sexual experience. By using experienced males, it may be difficult to determine whether experimental manipulations affect the unlearned or *unconditioned* incentive value of primary female cues.

In the current study, we attempted to replicate and extend our earlier findings on the inherent attractiveness of estrous female cues by examining the ability of a dopamine-receptor antagonist to alter this incentive-motivational process. We hypothesized that haloperidol would dose-dependently attenuate the incentive value of estrous female cues, such that male subjects would run equally fast for a nonestrous and estrous female in an operant runway. The effect of drug administration on running for an empty goalbox would serve as a control for the potential motor-debilitating activity of haloperidol.

Method

Animals

A total of 38 male and 3 female Long-Evans rats were obtained from Charles Rivers Laboratories (Wilmington, MA). The males were approximately 100 days old and the females 100-150 days old at the start of testing. All animals were housed individually in hanging wire cages within a 22°C vivarium environment maintained under a reverse 14:10 light-dark schedule (lights on 2300 h-1300 h). Food and water were provided on an *ad libitum* basis. Prior to arrival in the vivarium, the males were

group housed but did not have access to females. Therefore, they were sexually-naive insofar as they lacked heterosexual copulatory experience.

Surgery

All females were ovariectomized (OVX) through a single lower abdominal incision 1-6 weeks prior to testing using standard aseptic surgical techniques and under deep anesthesia. Anesthesia was induced by IP administration of 90 mg/kg ketamine and 2 mg/kg xylazine, in a volume of 1 ml/kg. All females received at least one week post-operative care prior to initiation of the experiment.

Apparatus

The apparatus used in this experiment was the same straight-arm runway described in Chapter IIA.

Procedure

All 38 male subjects were allowed to individually explore the empty runway apparatus for 5-7 minutes on each of two initial trials. The three female targets were also individually placed within the goalbox for 10 minutes on two days. This was done to acclimate the animals to the runway environment. All testing took place under red light conditions during the dark portion of the rats' photoperiod.

On any given test day, all 38 subjects ran for the same target in the goalbox; only one trial per day per subject was conducted. Before a day's trials, the designated target female was placed into the goalbox for 2-3 minutes. The partition was then introduced into the goalbox, with the target female placed on the side farthest from the goalbox entrance. At this point, the trials began: first, a subject male was placed

into the goalbox on the opposite side of the partition from the target female for four minutes. The subject was then removed and immediately placed into the startbox. After 10 seconds, the goal-door and start-door were lifted, and the time required for the subject to traverse the alley was recorded. Once the subject had entered the goalbox, the door was closed and the animal was left for one minute before being removed and returned to his home cage. The next subject's trial was then initiated. This procedure continued, one animal at a time, until all 38 subject males were tested within the runway for their motivation to approach the female target. The order of subjects run was held constant throughout the experiment. The dependent measure of interest was run time - i.e., the time elapsed between the subject's leaving the startbox and entering the goalbox. Shorter run times presumably reflect a greater motivation to approach the goalbox target.

Subjects ran for three different targets: an empty goalbox, a nonestrous female (OVX female) or an estrous female. Estrus was induced via subcutaneous (SC) administration of 15 μg of estradiol benzoate (in 0.1 ml sesame oil) 48 and 24 hours before testing, with an additional SC injection of 500 μg progesterone (in 0.1 ml propylene glycol) 3-5 hours before testing. Steroid hormones were purchased from Sigma Chemical Company, St. Louis, MO. Behavioral estrus was confirmed prior to the days' trials during a brief one minute pretest conducted in another room in which the target female was paired with an adult Long-Evans male (taken from another experiment). Each of the three target females was rotated through each hormonal condition three to four times over the course of the experiment.

Subjects initially ran a total of nine trials (one trial per day), three for each goalbox target, to establish baseline run times. Following these nine trials, the subjects were divided into three groups such that the mean run times for the three baseline goalbox conditions were approximately the same for all groups. Subjects were then re-tested within the runway for their motivation to approach the three goalbox targets, under differing drug conditions. Subjects within the vehicle control group (N=12) were given intraperitoneal (IP) injections of 0.002 M lactic acid vehicle 45 minutes prior to testing. Subjects in the second group (N=13) were pretreated with IP injections of 0.075 mg/kg haloperidol (dissolved in lactic acid vehicle), and subjects in the third group (N=13) were pretreated with IP injections of 0.15 mg/kg haloperidol. All injections were administered in a volume of 1 ml/kg. Subjects were tested under these drug-conditions once for each goalbox target (yielding a total of 3 drug-trials/subject). In between drug trials, subjects were tested under non-drug conditions for each of the three goalbox targets. Thus, the testing schedule following establishment of baseline run times was as follows: drug-day, 3 non-drug days, drug-day, 3 non-drug days, drug-day. The order of haloperidol trials was counterbalanced between groups, as was the goalbox condition, such that one-third of the subjects within each group experienced a different order (empty/nonestrous/estrous, nonestrous/estrous/empty, estrous/empty/nonestrous).

Results

The baseline run times for each group of subjects were nearly equivalent, hence the data were collapsed in order to simplify statistical analysis. Baseline mean (+SEM) run times for all 38 male subjects are displayed in Figure 2, panel A.

A repeated measures one-way ANOVA on the data in panel A revealed a significant effect of goalbox target on subjects' run time, $F(2,37)=30.177$, $p<0.001$. A series of planned comparisons using one-tailed, paired-sample *t*-tests revealed significant differences between subject run times for the empty goalbox and nonestrous female target, $t(37)=3.835$, $p<0.001$, between the empty goalbox and estrous female target, $t(37)=5.493$, $p<0.001$, and between the nonestrous and estrous female targets, $t(37)=3.362$, $p<0.001$.

The mean (+SEM) run times for the three experimental groups while under drug-treatment are displayed in Figure 2, panels B-D. A repeated measures one-way ANOVA comparing run times for the three goalbox targets was conducted on the data depicted in each of these panels. The vehicle group (panel B) behaved comparably to baseline, with the ANOVA confirming a significant main effect of goalbox target, $F(2,11)=5.986$, $p=0.03$, and the pattern of results comparable to that in Figure 2, panel A. For both the 0.075 mg/kg and 0.15 mg/kg haloperidol groups, there was no significant effect of goalbox target on subject run time, $F(2,12)=1.454$, $p=0.251$ and $F(2,12)=0.207$, $p=0.657$ respectively.

Figure 2 seems to indicate that the primary effect of haloperidol was to decrease the subjects' motivation to approach estrous female targets. Three one-way

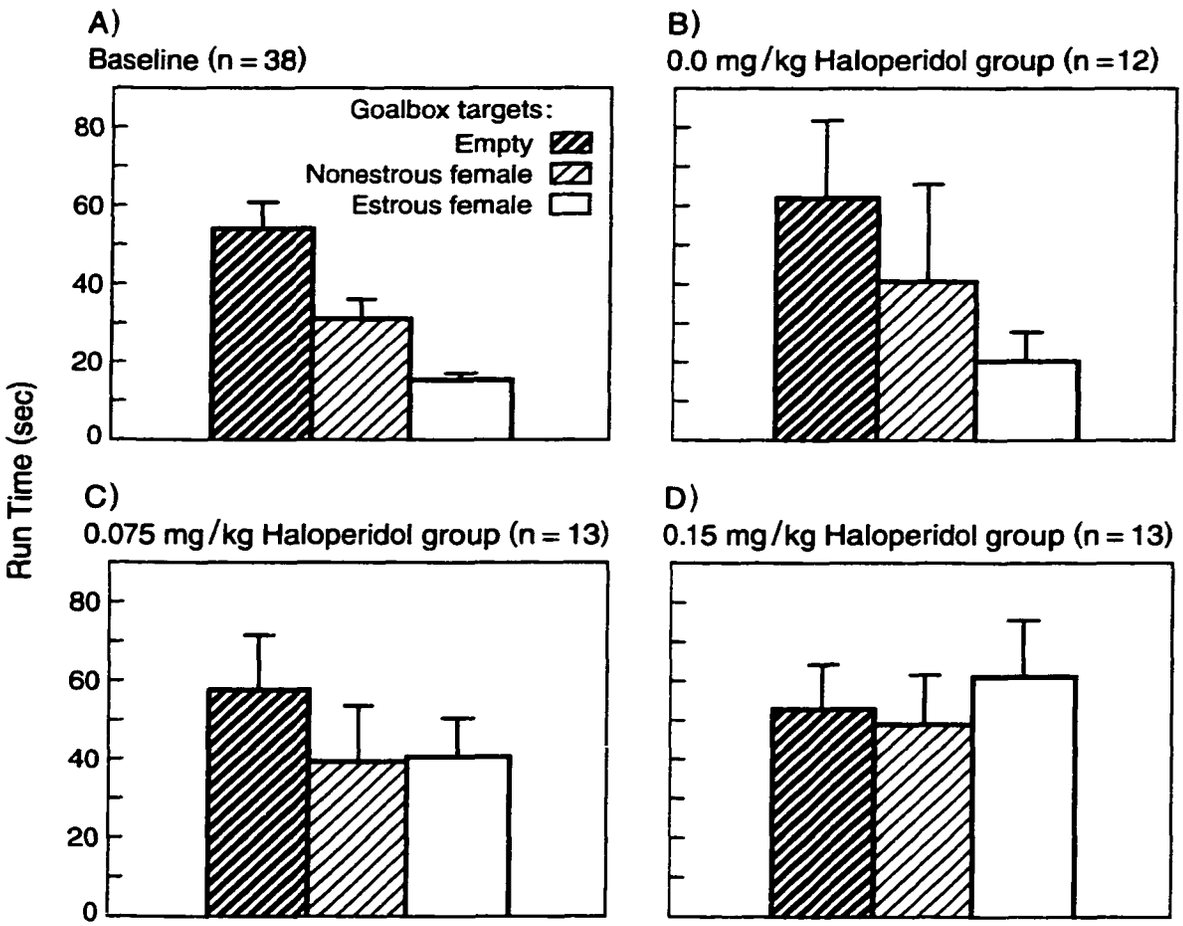

Figure 2. Mean (+SEM) run times of sexually-naïve male rats running for each of three goalbox targets: an empty goalbox, a nonestrous female, and an estrous female. Panel A depicts the baseline run times for all 38 subjects for each of the three goalbox targets. Panels B-D depict run time data from three sub-groups of subjects given haloperidol pre-treatment (0.0, 0.075, or 0.15 mg/kg) 45 minutes prior to testing. Vehicle-treated controls (panel B) continued to motivationally differentiate between the goalbox targets, while drug-treated subjects (panels C and D) experienced a reduction in motivation to approach estrous female targets.

ANOVA's, testing the main effect of drug-dose on run time for each goalbox target, were conducted to test this possibility. There was no significant effect of haloperidol dose on subjects' run times for either the empty goalbox, $F(2,35)=0.089$, $p=0.91$, or the nonestrous female target, $F(2,35)=0.092$, $p=0.91$. However, there was a main effect of drug-dose on mean run time for the estrous female target, $F(2,35)=3.204$, $p=0.05$, indicating that haloperidol reduced the incentive value of estrous female cues. The fact that subjects' run times for the empty goalbox were unaffected by haloperidol pretreatment implies that the drug did not significantly reduce the motoric capacity of the subjects.

Discussion

Sexually-naïve male rats expressed an increased motivation to approach estrous females over nonestrous females, and an increased motivation to approach a female target over an empty goalbox, as reflected by run times in an operant runway. These data replicate prior work done in our laboratory demonstrating an inherent male tendency to be motivated by primary female cues. Our results also reveal that male rats are less motivated to approach female targets when pretreated with the dopamine receptor antagonist, haloperidol. Furthermore, the slowed approach behavior during haloperidol challenge was restricted to the estrous female target condition – there were no changes in the subjects' response to the empty goalbox or nonestrous female.

The fact that subject run times for the empty goalbox were unaffected by haloperidol treatment reduces the likelihood that the observed changes in runway behavior were a consequence of the potential motor-debilitating effects of haloperidol. However, it is possible that haloperidol differentially impaired faster running (for the estrous target) versus slower running (for the empty goalbox). There are several reasons to suspect that this is not the case. First, a number of studies (see Wise, 1982, for a review) have dissociated the performance-debilitating effects of neuroleptics from their motivational or reward actions. For example, the administration of such drugs does not influence the response-initiation latencies nor running speeds of subjects working in an operant runway for access to self-stimulation or food reward on the first few days of training (Horvitz & Ettenberg, 1989; Wise et al., 1978). Slowed running only appears once subjects have had repeated experience with the goalbox reward while drugged, suggesting an extinction-like effect. Indeed, this work was crucial in demonstrating the role of dopamine in reward processes (Wise, 1982). Second, prior work in our laboratory has shown that at the doses used in the current study, haloperidol does not compromise a rat's ability to respond normally in the alley on a single trial (McFarland & Ettenberg, 1995, 1998, 1999). Lastly, during pilot studies aimed at determining an appropriate dosage regimen for this research, we found that higher doses of haloperidol (i.e., 0.30 mg/kg) dramatically retarded operant running, even for an empty goalbox. Thus, the reported baseline run times for an empty goalbox in the current study do not represent a "ceiling" that prevents the detection of motor

impairment. Collectively, these considerations increase our confidence that the effect of haloperidol on the subjects' run times for the nonestrous and estrous female targets in the current study was primarily due to a motivational and not motoric impairment.

This study supports prior conclusions on dopamine's role in the generation of male sexual motivation (Bitran & Hull, 1987; Everitt, 1990, 1995; Hull et al., 1999; Melis & Argiolis, 1995; Pfaus & Everitt, 1995; Wilson, 1993). However, since our male subjects did not receive sexual experience within the test apparatus, we were able to dissociate dopamine's role in regulating the incentive value of primary female cues from that of secondary conditioned incentives (e.g., environmental stimuli) established via sexual reinforcement. Similarly, we were not faced with the need to control for the effects of sexual experience upon sexual motivation, which we have previously shown to dramatically alter male motivation to seek female targets. Thus, by keeping subjects sexually naïve throughout the experiment, and by adopting an operant paradigm that allows for the examination of motivational processes without the introduction of reinforcement, we can safely conclude that haloperidol reduces male sexual motivation by decreasing the unconditioned incentive value of female cues.

This conclusion is supported by recent *in vivo* analyses, utilizing both microdialysis and voltammetry, showing an increase in dopamine levels within the nucleus accumbens during a naïve male rat's *first* exposure to a sexually receptive female (Louilot et al., 1991; Wenkstern, Pfaus & Fibiger, 1993). This response occurs even if the male remains behind a wire-mesh screen and is not allowed to

initiate copulation. Such data strongly suggest that enhanced dopaminergic activity within the nucleus accumbens prior to copulation is an innate, unconditioned neurochemical event. However, *in vivo* observations do not explicitly identify a functional role for this dopaminergic response. Our experiment is the first to illustrate that blocking the postsynaptic effect of dopamine in response to the perception of primary female cues reduces sexual motivation in naïve males, as measured by approach behavior within a runway.

It should be noted that there is at least one alternative explanation of these results. It is not clear whether administration of systemic haloperidol prior to runway trials directly inhibits the sexual motivation of male rats, or indirectly influences it through action on other cognitive systems. For example, it could be the case that dopamine-receptor blockade attenuates the attentional capacities of the subjects such that the salience of the available female cues is reduced (Clark, Geffen, & Geffen, 1987; Horvitz, 2000; Matthysse, 1978; Ragozzino, 2000). In order to rule out this hypothesis, it would be necessary to demonstrate that subjects retained the ability to discriminate between sexual and non-sexual environmental stimuli under haloperidol-challenge.

Chapter III: The Role of Sexual Experience

A. Enhancement of Primary Female Incentives

Compared to sexually-naïve males, experienced male rats are more likely to initiate sexual behavior (Dewsbury, 1969; Fleming & Kucera, 1991) and express shorter mount latencies (Rabedeau & Whalen, 1959) when in the vicinity of a receptive female. One interpretation of these observations is that sexual experience enhances the incentive value of primary female cues through reward-mediated associations such that on subsequent sexual encounters, sexually-experienced males will be more motivated to approach an estrous female and initiate copulation. In order to test this hypothesis, we conducted an experiment comparing the sexual motivation of male rats, when sexually-naïve and when experienced. As in Chapter II, motivation was operationalized as the time taken to traverse an alley and enter a goalbox containing either a nonestrous or estrous female. Sexual experience consisted of a single copulatory experience with a receptive female culminating in either one ejaculation or one mount with intromission. Based on the results of our earlier experiments, we expected that subjects would be more motivated to approach an estrous female versus a nonestrous female when both naïve and experienced. However, experienced subjects should run faster for an estrous female target when compared to naïve males. Presuming that ejaculation is a necessary component of sexual reward, we also hypothesized that only those subjects given one complete copulatory episode with a receptive female, and not those given merely one intromission, would express an increase in sexual motivation following sexual experience.

Method

Animals

A total of 30 male and 11 female Long-Evans rats were obtained from Charles Rivers Laboratories (Wilmington, MA). The males were 70-90 days old and 6 target females 90-120 days old at the start of testing. An additional 5 females (3 to 7 months of age) were used to provide sexual experience for the male subjects. All animals were housed individually in hanging wire cages within a 22°C vivarium environment maintained under a reverse 14:10 light-dark schedule (lights on 2300 h-1300 h). Food and water were provided on an *ad libitum* basis.

Prior to arrival at our vivarium, the males were group housed but did not have access to females. Therefore, they were sexually-naive insofar as they lacked heterosexual (but possibly not homosexual) copulatory experience.

Surgery

Females were ovariectomized (OVX) through a single lower abdominal incision two months prior to testing using standard aseptic surgical techniques and under deep anesthesia. Atropine (0.3 mg/kg; Pittman-Moore, Washington Crossing, NJ) was administered intraperitoneally (IP) before anesthesia to reduce potential respiratory problems. Each animal received 30 mg/kg IP sodium pentobarbital (Nembutal: Pittman-Moore) fifteen minutes after atropine treatment. Supplementary doses of Nembutal and methoxyflurane (Metofane: Pittman-Moore) were administered during the surgery as needed.

Apparatus

The apparatus used in this experiment was the same straight-arm runway described in Chapter IIA.

Procedure

Thirty sexually-naive males were used as subjects, running for one of three different target females, using an identical runway procedure as that described in Chapter IIA. On a given day, all 30 males traversed the alley for the same target. Target females were either nonestrous (OVX and given vehicle injections as in Chapter II) or estrous (OVX and given SC injections of both estradiol and progesterone as in Chapter II). Receptivity of the female target was confirmed during a brief one-minute pretest. Subject males ran a total of six trials, one trial per day (three for a nonestrous target and three for an estrous target) before they were provided with sexual experience. These trials not only established a baseline with which to compare subsequent run times, but also permitted subjects to learn the consequences of the operant task without the introduction of copulatory experience. Following these six trials, the subjects were divided into three groups such that the mean run time for nonestrous targets was approximately the same for all three groups, and the mean run time for estrous targets was approximately equivalent between groups.

Over the course of two days, all three groups were taken to a separate testing arena in order to provide them with a particular experience. Each subject male was tested individually under red light during the dark portion of the photoperiod. The

testing arena was composed of cylindrical Plexiglas, 45 cm diameter by 60 cm height, with the floor of the arena covered with wood-chips. A total of five OVX Long-Evans females, given estradiol and progesterone prior to testing, were used. For one group, each male was individually paired with a receptive female until he achieved one mount with intromission ("One Intromission" group). For another group, each male was paired with a receptive female until he achieved an ejaculation ("One Ejaculation" group). For the last group, each male was placed within the testing arena alone, without a female present, for a total of five minutes ("No Sexual Experience" group). Each subject male was returned to his home cage following his particular experience.

Two days after the final "experience" day, subjects were again tested within the runway for their motivation to approach either nonestrous or estrous female targets (note that while the same three females were used as targets throughout the runway portions of the experiment, none of these target females were used during the sex experience episodes). A total of eight post-sexual experience trials were conducted, one trial per day, four for a nonestrous target, four for an estrous target. Thus, over the course of the entire experiment, each of the thirty subjects ran a total of 14 trials.

Results

Prior to receiving any sexual experience, the mean (±SEM) run time of all 30 male subjects for the nonestrous female target was 53.6 (±7.6) and 32.7 (±4.4)

seconds for the estrous female target. A one-tailed, student *t*-test comparing these means revealed a significant difference, $t(29)=2.670$, $p=0.012$. The mean (+SEM) run times for the three experimental groups are displayed in Figure 3. A mixed two-factor (Pre/Post vs. Receptivity) ANOVA was conducted on the data for each sexual experience condition (i.e., on the data depicted in each panel of Fig 3). No reliable effects were obtained for either the No Sexual Experience group (top panel) or the One Intromission group (middle panel). Most significantly, there were no changes in mean run times, pre- to post-sexual experience. In contrast, the ANOVA conducted on the One Ejaculation group revealed a significant reduction in mean run time following sexual experience, $F(1,9)=5.31$, $p=0.047$. Subjects expressed this post-ejaculation increase in motivation when running for both nonestrous and estrous targets (see Fig 3, bottom panel); therefore, there was no Pre/Post x Receptivity interaction.

Discussion

The results of this experiment replicated the central finding of our initial work on the sexual motivation of male rats (Chapter IIA): sexually-naive males are more motivated to approach an estrous female over a nonestrous female. In addition, this experiment demonstrated that a single ejaculation is sufficient sexual experience to further enhance a male rat's sexual motivation. Only males that experienced an ejaculation, but not those who merely mounted and intromitted, reduced their mean

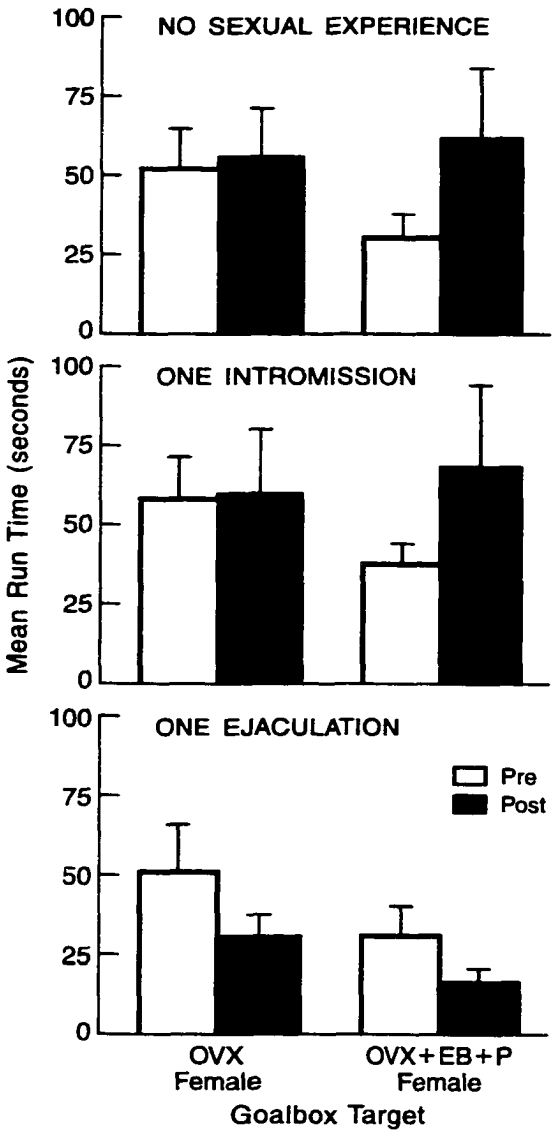

Figure 3. Mean (+SEM) run times for three groups of subject males (N=10 each): the "No Sexual Experience" (top), "One Intromission" (middle), and "One Ejaculation" (bottom) groups. Within each graph, the two bars to the left are subject run times when the goalbox contained a nonestrous (OVX) female, while the two bars to the right are subject run times when the goalbox contained an estrous (OVX+EB+P) female. The clear bars are mean run times prior to sexual experience (averaged across 3 trials), while the shaded bars represent run times following the groups' respective experience (averaged across 4 trials).

run times for both nonestrous and estrous female targets. Presumably, ejaculatory reinforcement alters subsequent motivation by enhancing the incentive value of primary female cues. These cues include, but are not limited to, precopulatory cues emitted by females in estrus. It is noteworthy that males in the One Ejaculation group were also more motivated to encounter nonestrous female targets following their copulatory experience, suggesting that general feminine cues also obtained a higher incentive value.

While this and other experiments have demonstrated that ejaculation serves as an effective reward (e.g., Agmo & Berenfeld, 1990), it cannot be concluded that ejaculation is necessary for copulation to be reinforcing, or that a single ejaculation is the minimal amount of copulatory experience sufficient to influence subsequent motivation. Prior experiments have demonstrated that intromissions without ejaculation are capable of inducing learning in a T-maze (Kagan, 1955; Whalen, 1961) and shorter run times in an alley (Sheffield, Wulff, & Backer, 1951; Ware, 1968). However, in these experiments subjects received two intromissions per trial over a number of trials, rather than receiving sexual experience on a single occasion. The present results show that a single intromission is not rewarding enough to enhance the incentive strength of female cues. It is possible that if subjects had performed numerous intromissions without ejaculating, they would have manifested shorter run times in subsequent runway trials.

Figure 3 reveals an intruiguing pre-post effect among those subjects within the "no experience" and "one intromission" groups when running for an estrous female

target. In contrast to the "one ejaculation" subjects that subsequently decreased their mean run time following sexual experience, subjects within the other two groups increased their run times - i.e., expressed a decreased motivation to approach estrous females pre-to-post. This finding may be interpreted as a form of learning on the subjects' part, related to their diminishing expectancy of sexual reward in the context of the runway. Over the course of the experiment, they never receive sexual reinforcement within the runway and so the predictive value of the perceived sexual incentives extinguishes with time. Subjects that copulate to ejaculation with a receptive female midway through the experiment experience an enhancement in the incentive value of female cues such that their subsequent presence within the runway continues to be sexually motivating – indeed, even more so.

It is unlikely that sexual experience influenced subsequent running behavior due to a change in the incentive value of response variables or contextual cues (Domjan, Akins, & Vandergriff, 1992). Copulatory experience was not contingent upon the experimental operant response (running behavior within the alley). Providing subjects with copulatory experience in a different environment from the runway apparatus precluded the establishment of sexually-conditioned contextual cues. In addition, because the females used for copulation were different from the ones used as targets within the goalbox, it cannot be argued that males were more motivated to engage the target females because they had copulated with those particular females. Rather, subjects associated female cues with their sexual

experience and generalized to other females exhibiting comparable cues when tested later during the post-experience trials.

Chapter III: The Role of Sexual Experience

B. Blocking of Incentive Enhancement by Haloperidol

The results of the previous experiment suggest that sexual experience enhances the incentive value of primary female cues through associative mechanisms dependent upon the rewarding nature of male ejaculation. Midbrain dopamine systems have been implicated in the processing of numerous rewarding events, including food and water, drugs of abuse, and intracranial self-stimulation (for reviews, see Ettenberg, 1989; Koob, 1992; Koob & Goeders, 1989; Phillips, 1984; Wise, 1982; Wise & Rompre, 1989). These conclusions are based in part upon the finding that systemic administration of dopaminergic antagonist drugs, such as haloperidol, effectively blocks the reinforcing consequences of such events. Thus, it seems plausible that the reward value of copulation and ejaculation may also be mediated by dopaminergic transmission, and subject to alteration via dopamine receptor blockade. If this were correct, then one would hypothesize that haloperidol challenge during sexual experience would prevent the subsequent expected increase in sexual motivation, by preventing reward-based incentive enhancement. The current experiment was devised to test this hypothesis.

Method

Animals

The subjects consisted of 34 male and 20 female Long-Evans rats obtained from Charles Rivers Laboratories (Wilmington, MA). Three of the females were used as runway targets, while the remaining 17 were paired with the males in order to provide them with sexual experience. The males were 70-100 days old and the

females 90-180 days old at the start of testing. All animals were housed individually in hanging wire cages within a temperature-controlled 22°C vivarium environment maintained under a reverse 14:10 light-dark schedule (lights on 2300 h-1300 h). Food and water were provided on an *ad libitum* basis.

Prior to arrival at our vivarium, the males were group housed but did not have access to females. Therefore, they were sexually-naive insofar as they lacked heterosexual (but possibly not homosexual) copulatory experience.

Surgery

All females were ovariectomized (OVX) through a single lower abdominal incision 1-6 weeks prior to testing, using standard aseptic surgical techniques conducted under deep anesthesia. Each animal was pretreated with 0.3 mg/kg IP atropine (Pittman-Moore, Washington Crossing, NJ) 15 minutes prior to the induction of anesthesia in order to reduce potential respiratory problems. Anesthesia was then induced by IP administration of 90 mg/kg ketamine and 2 mg/kg xylazine. All females received at least one week post-operative care prior to use within the experiment.

Apparatus

The apparatus used in this experiment was the same straight-arm runway described in Chapter IIA.

Procedure

On two separate days, each of the male subjects was allowed to individually explore the runway apparatus for 5-7 minutes. The three female targets were also

individually placed within the goalbox for 10 minutes each on two days. This was done to acclimate the animals to the novel runway environment. All testing took place under red light conditions during the dark portion of the rats' photoperiod.

On any given test day, all 34 male subjects ran for the same target in the goalbox; only one trial per day per subject was conducted. The procedure for individual trials in this experiment was identical to that described in Chapter IIIA. On different days/trials, subjects ran for one of two different targets, randomly determined: either a nonestrous female (OVX female) or an estrous female. Estrus was induced via subcutaneous (SC) administration of 15 μg of estradiol benzoate (in 0.1 ml sesame oil) 48 and 24 hours before testing, with an additional SC injection of 500 μg progesterone (in 0.1 ml propylene glycol) 3-5 hours before testing. Steroid hormones were purchased from Sigma Chemical Company, St. Louis, MO. Behavioral estrus was confirmed prior to the days' trials during a brief one minute pretest. Each of the three target females was rotated through both hormonal conditions three to four times over the course of the experiment.

Subject males ran a total of ten trials, one trial per day (five for a nonestrous target and five for an estrous target) before they were provided with sexual experience. Following these ten trials, each subject was assigned to one of four groups (N = 8-9/group) such that the mean baseline run times for both nonestrous and estrous targets was approximately the same for all four groups. Over the course of two days, all four groups were taken to a separate testing arena in order to provide them with sexual experience. Each subject male was tested individually under red

light during the dark portion of the photoperiod. The testing arena was composed of cylindrical Plexiglas, 45 cm diameter by 60 cm height, with the floor of the arena covered with wood-chips. A total of seventeen OVX Long-Evans females, given estradiol and progesterone to induce robust receptivity, were used. It should be noted that while the same three females were used as targets throughout the runway portions of the experiment, none of these target females were used to provide sexual experience. Each male was individually paired with a female until he achieved one ejaculation. Males within the control group were given intraperitoneal (IP) vehicle injections of 0.002 M lactic acid 45 minutes prior to testing. Subjects in the remaining three groups were given an IP injection of haloperidol (0.05, 0.075, or 0.10 mg/kg) dissolved in 0.002 M lactic acid, 45 minutes prior to testing. All injections were made in a volume of 1 ml/kg.

When the males were provided with sexual experience, two measures of copulatory performance were recorded for each subject: mount latency (ML) and ejaculation latency (EL). Mount latency is defined as the time between introduction of the receptive female and the first successful mount conducted by the male. Ejaculation latency is the time between introduction of the female and ejaculation.

Each subject male was returned to his home cage following sexual experience. Two days later, subjects were re-tested within the runway for their motivation to approach either a nonestrous or an estrous female target. A total of ten post-sexual experience trials were conducted, one trial per day, five for a nonestrous target, five for an estrous target. Thus, over the course of the entire experiment, each of the 34

subjects ran a total of 20 trials within the runway, 10 prior to sexual experience and 10 afterwards.

Results

Baseline Run Times

Prior to receiving any sexual experience, the mean (±SEM) run time (over five trials) of all 34 male subjects was faster for the estrous female target than for the nonestrous female target: 35.8 (±5.9) and 59.4 (±6.9) seconds, respectively. A one-tailed, paired-sample *t*-test comparing these means revealed a significant difference, $t(33)=3.915$, $p<0.001$. Thus, even prior to any sexual experience, male rats are more motivated to approach an estrous female versus a nonestrous female.

Effect of Haloperidol on Sexual Behavior

The mean (±SEM) mount latencies for the four experimental groups (vehicle, 0.05, 0.075, and 0.10 mg/kg haloperidol) were: 39.4 (±10.5), 56.7 (±26.9), 181.1 (±110.2), and 67.5 (±24.3) seconds, respectively. The mean (±SEM) ejaculation latencies were: 693.8 (±117.5), 426.7 (±71.0), 544.4 (±115.7), and 531.2 (±55.3) seconds. A one-way ANOVA comparing the mean mount latencies between the four groups and another ANOVA comparing the mean ejaculation latencies were conducted to determine whether haloperidol had an inhibitory effect upon copulatory performance. At the doses used, there was no significant effect of haloperidol on mount latency, $F(3, 30)=1.131$, $p=0.35$, nor on ejaculation latency, $F(3,30)=1.341$,

p=0.28. Hence, haloperidol did not reliably alter these measures of male sexual performance.

Effect of Sexual Experience + Haloperidol on Run Times

The male subjects' performance in the runway (mean+SEM run times) during pre- and post-sexual experience trials is depicted for each group in Figure 4 (panels A-D). A mixed two-factor (Sexual Experience x Target Receptivity) ANOVA was conducted on the data within each group. For the vehicle-group, there was a significant main effect of sexual experience, $F(1,7)$=5.178, p=0.05, and a significant main effect of the target's sexual receptivity, $F(1,7)$=9.024, p=0.02, but no interaction between these two factors. Thus, subjects in this group ran reliably faster for target females following sexual experience, and faster for estrous females over nonestrous females. For the 0.05 mg/kg and 0.075 mg/kg haloperidol groups, there were no significant main effects of sexual experience or receptivity, nor a reliable interaction. For the 0.10 mg/kg haloperidol group, there was a main effect of sexual experience, $F(1,7)$= 7.561, p=0.03, but no effect of receptivity nor an interaction. However, in contrast to the vehicle group, the high-dose subjects took significantly longer to approach target females following sexual experience.

In order to better visualize the effect of sexual experience (with and without haloperidol challenge) upon sexual motivation, difference scores were calculated by subtracting the post-sexual experience run time of each subject from his pre-sexual experience run time. The mean (+SEM) differences, plotted by the hormonal status of the target female, are depicted in Figure 5 (panels A and B). Large differences

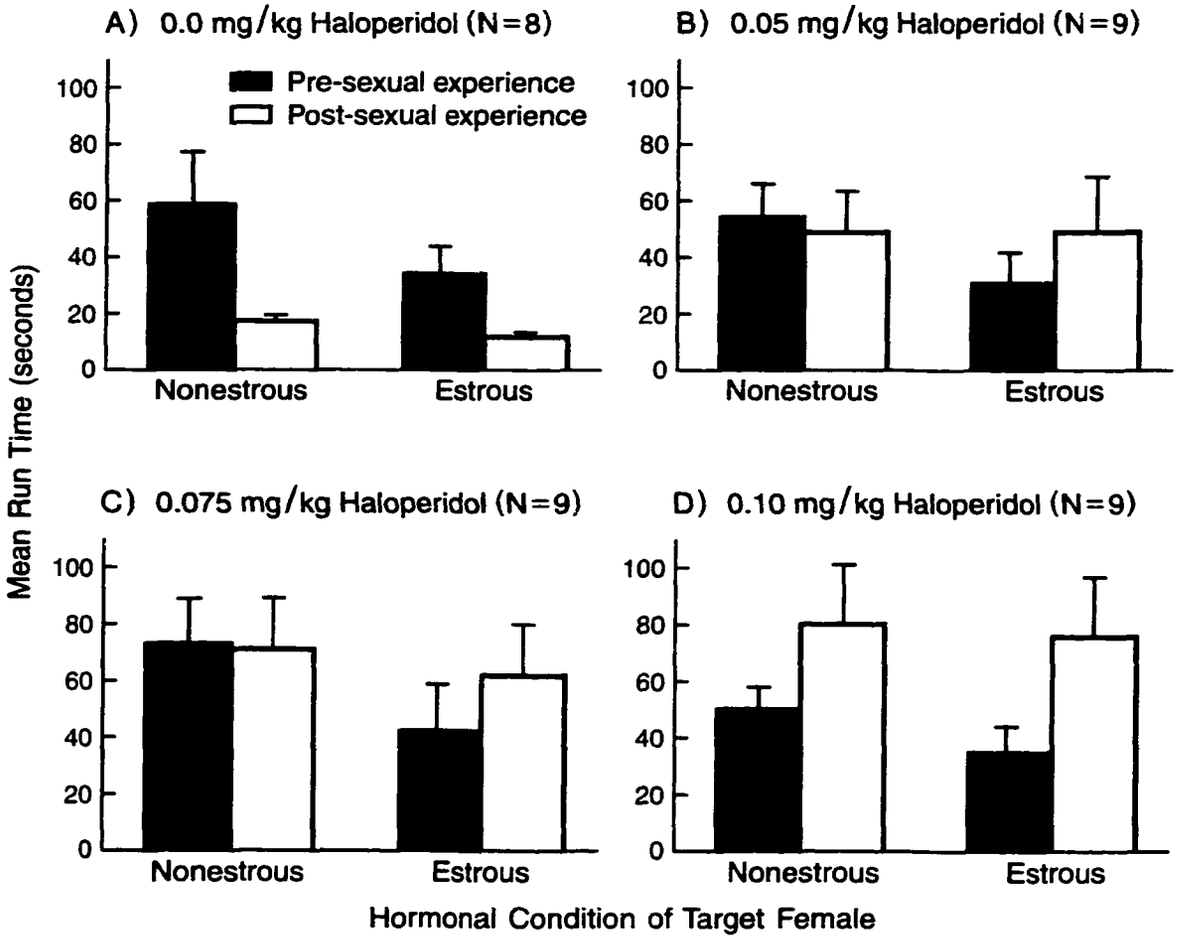

Figure 4. Mean (+SEM) run times, pre- and post-sexual experience, for the four groups of experimental subjects. Panel A represent the vehicle controls, panels B-D depict data from subjects given haloperidol 45 minutes prior to sexual experience. Black bars represent mean run times prior to sexual experience, while white bars represent mean run times following sexual experience. Within each panel, the two leftmost bars are subject run times when the goalbox contained a nonestrous female target, and the two rightmost bars are subject run times for estrous female targets.

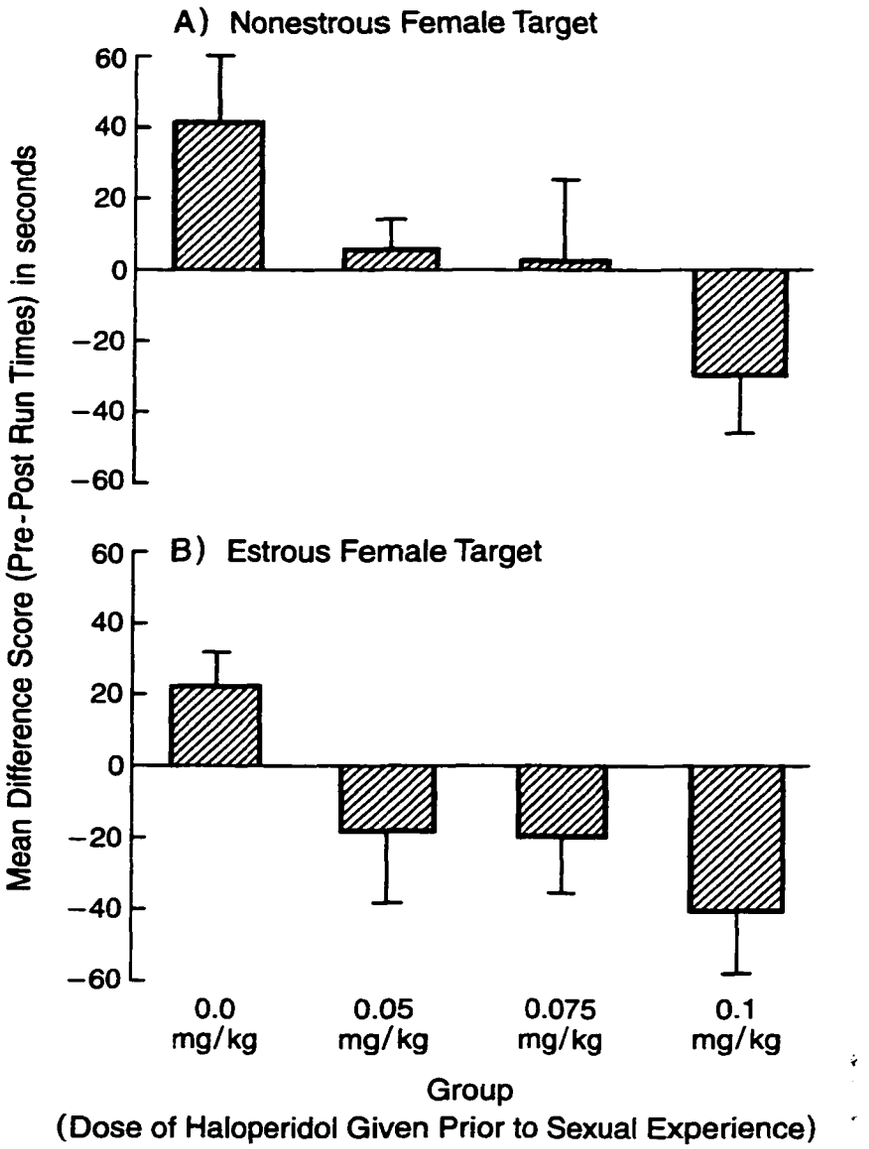

Figure 5. Mean (+SEM) difference in run time from pre-sexual experience trials to post-sexual experience trials, for four groups of experimental subjects. Top panel A depicts difference scores when subjects were running for nonestrous female targets, and bottom panel B depicts these data when subjects were running for estrous female targets. Positive difference scores represent faster running following sexual experience (i.e., an increase in motivation), while negative difference scores indicate that subjects ran slower for the target female following sexual experience.

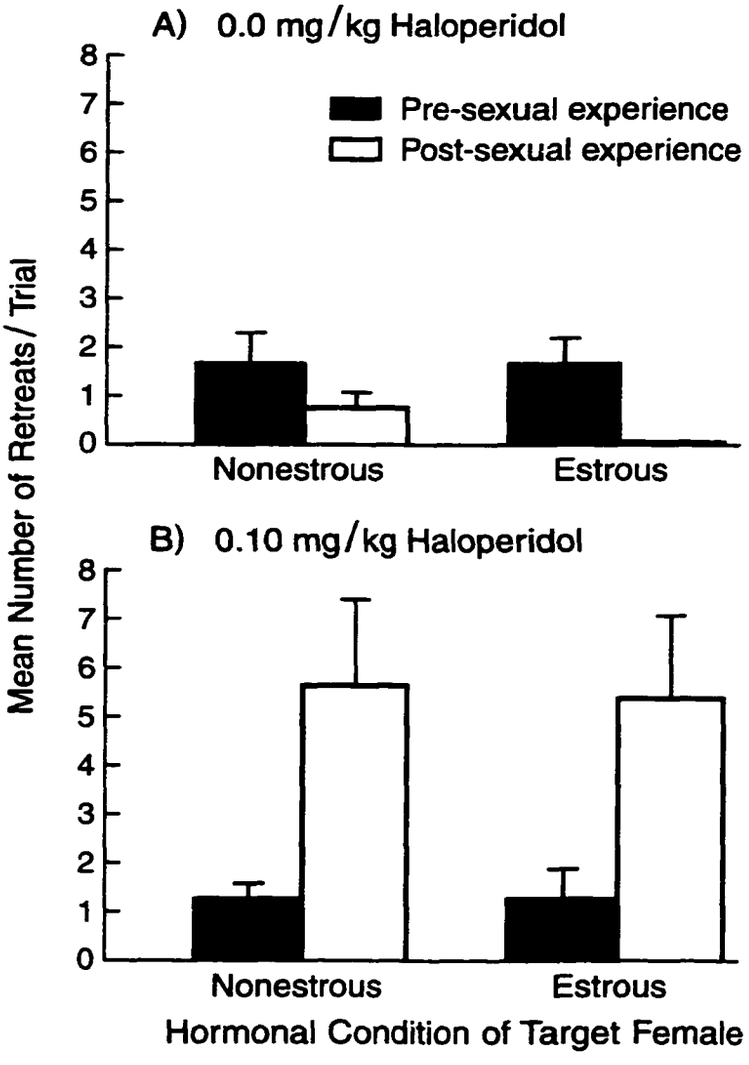

Figure 6. Mean (+SEM) retreats per trial, pre and post-sexual experience, for two groups of experimental subjects. Panel A depicts the retreat behavior of the vehicle controls, and panel B displays the retreat data of the 0.10 mg/kg haloperidol-treated subjects. Black bars represent mean number of retreats prior to sexual experience, while white bars are mean retreats following sexual experience. Within each panel, the two leftmost bars are group retreats when the goalbox contained a nonestrous female target, and the two rightmost bars are retreats during trials when an estrous female target was in the goalbox.

(deviations from zero) indicate changes in motivation following sexual experience; positive differences reflect an increase in motivation, while negative differences reflect a decrease. A two-way (Group x Target Receptivity) ANOVA comparing the difference scores across groups revealed a main effect of haloperidol dose, $F(3,30)=3.476$, $p=0.028$. A series of eight planned, one-tailed *t*-tests were conducted to determine whether each bar displayed in Figure 5 was significantly different from zero (i.e., was there a reliable change in run time following sexual experience). These analyses revealed a significant decrease in run time (increase in motivation) following sexual experience for subjects within the control condition, when running for both a nonestrous female, $t(7)=2.221$, $p<0.05$, and estrous female, $t(7)=2.256$, $p<0.05$. There were no significant pre-post differences for either the 0.05 or 0.075 mg/kg dose groups, when running for either nonestrous or estrous female targets. Subjects within the 0.10 mg/kg dose group took significantly more time (expressed decreased motivation) to approach both nonestrous females, $t(7)=-1.849$, $p<0.05$, and estrous female targets, $t(7)=-2.329$, $p<0.05$, following sexual experience.

Effect of Sexual Experience + Haloperidol on Retreat Behavior

To further examine whether haloperidol influenced the motivational impact of sexual experience, we examined the retreat behaviors of the two experimental groups that expressed a significant change in run time following sexual experience: the vehicle group and the high haloperidol dose (0.10 mg/kg) group. The mean (+SEM) number of retreats displayed by these two groups, pre- and post-sexual experience, are shown in Figure 6. An overall three-way (Group x Sexual Experience x Target

Receptivity) ANOVA on these data revealed a significant main effect of group, $F(1,14)=5.932$, $p=0.029$, and a significant interaction between group and sexual experience, $F(1,14)=13.498$, $p=0.003$. This suggests that those subjects given the 0.10 mg/kg dose of haloperidol prior to sexual experience associated both positive and negative experiences with the female, later expressed as approach/avoidance conflict behavior (i.e., retreats) within the runway.

Discussion

This experiment successfully replicated several results from our earlier work on the sexual motivation of male rats. First, prior to receiving any sexual experience, male subjects were more motivated to approach estrous females over nonestrous females, reflecting an inherent motivational bias toward the former. Second, male subjects within the control group that received vehicle injections prior to copulating to ejaculation expressed an increased motivation to approach both nonestrous and estrous female targets in subsequent motivational testing. Thus, sexual experience consisting of one ejaculation is sufficient to enhance the incentive value of primary female cues.

Haloperidol challenge during copulation dose-dependently attenuated this effect. In particular, subjects within the 0.05 and 0.075 mg/kg groups did not experience an increase in sexual motivation following sexual experience. Their pattern of pre-to-post sexual experience run times mirrors that of subjects not given any sexual experience at all or merely one intromission without ejaculation. Based

upon these results, we suggest that the positive, rewarding properties of sexual behavior and ejaculation enhance the incentive value of female cues associated with copulatory experience through dopaminergic release.

Subjects within the high haloperidol dose group (0.10 mg/kg) expressed a decreased motivation to approach female targets following their sexual experience. There are at least three possible explanations for this. First, the haloperidol itself might have been punishing, causing the subjects to associate a negative drug experience with female cues. However, similar doses of haloperidol do not produce place aversions when paired alone with one side of a preference chamber, indicating that environmental cues associated with a haloperidol experience do not acquire negative valance (Spyraki, Fibiger, & Phillips, 1982ab). A second possibility is that the haloperidol compromised the sexual performance of the treated males, such that they were not able to engage in a similar degree of sexual behavior as control subjects. The data collected on mount and ejaculation latencies do not support this interpretation; there were no significant group differences on these measures, and all drug-treated males successfully achieved ejaculation following repeated mounting and intromission.

Although speculative, a third hypothesis is that the highest dose of haloperidol not only negated the positively reinforcing properties of sexual activity, but also caused the subjects to experience copulation as an aversive form of physical stimulation. This was not a consequence of either the haloperidol or the sexual behavior *per se*, but rather a negative result of the interaction or combination of the

two (at least at the highest haloperidol dose). If this were true then one might expect subjects, on subsequent trials, to approach the female targets because of their aforementioned inherent sexual attractiveness, but also avoid the targets because of the associated negative sexual experience. This approach-avoidance conflict would account for the increased frequency of post-experience runway retreats observed in the high-dose group (see Figure 6), particularly in view of the fact that such "retreat" behaviors have been shown to reflect motivational conflict under other circumstances (Ettenberg & Geist, 1993; Geist & Ettenberg, 1997).

As noted, at the doses administered, haloperidol had no significant effect upon either the mount or ejaculation latencies of the subject males. This finding is partly in contrast with earlier work demonstrating an inhibitory effect of dopamine antagonists on mount latency (Everitt, 1990; Pfaus & Phillips, 1989, 1991). However, in these earlier studies, decrements in sexual performance generally occurred following administration of higher haloperidol doses (0.1, 0.2, 0.5 mg/kg). The fact that haloperidol did not affect the sexual performance of our subjects diminishes the possibility that the subsequent differences in sexual motivation resulted from differences in the quantity of sexual experience received, rather than differences in the reinforcing quality of the act. Nevertheless, only two measures of sexual performance (mount and ejaculation latency) were examined. Hence, a more detailed observational analysis of the copulatory sessions, involving alternate behavioral measures such as copulatory hit rate, might have revealed subtle decrements in sexual function during dopamine antagonist challenge.

Surprisingly, evidence supporting a dopaminergic basis to sexual reward has been sparse. If true, one would expect that dopamine receptor antagonists would be capable of extinguishing operant responses maintained via sexual reinforcement, and prevent sexually-conditioned place preferences. While it has been reported that the mixed D1/D2 antagonist, alpha-flupenthixol, dose-dependently decreased responding for access to a receptive female under a second-order schedule (Everitt, 1990), Agmo and Berenfeld (1990) failed to block a sexually-conditioned place preference with a 1 mg/kg systemic dose of the dopamine antagonist, pimozide. However, their experiment more specifically focused upon the rewarding properties of the post-ejaculatory interval: males were placed in the preference chamber following copulation. Thus, pimozide did not challenge the reinforcing consequences of sexual behavior and ejaculation *per se*.

In contrast, numerous studies utilizing *in vivo* neurochemical techniques such as microdialysis and voltammetry, have documented a significant correlation between sexual activity and central dopamine release. Dopamine concentrations within the nucleus accumbens (Pfaus et al., 1990; Pleim et al., 1990) and the medial preoptic area of the hypothalamus (Hull et al., 1993, 1995; Sato et al., 1995) rise prior to (following exposure to estrous female cues) and during copulation. Moreover, repeated exposure to estrus female bedding leads to an increased, or sensitized, dopaminergic response within the nucleus accumbens (Mitchell & Gratton, 1991). It seems plausible then that dopaminergic activity during sexual behavior modulates the incentive value of primary female cues through this process of sensitization

(Wenkstern, Pfaus, & Fibiger, 1993). Increased mesolimbic dopamine transmission during copulation could serve both to reward ongoing sexual activity and to facilitate subsequent preparatory behaviors elicited by primary female incentives

These considerations attest to the difficulty in identifying the exact mechanism by which dopamine mediates changes in incentive value. While it is clear that dopaminergic activity during copulation induces learning, there are several potential explanations for this process. One possibility is dopamine specifically mediates the rewarding aspects of copulation but does not play a direct role in the associative process, which is controlled by non-dopaminergic pathways. In support of this possibility, Fleming and Kucera (1991) have noted that administration of the protein synthesis inhibitor, cycloheximide, or the noncompetitive NMDA antagonist, MK-801, during sexual behavior blocks the subsequent facilitation of male mounting behavior following sexual experience. This suggests that glutamatergic activity is also central to copulation-induced learning.

Schultz (1998) has suggested that dopamine mediates both the reward value of sexual experience and association formation itself, citing evidence that dopamine neurons activated by rewarding events are also implicated in the modification of synaptic transmission. Thus, sexual activity may increase the future response-properties of neurons that signal the presence of sexually-relevant, female cues. Under this conception, dopamine-receptor antagonism during sexual activity would interfere with the modulation of female incentive value by preventing synaptic change. These considerations will be discussed further in Chapter V, in which an

experiment using immunocytochemistry to identify differences in *c-fos* expression between naïve and experienced male rats is discussed.

Chapter IV: Sexually-Conditioned Incentives

Primary female incentives form only one class of stimuli males find sexually inviting. Environmental stimuli that become associated with successful copulation, known as secondary or conditioned incentives, can also increase sexual motivation (Agmo, 1999; Stewart, 1995). For instance, male rats trained under second-order instrumental conditions learn to bar-press for presentation of a light previously associated with copulation (Everitt, 1990). Subjects allowed to periodically copulate with receptive females in a bi-level chamber eventually become behaviorally activated (display multiple level-changes) when placed into the chamber, indicating that the local environment has acquired motivational significance (Mendelson & Pfaus, 1989). Additionally, male rats spend a majority of their time on a sex-paired side of a conditioned place-preference apparatus, even if conditioning consists of only a single ejaculation (Agmo & Berenfeld, 1990). Males also demonstrate an ejaculatory-preference for receptive females marked with a scent previously paired with successful copulation (Kippin et al., 1998). Lastly, both male rats and Japanese quail initiate copulation and achieve ejaculation sooner with receptive females if a conditioned incentive is present (Domjan et al., 1986; Zamble, Mitchell, & Finlay, 1986).

As noted in Chapter I, there is growing evidence that dopamine plays a crucial role in mediating the behavioral activating effects of both primary and secondary incentives across a variety of motivational domains, including sex (Berridge & Robinson, 1998; Blackburn et al., 1987; Blackburn et al., 1989; Blackburn et al., 1992; Horvitz, 2000; Ikemoto & Panksepp, 1999; Kiyatkin, 1995; Mogenson et al.,

1980; Phillips et al., 1991; Robbins & Everitt, 1996; Salamone, 1994, 1996; Schultz, 1998; Schultz et al., 1997). However, many of the experimental methodologies employed in this research area confound the relative motivational impact of primary and secondary sexual incentives, making the interpretation of dopamine's exact effect somewhat difficult. For instance, subjects are often trained to emit an operant response (such as press a lever or traverse an alley) in order to gain access to a receptive female (Everitt, 1990; Moses et al., 1995; Warner et al., 1991). Administration of dopamine receptor antagonists under these conditions decreases the rate and/or intensity of operant responding. It is uncertain as to whether this reduced responding occurs because of a reduction in the incentive value of the female herself (who resides within the test-apparatus), the value of local conditioned cues, or the value of the operant response itself (Domjan, Akins, & Vandergriff, 1992). Additionally, dopamine receptor antagonism may block the reinforcing nature of the sexual encounter and thus lead to an extinction of operant responding (Ettenberg, 1989; Wise, 1982).

In order to address these issues, the current experiment was designed with the following goals in mind: 1) to establish a sexually-conditioned incentive following a minimum of sexual experience, 2) to experimentally isolate the motivational impact of a conditioned incentive, independent of other external factors, and 3) to examine dopamine's role in mediating the positive value and behaviorally-activating effects of a sexually-conditioned cue. In order to isolate the effect of a secondary incentive, we utilized an experimental protocol in which subjects do not receive sexual experience

within the same apparatus used to test their sexual motivation. Thus, a sexually-reinforced operant response is not established. Male rats are conditioned to associate two neutral olfactory cues with copulation and social isolation respectively, within a Plexiglas arena. The incentive value of these cues is subsequently tested by presenting each of them individually to subjects within a straight-arm operant runway. The subjects' approach behavior towards the scent (located within the goalbox) is taken as an objective measure of its motivational value. This procedure allows for the examination of sexual motivation induced by sexually-conditioned cues independent of the effects of primary female incentives, as subjects never perceive nor encounter a female within the runway apparatus. Through administration of haloperidol prior to runway trials, the effects of dopamine receptor antagonism on the incentive value of a previously established conditioned cue can also be tested.

Method

Animals

A total of 93 male and 50 female Long-Evans rats were obtained from Charles Rivers Laboratories (Wilmington, MA). The males ranged from 80-100 days old and the females from 80-150 days old at the start of testing. All animals were housed individually in hanging wire cages within a 22°C vivarium environment maintained under a reverse 14:10 light-dark schedule (lights on 2300 h-1300 h). Food and water were provided on an *ad libitum* basis. Prior to arrival in the vivarium, the males were

group housed but did not have access to females. Therefore, they were sexually-naive insofar as they lacked heterosexual copulatory experience.

Surgery

All females were ovariectomized (OVX) through a single lower abdominal incision 1-8 weeks prior to testing using standard aseptic surgical techniques and under deep anesthesia. For 30 females, anesthesia was induced by intraperitoneal (IP) administration of a 90 mg/kg ketamine, 2 mg/kg xylazine mix, in a volume of 1 ml/kg. For 20 females, isoflurane gas anesthesia (4% induction, 2% maintenance) was given. All animals were pretreated with 0.3 mg/kg IP atropine (Pittman-Moore, Washington Crossing, NJ) 15 minutes prior to the induction of anesthesia in order to reduce potential respiratory problems. Females received at least one week of post-operative care prior to initiation of the experiment.

Inducing Female Sexual Receptivity

Receptivity was induced in the female rats via hormonal administration consisting of subcutaneous (SC) administration of 15 μg of estradiol benzoate (in 0.1 ml sesame oil) 48 and 24 hours before testing, with an additional SC injection of 500 μg progesterone (in 0.1 ml propylene glycol) 3-5 hours before testing. Steroid hormones were purchased from Sigma Chemical Company, St. Louis, MO.

Apparatus

Sexual conditioning took place within three cylindrical Plexiglas arenas (45 cm diameter, 40 cm height). Motivational testing occurred within the same straight-

arm runway described in Chapter IIA. However, neither of the experiments described here utilized a Plexiglas partition to divide the goalbox into two semi-circular halves.

Procedure

Phase 1: Conditioning

Two experiments were conducted, each employing an identical conditioning phase. On alternating days, subjects were given copulatory sessions in the presence of one of two distinct scents, either orange or almond extract (Felbro Food Products, Inc.), and isolation sessions in the presence of the other scent. Only one session per subject occurred each day. The scent paired with copulation will henceforth be referred to as the CS+, while the scent paired with social isolation will be referred to as the CS-. For half the subjects, the orange scent was the CS+ and almond the CS-, with the opposite being true for the other half. A total of five copulatory and five isolation sessions were given over the course of ten days. This ten-day period made up the entire conditioning phase of both experiments for the majority of subjects.

On each day, half of the male subjects were paired with a sexually receptive female until they achieved one ejaculation or until 30 minutes passed, whichever came first. Those subjects not given sexual experience spent an individual amount of time in the arena alone. The length of each subject's isolation period was equal to the amount of time it took that same subject to copulate to ejaculation on the preceding day. For the first day of conditioning, non-copulating subjects were arbitrarily isolated for 10 minutes. On half of the days (determined randomly), copulatory

sessions were conducted prior to the isolation sessions; on the remaining days, isolation sessions preceded the copulatory ones.

Immediately prior to each session, that day's scent was applied using a disposable wipe drawn along the top edge of the Plexiglas arena. On any given day, both copulatory and isolation sessions occurred in the presence of the same scent, such that the use of the two scents alternated day to day. At the end of all subject sessions each day, all three arenas were thoroughly cleaned with a 30% alcohol solution to remove any scent traces.

Periodically, some males would not immediately copulate with the introduced female, especially if it was their first conditioning session. In order to stimulate copulation, the initial female was replaced with a second, and sometimes third receptive female. If the male failed to copulate for 30 minutes, the session was terminated. At the end of the 10-day conditioning period, an 11^{th} day of conditioning occurred during which all previously-noncopulating males were given an additional opportunity to copulate with a receptive female under identical circumstances as their earlier failure. If a subject failed to copulate on two or more conditioning sessions, including this 11^{th} day, he was dropped from the experiment. A total of four males were dropped from Experiment 2 for this reason. No subjects were dropped from Experiment 1.

Female rats were rotated through hormone treatments such that receptivity was induced every four days. A different female was paired with a subject male each time he was given a copulatory session, such that over the course of the conditioning

phase he was exposed to five different females (possibly more, if female replacement had occurred due to lack of sexual activity). If a female was placed with a male but did not demonstrate immediate and sufficient receptivity for successful copulation, it was removed and replaced with another female.

Phase 2: Motivational Testing

Experiment 1:

All runway testing took place under red light conditions during the dark portion of the rats' photoperiod. Following the 10-day conditioning regimen, 16 male subjects were allowed to individually explore and habituate to the empty runway apparatus for 10 minutes on two consecutive days. Over the next four days, subjects were tested for their motivation to approach a goalbox placed under one of three conditions: unscented, CS+ scented, or CS- scented. Subjects were tested for the unscented goalbox (control condition) twice. For the scented-goalbox trials, a small glass container of the extract (holding approximately 30 ml) was placed, uncovered, at the far end of the goalbox 15 minutes prior to testing. On any given test day, all 16 subjects ran for the same goalbox condition; only one trial per subject per day was conducted. The order of trials across the four-day testing period was randomized for three separate groups of subjects.

Individual trials were conducted using the following procedure: first, a subject male was placed into the startbox for 15 seconds. The start-door was lifted and the subject was given access to the alley. Leaving the startbox interrupted an infrared photocell that triggered a timer, which stopped once the subject entered the

goalbox. At this point, the trial was ended and the subject returned to his home cage. The next subject's trial was then initiated. This procedure continued, one animal at a time, until all 16 subjects were tested. The order of subjects run was held constant throughout the experiment.

The dependent measure of interest was run time - i.e., the time elapsed between the subject's leaving the startbox and entering the goalbox. Thus, we view run time as an objective index of each subject's motivation to approach the goalbox stimuli; shorter run times presumably reflect greater motivation.

Experiment 2:

As in Experiment 1, all runway testing took place under red light conditions during the dark portion of the rats' photoperiod. After conditioning, 77 male subjects were habituated to the empty runway apparatus for 10 minutes on two consecutive days. On the following day, all subjects were given a baseline test to measure their motivation to approach an empty, unscented goalbox. Individual trials were conducted using an identical procedure as in Experiment 1. Subjects were then assigned to one of four haloperidol dosage groups.

The next day, each subject was tested within the runway for his motivation to approach either the CS+ or CS-, as described in Experiment 1. On this "test day", all subjects ran for the same goalbox condition. Half of the subjects within each dosage condition ran for their CS+ and half for their CS-, making a total of eight independent groups. Forty-five minutes prior to this test, subjects within each group were pre-treated with either a vehicle injection or one of three doses of haloperidol, a

dopamine-receptor antagonist. Subjects in groups 1 and 2 were given IP vehicle injections of 0.002 M lactic acid. Subjects in groups 3 and 4, 5 and 6, and 7 and 8 were given IP injections of 0.075, 0.15, and 0.30 ml/kg haloperidol respectively. All injections were given in a volume of 1 ml/kg. To clarify, half the subjects in each dosage condition were pretreated with a given dose of haloperidol and then exposed to the CS+ on test day; the other half received the same treatment but were exposed to the CS-.

Results

Experiment 1

When tested for their motivation to approach the CS+, unexpected noises disrupted the trials of two subjects. Thus, the run times of these two subjects, but only for the CS+ goalbox condition, were excluded from analysis. Figure 7 shows the mean (+SEM) run time for all 16 subjects running for the three goalbox conditions (No Scent, CS+, and CS-). For the reason mentioned above, the mean for the CS+ condition only contains data from 14 subjects. A one-way repeated-measures ANOVA conducted on these data revealed a significant difference in run times across conditions, $F(2, 26)=4.860, p=0.016$. Three one-tailed, paired-sample *t*-tests compared the mean run times between each of the three conditions. There was no significant difference in subject run times between the No Scent and CS- condition, $t(15)=-0.916, p=0.18$. However, the difference between the No Scent

Figure 7. Mean (+SEM) run times for 16 male subjects tested for their motivation to approach an unscented (2 trials), an S+ scented (1 trial), and an S- scented goalbox (1 trial). Subjects took significantly less time to enter an S+ scented goalbox versus an S- scented or unscented goalbox.

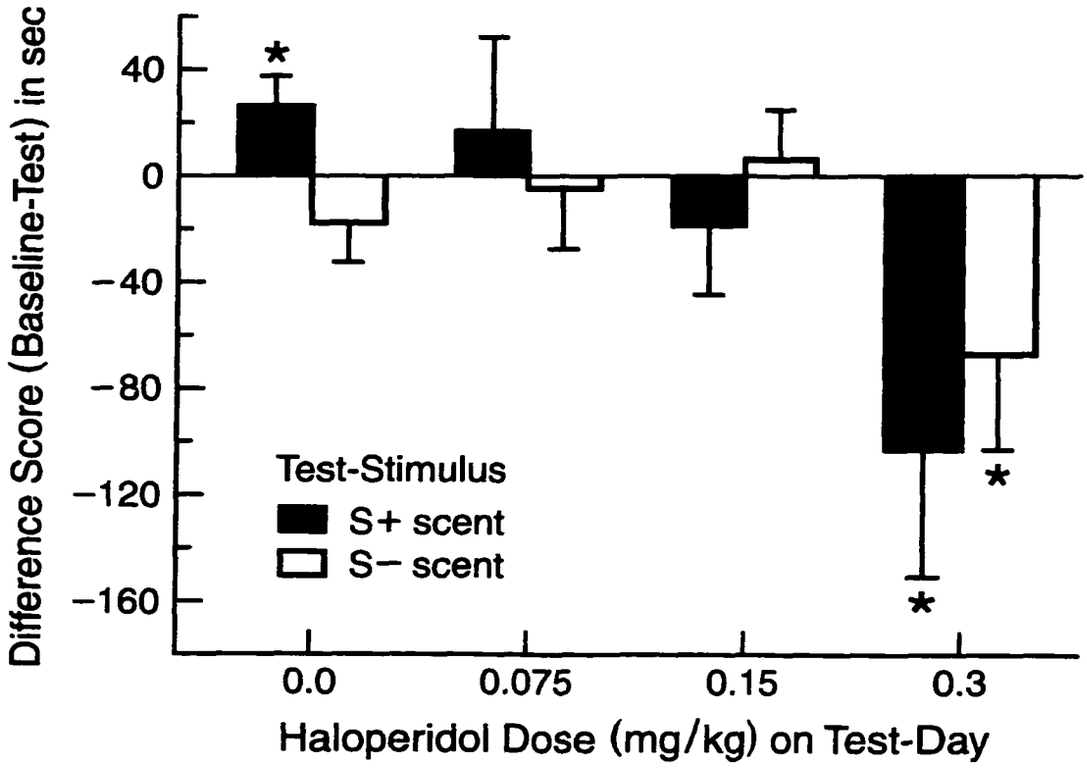

Figure 8. Mean (+SEM) difference scores (test-baseline) for the eight groups of experimental subjects. Black bars represent difference scores for groups exposed to the S+ on test-day, while white bars represent difference scores for groups exposed to the S-. Positive difference scores indicate that subjects ran faster on test-day compared to baseline. Negative scores reflect slower run times on test-day compared to baseline. On test day, all subjects were pretreated with either vehicle or one of three doses of haloperidol 45 minutes prior to testing.

and CS+ condition was statistically significant, $t(13)=2.206$, $p=0.023$, as was the difference between the CS+ and CS- conditions, $t(13)=-2.732$, $p=0.008$.

Experiment 2

Figure 8 shows the mean (+SEM) difference scores (baseline-test run times) for all 8 groups of subjects. An overall 4 (dose) x 2 (test stimulus: CS+/CS-) ANOVA conducted on the data across all groups revealed a significant effect of dose, $F(3, 69)=4.947$, $p=0.004$. There was no overall effect of test stimulus nor a significant dose x stimulus interaction. To assess whether test-day performance reliably differed from baseline, individual one-tailed, one-sample *t*-tests were conducted on the data within each group, comparing the mean difference scores to zero. Subjects given vehicle injections and exposed to the CS+ on test-day ran significantly faster compared to baseline, $t(10)=2.639$, $p=0.012$. Subjects within both 0.30 mg/kg haloperidol dosage groups ran significantly slower on test-day compared to baseline, $t(7)=-2.203$, $p=0.032$ for the CS+ group, and $t(8)=-1.932$, $p=0.045$ for the CS- group.

Discussion

The results of both Experiment 1 and 2 indicate that male rats given five copulatory episodes in the presence of a distinct scent learned to associate it with sexual reward. In subsequent testing, subjects expressed a stronger motivation (as reflected by shorter run times) to approach the sexually-conditioned scent in an operant runway over a scent previously paired with isolation or an unscented goalbox.

This approach behavior occurred even though subjects perceived the olfactory cue in a different environment from the one that they were conditioned in, and even though they did not experience sexual reinforcement within the runway itself. Thus, this methodology allows one to study the motivational impact of a secondary, conditioned incentive independent of other factors, including primary incentives and previously learned operant behaviors.

In addition, Experiment 2 provided evidence for a dopaminergic role in mediating these motivational effects. Vehicle-treated subjects exhibited run times for an CS+ scented goalbox that were significantly faster than times for an unscented goalbox (baseline), thereby replicating the data from Experiment 1. Subjects given the two lower doses of haloperidol (0.075 and 0.15 mg/kg) did not differentiate between an unscented goalbox and the CS+, signifying that the cue's incentive value had been abolished. The runway behavior of subjects given these same doses and presented with the CS- also did not differ from baseline. We believe this pattern of results indicates that haloperidol caused a selective motivational deficit, and not a general loss of motor ability. In contrast, subjects given the highest dose of haloperidol (0.30 mg/kg) ran significantly slower for both the CS+ and CS- in comparison to baseline, and their ability to initiate movement and traverse the runway appeared severely compromised. It is likely then that this dose of haloperidol caused both a motivational *and* motoric impairment.

It is conceivable that the two lower doses of haloperidol differentially impaired subjects' faster running for the CS+ versus their slower running for the CS-.

However, there are reasons to suspect that this explanation is inadequate. A number of studies have successfully dissociated the performance-debilitating effects of neuroleptics from their capacity to attenuate motivation (see Wise, 1982, for a review), and have shown that dopamine receptor antagonists do not necessarily compromise a rat's ability to respond normally on a single trial. In fact, prior research conducted in our laboratory has shown that haloperidol, within the dosage range adopted in the current study, does not affect the response-initiation latencies nor running speeds of subjects working in an operant runway for access to self-stimulation on the first few days of training (Horvitz & Ettenberg, 1989) or food or heroin during relapse-reinstatement (McFarland & Ettenberg, 1995, 1998, 1999). Our own previous work has shown that while doses of 0.075 and 0.15 mg/kg haloperidol slow a subject's approach behavior for a goalbox containing an estrous female (Lopez & Ettenberg, 2001), the same two doses do not affect subject run times when the goalbox contains a nonestrous female or is empty, again suggesting that haloperidol's actions can be specifically motivational and not motoric. Interestingly, pilot studies preceding that work also showed that a 0.30 mg/kg dose of haloperidol slowed subject run times for all targets, including an empty goalbox. Thus, taken together with our previous findings, the current experiment's pattern of results indicates that haloperidol doses of 0.15 mg/kg and below are capable of specifically targeting motivational systems, while those of 0.30 mg/kg (and presumably higher) tend to inhibit voluntary movement.

Our results support the large body of evidence implicating dopamine as a biochemical signal of motivationally significant stimuli (Blackburn et al., 1987; Blackburn et al., 1989; Blackburn et al., 1992; Kiyatkin, 1995; Mogenson et al., 1980; Phillips et al., 1991; Salamone, 1994; Schultz, 1998; Schultz et al., 1997). This dopaminergic signal can occur in response to the perception of primary incentives such as estrous female cues, even within a sexually-naïve male (for reviews, see Mitchell & Gratton, 1994; Phillips et al., 1991; for specific studies, see Louilot et al., 1991; Wenkstern, Pfaus, & Fibiger, 1993). The dopamine response may also become conditioned, tied to the perception of a stimulus that predicts the presence of a primary goal (Schultz, 1998; Schultz et al., 1997). West et al. (1992) noted that sexually-conditioned incentives increase the firing rate (percentage and magnitude) of cells within the nucleus accumbens, hypothesizing that this effect may be mediated by mesolimbic dopamine activation.

The two experiments described here successfully demonstrated that a conditioned incentive can be established through five pairings of sexual access with the presence of a distinct olfactory scent. This sexually-conditioned incentive is capable of eliciting behavioral approach within an operant runway, independent of other motivational factors (such as primary female incentives), and even though the scent had not been previously perceived within the runway apparatus. In addition, Experiment 2 illustrated that administration of the dopamine-receptor antagonist, haloperidol, prior to testing within the runway attenuates the incentive value and behavioral-activating effects of a sexually-conditioned cue. These results, along with

those presented in Chapters II and III, suggest that dopamine not only mediates the ability of sexual reward to enhance and establish incentives, but also the motivational impact of both primary and secondary sexual incentives.

Chapter V: Sexual Incentive-Induced *c-fos* Expression

Numerous studies have documented copulation-induced expression of immediate early genes (IEG), such as *c-fos*, within a variety of brain regions and across a number of species (reviewed in Bialy & Kaczmarek, 1996; Pfaus & Heeb, 1997; Veening & Coolen, 1998). In the male rat, *c-fos* expression following sexual activity has been noted within the nucleus accumbens (NA) (Robertson et al., 1991), the medial preoptic area of the hypothalamus (MPOA) (Baum & Everitt, 1992; Baum & Wersinger, 1993; Coolen, Peters, & Veening, 1996; Greco et al., 1998ab; Lumley & Hull, 1999; Robertson et al., 1991), the bed nucleus of the stria terminalis (BNST) (Baum & Everitt, 1992; Baum & Wersinger, 1993; Coolen, Peters, & Veening, 1996; Greco et al., 1998ab; Robertson et al., 1991), and the amygdala (Baum & Everitt, 1992; Baum & Wersinger, 1993; Coolen, Peters, & Veening, 1996; Greco et al., 1998ab; Lumley & Hull, 1999), among others. These observations, along with the results of lesions studies and *in vivo* neurochemical analyses (reviewed in Bitran & Hull, 1987; Mitchell & Gratton, 1994; Sachs & Meisel, 1988), suggest that these regions comprise a neuroanatomic circuit dedicated to the generation of male sexual behavior.

However, the interpretation of these results is problematic, given the many potential causes of neuronal activation associated with male sexual behavior. Increased *c-fos* expression may be induced by the perception of sexually-relevant female cues, by changes in sexual motivation, or by the physical experience of copulation, including genital stimulation and ejaculation. Hence, it has been difficult to dissociate the relative contribution of appetitive versus consummatory components

(Beach, 1956; Everitt, 1990; Konorski, 1967) to neuronal activation when male subjects are allowed to engage in actual copulation. In order to address this concern, some experimenters have attempted to examine *c-fos* expression in males following the presentation of estrous cues (Baum & Everitt, 1992; Bressler & Baum, 1996; Greco et al., 1998b; Kollack-Walker & Newman, 1997; Yokosuka et al., 1999) or anogenital investigation of a nonestrous female (Coolen, Peters & Veening, 1997), independent of copulation. The degree of *c-fos* expression following mere exposure to a female presumably reflects the activation of precopulatory, incentive-motivational processes ultimately responsible for the initiation of goal-directed behavior.

Certain limitations within these studies, however, have prevented a complete portrayal of neural activity correlated with sexual desire. First, anogenital investigation of nonestrous females, while behaviorally similar to investigation of estrous females, does not provide equivalent, chemosensory input (Coolen, Peters & Veening, 1997). Certainly, male rats are more motivated to approach and engage estrous females over nonestrous females (e.g., Lopez, Olster, & Ettenberg, 1999); thus, *c-fos* induction following behavioral interaction with a nonestrous female does not reflect the full activation of sexually-specific motivational processes. Second, the presentation of female incentives is sometimes limited to pheromonal odors derived from the urine and feces of estrous females. While this technique has successfully induced *c-fos* expression within the MPOA and BNST of male hamsters (Kollack-Walker & Newman, 1997) and the premammillary nucleus of male mice (Yokosuka

et al., 1999), it has failed to induce greater *c-fos* expression in male rats compared to that seen following exposure to nonestrous odors (Bressler & Baum, 1996). Similarly, Baum and Everitt (1992) reported no difference in Fos-like immunoreactivity (Fos-Li) between male rats allowed to perceive an estrous female behind a mesh barrier, and isolated control males. In contrast, Greco et al. (1998b) not only found differences in *c-fos* induction between mated males and social controls (exposed to an estrous female, but prevented from physically interacting with them by a mesh barrier), but also between social controls and isolated controls, specifically within the MPOA, BNST, and dorsomedial amygdala.

In addition, all experiments that have looked at copulation- or cue-induced *c-fos* expression in rats have utilized sexually-experienced males. Previous work by our laboratory and others has demonstrated that sexually-naïve male rats are more motivated to approach an estrous female over a nonestrous female, indicating that estrous cues are inherently attractive (Beach, 1942; Landauer, Wiese, & Carr, 1977; Lopez & Ettenberg, 2001; Lopez, Olster, & Ettenberg, 1999; Merkx, 1983). Thus, one might expect increased neuronal activation within sexually-significant brain regions in response to primary female incentives in naïve, as well as experienced males. Indeed, Wenkstern, Pfaus, and Fibiger (1993) have documented increased dopaminergic release within the nucleus accumbens of naïve male rats during their first exposure to an estrous female. One goal of the current experiment was to measure sexual incentive-induced *c-fos* activation in naïve male rats, with the

expectation that greater immunoreactivity would be observed following presentation of estrous versus nonestrous cues.

A second goal was to directly compare *c-fos* expression in naïve males to that in experienced males, and thus determine whether sexual experience induces measurable, long-term changes in neuronal reactivity to female incentives. Such changes might explain the enhanced sexual motivation of experienced males, as reflected by their greater propensity to initiate sexual behavior with a receptive female (Dewsbury, 1969; Fleming & Kucera, 1991), shorter mount latencies (Rabedeau & Whalen, 1959), and faster female-induced run times in an operant runway (Lopez, Olster, & Ettenberg, 1999), in comparison to naïve males. Experienced males are also less sensitive to losses of sensory information (Beach, 1942; Larsson, 1975; Saito & Moltz, 1986; Thor & Flannelly, 1977), to the anxiety-provoking effects of novel environments (Pfaus & Wilkins, 1995), to the loss of testosterone due to castration (Mitchell & Stewart, 1989), and to lesions of the MPOA (Allendash & Gorski, 1983; DeJonge et al., 1989). These observations collectively suggest that sexual experience modifies synaptic organization within motivationally-relevant neural loci, such that the incentive value of estrous female cues is subsequently enhanced.

Based upon the conclusions of our earlier work (Lopez & Ettenberg, 2000; Lopez, Olster, & Ettenberg, 1999), we hypothesized that male subjects given one copulatory episode with a receptive female would subsequently demonstrate enhanced *c-fos* expression in response to the presentation of estrous female cues,

compared to that seen in naïve males. We focused our assessment on six specific neuronal populations, previously implicated in male sexual behavior: the NA (both core and shell), the MPOA, the BNST, and the amygdala (both basolateral and central regions).

Methods

Animals

A total of 24 male and 10 female Long-Evans rats were obtained from Charles Rivers Laboratories (Wilmington, MA). The males ranged from 80-100 days old and the females from 80-150 days old at the time of testing. All animals were housed individually in hanging wire cages within a temperature-controlled 22°C vivarium environment maintained under a reverse 14:10 light-dark schedule (lights on 2300 h-1300 h). Male and female rats were housed in separate rooms so as to prevent male perception of female incentives (e.g., olfactory and auditory cues) prior to testing. Food and water were provided on an *ad libitum* basis. Prior to arrival in the vivarium, the males were group housed but did not have access to females. Therefore, they were sexually-naive insofar as they lacked heterosexual copulatory experience.

Surgery

All females were ovariectomized (OVX) through a single lower abdominal incision 1-8 weeks prior to testing using standard aseptic surgical techniques and under deep anesthesia induced by inhalation of isoflurane gas (4% induction, 2% maintenance; Western Medical Supply, Arcadia, CA). All animals were pretreated

with 0.3 mg/kg IP atropine (Pittman-Moore, Washington Crossing, NJ) 15 minutes prior to the induction of anesthesia in order to reduce potential respiratory problems. Females received at least one week of post-operative care prior to initiation of the experiment.

Inducing Female Sexual Receptivity

Within this paper, "nonestrous female" refers to an OVX female not given any hormonal treatments. An "estrous female" is an OVX female in which receptivity has been induced via hormonal treatment consisting of subcutaneous (SC) administration of 15 μg of estradiol benzoate (in 0.1 ml sesame oil) 48 and 24 hours before testing, with an additional SC injection of 500 μg progesterone (in 0.1 ml propylene glycol) 3-5 hours before testing. Steroid hormones were purchased from Sigma Chemical Company, St. Louis, MO. Hormonally-treated females were screened prior to use in either copulatory experience or exposure tests to confirm receptivity.

Apparatus

Exposure tests occurred within a single Plexiglas cylinder (45 cm diameter, 40 cm height). Within this arena, a removable Plexiglas partition divided the area into two semi-circular halves. Sixteen 1.2 cm diameter holes drilled into the partition and spaced 8 cm apart from one another allowed air to pass between the two sides. Thus, the partition prevented even minimal tactile contact between subject and target, although visual, auditory and olfactory cues were accessible.

Procedure

Upon arrival, all subjects were handled for several days by the primary experimenter, and given three days of pre-exposure to the empty apparatus (10 minutes per day per animal). These steps were conducted in order to acclimate the subjects to the "background" stimuli they would encounter on test-day, thus reducing any potential novelty- or stress-induced *c-fos* response.

Subjects were divided into six groups, four animals per group: three groups were given sexual experience 24 hours prior to testing, while the other three groups remained sexually-naïve. Sexual experience consisted of a single pairing with a receptive female in an open-field (0.75m x 0.75m). Individual subjects were allowed to copulate with the female until they achieved a single ejaculation, at which point they were removed and returned to their home-cage. All copulatory episodes took place under red-light illumination, during the dark portion of the rats' photoperiod. All 12 males within the 3 sexually-experienced groups successfully achieved ejaculation with the female during this one pairing. In order to control for the experience of extra handling and transport, subjects in the sexually-naïve groups were individually placed within the open-field for 10 minutes each, without a female present.

The following day, subjects were tested in the exposure arena by pairing them with one of three stimulus targets: an uninhabited arena, a nonestrous female, or an estrous female. Prior to a subject's trial, the arena was prepared for use: a layer of bedding (Sanichips) was placed on the floor, the Plexiglas partition was introduced to

divide the arena into two halves, and a female target was placed into one half (for some test conditions). The stimulus properties of the bedding differed depending on the nature of the target. If the male was going to be exposed to an empty arena, then fresh, unscented bedding was used. If the male was going to be exposed to a nonestrous female, bedding that had been placed in holding-bin along with a different nonestrous female was used. Similarly, if the male was going to be exposed to an estrous female, bedding that had been scented by a different estrous female was used. The first and second males tested were always within the uninhabited-arena condition, the third and fourth within the nonestrous female target condition, and the fifth and sixth within the estrous female target condition. This order was maintained across test-days so as to prevent the possibility of lingering estrous odors influencing the *c-fos* expression in nonestrous-paired or uninhabited arena-paired subjects. However, the order of testing between sexually-naïve and sexually-experienced males was counterbalanced across test-days.

Each subject was exposed to their stimulus target for 15 minutes, during which time an experimenter recorded the number of genital grooms displayed by the subject. This was done in order to see whether subjects exposed to an estrous female engaged in a greater amount of physical self-stimulation, a potential confounding influence on *c-fos* expression in sexually-relevant brain systems. All testing occurred under red-light illumination during the dark portion of the photoperiod. Following the completion of each trial, the subject male was returned to his holding tub, the female target (if present) was either returned to her home-cage or prepared for use in

the next trial, and all bedding was removed from the floor of the arena. In addition, the experimenter wiped the entire arena with a moistened paper-towel between trials in order to remove any male and/or female odors.

Perfusion, tissue processing, and immunocytochemistry

Exactly one hour following the completion of each exposure trial, the subject was deeply anesthetized with an intraperitoneal injection of 1 ml sodium pentobarbital (Nembutol: Pittman-Moore, Washington Crossing, NJ) and perfused transcardially with 200 ml of 0.1 M sodium phosphate buffer (PB; pH 7.2) followed by 400 ml of 4% paraformaldehyde in PB. Peak *c-fos* expression occurs approximately one hour following a significant stimulus event and persists for several hours (Baum & Everitt, 1992). Following perfusion, brains were removed, and cryoprotected for at least 24 hours in a 20% sucrose solution. Within five days, brains were disected into separate hemispheres, each right hemisphere was sliced into 40 μm coronal sections using a cryostat, and sections were immediately mounted onto slides. Slides were stored at -20° C until immunocytochemical staining.

The elite Vectastain Kit (Santa Cruz Biotech, Santa Cruz, CA) was used for the avidin-biotin peroxidase method of immunostaining. Sections were washed 2 X 10 min with 0.05 M tris-buffered saline (TBS), and then once for 5 min in 0.25% Triton-X-100, followed by 2 more 10 min washes with TBS. This was followed by a 5% dimethyl sulfoxide wash for 10 min, and 2 X 10 min TBS washes. To serve as a blocking step, each slide was incubated for one hour in a sealed, humidified chamber in 300 μl of a mixture containing 20% Normal Goat Serum (NGS), 1% Bovine Serum

Albumin, and TBS. Blocking was followed by a 24-hour incubation period with the primary antibody, also within sealed, humidified chambers. Primary antibody mixture consisted of rabbit anti-*c-fos* polyclonal (diluted 1:1000) in TBS, NGS, and 10% Triton. Sections were then washed 2 X 10 min in TBS before being incubated for 1 hour with the secondary antibody, consisting of anti-rabbit IgG, TBS, and NGS. Two more 10 min TBS washes followed. Sections were then incubated for 35 min in TBS containing 0.5% avidin-biotinylated horseradish peroxidase complex. Sections were washed 2 X 10 min in TBS prior to staining in a solution containing 3'3'-diaminobenzidine (DAB) and hydrogen peroxide in TBS for 20 min. Following a final 10 min wash in TBS, sections were dehydrated via a 70%, 95%, 100% ethanol series, cleared in a 30 min HemoDe soak, and lastly coverslipped using DPX.

In addition, a set of control slides were tested to ensure the specificity of the primary antibody. These slides were included in every step of the staining protocol with the following exception: during the primary antibody incubation, a mixture containing only TBS, NGS, and 10% Triton was applied. Later analysis of these slides revealed virtually no staining.

Cell Count

Numbers of positively stained nuclei, indicative of Fos-like immunoreactivity (Fos-Li), were counted using a microscope equipped with an eye-piece reticle that divided the field of view into a 10x10 grid (0.25 mm^2). Initial positioning was accomplished at 10X magnification; the central four squares of the viewing-grid were placed over the targeted region. Magnification was then increased to 40X, and the

total number of positively-stained cells within the entire grid was counted. A research assistant, blind to each slide's experimental grouping, conducted all counting.

A total of six different brain regions were targeted for analysis, and an additional seventh brain region was included as a control. Both NA core (bregma +1.00, DV 7.3, ML 1.0) and shell (bregma +1.00, DV 6.8, ML 1.6) regions were examined, and were located using the position of the anterior commisure and third ventricle. The MPOA (bregma –0.92, DV 8.6, ML 0.3) and BNST (bregma –0.92, DV 7.6, ML 1.0) were identified using the positions of the anterior commisure, third ventricle, and optic chiasm. Both the basolateral (bregma -2.30, DV 8.5, ML 4.9) and central (bregma –2.30, DV 8.1, ML 4.0) nuclei of the amygdala were inspected, and located using the positions of the internal capsule and optic tract. Lastly, the ventral pallidum (VP; bregma -0.30, DV 7.8, ML 2.7) was examined as a control region. All brain areas were localized by referring to Paxinos and Watson (1986). Figure 9, panels A-C shows schematic diagrams of the areas under question.

Results

A preliminary analysis, consisting of a 2 (naïve/experienced) x 3 (test-stimulus) ANOVA, revealed that there were no significant differences in the number of genital grooms displayed by subjects across all experimental conditions.

Figure 10, panels A-F, displays the mean (+SEM) number of positively stained, Fos- Li nuclei counted within a 0.25 mm^2 area in each of the six experimental

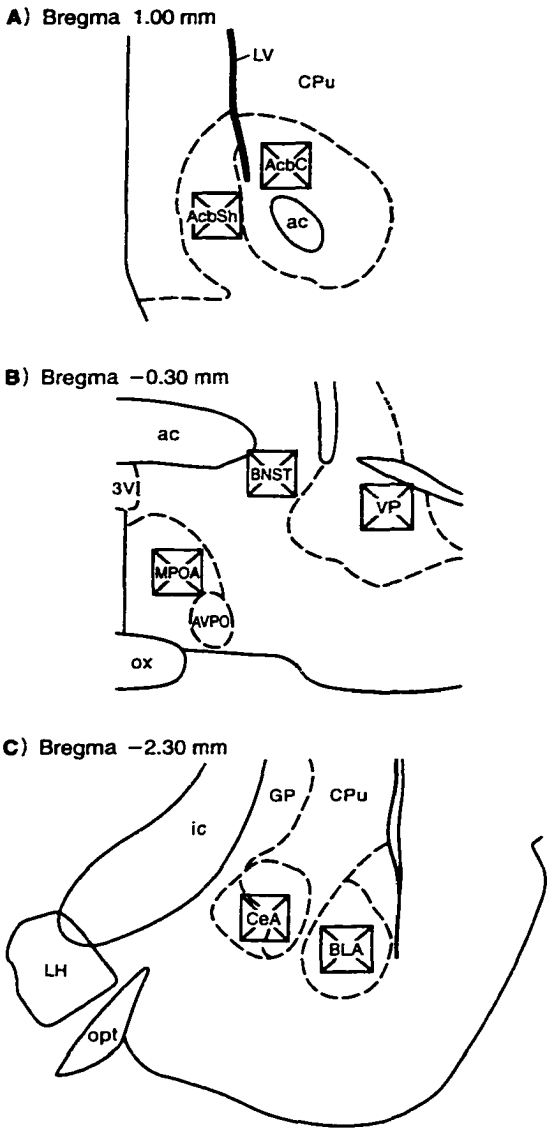

Figure 9. Location of seven neural regions that were targeted for Fos-Li analysis. These schematics were adapted from Paxinos & Watson (1986). 3V = 3rd ventricle, ac=anterior commissure, AcbSh = nucleus accumbens shell, AcbC = nucleus accumbens core, AVPO = anteroventral preoptic nucleus, BLA = basolateral amygdala, BNST = bed nucleus of stria terminalis, CeA = central amygdala , Cpu = caudate putamen, ic = internal capsule, GP = globus pallidus, LH = lateral hypothalamus, LV = lateral ventricle, MPOA = medial preoptic area, opt = optic tract, ox = optic chiasm, VP = ventral pallidum.

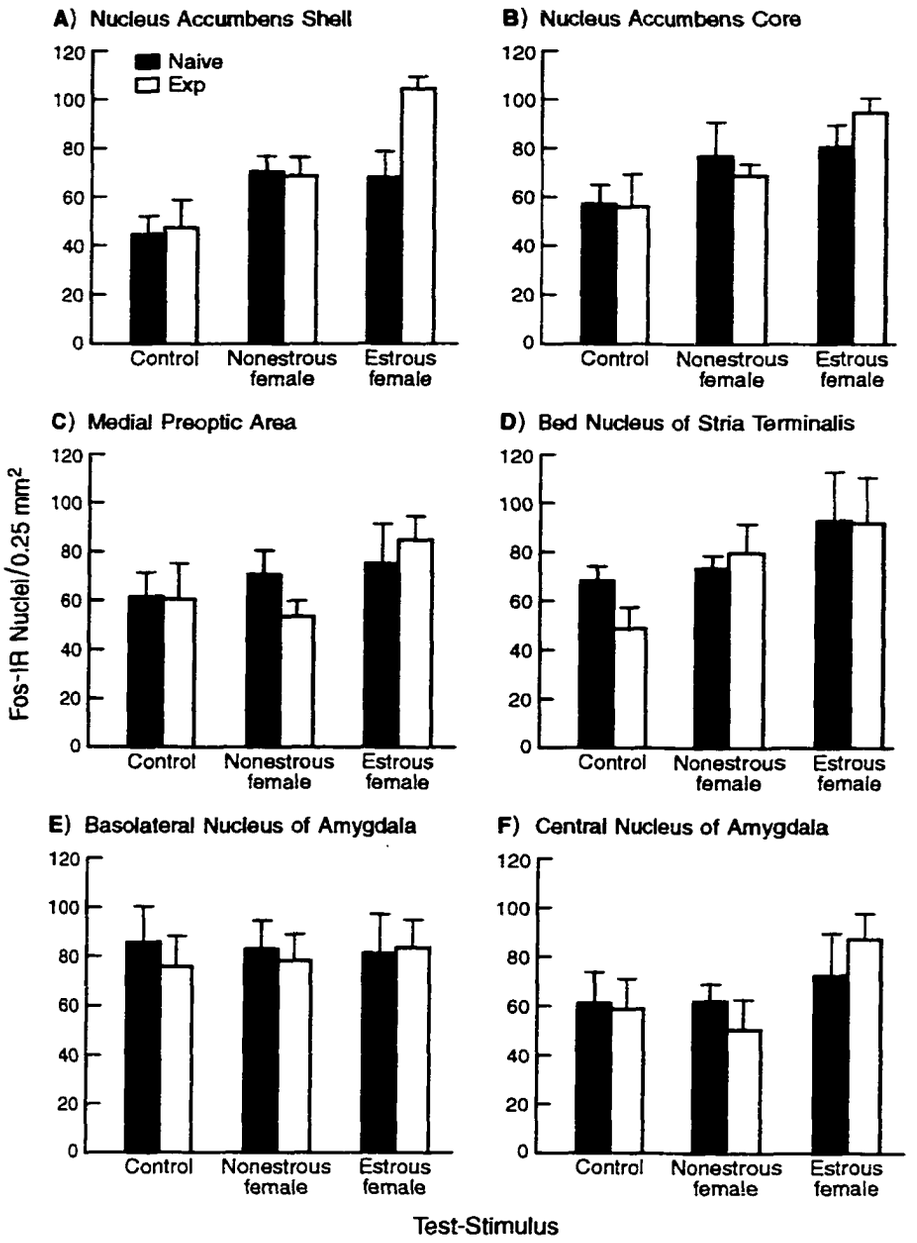

Figure 10. Mean (+SEM) number of positively stained, Fos-Li nuclei counted within a 0.25 mm² area in each of the six experimental brain regions, for subjects exposed to each of the three test-stimuli. Panels A-F represent means from the a) NA shell, b) NA core, c) MPOA, d) BNST, e) basolateral amygdala, and f) central amygdala respectively. Black bars are sexually-naïve subjects and white bars are experienced subjects.

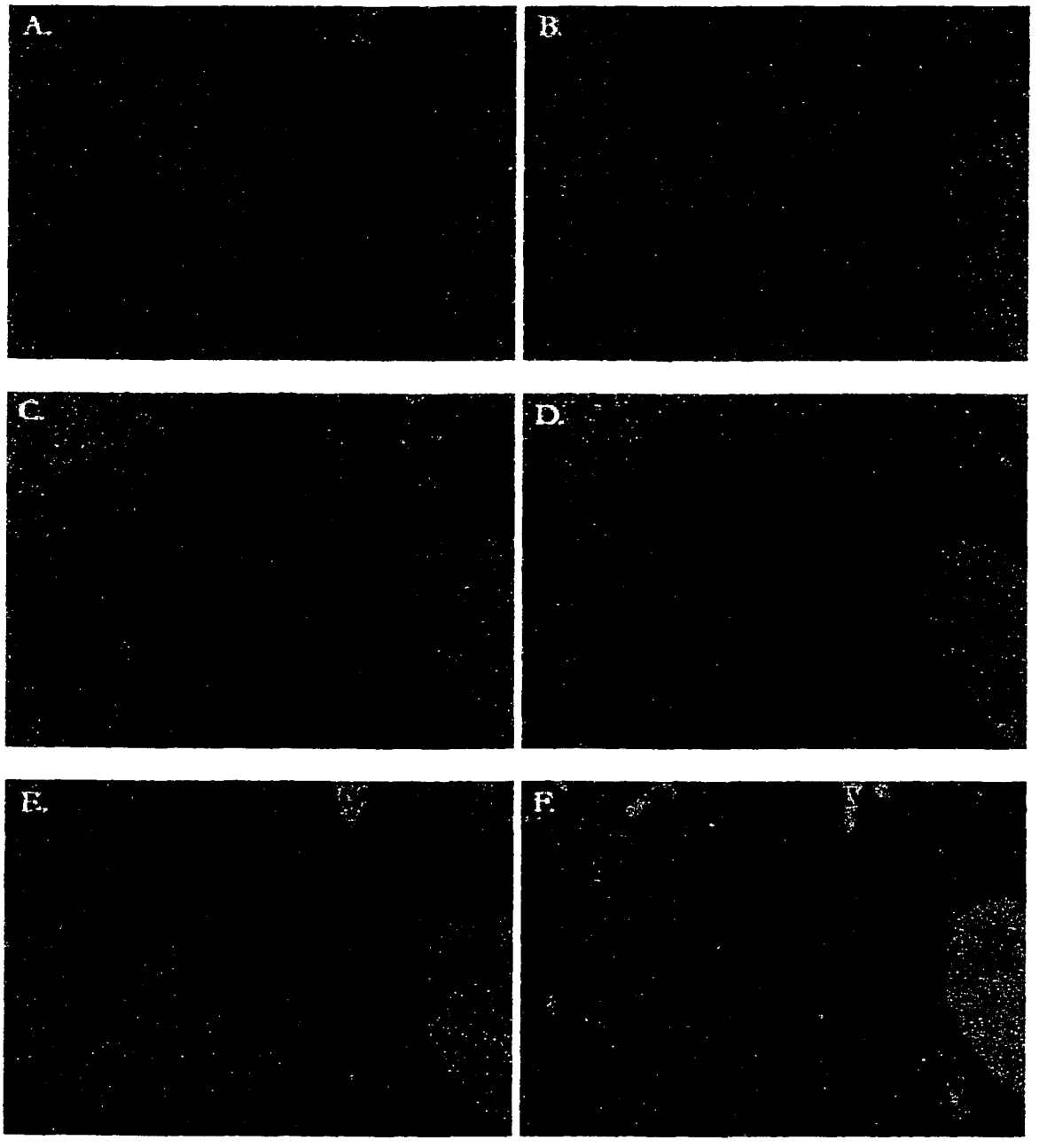

Figure 11. Photomicrographs of representative Fos-Li within the NA shell of naïve and experienced male rats exposed to the three test-stimuli. Panels A, C, and E are from naïve subjects, while panels B, D, and F are from experienced subjects. Panels A&B, C&D, and E&F are from subjects exposed to an uninhabited arena, a nonestrous female, and an estrous female, respectively.

brain regions. An overall 2 (naïve/experienced) x 3 (test-stimulus) x 6 (brain region) ANOVA revealed a significant effect of test-stimulus, $F(2)=13.80$, $p<0.005$. A total of six 2 x 3 (naïve/experienced x test-stimulus) ANOVA's were then conducted on the data presented in each of the panels of Figure 10, i.e. on the data for each brain region. These analyses revealed a main effect of test-stimulus within the NA shell, $F(2)=11.81$, $p<0.005$, and NA core, $F(2)=5.13$, $p=0.017$. There were no other significant main effects or interactions within any brain region.

A set of seven post-hoc, contrast-comparisons were then conducted on the data within panels A and B (NA shell and core), using Bonferroni's adjustment to achieve an alpha of 0.007 (comparisons between subjects placed within an uninhabited arena and subjects exposed to an estrous female were excluded because of their theoretical irrelevance). Within the NA shell, these analyses revealed a significant difference between naïve and experienced subjects exposed to an estrous female, $p=0.006$, and between experienced subjects exposed to a nonestrous versus estrous female, $p=0.007$. These differences in Fos-Li expression in the NA shell can be observed in the photomicrographs of Figure 11, panels A-F. There were no significant contrasts in the data obtained from the NA core.

Lastly, it should be noted that within the designated control region, the ventral pallidum, there were no differences in Fos-Li across all conditions. The mean (+SEM) numbers of stained nuclei noted within this brain region were 16.75 (+2.66), 18.50 (+3.69), 23.25 (+4.09), 13.00 (+5.70), 13.25 (+4.53), and 17.50 (+4.09) for the

naïve/control, naïve/nonestrous, naïve/estrous, experienced/control, experienced/ nonestrous, experienced/estrous conditions respectively.

Discussion

The results of this experiment indicate that the perception of primary female incentives, exclusive of copulatory activity, elicits *c-fos* activation within the nucleus accumbens of male rats. As hypothesized, incentive-induced *c-fos* expression within this region was enhanced by previous sexual experience; subjects allowed to copulate with a receptive female (24 hrs prior to testing) demonstrated greater Fos-Li when presented with an estrous female, compared to naïve subjects. In addition, there was greater Fos-Li within the NA shell of experienced males when exposed to an estrous female versus a nonestrous female. This suggests that the NA may be a critical processing center in the generation of male sexual motivation, a hypothesis that has already received a significant amount of support (Everitt, 1990; Pfaus & Everitt, 1995; Pfaus & Phillips, 1991). Thus, the measurement of incentive-induced Fos-Li may be a useful means of localizing areas dedicated to filtering motivationally-relevant sensory input and/or those responsible for translating such information into appropriate behavioral responses (Mogenson et al., 1980).

Furthermore, given the evidence tying dopamine to incentive-motivational processes (Berridge & Robinson, 1998; Blackburn et al., 1987; Blackburn et al., 1989; Blackburn et al., 1992; Horvitz, 2000; Ikemoto & Panksepp, 1999; Kiyatkin, 1995; Mogenson et al., 1980; Phillips et al., 1991; Robbins & Everitt, 1996;

Salamone, 1994, 1996; Schultz, 1998; Schultz et al., 1997), it seems reasonable to hypothesize a link between dopamine release caused by the perception of primary female incentives and the enhanced Fos-Li noted in this study. Increases in extracellular dopamine within both the NA (Pfaus et al., 1990; Pleim et al., 1990; Wenkstern, Pfaus, & Fibiger, 1993) and MPOA (Hull et al., 1995) have been noted prior to and during copulation. These increases occur when males are simply allowed to perceive a receptive female from behind a mesh barrier, preventing physical interaction (Damsma et al., 1992; Fiorino, Coury, & Phillips, 1997; Hull et al., 1995), or when estrous pheromones are presented independent of an actual female (Louilot et al., 1991; Mitchell & Gratton, 1991, 1992). Moreover, repeated exposure to estrous female bedding leads to an increased, or sensitized, dopaminergic response within the NA (Mitchell & Gratton, 1991). These data collectively suggest that dopaminergic fluctuations within the NA and MPOA directly parallel changes in sexual motivation. The increased dopamine transmission during the preparatory and consummatory phases of sexual behavior in male rats could serve to both reward ongoing sexual activity and facilitate preparatory sexual behaviors that are elicited by primary or secondary incentives. The fact that the most significant Fos-Li seen in this study occurred within the nucleus accumbens shell, a primary terminal region for dopamine, certainly lends credence to the possibility that estrous cue-induced *c-fos* activity is correlated with dopaminergic release.

The observation that significant increases in Fos-Li were induced by estrous cues only in experienced males was somewhat surprising. Based on our previous

observations of the inherent incentive value of estrous cues (Lopez & Ettenberg, 2000, 2001; Lopez, Olster, & Ettenberg, 1999), and the fact that primary female incentives are capable of eliciting significant increase in dopamine release within the NA of naïve males (Wenkstern, Pfaus, & Fibiger, 1993), it was hypothesized that naïve males would also demonstrate enhanced Fos-Li in response to estrous versus nonestrous female cues. However, the *c-fos* IEG is only one member of a family of genes that are activated by neuronal stimulation, and its expression may not be involved in mediating unconditioned neurochemical responses to female incentives. Indeed, given the suspected role of *c-fos* and other IEG's in regulating long-term synaptic change due to experience, it is perhaps not surprising that the activity of this gene was more easily observed in sexually-experienced subjects. We have previously suggested that sexual experience, consisting of a single ejaculation, is capable of enhancing the incentive value of estrous cues (Lopez & Ettenberg, 2000; Lopez, Olster, & Ettenberg, 1999). The current experiment provided some evidence that such adjustments in incentive value may be related to activity of the *c-fos* gene, and specifically occur within the nucleus accumbens.

As stated in the introduction, copulation-inducted Fos-Li has been noted in the NA, as well as all of the other brain regions examined in this study. Perhaps such genomic activity during consummatory sexual behavior induces permanent synaptic changes that subsequently affect the sexual motivation and/or copulatory efficiency of experienced male rats. Lumley and Hull (1999) have noted that administration of a D1-receptor antagonist prior to copulation reduces the amount of Fos-Li observed in

the MPOA. This observation provides further evidence for a link between dopaminergic activity, induced by both appetitive and consummatory processes, and *c-fos* activation within sexually-relevant neural loci. We suspect that dopamine-receptor blockade during copulation would also prevent experience-mediated enhancement of incentive-induced Fos-Li, much as it prevents increases in male sexual motivation (Lopez & Ettenberg, 2000).

Chapter VI: Summary and Conclusions

The data presented in this dissertation both expand upon and modify current hypotheses regarding the biopsychological features of male sexual motivation. Perhaps most importantly, the experimental results presented here challenge a fundamental tenet of incentive-motivational theory: that incentives must be learned through associative processes. The data presented in both Chapters II and III clearly indicate that adult male rats are inherently more attracted to estrous females over nonestrous females or other males. The perception of estrous female cues elicits unconditioned approach behavior in an operant runway, even though subjects have never experienced heterosexual copulation with a receptive female and are not allowed to physically interact with the target over the course of the experiment. It is perhaps because the majority of researchers implicitly assume that sexual experience is necessary for sexual motivation to emerge that virtually all methodologies utilized to examine motivational processes in laboratory animals involve the application of reinforcement and conditioning. In contrast, our research program demonstrates that an extended training period is not necessary, that subjects need merely to be exposed to goal-stimuli in order to exhibit goal-directed behavior. This finding should provide encouragement to researchers who wish to study motivational processes within naïve subjects across a number of behavioral domains, and explore the relationship between evolved psychological architecture and modification through learning.

To many scientists, and lay-people, it is perhaps not surprising that male rats are inherently attracted to estrous females. Ethologists and psychologists have long discussed the positive reproductive benefits that accrue to females who display their

reproductive status to males, and to males who are capable of accurately interpreting such signals (Daly & Wilson, 1984; Symons, 1979). Periods of ovulation and possible fertilization occupy only small portions of the entire mammalian estrous/menstrual cycle. Sexual activity that occurs outside of this time frame is generally considered to be energetically wasteful and potentially dangerous, as copulation increases participants' risk of being the victim of predation or other environmental hazards. Females of many species evolved physical and behavioral signals that manifest themselves during this fertile period; in its characteristically efficient manner, natural selection causally tied such signals to the activity of ovarian hormones. Males who evolved the tendency to be attracted to and intiate copulation with those females displaying such cues possessed significant reproductive advantages over males that failed to distinguish between nonreceptive and receptive females.

Indeed, the alternative scenario, in which males must learn which stimuli predict possible copulation through processes of sexual reinforcement, is rife with contradiction. First and foremost, it fails to explain how or why a male would be capable of engaging in sexual activity with a receptive female during his first encounter. Some researchers have suggested that such an episode could occur spontaneously and accidentally (Agmo, 1999; Pfaus, 1996), but upon deeper consideration, the chances of an accidentally successful penile intromission into a waiting vagina are infinitesimally small. Second, even this scenario requires the existence of certain motivational biases, such as an attraction towards members of

one's species. The decision to draw the line of attraction arbitrarily between nonestrous and estrous female seems particularly nonsensical.

Having said this, it is also likely that certain non-copulatory, social interactions over the course of early development are essential to the formation of a "normal" motivational predisposition to engage estrous females. The pioneering work of Harlow stressed the importance of maternal bonding and physical feedback in the emergence of a normal adult sexual response (Harlow, 1965; Harlow & Harlow, 1969). In addition, it has been shown that adult male rats prefer to ejaculate in a receptive female whose vagina has been scented with an olfactory cue previously paired with the male's dam during his infancy (Filllion & Blass, 1986). Many species learn what a prospective mate should look like during infancy through contact with their parents and/or siblings (Bateson, 1983). However, such observations do not fit easily within the prototypical incentive-learning framework, as those mechanisms that establish the incentive value of sexual cues under these circumstances are clearly not simple reinforcement-mediated associative processes. Indeed, it is an amazingly-specific learning process that allows an organism to learn certain attractiveness features from associations with kin while concurrently developing a functional incest-avoidance mechanism (Lieberman, personal communication).

A further conclusion generated from this dissertation is that sexual experience is capable of influencing subsequent sexual motivation in two primary ways. First, it enhances the inherent positive incentive value of estrous female cues. Thus, on

subsequent occasions, male rats with previous sexual experience demonstrate an increased desire to interact with receptive females, reflected in our experiments by faster approach behavior. This enhancement occurs even when sexual experience is limited to a single copulatory episode culminating in ejaculation; however, a single intromission with a receptive female is inadequate. We interpret these findings to signify that the rewarding consequences of copulation, and specifically ejaculation, strengthen the incentive value of those stimulus properties associated with female rats (both general feminine cues and specifically estrous cues). Thus, contrary to what has been suggested in the literature, primary incentives are not established by copulatory experience, but their value can be modified by it.

Whether this enhancement plays a functional role in the natural mating patterns of male and female rats is questionable. Wild rats live together in burrow colonies, generally consisting of a few unrelated adult males and several related females and their offspring (Barnett, 1963; Calhoun, 1962). Females that inhabit common burrows tend to experience estrous cycle synchronization, such that they enter "heat" concurrently. When mating occurs, numerous females and males copulate with one another, often switching mates between intromissions, until the estrous period terminates. This relatively unique mating system has been termed "panogamy" (McClintock, 1984). Assuming that a burrow might consist of both sexually naïve and experienced males, it is unclear what reproductive advantage experienced males would hold over their virgin counterparts, given that all males within the burrow tend to participate in mating once females come into estrus. It is

more likely that the enhanced sexual motivation of experienced males is a bi-product resulting from the activation of learning mechanisms that allow for the establishment of conditioned incentives.

This, as demonstrated in Chapter IV, is the second motivationally-significant consequence of sexual experience. Copulatory activity culminating in ejaculation is capable of transforming particular environmental stimuli, detached from the actual goal, into conditioned incentives that subsequently influence motivational systems. Again, it is likely that the rewarding aspects of copulation and ejaculation allow for such learning to take place. However, the exact nature of this learning process is far from clear. In particular, our results do not reveal whether the neutral stimulus becomes associated with the goal-stimulus (US) or the actual outcome (copulation). This question could be tested fairly easily: if a CS-US association is being formed, devaluing the US (through negative conditioning) following the establishment of the CS+ should reduce the motivational impact of the CS+. This assumes that perception of the CS triggers activation of an associated US representation that is ultimately responsible for stimulating motivation. If US devaluation had no effect on the motivational impact of the CS, this would provide evidence for a stimulus-outcome association (Dickinson & Balleine, 1994). This hypothesis has been tested in male Japanese quail, and results indicated that US devaluation did indeed attenuate approach behavior normally elicited by the CS+ (Hilliard & Domjan, 1995). However, the fact that sexual satiation was used as the mechanism of US-devaluation

introduces a serious confound into the results, namely that subjects were not sexually motivated in general, and thus not interested in the predictive value of the CS+.

On a functional level, the ability to form motivationally-specific CS-US or stimulus-outcome associations allows individual organisms to accommodate their behavior to local conditions (that vary between members of a species) in an attempt to efficiently satisfy recurring physiological needs and maximize survival and reproductive success. More specifically, sexual-conditioning might allow males to recall, seek, and identify locations where female conspecifics regularly gather, and where copulation is more likely to occur. In addition, sexual predictive signals may facilitate behavioral interactions between males and females, and potentially stimulate critical aspects of reproductive physiology (Domjan, Blesboi, & Williams, 1998; Graham & Desjardins, 1980). For example, male Japanese quail release a greater volume of semen and greater numbers of spermatozoa over controls when allowed to copulate in the presence of a sexually-conditioned, secondary incentive (Domjan, Blesboi, & Williams, 1998). More generally, such incentives are also capable of causing significant increases in both testosterone and lutenizing hormone within male rats (Graham & Desjardins, 1980), thus providing a physiological mechanism behind potential functional benefits.

This learning process is not necessary to establish primary female incentives, because those cues directly tied with the goal object are stable, recurrent features of an organism's environment, and thus a suitable target for natural selection. We would also expect primary incentives associated with other natural rewards, such as

food and water, and natural punishments, such as common predators, to unconditionally activate goal-directed behavior in experimental subjects. However, the stimulus properties of the experimental goal must adequately match those associated with the naturally-occurring, species-typical goal. For example, we would not expect that the presentation of laboratory "rat-chow" to activate midbrain dopamine systems in "naïve" rats, since it does not share many stimulus properties with the food normally consumed by wild rats in their natural habitat. In other words, their biopsychology is not well-adapted to respond to this goal. With repeated consummatory experience, however, subjects will learn to associate rat-chow with positive nutritional consequences, such that its stimulus properties become secondary incentives. It is this failure to appreciate the importance of an organism's "natural architecture" within the experimental setting that has engendered the bias towards learning in both traditional and modern incentive-motivational theory. Indeed, choice of both subject and goal alike is often conducted arbitrarily, with the assumption that the results generated reflect some common, universal structure among species. While aspects of both instrumental and classical conditioning are probably shared between many organisms, such an approach certainly precludes the examination of inherent motivational predispositions that are fundamental in the generation of natural behavior.

Beyond these speculations, there remain some critical questions regarding the ability of organisms to form predictive associations. First and foremost, how does an organism "decide" upon the local environmental cues that will become secondary

incentives, given the infinite possibilities available? It is very likely that the choice of secondary incentives is not a random process, and depends upon non-neutral incentive properties of particular classes of stimuli. For example, it is perhaps not surprising that those subjects discussed in Chapter IV found it relatively easy to associate food-related (orange and almond) olfactory cues with the US or sexual outcome. Certainly, it would be interesting to test whether other classes of stimuli (particularly non-organic smells, such as those of metal) would be as effective in conditioning regimens.

Second, what is the range of possible temporal and spatial relationships that can exist between the stimulus and physiological event (e.g., ejaculation) for conditioning to occur? Pfaus (personal communication) has suggested that for successful conditioning to occur, the US and CS must be paired during the post-ejaculatory interval. In the procedures described in Chapters III and IV, those males given sexual experience were allowed to co-exist with the females they copulated with for approximately one minute following ejaculation. Thus, it is possible that both the incentive-enhancement and incentive-establishment effects of sexual experience are mediated during this post-ejaculatory period.

It has been suggested that the answers to these questions are quite different depending upon the motivational/behavioral domain under examination (Domjan & Hollis, 1988). Adaptive learning specializations and constraints certainly exist for individual species and sexes (e.g., song-learning in male birds, learning of stellar orientation cues in migratory birds, dead-reckoning in ants; Sherry & Shacter, 1987).

Specializations may also exist in copulation-mediated learning, dependent upon the nature of a species' mating system and typical ecological conditions (Domjan & Galef, 1983; Domjan & Hollis, 1988). For these reasons, it would doubtless behoove researchers within this field to adopt a more evolutionary, comparative approach in the study of motivational systems, while the search for a common neural substrate or neurochemical mediator of "desire" continues.

The second central goal of this dissertation was to more specifically identify the role of dopamine in generating male sexual motivation. Previous research has made it quite clear that dopaminergic manipulations affect several aspects of male sexuality, even when controlling for potential motoric confounds. The data presented in Chapters IIB, IIIB, and IV suggest that dopamine participates in a variety of central processes that both directly and indirectly impact male sexual motivation. Before discussing these hypotheses, is should be noted that the use of haloperidol within this series of dissertation experiments introduces a number of interpretive difficulties, revolving around the drug's specificity. Haloperidol not only acts as a competitive dopamine-receptor antagonist at both D1 and D2 subtypes (with a substantially greater affinity for the latter), but also affects serotoninergic and adrenergic activity to a lesser degree (Kinon & Lieberman, 1996). Thus, while use of haloperidol in behavioral pharmacology has a long and well-established history, these studies should be viewed as a first-step in the exploration of dopamine's role in motivational processes. Further studies could make use of more specific dopamine-antagonist

drugs, as well as localize crucial neuronal populations through central administration of these drugs to particular brain regions.

With these caveats in mind, the results presented in Chapter IIIB suggest that dopaminergic release during sexual activity mediates incentive-enhancement, and presumably incentive formation as well (although this was not explicitly tested). This dopaminergic activity is probably also associated with the experience of sexual reward and the ability of copulation to reinforce operant behaviors, although the former is untestable and the latter, not applicable to the current research. Indeed, there are several possible means by which dopamine release during copulation could mediate incentive-learning or enhancement. Dopamine may be released during adaptively significant activities as a reflection of increased attention (Clark et al., 1987; Matthysse, 1978; Ragozzino, 2000). Attentional enhancement may, in turn, increase the probability of associative learning, and potentially focus such conditioning processes on relevant environmental stimuli. Dopamine may also mediate the learning process itself, such that haloperidol directly prevents incentive-formation. However, this hypothesis is less likely, considering the significant evidence documenting the continued capacity of organisms to learn even when under dopamine-receptor antagonist challenge (e.g., Spyraki, Fibiger, & Phillips, 1982ab). Finally, dopamine release may mediate a conscious hedonic experience that rewards organisms for engaging in adaptive behavior and simultaneously acts as a necessary catalyst for the establishment of stimulus-outcome associations. This explanation,

while intuitively reasonable, is also the most difficult to prove, given the inherently private nature of conscious experience.

In addition to mediating incentive-enhancement, dopamine also appears to serve as a signal of potential reward by responding to the presence of both primary and secondary incentives. Chapter IIB described an experiment in which haloperidol attenuated the unconditioned approach behavior that male rats display when presented with an estrous female, but did not affect run times for either nonestrous females or an empty goalbox. These results tie in quite well with previous *in vivo* demonstrations of an innate dopaminergic response to the presentation of estrous female cues within the nucleus accumbens (Louilot et al., 1991; Wenkstern, Pfaus, & Fibiger, 1993), with our data providing evidence that this response plays a functional role in mediating approach behavior towards a desired goal. However, it is possible that haloperidol treatment indirectly affected motivational activation via inhibition of sensory processes and/or attention.

Chapter IV presented an experiment in which haloperidol attenuated the motivational impact of secondary, conditioned incentives, previously established via copulatory experience. This result was somewhat surprising, for while several researchers had previously documented an inhibitory effect of dopamine-receptor antagonists on sexually-conditioned behaviors, such as lever-pressing for presentation of a conditioned incentive (Everitt, 1990) and level-changing behavior in a bi-level chamber (Pfaus & Phillips, 1989, 1991), it seemed possible that these earlier results were confounded by the involvement of multiple motivational factors. In addition,

our laboratory had previously shown that haloperidol does not reduce subjects' motivation to approach an olfactory discriminative stimulus (S+) predictive of either heroin or food reward (McFarland & Ettenberg, 1995, 1997, 1998).

However, a number of methodological differences between the McFarland & Ettenberg (hereforward, M&E) studies and those included in this dissertation may explain the discrepancy in results. Most significantly, in the M&E experiments, subjects were trained to traverse a runway through repeated trials of partial reinforcement. The discriminative stimuli placed within the runway (almond or orange extract) predicted the presence or absence of the goalbox reward. Motivational testing therefore took place within the same apparatus that conditioning was concurrently occurring. The subjects' motivation to approach the goalbox was mediated not only by the presence of secondary incentives (including the S+), but also the establishment of a stimulus-response association (and/or action-outcome association; Dickinson & Balleine, 1994). In addition, because reinforcement occurred within the runway, the many contextual cues available (i.e., the apparatus itself) most likely became salient secondary incentives over repeated testing. In contrast, those subjects in Chapter IV never received sexual reinforcement within the runway, and thus their motivation to approach the goalbox was based purely on the incentive value of the CS+ or CS-. One might expect that haloperidol would have less of a behavioral effect in the presence of multiple motivational inputs, including a strong S-R habit.

A second possibility emerges from the recent work of Shultz and colleagues (Schultz, 1998; Schultz et al., 1997) who have demonstrated that dopaminergic neurons respond to unexpected, but not expected, incentives. Perhaps then in the M&E studies, the subjects' increasing expectation of heroin or food reward upon presentation of the S+ led to an eventual decline in dopaminergic mediation of the incentive-motivational processes underlying their approach behavior. In contrast, for subjects in Chapter IV, the CS+ may have been an unexpected incentive in the context of the runway, and thus stimulated dopaminergic pathways. Haloperidol pre-treatment annulled this response and prevented motivational activation.

Taken together, the data presented in Chapters IIB and IV suggest that sexual incentives, both primary and secondary, activate dopaminergic pathways that in some way are causally responsible for goal-directed, precopulatory behavior. This conclusion coincides with what other researchers have noted in different motivational domains, such as feeding, aggression, and the avoidance of a potentially dangerous stimulus (Berridge & Robinson, 1998; Blackburn, Pfaus, & Phillips, 1992; Blackburn, Phillips, & Fibiger, 1987; Blackburn et al., 1989; Ikemoto & Panksepp, 1999; Phillips, Pfaus, & Blaha, 1991; Robbins & Everitt, 1996; Salamone, 1994, 1996; Schultz, 1998; Schultz, Dayan, & Montague, 1997; Schultz et al., 1992). Interestingly, it seems that incentives may only activate dopaminergic pathways, and consequently stimulate goal-directed behavior, when an organism exists within a strong "drive-state." For example, Wilson et al. (1995) noted that a conditioned environment predictive of food-delivery induced accumbal dopamine release only

when animals were in a state of nutrient deprivation. Thus, motivational intensity seems to be directly correlated with the degree of midbrain dopamine activation. Given that sexual deprivation does lead to intensified sexual motivation under certain experimental circumstances (Lorenz & Leyhausen, 1973; Singer & Toates, 1987; Warner, 1927), we would predict that such deprivation would also lead to a greater dopaminergic response to the perception of both primary female incentives and sexually-conditioned incentives.

This potential interaction between drive and incentive value likely reflects a more general interaction between internal and external factors in the modulation of motivational states. Increased drive, as represented by the activation of homeostatic mechanisms and changing hormonal activity, may enhance the incentive value of goal-related stimuli via an interaction with midbrain dopaminergic pathways. Hull and colleagues (1997, 1999) have suggested that male sexual desire is heavily dependent upon testosterone-dopamine interactions within the MPOA. Similarly, within female rats, changing levels of ovarian hormones across the estrous cycle may act within other hypothalamic nuclei, such as the ventromedial nucleus, to alter the incentive value of male cues. Our data suggest that the internal, stored experience of past copulatory activity modulates dopaminergic activation of the nucleus accumbens (see Chapter VI). Thus, mesolimbic dopamine activity may indeed represent the final product of a crucial process whereby internal factors modify the incentive value of incoming representations of relevant environmental stimli.

If intensity of midbrain dopamine release is indicative of motivational strength and persistence, than a number of intriguing hypotheses present themselves. First, assuming that the intensity of male sexual desire is positively correlated with the attractiveness of a perceived female, then females with greater mate-value should stimulate a stronger dopaminergic response in the male brain. Additionally, for species that tend to establish long-term pair-bonds for reproductive purposes, perhaps the perception of one's mate would also stimulate greater dopamine release than perception of another conspecific. However, we have already noted how the opposite process, namely the perception of a novel female verus one that has been previously mated with, induces greater accumbens dopamine release in male rats (Fiorino, Coury, & Phillips, 1997). Would we see this response in species that do not exhibit strong Coolidge effects? These hypotheses are purely suggestive, and this discussion is not meant to indicate that dopamine is the only neurochemical mediator of sexual motivation. By necessity, this dissertation has focused on one neurotransmitter system, ignoring the role of serotonin, norepinephrine, and other chemicals that have been implicated in male sexual behavior (Pfaus & Everitt, 1995; Wilson, 1993).

From an information-processing standpoint, dopamine's dual role in mediating both reward-related learning processes and incentive-motivation is not illogical. An essential aspect of adaptive behavior is the translation of experienced events into motivational adjustment. In other words, an organism requires some means of integrating the results of behavioral choices into decision-making routines that will determine subsequent responses. Feedback regarding positive benefits (i.e.,

reward) and negative consequences (i.e., punishment) should not only create "cognitive expectancies" under similar environmental conditions (Tolman, 1932), but also modify pre-existing behavioral strategies in order to maximize future success in attaining relevant goals. Dopamine may be a biochemical substrate for mapping the positive effects of rewarding experience onto motivationally-appropriate algorithms, perhaps by simply increasing the probability of "approach" decisions when positive incentives are perceived.

The data presented in Chapter V provide some suggestive evidence regarding a potential neural locus of male sexual motivation. The observation of increased Fos-Li within the nucleus accumbens in response to estrous female cues (in comparison to nonestrous cue-induced activation) supports the hypothesis that this region is involved in appetitive sexual processes (Everitt, 1990; Pfaus & Phillips, 1991). The results also provide further support to a proposed link (Lumley & Hull, 1997) between dopaminergic release and *c-fos* activation. A variety of experimenters have recorded increases in dopamine concentration within the nucleus accumbens of male rats exposed to estrous female, using *in vivo* neurochemical techniques (Damsma et al., 1992; Fiorino, Coury, & Phillips, 1997; Pfaus et al., 1990; Pleim et al., 1990; Wenkstern, Pfaus, & Fibiger, 1993). A conjunction of these prior observations with the results from our *c-fos* study and the data presented in Chapter IIB, provide fairly compelling evidence that dopaminergic activation in the nucleus accumbens is a central causal factor in the generation of male sexual motivation.

The difference in incentive-induced Fos-Li between naïve and experienced males was particularly impressive since experienced subjects only received one copulatory episode to ejaculation prior to testing. However, the failure to note enhanced Fos-Li in response to estrous cues within sexually-naïve males was surprising, given our previous success in demonstrating the inherent attractiveness of primary female incentives. As suggested in the Chapter V discussion, activity of the *c-fos* gene may be more involved in mediating long-term changes in neuronal responsiveness based upon the effects of a previously rewarding experience. The fact that the strongest differences between naïve and experienced males were noted within the nucleus accumbens shell matches well with the results discussed in Chapter IIIB, in which dopaminergic blockade during copulation prevented subsequent increases in male sexual motivation. It is possible that increased dopaminergic activity within the nucleus accumbens during sexual activity may initiate a sequence of neurochemical events, involving *c-fos*, that subsequently lead to an enhanced, or sensitized neural response to the presenation of sexual incentives.

In summary, this dissertation has provided converging evidence in support of 1) an inherent male, motivational bias towards estrous incentives and 2) a role for dopaminergic release in mediating the behavioral-activating effects of both primary and secondary sexual incentives, and 3) a role for dopamine in copulation-mediated incentive-establishment and enhancement. These observations became possible through adoption of a modified operant paradigm in which subjects were not trained

to emit an operant response nor allowed consummatory sexual contact within the motivational-testing apparatus. Along with providing a new blueprint towards studying motivational processes in different behavioral domains, this dissertation also suggests that a more ethological, evolutionary approach towards studying appetitive processes is not only fruitful but necessary given the many potential species-specific motivational and learning predispositions. These considerations are particularly important in reference to sexual behavior, which may be regulated by quite different systems in males versus females, and across males of different mammalian species.

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