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**The Involvement of the Mesolimbic Dopamine System in the
Sexual Behavior of the Male Rat**

John B. Mitchell

A Thesis

in

The Department

of

Psychology

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

December, 1987

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ABSTRACT**The Involvement of the Mesolimbic Dopamine System in the Sexual Behavior of the Male Rat**

John B. Mitchell, Ph.D.
Concordia University, 1988.

Data from a variety of sources indicate that the neurotransmitter dopamine is involved in sexual behaviors, and suggest that the mesolimbic dopamine system is involved in mediating sexual arousal. Castration results in an immediate and progressive decline in sexual arousability. Therefore, it was predicted that castration would influence activity within the mesolimbic dopamine system, and furthermore, that activation of this system would facilitate sexual arousability in both gonadally intact and castrated males.

Experiments 1-3 investigated the effects of castration, steroid replacement, and post-castration sexual experience upon sexual behaviors and the concentrations of amine and amine metabolites in the mesolimbic and nigrostriatal dopamine systems. Castration produced a significant decrease in the concentrations of dopamine and a dopamine metabolite in a terminal field of the mesolimbic dopamine system, the nucleus accumbens. This decrease was prevented by post-castration treatment with testosterone or estradiol. Changes in dopamine and dopamine metabolite concentrations in the nucleus accumbens coincided with changes in measures of sexual arousal. Sexual experience did not affect dopamine concentrations, but did increase dopamine metabolism in the nucleus accumbens, and also increased the frequency of female-directed behaviors in castrated males.

Morphine is known to influence the activity of dopaminergic cells; Experiment 4 investigated the effects of intracranial opiate and opioid infusions on sexual behaviors. Morphine and the opioid peptide, dynorphin, was applied directly to the region of the mesolimbic dopamine cell-bodies, the ventral tegmental area, in castrated males.

maintained on behaviorally subthreshold doses of testosterone, and animals were tested for sexual behaviors. Both morphine and dynorphin produced a dose-dependent increase in the number of males that mounted, and dynorphin also increased the display of female-directed behaviors. In Experiments 5 and 6, animals were given repeated systemic injections of morphine in a distinctive environment; treatment thought to produce a conditioned increase in activity within the mesolimbic dopamine system. Both gonadally intact and castrated males showed an increase in measures of sexual arousal when presented with a sexually receptive female in an environment previously paired with repeated injections of morphine.

Taken as a whole, these experiments support the hypotheses that castration influences the mesolimbic dopamine system, and that this system is involved in mediating sexual arousability.

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I would like to dedicate this thesis to my wife, Debra Jared. Debra's unwavering support, encouragement, and understanding have been invaluable to me. With love.

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In 1849 Professor A. A. Berthold of Göttingen described the results of a study in which he transplanted testes into the abdominal cavities of two castrated cocks. He noted that both the appearance and the sexual behavior of these two birds developed normally, and concluded that testicular secretions were carried by the blood to act on the nervous system (Berthold, 1849). Since that time, research has been conducted to determine where gonadal steroid hormones act in the brain and which neurotransmitter systems and brain areas control male sexual behavior. Accumulating evidence suggests that one of the neurotransmitters critically involved is dopamine (DA); DA has been implicated in the control of both the performance of copulation and the arousal or motivation that underlies sexual behaviors.

The present thesis concentrates on evidence for dopaminergic involvement in the control of male sexual behavior, and especially sexual arousability. It includes a description of copulation and the hypothetical mechanisms that have been used to explain copulation, the effect of hormone removal by gonadectomy, the evidence for dopaminergic involvement in male sexual behavior, the role of different DA systems, and, finally, evidence for hormonally-mediated changes in DA concentrations and utilization.

Male copulatory behavior. Tests of male copulatory behavior are usually conducted by placing a sexually receptive female rat in a mating arena containing a sexually active male rat. The male rat will pursue the female, climb over or under the female, lick or sniff the female's genitals, and manipulate the female's flanks. The frequency of these precopulatory behaviors depends on the sexual experience of the male; experienced, vigorous copulators typically display little, if any, precopulatory behavior (Beach, 1956), while less experienced males may continue to display these behaviors even after copulation has begun (Pottier & Baran, 1973). The female will display ear wiggling, hopping and darting (Ball, 1937), and these solicitation or

proceptive behaviors (Beach, 1976a) potentiate mounting by the male.

When the female stops in a crouching position, the male mounts the female from the rear, grasping her flanks with his forelimbs, and then displays a series of rapid, shallow pelvic thrusts. In response to stimulation of the flanks, the female adopts a characteristic sway-backed posture, lordosis. If the male then dismounts from the female, the encounter is termed an incomplete mount (i.e., without penile insertion), or simply a mount.

A mount may be terminated by an intromission; a longer, slower and deeper thrust, often accompanied by a rapid kick with one hindleg, and a characteristic rapid back-stepping dismount. Normally, penile insertion accompanies the final deep thrust, but an intromission is identified by the body movements of the male and not by vaginal penetration (Sachs & Barfield, 1976). Verification of penile insertion is impossible due to the positioning of the male and female, and furthermore, performance of this motor pattern does not require a penis; females, for example, can display the full intromission pattern although there is obviously no penile insertion (Emery & Sachs, 1975).

Autogenital grooming follows some mounts and almost all intromissions.

After a number of intromissions the male will mount the female and display a third distinct behavior, ejaculation. Ejaculation is characterized by a final pelvic thrust that is slower and deeper than that accompanying an intromission, a reduction in the elevation of the hindleg, and removal of the forelimbs from the female that is slower than for a mount or intromission and that is held momentarily at the apex. A series of brief, spasmodic muscle contractions in the male's hindquarters may be visible. There is also an absence of back-stepping before genital grooming. Although the ejaculatory pattern is commonly associated with seminal emission, it can be observed in animals that are not capable of ejaculating, such as castrates (e.g. Bloch & Davidson, 1968; Davidson, 1966a; Davidson & Bloch, 1969) or perinatally untreated females on long-term estradiol

treatment (Emery & Sachs, 1972).

After ejaculation there is a refractory period of 4 to 8 min during which there is no sexual activity and the male is quiescent. The male vocalizes ultrasonically (22 to 23 kHz) during the first few minutes of the postejaculatory interval. The postejaculatory period appears to encompass both an absolute refractory phase, during which the male is incapable of being aroused to renewed copulation, and a relative refractory phase, during which supranormal levels of stimulation are required to bring about the resumption of copulation (Beach & Holz-Tucker, 1949; Sachs & Barfield, 1976). The entire sequence repeats until the male becomes sexually exhausted, usually after six to ten ejaculations. The period between ejaculation and the initiation of the next copulatory sequence is termed the postejaculatory interval. Each of the three copulatory behaviors, mounts, intromissions and the ejaculatory pattern, are easily distinguished by an experienced observer.

Controlling mechanisms. Evidence from studies of sexual behavior led Frank Beach to reject the notion of a unitary sex drive and to propose, instead, that two mechanisms are involved in the control of male sexual behavior (Beach, 1956; Beach & Jordan, 1956). Beach had noted, for example, that the number of intromissions preceding successive ejaculations declined while the latency to resume copulation after each ejaculation increased. The different slopes of these two functions suggested control by two different mechanisms (Beach, 1956; and see Sachs & Barfield, 1976 for a detailed review). The effects of a variety of manipulations are consistent with the view that different aspects of copulation can vary independently (see below).

Beach (1956; Beach & Jordan, 1956) proposed that the sexual arousal mechanism (SAM, or AM) controlled the period prior to the initiation of copulation, and functioned to bring the male to the threshold necessary for the initiation of copulation. The AM is, therefore, primarily concerned with sexual motivation. The state of the AM is

determined both by the male's intrinsic arousability and by extrinsic sources of arousal. The AM is susceptible to habituation; the likelihood of initiating copulation decreases with time, and the male becomes less attentive to the receptive female (Beach, 1956). Disrupting habituation to sexually relevant cues by changing the stimulus female can elicit copulation (Bermant, Lott, & Anderson, 1968; Stone & Ferguson, 1940).

Because the AM controls behavior up to the initiation of copulation, mount and intromission latencies reflect the state of the AM. Factor analysis of a variety of measures of male copulation found that mount and intromission latencies contributed to a factor similar to the AM (Sachs, 1978), supporting the use of these measures as indices of the state of the AM. The activity prior to the initiation of copulation (precopulatory behaviors) and the mount latency, however, have been considered 'purer' measures of motivation; both motivational and performance factors determine the intromission latency (Cherney & Bermant, 1970; Pfaff & Zigmond, 1971). Although the length of the postejaculatory interval has also been used to indicate the state of the AM (Beach, 1956), initiation latencies and the rate of recovery from postejaculatory refractoriness do not necessarily covary (Beach & Whalen, 1959). The absolute refractory phase is thought to derive from activity in an inhibitory system, and the relative refractory phase from inactivity in an arousal system; only the relative refractory phase, which represents the last 25-35% of the postejaculatory interval, would be expected to vary with the state of the AM (Sachs & Barfield, 1976). Factor analysis has indicated that only a measure of the relative refractory phase (the postejaculatory interval minus the duration of ultrasonic vocalization) loads into a factor analogous to the AM (Sachs, 1978).

The pacing of copulation, although occurring during copulation itself, may also reflect the state of the AM. In general, treatments that serve to increase arousal, such as amphetamine (Butcher, Butcher, & Larsson, 1969) or changing the stimulus female (Bermant, et al., 1968) reduce the mean time between intromissions, the

interintromission interval. There are, however, manipulations that would be expected to affect arousal, such as electrical brain stimulation (Eibergen & Caggiula, 1973) or hypothalamic lesion (Caggiula, Antelman, & Zigmond, 1973), that do not affect the interintromission interval. The interintromission interval also neglects the temporal clustering of mounts and intromissions (Sachs & Barfield, 1976). For these reasons, the interintromission interval may be a somewhat less reliable measure of sexual arousal than initiation latencies and precopulatory or female-directed behaviors.

According to Beach's model (Beach, 1956; Beach & Jordan, 1956), control over behavior passed from the AM to a second mechanism, the intromission and ejaculatory mechanism (later simply the copulatory mechanism or CM) after copulation was initiated. The CM controlled copulation once it had begun and functioned to bring the animal to the threshold for ejaculation. Measures of copulation itself were considered functions of the CM (Beach & Jordan, 1956). Facilitation of the CM would bring the male to the threshold for ejaculation more quickly; the number of intromissions and the ejaculation latency, therefore, vary inversely with the state of the CM.

These two mechanisms, however, did not accommodate all of the data, and a separate ejaculatory mechanism (EM) was proposed (Beach, Westbrook, & Clemens, 1966; McGill, 1965). When excitation reached the threshold for ejaculation, control passed from the CM to the EM, ejaculation accompanied the next intromission, and the EM discharged, delaying re-activation of the AM. A recent factor analysis has suggested that a minimum of four mechanisms may be required to explain control of masculine sexual behavior (Sachs, 1978). Three of these factors are similar to the previously proposed AM, CM and EM, and the fourth appears to be primarily concerned with the efficiency of copulation.

The division between the AM and CM is similar to a dissociation between arousal and performance. Arousal refers to the animal's level of excitation or readiness to

respond (Beach, 1956; Sachs & Barfield, 1976). Because copulatory behavior and general locomotor activity can vary independently (e.g., Foreman & Hall, 1987; Malmnas, 1973), the modifying word sexual will be retained when discussing the arousal that underlies the initiation of copulation, rather than assuming it necessarily reflects some form of general, nonspecific arousal. Although performance presupposes adequate arousal, arousal does not necessarily lead to performance. A failure to copulate could reflect an inability to perform the necessary motor sequences, inadequate sexual arousal, or an inability to channel adequate sexual arousal into the performance of copulation.

The distinction between motivation and performance, or between hypothetical controlling mechanisms, such as the AM and CM, is useful in assessing the role of gonadal steroid hormones in sexual behavior. A common strategy in assessing steroid action is to remove the source of endogenous hormone and measure behavior with or without exogenous hormone. The following section will describe the behavioral effects of castration.

Effects of castration on sexual behavior. The copulatory performance of a mammalian male is well known to be dependent on testicular androgens. Male castrates show a gradual decline in sexual behavior, and sexual behavior can be maintained or restored by treatment with testosterone (T) (e.g. Bloch & Davidson, 1968; Phoenix, Slob, & Goy, 1973). Many of T's actions are mediated by its conversion to estradiol (E_2) and 5α -dihydrotestosterone (DHT). Both aromatized (E_2) and 5α -reduced (DHT) metabolites of T are present in the rat brain (Jaffe, 1969; Mainwaring, 1977; Martini, 1982; Naftolin, Ryan, & Petro, 1972). E_2 will maintain most aspects of sexual behavior in castrates, but is less effective than T in maintaining intromissions and the ejaculatory pattern (Davidson, 1969; Paup, Mennin, & Gorski, 1975; Pfaff, 1970; Sodersten,

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1973). Concurrent treatment with E₂ and DHT is as effective as T in maintaining all aspects of sexual behavior in castrates (Sodersten, Hansen, Eneroth, Wilson, & Gustafsson, 1980), and there is evidence that the synergism between the T metabolites is central (Baum, Sodersten, & Vreeburg, 1974; Baum, Tobet, Starr, & Bradshaw, 1982).

One of the oldest problems of behavioral endocrinology is the reason for the persistence of copulation in male castrates despite the disappearance of testicular androgens within hours of castration (Gupta, Zaraycki, & Rodger, 1976). Many males continue to mount and intromit for several weeks after castration, and it has been reported that an occasional male will show the ejaculatory pattern as long as 10 weeks after castration (Davidson, 1966a).

The decline in male sexual behavior after castration shows a characteristic pattern. Intromission and the ejaculatory pattern are the first to disappear. Some investigators have reported that intromissions disappear before the ejaculatory pattern (Beach, 1942; Stone, 1939), but the occurrence of intromission without the ejaculatory pattern is rare (Beach & Nucci, 1970; Bloch & Davidson, 1968; Davidson, 1966a; Madlafousek, Hlinka & Beran, 1976; Whalen, Beach & Kuehn, 1961). Mounts persist longer than intromission and ejaculation, and precopulatory and female-directed behaviors persist the longest (Madlafousek, et al., 1976). When sexual behavior is restored by T administration, the behaviors reappear in the reverse order; female-directed behaviors are restored first, then mounts, intromissions and the ejaculatory pattern (Singer, 1972; Larsson & Sodersten, 1973).

The post-castration decline in sexual behaviors cannot be explained by morphological changes at androgen sensitive peripheral sites, such as the penis. A change in penile sensitivity cannot explain a decline in sexual behavior; male rats will continue to mount after complete denervation of the penis (Lodder & Zeilmaker, 1976), and male castrates may continue to mount and intromit even though the number of

epidermal papillae or spines covering the penis decline after castration (Beach & Levinson, 1950). Furthermore, it has been reported that erection is locally evocable in castrated males after all mount attempts have stopped (Rodgers & Alheid, 1972). The T metabolite DHT and the androgen fluoxymesterone are capable of maintaining androgen-sensitive tissue, but not sexual behavior (Beach & Westbrook, 1968; Butera & Czaja, 1985; Feder, 1971). Similarly, DHT maintains spinally mediated penile reflexes (Hart, 1979) but not copulation.

A more detailed analysis of sexual behavior after castration indicates that the proposed controlling mechanisms, the AM and CM, are differentially affected. Among males that continue to copulate after castration, the number of mounts increases, the number of intromissions decreases, and ejaculation latency shows an initial decrease followed by an increase (Bloch & Davidson, 1968; Davidson, 1966a). Because, theoretically, the number of intromissions to ejaculation and ejaculation latency vary inversely with the state of the CM, these data imply, perhaps surprisingly, an initial potentiation of the CM after castration. Despite this apparent facilitation there is a steady increase in intromission latency, a measure of sexual arousal (mount latency was not reported). This led Davidson to conclude that '... while the efficiency of the "copulatory mechanism" appears enhanced for some time after castration, measures related to sexual "arousal" show immediate and continuing deterioration' (Davidson, 1966a, p. 271). Thus, a castration-induced decrease in sexual arousability appears to precipitate the decline and eventual cessation of copulation.

In free choice tests that do not involve copulation, a sexually experienced male will spend significantly more time in the vicinity of a sexually receptive female than in the vicinity of a non-receptive female or an intact male. This difference is abolished by castration and reinstated by T (Hetta & Meyerson, 1978). Thus, even without copulation, the castrate has diminished interest in the sexually receptive female that may

be related to sexual motivation or arousal.

Extrinsic sources of sexual arousal. If the post-castration decline in copulation arises from decreased arousability, extrinsic sources of arousal may be particularly important for the display of sexual behavior by castrates; it has been found that the number of castrates that mount and intromit is increased by mildly painful flank shock (Barfield & Sachs, 1970). An obviously important source of arousal in copulation tests is the receptive female. Madlafousek, et al. (1976) tested castrated males with females that had been primed, using different estrogen and progesterone treatments, to display different levels of proceptive or solicitation behaviors. The intensity of the female's proceptive behavior was an important factor in determining the rate at which the male's sexual behavior declined. The typical sequence of behavioral changes was observed, but all components persisted longer if the male was paired with a highly proceptive female; that is, the sexual behavior of the male varied with the incentive qualities of the female.

Extrinsic sources of arousal are important in the sexual behavior of gonadally intact males, as well as castrates. More males will mate on their first exposure to a receptive female if they are paired with a highly proceptive female (Madlafousek & Hlinak, 1983), or if intermittent tail shock is applied (Caggiula, 1972; Caggiula & Eibergen, 1969). Furthermore, when applied at regular intervals, peripheral shock can pace copulation; that is, most shocks are followed by a copulation attempt (Barfield & Sachs, 1968; Sachs & Barfield, 1974). Other manipulations that increase arousal, such as repeated handling (Larsson, 1963), flashing lights and intermittent tones (Fisher, 1962), or changing the stimulus female (Bermant et al., 1968), also facilitate male copulation.

Examination of gonadally intact non-copulating animals is germane to the question of the mechanisms underlying sexual arousal. A large percentage of males (20 to 40 %) fail to copulate despite repeated exposure to sexually receptive females (Whalen, 1964). Among at least some noncopulators, failure to mate cannot be attributed to abnormalities

in androgen target organs (Whalen et al., 1961), inadequate androgen levels, or androgen insensitivity (Whalen et al., 1961; Whalen, 1964). These spontaneous noncopulators initially spend less time in female-directed behaviors than copulators, but increase to higher than normal levels after three or four tests (Pottier & Baran, 1973). Noncopulators appear to be intrinsically less arousable; in tests of nonsexual behavior, they are less active, less responsive to novel stimuli, and habituate to stimuli more slowly (Pottier & Baran, 1973). Sexual activity can be induced in persistent noncopulators by peripheral shock (Caggiula & Eibergen, 1969; Malsbury & Pfaff, 1974), and a stimulus previously paired with shock will itself induce copulation in persistent noncopulators (Crowley, Popolow, & Ward, 1973).

One of the manipulations that increases sexual activity, tail pinch, has received extensive study. Tail pinch can induce feeding and drinking, as well as copulation (Antelman, Rowland & Fisher, 1976; Antelman, Szechtman, Chin & Fisher 1975; Rowland & Antelman, 1976). The effect of tail pinch can be attenuated by pretreatment with the DA receptor blockers haloperidol, spiroperidol, or pimozide, but not by α -adrenergic (phentolamine) or β -adrenergic (sotalol) antagonists (Antelman, Szechtman, Chin & Fisher, 1975). Similarly, tail shock-induced facilitation of copulation is impaired by a variety of DA receptor blockers, whereas even extremely high doses of noradrenergic blockers have little effect (Antelman, Herndon, Caggiula, & Shaw, 1975). The results of these studies suggest dopaminergic mediation of sexual arousability. The role of DA in sexual behavior will be more fully explored in the following section.

Dopamine and masculine sexual behavior. When castrated males, maintained on behaviorally subthreshold doses of T, are treated with the DA agonist apomorphine (Malmnas, 1973) or with the DA precursor L-DOPA (Malmnas, 1976) the number of males initiating copulation increases. L-DOPA will also increase the display of female-directed behaviors and the DA receptor blocker pimozide will antagonize this

effect. In castrates without exogenous T, Malmnas (1977) found that apomorphine increased the number of castrated, or castrated and adrenalectomized, animals that displayed mounts, intromissions and the ejaculatory reflex, and this effect was again blocked by pimozide. The time allowed for the initiation of copulation in these studies was unusually short (3-5 min) and more control animals might have initiated copulation if allowed more time. Nonetheless, increases in female-directed behaviors and in the percentage of castrates that initiate copulation indicate that DA has a role in mediating sexual arousal.

Accruing evidence suggests that DA also facilitates the sexual behavior of gonadally intact animals, regardless of their level of sexual activity. Moderate doses of apomorphine and amphetamine reduce the interintromission interval and ejaculation latency (Butcher et al., 1969). Dallo, Lekka and Knoll (1986) tested sexually sluggish male rats with amphetamine, apomorphine or bromocriptine and found that all three treatments increased the percentage of animals that ejaculated. They also administered (-)deprenyl, a type B monoamine oxidase (MAO) inhibitor, and found a long-lasting (3 week) increase in the percentage of animals that ejaculated. Tagliamonte, Fratta, Del Fiacco and Gessa (1974) found that among males that had not ejaculated in three of four pretests, treatment with either apomorphine or L-DOPA increased the number of mounts, intromissions and ejaculations. Animals selected as good copulators ejaculated sooner, required fewer mounts and intromissions to ejaculate, and had shorter postejaculatory intervals after treatment with L-DOPA. The effects of apomorphine and L-DOPA were blocked by haloperidol, suggesting that the important consequence of each treatment was increased activity at DA receptors. Unfortunately, mount and intromission latencies, measures of sexual arousal, were not reported. Intromission latency was reported by Paglietti, Pellegrini Quarantotti, Mereu and Gessa (1978), and treatment with both apomorphine and L-DOPA significantly reduced intromission

latency, as well as facilitating several other measures of copulation. The apomorphine-induced facilitation was blocked by pimozide. Thus, this set of pharmacological studies lend support to the view that DA is important for both sexual arousal and the performance of copulation.

Several reports do not support an excitatory role for DA in sexual behavior. Treatment with L-DOPA, for example, has been found by some investigators to inhibit male copulation (Gray, Davis & Dewsbury, 1975; Hyypa, Lehtinen, & Rinne, 1971); Malmnas (1976), however, has argued that in his studies high doses of L-DOPA inhibited the display of copulatory behaviors by causing motor disturbances. A putative DA autoreceptor agonist (RDS-127), which might be expected to decrease DA neurotransmission, has been reported to facilitate mounting and ejaculating in gonadally intact male rats (Clark & Smith, 1986; Clark et al., 1982). Pimozide, however, did not affect the agonist-induced facilitation (Clark & Smith, 1986), making it unlikely that DA receptor activation was involved. There are, however, several other reports that autoreceptor activation facilitates male sexual behavior (Alhenius & Larsson, 1984; Gower, Berendson, Princen, & Broekkamp, 1984; Napoli-Farris, Fratta & Gessa, 1984). A very narrow range of doses, and dose and treatment schedules different from those used in those studies reporting dopaminergic facilitation of sexual behavior have been employed in these experiments. Furthermore, the specificity for DA autoreceptors of the agonists employed, critical for interpretation of these findings, has been questioned (Hull, et al., 1986).

Foreman and Hall (1987) appear to have resolved some of these conflicting findings in a recently reported series of experiments. They administered the specific D2 receptor agonist LY163502 to persistent noncopulators, animals that had copulated without ejaculating in four pretests, and to good copulators. Moderate doses of LY163502 produced a dose-dependent facilitation of copulation in all groups of males.

The D2 agonist, in doses between 25 ng/kg and 25 µg/kg, increased the number of noncopulators that mounted and ejaculated, increased the number of previous non-ejaculators that ejaculated, and decreased ejaculation latency and the number of mounts to ejaculation in good copulators.

The effect of D2 receptor stimulation, however, was not uniform across a wider dose range. Selective activation of high affinity autoreceptors by the lowest doses (25 pg to 10 ng) inhibited sexual behavior. Moderate doses (25 ng to 2.5 mg), which would stimulate post-synaptic receptors as well, facilitated sexual behavior. The highest dose (25 mg) led to the emergence of stereotypy that interfered with the performance of copulation. The facilitatory effect of the D2 agonist was blocked by prior treatment with the DA receptor blockers Ro 221319 and sulpiride, but not by the peripherally acting antagonist domperidone. It is interesting to note, also, that lower doses of the D2 agonist were required to demonstrate a significant facilitation of sexual behavior than to produce changes in general locomotor activity.

Neural substrates. Systemically applied DA agonists would be expected to affect a number of different DA systems and there have been few attempts to localize the neuron group(s) critical for the facilitation of male sexual behavior by dopaminergic receptor stimulation. Most of the relevant studies involve either lesions or electrical brain stimulation; there are only two reports of intracerebrally applied DA agonists or antagonists. These studies have concentrated on the preoptic area-anterior hypothalamus continuum, specifically the medial preoptic area (mPOA).

Medial preoptic area. The mPOA is a principle binding site for gonadal steroids (Sar & Stumpf, 1975), and T implanted in the mPOA will restore sexual behavior in castrated males (Lisk, 1967; Davidson, 1966b). Furthermore, the mPOA is a terminal field of the rostral incertohypothalamic DA system that has cell bodies in the rostral periventricular hypothalamus (A14 cell group) (Bjorklund & Lindvall, 1984). The

effects of mPOA lesions on sexual behavior are dramatic. Heimer and Larsson (1966/67) lesioned the mPOA-anterior hypothalamus region and found that mounts, intromissions and ejaculation were eliminated in sexually experienced males. They also reported that these animals, although sexually inactive, did approach and pursue the female and engaged in genital exploration. Similarly, Giantonio, Lund and Gerall (1970) found that lesioning the mPOA-anterior hypothalamus completely abolished copulation in most males while leaving female-directed behavior intact. The lesioned animals pursued and sniffed the female, and engaged in partial mounts with shoulder palpation but without copulatory thrusting. Thus, mPOA lesions appear to abolish the performance of copulation leaving sexual arousal relatively intact.

The abolition of copulation after mPOA lesion is well established (Chen & Bliss, 1974; Hendricks & Scheetz, 1973; Van de Poll & Van Dis, 1979). mPOA lesions do not induce gonadal atrophy (Heimer & Larsson, 1966/67), affect basal luteinising hormone (LH), T, or prolactin levels, nor do they interfere with the release of LH induced by exposure to a sexually receptive female, although they do block the release of prolactin and T normally induced by such exposure (Kamel & Frankel, 1978). The absence of exposure-induced T and prolactin release could be the result of the mPOA lesion, per se, or to the absence of copulation.

The preference of male rats for a sexually receptive female over a non-receptive female is significantly reduced by lesions of the mPOA (Edwards & Einhorn, 1986), suggesting, unlike what was said previously, a deficit in motivation. Interpretation of these data, however, is complicated by the fact that males had access to the females and could copulate with them, as the intact males were reported to do. The mPOA lesion may, therefore, have resulted in relatively less time in the vicinity of the female because there was no time devoted to copulation. In support of this interpretation is the finding of Szechtman, Caggiula, and Wulken (1978) that if copulation is not allowed, isolation

of the mPOA by sagittal knife cuts does not affect the male's preference for the odor of a sexually receptive female over the odor of a non-receptive female.

Lesions induced by the DA neurotoxin N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) suggest that a disruption of mPOA DA is responsible for some of the effects of electrolytic lesion. MPTP infused into the third cerebral ventricle of sexually experienced males resulted in a dose-related suppression of sexual behavior (Sirinathsinghi, 1987). The effects of MPTP were all on the performance of copulation (mounts, intromissions, and ejaculation); it did not affect measures of sexual arousal (mount and intromission latencies and the number of animals that initiated copulation). The effects of the infusion were not localized, but mediation by the periventricular hypothalamus, the location of DA cell bodies that project to the mPOA, is probable; the periventricular hypothalamus has access to the ventricular circulation, has the highest density of MPTP binding sites in the rat brain (Javitch, Uhl, & Snyder, 1984; cited in Sirinathsinghi, 1987), and is the location of DA cell bodies (Bjorklund & Lindvall, 1984; Bjorklund, Lindvall, & Nobin, 1975).

Studies of electrical stimulation of the mPOA have found effects that complement the lesion studies. Roberts, Steinberg, and Means (1967) were able to elicit male sexual behavior by electrical stimulation of the mPOA in both the male and female opossum. Malsbury (1971) found that electrical stimulation of the mPOA decreased the number of mounts and intromissions to ejaculation, and, in some cases, reduced the intromission latency and the length of the postejaculatory interval. A facilitation of male sexual behavior by electrical stimulation of the mPOA-anterior hypothalamus has become well established (Merari & Ginton, 1975; Van Dis & Larsson, 1971). Interestingly, Malsbury and Pfaff (1974) have reported that electrical stimulation of the mPOA-anterior hypothalamus did not elicit copulation unless the subjects were sexually experienced, although it did potentiate penile responses ex copula in all males. The sexual behavior of

persistent noncopulators is not affected by electrical stimulation of the mPOA-anterior hypothalamus (Malsbury & Pfaff, 1974). These results suggest that electrical stimulation of the mPOA-anterior hypothalamus cannot affect copulation if the male is sexually naive, or suffers a deficit in arousal.

Hull et al. (1986) applied the DA agonist, apomorphine, to the mPOA, rather than nonspecifically activating the mPOA via electrical stimulation. Apomorphine infused into the mPOA increased the number of ejaculations and the efficiency of copulation (the ratio of intromissions to all mounts), and decreased the mean time between intromissions. The intra-mPOA infusions of apomorphine did not affect the latency to initiate copulation. Facilitation of copulation by intra-mPOA infusions of apomorphine was found to be blocked by the DA receptor blocker cis-flupenthixol (Hull, et al., 1987). The mPOA, therefore, was important for the performance of copulation, functioning analogously to Beach's proposed CM, but there was no evidence that it was involved in the control of sexual arousal.

Research has extended the study of the preoptic area to afferent and efferent pathways. Transverse knife cuts either anterior or posterior to the mPOA have no effect on copulation (Szechtman et al., 1978). Horizontal knife cuts dorsal to the mPOA do not change the likelihood of copulation, although they do alter the pattern of copulation. The dorsal cuts would sever several fiber systems, including connections with the amygdala via the stria terminalis and with the hippocampus via the corticohypothalamic tract.

On the other hand, sagittal knife cuts lateral to the mPOA, which would disconnect the mPOA from the medial forebrain bundle (MFB), have been reported to decrease the number of animals that ejaculate (Paxinos & Bindra, 1972), and greatly reduce the number of animals that copulate at all (Szechtman, et al., 1978). These latter investigators found, however, that if copulation did occur the lesioned male was as likely

to ejaculate as an intact male. Interestingly, it has been found that although knife cuts sagittal to the mPOA reduce the likelihood of copulation, they do not affect the frequency of female-directed behaviors or the male's preference for the odor of a sexually receptive female over a non-receptive female (Szechtman et al., 1978). Thus, even if copulation does not occur, animals with the mPOA isolated from the MFB maintain interest in the sexually receptive female. If unilateral mPOA lesion or sagittal knife cuts are combined with contralateral MFB lesion copulation is abolished or greatly reduced (Hendricks & Scheetz 1973; Paxinos, 1974) Interestingly, Hendricks and Scheetz (1973) noted that pursuit of the female and partial mounts were common among animals with bilateral mPOA lesions, but were much less common among males with unilateral mPOA lesion and contralateral MFB lesion.

Medial forebrain bundle. The MFB, a major fiber tract connecting telencephalon and midbrain via both ascending and descending fibers, is necessary for sexual behavior; bilateral lesions of the MFB reduce or abolish copulation in sexually experienced males (Caggiula, Antelman, & Zigmond, 1973; Hendricks & Scheetz, 1973; Hitt, Hendricks, Ginsberg, & Lewis, 1970). Unlike animals with mPOA lesions, animals with MFB lesions do not pursue the female, or display partial mounts (Hendricks & Scheetz, 1973) After MFB lesion, males cannot be induced to copulate by peripheral shock, handling, or the introduction of new females (Caggiula, Antelman, & Zigmond, 1974), indicating that these lesions interrupt a set of fibers necessary for increases in sexual arousal by peripheral stimulation.

Whereas MFB lesions interfere with copulation, electrical stimulation of the MFB in the region of the lateral hypothalamus or posterior hypothalamus results in stimulation-bound copulation (Caggiula, 1970; Caggiula & Hoebel, 1966; Vaughan & Fisher, 1962), and a reduction in the number of intromissions to ejaculation (Caggiula & Szechtman, 1972; Vaughan & Fisher, 1962). The MFB is important for the display of

sexual behavior, but mPOA and other descending and ascending pathways cannot be differentiated on the basis of MFB lesion or electrical stimulation. Two of the systems that would be affected by MFB lesion are major dopaminergic systems; the nigrostriatal DA system, that has cell bodies in the substantia nigra (A9 cell group) and terminals in the caudate putamen, and the mesolimbic DA system, that has cell bodies in the VTA (A10 cell group) and an important terminal field in the nucleus accumbens (NAS) (Bjorklund & Lindvall, 1984; Dahlstrom & Fuxe, 1964).

Mesolimbic and nigrostriatal dopamine systems. In order to selectively impair catecholaminergic systems, including the mesolimbic and nigrostriatal DA systems, Caggiula and co-workers (Caggiula, Shaw, Antelman, & Edwards, 1976) injected the neurotoxin 6-hydroxydopamine (6-OHDA) into the lateral cerebral ventricles. Animals did copulate after subtotal 6-OHDA lesions (less than 85 % catecholamine depletion). Catecholaminergic activity was further challenged in these animals by treatment with the tyrosine hydroxylase inhibitor α -methyltyrosine (α -MT or α -MPT). Very few 6-OHDA lesioned animals copulated after α -MT treatment; those that did copulate had a greatly lengthened intromission latency and most stopped copulating after achieving a few intromissions. The lesioned animals could, however, be induced to resume copulation and to ejaculate by tail pinch. Although non-lesioned animals did copulate after α -MT treatment, measures of sexual arousal were affected: intromission latency increased, the rate of intromitting decreased, and the length of the postejaculatory interval increased.

To further examine the effect of 6-OHDA lesion on arousability, Caggiula et al. (1976) eliminated solicitation by the female. Female rats treated with haloperidol will remain receptive (i.e. reliably display lordosis) but will not display proceptive behaviors. When tested with non-proceptive females, mount, intromission, and ejaculation latencies and the length of the postejaculatory interval all increased in 6-OHDA lesioned subjects.

Thus, intraventricular 6-OHDA decreased the sexual arousability of the male, an effect that could be exacerbated by α -MT treatment or by non-proceptive females, and that could be reversed by tail pinch.

Intracerebral 6-OHDA affects catecholaminergic systems in general and does not differentiate between noradrenergic and dopaminergic systems. Although noradrenergic systems may have a role in a behavior as complex as male copulation, the data are equivocal. Manipulation of noradrenergic activity has been reported to produce no effect, facilitation, and inhibition of sexual behaviors (Caggiula et al., 1973; Malmnas, 1973; Clark, Caggiula, McConnell, & Antelman, 1975). On the other hand, the pharmacological data consistently indicates an important role for DA, suggesting that the effects of intraventricular 6-OHDA were mediated by lesioning dopaminergic cells.

Although intraventricular 6-OHDA lesions, and electrolytic MFB and mPOA lesions all result in a reduction in the number of animals that copulate, there are differences in their effects on sexual behaviors. Animals with mPOA lesions will copulate to ejaculation if copulation is begun, and initiation latencies are not consistently affected. In contrast, 6-OHDA lesioned animals stop intromitting before reaching ejaculation and initiation latencies are lengthened. Furthermore, mPOA, but not MFB, lesioned males will pursue the female, and display partial mounts. These data suggest that one of the ascending dopaminergic systems is responsible for mediating sexual arousability; relatively little research, however, has been devoted to trying to specify whether it is the mesolimbic or nigrostriatal DA system that is involved.

McIntosh and Barfield (1984) assessed the role of nigrostriatal DA in male copulation by producing electrolytic or chemical lesions of the substantia nigra, the location of the DA cell bodies. Unilateral chemical (6-OHDA) lesions lengthened the postejaculatory interval. Unilateral electrolytic lesions both lengthened the postejaculatory interval and increased initiation latencies and decreased the number of

intromissions to ejaculation. Because the only consistent effect of unilateral substantia nigra lesion was an increase in the length of the postejaculatory interval, McIntosh and Barfield concluded that the nigrostriatal system was important for the control of the postejaculatory refractory period.

Eibergen and Caggiula (1973) have investigated the effect of electrical stimulation of the VTA, the location of mesolimbic DA cell bodies, on male copulation. Electrical stimulation of the VTA elicits stimulation-bound copulation, and reduces the number of intromissions to ejaculation, ejaculation latency, and the length of the postejaculatory interval. Unfortunately, initiation latencies were not reported, though they might not have been meaningful in this paradigm without an investigation of thresholds. There are no published reports of the effects of VTA lesions on male sexual behaviors.

Terminal fields of the mesolimbic and nigrostriatal DA systems were included in the study by Hull et al. (1986) that tested male sexual behavior after intracerebral apomorphine infusions and found that intra-mPOA apomorphine facilitated only the performance of copulation. Apomorphine infused into a terminal field of the nigrostriatal DA system, the caudate-putamen, had no effect on sexual behavior. Apomorphine infusions into a terminal field of the mesolimbic DA system, the NAS, produced a dose-related decrease in intromission latency, but the effect was small ($p = .058$); mount latency was not reported. The subjects in this study were very experienced copulators, having copulated eight to ten times, and all tests were performed in the same environment. Because repeated testing of gonadally intact males in the same environment results in a decrease in the latency to initiate copulation (Dewsbury, 1969), greater facilitation of intromission latency by apomorphine may have been difficult to demonstrate.

Taken together, these data suggest that DA is critically involved in male sexual behavior, and that dopaminergic agonists facilitate performance at the mPOA and sexual

arousability at another site. The effects of MFB lesions, intraventricular 6-OHDA, VTA stimulation, and NAS apomorphine infusions all imply an important contribution of the mesolimbic DA system in mediating sexual arousability.

Castration effects on dopamine. Castration could affect dopaminergic neurotransmission by changing either the availability or utilization of DA. The dopaminergic systems most studied for steroid effects on DA content and turnover have been the incertohypothalamic DA system, the source of most, if not all, preoptic area DA (Bjorklund & Nobin, 1973; Bjorklund & Lindvall, 1984), and the tuberoinfundibular DA system. The tuberoinfundibular DA system has cell bodies in the arcuate nucleus and the adjacent part of the periventricular nucleus (A12 cell group), and terminals in the median eminence near the primary capillary plexus of the hypophysial portal system (Bjorklund, Moore, Nobin & Stenevi, 1973; Bjorklund & Lindval, 1984). Neuroendocrine functions have been suggested for both the incertohypothalamic and tuberoinfundibular DA systems (Bjorklund et al., 1973).

Bernard and Paolino (1974) evaluated hypothalamic DA concentrations and DA turnover in male rats after α -MPT injection. They reported an increase in hypothalamic DA content and utilization 3 weeks, but not 6 weeks, after castration. Their dissection would have included incertohypothalamic as well as tuberoinfundibular DA (Bjorklund & Lindvall, 1984). Incertohypothalamic DA concentrations have been studied using more specific dissections. Donoso, Stefano, Biscardi, and Cukier (1967) reported that DA concentrations in the anterior region of the hypothalamus were reduced 10 and 20 days after castration. Simpkins, Kalra, and Kalra (1980a, 1980b, 1983), on the other hand, have reported no change in DA concentrations in the mPOA or anterior hypothalamus 2 weeks after castration.

Gunnet, Lookingland and Moore (1986) found that DA turnover in the medial preoptic area was reduced 2 weeks after castration; an effect that was prevented by T

administration. Simpkins et al. (1980a, 1980b), however, found that DA turnover in combined preoptic area-anterior hypothalamus was higher in castrates than in castrates treated with either preoptic area DHT implants or systemic T. Simpkins et al. (1983) used a more specific tissue dissection and found that DA turnover in both the mPOA and the anterior hypothalamus was higher in castrates than in castrates treated with androgen; T administration resulted in an apparent total cessation of DA turnover. DA concentrations and turnover in the periventricular nuclei, the location of the rostral incertohypothalamic DA cell bodies, did not differ between castrates and castrates treated with T. The finding that T decreased DA turnover in castrates (Simpkins et al., 1980a, 1980b, 1983) is in the opposite direction to what would be predicted from the effects of DA agonists and antagonists on sexual behaviors; a castration-induced decrease and T-induced increase would be expected.

Castration also influences the tuberoinfundibular DA system. Castration increases the rate of DA synthesis and turnover in the median eminence of male rats (Demarest, Moore & Riegle, 1985; Gunnet et al., 1986; Kizer, Humm, Nicholson, Greely, & Youngblood, 1978). Simpkins et al., (1983), however, have reported that DA turnover in the median eminence was lower in castrates than in castrates given T.

A number of studies have found evidence that the presence or absence of T influences DA turnover and content in several terminal regions of the incertohypothalamic and tuberoinfundibular DA systems. Biochemical studies of these systems have, however, yielded inconsistent results, and some of the discrepancies may arise from important differences in tissue dissection, time after castration selected for sacrifice, and the methods used to evaluate DA content and turnover.

Very little attention has been directed to the mesolimbic or nigrostriatal DA systems, despite evidence that they are involved in male sexual behavior and might, therefore, be affected by gonadal steroids. The NAS has been reported to concentrate

the neural metabolites of T, DHT and E₂, although the amount of steroid binding is small, whereas no steroid binding has been reported for the caudate-putamen (Pfaff & Keiner, 1973; Sar & Stumpf, 1975).

Gunnert et al. (1986) found only a small, not statistically significant decrease in the DA content of the NAS 2 weeks after castration. Alderson and Baum (1981) measured the concentrations of DA and the DA metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) 30 days after castration in terminal regions of the mesolimbic and nigrostriatal systems. They found that DA content was significantly lower in the NAS and septum 30 days after castration; the DA metabolites exhibited a similar trend. The castration-induced decreases in DA content were prevented by steroid administration. Castration and steroid administration had no effect on DA concentrations in the caudate-putamen.

It is not known if any of the castration-induced changes in DA content or turnover coincided with changes in sexual behavior. In a number of studies animals were sacrificed 2 weeks or less after castration (Gunnert et al., 1986; Kizer, 1978; Simpkins et al., 1980a, 1980b, 1983), and, depending on sexual experience and strain, many males could still be sexually active. Alderson and Baum (1981), on the other hand, sacrificed their subjects at a time when very few subjects would be expected to engage in sexual behavior. In none of these studies, however, was sexual behavior measured.

Baum, Melamed and Globus did measure sexual behavior as well as DA concentrations after castration in a more recent study (1986), but found no changes in DA, DOPAC, or HVA concentrations in the NAS, caudate-putamen, preoptic area/anterior hypothalamus, or septum. In both studies by Baum and co-workers (Alderson & Baum, 1981; Baum et al., 1986) brain areas were dissected from 2 mm thick coronal sections, and differences in tissue dissection combined with highly localized neurochemical changes could have accounted for the different results.

The present experiments. Analysis of evidence from a wide variety of experiments leads to the hypotheses that DA is important for sexual arousal in the male rat, that castration of the male leads to a deficit in sexual arousability mediated by decreased dopaminergic neurotransmission, and that changes in the mesolimbic DA system, in particular, are critically involved.

The first series of experiments to be reported in this thesis was conducted to assess the effects of castration and steroid replacement on biochemical changes in the mesolimbic DA system and on concomitant changes in sexual behavior. If steroid hormones do act, directly or indirectly, on this system, then castration might be expected to produce a steroid-reversible change in the concentration of DA or its metabolites. In order to make any statements about the relation between activity in the mesolimbic DA system and sexual behavior, it was important to demonstrate that changes within the system are systematically related to some aspect of change in sexual behavior.

In a second series of experiments, the influence of the mesolimbic DA system on sexual arousal in the male rat was assessed by directly activating this system in the presence of a sexually receptive female. In these experiments, advantage was taken of the fact that opiate compounds applied directly to the cell body region of the mesolimbic DA-neurons in the VTA activate DA neurons (Gysling & Wang, 1983). It was hypothesized that activation of mesolimbic DA neurons would facilitate sexual arousal in the male rat.

A final set of experiments was concerned with assessing the effects on sexual behavior of presenting the male rat with conditioned stimuli that could be expected to bring about conditioned increases in DA activity. In these experiments, male sexual behavior was studied when animals were presented with a sexually receptive female in an environment where the male had previously experienced repeated injections of the opiate drug morphine, a treatment known to sensitize animals to the behavioral effects of

opiates and to other inducing stimuli (Vezina, Kalivas, & Stewart, 1987). It was hypothesized that if increases in DA activity within the mesolimbic system were important in sexual arousal, then stimuli that have been shown to lead to sensitization of this system or to conditioned activation of it should facilitate sexual behavior in the male rat.

Studies of the Effects of Castration and Hormone Replacement on Dopaminergic Activity and Male Sexual Behavior

In the following experiments the effects of castration and steroid replacement on the mesolimbic and nigrostriatal DA systems were investigated. In all of the work, brain tissue was prepared using the microdissection technique of Palkovits (Palkovits & Brownstein, 1983), and amine concentrations were estimated using high performance liquid chromatography with electrochemical detection (HPLC-EC). In the neurochemical assays, special attention was paid to the concentrations of DA and DOPAC. DOPAC concentrations provide an estimate of DA utilization or release because it is thought to be mostly formed from DA after reuptake into the presynaptic terminal (Korf, Grasdijk, & Westerink, 1976; Lavielle et al., 1978; Melamed, Hefti, & Wurtman, 1980; Roth, Murrin, & Walters, 1976, but see Commissiong, 1985).

In the measurement of sexual behavior special attention was paid to indices of sexual arousal. These included the latency to initiate copulation and female-directed behaviors (Madlafousek et al., 1976; Michal, 1973; Stewart & Kaczender-Henrik, 1971). Female-directed behaviors provide a measure of interest in, or attention to, sexually relevant cues. Both gonadally intact and castrated male rats display female-directed behaviors, and they are usually the last sexual behaviors to disappear after castration (Madlafousek et al., 1976).

EXPERIMENT 1

In Experiment 1, animals were tested for sexual behaviors 2, 4, or 8 weeks after castration and then sacrificed for amine determination. These time intervals were chosen to include the time at which Gunnet et al., (1986) failed to find an effect of castration (2 weeks), and the time at which Alderson and Baum (1981) reported a decrease in NAS DA content (4 weeks). The use of a range of times allowed for the detection of any temporary neurochemical changes, such as that reported for hypothalamic DA (Bernard & Paolino, 1974). These times also encompass the period during which most castrates decline from displaying relatively complete sexual behavior to exhibiting little, if any, copulation (Davidson, 1966a).

Method

Subjects. Long-Evans male hooded rats (Charles River Canada) were used in the experiment. The males weighed 250-275 g at the start of the experiment, and were selected from a larger group on the basis of a single test of copulatory behavior. Initially, 42 males were screened for sexual behavior, and only those males that mounted within 20 min and ejaculated within 30 min of the first intromission were included as subjects. After pretesting, 24 males were bilaterally castrated via a single incision along the midline of the scrotum. Sham castrates (n=12) received only the scrotal incision. In this, and all subsequent experiments, surgery was performed under a methoxyflurane anesthetic (Metofane, Pitman-Moore Ltd./M.T.C. Pharmaceuticals, Mississauga, Ont.).

Female Long-Evans rats were used, in this and subsequent experiments, as target females. The females had been ovariectomized under Metofane anesthesia. Sexual receptivity was induced by subcutaneous (s.c.) injections of 10 μ g estradiol benzoate (Sigma, St. Louis, Mo.) in 0.1 ml peanut oil 72 and 24 h before use, and 0.5 mg progesterone (Sigma) in 0.2 ml peanut oil 4 to 6 h before use. Female sexual receptivity was verified by placing a female with a vigorous copulator just prior to use with an

experimental male.

All animals were housed in standard wire mesh cages with Purina Rat Chow and water available ad libitum. The animal colony was maintained on a 12 h light: 12 h dark reverse light cycle with lights off at 930 h.

Apparatus. A bank of six semicircular mating arenas (61 cm diameter x 36 cm deep) were used for tests of sexual behavior. Each mating arena had a plywood floor covered by 1-2 cm of bedding, a Plexiglas front and a curved tin back. The arenas were in a room dimly lit by four, 40 watt red light bulbs. A red-light sensitive camera (Panasonic CCTV camera, model WV-1460), and a video cassette recorder (Sony Betamax VCR, model SLO-420 or SL-HFR30) was used to record the sessions for future scoring. The mating arenas and video equipment were used in subsequent experiments.

Behavioral tests. Groups of castrated males ($n=8$) were tested for sexual behavior 2, 4, or 8 weeks after castration. To control for the time since surgery, six sham-castrated males were tested 2 weeks after surgery, and six after 8 weeks. Surgery was timed so that all animals were the same age at the time of testing. For each test, a male was placed into the mating arena for 5 min and then a sexually receptive female was introduced. Tests lasted 30 min from the time of the introduction of the female, and all behavioral testing was conducted between 1200 and 1700 h.

The behavioral categories scored included the following:

Mounts. Identified by the male mounting the female from the rear and displaying a number of rapid, shallow pelvic thrusts.

Intromissions. Similar to a mount, but included a long, deep thrust after the rapid shallow thrusts, a rapid kick with one hindleg, and a rapid short-stepped withdrawal from the female.

Ejaculatory pattern. Identified by a final pelvic thrust that was slower and deeper

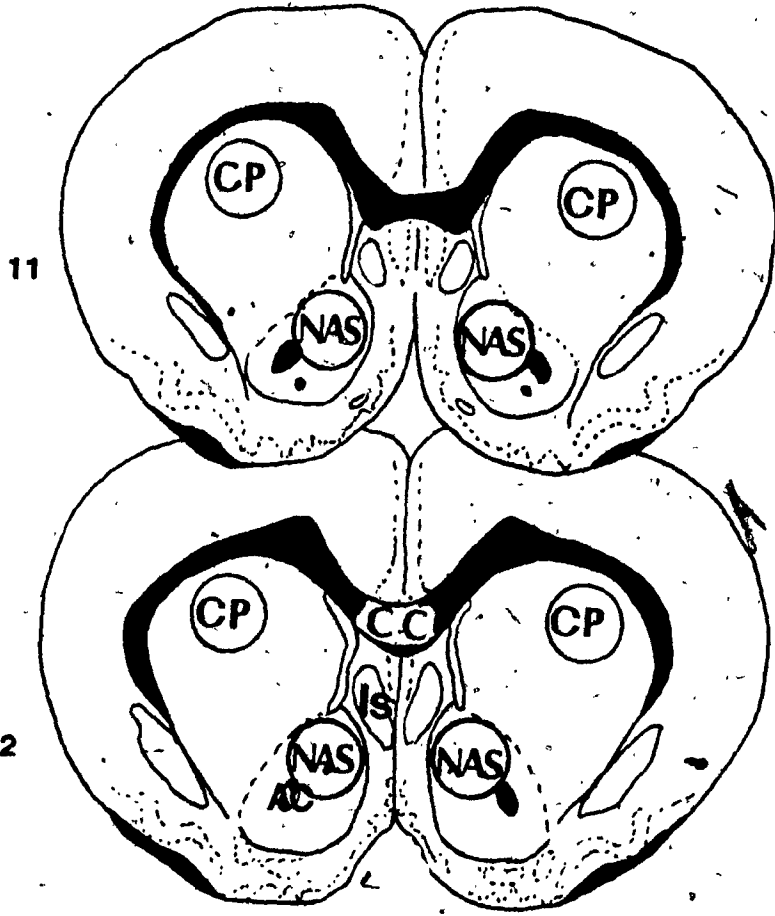
than of an intromission, a lateral removal of the forelimbs from the female that was held momentarily at the apex, and an absence of back-stepping before genital grooming.

The videotapes were then scored for female-directed behaviors using a time sampling procedure. Female-directed behaviors were defined as activities directed toward the female: pursuing, sniffing, grooming, anogenital exploration, climbing over, and manipulating the flanks (Madlafousek et al., 1976; Michal, 1973). During the 30 min test, each male was observed for 2-3 s every 30 s and the predominant behavior noted. Thus, there were 60 observations per animal in each test. An animal's overall score was expressed as the percent of observations in which female-directed behaviors occurred.

After removal from the mating arenas, the males were taken to a nearby room and sacrificed for amine determination.

Brain dissection and amine assay. Rats were sacrificed by decapitation and the brains rapidly removed and placed on dry ice. Whole brains were stored at -70°C until processing. The brains were sectioned into $300\ \mu$ slices at -10°C using a refrigerated cryostat (Ames), and the sections thaw-mounted on gel-coated glass slides. Specific brain areas were dissected using the brain microdissection technique of Palkovits (Palkovits & Brownstein, 1983) and the atlas of Palkovits (1980). The NAS and caudate-putamen were dissected using a 1 mm diameter tissue punch (Figure 1), and a 0.5 mm diameter punch was used for the mPOA (Figure 2) and for the VTA and substantia nigra (Figure 3). A number of other areas were assayed; for the sake of clarity, however, only the data from the areas mentioned above will be included in this thesis.

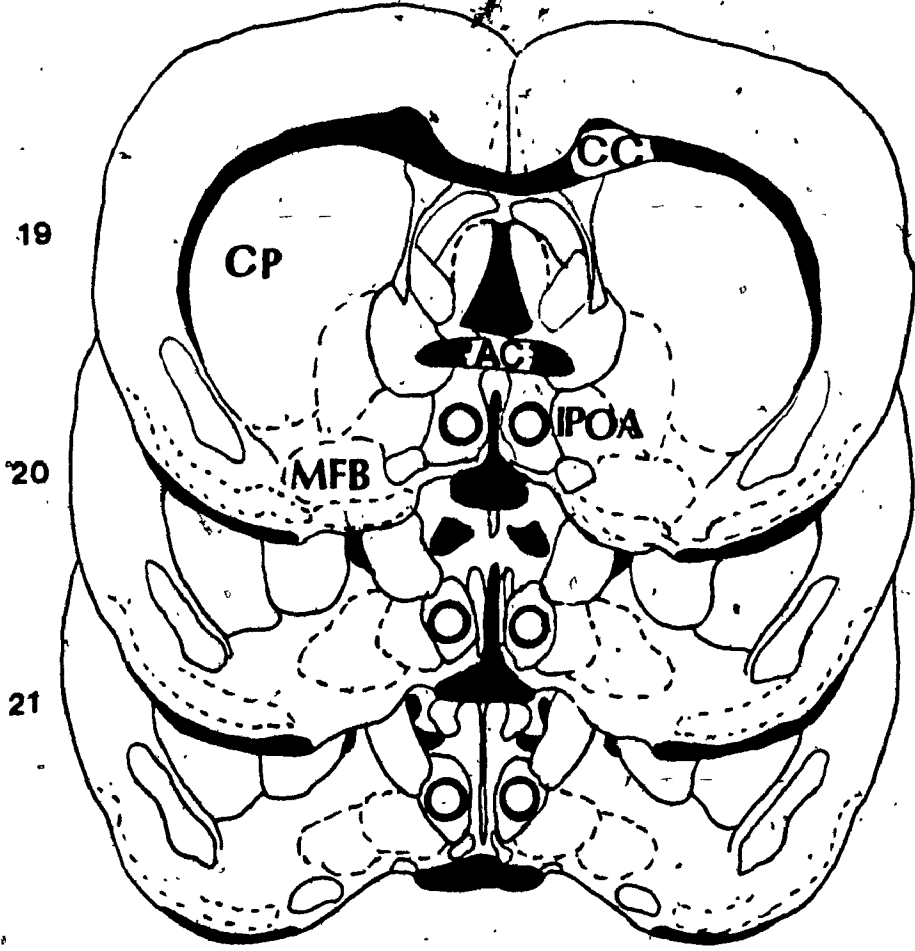
Figure 1. Dissection of the NAS and caudate-putamen for Experiments 1-3. Circles represent the tissue samples removed for HPLC-EC analysis. Each tissue section was 300 μ thick, and tissue samples for both the NAS and caudate-putamen had a diameter of 1 mm. Abbreviations: AC: anterior commissure; CC: corpus callosum, CP: caudate-putamen; ls: lateral septum; NAS: nucleus accumbens. Numbers to the left of each section represent the section number according to Palkovits (1980). Adapted from Palkovits (1980).



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Figure 2. Dissection of the mPOA for Experiments 1-3. Circles represent the tissue sample removed for HPLC-EC analysis of the mPOA. Sections were 300 μ thick, and each tissue sample had a diameter of 0.5 mm. Abbreviations: AC: anterior commissure; CC: corpus callosum; CP: caudate-putamen; IPOA: lateral preoptic area; MFB medial forebrain bundle. Numbers to the left of each section represent the section number according to Palkovits (1980). Adapted from Palkovits (1980).




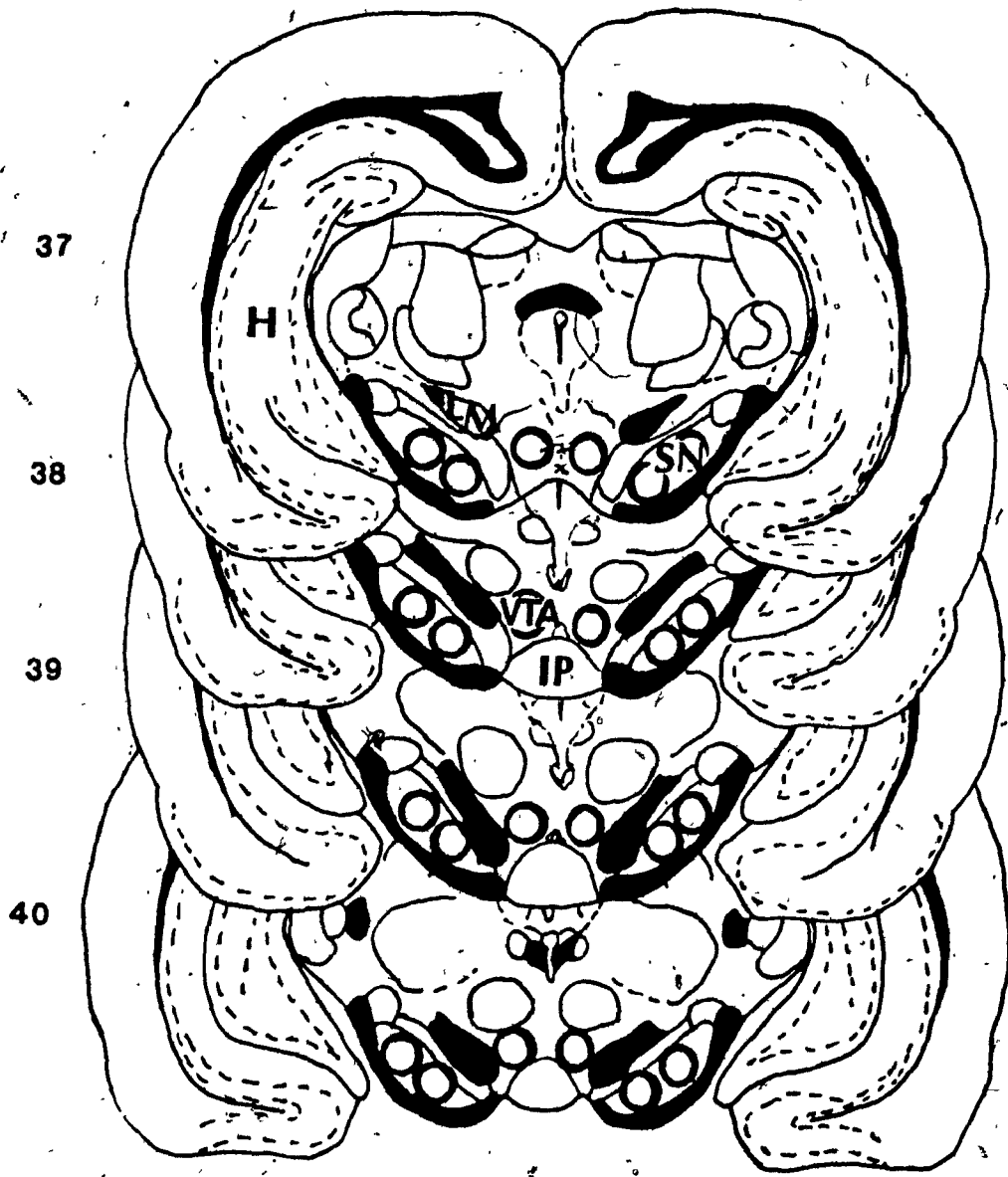


Figure 3. Dissection of the VTA and substantia nigra for Experiments 1-3. Circles represent the tissue removed for analysis by HPLC-EC. Tissue sections were 300 μ thick, and tissue samples for both the VTA and substantia nigra had a diameter of 0.5 mm. Abbreviations: LM: medial lemniscus; H: hippocampus; IP: interpeduncular nucleus; LM: medial lemniscus; SN: substantia nigra; VTA: ventral tegmental area. Numbers to the left of each section represent the section number according to Palkovits (1980). Adapted from Palkovits (1980).



Tissue was expelled into 100 μ l of ice-cold medium containing 0.15 M sodium acetate (Fisher, Fairlawn, NJ), 0.1 mM EDTA (Fisher), and 4.3 ml/l glacial acetic acid (Fisher), pH 5.0. Tissue punches were disrupted by freeze thawing, and 5 μ g ascorbat-oxidase (Boehringer Mannheim, W. Germany) was added upon thawing. Samples were then vortexed and centrifuged at 13,000 x g for 5 min. The supernatant was analysed for amine content using HPLC-EC. Pellets were dissolved in 0.1 N NaOH (Fisher) for protein determination (Bradford, 1976).

The chromatographic system consisted of a 15 cm x 3.9 mm Bondapak C18 column (Waters Scientific Ltd., Mississauga, Ont.) with 4 μ packing, and a programmable sample processor (Waters 710-B WISP). An amperometric electrochemical detector (Bioanalytical Systems Inc., Lafayette, IN, model LC4B) with a glassy carbon electrode (Bioanalytical Systems, model TL-5A) was used for electrochemical detection. The electrode potential was set at +0.76 V versus a Ag/AgCl reference electrode.

Samples were eluted with a 0.15 M sodium acetate mobile phase containing 0.1 mM EDTA, 80 mg/l octyl sodium sulfate (Kodak, Rochester, NY), and 3.3 % acetonitrile (Fisher), pH of 3.8 (adapted from Renner & Luine, 1984). The mobile phase was pumped at 1.3 ml/min (2000 psi back pressure) by a Waters model 510 pump.

Sample catecholamine and metabolite concentrations were estimated from the peak heights by comparison with injections of known amounts of pure standards (Sigma). The detection limit, as defined by a minimum signal to noise ratio of 3:1, was : DA 22 pg (0.15 pmol); DOPAC 16 pg (0.1 pmol); norepinephrine (NE) 24 pg (0.14 pmol); serotonin (5-HT) 34 pg (0.19 pmol); 5-hydroxyindoleacetic acid (5-HIAA) 28 pg (0.15 pmol). Dihydroxybenzylamine (DHBA; Sigma) was used as an internal standard. The response of the chromatographic system was linear between amine concentrations of 100

pg and 1000 pg (r 's $\geq .98$). Intra-assay variability was $< 5\%$.

Statistics. In this and subsequent experiments, the amount of female-directed behavior and the neurochemical assay data were analysed by analysis of variance, and subsequent post hoc comparisons were made using Tukey's HSD. The proportion of animals in each group mounting, intromitting, or displaying the ejaculatory pattern were analysed by χ^2 tests.

Results

Table 1 presents the assay results for the three castrated groups and the sham-operated animals. Because the two sham-castrated groups did not differ on any measure their data were combined to form a single gonadally intact group. Data for each brain area were analysed using a one factor between-groups analysis of variance. The analysis of variance yielded a significant group effect for NAS DA concentrations, $F(3,30) = 3.8, p < .05$. Post hoc comparisons showed that the 4 week group had significantly lower DA levels than the gonadally intact group ($p < .05$). Neither of the other castrated groups differed significantly from the intact group (p 's $> .1$).

The analysis of the DOPAC concentrations in the NAS also yielded a significant group effect, $F(3,31) = 6.36, p < .01$. Post hoc comparisons showed that the 4 week group had significantly lower DOPAC concentrations than the intact control group ($p < .05$). Although the 8 week group appeared to have lower DOPAC concentrations than the intact group, this comparison did not quite attain statistical significance. Figure 4 shows the NAS DOPAC/DA ratios for the three castrated groups and the intact controls. The statistical analysis yielded a significant group effect, $F(3,30) = 6.08, p < .01$, and post hoc comparisons showed that the DOPAC/DA ratio was lower both 4 weeks and 8 weeks after castration (p 's $< .05$), suggesting lower DA metabolism in these two groups.

DA concentrations in the mPOA were higher after castration, and the analysis of

Table 1. Monoamine and amine metabolite concentrations for groups sacrificed at different times after castration. Animals were either sham-castrated (Intact), or castrated and sacrificed for amine determination 2, 4, or 8 weeks post-castration. Values shown are the mean \pm the S.E.M. and are expressed as pg/ μ g protein.

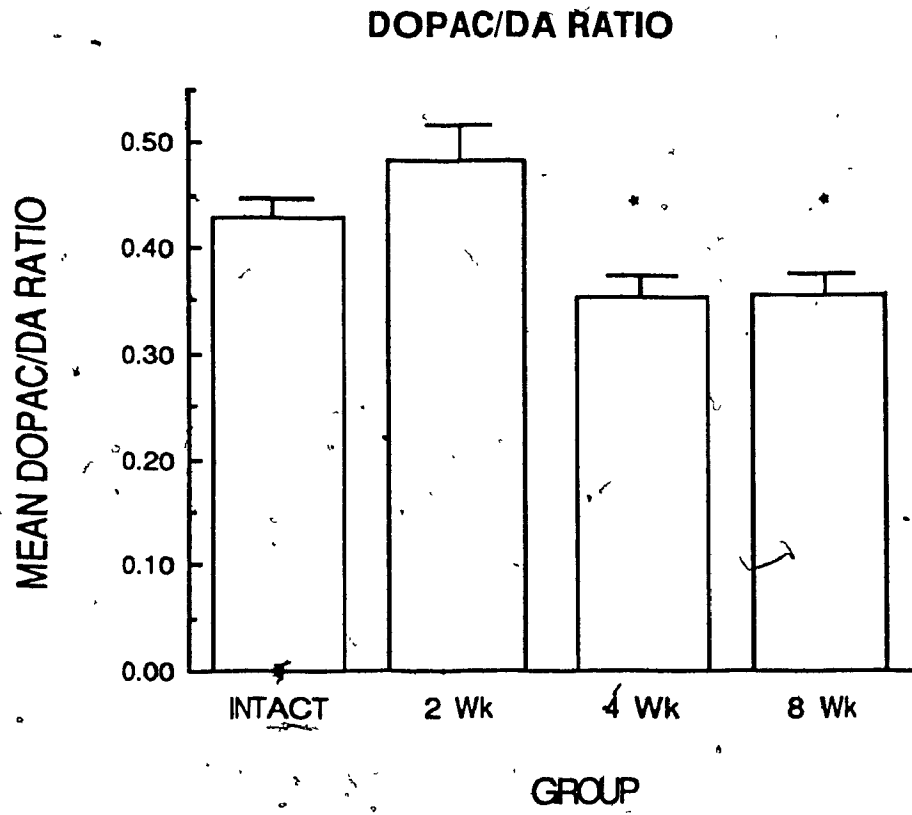
Area ¹	Group	DA	DOPAC	NE	5-HT	5-HIAA
NAS	Intact	128.3 \pm 5.5	54.9 \pm 3.3	8.2 \pm 1.2	11.5 \pm 1.3	14.8 \pm 0.9
	2 Wks	125.2 \pm 8.4	56.9 \pm 5.4	8.8 \pm 1.2	9.1 \pm 0.8	12.4 \pm 0.9
	4 Wks	103.9 \pm 3.4*	37.4 \pm 3.1*	8.8 \pm 0.8	11.7 \pm 0.9	13.9 \pm 0.9
	8 Wks	117.5 \pm 4.0	41.9 \pm 2.9*	7.7 \pm 0.9	8.6 \pm 1.5	13.0 \pm 1.2
VTA	Intact	16.4 \pm 2.2	4.2 \pm 0.3	13.0 \pm 0.7	16.1 \pm 1.2	20.5 \pm 0.9
	2 Wks	13.1 \pm 3.7	4.8 \pm 1.6	12.7 \pm 1.0	14.6 \pm 1.4	19.3 \pm 1.0
	4 Wks	17.9 \pm 3.2	4.4 \pm 0.7	12.7 \pm 1.0	16.9 \pm 1.4	21.8 \pm 2.3
	8 Wks	19.9 \pm 5.5	5.4 \pm 1.3	12.3 \pm 1.1	17.6 \pm 2.1	21.4 \pm 2.1
CP	Intact	167.2 \pm 6.2	29.6 \pm 2.6	1.4 \pm 0.1	2.6 \pm 0.1	6.1 \pm 0.2
	2 Wks	157.5 \pm 6.0	33.3 \pm 1.7	1.9 \pm 0.3	2.5 \pm 0.2	6.7 \pm 0.4
	4 Wks	142.3 \pm 5.0	24.8 \pm 1.5	1.2 \pm 0.1	2.4 \pm 0.2	6.0 \pm 0.2
	8 Wks	154.4 \pm 6.3	30.0 \pm 1.9	1.6 \pm 0.2	2.4 \pm 0.2	6.5 \pm 0.4
SN	Intact	13.5 \pm 1.2	3.3 \pm 0.4	2.3 \pm 0.3	19.5 \pm 0.8	15.9 \pm 0.6
	2 Wks	10.5 \pm 1.6	2.9 \pm 0.6	2.1 \pm 0.4	17.0 \pm 0.8	13.4 \pm 0.4*
	4 Wks	12.7 \pm 0.6	2.6 \pm 0.1	2.4 \pm 0.2	19.7 \pm 0.6	15.8 \pm 0.5
	8 Wks	12.9 \pm 1.8	3.3 \pm 0.5	2.2 \pm 0.4	17.8 \pm 1.2	15.6 \pm 0.7
mPOA	Intact	3.7 \pm 0.4	n.d.	30.4 \pm 2.8	14.9 \pm 1.2	15.0 \pm 1.2
	2 Wks	5.6 \pm 0.5*	n.d.	37.8 \pm 2.3	15.3 \pm 1.0	18.9 \pm 1.0
	4 Wks	5.4 \pm 0.6*	n.d.	40.6 \pm 4.2	17.5 \pm 1.9	16.3 \pm 1.2
	8 Wks	6.1 \pm 0.5*	n.d.	40.5 \pm 4.0	14.1 \pm 1.4	17.3 \pm 0.7

¹Abbreviations: NAS: nucleus accumbens; VTA: ventral tegmental area; CP: caudate-putamen; SN: substantia nigra; mPOA: medial preoptic area.

* significantly different from Intact, $p < .05$.

n.d.: not detectable.

Figure 4. The mean (+ 1 S.E.M.) DOPAC/DA ratio for each group in Experiment 1. Subjects were either gonadally intact (INTACT), or had been castrated 2, 4, or 8 weeks prior to testing and sacrifice for amine determination. * significantly different from INTACT, $p < .05$. Each column represents the mean of 8 determinations, except 2 Wk (n=7).



variance yielded a significant group effect, $F(3,28) = 6.09$, $p < .01$. All castrated groups had higher DA levels than the intact control group (p 's $< .05$). Castration appeared to lower DA concentrations in the caudate-putamen, but the change was not statistically significant, $F(3,30) = 2.73$, $.05 < p < .1$. The only other statistically significant change after castration was in the concentration of 5-HIAA in the substantia nigra, $F(3,31) = 4.55$, $p < .01$.

Figure 5 shows the percent of observations in which female-directed behaviors occurred during the 30 min test for sexual behaviors. A two factor analysis of variance (group x time) yielded significant group, $F(3,32) = 10.36$, $p < .001$, and time, $F(3,32) = 43.73$, $p < .001$, effects. It can be seen that the group effect can be attributed primarily to the 8 week and 4 week groups' low levels of female-directed behaviors, especially in the latter part of the session. Because, however, female-directed behaviors decreased across time for all groups, the group x time interaction was not statistically significant ($p > .1$).

Figure 6 presents the proportion of animals in each group that engaged in the three measures of copulatory behavior: mounts, intromissions, and the ejaculatory pattern; it can be seen that after castration there was a progressive decline in the number of animals that copulated. Groups differed in the number of animals displaying mounts, $\chi^2(3) = 16.73$, $p < .001$, intromissions, $\chi^2(3) = 22.88$, $p < .001$, and the ejaculatory pattern, $\chi^2(3) = 15.29$, $p < .01$.

Discussion

In the present experiment, it was found that castration significantly reduced DA and DOPAC concentrations and a measure of DA metabolism, the DOPAC/DA ratio, in the NAS. The decrease in NAS DA was slow to emerge after castration; it was not statistically significant until 4 weeks after surgery. Furthermore, the decrease in DA, DOPAC, and the DOPAC/DA ratio, was restricted to a terminal field of the mesolimbic

Figure 5. Mean (± 1 S.E.M.) percent of observations during which female-directed behaviors were observed as a function of time during the test for sexual behavior in Experiment 1. Subjects were gonadally intact (INTACT), or had been castrated for 2, 4, or 8 weeks at the time of testing.

FEMALE-DIRECTED BEHAVIORS

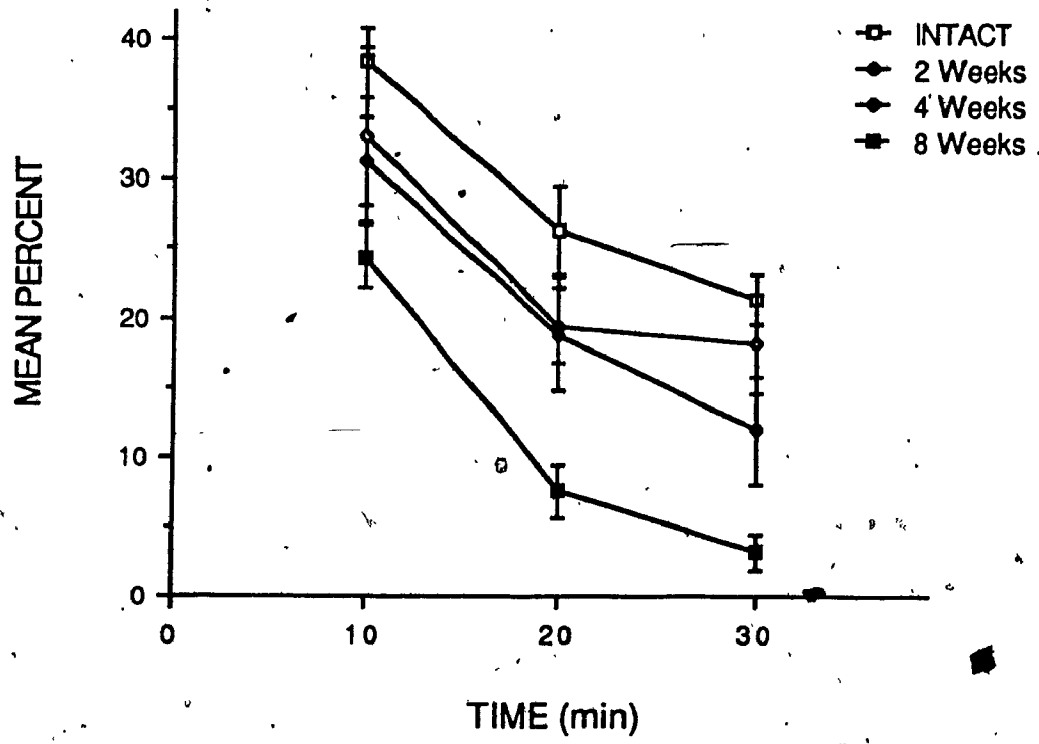
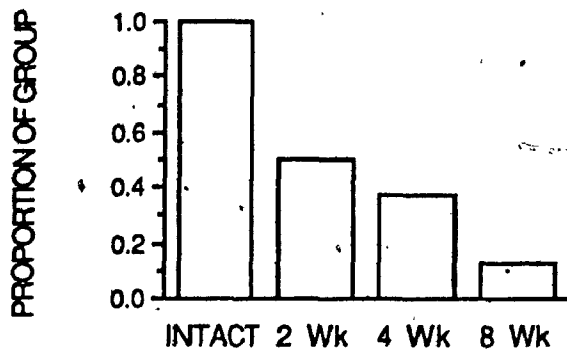
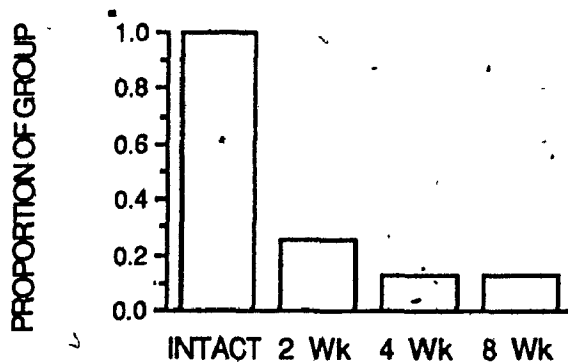
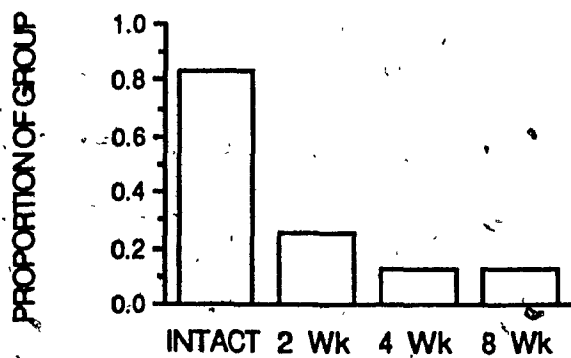


Figure 6. The proportion of animals in each group in Experiment 1 that displayed mounts (top panel), intromissions (middle panel) or the ejaculatory pattern (bottom panel) in the test for sexual behaviors. Subjects were gonadally intact (INTACT) or had been castrated 2, 4, or 8 weeks prior to testing.

MOUNTS**INTROMISSIONS****EJACULATORY PATTERN**

Group

DA system; nigrostriatal DA and DOPAC levels were not significantly affected by castration. The results of this experiment replicate those of Alderson and Baum (1981) who showed a castration-induced decrease in DA and DOPAC concentrations in the NAS.

In tests of copulatory behaviors, it was found, as in previous studies (Beach & Nucci, 1970; Davidson, 1966a; Madlafousek et al., 1976; Whalen et al., 1961), that the intromission and ejaculatory patterns disappeared simultaneously after castration, and that some castrates continued to mount after intromission and ejaculation had stopped. More important to the present experiments, however, was the fact that the display of female-directed behaviors decreased after castration, indicating that the castrates were less responsive to a sexually receptive female and apparently less sexually aroused.

The finding of a progressive decline in female-directed behaviors after castration may appear to contradict that of Madlafousek et al. (1976) who reported no change in female-directed behaviors as long as 10 weeks after castration. They scored, however, only the occurrence or non-occurrence of female-directed behaviors using a criterion of three female-directed acts, rather than analysing the frequency of these behaviors within a test session. In the present experiment it was found that the frequency of female-directed behaviors decreased after castration; by the criterion of Madlafousek et al. (1976) no change would have been recorded.

Two weeks after castration, subjects engaged in as much female-directed behavior as did intact animals, and did not differ from intact animals in mesolimbic DA levels or metabolism. By 4 weeks post-castration, fewer animals copulated, they exhibited low levels of female-directed behaviors late in the 30 min test, and were also found to have decreased mesolimbic DA levels and metabolism. Eight weeks after castration, subjects exhibited the lowest levels of copulatory and female-directed behaviors, and although NAS DA content did not differ significantly from the intact controls, the DOPAC/DA

ratio continued to differ from controls, suggesting that activity in the mesolimbic DA system was still suppressed. The time-limited nature of the reduction in DA concentrations suggests a slow readjustment of the system to compensate for the absence of gonadal steroids, although the nature of any compensatory changes is presently unknown and was not reflected in behavior.

The mPOA also showed changes in DA levels after castration, but in the opposite direction and with a different time course from the changes found in the NAS. Unfortunately, in the present experiment mPOA DOPAC levels were below detection limits, even in controls, and no measures of DA metabolism were available. Simpkins et al. (1983) reported that DA concentrations in the mPOA did not differ between castrates and castrates given T. Bernard and Paolino (1974), however, reported an increase in hypothalamic DA levels, fractional rate constants, and utilization three weeks post-castration, and although their tissue dissection was not specific, some of the DA they measured, like that of the mPOA, would have been of incertohypothalamic origin. Recall, however, that pharmacological treatments that increase dopaminergic activity, such as L-DOPA treatment, increase the display of sexual behavior. The increase in mPOA DA after castration, therefore, would not seem to coincide with changes in sexual behavior. It is possible, however, that the greater DA levels found in the mPOA after castration reflected a reduced DA release and, therefore, less receptor stimulation.

The decrease in amine levels that coincided with the castration-induced decline in sexual behaviors was both chemically specific, being restricted to DA and a DA metabolite within the NAS, and anatomically specific, occurring within the mesolimbic, but not the nigrostriatal, DA system.

EXPERIMENT 2

The previous experiment demonstrated a castration-induced decrease in both DA content and a measure of DA metabolism in the mesolimbic, but not the nigrostriatal, DA system. If this change was due to the loss of gonadal steroids, then such decreases should be preventable by post-castration steroid replacement. The present experiment was undertaken to determine whether the effect of castration on the mesolimbic DA system could be prevented by testosterone replacement. It also offered an opportunity to compare the effects of T and its metabolites, E₂ and DHT, on amine content. T, E₂ and DHT differ in their effectiveness in maintaining or restoring sexual behavior in castrates, and, therefore, might be expected to differ in their effectiveness in maintaining mesolimbic DA levels.

The steroid treatment selected was similar to that used by Alderson and Baum (1981). The post-castration interval selected for testing and sacrifice was 4 weeks; the time at which NAS DA content had been the lowest in Experiment 1.

Method

Subjects. Forty-eight male Long-Evans rats (Charles River Canada), screened for sexual behavior, were randomly assigned to one of six groups. Five groups were castrated and, at the time of castration, had silastic capsules implanted s.c. via an incision in the dorsal flanks. The sixth group was sham-castrated and served as an intact control group.

Steroid treatment and testing. Groups of 8 animals each received implants containing either cholesterol (Chol), T, DHT, or E₂, or received both DHT and E₂ implants (DHT+E₂). The T group was implanted with a 45 mm length of silastic tubing (1.57 mm i.d. x 3.18 mm o.d.) filled with testosterone; an implant previously shown to produce circulating T levels of approximately 2.5 ng/ml (Damassa, Smith, Tennent, &

Davidson, 1977). A similar 45 mm length of silastic tubing filled with cholesterol was implanted in the Chol group. Crystalline E_2 was diluted 10:1 with cholesterol and packed into 30 mm lengths of silastic tubing (1.47 mm i.d. x 1.96 mm o.d.); these implants were shown by Damassa et al. (1977) to produce circulating levels of E_2 between 90 and 120 pg/ml. The DHT implants were 60 mm long (1.57 mm i.d. x 3.18 mm o.d.), and were similar to those used by Alderson and Baum (1981). Subjects in the DHT+ E_2 group received one DHT and one E_2 implant. All silastic capsules were sealed with silastic elastomer (Dow Corning, Midland, Mich.), and incubated in sterile 0.9 % saline for 24 h and 70 % ethanol for 1 h prior to use. All compounds, except T (Steraloids, Wilton, NJ), were obtained from Sigma.

Four weeks after castration and silastic capsule implantation, all subjects were observed during a 30 min test for sexual behavior, and then sacrificed for amine determination. In addition to the behavioral measures outlined in Experiment 1, mount latency (the latency from the introduction of the female to the first mount), intromission latency (the latency from the introduction of the female to the first intromission), and ejaculation latency (the length of time from the first intromission to the first ejaculation), were recorded.

Brain dissection and HPLC-EC analysis was conducted according to the protocol outlined in Experiment 1.

Results

The results of the neurochemical assay are presented in Table 2. No data for the mPOA were available because of technical difficulties. The analysis of variance of NAS DA concentrations yielded a significant group effect, $F(5,41) = 5.09$, $p < .01$. It can be seen that both the Chol and DHT groups had lower DA levels than the intact group (p 's $< .05$), whereas none of the other groups differed significantly from the intact group or

Table 2. Monoamine and amine metabolite concentrations after castration and steroid replacement. Groups were either sham-castrated (Intact), or castrated and implanted with silastic capsules containing cholesterol (Chol), testosterone (T), 5 α -dihydrotestosterone (DHT), estradiol (E2), or both estradiol and dihydrotestosterone (DHT+E2). Values shown are the mean \pm the S.E.M. and are expressed as pg/ μ g protein.

Area ¹	Group	DA	DOPAC	NE	5-HT	5-HIAA
NAS	Intact	75.7 \pm 4.7	22.1 \pm 1.6	5.2 \pm 0.8	4.8 \pm 0.6	5.3 \pm 0.6
	Chol	53.2 \pm 4.3*	14.1 \pm 0.8*	3.2 \pm 0.5	3.5 \pm 0.6	5.4 \pm 0.3
	T	73.4 \pm 2.8	24.4 \pm 1.8	3.9 \pm 0.7	5.2 \pm 0.4	6.6 \pm 0.5
	DHT	63.1 \pm 2.7*	24.5 \pm 1.7	n.m.	3.7 \pm 0.4	4.7 \pm 0.3
	E2	71.5 \pm 3.9	24.1 \pm 2.4	4.6 \pm 2.7	3.7 \pm 0.4	5.4 \pm 0.5
	DHT+E2	68.3 \pm 1.8	20.8 \pm 0.9	n.m.	5.3 \pm 0.3	5.4 \pm 0.4
VTA	Intact	11.7 \pm 1.9	3.5 \pm 0.7	10.9 \pm 4.4	22.7 \pm 4.4	10.9 \pm 4.4
	Chol	7.2 \pm 1.2	2.3 \pm 0.3	9.8 \pm 1.3	18.3 \pm 3.1	8.6 \pm 1.5
	T	11.7 \pm 1.5	3.0 \pm 0.3	9.5 \pm 0.9	21.3 \pm 2.3	9.7 \pm 0.7
	DHT	13.0 \pm 1.6	3.1 \pm 0.2	10.9 \pm 0.8	25.5 \pm 1.4	9.2 \pm 0.4
	E2	10.7 \pm 1.0	3.1 \pm 0.4	10.8 \pm 1.7	24.9 \pm 2.7	9.1 \pm 1.1
	DHT+E2	9.8 \pm 1.3	3.5 \pm 0.4	11.9 \pm 1.9	25.8 \pm 3.4	10.1 \pm 1.4
CP	Intact	185.8 \pm 14.2	72.5 \pm 8.9	3.1 \pm 0.4	n.d.	4.3 \pm 0.5
	Chol	201.0 \pm 21.3	68.9 \pm 5.4	2.5 \pm 0.5	n.d.	4.2 \pm 0.5
	T	182.9 \pm 14.1	70.7 \pm 6.7	2.3 \pm 0.2	n.d.	3.6 \pm 0.3
	DHT	168.3 \pm 11.2	74.3 \pm 5.5	3.0 \pm 0.3	n.d.	3.8 \pm 0.3
	E2	169.4 \pm 9.4	66.5 \pm 4.6	3.3 \pm 0.4	n.d.	3.4 \pm 0.4
	DHT+E2	138.0 \pm 8.7*	53.3 \pm 5.4	2.6 \pm 0.5	n.d.	3.4 \pm 0.4
SN	Intact	26.5 \pm 2.2	4.0 \pm 0.5	6.8 \pm 1.4	60.0 \pm 7.8	19.1 \pm 2.8
	Chol	25.5 \pm 3.7	3.4 \pm 0.4	5.7 \pm 1.9	60.4 \pm 10.3	20.5 \pm 3.6
	T	28.9 \pm 2.5	3.7 \pm 0.3	6.7 \pm 0.9	52.0 \pm 7.7	18.4 \pm 7.7
	DHT	21.1 \pm 1.3	3.2 \pm 0.2	5.4 \pm 0.7	46.6 \pm 5.1	16.3 \pm 1.5
	E2	20.6 \pm 2.6	2.9 \pm 0.5	4.5 \pm 0.6	39.3 \pm 5.8	13.7 \pm 2.4
	DHT+E2	22.8 \pm 2.0	3.9 \pm 0.5	5.0 \pm 0.6	41.6 \pm 5.6	12.8 \pm 1.3

¹ Abbreviations: NAS: nucleus Accumbens; VTA: ventral tegmental area; CP: caudate-putamen; SN: substantia nigra.

* significantly different from Intact, $p < .05$.

n.m.: not measureable.

n.d.: not detectable.

from each other (p 's $> .05$). The statistical analysis of NAS DOPAC concentrations also yielded a group effect, $F(5,40) = 5.4$, $p < .01$. In this case, all steroid treatments were effective in maintaining DOPAC levels; only the group that was castrated and implanted with Chol differed from the intact control group ($p < .05$). The DOPAC/DA ratio did not differ significantly between groups.

There was a statistically significant group effect for DA concentrations in the caudate-putamen, $F(5,40) = 2.59$, $p < .05$. In this case, however, the only group that differed significantly from the intact control group was the DHT+E₂ group ($p < .05$), whose levels were extremely low relative to the other groups. The reason for this effect is not clear. There were no other statistically significant differences in amine concentrations (all other p 's $> .1$, except NAS 5-HT, $p = .07$, and substantia nigra 5-HIAA, $p = .06$).

The analysis of variance of female-directed behaviors yielded group, $F(5,42) = 2.73$, $p < .05$, and time, $F(2,84) = 216.6$, $p < .001$, effects. As shown in Figure 7, the group effect can be attributed to low levels of female-directed behaviors in the Chol and DHT groups. Scores in these groups differed significantly from those in the intact group (p 's $< .05$); the T, E₂, DHT+E₂, and intact groups all displayed similar levels of female-directed behaviors.

Castration reduced the number of animals that exhibited the different copulatory behaviors. Groups differed in the number of animals that exhibited mounts, $\chi^2(5) = 16.0$, $p < .01$; intromissions, $\chi^2(5) = 25.33$, $p < .01$; and the ejaculatory pattern, $\chi^2(5) = 25.03$, $p < .01$. It can be seen from Figure 8 that replacement treatment with T, E₂ and DHT+E₂ were equally effective in maintaining mounts and intromissions, but that only T successfully maintained the ejaculatory pattern. Potential differences between steroid treatments were further explored by analysing mount, intromission and

Figure 7. Mean (+ 1 S.E.M.) percent of observations during which female-directed behaviors were observed during the test for sexual behavior in Experiment 2. Four weeks prior to testing subjects in different groups had been sham-castrated (INTACT), or castrated and implanted with silastic capsules containing cholesterol (CHOL), testosterone (T), 5 α -dihydrotestosterone (DHT), estradiol (E2), or capsules of both 5 α -dihydrotestosterone and estradiol (DHT+E2). n = 8 for each group. * significantly different from the INTACT group (p < .05).

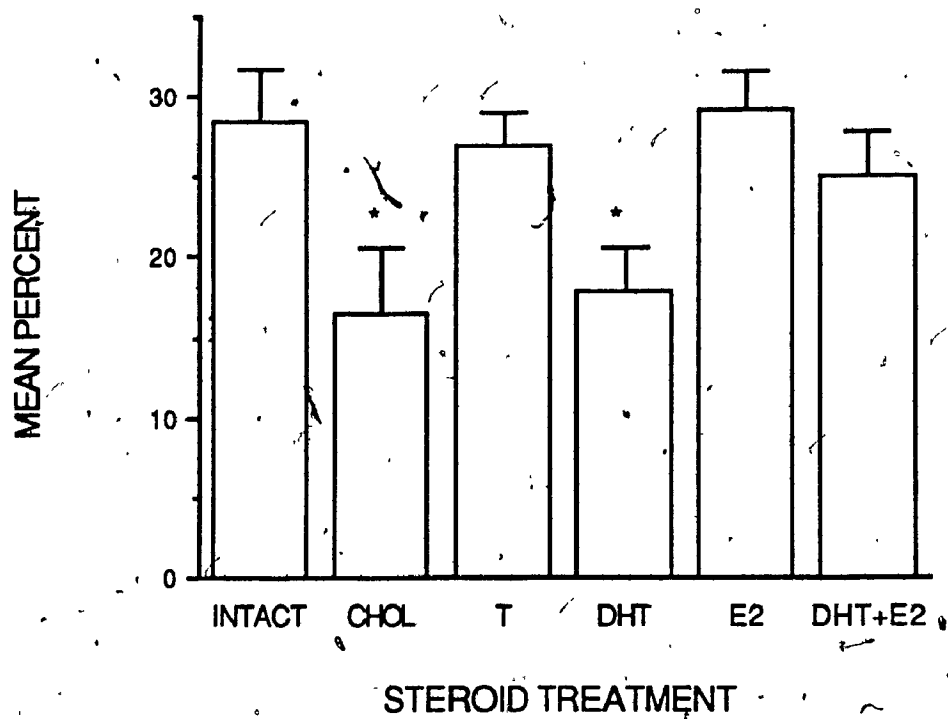
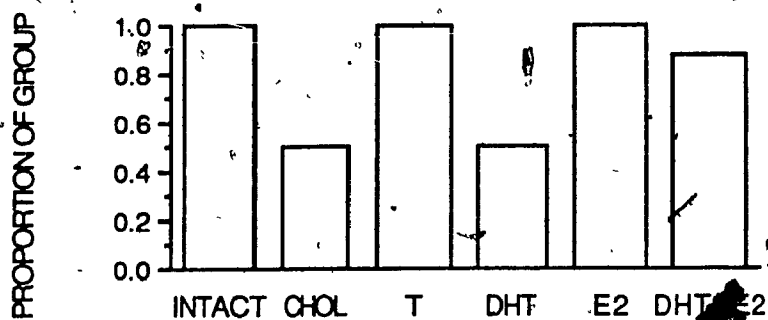
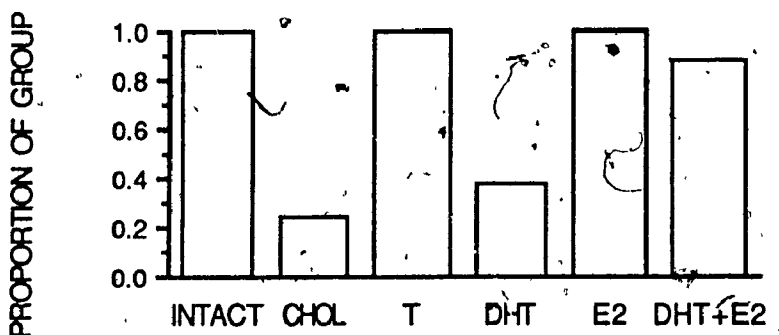
FEMALE-DIRECTED BEHAVIORS

Figure 8. The proportion of animals in each group that mounted (top panel), intromitted (middle panel), or displayed the ejaculatory pattern (lower panel), during the test for sexual behavior in Experiment 2. Four weeks prior to testing subjects in different groups had been sham-castrated (INTACT), or castrated and implanted with silastic capsules containing cholesterol (CHOL), testosterone (T), 5 α -dihydrotestosterone (DHT), estradiol (E2), or capsules of both 5 α -dihydrotestosterone and estradiol (DHT+E2). n = 8 for each group.

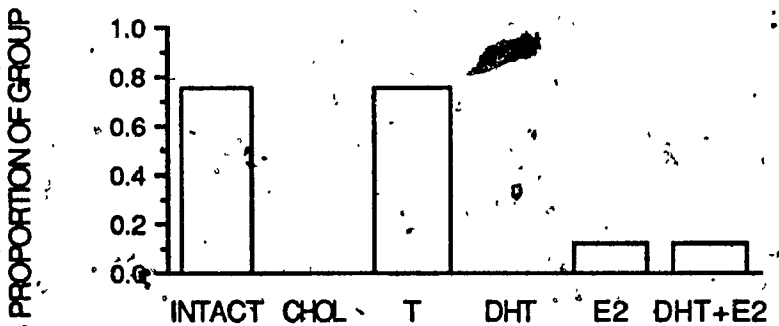
MOUNTS



INTROMISSIONS



EJACULATORY PATTERN



STEROID TREATMENT

ejaculatory latencies, and the number of mounts and intromissions.

It can be seen from Figure 9 that animals in both the Chol and DHT groups were slower than the other groups to initiate mounting. Mount latencies within groups, however, were highly variable and an analysis of variance carried out on the logarithmically transformed scores (Myers, 1979) showed that the group effect only approached statistical significance, $F(5,33) = 2.05$, $.05 < p < .1$. The number of mounts for each group is shown in Figure 10. The analysis of variance yielded a significant group effect, $F(5,33) = 2.77$, $p < .05$, and post hoc comparisons showed that both the Chol and DHT groups exhibited fewer mounts than the intact control group (p 's $< .05$).

The Chol group was excluded from further analyses because too few animals intromitted to allow meaningful comparisons. Among animals that intromitted, all steroid treatments were effective in maintaining intromission; groups did not differ significantly in either intromission latency, or the number of intromissions (p 's $> .1$).

T was the only steroid treatment that maintained the ejaculatory pattern, and a t-test comparing the T-treated group and the intact control group found that they did not differ significantly in ejaculation latency ($p > .1$).

Discussion

Administration of T, E_2 , or the combination of DHT and E_2 after castration prevented the decrease in DA and DOPAC levels, and also maintained female-directed behaviors, mount latency, and the number of mounts. On the other hand, the performance of intromissions and the ejaculatory pattern did not coincide with steroid effects on the mesolimbic DA system; only T maintained ejaculation and none of the steroid treated groups differed significantly in the number of intromissions or intromission latency. Thus, there was a good correspondence between steroid effects on mesolimbic DA and several measures of sexual arousal.

Figure 9. Mean (+ 1 S.E.M.) mount latency for each group in Experiment 2 in the test for sexual behavior. Four weeks prior to testing subjects in different groups had been sham-castrated (INTACT), or castrated and implanted with silastic capsules containing cholesterol (CHOL), testosterone (T), 5 α -dihydrotestosterone (DHT), estradiol (E2), or capsules of both 5 α -dihydrotestosterone and estradiol (DHT+E2). The number at the base of each column represents the number of animals contributing to the mean.

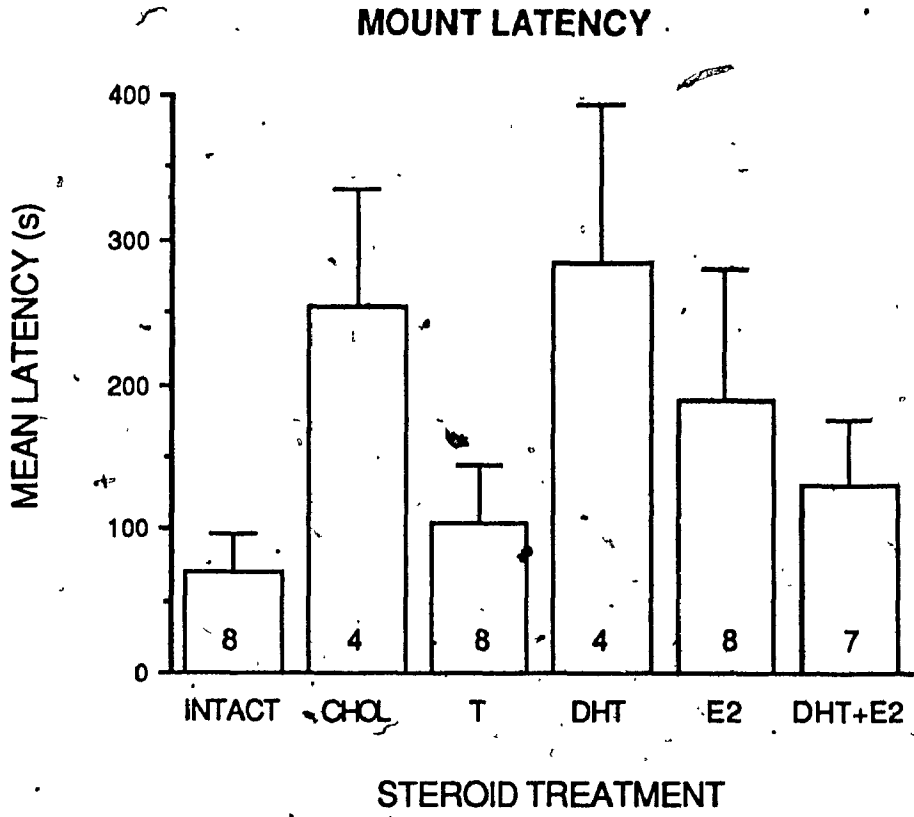
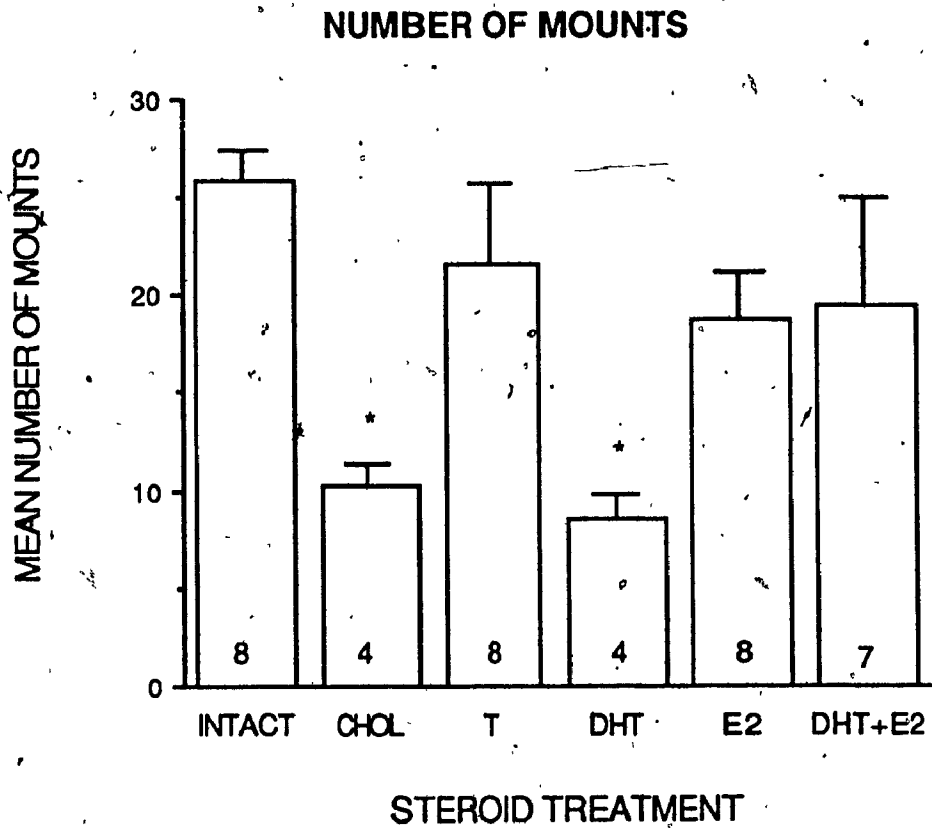


Figure 10. Mean (+1 S.E.M.) number of mounts for each group during the test for sexual behavior in Experiment 2. Four weeks prior to testing subjects in different groups had been sham-castrated (INTACT), or castrated and implanted with silastic capsules containing cholesterol (CHOL), testosterone (T), 5 α -dihydrotestosterone (DHT), estradiol (E2), or capsules of both 5 α -dihydrotestosterone and estradiol (DHT+E2). * significantly different from the INTACT group ($p < .05$). The number at the base of each column represents the number of animals contributing to the mean.



In this experiment, replacement T and E₂ were more effective than DHT in preventing the decrease in NAS DA concentrations after castration. Alderson and Baum (1981), however, reported that E₂ and DHT were more effective than T in maintaining NAS DA after castration. The reason for this difference is not clear; similar steroid replacement regimens and time courses were used in both studies. The results of the present experiment, however, are in agreement with the aromatization hypothesis (McDonald et al., 1970), and offer a good correspondence with behavior. DHT does not prevent the decline of copulation after castration, and it did not maintain either indices of sexual arousal or NAS DA concentrations. T, on the other hand, does maintain copulation after castration, and it maintained both sexual arousal and NAS DA concentrations.

In tests of copulation, it was found, as in a previous study (Chambers & Phoenix, 1986), that replacement with both E₂ and DHT was not as effective as T in maintaining the ejaculatory pattern. A number of studies, however, have found that concurrent E₂ and DHT is as effective as T in maintaining the ejaculatory pattern in castrates (Baum & Vreeburg, 1973; Larsson & Sodersten, 1973; Sodersten et al., 1980). It is unlikely that the DHT implants provided too little steroid to effectively synergize with E₂; similar implants have been found to maintain ejaculation (Darnassa et al., 1977). The differences in the maintenance of ejaculation could reflect strain, subject selection, or testing procedure differences. There is also the possibility that T's effects are not mediated solely by the interaction of aromatized metabolites with estrogen receptors and 5 α -reduced metabolites with androgen receptors. Whalen, Battie, and Luttge (1972) found that an anti-estrogen did not block the effects of T on male sexual behavior in male rats. Furthermore, there is evidence (reviewed by Sheridan, 1983) for at least two

androgen receptors, one that preferentially binds T and one that preferentially binds DHT. Although the behavioral effects of T and its metabolites have been investigated, further research is needed to determine if T has a unique action.

EXPERIMENT 2b

In Experiment 2, steroids were administered by silastic implants and would be present throughout the post-castration interval, including the time of testing and sacrifice. DA and DOPAC concentrations in the NAS did not decrease until 4 weeks after castration, however (Experiment 1), suggesting that the availability of steroids must be long-term, not acute, in order to maintain mesolimbic DA. The following experiment assessed the effect of acute T treatment in long-term castrates to verify this interpretation. The amount of T administered was well above the threshold necessary to maintain or restore sexual behavior after castration (Davidson & Bloch, 1969).

Method

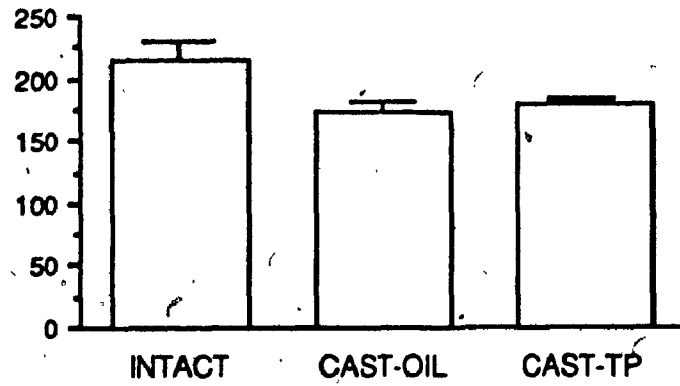
Subjects. Twenty-one Long-Evans male rats, screened for sexual behavior, were randomly assigned to one of three groups. Two groups were castrated via a scrotal incision, and the third group was sham-castrated.

Procedure. Four weeks after castration, males in one group received an injection of 250 μ g testosterone-propionate (TP; Sigma) in 0.1 ml peanut oil. The other group of castrates and the intact control group received 0.1 ml injections of the oil vehicle. All injections were administered s.c. One hour after injection, the males were exposed to a sexually receptive female for 30 min, and then sacrificed for amine determination using the protocol outlined above.

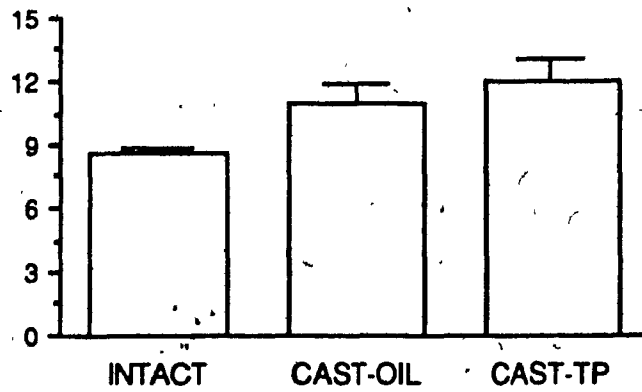
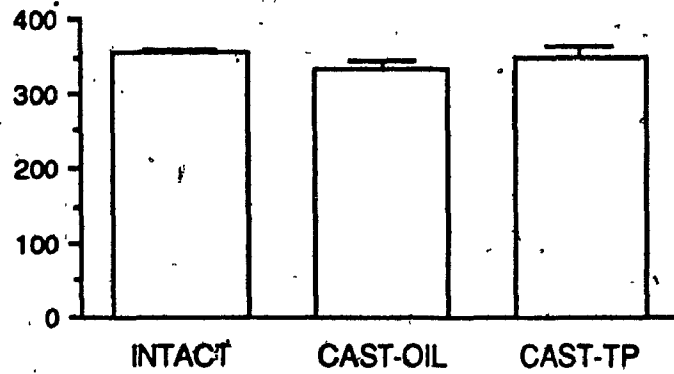
Results and Discussion

The DA concentrations in the NAS, caudate-putamen, and mPOA are shown in Figure 11. Castration reduced DA concentrations in the NAS, and the analysis of variance yielded a significant group effect, $F(2,17) = 4.05$, $p < .05$. TP injection did not increase DA concentrations; oil and TP injected castrates did not differ significantly in DA levels ($p > .1$). Nigrostriatal DA, as measured in the caudate-putamen, did not differ between groups ($p > .1$). The DA content of the mPOA was increased by castration,

Figure 11. Mean (+ 1 S.E.M.) concentrations of DA in the NAS (top panel), mPOA (middle panel), and caudate-putamen (lower panel) for the three groups in Experiment 2b. Subjects were gonadally intact and injected with 0.1 ml of oil 1 h prior to sacrifice (INTACT), or had been castrated 4 weeks previously and injected with 0.1 ml oil (CAST. OIL) or 250 μ g testosterone propionate (CAST. TP) 1 h prior to sacrifice. Each column represents the mean of 8 determinations.

DOPAMINE CONCENTRATIONS**NUCLEUS ACCUMBENS**

MEAN CONCENTRATION (pg/μg protein)

MEDIAL PREOPTIC AREA**CAUDATE-PUTAMEN**

GROUP

$F(2,18) = 4.06, p < .05$, and acute TP administration was without effect.

As suggested by the time course of the decrease in mesolimbic DA after castration (Experiment 1), the effectiveness of the steroid replacement in Experiment 2 appears to be due to the long-term presence of the steroid.

EXPERIMENT 3

Sexual behavior of castrates is influenced by pre-castration sexual experience. Cats, the most thoroughly investigated species, will not engage in sexual behavior if they are sexually naive at the time of castration, and, furthermore, T is effective in eliciting sexual behavior only if steroid treatment coincides with exposure to a receptive female (Rosenblatt & Aronson, 1958a, 1958b; Rosenblatt, 1965). Similarly, sexually experienced rats will copulate for some time after castration while sexually naive castrates are less likely to copulate (Larsson, 1978; but see Bloch & Davidson, 1968 for a conflicting report). The amount of pre-castration sexual experience does not appear to be important. Davidson (1966a) found no correlation between the amount of pre-castration sexual experience and the persistence of sexual behavior.

Although Beach (1976b) has reported that copulation persists the longest in male dogs that have periodic post-castration exposure to a female in heat, very little is known about the effect of post-castration sexual experience on the maintenance of sexual behaviors. The following experiment assessed the effect of repeated exposure to a receptive female on the sexual behaviors of castrates.

Sexual experience may affect the mesolimbic DA system as well as sexual behaviors, and differences in sexual experience may account for differences in the results of studies that have measured mesolimbic DA concentrations in castrates. A castration-induced decrease in NAS DA concentrations has been found in subjects with no sexual experience (Alderson & Baum, 1981), and in subjects with only one pre- and one post-castration opportunity to copulate (Experiments 1 and 2); in contrast, no change was reported for subjects with two pre- and four, weekly post-castration opportunities to copulate (Baum et al., 1986), suggesting that repeated post-castration exposure to a sexually receptive female and the opportunity to engage in sexual behaviors might maintain mesolimbic DA levels. The following experiment explored this possibility.

Method

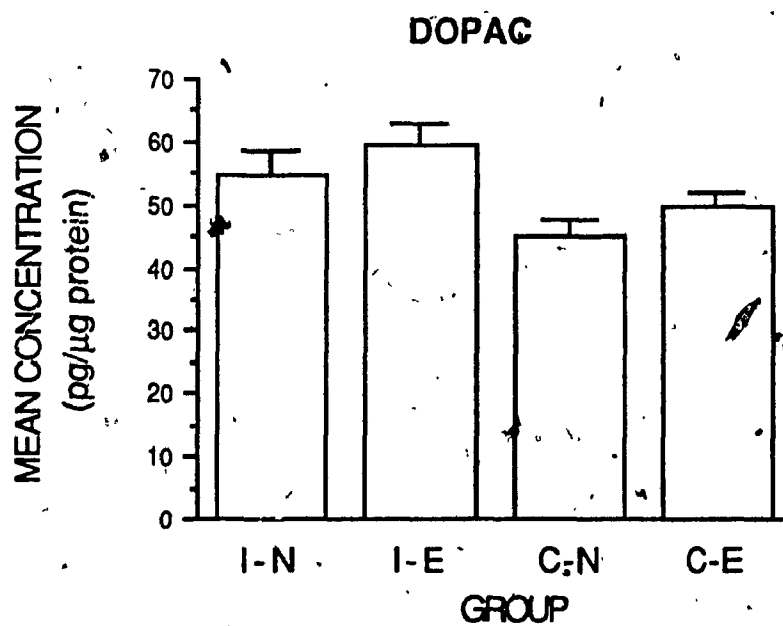
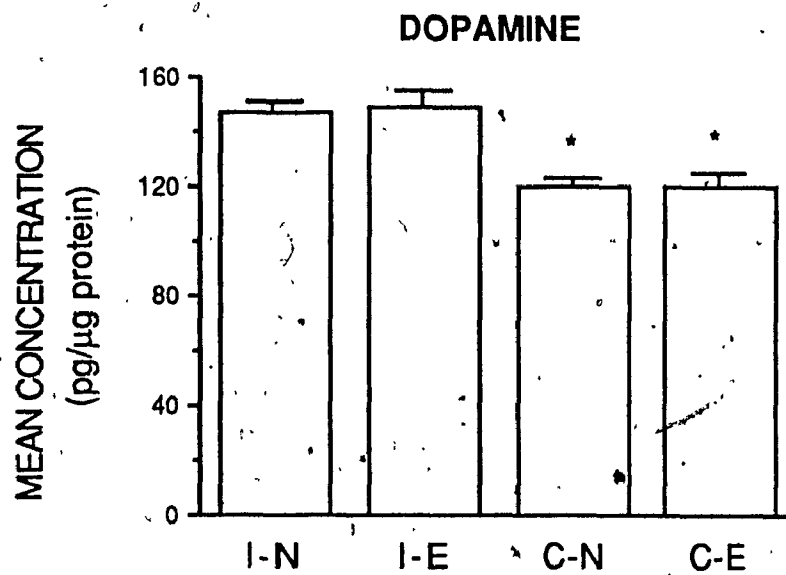
Subjects. After screening for sexual activity, 32 Long-Evans male rats were randomly assigned to one of four groups. Two groups of animals were bilaterally castrated, and two sham-castrated as described in Experiment 1.

Procedure. Beginning on the fourth day after surgery, one group of castrates and one group of intact males were given opportunities to copulate twice weekly. On each occasion, the male was placed in the mating arena and 5 min later a sexually receptive female was introduced. Thirty min later, the male was returned to the animal colony. To control for familiarity with handling, transportation, and the mating arenas, the other two groups were placed in the mating arenas, with no female present, for 30 min twice weekly. The four groups were: castrated with post-castration sexual experience (C-E), gonadally intact with experience (I-E), castrated without experience (C-N), and intact without experience (I-N). Four weeks after surgery, the animals were placed individually in mating arenas; 5 min later a sexually receptive female was introduced, and animals were allowed to copulate for 30 min. Behavioral measures were obtained as described in Experiments 1 and 2. After the behavioral test, animals were sacrificed for amine determination using HPLC-EC.

Results

The mean concentrations of DA and DOPAC in the NAS for each of the groups are presented in Figure 12. Two-factor analyses of variance (experience \times castration) were carried out on the DA and DOPAC concentrations and the DOPAC/DA ratio. It can be seen that castration reduced the concentrations of both DA and DOPAC in the NAS, and the statistical analysis yielded a significant castration effect for DA, $F(1,28) = 34.05$, $p < .001$, and for DOPAC, $F(1,28) = 9.6$, $p < .01$. Although sexual experience did not have a statistically significant effect on either DA or DOPAC concentrations (p 's $> .1$), the analysis of variance of the DOPAC/DA ratio yielded a small, but significant effect of

Figure 12. Mean (+ 1 S.E.M.) concentrations of DA (upper panel) and DOPAC (lower panel) for the groups in Experiment 3. Subjects were either gonadally intact (I) or castrated (C), and had been allowed to copulate twice a week for 4 weeks (E), or had no copulatory experience between castration and the final test for sexual behavior (N). Each column represents the mean of 8 determinations. * significantly different from the corresponding I group ($p < .05$).



experience; $F(1,28) = 4.11$, $p = .05$. The subjects with post-castration sexual experience had a higher DOPAC/DA ratio than the subjects without sexual experience; the means were: with experience 0.41, and without experience 0.37.

The only other brain area in which DA content differed significantly between groups was the mPOA. Castrated animals had higher DA concentrations than did sham-castrated controls, and the effect of castration was statistically significant, $F(1,28) = 5.43$, $p < .05$; the means were: intact 4.02 pg/ μ g protein, and castrates 5.14 pg/ μ g protein. Sexual experience did not affect DA concentrations in the mPOA ($p > .1$).

Sexual experience had a significant effect on female-directed behaviors. It can be seen from Figure 13 that, overall, experienced animals exhibited higher levels of female-directed behaviors, and the analysis of variance yielded a significant experience effect, $F(1,28) = 6.85$, $p < .05$. The experience x castration interaction was also significant, $F(1,28) = 5.35$, $p < .05$; only castrated animals showed an effect of the additional sexual experience. Castrated animals without additional copulatory experience engaged in low levels of female-directed behaviors compared to the other three groups (p 's $< .05$). The occurrence of female-directed behaviors decreased with time for all groups, $F(2,56) = 45.3$, $p < .001$.

The proportion of animals in each group that mounted, intromitted, or displayed the ejaculatory pattern is shown in Figure 14, and it can be seen that groups differed in the number of animals displaying each of these behaviors (mount: $\chi^2(3) = 6.4$, $.05 < p < .1$; intromission: $\chi^2(3) = 15.94$, $p < .01$; ejaculatory pattern: $\chi^2(3) = 9.6$, $p < .05$).

The additional sexual experience increased the proportion of castrates that mounted (.5 to .75) and the proportion of intact animals that ejaculated (.5 to .75). Other differences between groups were attributable to the effect of castration.

Among animals that did mount the female, sexual experience decreased mount latency. An analysis of variance of logarithmically transformed latency scores yielded a

Figure 13. Mean (± 1 S.E.M.) percent of observations during which female-directed behaviors were observed as a function of time during the test for sexual behavior in Experiment 3. Subjects were either gonadally intact (I) or castrated (C), and were then allowed to copulate twice a week for 4 weeks (E), or had no copulatory experience between castration and the final test for sexual behavior (N). $n = 8$ for each group.

FEMALE-DIRECTED BEHAVIORS

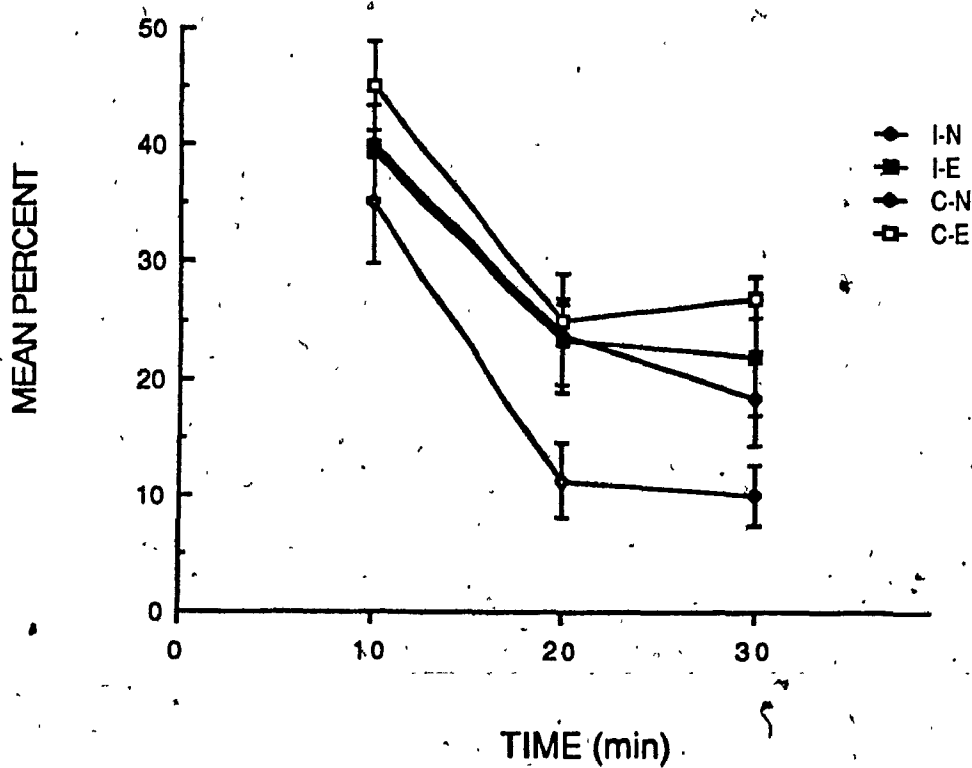
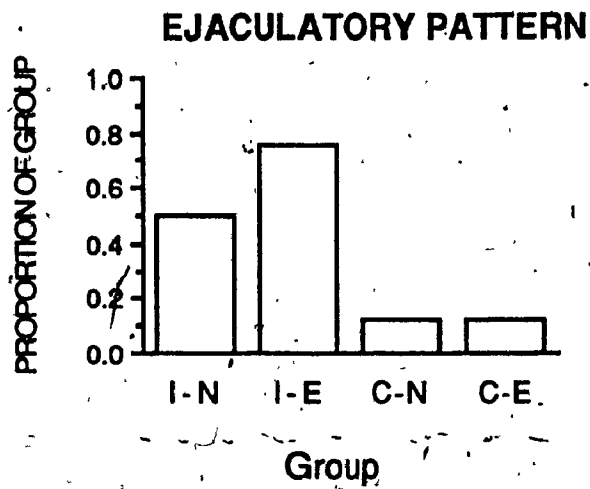
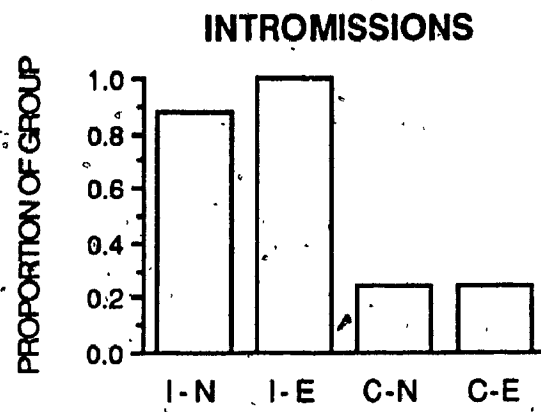
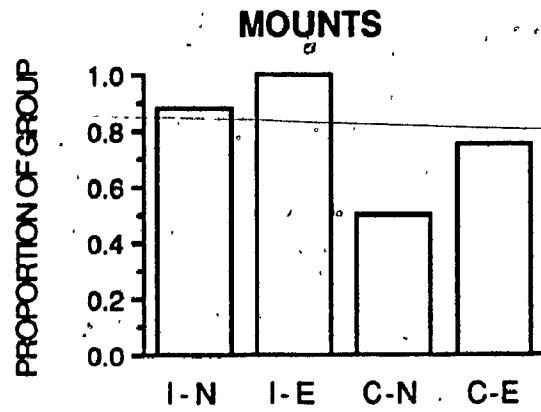


Figure 14. The proportion of animals in each group in Experiment 3 that mounted (top panel), intromitted (middle panel), or displayed te ejaculatory pattern (lower panel) during the test for sexual behavior in Experiment 3. Subjects were either gonadally intact (I) or castrated (C), and were then allowed to copulate twice a week for 4 weeks (E), or had no copulatory experience between castration and the final test for sexual behavior (N).



statistically significant experience effect, $F(1,21) = 6.19$, $p < .05$. Although, as can be seen from Figure 15, the C-E group had a shorter mean mount latency than the C-N group, post hoc comparisons between individual groups did not attain statistical significance. Groups did not differ significantly in the total number of mounts (p 's $> .1$).

The two sham-castrated groups were compared on the intromission and ejaculation measures; too few castrates performed these behaviors to be included in the analyses. Among gonadally intact animals that intromitted, the experienced group had a lower intromission latency than the inexperienced group (47.1 s versus 137.2 s), and this difference was statistically significant, $t(13) = 4.19$, $p < .05$. The two intact groups did not differ significantly in the number of intromissions, or in ejaculation latency (p 's $> .05$).

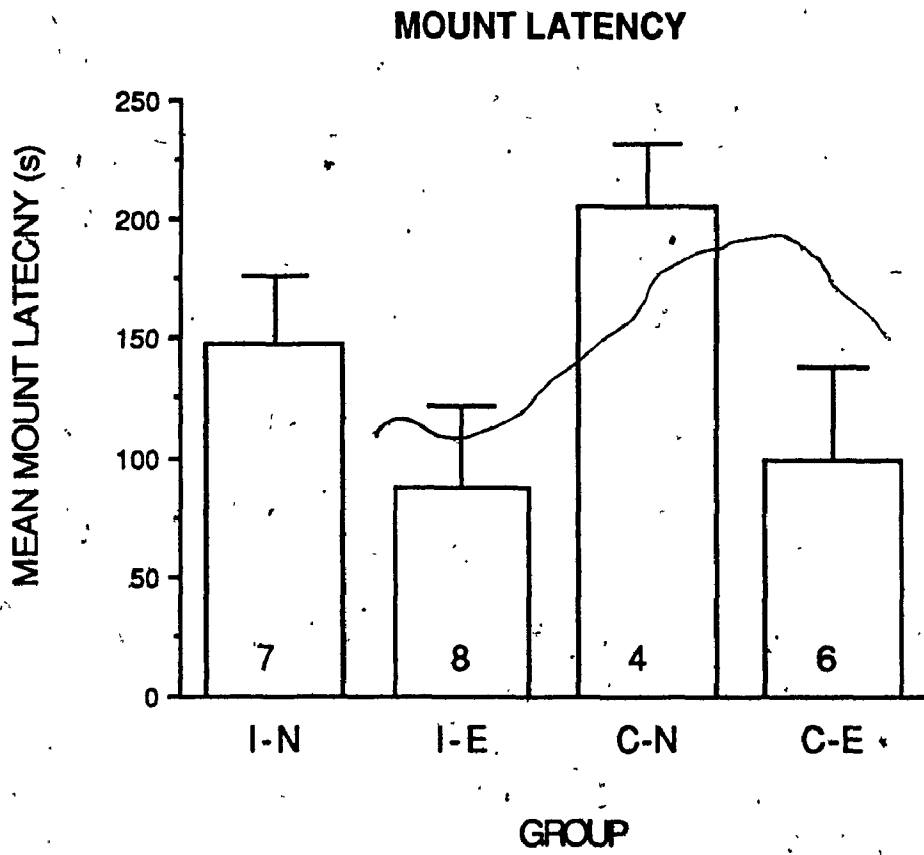
Discussion

In this experiment, it was found that the repeated presentation of a receptive female and the opportunity to copulate after surgery facilitated sexual behavior.

Gonadectomized animals given post-castration experience maintained a higher level of female-directed behaviors, and were somewhat more likely to mount the female than those without post-castration experience. Among sham-castrated animals, mount and intromission latencies were shorter when there had been repeated sexual experience in that environment. Among all of the groups, the additional sexual experience had little effect on copulation once it had begun. Thus, the additional sexual experience appeared to affect measures of sexual arousal while having little effect on the performance of copulation.

The two intact groups differed in both the total amount of sexual experience and in the amount of experience in the mating arena. Although no attempt was made to explicitly measure the conditioning of sexual arousal, it is interesting to note that the

Figure 15. Mean (+ 1 S.E.M.) mount latency for the groups in Experiment 3. Subjects were either gonadally intact (I) or castrated (C), and had been allowed to copulate twice a week for 4 weeks (E), or had no copulatory experience between castration and the final test for sexual behavior (N). The number at the base of each column represents the number of animals contributing to the mean.



differences between the two intact groups are similar to those reported by Zamble, Hadad, Mitchell, and Cutmore (1985) for tests of conditioned sexual arousal.

As mentioned earlier, pre-castration sexual experience has been reported to affect the maintenance of sexual behaviors, and in the present experiment it was found that post-castration sexual experience also helped maintain some aspects of sexual behavior. This happened in spite of the fact that female-directed behaviors were not maintained by copulation during the final test; castrates with post-castration sexual experience were no more likely to intromit or ejaculate than castrates without additional sexual experience. Furthermore, it is interesting to note that castrates given post-castration exposure to a sexually receptive female continued to engage in female-directed behaviors even while their sexual performance during the experience trials was declining. That is, rather than leading to a more rapid loss of interest in the female because of repeated failure to copulate successfully, post-castration exposure to the female appeared to maintain attention to sexually relevant stimuli and sexual arousability. It is possible that exposure to a receptive female after castration reinstated, or perhaps even promoted the development of conditioned sexual arousal to the test environment. Although the present experiment was not designed to directly assess conditioned factors, it might be profitable to explore the conditioning of sexual arousal among recent and long-term castrates in future studies.

The results of the neurochemical analyses replicate the previous finding of a castration-induced decrease in NAS DA and DOPAC concentrations and increase in mPOA DA concentrations. The absence of an experience x castration interaction for NAS DA levels, however, suggests that sexual experience cannot account for the difference between the present results together with those of Alderson and Baum (1981), and those reported by Baum et al. (1986). Sexual experience, although it did not significantly affect the amount of DA available, did affect a measure of DA metabolism,

the DOPAC/DA ratio. Repeated exposure to a sexually receptive female increased activity in the mesolimbic DA system, whether the animal was castrated or gonadally intact.

In summary, castration reduced mesolimbic DA and DOPAC concentrations and sexual behaviors; repeated exposure to a receptive female increased mesolimbic DA metabolism and increased sexual arousal. The behavioral and neurochemical data are consistent with the hypothesis that the mesolimbic DA system mediates sexual arousability.

General Discussion

In these experiments it was found that a terminal field of the mesolimbic DA system was affected by castration, and the biochemical changes were systematically related to some changes in sexual behavior. The behaviors that consistently coincided with the biochemical data were all measures of sexual arousal or motivation:

- female-directed behaviors, and the likelihood of mounting and intromitting. Changes in the performance of copulation did not vary with changes in the mesolimbic DA system.

Relatively few studies of male sexual behavior have measured female-directed behaviors. Consistent with the current hypothesis, Malmnas (1976) reported an increase in female-directed behaviors after L-DOPA treatment that was blocked by pimozide. Madlafousek et al. (1976) reported that female-directed behaviors did not decrease after castration, but they used a very lenient criterion. The present experiments found that the amount of female-directed behaviors decreased after castration, and the nature of this decline is interesting. All males displayed high levels of female-directed behaviors during the initial part of the test, and all showed a decline during the 30 min test. For intact animals, this decline coincided with the initiation and performance of copulation. Long-term castrates showed a greater decrease in female-directed behaviors, and female-directed behaviors were replaced by exploration of the general environment and inactivity. That is, long-term castrates, without steroid replacement, appeared to be aroused by the female, but this arousal was not sufficient to support copulation and was not maintained for the duration of exposure to the female.

The decrease in DA concentrations after castration most likely reflected a decrease in the amount of DA present, and not increased utilization. If the decrease in DA concentrations were due to increased utilization, then DOPAC concentrations should have been increased. DOPAC concentrations, and the DOPAC/DA ratio, however, were, like DA, lower 4 weeks after castration. Thus, these findings suggest that there

were two effects of castration on the mesolimbic DA system; DA concentrations declined, and, in addition to this, dopaminergic activity in the NAS decreased.

The underlying mechanism(s) responsible for the change in DA concentrations was not explored, but research on other steroid effects offers several possibilities. The changes in DA concentrations may have been mediated by steroid effects on metabolizing enzymes; E_2 affects type A MAO and acetylcholine esterase (AChE) in female rat brain (Luine & Rhodes, 1983), as well as catechol-O-methyltransferase (COMT) (Breuer, Schneideer, Wandschoer, & Ladosky, 1978). Steroid hormones may also act to modulate the amount or activity of synthesizing enzymes. Castration affects the activity (V_{max}) of tyrosine hydroxylase in the median eminence of male rats without changing the affinity of the enzyme for either substrate or cofactor (Kizer et al., 1978), and the amount of tyrosine hydroxylase present in the median eminence and superior cervical ganglion of the female rat changes with the estrus cycle (Porter, 1986). Estrogens also affect the activity of glutamate decarboxylase, a GABA synthesizing enzyme, in male rat brain (Nicoletti & Meek, 1985). The effect of castration could also be mediated post-synaptically, and operate via feedback to the mesolimbic system; E_2 , for example, has been reported to induce hyposensitivity of DA receptors in the male rat (Piccardi, Bernardi, Rossetti, & Corsini, 1983). The regional and chemical specificity of the decrease in DA concentrations would appear to exclude nonspecific effects, such as changes in precursor availability, or general metabolic processes.

Post-castration decreases in NAS DA and DOPAC concentrations coincided with a decrease in behaviors that reflect sexual arousal. Maintaining sexual arousal by steroid administration also maintained DA levels, and post-castration sexual experience affected both measures of sexual arousal and mesolimbic DA activity. These experiments, therefore, support the hypothesis castration of the male leads to a deficit in sexual

arousability, and that changes in the mesolimbic DA system are critically involved.

These data are, of course, correlational, and it is necessary to show that specific activation of the mesolimbic DA system activates male sexual behaviors. The following experiments addressed this possibility.

Effects of Morphine and Dynorphin Infusions into the VTA on Male Sexual
Behaviors

EXPERIMENT 4

The VTA, location of mesolimbic DA cell bodies, has a high concentration of both enkephalinergic terminals (Sar, Stumpf, Miller, Chang, & Ciatrecasas, 1978; Wamsley, Young, & Kuhar, 1980) and opiate receptors (Goodman, Snyder, Kuhar, & Young, 1980; Hong, Yang, Fratta, & Costa, 1977; Sar et al., 1978), suggesting that endogenously released opioids might influence the activity of the DA cells. Data from a variety of sources indicate that exogenously administered opiates increase activity in the A10 DA neurons. Peripheral and iontophoretic administration of morphine, a mu opioid receptor agonist, alters the firing frequency of DA and non-DA neurons in the VTA (Gysling & Wang, 1983). Infusions of morphine and analogues of enkephalin peptides into the VTA increase DA metabolism in mesolimbic terminal fields, including the NAS (Kalivas, Widerlov, Stanley, Breese, & Prange, 1983), and produce a dose-dependent increase in locomotor activity that is blocked by DA antagonists (Joyce & Iversen, 1979; Kalivas et al., 1983; Vezina & Stewart, 1984). Both morphine and the endogenous opioid peptide, dynorphin, (Goldstein, Tachibana, Lowney, Hunkapiller, & Hood, 1979) injected into the VTA elicit feeding in satiated rats (Hamilton & Bozarth, 1986, 1987), and feeding induced by VTA morphine infusion is attenuated by the opioid receptor blocker naloxone (Wise, Jenck, & Raptis, 1985). Thus, the activation of opioid receptors in the VTA increases mesolimbic DA neurotransmission and elicits appetitive approach behaviors. Although opioid agonists applied to the VTA increase locomotion and elicit feeding, tests for copulation have not been reported.

In Experiment 4, the effects of different doses of morphine and of the peptide fragment dynorphin[1-13] upon male sexual behavior were examined. The arousability

of the males was reduced by castrating them and then maintaining them on behaviorally subthreshold doses of T (Davidson & Bloch, 1969; Mitchell, 1986, unpublished observations). The doses of morphine and dynorphin[1-13] used were selected on the basis of their efficacy in eliciting feeding (Hamilton & Bozarth, 1986, 1987; Wise et al., 1985) and from a pilot study of male sexual behavior (Mitchell, Stewart, & Vezina, 1987, unpublished observations). To demonstrate receptor mediation of any observed effects, all males were also tested for sexual behavior following infusion of a previously effective dose of morphine or dynorphin[1-13] together with systemic naloxone.

It was predicted that morphine, and perhaps dynorphin[1-13], would increase sexual activity and would especially facilitate measures of sexual arousal.

Method

Subjects. Male Long-Evans rats (Charles River Canada Ltd.) weighing 280-300 g were used. The males had been screened for sexual activity, castrated, and received daily injections of 5 µg TP, s.c., in peanut oil vehicle throughout the experiment.

Surgery. Animals were injected with 0.1 ml atropine sulfate, s.c., (Glaxo Laboratories, Montreal, Que.), and anesthetized with sodium pentobarbital, i.p., (0.85 ml/kg Somnotol, M.T.C. Pharmaceuticals Ltd.). Subjects were stereotaxically implanted with chronic bilateral guide cannulae (22 gauge, Plastic Products) aimed at the VTA or substantia nigra. Blocker and injection cannulae (28 gauge, Plastic Products) extended 1 mm beyond the guide cannulae. The VTA coordinates were: A/P -3.6, L ±0.6, and D/V -8.9 from skull (Pelligrino, Pellegrino, & Cushman, 1979). The guide cannulae were implanted at 16 degrees to the vertical to avoid the periventricular gray (PVG) and penetration of the cerebral aqueduct. The substantia nigra coordinates were: A/P -3.8, L ±2.5, and D/V -8.9 (Pelligrino et al., 1979). The substantia nigra cannulae were implanted at 8 degrees to the vertical, permitting the use of Plastic Products blocker cannulae. Guide cannulae were secured by dental cement molded around 4-5 stainless

steel screws imbedded in the skull. Animals were allowed 10-14 days to recover from surgery.

Apparatus. Subjects were screened for sexual behavior in the semi-circular boxes used in Experiments 1-3, and a different set of boxes was used for subsequent tests. Tests for sexual behavior after drug delivery were conducted in Plexiglas-fronted boxes, 36 cm x 50 cm x 28 cm deep, individually lit by a 7w red light bulb. A red-light sensitive camera and video cassetted recorder, described earlier, was used to video tape the tests for future scoring.

Procedure. Morphine sulfate (BDH Chemicals, Toronto, Ont.) and dynorphin[1-13] (Sigma) were dissolved in sterile 0.9 % saline. All injections were a volume of 0.5 μ l/side and were made over 45 s. with 28 gauge injection cannulae connected to 1 μ l microsyringes (Hamilton, Reno, NV) by PE-20 tubing. Bilateral injections were made simultaneously in unrestrained animals. Seventy-five seconds after the end of the injection, the injection cannulae were removed, obturators replaced, and the animal immediately placed in a mating arena. Groups of 6-8 males were tested concurrently. Five minutes after the last male had been placed in a mating arena, sexually receptive females were introduced. Tests of sexual behavior lasted 30 min and were conducted during the animal's dark cycle every 2-3 days.

Animals with VTA cannulae were randomly assigned to one of two groups. One group of 10 animals (MOR) received 0.1, 1, 10 and 30 nmoles morphine, and the other group (DYN) 0.03, 0.1, 0.3 and 3 pmoles of dynorphin[1-13]; both groups also received a vehicle injection. Dose order was randomized with the restriction that some animals in each group received the vehicle injection on the first, third and fifth test. Three days after the dose-response investigation was completed, a test for naloxone antagonism was conducted; each animal received an i.p. injection of 1 mg/kg naloxone HCl (Endo Laboratories Inc., Garden City, NY), and a VTA infusion of either 10

nmoles morphine or 0.3 pmoles dynorphin[1-13] and were tested for sexual behavior.

Animals with substantia nigra cannulae were tested for sexual behavior after injection with vehicle, 10 nmoles morphine and 0.3 pmoles dynorphin[1-13], in randomized order.

After completion of the experiment, all subjects were deeply anesthetized and perfused transcardially with saline and 10 % formalin (Fisher), and the brains stored for 7-10 days in 10 % formalin. Histological verification of cannulae tip placements were made on 40 μ thionin-stained coronal sections.

A measure of locomotor activity was obtained by recording the number of center-crossings during the 5 min prior to the introduction of the female. After the female had been placed in the mating arena, the occurrence of mounts, intromissions, and the ejaculatory pattern was recorded. Female-directed behaviors were scored using the time sampling procedure described in Experiment 1.

Results

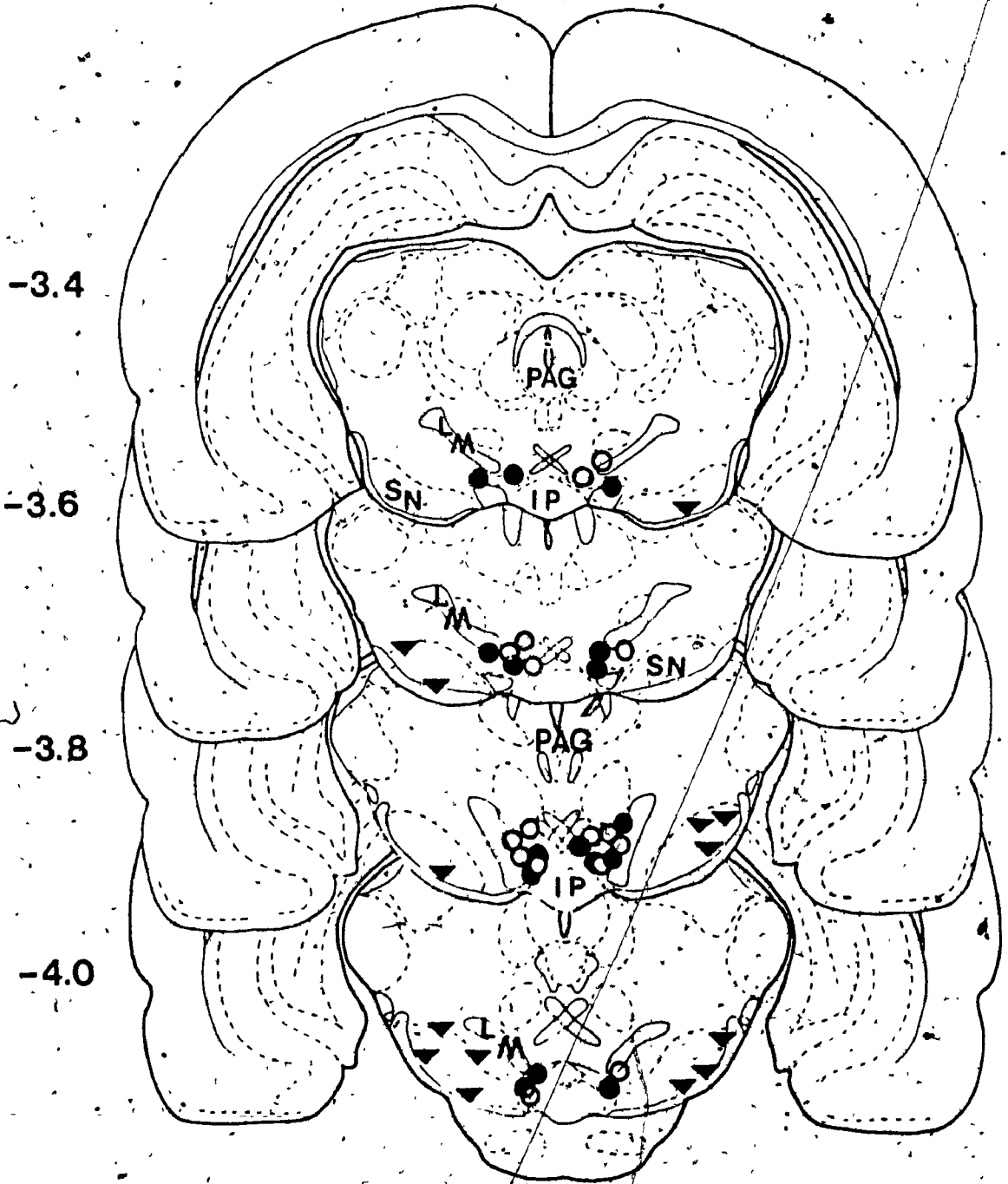
Injector cannulae tip placements are illustrated in Figure 16. Most VTA cannulae placements were lateral of the interpeduncular nucleus and medial of the medial lemniscus; this area corresponds to the approximate location of the mesolimbic DA cell bodies (Bjorklund & Lindvall, 1984; Dahlstrom & Fuxe, 1964). The data for two subjects in the DYN group and one in the MOR group were excluded because of inaccurate cannulae tip placements, and the data for a second MOR subjects were dropped because the dental cement securing the guide cannulae became loose, precluding accurate histology. The substantia nigra injector cannulae tips were located in the substantia nigra for all but one subject; the data for this subject were excluded.

Sexual behaviours. An analysis of variance (group x dose x time) of the percentage of observations during which female-directed behaviors occurred was performed. The analysis of variance yielded significant effects for dose,

Figure 16. Injector cannulae tip placements for the subjects in Experiment 4 that received injections of morphine (filled circles; $n = 8$), and dynorphin[1-13] (open circles; $n = 8$). Subjects that received injections of saline, morphine and dynorphin[1-13] had cannulae aimed at the substantia nigra, and are represented by filled triangles ($n=7$).

Abbreviations: LM: medial lemniscus; PAG: periaqueductal gray; IP: interpeduncular nucleus. Numbers to the left of the sections represent the distance from bregma.

Adapted from Pelligrino, Pelligrino and Cushman (1979).



$F(4,56) = 16.99, p < .001$, and time, $F(2,28) = 141.92, p < .001$, reflecting the fact that the amount of female-directed behaviors varied with drug dose and decreased across the test session. More importantly, there were also statistically significant group x dose, $F(4,56) = 2.87, p < .05$, dose x time, $F(8,112) = 4.69, p < .01$, and group x dose x time, $F(8,112) = 2.53, p < .05$, interactions. Figure 17 illustrates the group x dose interaction; it can be seen that the frequency of occurrence of female-directed behaviors increased with increasing doses of dynorphin[1-13], up to the highest dose at which the amount of female-directed behavior decreased. Among morphine-infused subjects, however, the frequency of female-directed behaviors was relatively unchanged by morphine, except at the highest dose where the behaviors decreased significantly ($p < .05$). As can be seen from Figure 17, however, female-directed behaviors tended to be more frequent in the MOR group after vehicle and low dose infusions than for the DYN group after corresponding infusions. The frequency of female-directed behaviors after saline infusions was further explored by separating subjects within groups on the basis of the trial during which they received the saline infusions. As can be seen from Figure 18, in the MOR group, the frequency of female-directed behaviors after saline was higher if the saline infusion occurred during the fifth trial than if it occurred during the first or third trial. In the DYN group, on the other hand, the frequency of female-directed behaviors after saline infusion did not vary as a function of the trial during which saline was infused.

Figure 19 presents the amount of female-directed behavior for each group at each drug dose at different times during the copulation tests, illustrating the three-way interaction. As can be seen from Figure 19, 30 nmoles of morphine significantly reduced the frequency of female-directed behaviors relative to all other morphine doses throughout the 30 min test ($p's < .05$), but none of the other morphine doses differed from each other or from the saline injection at any time ($p's > .05$). On the other hand,

Figure 17. Mean (± 1 S.E.M.) percent of observations during which female-directed behaviors were observed after infusions of saline, different doses of morphine (filled symbols) and dynorphin[1-13] (open symbols), and after an infusion of morphine or dynorphin[1-13] preceded by 1 mg/kg naloxone (Nal). $n = 8$ for each group.

FEMALE-DIRECTED BEHAVIORS

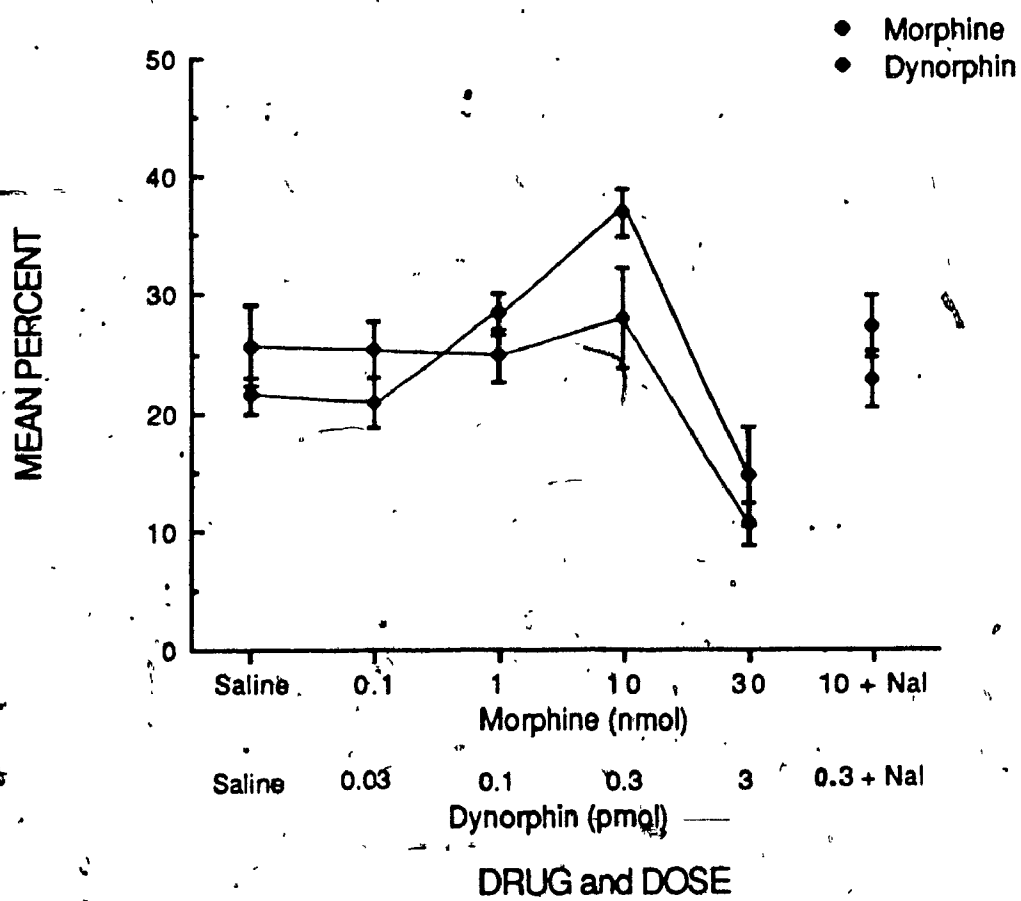


Figure 18. Mean percent of observations during which female-directed behaviors were observed after saline infusions as a function of the trial on which the saline infusion was given. During drug trials, subjects received infusions of either morphine (MOR) or dynorphin[1-13] (DYN).

FEMALE-DIRECTED BEHAVIORS

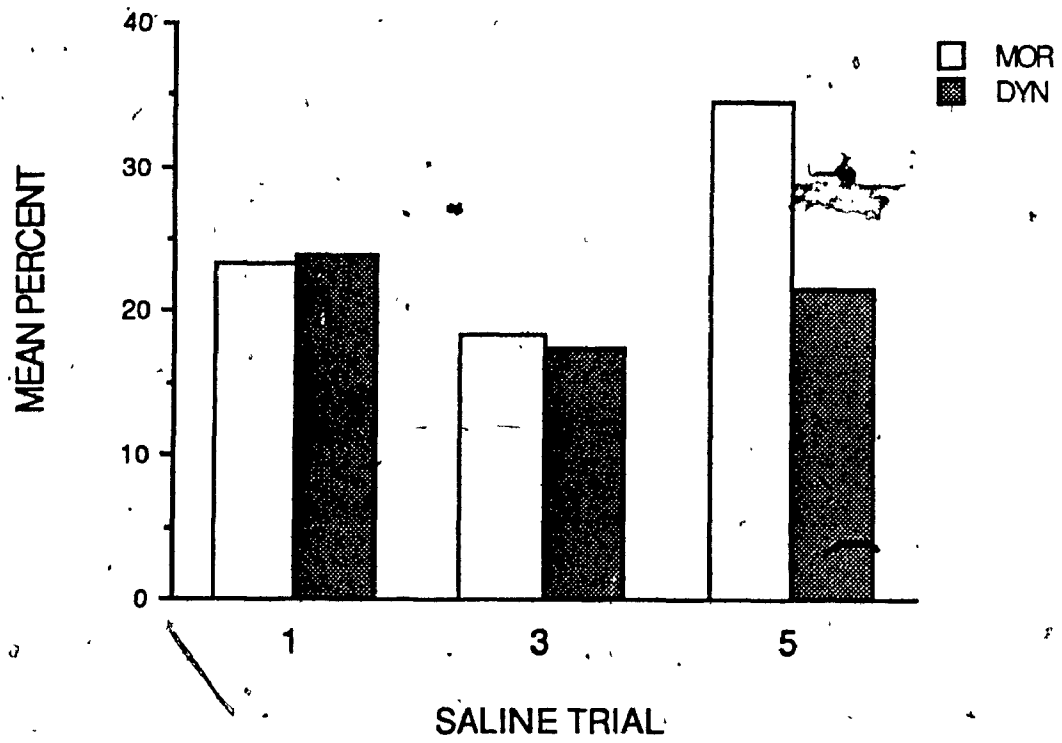
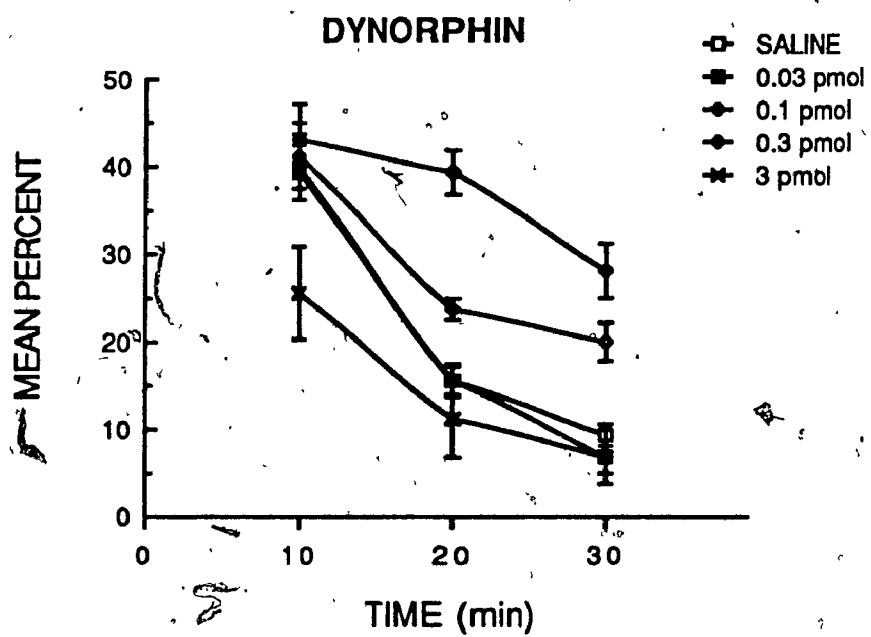
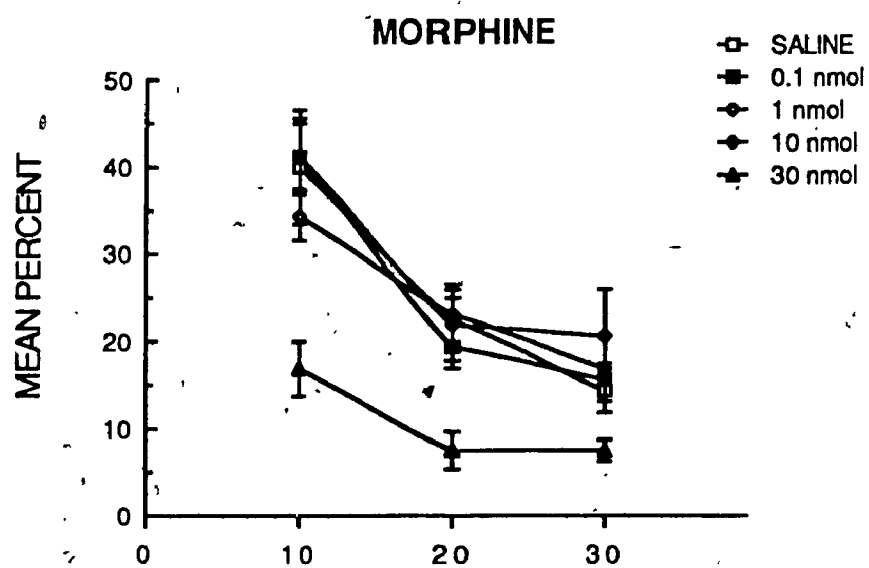


Figure 19. Mean (± 1 S.E.M.) percent of observations during which female-directed behaviors were observed as a function of time after different doses of morphine (upper panel) and dynorphin[1-13] (lower panel). $n = 8$ for each group.

FEMALE-DIRECTED BEHAVIORS



increasing doses of dynorphin[1-13] increased the frequency of female-directed behaviors; at 20 min, 0.3 pmoles of dynorphin[1-13] increased the frequency of, female-directed behaviors in comparison to all other doses of dynorphin[1-13] and the vehicle injection (p 's $< .05$); at 30 min, 0.3 pmoles resulted in more frequent female-directed behaviors than all other infusions except 0.1 pmoles (p 's $< .05$), and the 0.1 pmole infusion differed from both 0.03 and 3 pmole infusions (p 's $< .05$). The highest dose of dynorphin[1-13], however, suppressed female-directed behaviors during the first 10 min of the session in comparison to all other treatments (p 's $< .05$), and during the middle 10 min of the session in comparison to the 0.1 and 0.3 pmole infusions.

Figure 20 presents the proportion of animals in each group that mounted, intromitted or displayed the ejaculatory pattern after each drug and vehicle infusion. There was a dose-related increase in the number of animals that mounted in both groups; the highest dose of both morphine and dynorphin[1-13], however, decreased the number of animals that mounted. The number of animals that mounted differed significantly between infusions for both the MOR, $\chi^2(5) = 14.29$, $p < .05$, and DYN, $\chi^2(5) = 16.64$, $p < .01$, groups. In the MOR group there was no effect of drug on the number of animals intromitting or displaying the ejaculatory pattern (p 's $> .1$); in the DYN group, only the occurrence of the ejaculatory pattern was affected, $\chi^2(5) = 9.44$, $.05 < p < .01$. Too few animals mounted after infusions other than 10 nmoles morphine or 0.3 pmoles dynorphin[1-13] to allow a meaningful comparison of mount latencies between drug infusions.

Activity. A between-within analysis of variance (group x dose) of the number of center-crossings occurring in the 5 min prior to the introduction of the female yielded group, $F(1,14) = 13.98$, $p < .01$, and dose, $F(4,56) = 10.54$, $p < .01$, effects, and a significant group x dose interaction, $F(4,56) = 4.13$, $p < .01$. As can be seen from Figure 21, there was a dose-related increase in activity for the MOR, but not the DYN,

Figure 20. Proportion of animals in each group that mounted, intromitted, or displayed the ejaculatory pattern after different doses of morphine (upper panel) and dynorphin[1-13] (lower panel) in Experiment 4.

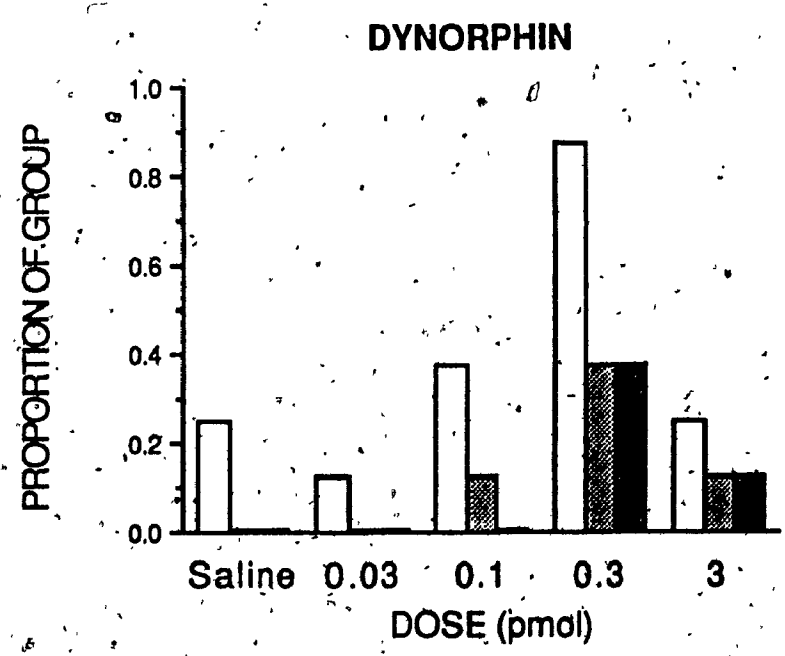
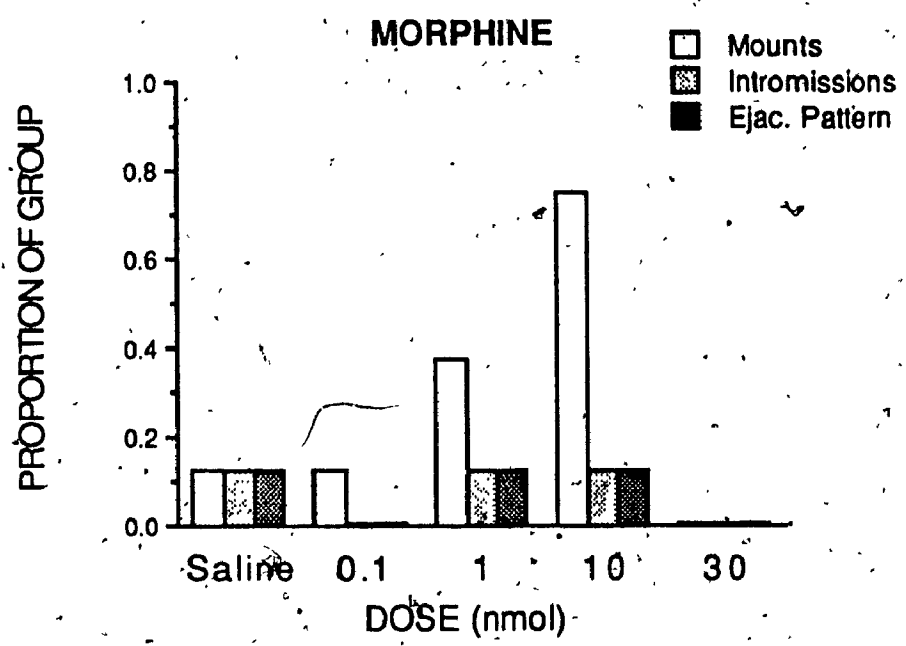
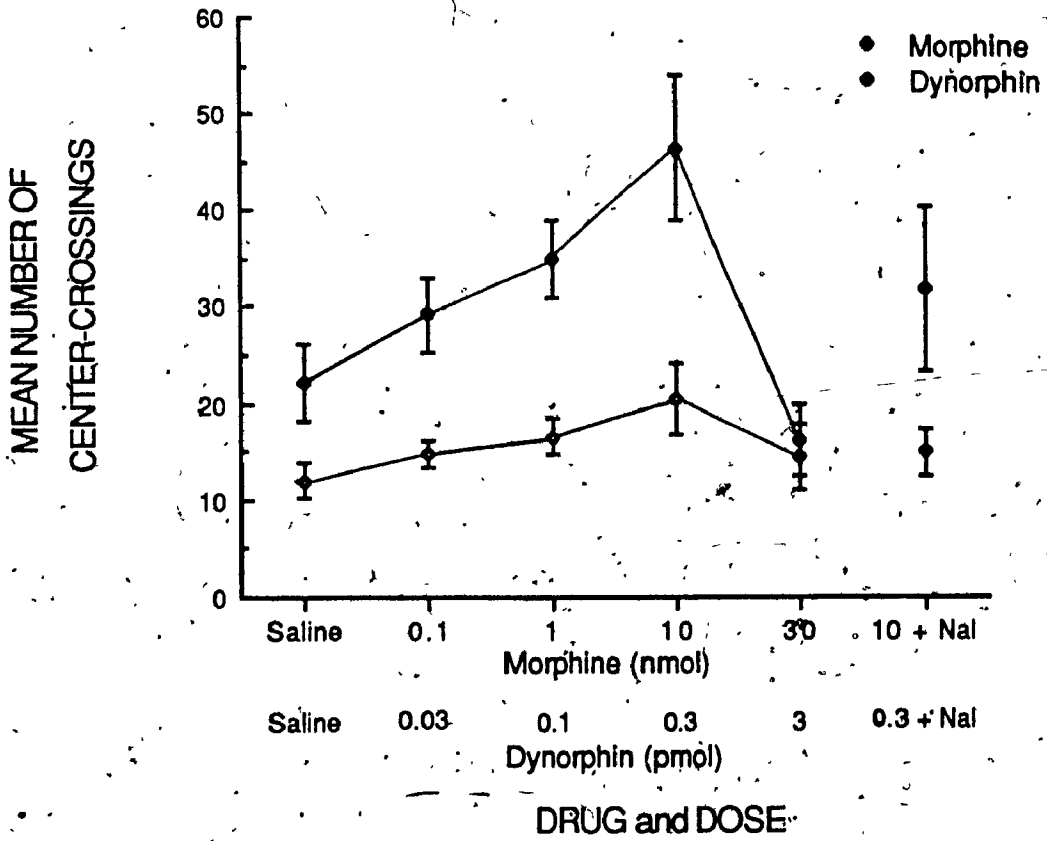


Figure 21. Mean (± 1 S.E.M.) number of center-crossing during the 5 min prior to the introduction of the female and the tests for sexual behaviors in Experiment 4. Subjects in different groups received infusions of saline and different doses of morphine (filled symbols), or saline and different doses of dynorphin[1-13] (open symbols). The mean number of center-crossings after pretreatment with naloxone (NAL) are also shown for each group. $n = 8$ for each group.

LOCOMOTOR ACTIVITY



group. Among the MOR subjects, there were more center-crossings after 1 and 10 nmoles morphine than after vehicle, and more after 10 nmoles than after 0.1 nmoles (p 's $< .05$). After the 30 nmole infusion of morphine, activity was decreased relative to all other morphine doses (p 's $< .05$), although the comparison with the vehicle injection was not statistically significant ($p > .05$). None of the dynorphin[1-13] doses differed from each other, or from the vehicle infusion (p 's $> .05$).

Naloxone challenge. The total amount of female-directed behaviors after naloxone was compared with the same drug dose without naloxone pretreatment using a t -test for repeated measures (two-tailed). Naloxone significantly decreased the amount of female-directed behaviors seen after 10 nmoles morphine, $t(7) = 2.67$, $p < .05$; the means were: 10 nmoles morphine 27.9, and 10 nmoles morphine plus naloxone 22.7 percent of observations. Naloxone also significantly decreased the amount of female-directed behaviors displayed after 0.3 pmoles dynorphin[1-13], $t(7) = 3.41$, $p < .05$; the means were: 0.3 pmoles dynorphin[1-13] 36.9, and 0.3 pmoles dynorphin[1-13] plus naloxone 27.3 percent of observations.

Naloxone blocked the facilitation of mounting previously seen after both morphine and dynorphin[1-13]; only one animal in the DYN group mounted if the 0.3 pmole infusion of dynorphin[1-13] was preceded by naloxone, whereas seven had mounted after the same dose of dynorphin[1-13] alone; after receiving 10 nmoles of morphine six animals had mounted, but none mounted if the same dose of morphine was preceded by naloxone. The naloxone-blockade of opioid-induced mounting was statistically significant for each group: MOR, $\chi^2(1) = 8.4$, $p < .01$, and DYN $\chi^2(1) = 9.0$, $p < .01$.

Pretreatment with naloxone also reduced the amount of locomotor activity otherwise seen after infusion of 10 nmoles of morphine, $t(7) = 2.81$, $p < .05$; the means were: morphine 46.4, and morphine plus naloxone 25.8 center-crossings. Naloxone did not affect activity after 0.3 pmoles of dynorphin[1-13] ($p > .1$).

Substantia nigra infusions. The amount of female-directed behavior displayed after infusions of vehicle, morphine or dynorphin[1-13] into the substantia nigra was analysed using a two-factor repeated measures analysis of variance (drug x time). The analysis of variance yielded a significant time effect, $F(2,12) = 64.29$, $p < .01$, indicating that female-directed behaviors became less frequent during the 30 min test. Neither the drug effect nor the drug x time interaction was statistically significant ($p's > .1$). Virtually no copulation occurred after substantia nigra infusions; one animal mounted after a vehicle infusion, and no other copulatory behaviors were observed. The number of center-crossings did not differ between the different infusions into the substantia nigra ($p > .1$).

Discussion

In this experiment, both morphine and dynorphin[1-13] infused into the VTA produced a dose-dependent increase in the number of animals that mounted, and dynorphin[1-13] also increased the display of female-directed behaviors. The effects of VTA infusions were receptor mediated; naloxone antagonized the effects of both morphine and dynorphin[1-13]. The activation of opioid receptors in the VTA, therefore, increased the display of male sexual behavior.

The likelihood of mounting increased with increasing doses of both morphine and dynorphin[1-13], and this facilitation was blocked by pretreatment with naloxone. Unfortunately, too few animals mounted after control or low dose infusions to allow comparison of mount latencies, a measure of sexual arousal. The increased likelihood of mounting, however, may be considered to vary with sexual motivation. Intromissions and the ejaculatory pattern were not affected, except for a small effect of dynorphin[1-13] on the ejaculatory pattern; the performance of copulation was relatively unaffected by opioid infusions into the area of the VTA.

Although both influenced mounting, there were important differences between the

effects of morphine and dynorphin[1-13]. Morphine produced a dose-dependent increase in locomotor activity, but not female-directed behaviors, whereas dynorphin[1-13] produced a dose-dependent increase in female-directed behaviors, but not locomotor activity. Dynorphin infusions resulted in a large, dose-dependent increase in the display of female-directed behaviors; at the most effective dose of dynorphin[1-13] (0.3 pmol) males maintained intense attention to the female throughout the 30 min test; except for the persistence of female-directed behaviors, however, the behavior of these animals appeared normal.

Dynorphin[1-13] did not significantly affect locomotor activity, consistent with the report that dynorphin injected unilaterally into the VTA does not elicit contraversive circling (Jenck, Bozarth, & Wise, submitted). There are reports that intraventricular injections of dynorphin suppress locomotor activity and cause motor disturbances, but these effects have been obtained with large doses of dynorphin (7.5 to 20 μ g), and cannot be antagonized by even large (20 mg/kg) injections of naloxone (Friedman, Jan, Chang, Lee, & Loh, 1981; Tilson, McLamb, & Hong, 1982; Walker, Katz, & Akil, 1980; Walker, Moises, Coy, Baldrighi, & Akil, 1982), indicating only that large physiological doses of dynorphin can produce non-receptor mediated effects. There is a report that systemic administration of the kappa agonists ketazocine and tifluadom has a biphasic effect on locomotor activity, first decreasing and then increasing locomotion (Beck & Krieglstein, 1986), but little is known about the effects of dynorphin over a broad range of doses. It should be noted that extremely small amounts of dynorphin were required to facilitate sexual behaviors, consistent with studies on dynorphin-induced feeding (Hamilton & Bozarth, 1986, 1987); the potency of dynorphin was apparent even when it was initially sequenced (Goldstein et al., 1979). The number of animals mounting and the frequency of female-directed behaviors decreased at the highest dose of dynorphin, suggesting a suppression of behavior at

higher doses; future research with dynorphin must, therefore, examine a very different dose range than typically used (fmole to pmole rather than nmole) to avoid potentially sedating overdoses and non-receptor mediated effects.

It was surprising, in view of the effect of morphine on mounting, that morphine infusions had little effect on female-directed behaviors up to the dose which produced a general suppression of behavior. It is interesting to note, therefore, that after both the vehicle and low dose infusions, the MOR group tended to display higher levels of female-directed behaviors than the DYN group after corresponding infusions. In this experiment, the order of infusions was randomized so that animals received the vehicle infusions on their first trial, or after two or four injections of morphine; MOR subjects that received the saline injection on the fifth trial displayed more female-directed behaviors than subjects that received the saline infusions on their first or third trial. The tendency for the morphine-treated subjects to display higher levels of female-directed behaviors after saline or low dose infusions, therefore, may have been due to sensitization and/or conditioning. It is known that repeated administration of morphine into the VTA leads to sensitization of the drug effects and to conditioned activation in the drug associated environment (Vezina & Stewart, 1984). Consistent with this interpretation was the finding that naloxone decreased significantly the amount of female-directed behaviors otherwise observed after infusions of 10 nmoles of morphine. Furthermore, in a pilot study using gonadally intact, sexually naive subjects (Mitchell, Stewart, & Vezina, 1987, unpublished observations), 10 nmoles of morphine injected into the VTA increased female-directed behaviors significantly compared to a vehicle injected control group.

Morphine infusions into the VTA resulted in a dose-dependent increase in locomotion, as has been reported previously (Joyce & Iversen, 1979; Kalivas et al., 1983; Vezina & Stewart, 1984). It is interesting to note that the MOR group was

consistently more active than the DYN group, even after infusions of drug vehicle. An explanation of this difference in locomotor activity between the two drug groups may be in the fact that previous infusions of morphine, but not dynorphin, lead to sensitization and/or conditioning of activity. Pairing a specific environment with intra-VTA morphine has been demonstrated to lead to conditioned increases in activity (Vezina & Stewart, 1984), and recent observations in this laboratory suggest that repeated infusions of dynorphin do not.

The differential effects of morphine and dynorphin on activity and sexual behaviors may reflect different affinities of these ligands for different opioid receptors. The existence of multiple opioid receptors is well documented (Childers, Creese, Snowman, & Snyder, 1979; Lord, Waterfield, Hughes, & Kosterlitz, 1977; Martin, Eades, Thompson, Huppler, & Gilbert, 1976). Morphine binds preferentially at the mu-binding site, whereas dynorphin binds preferentially at the kappa site (Paterson, Robson, & Kosterlitz, 1984), and is thought to be the endogenous kappa-ligand (Chavkin, James, & Goldstein, 1982). Although both mu and kappa agonists are analgesics (Iwamoto, 1981), and when infused into the VTA both elicit feeding (Hamilton & Bozarth, 1986, 1987) and facilitate stimulation-induced feeding (Wise, Jenck, Gratton, & Quirion, 1986), differences between them have been reported. Infusion of the prototypical mu agonist, morphine, into the VTA elicits contraversive circling and facilitates brain stimulation reward, but the kappa agonist U-50,488H does not (Jenck, Bozarth, & Wise, submitted; Jenck, Gratton, & Wise, 1987). Interestingly, morphine and U-50,488H have been reported to have opposite effects on substantia nigra DA cell firing (Walker, Thompson, Frascella, & Friederich, 1987). Furthermore, the effects of morphine on DA metabolism are antagonized by kappa agonists; systemic morphine increases striatal, mesolimbic and mesocortical DA metabolism, and the synthetic kappa agonists ethylketocyclazone, MR-2034, U-50,488H, and tifluadom all block

morphine-induced increases in striatal and mesocortical DA metabolism, while having little effect on DA metabolism in these areas when administered alone (Kim, Iyengar, & Wood, 1987; Wood, 1983; Wood, Stotland, Richard, & Thakur, 1982).

Most of the studies that have attempted to differentiate the effects of mu and kappa receptor activation have used the synthetic kappa agonists ethylketocyclazone, MR-2034, U-50,488H, or tifluadom. It is somewhat unsettling, therefore, that synthetic kappa agonists have been found to antagonize mu agonist effects in the rat vas deferens, tissue reported to lack kappa receptors (Gillan, Kosterlitz, & Magnan, 1981). Furthermore, it has been reported that the ability of these kappa agonists to displace mu agonist binding, in vitro, correlates with their ability to antagonize morphine effects on nigrostriatal DA metabolism (Wood, 1981). Wood has suggested that at least some of the effects of synthetic kappa agonists are mediated by antagonism at the mu-2 receptor, rather than agonism at the kappa receptor, and that mu-2 antagonism is inherent to the synthetic kappa agonists (Kim et al., 1987; Pasternak & Wood, 1986; Wood, 1983; Wood et al., 1982). Indeed, the putative endogenous kappa agonist, dynorphin, and the synthetic kappa agonists may have different effects. Unlike the synthetic kappa agonists, dynorphin does not antagonize the action of morphine on nigrostriatal DA metabolism (Wood, Kim, Cosi, & Iyengar, in press; cited in Kim et al., 1987), it has no effect on contractions of the rat vas deferens ($IC_{50} > 10,000$ nmoles; Corbett, Paterson, McKnight, Magnan, & Kosterlitz, 1982), and it elicits contraversive circling when injected into the substantia nigra (Herrera-Marschitz, Hokfelt, Ungerstedt, Terenius, & Goldstein, 1985). The effects of dynorphin could be mediated by agonist activity at the kappa receptor without concomitant mu-2 antagonism, or by combined agonist activity at both kappa and mu sites; current data cannot discriminate between these, and other, possibilities.

Morphine is well known to increase DA metabolism in a number of different

terminal fields, but no conclusion about the involvement of DA in mediating the effects of dynorphin can be drawn from the currently available data. Nonetheless, differences found between the effects of morphine and dynorphin in the present experiment and those discussed above, suggest that the neural elements in the VTA that mediate sexual behavior and feeding differ from those that mediate locomotion and brain stimulation reward.

In the present experiment, it was found that infusion of both the μ opiate receptor agonist, morphine, and the endogenous opioid peptide, dynorphin, into the VTA increased mounting by male rats, and that dynorphin also significantly increased the display of female-directed behaviors. The opioid effects were antagonized by naloxone pretreatment, demonstrating pharmacological specificity, and were not obtained by infusions into the substantia nigra, demonstrating anatomical specificity. Although questions concerning opioid receptor type and dopaminergic involvement remain, these results demonstrated that activation of opioid receptors in the region of the VTA elicited sexual behavior.

Enhancement of Sexual Behavior in an Environment Previously Associated with Morphine

Systemic injections of moderate to high doses of morphine have a biphasic effect on locomotor activity, resulting first in decreased, and then in increased activity (Babbini & Davis, 1972; Sloan, Brooks, Eisenman, & Martin, 1962; Vasko & Domino, 1978). With repeated injections, however, only an increase in locomotor activity is found (Babbini & Davis, 1972; Vasko & Domino, 1978), and animals show a progressively increased, or sensitized, response (Hinson & Siegel, 1983; Mucha, Volkovskis, & Kalant, 1981). If care is taken to explicitly pair drug delivery with a distinctive environment, sensitization to the locomotor effects of morphine is manifested only in that environment (Hinson & Siegel, 1983; Mucha et al., 1981). Furthermore, even after injections of saline, increased locomotor activity is found if the test environment has previously been paired with morphine administration (Hinson & Siegel, 1983; Mucha et al., 1981).

Injections of morphine directly into the A10 cell group result in increased locomotor activity, and repeated administration leads to sensitization to the effects of morphine (Joyce & Iversen, 1979; Vezina & Stewart, 1984). Vezina and Stewart (1984) demonstrated that, if injections of morphine into the area of the VTA are paired with a distinctive environment, sensitization is environment-specific. Furthermore, even after injections of saline, animals that have previously received repeated intra-VTA injections of morphine in the test environment are more active than morphine-naive animals and animals that had received morphine in a different environment (Vezina & Stewart, 1984). These findings suggest that the sensitized and conditioned effects of morphine on locomotor activity, found after repeated systemic injections, are mediated by the mesolimbic DA system. Consistent with this view is the fact that systemically and

locally administered opiates, as mentioned earlier, affect the firing frequency of a sub-population of dopaminergic cells in the VTA (Gysling & Wang, 1983; Matthews & German, 1984), and lead to an increase in DA metabolism (Kalivas et al., 1983).

Furthermore, conditioned changes in DA turnover have been found in animals that had previously received repeated morphine administration (see Schiff, 1982).

If the mesolimbic DA system is involved in the mediation of sexual arousal, then conditioning thought to involve activity within this system might be expected to increase the display of sexual behaviors. In Experiment 4, it was found that the frequency of female-directed behaviors after saline infusions was greater if there had been repeated administration of morphine in the test environment, suggesting a conditioned effect of morphine on male sexual arousal. The following experiments were conducted to investigate whether male sexual behavior would be facilitated in an environment previously associated with injections of morphine.

EXPERIMENT 5

In Experiment 5, the effect on sexual behavior of testing the animal in an environment previously associated with repeated injections of morphine was assessed. In this experiment, one can consider the environment in which morphine was administered to be the conditioning stimulus (CS), and morphine the unconditioned stimulus (US). Mucha et al. (1981) and Hinson and Siegel (1983) have shown that after repeated systemic injections of morphine in a particular environment, the conditioned response (CR) to morphine is increased locomotor activity. Vezina and Stewart (1984) have shown that when locomotor activity is the unconditioned response directly elicited by morphine administration to the VTA, the CR is increased locomotor activity. Thus, if the effects of morphine on the mesolimbic DA system and the resulting behavioral activation can be thought of as constituting the unconditioned response, it would be expected that the CR would mimic some aspects of this response.

Method

Subjects. Twenty-seven male Long-Evans hooded rats were used. Subjects had been screened for sexual activity and weighed 250-275 g at the start of the experiment. Subjects were randomly assigned to one of three groups.

Procedure. The experiment included an initial conditioning phase followed by tests for sexual behavior. During the conditioning phase of the experiment, injections of 10 mg/kg (1 ml/kg) of morphine were given to one group of animals (Cond) immediately before they were placed in the mating arenas, and to another group (Pseudo) in the animal colony. Conversely, the Cond group received saline injections in the animal colony, and the Pseudo group received saline injections prior to placement in the mating arenas. The third group (Control) received injections of saline in both environments. In this and the subsequent experiment, animals were placed in the mating arenas, with no female present, for 1 h every other day, and received their animal colony injections on

the intervening days. In this, and the subsequent experiment, a total of four conditioning trials and four animal colony injections were administered.

Two days after the last injection of morphine, each animal received an injection of saline, was placed in a mating arena, and 5 min later a sexually receptive female was introduced. The test for sexual behavior lasted 30 min. Two weeks later, a second test for sexual behavior was given.

In addition to the measures described earlier, three other measures were taken. These included:

The postejaculatory interval: the time between ejaculation and the first intromission of the next copulatory series.

The interintromission interval: the average number of intromissions per min to the first ejaculation.

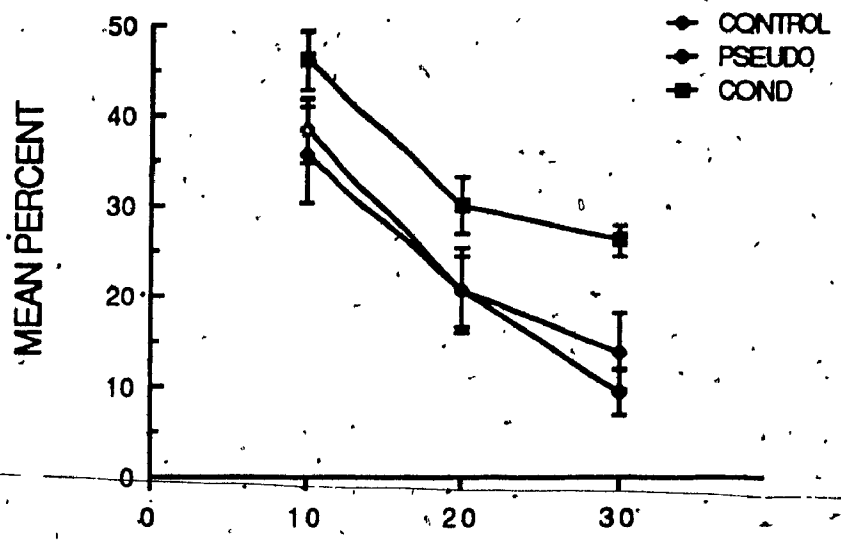
The intromission ratio: the ratio of intromissions to mounts plus intromissions.

Results

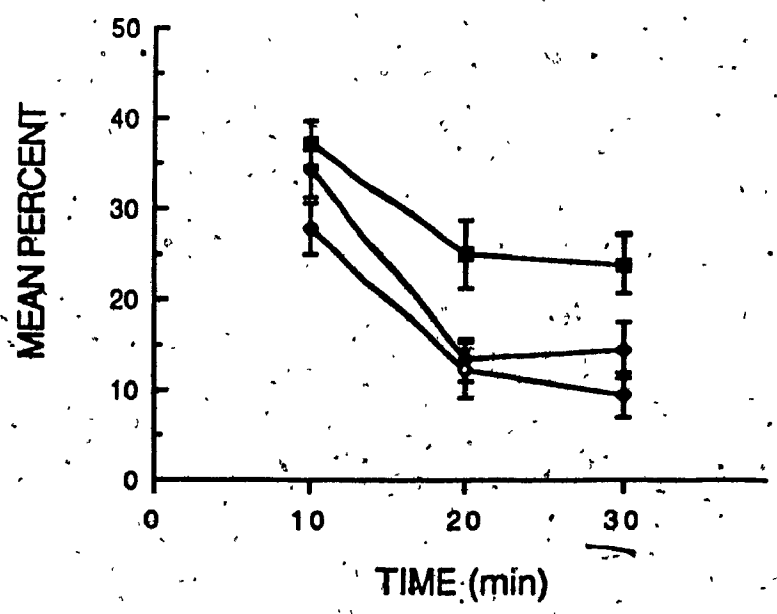
The mean percent of observations in which female-directed behaviors were observed for the three groups on both tests is shown in Figure 22. An analysis of variance (group x time x test) of these scores yielded a significant group effect, $F(2,24) = 13.85$, $p < .001$. Overall, the Cond group differed from both the Control and Pseudo groups (p 's $< .01$), whereas the Control and Pseudo groups did not differ from each other ($p > .1$). As can be seen from Figure 22, the differences between the Cond group and the Control and Pseudo groups were greater after the initial 10 min during both tests. Post hoc comparisons of scores from the first test showed that by 30 min the Cond group displayed significantly more female-directed behaviors than either the Control ($p < .01$) or the Pseudo ($p < .05$) groups. During the second test, the Cond group displayed significantly more female-directed behaviors than either the Control and Pseudo groups at 20 min (p 's $< .05$) and more than the Control group at 30 min ($p < .01$). Overall,

Figure 22. Mean (± 1 S.E.M.) percent of observations during which female-directed behaviors were observed as a function of time for groups with previous experience with morphine in the mating arenas (COND, filled squares), or in the animal colony (PSEUDO, filled circles), or that had received injections of saline in both the mating arenas and animal colony (CONTROL, open circles). $n = 9$ for each group.

TEST 1



TEST 2



female-directed behaviors were less frequent on the second test than on the first, $F(1,24) = 4.48$, $p < .05$, but the differences between the first and second test for individual groups were not significant (p 's $> .1$). Finally, the frequency of female-directed behaviors decreased across time in all groups, $F(2,28) = 82.49$, $p < .001$.

The measures of copulation are shown in Table 3. Because not all animals copulated on both tests, each test was analysed separately. The analysis of variance of mount latencies yielded significant group effects for both the first, $F(2,20) = 5.25$, $p < .05$, and second test, $F(2,24) = 4.30$, $p < .05$, of sexual behavior. As can be seen from Figure 23, the Cond group initiated mounting more quickly than either the Control or Pseudo groups during both tests (p 's $< .05$). The mean intromission latency of the Cond group in the first test was considerably shorter than either of the other two groups, but due to highly variable scores, the group effect was not significant ($p > .1$). During the second test, however, there was a significant group effect, $F(2,23) = 3.64$, $p < .05$, and post hoc comparisons showed that the intromission latency of the Cond group was significantly shorter than that of the Pseudo group ($p < .05$).

The interintromission and postejaculatory intervals both appeared to be shorter in the Cond group, especially during the first test, but the differences were small and not statistically significant (p 's $> .1$). Groups did not differ significantly on any other measure in either test (p 's $> .1$), nor did groups differ significantly in the number of animals that mounted, intromitted, or ejaculated during each test (p 's $> .05$).

Discussion

Female-directed behaviors were increased, and mount and intromission latencies shortened in the Cond group animals, suggesting that previous repeated injections of morphine in the test environment led to increased sexual arousability when animals were subsequently presented with a sexually receptive female in that environment.

Furthermore, the Control and Pseudo groups did not differ from each other on any

Table 3. Mean behavioral scores of subjects after repeated injections of morphine in the mating arena (Cond), or the animal colony (Pseudo), or injections of saline in both environments (Control). Columns represent the number of animals in each group that mounted (M), intromitted (I) or ejaculated (E), and the mean mount latency (ML), intromission latency (IL), ejaculation latency (EL), postejaculatory interval (PEI), number of mounts (NM), number of intromissions (NI), interintromission interval (III), and the intromission ratio (I ratio; NI/NI+NM). Mount, intromission, and ejaculation latencies, and the postejaculatory interval are in seconds. Interintromission interval is the number of intromissions/min. n=9 for all groups.

Group	M	I	E	ML	IL	EL	PEI	NM	NI	III	I ratio
TEST 1											
Control	7	6	5	140.6	183.5	475.2	322.2	23.3	14.7	1.43	.351
Pseudo	8	6	5	121.2	236.0	558.1	322.2	19.1	16.2	1.18	.410
Cond	8	7	6	41.6 ^{ab}	84.7	766.2	309.5	24.3	14.6	0.96	.347
TEST 2											
Control	9	9	5	47.7	76.7	639.2	307.2	30.2	18.9	1.26	.382
Pseudo	9	8	6	54.6	195.5	634.1	325.5	35.8	17.4	1.27	.382
Cond	9	9	8	17.0 ^{ab}	47.2 ^b	550.6	276.9	40.2	19.0	1.32	.338

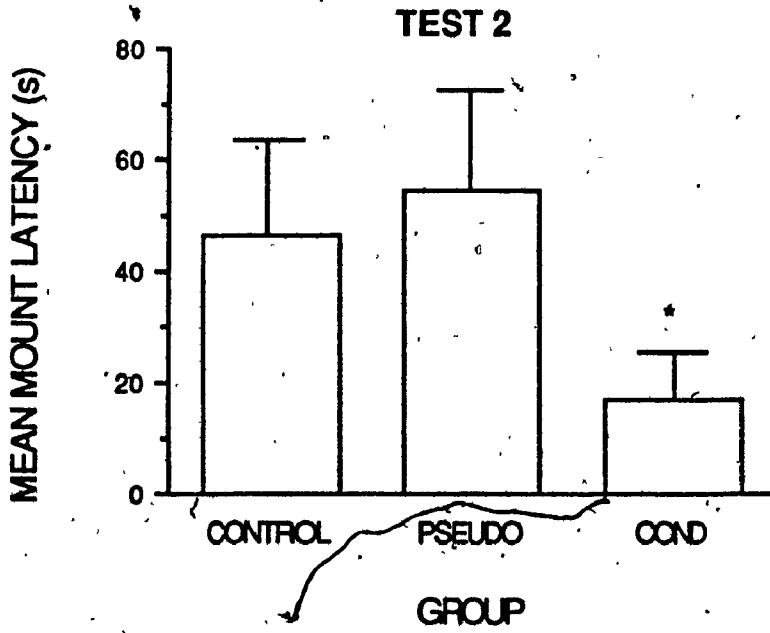
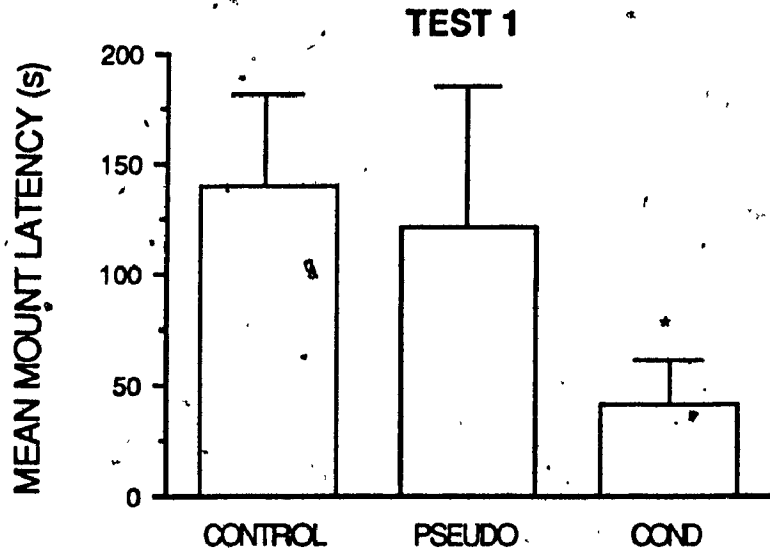
a: significantly different from Control, $p < .05$.

b: significantly different from Pseudo, $p < .05$.

Figure 23. Mean (+ 1 S.E.M.) mount latency for groups with previous experience with morphine in the mating arenas (COND), or in the animal colony (PSEUDO), or that had received injections of saline in both the mating arenas and animal colony (CONTROL).

* significantly different from Control and Pseudo groups ($p < .05$). $n = 9$ for each group.

MOUNT LATENCY



measure, indicating that the increased sexual arousal in the Cond group was due to conditioning to the stimuli associated with morphine administration, and not to the effects of previous morphine administration, per se.

Opiate agonists and antagonists are known to have a powerful effect on the hypothalamic-pituitary-gonadal axis; opiates reduce serum LH and, subsequently, T concentrations; naloxone increases LH concentrations (Bruni, van Vugt, Marshall, & Meites, 1977; Cicero & Badger, 1977; Cicero, Meyer, Gabriel, Bell, & Wilcox, 1980; McConnell, Baum, & Badger, 1981; Mendelson, Ellingboe, Kuehne, & Mello, 1980; Mirin, Meyer, Mendelson, & Ellingboe, 1980). Consequently, chronic opiate administration decreases the functioning of the accessory sex organs in both humans (Cicero, Bell, Wiest, Allison, Polakoski, & Robins, 1975; Mendelson, 1975), and rats (Cicero, Meyer, Wiest, Olney, & Bell, 1975; Cicero, Meyer, Bell, & Koch, 1976). In the present experiment, however, animals that had received injections of morphine in the animal colony and morphine-naive animals did not differ on any measure of copulation, suggesting that morphine effects on circulating hormone titers did not influence later sexual behavior. The absence of any behavioral evidence of morphine-induced suppression of hormone secretion and sexual functioning may have been due to the dose and injection schedule used. After administration of 20 mg/kg of morphine, twice the amount used in the present experiment, serum LH and T concentrations return to basal levels within 7 hours (Cicero & Badger, 1977). Therefore, it is unlikely that, in the present experiment, morphine-treated animals had LH or T concentrations suppressed chronically during the conditioning phase of the experiment.

The interintromission and postejaculatory intervals have been considered to be indices of the state of the AM, but, as discussed earlier, the utility of these measures for measuring sexual arousal has been questioned. The present experiment found that the interintromission and postejaculatory intervals were not affected by previous injections

of morphine in the test environment, suggesting that they are less sensitive measures of sexual arousal than either female-directed behaviors or initiation latencies.

These results suggest that an environment previously paired with morphine was able to facilitate sexual arousal. As discussed earlier, the mesolimbic DA system has been implicated in the mediation of morphine-induced behavioral activation, and recall that castration was found to decrease DA content and metabolism in the NAS (Experiments 1-3; Alderson & Baum, 1981). It was decided, therefore, to investigate whether an environment previously paired with morphine might facilitate some aspects of sexual behavior in castrated male rats.

EXPERIMENT 6

In Experiment 6, conditioning trials were timed to take place during the period after castration when DA content and metabolism would be declining. The first test for sexual behaviors was given at a time when DA concentrations and metabolism had been found previously to be significantly reduced, 28 days after castration (see Table 1). Because the pseudoconditioning and the saline control groups in Experiment 5 did not differ from each other on any measure, only pseudoconditioning control groups were included.

Method

Subjects. Thirty-six male Long-Evans rats, screened for sexual behavior, were randomly assigned to one of four groups. Two groups of animals were bilaterally castrated and two sham-castrated, as described in Experiment 1.

Procedure. Beginning 20 days after surgery, one group of castrates (Cast-Cond) and one-gonadally intact group (Intact-Cond) received injections of 10 mg/kg morphine immediately before being placed in the empty mating arenas, and injections of saline in the animal colony. The other groups of castrates (Cast-Pseudo) and sham-castrates (Intact-Pseudo) received 10 mg/kg injections of morphine in the animal colony and saline injections prior to being placed in the mating arenas. Conditioning trials lasted 1 h and were given every other day; animal colony injections were administered on the intervening days. Two and 16 days after the last conditioning trial (28 and 42 days after surgery) animals received injections of saline and were given 30 min tests for sexual behavior in the mating arenas.

Results

An overall analysis of variance (conditioning x castration x time x test) of the number of observation periods during which female-directed behaviors occurred yielded a significant effect of conditioning, $F(1,32) = 65.72$, $p < .001$, and, more importantly, significant conditioning x castration, $F(1,32) = 7.08$, $p < .05$, and conditioning x time,

$F(2,64) = 12.99, p < .001$, interactions. Figure 24 illustrates the conditioning x castration interaction. As can be seen, animals in the Cond groups increased female-directed behaviors whether they were intact or castrated; female-directed behaviors were least frequent in the Cast-Pseudo group. Both Cond groups differed significantly from their respective Pseudo groups (p 's $< .01$), but the Intact- and Cast-Cond groups did not differ from each other ($p > .05$). Although the Cast-Pseudo group appeared to display less female-directed behavior than the Intact-Pseudo group, this comparison was not statistically significant.

Figure 25 presents the mean percent of observations during which female-directed behaviors occurred for each group at three different times during each test. During the first test, the Cond groups tended to display more frequent female-directed behaviors than the Pseudo groups after the first 10 min, but at 20 min only the difference between the Cast-Cond and Cast-Pseudo groups was significant ($p < .05$). At 30 min, however, both Cond groups differed from their respective controls (p 's $< .05$). These differences were even more pronounced during the second test; at both 20 and at 30 min, both Cond groups differed from their Pseudo control group (p 's $< .05$). At 30 min in the second test, the difference between the Intact-Pseudo and Cast-Pseudo groups was significant ($p < .05$).

The proportion of animals in each group that mounted, intromitted, or displayed the ejaculatory pattern during each test is shown in Figure 26. Groups differed significantly in the number of animals that mounted, $\chi^2(3) = 26.19, p < .001$, intromitted, $\chi^2(3) = 23.84, p < .001$, or showed the ejaculatory pattern, $\chi^2(3) = 11.88, p < .01$, during the first test. Groups also differed on these measures during the second test; mount, $\chi^2(3) = 19.06, p < .001$, intromissions, $\chi^2(3) = 19.54, p < .001$, and the ejaculatory pattern, $\chi^2(3) = 24.0, p < .001$. The group differences were attributable to

Figure 24. Mean (+ 1 S.E.M.) percent of observations during which female-directed behaviors were observed collapsed across time and test, for Experiment 6. Groups (n=9) were gonadally intact (INTACT; filled symbols) or had been castrated for 4 weeks at the time of the first test (CAST; open symbols), and had previously received morphine in the mating areans (COND; squares) or the animal colony (PSEUDO; circles).

* significantly different from the corresponding Pseudo group ($p < .05$).

FEMALE-DIRECTED BEHAVIORS

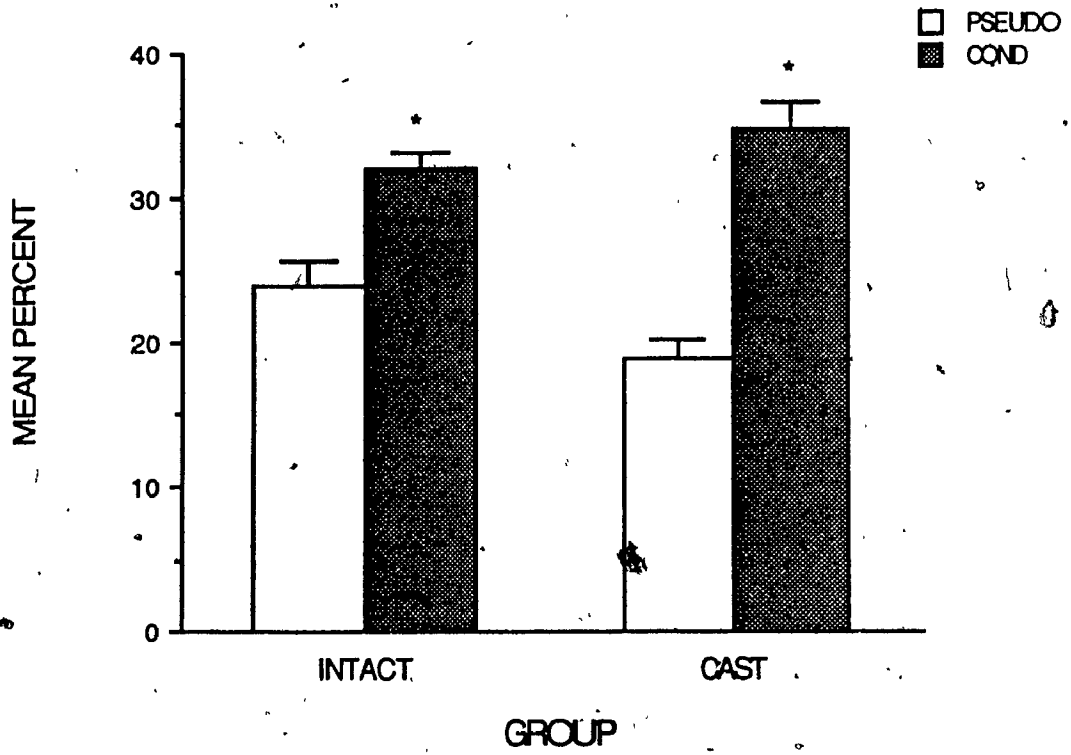
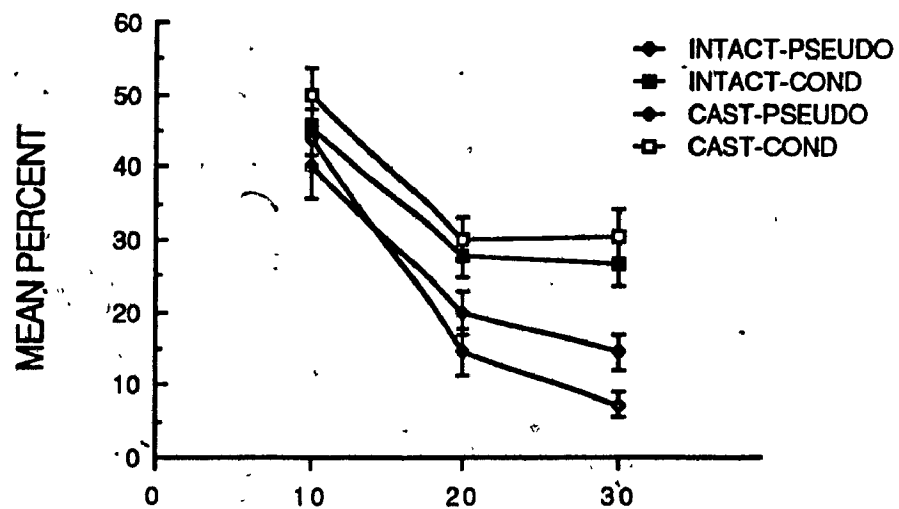


Figure 25. Mean (± 1 S.E.M.) percent of observations during which female-directed behaviors were observed during each test as a function of time. Groups (n=9) were gonadally intact (INTACT; filled symbols) or had been castrated for 4 weeks at the time of the first test (CAST; open symbols), and had previously received morphine in the mating areas (COND; squares) or the animal colony (PSEUDO; circles).

FEMALE-DIRECTED BEHAVIORS

TEST 1



TEST 2

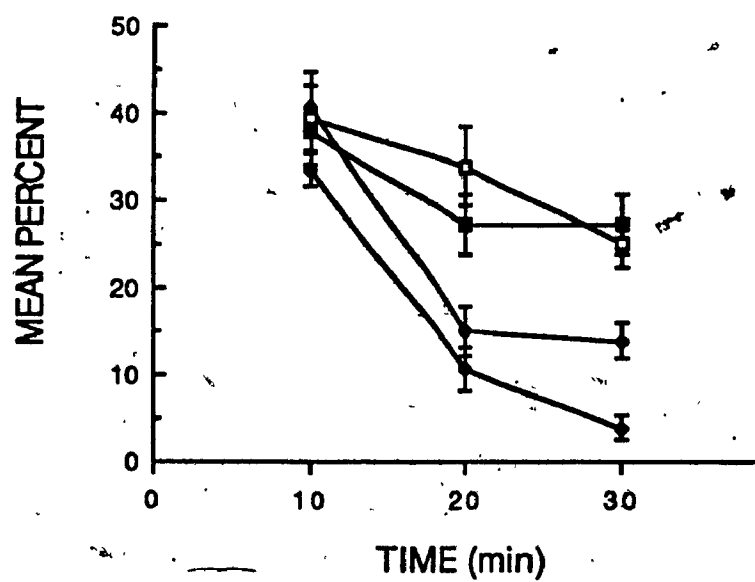
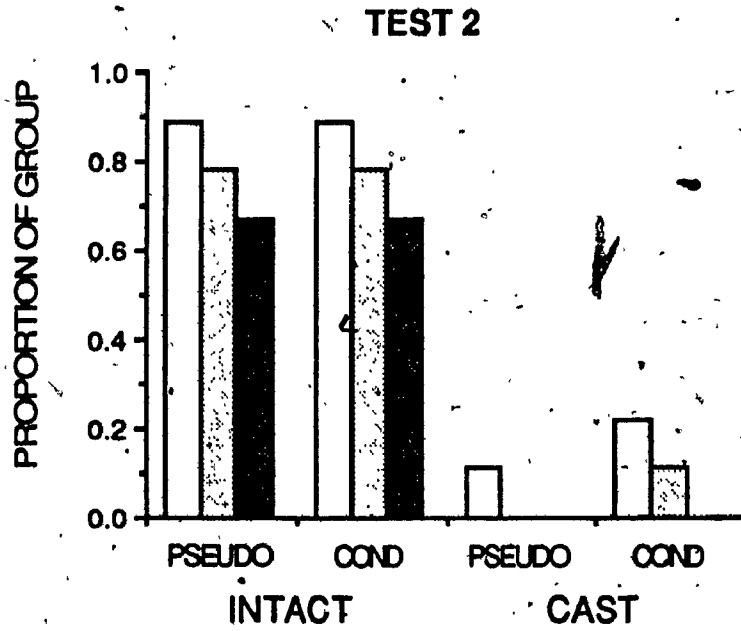
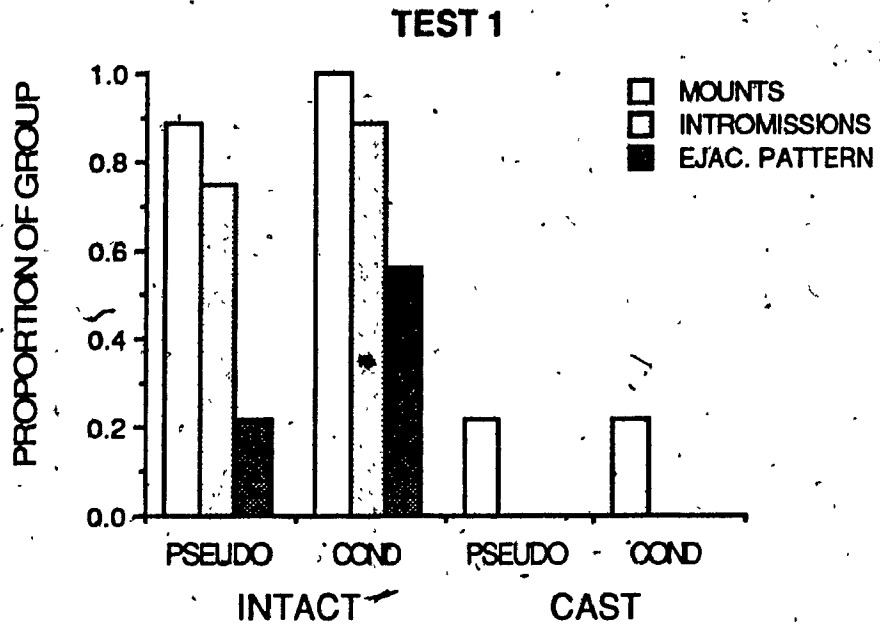


Figure 26. Proportion of animals in each group in Experiment 6 that mounted, intromitted and displayed the ejaculatory pattern during the first (upper panel) and second (lower panel) test for sexual behaviors. Groups (n=9) were gonadally intact (INTACT) or had been castrated for 4 weeks at the time of the first test (CAST), and had previously received morphine in the mating areans (COND) or the animal colony (PSEUDO).



the effects of castration, except that, perhaps surprisingly, previous experience with morphine in the mating arena increased the proportion of Intact animals that ejaculated during the first test.

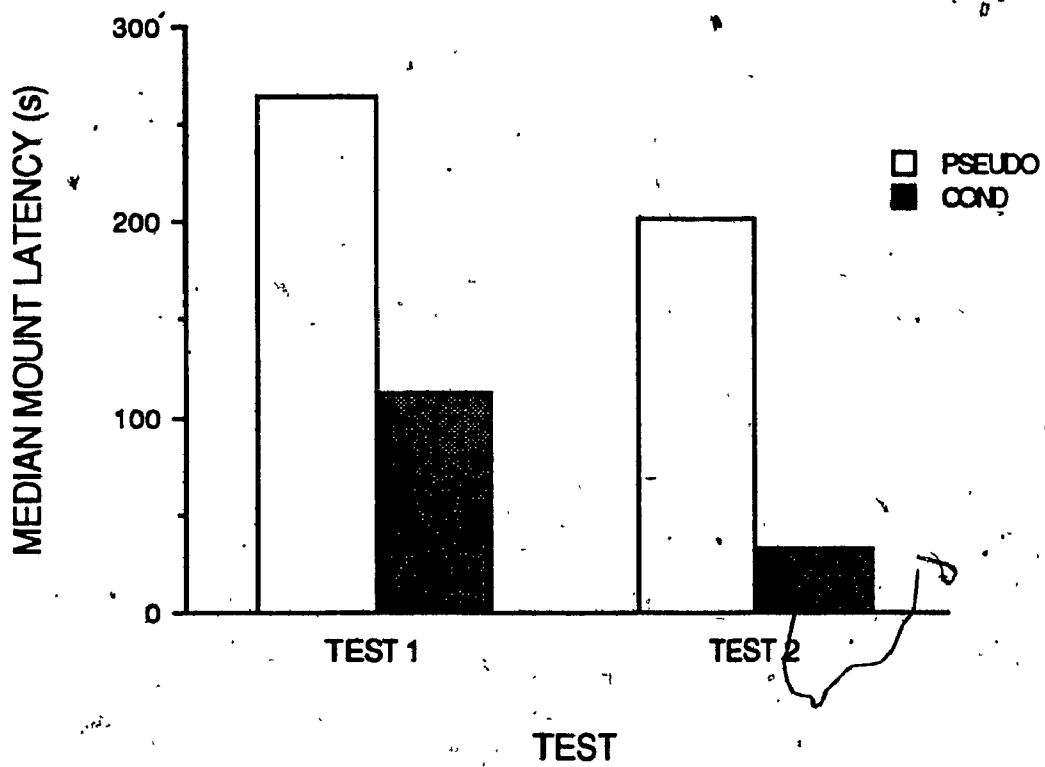
Because so few castrated animals copulated, analyses of copulation measures were done for the Intact groups only. Because significant heterogeneity of variance was found for the mount latency scores ($p < .05$, Cochran's test for homogeneity of variance), these scores were analysed using Mann-Whitney U tests. Figure 27 shows the median mount latency for each gonadally intact group in each test, and it can be seen that the Intact-Cond group initiated mounting more quickly than the Pseudo group. The statistical analysis found that, although the difference was not significant for the first test ($.05 < p < .1$), it was for the second test, $U(8,8) = 58, p < .05$. None of the other comparisons made between the Intact-Cond and Intact-Pseudo groups were significant (t-tests for independent groups, two-tailed).

Discussion

Among intact animals tested for sexual behavior in the conditioning environment (Intact-Cond), female-directed behaviors were more frequent and mount latency was shortened, compared to the Intact-Pseudo group. These findings replicate those of Experiment 5. In the present experiment, it was also found that castrated animals tested for sexual behavior in the conditioning environment showed higher frequencies of female-directed behaviors than castrated pseudoconditioned animals. The persistence of female-directed behaviors among Cast-Cond animals, especially during the second test six weeks after castration, was surprising. These animals continued to pursue, sniff and climb on the female despite the fact that they engaged in virtually no copulation. These results suggest that conditioning thought to involve activity within the mesolimbic DA system can facilitate sexual arousal among castrated male rats.

Figure 27. Median mount latency during the first and second test of sexual behaviors for gonadally intact subjects that had previously received morphine in the mating arenas (COND, shaded columns), or the animal colony (PSEUDO, open columns).

MOUNT LATENCY



General Discussion

In Experiments 5 and 6, animals that received injections of morphine paired with a distinctive environment displayed more frequent female-directed behaviors, and, if they were gonadally intact, reduced initiation latencies when later tested for sexual behavior in that environment. The performance of copulation was not affected by previous repeated injections of morphine.

These findings may appear to conflict with those of previous studies of opiates and male sexual behavior. Despite the fact that heroin addicts often describe the immediate postinjection sensations in sexual terms (Mirin et al., 1980), male heroin addicts and patients on methadone maintenance programs commonly report decreased sexual desire or interest, impotence, and delayed ejaculation (Cushman & Dole, 1973; Mirin et al., 1980). Systemic injections of morphine to gonadally intact or castrated, T-treated male rats has been reported to inhibit sexual behavior (McIntosh, Vallano, & Barfield, 1980; Mumford & Kumar, 1979).

When injected intraventricularly, morphine has been reported to decrease the rate of intromitting and reduce the ratio of intromissions to mounts, indicating less efficient copulation, and it was suggested that these changes were primarily attributable to an increase in mounting (Band et al., 1986). Intraventricular injections of b-endorphin, however, have been reported to reduce the number of males that mount (Meyerson & Terenius, 1977), and intraventricular injections of the enkephalin analogue, DALA, lengthen initiation latencies (Gessa, Paglietti, & Pellegrini Quarantotti, 1979; Pellegrini Quarantotti, Corda, Paglietti, Biggio, & Gessa, 1978). Intraventricular injections, although perhaps more specific than systemic injections, would be expected to affect any opioid sensitive site with access to ventricular circulation, and would affect, therefore, a number of systems in addition to the mesolimbic DA system. When applied directly to the region of the mPOA, morphine has been reported to decrease the number of

ejaculations and increase the length of the postejaculatory interval (Band et al., 1986).

In summary, most research has found that systemic and intraventricular injections of opiates and opioid agonists inhibit sexual behavior. Few studies using intracerebral administration have been performed, but the available data suggests an inhibitory effect of mPOA infusions of morphine on male sexual behavior.

Opiate receptor antagonists, on the other hand, have been reported to decrease the number of intromissions to ejaculation and shorten ejaculation latency (McConnell et al., 1981; McIntosh et al., 1980; Myers & Baum, 1979, 1980; Pellegrini Quarantotti, Paglietti, Bonanni, & Gessa, 1979), suggesting facilitation of the performance of copulation. There are, however, reports that naloxone does not affect copulation among sexually experienced, vigorous copulators (Gess et al., 1979; Miller & Baum, 1987; Pellegrini Quarantotti, Corda, Paglietti, Biggio, & Gessa, 1978), but that it does increase the number of persistent noncopulators that mount, intromit, and ejaculate (Gessa et al., 1979), suggesting facilitation of sexual arousal. In apparent contrast to these findings, naloxone has also been reported to lengthen the postejaculatory interval (McConnell et al., 1981; Myers & Baum, 1979, 1980; Sachs, Valcourt, & Flagg, 1981). McConnell et al. (1981), however, found that naloxone lengthened the postejaculatory interval by the same amount in animals that had tail pinch applied after ejaculation as it did in animals without tail pinch, and from this they argued that naloxone lengthened the absolute, and not the relative, postejaculatory refractory period. As discussed earlier, the length of the absolute refractory period does not vary with sexual arousal; the absolute refractory period and sexual arousal are thought to be mediated by different mechanisms.

In contrast to these findings, Miller and Baum (1987) have reported that doses of naloxone that did not affect copulation among gonadally intact, sexually rested male rats abolished mounting and ejaculation 14 days after castration, and lengthened initiation latencies and inhibited copulation if the male was sexually sated. That is, low doses of

naloxone inhibited sexual behaviors, including measures of sexual arousal, only when the male's sexual arousability was low due to castration or sexual exhaustion.

There are two reports of the effects of intracerebral naloxone injections on male sexual behavior. When injected into the mPOA, naloxone has been reported to shorten ejaculation latency and the postejaculatory interval (Band et al., 1986), indicating facilitation of copulation, and, in contrast, to lead to a cessation of copulation (Band & Hull, 1987). It has been suggested that the contradictory effects found with intra-mPOA injections of naloxone may be related to differences in baseline sexual performance (Band & Hull, 1987). The results of these two studies, however, must be viewed with caution; naloxone is somewhat lipophilic (Greenshaw, 1985), and is unsuitable for localized intracerebral administration (Britt & Wise, 1983).

In all of the above mentioned studies, animals were tested for sexual behavior while the opiate agonists and antagonists were pharmacologically active. In Experiments 5 and 6, on the other hand, animals were tested for sexual behavior at least 48 hours after the last injection of morphine to investigate whether male sexual behavior would be facilitated in an environment previously associated with injections of morphine. Thus, although systemic injections of morphine have been reported to inhibit sexual behavior (e.g. McIntosh, et al., 1980), the conditioned effect seen in the present experiments was facilitation of some aspects of sexual behavior. These findings parallel those from studies of morphine effects on locomotor activity; although acute systemic injections of moderate to high doses of morphine initially suppress locomotion (e.g. Babbini & Davis, 1972), the conditioned effect is increased locomotor activity (Hinson & Siegel, 1983; Mucha et al., 1981).

It is important to note, furthermore, that most studies of opiate agonist and antagonist effects on male sexual behavior have used either systemic or intraventricular injections; apart from Experiment 4 in this thesis, there are only two reports of injections

into specific brain areas (Band et al., 1986; Band & Hull, 1987). Opiates have a number of different effects mediated by interactions with opiate receptors at different brain, spinal, and peripheral sites, and some of these effects may be incompatible. Injections of morphine into the periaqueductal gray area, for example, have sedative actions (Broekkamp et al., 1976), whereas intra-VTA injections of morphine increase locomotor activity (Joyce & Iversen, 1979; Vezina & Stewart, 1984). Thus, although morphine may act at VTA sites to facilitate some aspects of sexual behavior (Experiment 4), when systemic or intraventricular injections are given concurrent incompatible actions at other sites may mask this facilitation.

Pertinent to a discussion of opiate effects is the finding that castration results in an increase in naloxone and naltrexone binding in rat brain (Hahn & Fishman, 1979, 1985). Several investigators have reported failures to replicate this finding (Cicero, Newman, & Meyer, 1983; Diez & Roberts, 1982; Wilkinson, Herdon, & Wilson, 1981), but methodological differences may account for these discrepant results (Hahn & Fishman, 1985). The increase in naloxone and naltrexone binding after castration appears to be mediated by a change in the concentration of binding sites, and not by changes in the concentrations of competing, endogenous ligands (Hahn & Fishman, 1985). Based on these findings, it might be expected that castrates would be more sensitive to the effects of opiates, and, in fact, Lieblich et al. (1985) found that doses of either morphine or naloxone, that did not influence the sexual behavior of gonadally intact males, decreased mount rate and the number of animals ejaculating among castrated male rats, suggesting greater sensitivity to opiates among castrates. The results obtained by Lieblich et al. (1985), however, are difficult to interpret; it is somewhat paradoxical that both morphine and naloxone had similar inhibitory effects on copulation.

Changes in opiate receptors would not appear to account for the findings of the present experiments; both gonadally intact and castrated males showed evidence for

conditioning at the dose of morphine used, indicating that differences in opiate receptor concentrations were probably not important.

In summary, when animals were tested for sexual behavior in an environment previously associated with morphine, sexual arousability was facilitated. Furthermore, the sexual arousability of castrates was increased when a receptive female was presented in an environment previously paired with repeated injections of morphine, suggesting that conditioning thought to involve the mesolimbic DA system may have compensated for the lower DA content and metabolism otherwise found at this time.

DISCUSSION

In the experiments reported in this thesis a systematic relationship was found between changes in the mesolimbic DA system and sexual arousability. First, changes in DA and DOPAC concentrations in a terminal field of the mesolimbic DA system, the NAS, coincided with the effects of castration and steroid administration on female-directed behaviors (Experiments 1-3). Second, opiate infusions into the region of the mesolimbic DA cell bodies, the VTA, facilitated mounting (Experiment 4). Third, conditioning thought to involve activity within the mesolimbic DA system facilitated measures of sexual arousal (Experiments 5 and 6).

Systemic DA agonists have been reported to facilitate sexual arousal and copulation in gonadally intact and castrated male rats (e.g. Foreman & Hall, 1987; Malmnas, 1973, 1976, 1977). Furthermore, castration decreases sexual arousal (e.g. Davidson, 1966a), and manipulations that increase dopaminergic neurotransmission can increase sexual behavior in castrates (e.g. Antelman, Hendron, Caggiula, & Shaw, 1975; Caggiula et al., 1976; Malmnas, 1973). The evidence that the mesolimbic DA system, specifically, mediates sexual arousal has, however, been indirect. The results of the experiments reported in this thesis are consistent with the results of these studies, and provides further evidence that the mesolimbic DA system is involved in mediating sexual arousal.

There were two manipulations that helped maintain the sexual arousability of castrates not treated with replacement steroids; repeated post-castration exposure to sexually receptive females (Experiment 3), and exposure to an environment previously associated with morphine (Experiment 6). In neither instance did a castration-induced decline in sexual arousal, as measured by female-directed behaviors, occur, and, in both, exposure to the stimuli alone maintained female-directed behaviors. Repeated post-castration sexual experience led to an increase in the DOPAC/DA ratio in the NAS

measured after a final test exposure to a receptive female, suggesting that repeated exposure to a female in that environment had influenced the response of the mesolimbic DA system. Post-castration sexual experience may, therefore, induce a conditioned and/or sensitized response to a receptive female involving the mesolimbic DA system.

Female-directed behaviors were also maintained by conditioning thought to involve activation of the mesolimbic DA system. Repeated injections of morphine into the VTA have been demonstrated to lead to both conditioned activity and to environment-specific sensitized activity responses to a subsequent injection of morphine (Vezina & Stewart, 1984). Furthermore, injections of morphine increase DA metabolism in the NAS (Kalivas et al., 1983), and, after repeated injections, conditioned increases in DA turnover are found (Schiff, 1982). Thus, it seems possible that repeated sexual experience and repeated injections of morphine in one environment may not only have similar effects on subsequent sexual arousability, but the effects may be mediated by similar neural mechanisms. It may be profitable, therefore, to compare directly the development of sensitization and the effects of repeated sexual experience. Experiments 5 and 6, for example, could be considered to be demonstrations of environment-specific cross-sensitization between the effects of morphine and the effects of a receptive female; the converse should also occur. Furthermore, DA receptor antagonists that block the development of environment-specific sensitization to intra-VTA infusions of morphine (Vezina & Stewart, 1984) might also be expected to attenuate the effects of post-castration sexual experience if administered during the repeated exposure to a receptive female.

Mechanisms underlying copulation. Behavioral studies of male sexual behavior have led to the proposal that several independently influenceable mechanisms control the occurrence of sexual behaviors. An arousal mechanism, the AM, is thought to control behavior prior to the initiation of copulation and to support copulation once it has begun, whereas a copulatory mechanism, the CM, is thought to control copulation, itself (Beach,

1956; Beach & Jordan, 1956; Sachs & Barfield, 1976). In the present experiments, sexual arousal, not the performance of copulation, was found to vary with steroid-reversible changes in NAS DA and DOPAC concentrations, post-castration sexual experience, intra-VTA opioid and opiate administration, and conditioning to morphine. Thus, the results of the present experiments, together with those of studies of the mPOA (e.g. Hull et al., 1986; Malsbury & Pfaff, 1974) indicate that it is possible to specify the neural mechanisms that might underlie the heretofore conceptually defined mechanisms for the control of arousal and copulation. That is, accruing evidence suggests that the mPOA is critically involved in controlling the performance of copulation (e.g. Giamonio et al., 1970; Hull et al., 1986; Malsbury & Pfaff, 1974; Szechtman et al., 1978), and the data from the studies reported in this thesis together with those from several other sources (e.g. Caggiula et al., 1974; Caggiula et al., 1976; Eibergen & Caggiula, 1973) indicate that the mesolimbic DA system is critically involved in mediating sexual arousal.

It should be apparent, however, that the complex sequence of actions that constitutes male sexual behavior cannot be fully understood in terms of the separate actions of two neural systems. Although the AM and CM are theoretically distinct, autonomous mechanisms, the full expression of sexual behavior depends on their integrated functioning. Similarly, it is obvious that the mesolimbic DA system, or the mPOA, does not function in isolation. A fuller understanding of sexual behavior will require a consideration of the interactions of these and other relevant systems in the context of external, sexually relevant stimuli. It will be necessary to delineate the connectivity of the relevant systems, and determine where and how they interact.

The mesolimbic dopamine system and behavior. Schneirla (1959) proposed a theory of behavior based on the distinction between approach and withdrawal, and accruing evidence, briefly reviewed below, suggests that the mesolimbic DA system is a critical link in the circuitry that mediates approach to environmental stimuli.

Infusions of morphine into the area of the VTA elicits sexual behavior if a sexually receptive female is present (Experiment 4), and feeding if food is present (Hamilton & Bozarth 1986, 1987), and, if neither food nor a female is present, then increased locomotion is found (Joyce & Iversen, 1979; Vezina & Stewart, 1984). Electrical stimulation of the MFB indirectly activates the mesolimbic DA system (Wise & Bozarth, 1984), and elicits sexual behavior (Caggiula, 1970; Caggiula & Hoebel, 1966; Vaughan & Fisher, 1962) and feeding (Glickman & Schiff, 1967); the behavior elicited is appropriate to the stimuli confronting the animal. Electrical stimulation of the VTA, itself, also elicits sexual behavior (Eibergen & Caggiula, 1973). Finally, conditioning thought to involve activity within the mesolimbic DA system has been found to increase sexual arousability (Experiments 5 and 6), and general locomotion if no female is present (Vezina & Stewart, 1984). Thus, activation of VTA cells elicits approach to environmental stimuli, or locomotion if localized stimuli are not present in the environment, and the ensuing behaviors are appropriate to the stimuli present.

Recent research, however, suggests that this interpretation of the function of the VTA is incomplete. Putative activation of kappa receptors in the region of the VTA elicits sexual behaviors (Experiment 4), and feeding (Hamilton & Bozarth, 1986, 1987), and facilitates stimulation-induced feeding (Wise et al., 1986), but does not facilitate brain stimulation reward or lead to increased locomotion (Jenck, Bozarth, & Wise, submitted; Jenck, Gratton, & Wise, 1987), suggesting at least some separation of the neural elements in the VTA that mediate feeding and sexual arousability, on the one hand, and locomotion and brain stimulation reward, on the other. It will be important, therefore, to directly compare the effects of injections of different opioid receptor agonists into the VTA on different behaviors, and to assess the involvement of DA in each instance. The VTA appears to mediate arousal or motivation, but it may be functionally heterogeneous and more research is needed to address this issue.

Several lines of evidence suggest that activation of DA cells in the VTA is reinforcing; animals will self-administer opiates into the VTA (Bozarth & Wise, 1981), and will lever press for electrical stimulation of the VTA (Crow, 1972; Routtenberg & Malsbury, 1969), and the MFB (Glickman & Schiff, 1967; Hoebel, 1969). That is, not only does activation of the mesolimbic DA system elicit approach, such activation is rewarding. If exposure to a sexually receptive female activates this system, then reinforcing effects of sexual behaviors would be expected to occur. Animals will learn a T-maze task for the opportunity to copulate (Kagan, 1955). Miller and Baum (1987) found that male rats increased the amount of time that they spent in an initially non-preferred compartment in which they had been allowed to copulate. Furthermore, if the mesolimbic DA system mediates sexual arousal, then exposure to a sexually receptive female and the ensuing sexual arousal, even without copulation, should be effective. Several findings suggest that this is true. Exposure to a sexually receptive female and the performance of only mounts or intromissions are effective reinforcers in the acquisition of a runway response (Sheffield, Wulff, & Backer, 1951) and of T-maze learning (Kagan, 1955; Whalen, 1961). Conditioned sexual arousal can be developed by using exposure to a sexually receptive female as a UCS (Zamble, Hadad, & Mitchell, 1985; Zamble, Hadad, Mitchell, & Cutmore, 1985). Further research, however, is needed to demonstrate that conditioning supported by exposure to a sexually receptive female involves mesolimbic dopaminergic activity.

Non-sexual behavior and castration. Because only sexual behaviors were tested in these experiments, and amine and amine metabolite concentrations were measured after exposure to a sexually receptive female, it is not known if the current findings generalize to other behaviors that involve activity within the mesolimbic DA system. Alderson and Baum (1981), however, used sexually naive animals and also reported a castration-induced decrease in NAS DA concentrations, suggesting that the effects of

castration are not restricted to tests of sexual behaviors. Of those studies that have investigated the effects of castration on non-sexual behaviors (see Beatty, 1979, for a review), relatively few have tested behaviors thought to be sensitive to activity within the mesolimbic DA system.

There are several reports that self-stimulation at some brain sites is affected by castration (Olds, 1958; Herberg, 1963), and that replacement treatment with T increases the response rate of castrated male rats (Caggiula, 1970). These studies, however, have reported the data for very few subjects, and more research is needed to explore electrical brain stimulation in castrated males.

There is evidence that T affects the persistence of behavior; compared to untreated controls, animals treated with T continue to respond after stimulus conditions are changed (Andrew, 1972; Archer, 1974, 1977; Thompson & Wright, 1979), and, if partial reinforcement was used during acquisition, responding during extinction is affected (Gray, Rickwood, Drewett, & Dunne, 1977). It has been proposed that T increases the persistence with which an animal attends to preferred stimuli (Andrew, 1972; Archer, 1974). The results of studies on behavioral persistence are consistent with the results of the experiments reported in this thesis; castration reduced attention to a sexually receptive female (Experiments 1-3, 6), and this was prevented by treatment with T (Experiment 2).

Bancroft (1980) found that replacement and withdrawal of T in castrated or hypogonadal men coincided with increases and decreases in sexual thoughts, and that changes in sexual thoughts preceded effects on erectile problems and ejaculation, and concluded that androgens act on sexuality by improving maintenance of attention (Bancroft, 1980; Bancroft & Wu, 1982). This suggestion is consistent with the effects of T on behavioral persistence (Andrew, 1972; Archer, 1974, 1977), and with the effects of castration and steroid treatment on the persistence of female-directed behaviors found in the experiments reported in this thesis. Thus, castration decreases the attention paid to

relevant stimuli, and the persistence of behavior, in tests of both sexual and nonsexual behaviors.

As discussed above, activation of the mesolimbic DA system results in forward locomotion. The finding that castration decreased NAS DA and DOPAC concentrations may, therefore, suggest that castrates would display less locomotor activity than gonadally intact males. There is, however, no evidence that adult castration influences locomotion; Menniti and Baum (1981) found that castrates and castrates implanted with T-containing silastic capsules showed similar amounts of locomotor activity when tested after systemic injections of amphetamine and when tested in a novel environment, and Savageau and Beatty (1981) reported that castration did not affect the amount of locomotor activity displayed after injections of amphetamine. Menniti and Baum (1981) tested animals 2-3 weeks after surgery, and this may not have been sufficient time to allow the development of castration-induced changes in NAS DA and DOPAC concentrations (see Table 1). Savageau and Beatty (1981), however, measured locomotor activity 7 weeks after surgery, and found that castrated and intact male rats displayed similar levels of locomotion after systemic injections of amphetamine.

It is possible that amphetamine (Menniti & Baum, 1981; Savageau & Beatty, 1981) and a novel environment (Menniti & Baum, 1981) were sufficiently potent in eliciting locomotion that differences between castrates and intact males resulting from differences in mesolimbic dopaminergic activity were obscured. It should be recalled that castrates given repeated post-castration sexual experience showed high levels of female-directed behaviors despite lower NAS DA concentrations, and that the repeated sexual experience led to an increase in a measure of DA metabolism (Experiment 3). Thus, although castration did affect the mesolimbic DA system, repeated sexual experience, as well as activation of the mesolimbic DA system by other means (see Experiments 4 and 6), compensated for this. It might be somewhat surprising, therefore, to find an effect of

castration on amphetamine- or novelty-elicited locomotion. Nonetheless, the results of the experiments reported in this thesis and those of Alderson and Baum (1981) would suggest that castrates and gonadally intact males might show subtle differences in locomotion, but that this may be manifested only in tests in which the mesolimbic DA system is not strongly activated, or after very low doses of DA mimetics.

Also pertinent to the present discussion is the effect of castration upon rotation after repeated systemic injections of amphetamine. After a systemic injection of amphetamine, animals will preferentially circle in one direction when placed in a small circular container (Robinson, Becker, & Pesty, 1982; Becker & Beer, 1986; Robinson, Becker, & Ramirez, 1980; Becker, Robinson, & Lorenz, 1982; Robinson, 1984). With subsequent injections of amphetamine, a sensitized, or increased, response is found. The presence of testicular hormones retards the development of the sensitization of rotation to amphetamine; gonadally intact males show significantly less sensitization than gonadally intact females, ovariectomized females, or castrated males (Robinson et al., 1982; Robinson, 1984). The effect of castration on the development of sensitization to amphetamine, together with the effect of castration on the mesolimbic DA system (Experiments 1-3; Alderson & Baum, 1981), suggests that if T is present the mesolimbic DA system can be more strongly activated, and that it may also be less modifiable. Furthermore, the finding that T decreases the modifiability of a dopaminergic system(s) is consistent with the findings that T increases the persistence of behavior and attentiveness to stimuli.

Conclusion. Castration results in a gradual decrease in sexual arousal that is important for the post-castration decline in sexual behaviors, and treatment with DA agonists increases sexual behavior in castrates, as well as in gonadally intact males. The results of the experiments reported in this thesis indicate that these effects involve the mesolimbic DA system; castration influenced the mesolimbic DA system, and,

conversely, treatments that increased activity within this system facilitated sexual arousability whether the animal was gonadally intact or castrated. Taken together these findings indicate that the mesolimbic DA system is involved in mediating sexual arousal.

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