NEURAL - BIOCHEMICAL CONTROL OF MASCULINE SEXUAL BEHAVIOR IN THE LABORATORY RAT (RATTUS NORVEGICUS)

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NEURAL-BIOCHEMICAL CONTROL OF MASCULINE SEXUAL BEHAVIOR IN THE LABORATORY RAT (RATTUS NORVEGICUS)

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ABSTRACT

NEURAL-BIOCHEMICAL CONTROL OF
MASCULINE SEXUAL BEHAVIOR IN THE
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By

Larry Wayne Christensen

Four experiments were carried out to investigate possible neurobiochemical events associated with the stimulation of masculine sexual behavior by testosterone. Castrated adult male rats were used in all experiments to investigate 3 problems; 1) Is testosterone necessarily converted to an estrogen before stimulating the resumption of mating behavior in long term castrated rats? 2) Is 3'5' cyclic adenosine monophosphate involved as an intracellular mediator in the action of testosterone on the brain in stimulating sexual behavior? and 3) What influence do drugs designed to alter the adrenergic or cholinergic milieu of the nervous system, have on copulatory performance, when they are applied directly to the preoptic area (POA) of the brain?

In Experiment I adult male rats that had been screened for copulatory behavior and subsequently castrated, where bilaterally implanted with stainless steel cannulae in the brain after meeting a criterion for the loss of sexual behaivor. The cannulae were placed so the tips rested in one of two areas, the preoptic area (POA) or the posterior hypothalamic area (PHA). Steroid hormones (ie. testosterone, estradiol or cholesterol) where then applied to either the POA or the PHA in an attempt to reinstate copulatory behavior. The application of testosterone or estradiol to the POA significantly increased sexual responses compared to scores exhibited by animals receiving the control steroid, cholesterol, or animals receiving either testosterone or estradiol in the PHA. Estradiol

was more effective than testosterone in stimulating the mean number of mounts and intromissions when applied to the POA.

Experiment II tested the hypothesis that the aromatization of testosterone to estradiol is necessary for the stimulation of masculine sexual behavior. This hypothesis was tested by administering directly to the POA, in combination with systemically administered testosterone, a compound (metapirone) that is known to inhibit the conversion of testosterone to estradiol. When animals were treated with systemic testosterone + intracerebral metapirone (infused directly into the POA) a significant increase in mounting was not observed, as was the case when animals received systemic testosterone + an intracerebral control solution or systemic estradiol + intracerebral metapirone.

In Experiment IV long term castrated male rats that showed no ejaculatory response on 3 consecutive weekly tests, were bilaterally implanted with cannulae resting in the POA. Solutions of cAMP, 5'AMP(the inactive metabolite of cAMP) or Dibutyryl cAMP (Db-cAMP) were then administered in an attempt to mimic the effects of testosterone on sexual behavior. None of the subjects showed any sexual responses during the 13 days of nucleotide treatment. However, when subsequently combined with systemic testosterone treatment 5'AMP but not cAMP or Db-cAMP significantly elivated the mean number of mounts and intromissions exhibited.

In Experiment IV drugs were administered via cannulae, directly to the POA of sexually active male rats in an attempt to determine the influence of adrenergic and cholinergic neural systems on the control of sexual behavior. Applications of norepinephrine (an adrenergic neurotransmitter) retarded the temporal pattern of copulation, the animals taking longer to initiate copulation and likewise longer between events in the

pattern once it was begun. Alpha-methyl-tyrosine (an inhibitor of norepinephrine synthesis) significantly decreased the number of ejaculations exhibited during a test for sexual behavior given 4 hrs after the initial application to the POA. Alpha-methyl-tyrosine affected no other component of sexual beahvior when compared to the sham test, when no drug was given. When carbachol (a cholinomimetic) was applied to the POA of copulating rats, all components of the copulatory pattern were eliminated. Application of a compound to the POA that blocks the synthesis of achetylcholine (hemicholinium-3) decreased the mean number of mounts, intromissions and ejaculations in a test given 4 hrs after the initial application. However, it had no effect on any component of copulation in a test given 15 minutes after the initial exposure. The decrease in copulation at 4 hrs was due to a prolonged period of sexual quiescence exhibited in a significant percentage of the hemicholinium-3 treated animals, after achieving 1 or 2 ejaculatory responses.

The data presented in this dissertation suggest: 1) the aromatization of testosterone to estradiol is necessary for the stimulation of masculine sexual behavior; 2) cAMP is involved in regulating the display of masculine copulatory responses, perhaps through an inhibitory mechanism; and 3) adrenergic neural systems are involved in temporally patterning copulation, where as cholinergic systems regulate the occurrence of the pattern.

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By

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"In scientific research there is no such thing as bad results, just unexpected ones."

R. Buckminster Fuller

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LIST OF ABBREVIATIONS

Ach	Acetylcholine
α-MT	Alpha methyl tyrosine
5'AMP	5' Adenosine monophosphate
cAMP	3'5' Cyclic adenosine monophosphate
Db-cAMP	Dibutyryl 3'5' cyclic adenosine monophosphate
DHT	Dihydrotestosterone
DNMR	Duncan's new multiple range test
HC-3	Hemicholinium
ICT	Intracerebral treatment
IF	Intromission frequency
IL	Intromission latency
IM	Intramuscular
MF	Mount frequency
MFB	Median forebrain bundle
MIII	Mean inter intromission interval
ML	Mount latency
NE	Norepinephrine
PEI	Post ejaculatory interval
PHA	Posterior hypothalamic area
POA	Preoptic area
TP	Testosterone propionate

INTRODUCTION

The importance of testicular hormones, especially testosterone, for the development and display of masculine copulatory behavior in mammalian species is well documented (see Young 1961 for review). The development and maturation of sexually dimorphic morphology and behavior depend upon the presence or absence of gonadal hormones during two critical periods in the animal's life, once around the time of sexual anatomical and behavioral differentiation and again during adulthood (postpuberal). When rats (Gerall and Ward 1966), guinea pigs (Phoenix, Goy, Gerall and Young 1959), hamsters (Eaton 1970) or mice (Edwards and Burge 1971) are exposed to testosterone during sexual differentiation and again postpuberally (in the form of testicular secretion in males and injected testosterone propionate in females) they develop the sexual morphology and sexual behavior patterns characteristic of normal males. On the other hand, lack of testosterone or certain of its metabolites, during either period, prevents the activation of masculine behavior and morphology.

The mechanisms by which testosterone influences the display of masculine sexual behavior in the adult animal are for the most part unknown. However, evidence suggests that in the male rat, the preoptic area (POA) of the brain is one site of testosterone action which is involved in the regulation of male copulatory behavior. Autoradiographic studies using castrated male rats have shown a high concentration of radioactivity in this neural area an hour after the administration of tritiated testosterone (Pfaff 1968, Resko, Goy and Phoenix 1967, Sar and Stumpf 1972).

Likewise, direct application of crystalline testosterone propionate to the POA of long term castrated rats resulted in an increase of sexual behavior (Davidson 1966, Lisk 1967, Johnston and Davidson 1972).

The present study was designed to provide information on possible neurobiochemical mechanisms involved in the reactivation of masculine sexual behavior in the castrated male rat (Rattus norvegicus). Investigation has centered around a working hypothesis which conceptualizes the biochemical events occurring in the POA during testosterone stimulation. In this hypothesis testosterone (4-androsten-17βol-3-one) reaches a nerve cell via the blood and is bound to the cell by a protein receptor at the plasma membrane (see Figure 1). The testosterone-receptor complex is taken into the cell where it is metabolized to an estrogenic form, probably estradiol (1,3,5(10)-estratrien-3,17β-diol). The estradiol now bound to the same or a different receptor protein enters the nucleus where it has anabolic effects, the products of which may be directly involved in preparing the cell for activity. Another intracellular pathway important in this working hypothesis involves the synthesis of cyclic 3'5'-adenosine monophosphate (cAMP). Cyclic AMP stimulates nuclear controlled anabolic pathways, the products of which may either be involved directly in the activation of the nerve cell or may be important indirectly in processes such as the conversion of testosterone to estradiol or in carbohydrate metabolism.

One obvious class of anabolic products needed by the nervous system for successful operation is neurotransmitter material. In the present working hypothesis it is suggested that testosterone restores the ability of the nerve cells to function by increasing the synthesis and availability of neurotransmitters.

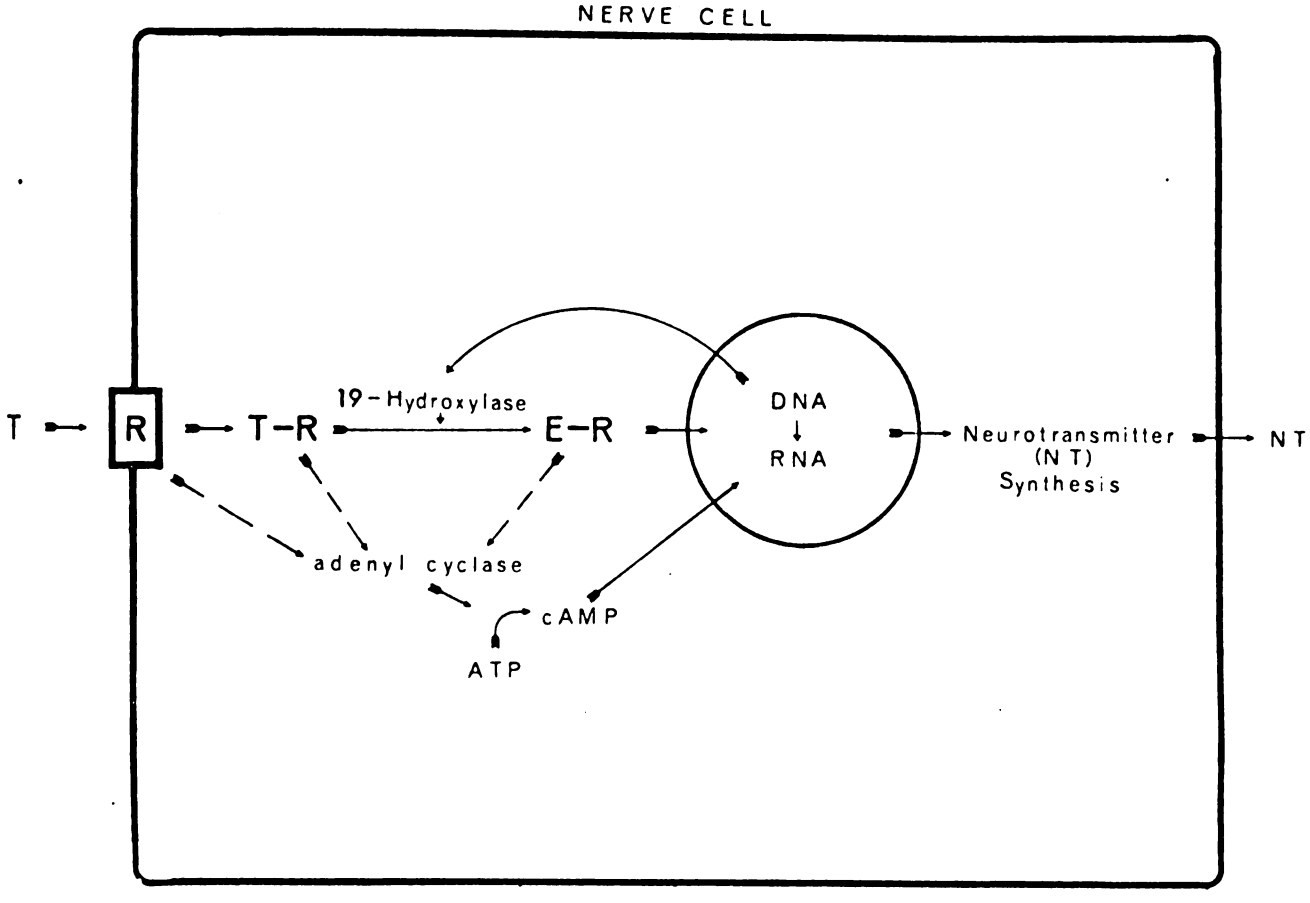


Figure 1. A working hypothesis of possible biochemical mechanisms involved in testosterone activation of the nervous system. In this working hypothesis testosterone (T) bound to an intracellular receptor (R) is converted to an estrogen (E). The estrogen then has effects on anabolic processes the products of which may be important in stimulating the nerve cell (ie. neurotransmitters). CyclicAMP is also involved in this hypothesis either directly or indirectly.

The experiments reported here concern three aspects of this working hypothesis outlined above. Experiments I and II test the hypothesis that conversion of testosterone to an estrogen in the POA of castrated male rats is necessary for the resumption of mating behavior. Experiment III tests the hypothesis that cAMP acts as an intracellular mediator of testosterone in activating the resumption of masculine sexual behavior. Experiment IV tests the hypothesis that within the POA the synthesis or presence of two neurotransmitters, norepinephrine and acetylcholine, are essential for the display of masculine mating behavior.

BACKGROUND

Sexual behavior is essential for successful reproduction and species survival in all vertebrate species. Through the use of various species specific signals (visual, olfactory and auditory) the process of mate selection insures that prospective participants are of the same species and are in mature reproductive condition. Other mechanisms involved in the mechanics of copulation are also important in isolating undesirable mates as well as activating specific physiological events necessary for reproductive success. For example, hormonal changes in the female ring dove in response to visual cues from the male integrate her behavior with that of the male (Lott, Scholz and Lehrman 1967); intromissions in the rat determine hormonal changes in the female necessary for implantation success (Adler, Resko and Goy 1970) and also trigger ovulation in cats and rabbits (Heape 1905). Thus, sexual behavior, from an evolutionary standpoint, is important in that it serves as an isolating mechanism valuable for the preservation of species identity. Likewise, the mechanics of courtship and copulation are important in activating physiological responses necessary for the propogation of offspring and hence the species.

The study of sexual behavior in laboratory mammals (ie. rats, hamsters, guinea pigs and mice) has proven extremely productive, largely due to their ease in domestication and ease in handling. In the following sections a description of female as well as male sexual behavior in the laboratory rat is presented to provide a wider reference base to which the present research must be related.

Hormonal Concomitants of Female Sexual Behavior

Many mammals (eg. deer, wolves, prairie dogs and bear) engage in mating behavior only during specific periods of the year (seasonal breeders), whereas others are sexually active throughout the entire year (eg. rabbit, hamster and man). Males of species sexually active throughout the entire year will generally mate on any occasion with a receptive female. However, females of nonseasonal species are only receptive during a particular phase of the estrous cycle. The estrous cycle of the laboratory rat for instance, is usually four days with the period of behavioral heat or sexual receptivity, lasting about 12 hours. The onset of heat occurs just prior to ovulation, and is a result of two days exposure to estrogen and a surge of progesterone on the night of proestrus (Barraclough, Collu, Mussa and Martini 1971, Feder, Resko and Goy 1968). Thus, sexual behavior in the intact female rat is dependent upon the ovarian release of estrogen and progesterone. The removal of the ovary abolishes sexual behavior (Ball 1936, Beach 1942). However, sexual receptivity may be induced in ovariectomized female rats by injections of estrogen and progesterone (Beach 1942).

Two doses of estradiol benzoate (4 μg) given 24 hours apart will induce estrus when synergized with an injection of progesterone (500 μg), given 24 hours after the second estrogen injection. One to four hours after progesterone treatment the female will respond sexually to the male. Estradiol benzoate will, by itself, induce receptivity if administered daily for a week or more (Davidson, Rodgers, Smith and Bloch 1968). However, high estrogen levels in the intact cycling female are never maintained for long periods, hence, the surge of progesterone is needed (Powers 1970). The mechanisms involved in estrogen priming and proges-

terone synergism are unknown.

Female Sexual Responses

Sexual behavior in the female rat consists of two main components 1) hopping and darting and 2) presentation or lordosis. The hopping and darting component of receptive behavior is believed to be involved in stimulating the male to mount (Coniglio and Clemens 1972). Hopping and darting consists of a short rapid run (1-3 feet), with the female usually coming to an abrupt halt in a crouch. A sexually active male will usually follow closely during the run and mount the female when she stops. During this quick run, short hops may be exhibited, especially at the beginning or end.

Lordosis usually occurs at the end of a run in response to the male's mount. The lordotic response consists of a concave arching of the back; the head and rump being raised with respect to the back. The complete response, with extreme curvature of the back, will only occur when the female is mounted by a male. However, slight lordotic responses may be exhibited during investigative sniffing or licking of the perineum by the male.

Lordosis in the rat is not limited to the female. Estrogen primed males will also display the lordosis posture when mounted (Beach 1938, 1945), although the amount of estradiol needed to prime the male is much greater than in the case of the female (Davidson 1969).

Masculine Sexual Pattern

In general, three separate components of male sexual behavior can be recognized in the rat: 1) nuzzling and chasing the female 2) mount

bouts and 3) intermount bout grooming. The nuzzling and chasing consists of anogenital licking and rooting of the female. This activity usually elicits a short run by the receptive female. When the female ends her run, the sexually vigorous male may engage in any one of three mount bout activities: 1) mount (without intromission), 2) intromission (mount with vaginal penetration) or 3) ejaculation. A mount bout is defined as a sequence of mounts (one or more) with or without intromission, uninterrupted by any behavior that is not oriented toward the female or the male's own genitalia (Sachs and Barfield 1970). Each of the three possible activities occurring in a mount bout are behaviorally identifiable and easily distinguished by the experienced observer.

During a mount, the male, approaching from the rear of the female, clasps his forelegs around the female's laterolumbar region, in what is called, a mount without palpation. While clasping the female, the male may palpate her sides with rapid movements of his forelimbs and simultaneously move his pelvic region in rapid piston-like thrusts (mount with palpation and thrusting). The male then slips off the female rather weakly. This type of dismount invariably signifies there was no vaginal penetration or "intromission" (Beach 1944). In the intromission pattern the palpation with thrusting does occur, but in addition, a final and forceful thrust is observed during which time vaginal penetration is achieved. Stone and Ferguson (1940) estimated from film studies that vaginal intromission lasts 1/3 to 2/3 of a second during which time 2-9 pelvic thrusts occur. Vaginal penetration is terminated after this short period with a "forceful backward lunge which carries the male several inches from the female" (Young 1961). If an ejaculation accompanies the intromission, the backward lunge is absent. Instead, the male stays in

contact with the female and slowly raises his front paws laterally until almost in an upright position. The ejaculate is expelled during this response. In castrated males given TP replacement, sperm are absent but the gross motor responses still occur. The penis is withdrawn following ejaculation and the male lowers himself to all fours.

Ejaculation is usually preceded by several mounts (without intromission) and 3 to 14 intromissions (Young 1961). The sequence of mounts and intromissions preceding an ejaculation is termed an ejaculatory series. Several ejaculatory series may be achieved by one male in a single 20 minute test. Several time dependent measures are commonly used in quantifying masculine sexual behavior. Mount and intromission latencies is the time measured in seconds from the introduction of a stimulus female into the testing area, until the occurrence of the first mount and intromission, respectively. The ejaculation latency is defined as the time from the first intromission in a series until the occurrence of the ejaculatory pattern. Mean Inter-Intromission-Interval (MIII), gives the mean time between intromissions and is calculated by dividing the ejaculation latency by the number of intromissions preceding the ejaculation. Following an ejaculation a period of sexual inactivity is seen, lasting 5-10 minutes. This period is termed the Post Ejaculatory Period (PEI) and is defined as the time from the ejaculatory response until the next intromission. During the PEI the male is not influenced by sexual stimuli (Larsson 1956). The length of the PEI increases with each successive ejaculatory response. After 3-10 ejaculatory series the PEI may extend over 30 minutes (an arbitrary criterion frequently used for defining sexual exhaustion or satiation) (Beach and Jordon 1956).

Beach and Jordon (1956) also reported changes in behavior preceding

each successive ejaculation. The number of intromissions in an ejaculatory series decreased from an average of 10.6 intromissions in the first ejaculatory series to 4.1 in the sixth. Ejaculation latency (here measured in seconds as the interval from the first mount to ejaculation) decreased from a mean of 450 to 130 seconds. The PEI increased from an average after the first ejaculation of 324 to 818 seconds after the 6th ejaculation. Sexual satiety was reached after 3-10 ejaculations, and a recovery period of 24 hours or more usually followed, although effects of sexual satiation could sometimes be noticed up to 7-8 days later in some measures.

Hormonal Control of Masculine Mating Behavior

The development and display of masculine sexual behavior depends upon the presence of testosterone during two critical periods in the life of a rodent; once very early in life, near the time of sexual differentiation, and again as an adult (postpuberal). In the absence of testosterone exposure during either period, an animal will exhibit very little adult sexual behaivor.

An hypothesis first expounded by Young and his co-workers (Phoenix, Goy, Gerall and Young 1959) to explain the sexual differentiation of male copulatory behavior, emphasized the necessity of testosterone exposure during early development. When the developing organ systems (genital and neural) of the young male animal are exposed to testosterone normally secreted from its own testes during sexual differentiation, these organ systems develop the capacity for male sexual behavior to be exhibited subsequent to testosterone exposure in adulthood. On the other hand, if testosterone is not present during sexual differentiation,

as is the case of genetic females, the animal will not exhibit masculine sexual behavior as an adult, even if given massive doses of testosterone.

The necessity of testosterone for the development and display of masculine sexual behavior in rodents has been well documented. When male hamsters (Swanson 1970, Coniglio, Paup and Clemens 1973) and rats (Beach and Holtz 1946) were castrated on the day of birth, they exhibited less male copulatory responses in adulthood than adult castrates even though both groups were given large doses of testosterone propionate (TP). Conversely, when female rats (Gerall and Ward 1966), guinea pigs (Phoenix, Goy, Gerall and Young 1959), hamsters (Eaton 1970) or mice (Edwards and Burge 1971) were exposed to testosterone propionate during the early critical period (the first 5 days after birth for the mouse, rat and hamster, and days 30-35 of gestation for the guinea pig) they displayed an increased frequency of masculine copulatory behavior when injected with TP postpuberally.

Thus, exposure to testosterone during early development sensitizes the animal (be it genetic male or female) so that when exposed to testosterone in adulthood the animal will display masculine mating patterns in appropriate stimulus conditions. Likewise, low levels of male mating responses will be exhibited if testosterone is absent perinatally or during testing postpuberally.

Neuro-hormonal Control of Adult Male Sexual Behavior

Neural Control:

Results of several studies point to the preoptic area (POA) of the brain as an important neural site for testosterone action in the adult male rat. Fisher (1955), Davidson (1966), Lisk (1967) and Johnston and

Davidson (1972) have reported increases in the sexual behavior of castrated male rats when pellets of TP were implanted into the POA. Autoradiographic techniques have also identified the POA as a neural site which concentrated large amounts of radioactivity when tritiated testosterone was given to castrated male rats (Pfaff 1968, Resko et al 1967, Sar and Stumpf 1972). Lesion and stimulation experiments have also implicated the POA in the control of masculine sexual behavior. Lesions which interfere with the median forebrain bundle (MFB) disrupt male sexual behavior in the adult rat (Hitt, Hendrics, Ginsburg and Harris 1970, Rogers and Law 1967). Likewise, lesions which destroyed the anterior portion of the POA, which is anatomically related to the MFB, disrupted male sexual behavior (Singer 1968). Electrical stimulation of the POA, on the other hand, accelerated masculine copulatory performance (Caggiula and Szechtman 1972, Malsbury 1971).

Hormonal control:

Studies investigating the influence of testosterone on ventral prostate and seminal vesicles suggest that testosterone serves as a "pre-hormone" for these tissues. That is to say, testosterone undergoes intracellular metabolism before many of the physiological responses associated with testosterone action are initiated. In these tissues, testosterone is converted to a reduced product, 5 -dihydrotestosterone (DHT) (5 -androstan-17 -ol-3-one) after being transported into the cell (Baulieu and Lasnitzki 1968, Bruchovsky and Wilson 1968). The intracellular DHT is then bound to the nucleus along with a protein receptor, specific for the DHT configuration. At the nucleus the receptor-DHT complex presumably initiates synthetic processes involving DNA-RNA

transcription, which eventually leads to the observed physiological responses (Main-Waring and Mangon 1970).

Similar processes may be involved in androgen stimulation of the nervous system. Testosterone bound at the plasma membrane to a protein receptor would be taken into the cell and metabolised to the effective configuration. In fact, the conversion of testosterone to DHT was demonstrated in slices of brain tissue (Jaffe 1969, Kniewald, Mussa and Martini 1971). DHT was also effective in inhibiting gonadotropin release from the pituitary of rats (Feder 1971). In the same study, Feder showed an accumulation of radioactivity in the brain following administration of tritiated DHT. However, DHT failed to induce masculine sexual behavior, either when given systemically (McDonald et al 1970, Feder 1971, Whalen and Luttge 1971) or intrahypothalamically (Johnston and Davidson 1972) to castrated male rats. Therefore, while DHT may be an important metabolite of testosterone in the stimulation of systemic tissues or regulation of gonadotropin activity, it is less involved in the control of masculine sexual behavior.

A number of investigators have suggested testosterone aromatization to an estrogen as a possible step in the hormonal stimulation of male copulatory responses (Young 1961, McDonald et al 1972, Beyer and Komisarak 1971). Data from several behavioral experiments lend support to the necessity of testosterone aromatization. Non-aromatizible androgens (DHT and androsterone) failed to stimulate masculine sexual behavior in the castrated male rat (McDonald et al 1970, Whalen and Luttge 1971) or hamster (Christensen, Coniglio, Paup and Clemens 1973). Furthermore, small quantities of estradiol benzoate (10 ug/day) were more effective than oil in inducing mounting and intromissions behavior in long-term castrated rats (Pfaff 1970). Systemically injected estradiol benzoate was also more

effective than oil, but less effective than testosterone, in prolonging the ejaculatory pattern in castrated male rats (Davidson 1969). Support for this concept of hormone conversion also comes from work in which androstenedione, a precursor to testosterone, was aromatized to estrone in the diecephalon of adult male rats (Naftolin, Ryan and Petro 1972). It thus appears as though androgens, at least those which may be aromatized to estrogens, or estrogens themselves are effective in stimulating masculine sexual behavior.

Aromatization process:

The proposed biochemical pathway of gonadal steroid synthesis is illustrated in Figure 2. The conversion of either androstenedione or testosterone to estrone or estradiol respectively, involves the hydroxylation of the C-19 carbon and aromatization of the A ring (removal of 1α and 2α hydrogens) (Townsley and Brodie 1968, Brodie, Kripalani and Possanza 1969). Although the aromatization process has not been completely delineated, the hydroxylation and oxidation steps appear to be temporally seperate (Brodie et al., 1969). Presummably, the conversion process occurs intracellularly at the level of the POA. In a study by Naftolin and co-workers (1972) they reported a .17% conversion of androstenedione to estrone in the diencephalon of adult male rats. At this conversion rate, one would expect that if estradiol were the active metabolite, it would have to be more potent than testosterone for inducing male behavior.

Thus a summary of the "aromatization hypothesis" suggests that testosterone undergoes C-19 hydroxylation and aromatization to an estrogen form, presumably estradiol. Estradiol, therefore, would be the steroid affecting intracellular anabolic processes involved in restoring masculine behavior. One way of testing this hypothesis would be to block the conversion of

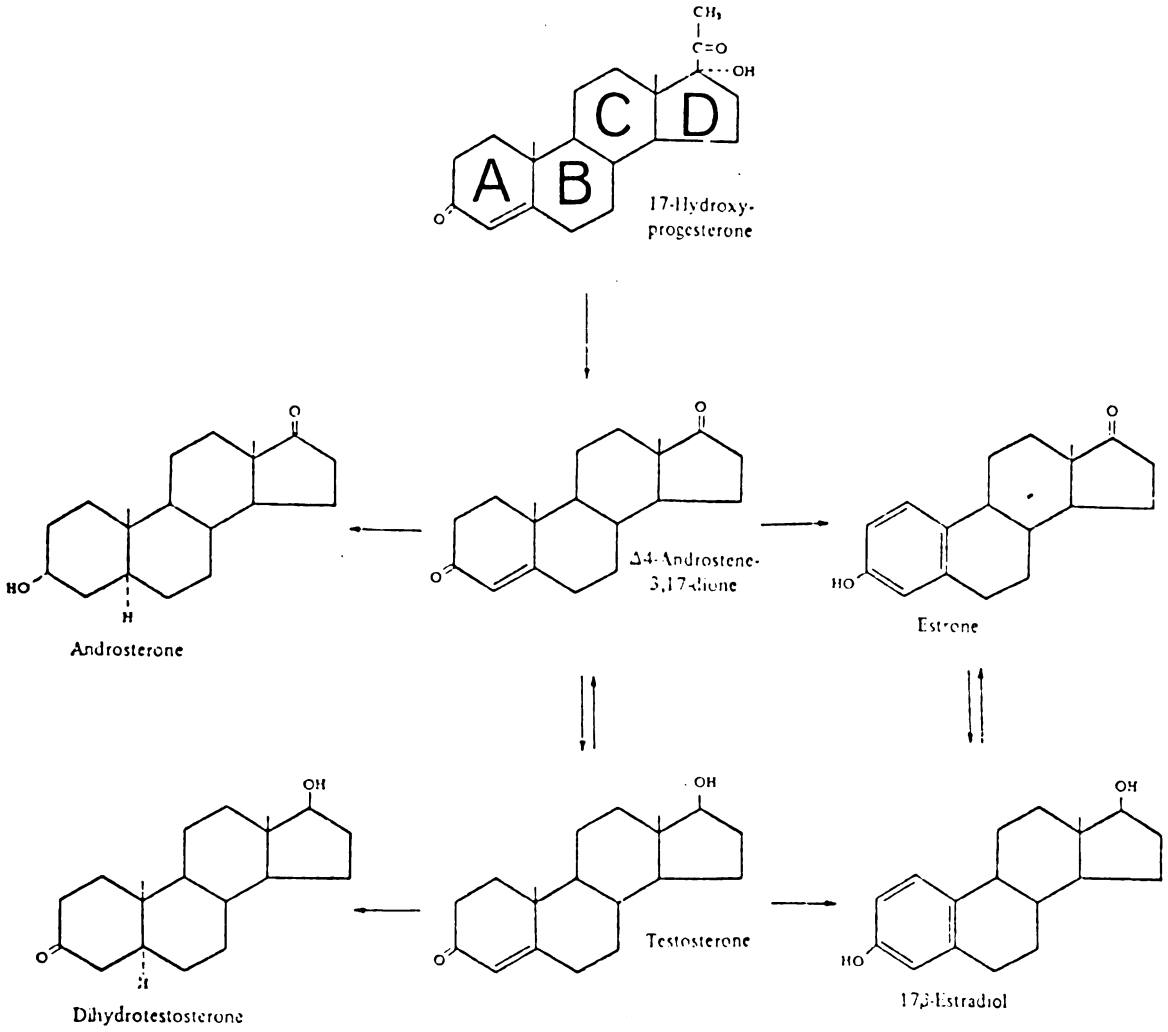


Figure 2. A hypothesized pathway for the aromatization of androgen to estrogen. In the aromatization process two mechanisms are involved: 1) the removal of two hydrogens from ring A and 2) the aromatization of ring A.

testosterone to estradiol.

Biochemical mechanisms involved in sexual behavior:

Little information is available on how steroid hormones activate nervous tissue in the restoration of masculine sexual behavior. However, recent studies of testosterone action on seminal vesicles and ventral prostate in rats (Singhal, Parulekas, Vijayvargiya and Robinson 1971) indicate that the nucleotide 3'5'-cyclic adenosine monophosphate (cAMP) may function as an intracellular mediator for testosterone. When testosterone was administered to castrated male rats, increases of cAMP were detected in the seminal vesicles and ventral prostate gland. Likewise, when cAMP was injected into castrated male rats it increased certain carbohydrate metabolizing enzymes which are also increased by testosterone administration. Presumably, a build-up in these enzymes increases the available stores of energy needed by the cell for testosterone-induced functions. In the same study, effects of submaximal doses of testosterone were potentiated by simultaneous injections of theophylline, an inhibitor of phosphodiesterase (the catabolic enzyme for deactivating cAMP). Theophylline plus submaximal doses of testosterone resulted in maximal dose responses (ie. increased enzyme levels). Inhibition of phosphodiesterase by theophylline presumably prevented the degredation of small amounts of cAMP activated by low levels of the steroid. The subsequent build-up in cAMP resulted in intracellular responses normally associated with maximal testosterone stimulation.

The possibility of cAMP being involved in the regulation of masculine sexual behavior was demonstrated in recent experiments by Christensen and Clemens (in press). Submaximal doses of testosterone propionate (TP) (5ug/day) were ineffective in maintaining sexual behavior of castrated male rats. However, when this same dosage was administered to castrated males in combination with 10mg/day of theophylline, a significant maintenance of

intromission and ejaculatory patterns was achieved. Similarly, 25ug of TP injected daily was ineffective in restoring sexual behavior of 6 long term castrated male rats. However, this dose of TP, when injected in combination with 10 mg of theophylline daily, restored the ejaculatory response in 60% of the castrated males. Intromission and mounting patterns were restored in 80% of the animals receiving TP and theophylline. Genital stimulation (measured as the number of penile spines and the length of penile papillae) was similar in the TP + saline and TP + theophylline groups, suggesting potentiation of testosterone effects in the central nervous system as a possible explanation of the observed behavioral differences rather than effects of these compounds upon genital morphology.

Cyclic-AMP may be involved in the regulation of masculine sexual behavior by affecting 1) all the intracellular biochemical events necessary for nerve cell activation or 2) specific aspects of testosterone activation (eg. stimulating enzymes needed for intracellular steroid or carbohydrate metabolism).

The brain has the highest capacity to make cAMP of any mammalian tissue (Sutherland, Rall and Menon 1962). Various neurohumors have been shown to increase the concentration of cAMP when applied to in vitro nerve preparations: histamine (Kakiuchi and Rall 1968), dopamine (Kebabian and Greengard 1971), norepinephrine (Huang, Shimizu and Daly 1971) and serotonin (Kakiuchi and Rall 1968). However, no definitive role of cAMP has emerged in the regulation of behavior. In general, high doses of dibutyryl-cAMP (Db-cAMP), a butyrized form of cAMP apparently resistant to metabolic inactivation, when injected into some cerebral areas, may induce general motor hyperactivity and convulsions (Gessa, Krishna, Forn Tagliamonte and Brodie 1970), hyperphagia, hyperthermia and prolonged estrous cycle (Breckenridge and Lisk 1969).

Studies carried out in our laboratory (unpublished) and by Breckenridge and Lisk (1969), suggest that cAMP is not involved in the activation of female receptivity. Likewise, direct application of cAMP or Db-aCMP to the anterior hypothalamus (a site for estrogen action) failed to stimulate increases in female receptivity (Breckenridge and Lisk 1969). Some of the possible roles for cAMP in testosterone stimulation may be: to increase the available energy via enhanced carbohydrate metabolism, to increase intracellular transport of synthesized products (ie. axoplasmic flow of neurotransmitters), or to increase the C-19 hydroxilase needed for the aromatization of testosterone.

Pharmacological Control of Masculine Sexual Behavior

Throughout the past decade, several neurotransmitters have been isolated in the vesicular fraction of brain homogenates: acetylcholine (Ach), serotonin, norepinephrine (NE), dopamine and histamine. Several studies utilizing systemically administered substances have implicated some of these transmitter substances in the control of masculine sexual behavior. Dewsbury and Davis (1970) have suggested that high levels of biogenic amines in the brain may retard copulatory behavior in the male rat. Such a hypothesis is consistent with the facilitory effects of monoamine depletors such as reserpine (Soulairac and Soulairac 1961, Soulairac 1963, Dewsbury and Davis 1970, and Dewsbury 1971a), tetrabenazine (Dewsbury1971b) and p-chlorophenylalanine (Shillito 1969, Sheard 1969, Tagliamonte, Tagiamonte, Gessa and Brodie 1969, Gessa 1970 and Salis and Dewsbury 1971). However, systemic administration of these drugs is known to affect the peripheral as well as the central nervous system. Therefore, systemic administration does not allow conclusions regarding behavioral effects in relation to changes in brain neurotransmitter levels. Likewise, general nonspecific

increases in neurohumoral agents throughout the entire brain offer little insight into what areas of the brain may be responsible for the observed effects. Experiments which alter the levels of neurotransmitters in localized regions of the brain are needed.

Fuxe (1965) has demonstrated through the use of histochemical fluoescence analysis that the POA has a high concentration of NE nerve fibers and cell bodies. Since the POA has been shown to be involved in sexual behavior, these data suggest that NE-containing nerve pathways may be involved in controlling masculine sexual behavior. Another line of evidence suggests that Ach-containing nerve pathways may be involved in the regulation of sexual responses. In studies conducted in this laboratory (Christensen and Clemens unpublished observation) carbamylcholine (carbachol), a sympathomimetic, when applied directly to the mesencephalic reticular formation of estrogen-primed female rats, greatly increased the number of lordoses. These results warranted investigation as to whether Ach was also involved in the control of masculine sexual behavior.

Following these lines of evidence, preliminary studies were carried out to investigate adrenergic and cholinergic control of masculine sexual behavior (Humphrys, Christensen and Clemens 1972). These preliminary studies utilized direct unilateral application of NE or carbachol to the POA. The effects of these treatments on sexual behavior suggested that cholinergic mechanisms may be involved in the inhibition of masculine copulatory behavior, while the control by adrenergic systems was less clear.

Objectives of the Present Study

The investigation reported in this dissertation was divided into three parts: A, B and C.

Part A. Investigation of the hypothesis that testosterone is converted to an estrogenic form in the activation of masculine sexual behavior.

Experiment I: The object of this experiment was to determine if estadiol is as effective as testosterone in inducing the resumption of masculine sexual behavior in long-term castrated rats. Pellets of crystalline estradiol or testosterone were applied directly to an area of the brain (POA) known to be responsive to testosterone implants. The data from this experiment provided information on the relative strength of the two steroids when applied directly to the brain. In addition, data on the importance of steroid stimulation of penile morphology for the resumption of copulatory behavior were collected.

Experiment II: The objective of this experiment was to determine if direct application of metapirone, a compound known to inhibit the conversion of testosterone to estradiol, to the POA of castrated male rats would block the activation of masculine sexual behavior by testosterone.

Part B. An investigation into the possible involvement of cAMP in the activation of masculine sexual behavior.

Experiment III: This experiment was designed to determine if direct application of cAMP or Db-cAMP to the POA of long-term castrated rats, would mimic the effects of testosterone in the resumption of copulatory behavior. This experiment also provided information relavant to the question of whether POA applied cAMP potentiates the behavioral effects of submaximal doses of testosterone.

Part C: An investigation into the neuropharmacological control of male copulatory behavior.

Experiment IV: In this study various chemicals designed to increase or decrease the level of adrenergic and cholinergic activity, were applied directly to the POA. The affect of these manipulations on the various parameters of masculine sexual behavior were measured.

EXPERIMENT I. DETERMINE IF ESTRADIOL IS AS EFFECTIVE AS TESTOSTERONE FOR INDUCING THE RESUMPTION OF MASCULINE SEXUAL BEHAVIOR.

Experiment I was divided into two parts (A&B). In Part A an area was located where testosterone implants would reactivate copulation in castrated male rats. In Part B estradiol was implanted in the same area to determine its effectiveness in stimulating masculine sexual behavior.

Part A. Determine a site of testosterone action.

Methods: Thirty male rats were used (see Appendix I, II and III for detailed methods for all experiments). All animals were selected on the basis of a screening test for sexual behavior and subsequently castrated and allowed to rest for 3 weeks. At the end of 3 weeks each subject was tested weekly for sexual behavior until they achieved a criterion of 3 consecutive tests without an ejaculatory response. Upon meeting this criterion all animals were bilaterally implanted with double-walled stainless steel cannula (26 gauge inserts) which permitted intracerebral treatment with crystalline hormones. Of the 30 males, 20 were implanted so the cannulae tips rested on the POA. The remaining 10 animals were implanted so the tips of the cannulae rested in the posterior hypothalamic area.(PHA). The methods of implantation are further detailed in Appendix.

Of the 20 males implanted in the POA, 10 received intracerebral treatment (ICT) of testosterone and the other 10 received cholesterol ICT. For the 10 animals with cannulae in the PHA, ICT consisted of testosterone. Each animal received one pellet of the respective steroid in each cannula 0,3,6,9 and 12 days after implantation. Thus each animal received a total of 10 pellets (5 on each side). (See Appendix II-a,b). The weight of a single pellet for each steroid used was 15 ug. All subjects were tested

for masculine sexual behavior 7, 10 and 13 days following the onset of treatment.

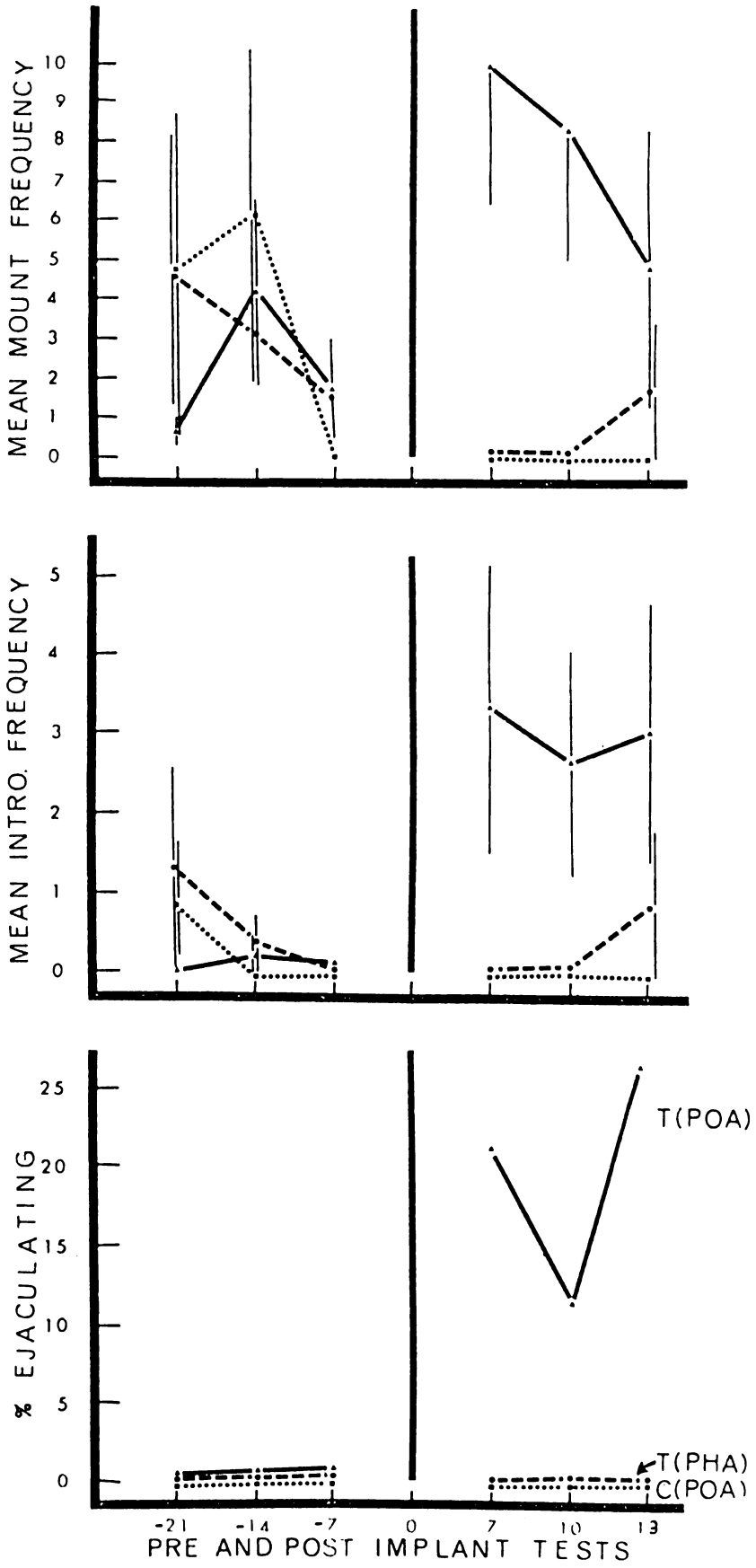
Twenty four hours after the last test, all animals were perfused and the brains and penises were prepared for histological examination. The location of the implants was determined and the number of penile spines and length of penile papillae were measured. Johnston and Davidson (1972) and Christensen and Clemens (in press) have shown that the development of these penile structures is a more sensitive index of circulating testosterone than is seminal vesicle weight or masculine sexual behavior. See Appendix III for histology.

Unless otherwise stated data from tests in this experiment and all subsequent experiments were analyzed using an analysis of variance F test for repeated measures (Winer 1962). The F statistic is given in the text of the result section along with the comparative F value and the .05 level of significance used. The degrees of freedom for the numerator and denominator of the calculated F ratio are also included with the comparative F statistic (ie. $F_{.05,2,27}$).

Results:

Masculine sexual behavior: As sumarized in Table 1 and Figure 3, intracerebral applications of testosterone into the POA of long term castrated rats were significantly more effective than treatments of testosterone in the PHA or cholesterol in the POA for stimulating increases in mounts, intromissions and ejaculations. An analysis of variance for repeated measures (Winer 1962) indicated a significant difference among the three treatment groups over the entire experiment ($F=6.12>F_{.05,2,27}$) and mean intromission frequency ($F=3.85>F_{.05,2,27}$). In addition, a G statistic (a variation of X^2) indicated a significant difference among the treatment groups in number of animals exhibiting the ejaculatory response ($G=10.60 >$

Figure 3. Represented for the three treatment groups are the mean number of mounts and intromissions ± SE as well as percentage of animals ejaculating. Animals receiving intracerebral applicaitons of testosterone to the POA exhibited significantly more mounts and intromissions as well as having a greater percentage of animals ejaculating when compared to animals treated with testosterone in the PHA or animals receiving cholesterol in the POA.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T(POA)
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-14
-7
0
7
10
13
PRE AND POST IMPLANT TESTS

Figure 3

Table 1. Effects of intracerebrally implanted testosterone or cholesterol on parameters of masculine sexual behavior.

	Test Days from Implantation	Testosterone (POA) $\bar{x} \pm SE$	Cholesterol (POA) $\bar{x} \pm SE$	Testosterone (PHA) $\bar{x} \pm SE$
Mean Mount Frequency	-21	0.6 ± 0.43	4.8 ± 3.62	3.1 ± 2.57
	-14	4.2 ± 2.43	6.2 ± 4.39	0.0
	-7	1.8 ± 1.06	0.0	0.0
	7	9.9 ± 4.09	0.0	0.0
	10	8.3 ± 3.90	0.0	0.0
	13	4.7 ± 3.77	0.0	1.8 ± 1.8
Mean Intromission Frequency	-21	0.0	1.3 ± 1.3	0.8 ± 0.8
	-14	0.2 ± 0.2	0.4 ± 0.4	0.0
	-7	0.0	0.0	0.0
	7	3.3 ± 1.87	0.0	0.0
	10	2.7 ± 1.75	0.0	0.0
	13	3.0 ± 2.03	0.0	0.8 ± 0.8
Percent Ejaculating	-21	0	0	0
	-14	0	0	0
	-7	0	0	0
	7	20	0	0
	10	10	0	0
	13	33	0	0

$X^2_{.05,2}$).

A priori comparisons on differences between groups in the mean number of mounts indicated significantly more mounts were exhibited by the Testosterone (POA) group than by the Testosterone (PHA) group ($F=10.04>F_{.05,1,27}$). Application of testosterone to the POA was also more effective in stimulating mounts than cholesterol (POA) ($p<.05$ by Duncan's new multiple range test [DNMR]). Testosterone, when placed in the PHA was no more effective than cholesterol in the POA in inducing mounting behavior ($F<1$).

Animals treated with testosterone in the POA were the only subjects to increase their intromission frequency scores over their pretest level ($t=1.83>t_{.05,9}$ by a paired students t test). The Testosterone (POA) group achieved more intromissions than Testosterone (PHA) group ($F=6.23>F_{.05,1,27}$) or the Cholesterol (POA) group ($F=7.54>F_{.05,1,27}$). There was no significant difference between the Testosterone (PHA) group or Cholesterol (POA) group ($p>.05$ DNMR) in the mean number of intromissions achieved.

Testosterone in the POA significantly increased the percentage of animals ejaculating when compared to the testosterone in the PHA and cholesterol in the POA ($G=5.45>X^2_{.05,2}$).

Part B. Determine if estradiol is as effective as testosterone when implanted in the same area.

Methods: Twenty sexually vigorous male rats were castrated and allowed to rest for three weeks. At the end of 3 weeks, all subjects were tested weekly for sexual behavior until the 3 test criterion for the loss of sexual behavior was met. All animals were then implanted bilaterally with double-walled stainless steel cannulae (21 gauge guide cannulae and 26 gauge inserts). Ten males were implanted with cannulae located in the POA and the remaining ten with cannulae in the PHA.

Beginning on the day of implantation all animals received ICT of estradiol (10ug per cannula) every 3 days. Thus each animal received one 10 ug pellet of estradiol through each cannula 0,3,6,9 and 12 days after implantation. Each animal received a total of 10 pellets. All subjects were tested for masculine sexual behavior 7,10 and 13 days following the onset of treatment.

At the conclusion of testing all animals were perfused and the brains and penises were prepared for histological examination. The location of the implants were determined and the number of penile spines and length of penile papillae were measured.

Results:

Masculine sexual behavior: Intracerebral applications of estradiol into the POA were effective in inducing mounting. intromitting and ejaculatory responses in long term castrated rats Figure 4 and Table 2. Estradiol when placed in the POA was significantly more effective than estradiol in the PHA in restoring mount ($F=8.19>F_{.05,1,27}$), intromission ($F=12.37>F_{.05,1,27}$) and ejaculatory responses ($X^2= 9.51>X^2_{.05,1}$). Estradiol when placed in the PHA was not significantly more effective than cholesterol in the POA for the induction of any of the sexual behavioral patterns ($p<.05$ in mean mounts and intromissions as well as percent ejaculating).

In order to evaluate the effectiveness of estradiol and testosterone as stimulatory agents of copulatory behavior the data from Parts A and B were analyzed together. It was found that the smaller amounts of estradiol placed in the POA were more effective than the larger implants of testosterone in restoring mount, intromission and ejaculatory responses. Analysis of variance utilizing all groups revealed that applying estradiol to the POA was significantly more effective than testosterone in stimulating

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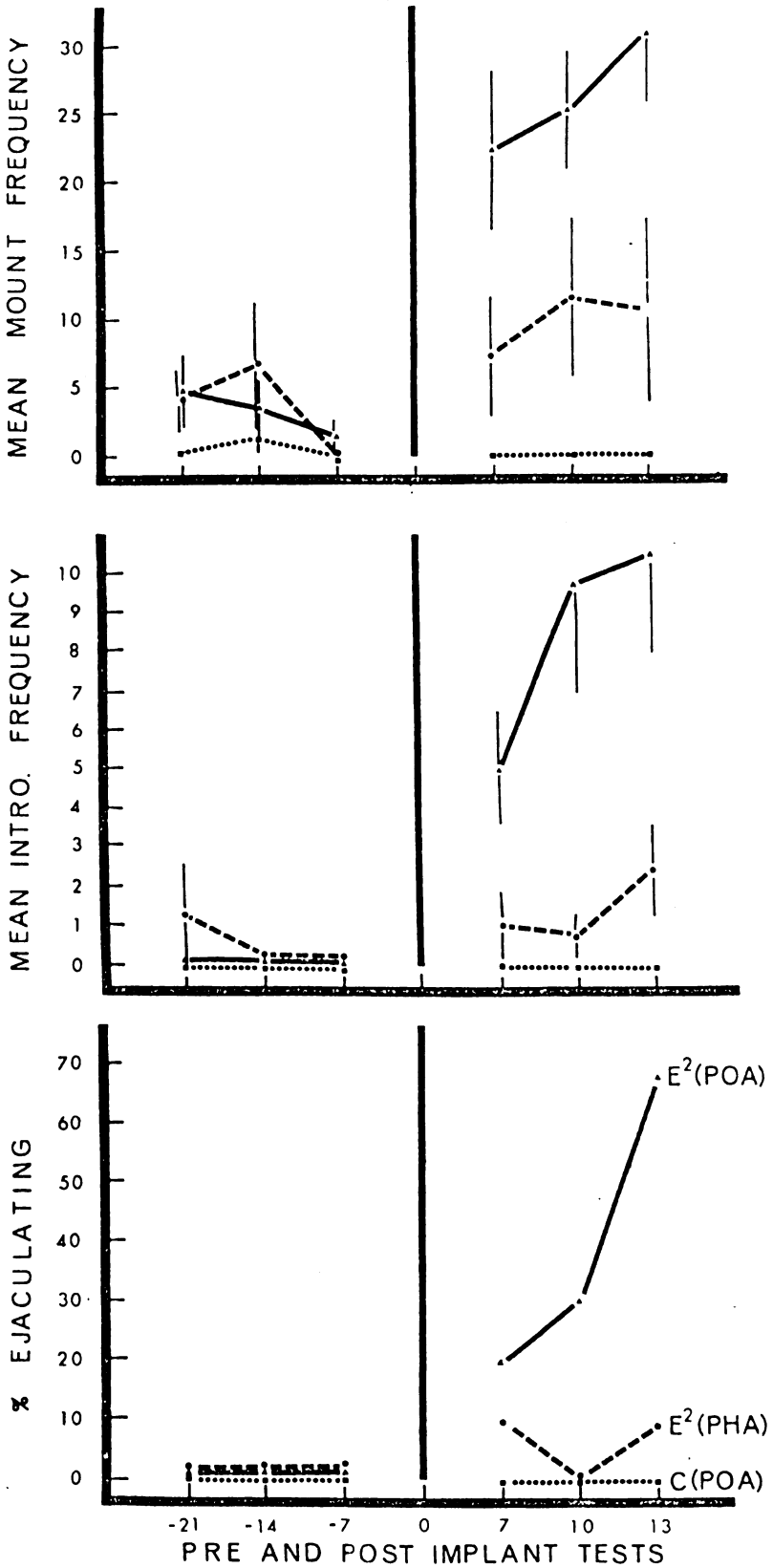
Figure 4. Represented for the three treatment groups are the mean number of mounts and intromissions ± SE as well as percentage of animals ejaculating in tests for sexual behaivor. Estradiol when placed in the POA was significantly more effective than estradiol placed in the PHA or cholesterol in the POA, in reinstateing mount, intromission and ejaculatory responses in long term castrated male rats.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E²(PHA)
C(POA)
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PRE AND POST IMPLANT TESTS

Figure 4.

Table 2. Effects of implanted estradiol or cholesterol on masculine sexual behavior.

	Test Days from Implantation	Estradiol (POA) $\bar{x} \pm SE$	Estradiol (PHA) $\bar{x} \pm SE$	Cholesterol (POA) $\bar{x} \pm SE$
Mean Mount Frequency	-21	4.9 ± 2.77	0.3 ± 0.2	4.8 ± 3.62
	-14	3.0 ± 1.74	1.5 ± 0.93	6.2 ± 4.39
	-7	1.6 ± 1.01	0.0	0.0
	7	22.0 ± 6.66	6.7 ± 4.40	0.0
	10	24.5 ± 5.37	12.3 ± 5.11	0.0
	13	29.4 ± 6.0	10.6 ± 6.67	0.0
Mean Intromission Frequency	-21	0.1 ± 0.1	0.0	1.3 ± 1.3
	-14	0.0	0.0	0.4 ± 0.4
	-7	0.2 ± 0.2	0.0	0.0
	7	5.0 ± 2.01	1.0 ± 0.1	0.0
	10	9.7 ± 3.10	0.9 ± 0.64	0.0
	13	10.1 ± 2.34	2.5 ± 1.36	0.0
Percent Ejaculating	-21	00	00	00
	-14	00	00	00
	-7	00	00	00
	7	20	10	00
	10	30	00	00
	13	66	10	00

mean number of mounts ($F=15.2>F_{.05,1,27}$) and intromissions ($F=9.87>F_{.05,1,27}$) per test. Estradiol (POA) was also more effective than testosterone (POA) in restoring the percent of tests during which ejaculation was achieved although this difference was not significant ($X^2=2.36<X^2_{.05,1}$).

Histological results: The location of the various steroid implants for both Parts A and B is represented in Figures 5 and 6 (POA) and Figures 7 and 8 (PHA). A statistical analysis of the anterior, vertical and lateral coordinates (according to Pellegreno's (1965) atlas) indicates no significant differences in the mean placement site between the 3 POA treated groups (1 way ANOVA $p<.05$ in all cases) or likewise between the 2 PHA implanted groups. Through the use of regression analysis the coordinates of maximum effectiveness were: estradiol; anterior 7.22, vertical 1.36, lateral 0.74 and testosterone; anterior 7.66, vertical 0.89, lateral 0.88. Comparison of the percent of preoptic nucleus destroyed at the point of maximum penetration using a plane planimeter, indicated there was no difference between the Testosterone (POA), Estradiol (POA) or Cholesterol (POA) groups in the percent of preoptic nucleus destroyed ($F<1$). Likewise, using regression analysis a coefficient of correlation was calculated for all the POA implanted groups using the percent of preoptic nucleus destroyed as the independent variable and mean mount score for the three post implant tests as the dependent variable. The coefficients are: Testosterone (POA) $r=-.47$, Estradiol (POA) $r=-.59$ and cholesterol (POA) $r=0$.

A gross microscopic examination of the penises from all groups, revealed the two testosterone implanted groups (POA and PHA) to be the only males exhibiting significant penile development (Table 3). The two testosterone treatment groups were not different from each other ($p>.05$ DNMR). Likewise, no significant differences in penile spines or papillae were detected between

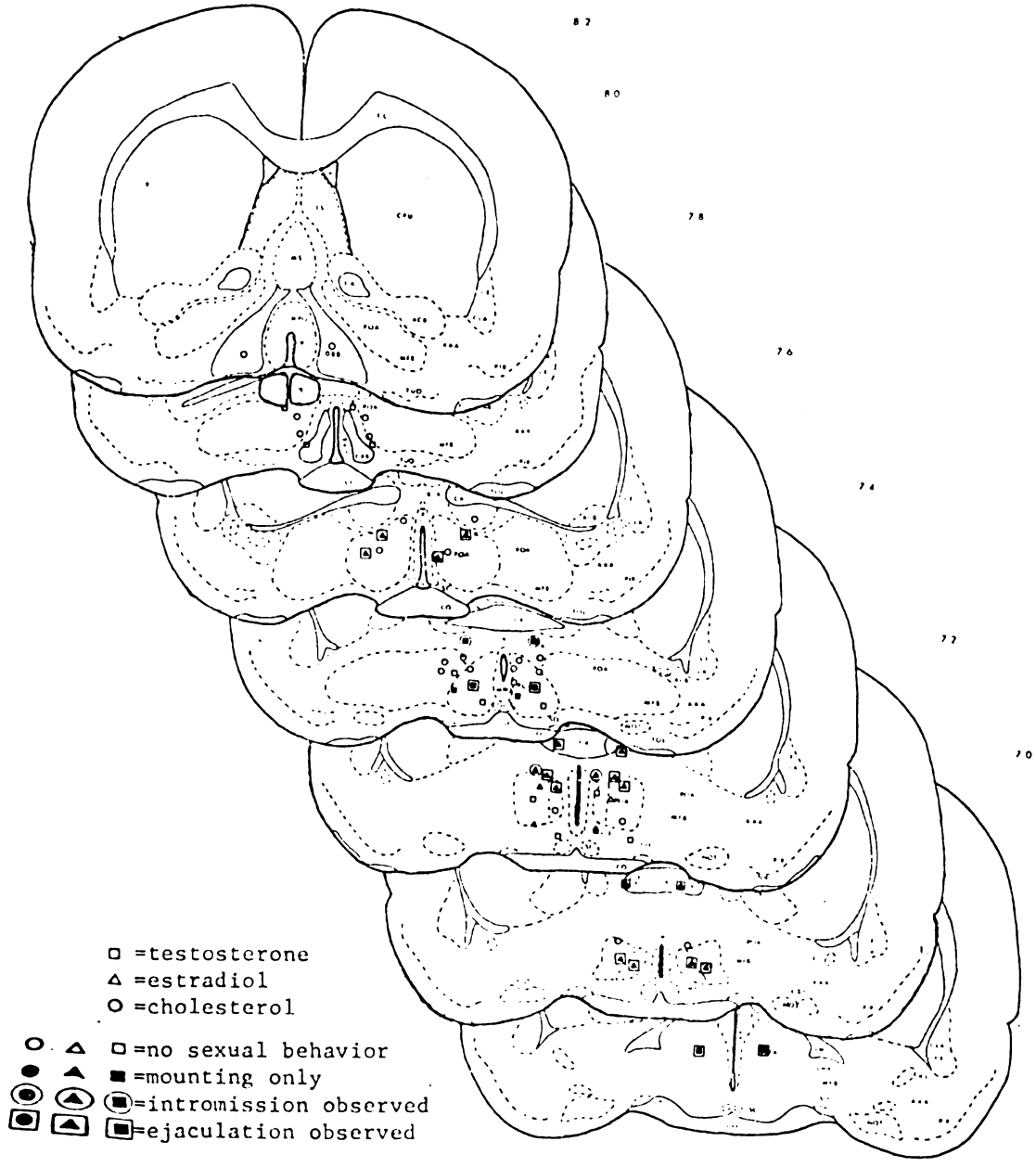


Figure 5. Locations of maximum POA cannulae penitration in Experiment I are represented in cross sectional maps (Pellegrino et al 1967).

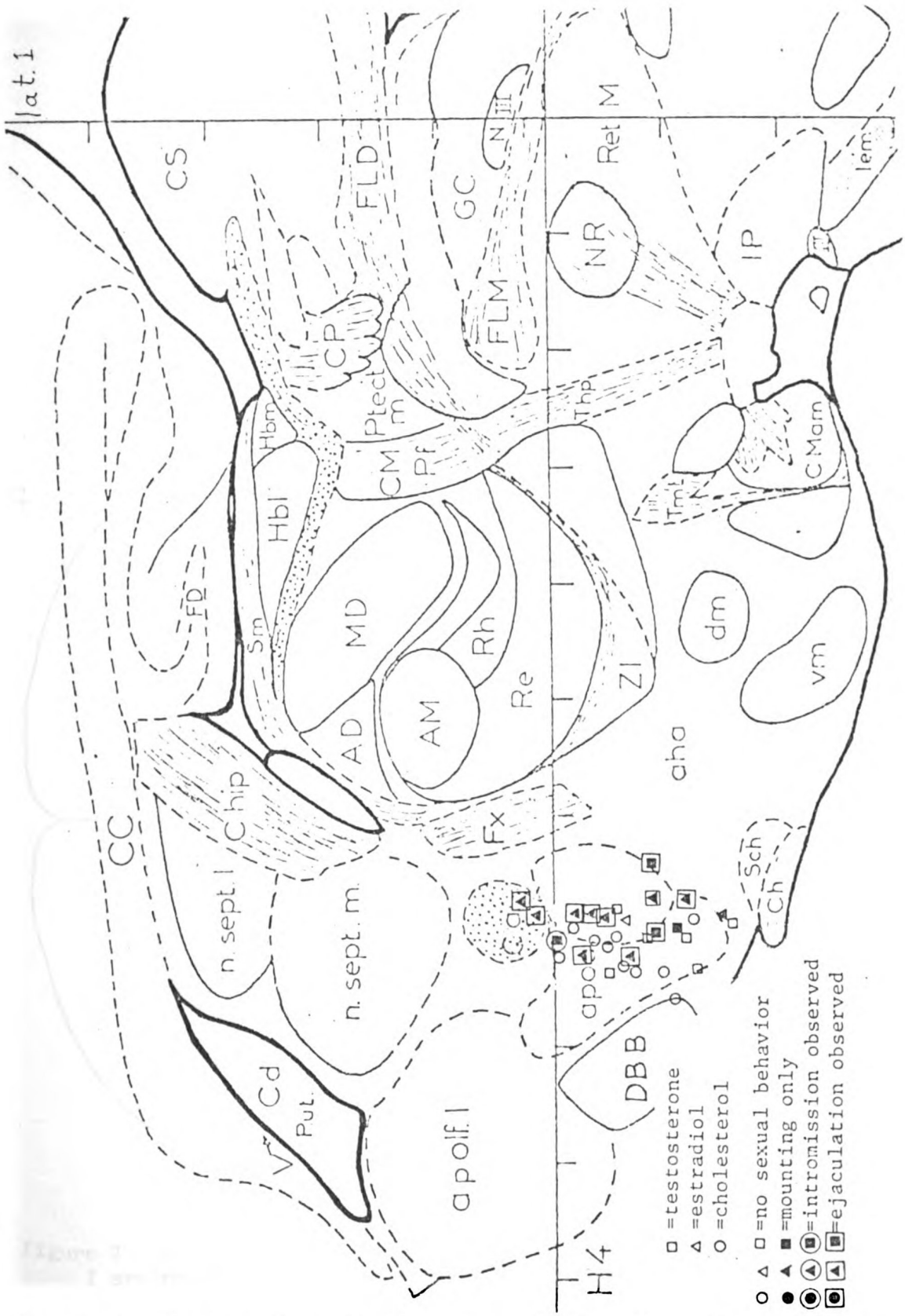


Figure 6. The locations of POA implants in Experiment I are represented in this longitudinal map (Able-Fessard et al 1967).

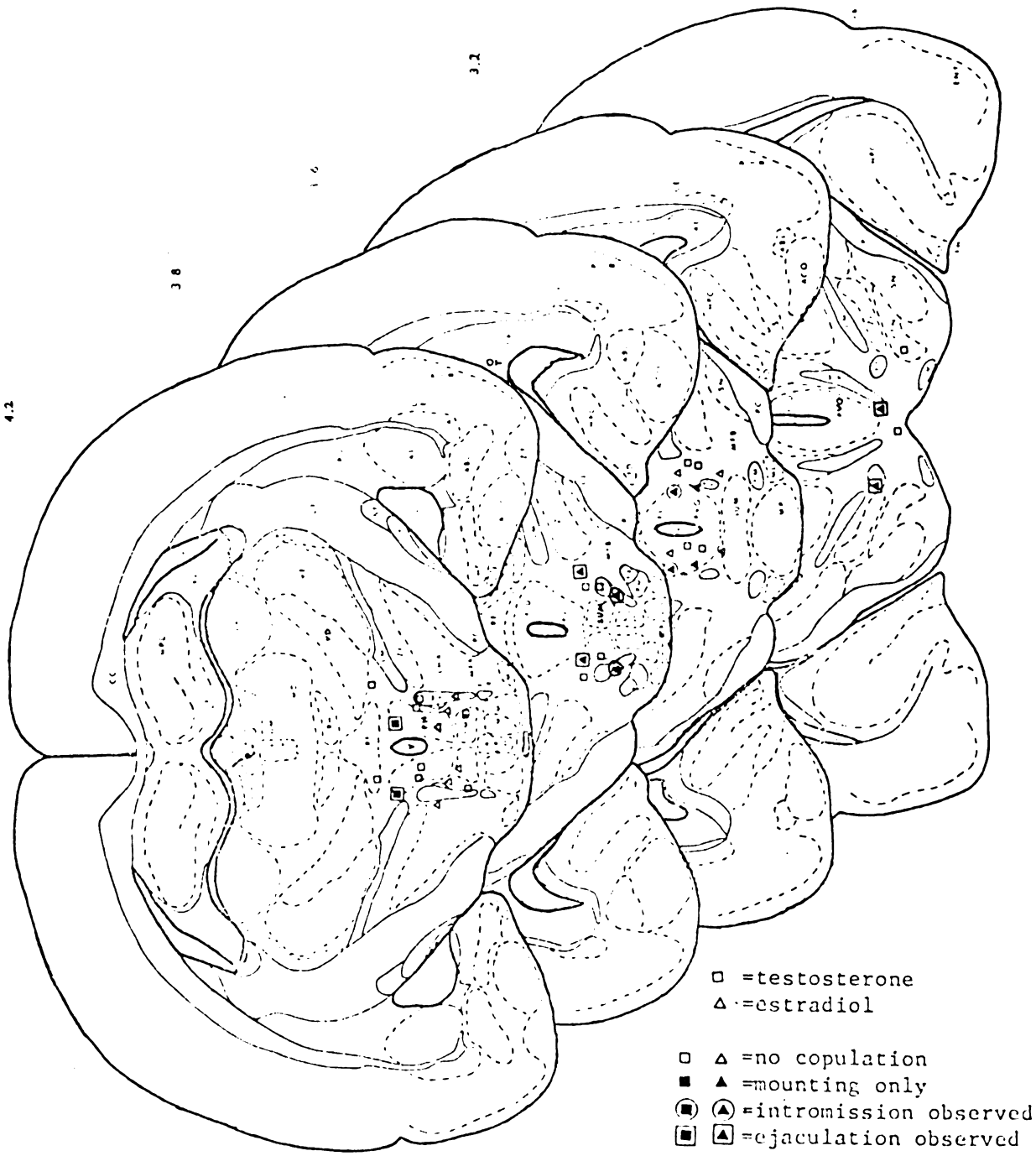


Figure 7. The locations of maximum PHA cannulae penitration in Experiment I are represented in these cross sectional maps (Pellegrino et al 1967).

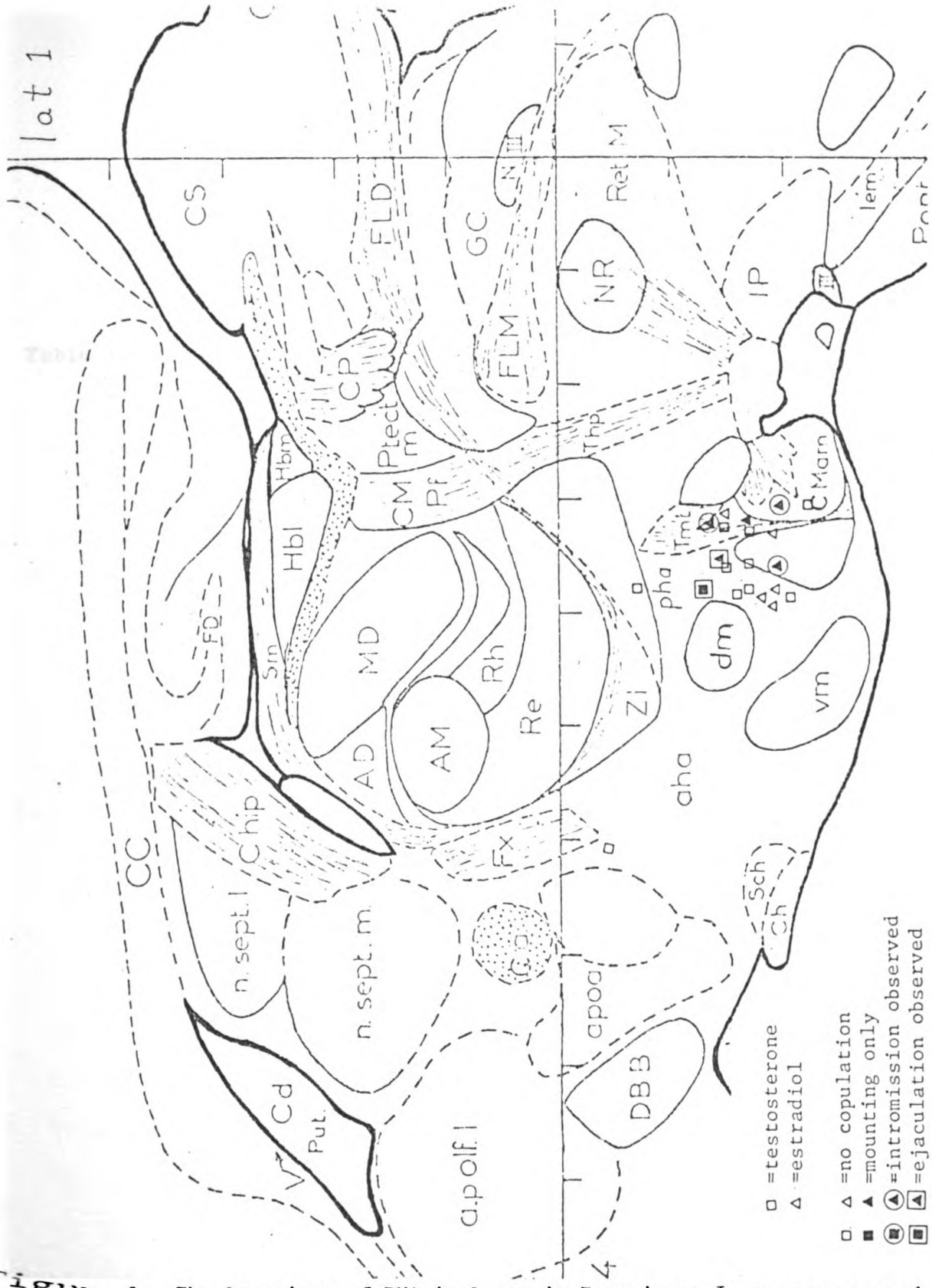


Figure 8. The locations of PHA implants in Experiment I are represented in this longitudinal map (Able-Fessard et al 1966).

Table 3. Effects of implanted steroids on the mean number of penile spines and the mean length of penile papillae.

	Penile Spines $\bar{x}$ ± SE	Papillae Length $\bar{x}$ ± SE
Testosterone (POA)	13.4 ± 1.81	.62 ± .02
Cholesterol (POA)	5.1 ± 1.23	.50 ± .02
Testosterone (PHA)	11.7 ± 1.42	.59 ± .01
Estradiol (POA)	4.3 ± 1.28	.48 ± .01
Estradiol (PHA)	4.5 ± 1.00	.48 ± .01

the estradiol (POA), estradiol (PHA) or cholesterol (POA) groups ($p>.05$ DNMR).

EXPERIMENT II. THE BLOCKING OF TESTOSTERONE METABOLISM TO AN ESTROGEN BY METAPIRONE AND ITS EFFECT ON THE INDUCTION OF MASCULINE SEXUAL BEHAVIOR.

The results of the previous experiment suggest that estradiol when applied to the preoptic area of castrated rats is more effective than testosterone in stimulating male copulatory behavior. Experiment II was subsequently conducted to determine if the conversion of testosterone to estradiol is a necessary step in the stimulation of masculine copulatory behavior by testosterone.

Methods

Thirty adult intact male rats selected for their sexual vigor, were castrated and bilaterally implanted in the POA with double-walled steel cannulae (23 gauge guide cannulae and 30 gauge obdurator) as explained in Appendix II. Ten males were then randomly assigned to each of the following treatment combinations: 1) 150ug of testosterone daily given intramuscularly (IM) + 150ug of sucrose ICT every 12 hr; 2) 150ug of testosterone (IM) daily + 150ug of metapirone ICT every 12 hr or 3) 50ug of estradiol(IM) daily + 150ug of metapirone ICT every 12 hr. Each group was administered their respective steroid intramuscularly for 13 days. The dose of estradiol was lowered from 50ug to 25 ug after 7 days of treatment, to prevent radical weight loss in the estrogen treated group. Metapirone and sucrose were dissolved in 0.9% NaCl solution and adjusted to PH 7.4 with $NaCO_2$. Metapirone and sucrose solutions were infused via the intracerebral cannulae in a volume of .005 ul/cannula. (See Appendix II). Metapirone or sucrose applications began on the evening of implatation and steroid treatment began the

next morning. Males were tested for masculine sexual behavior on the 7th, 10th and 13th day of steroid administration. Histological verification of implant sites was made as in Appendix III.

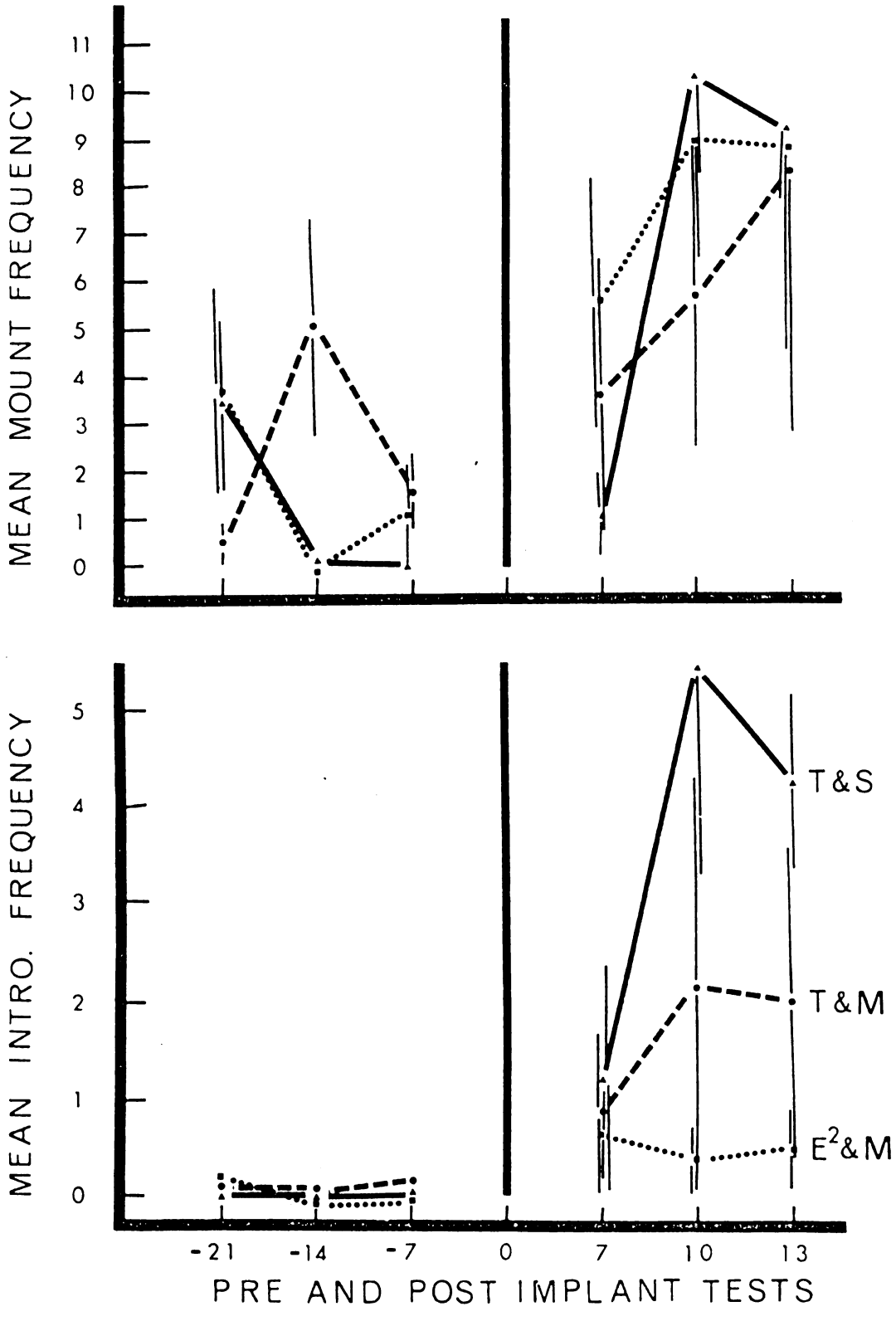
Results.

As seen in Figures 9 & 10 and Tables 4 & 5 intracerebral application of metapirone in combination with systemic testosteorne, prevented the increase in mounting activity exhibited by animals treated with sucrose + testosterone or metapirone + estradiol. While the testosterone + sucrose treated group showed more intromissions and ejaculations than the other treatment groups these differences were not consistent.

An over-all analysis of variance for repeated measures revealed no significant differences between the three treatment groups in mean mount frefrequency ($F<1$), even though a X^2 statistic revealed the groups to be different in the percent of tests during which mounting was exhibited ($X^2=6.01>X^2_{.05,2}$). An analysis over the entire treatment period indicated a significant difference among the treatment groups in the mean number of intromissions acheived ($F=3.61>F_{.05,2,27}$). A X^2 analysis indicated a difference among the groups in percent of tests during which an ejaculation was achieved ($X^2=21.18>X^2_{.05,2}$).

The treatment of castrated males with systemic testosterone + intrahypothalamic infused sucrose or treatment with systemically injected estradiol + intrahypothalamic metapirone significantly increased the mean number of mounts in the three postimplant tests, when compared to the three preimplant tests ($t=2.78$ and 1.84, respectively$>t_{.05,9}$; by paired students t test). However, treatment with systemic testosterone + intrahypothalamically infused metapirone failed to raise the mean number of mounts in the 3 post tests ($1.3<t_{.05,9}$). Likewise, the percent of tests during which mounting

Figure 9. The effects of testosterone + metapirone (T+M), testosterone + sucrose (T+S), or estradiol + metapirone (E^2+M) on the mean mount and intromission frequency as a function of the pre and post implant test days. Both T+S and E^2+M but not the T+M treated groups achieved significantly more mounts in the three post tests compared to the pre test scores. The T+S treated group also more intromissions than the other treatment groups although the difference was not significant in the case of the T+M treatment group.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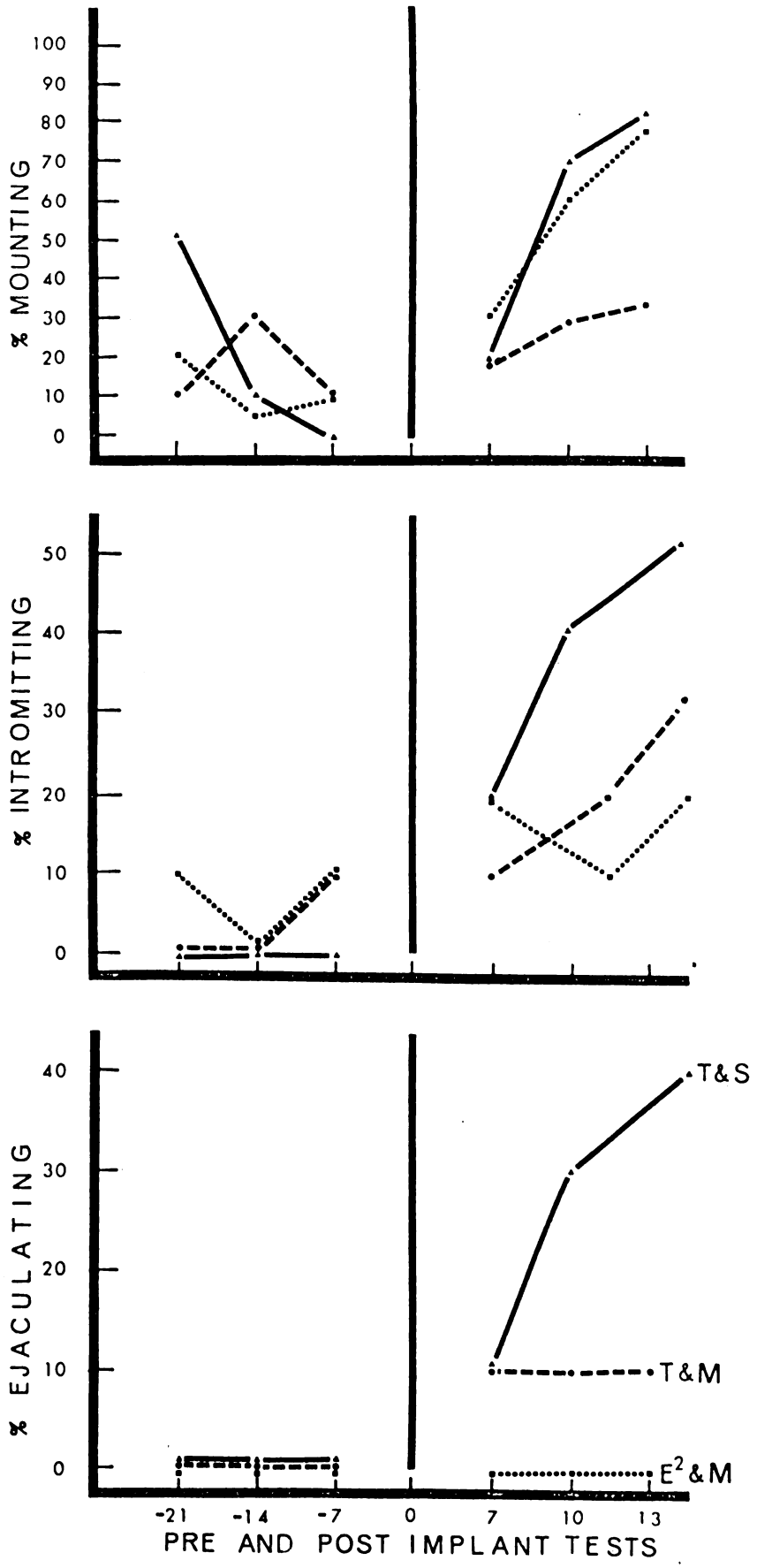
PRE AND POST IMPLANT TESTS

Figure 9.

Figure 10. Percentage of animals mounting, intromitting and ejaculating during the 3 pre implant tests and 3 post implant tests are shown. The following abbreviations are used: T+M, testosterone + metapirone; T+S, testosterone + sucrose; E^2+M, estradiol + metapirone. The T+S treated groups had significantly more animals mounting than the T+M treated group. The T+S treatment was significantly more effective than the E^2+M treatment but not so the T+M treatment in inducing intomissions. The percentage of animals ejaculating under T+S treatment was significantly greater than either of the other treatments.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PRE AND POST IMPLANT TESTS

Figure 10.

Table 4. The means ± SE of mount and intromissions scores for three groups of animals receiving testosterone or estradiol in combination with intracerebrally infused metapirone or sucrose.

	Test Days from Implantation	Testosterone + Metapirone $\bar{x}$ ± SE	Testosterone + Sucrose $\bar{x}$ ± SE	Estradiol + Metapirone $\bar{x}$ ± SE
Mean Mount Frequency	-21	0.4 ± 0.4	3.5 ± 1.88	3.7 ± 2.50
	-14	5.0 ± 3.29	0.1 ± 0.1	0.0
	-7	3.6 ± 2.83	0.0	1.1 ± 1.1
	7	3.6 ± 2.83	0.9 ± 0.8	5.5 ± 2.85
	10	5.1 ± 2.85	11.2 ± 3.40	8.8 ± 2.97
	13	8.4 ± 6.04	10.0 ± 3.00	9.3 ± 5.26
Mean Intromission Frequency	-21	0.0	0.0	0.2 ± 0.2
	-14	0.0	0.0	0.0
	-7	0.1 ± 0.1	0.0	0.2 ± 0.2
	7	0.8 ± 0.8	1.2 ± 1.2	0.6 ± 0.43
	10	2.2 ± 1.99	5.4 ± 2.46	0.3 ± 0.3
	13	2.0 ± 1.24	4.66± 1.5	0.3 ± 0.3

Table 5, Percentage of castrated male rats exhibiting mount, intromission or ejaculatory responses after receiving systemic testosterone or estradiol plus intracerebrally infused metapirone or sucrose.

	Test Days from Implantation	Testosterone + Metapirone	Testosterone + Sucrose	Estradiol + Metapirone
	-21	10	50	20
	-14	30	10	00
Percent	-7	10	00	10
Mounting	7	10	20	30
	10	30	70	60
	13	33	80	77
	-21	00	00	10
	-14	00	00	00
Percent	-7	10	00	10
Imtromitting	7	10	10	20
	10	20	40	10
	13	33	50	20
	-21	00	00	00
Percent	-14	00	00	00
Ejaculating	-7	00	00	00
	7	10	10	00
	10	10	30	00
	13	10	40	00

was exhibited, was significantly greater in the testosterone + sucrose and the estradiol + metapirone groups when compared to the testosterone + metapirone treated animals (X^2=5.10 and X^2=5.50, respectively>$X^2_{.05,1}$) (Figure 10 Table 5).

Treatment with testosterone + sucrose was significantly more effective in the induction of intromissions than was estradiol + metapirone ($p<.05$ DNMR). Testosterone + sucrose treatment tended to be more effective than testosterone + metapirone although the difference did not reach the level of significance 0.16 (F=2.7). Likewise, the percent of tests positive for intromissions was not significantly greater for the testosterone + sucrose groups when compared to the estradiol + metapirone (X^2=2.01<$X^2_{.05,1}$) or to the testosterone + metapirone group (X^2=1.2<$X^2_{.05,1}$).

Histological Results.

The location of the implantation sites for the three groups is shown in Figures 11 and 12. An analysis of variance revealed no statistical differences in the site of implantation between the three groups. The most effective location for metapirone inhibition of mounting was: anterior 7.43, vertical 0.92 and lateral 0.68 (multiple quadratic regression). An analysis of variance revealed that there was no difference between the three groups in the percent of preoptic nucleus destroyed by the implantation technique (F<1). The correlation coefficient between the percent of preoptic nucleus destroyed and the mean mount score per test was calculated for the three groups: Testosterone + Sucrose r=-.36, Testosterone + Metapirone r=-.35 and Estradiol + Metapirone -.38.

It is interesting that the sites of metapirone application least effective in blocking the resumption of sexual behavior, were also the farthest away from the most effective site for blockage. For instace, the three

Figure 11. The locations of maximum cannulae penitration in Experiment II are represented in these cross sectional maps (Pellegrino et al 1967).


O=testosterone + metapirone
□=testosterone + sucrose
△=estradiol + metapirone
△ □ O=no copulation
▲ ■ ●=mounting only
=intromission observed
=ejaculation observed

Figure 11.

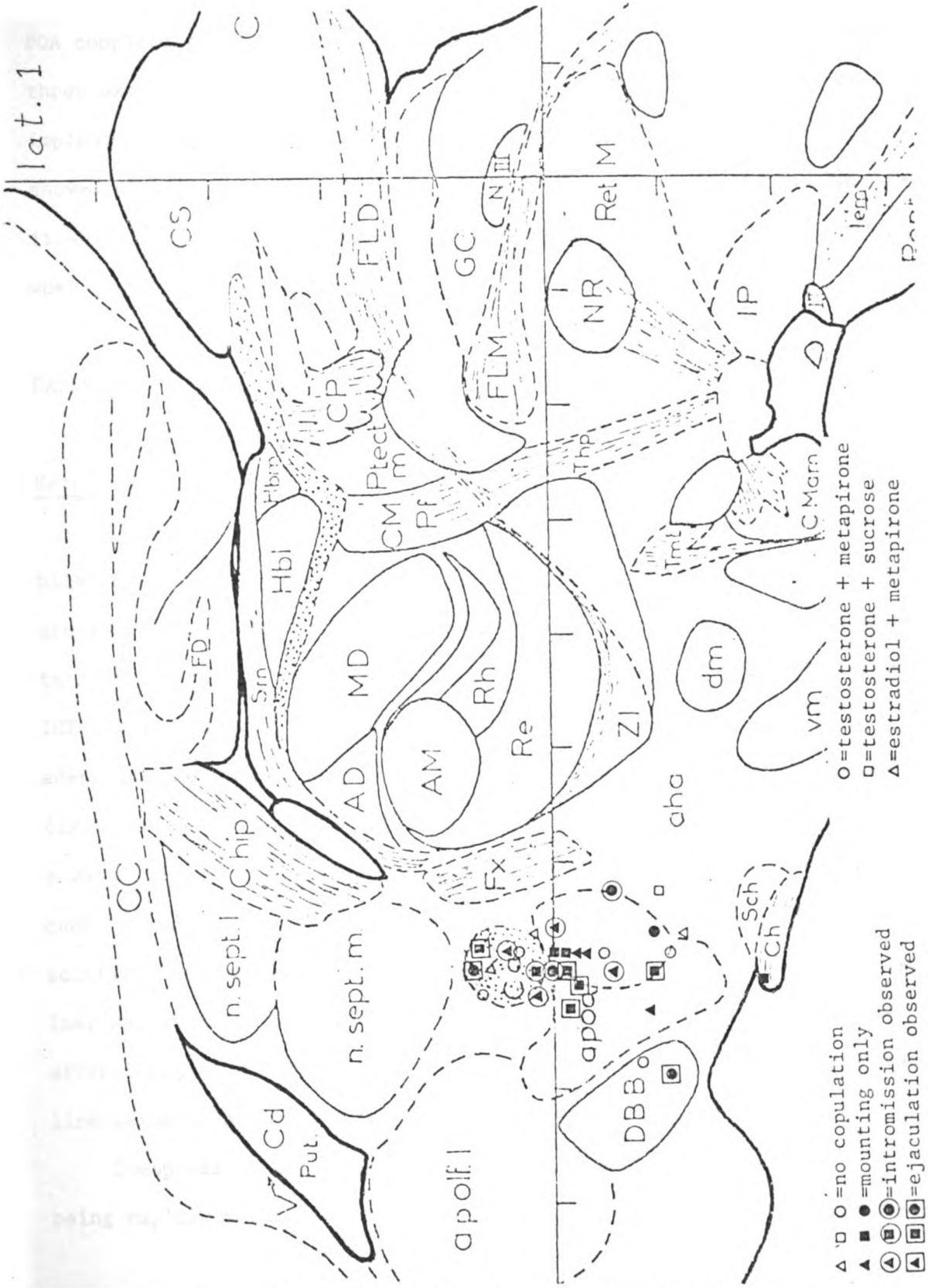

Figure 12. The locations of maximum cannulae penitration in Experiment II are represented in this longitudinal map (Able-Fessard 1966).

animals that exhibited the most mounts and intromissions were out of the POA completely. When an ANOVA was run on the three groups minus these three exceptions plus one in the testosterone + saline group that was implanted in the optic tract, the testosterone + metapirone treated group showed significantly fewer mounts ($F= 4.21 > F_{.05,1,22}$), intromissions ($F= 11.44 > F_{.05,1,22}$) and percent of tests showing ejaculations ($X^2=7.78 > X^2_{.05,2}$) when compared to the testosterone + sucrose group.

EXPERIMENT III. DETERMINE IF cAMP CAN MIMIC THE EFFECTS OF TESTOSTERONE IN INDUCING THE RESUMPTION OF MASCULINE SEXUAL BEHAVIOR.

Methods.

Procedure for selection, castration and subsequent implantation of bilateral cannulae into the POA (23 gauge guide and 30 gauge obdurator), are descrided in Appendix. Of 30 males, 10 each were randomly assigned to 1 of 3 treatment groups: 1) 25ug of cAMP administered intracerebrally ICT every 24 hours + 5 mg of theophylline (IM) every 12 hr; 2) 25ug of 5' adenosine monophosphate (5'AMP) ICT every 24 hr + 5 mg of theophylline (IM) every 12 hr; or 3) 25ug of Dibutryryl cAMP (Db-cAMP) ICT every 24 hr + 20% C_2OH_6 .9% NaCl solution (ethanoic saline) (IM) every 12 hours. The cAMP, 5'AMP and Db-cAMP were administered in a volume of .005 ml 0.9 NaCl solution per cannula. Theophylline or the control vehicle (ethanoic saline) was injected in a volume of 0.1 ml. All treatments began the day after implantation and lasted 10 days. All animals were tested for masculine sexual behavior on the 7th and 10th day of treatment.

Theophylline was given intramuscularly with cAMP to prevent cAMP from being rapidly metabolized and hence inactivated. Db-cAMP is a diacylated

derivative of cAMP which is apparently resistant to phosphodiesterase degradation (Posternak, Sutherland and Henion 1962, Menahan, Hepp and Weiland 1969, Drammand and Powell 1970). Five ' AMP is the inactive metabolite of cAMP.

Following the behavioral test at day 10, all IM treatments (ie. either theophylline or ethanoic saline) were terminated. On day 11 all animals began 8 days of daily testosterone (150 ug/day) injection (IM) in combination with their ICT (ie. cAMP, 5'AMP or Db-cAMP). Testosterone was administered for 8 days, but the intracerebral treatment lasted only 7 days. On the 8th day of testosterone treatment all animals were tested for masculine sexual behavior.

Results.

Masculine sexual behavior: As seen in Figure 13 and Table 6 none of the nucleotides by themselves restored any component of masculine sexual behavior. However, when testosterone was injected systemically along with the nucleotide, the 5'AMP treated animals achieved significantly more mounts intromissions and ejaculations. None of the animals receiving cAMP or Db-cAMP exhibited any sexual behavior. Only 19 animals were tested at the 8th day of steroid treatment (day 18 postimplant) due to death or overt illness in 11 animals (4 each in cAMP + Testosterone and 5'AMP + Testosterone and 3 in Db-cAMP + Testosterone). Four of the six animals in the 5'AMP group achieved at least one mount in the day 18 test. The mean mount frequency for this group being significantly greater than either cAMP + Testosterone or Db-cAMP + Testosterone ($F=6.89>F_{.05,1,16}$). The 5'AMP + Testosterone group also exhibited significantly more intromissions than the other groups ($F=5.59>F_{.05,1,16}$), but the percent of animals ejaculating did not reach significance ($X^2=3.01<X^2_{.05,2}$).

Figure 13. The effects of intracerebrally applied nucleotides on the mean number of mounts and intromissions as well as the percentage of animals ejaculating during 10 days of intracerebrally applied (POA) nucleotides + theophylline or saline and then 1 week of intracerebral nucleotide + testosterone. Testosterone when given with intracerebrally applied 5'AMP significantly increased the mean number of mounts and intromissions as well as increased the percentage of animals ejaculating when compared to cAMP or Db-cAMP in combination with testosterone.

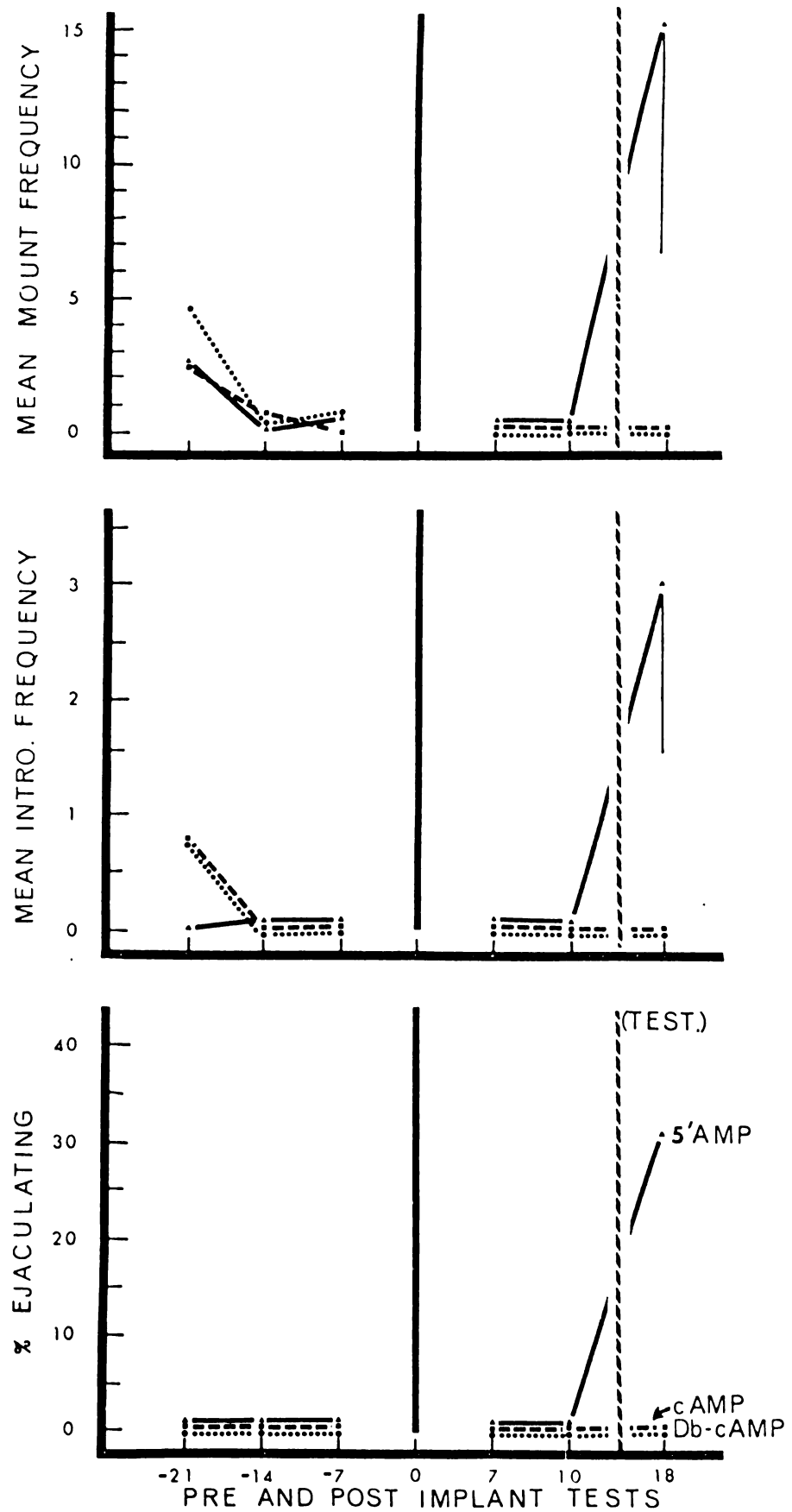
MEAN MOUNT FREQUENCY
15
10
5
0
MEAN INTRO. FREQUENCY
3
2
1
0
% EJACULATING
40
30
20
10
0
(TEST.)
5'AMP
cAMP
Db-cAMP
-21
-14
-7
0
7
10
18
PRE AND POST IMPLANT TESTS

Figure 13.

Table 6. Effects of intracerebrally infused 5'AMP, cAMP or Db-cAMP by themselves or with 8 days of testosterone (day 18), on male copulatory patterns.

	Test Days from Implantation	5'AMP $\bar{x} \pm$ SE	cAMP $\bar{x} \pm$ SE	Db-cAMP $\bar{x} \pm$ SE
Mean Mount Frequency	-21	2.7 ± 1.80	2.6 ± 2.49	4.5 ± 4.07
	-14	0.5 ± 0.27	1.0 ± 0.90	0.1 ± 0.1
	-7	0.9 ± 0.9	0.0	0.0
	7	0.0	0.0	0.0
	10	0.0	0.0	0.0
	18(+Test.)	15.0 ± 7.04	0.0	0.0
Mean Intromission Frequency	-21	0.0	0.7 ± 0.7	0.7 ± 0.7
	-14	0.0	0.0	0.0
	-7	0.0	0.0	0.0
	10	0.0	0.0	0.0
	18(+Test.)	3.0 ± 1.61	0.0	0.0
Percent Ejaculating	-21	00	00	00
	-14	00	00	00
	-7	00	00	00
	7	00	00	00
	10	00	00	00
	18(+Test.)	33	00	00

Histological results: The location of the treatment application sites are represented Figures 14 and 15. An analysis of variance reveals there were no significant differences among the three groups in location of implants (each dimension coordinate was analysized separately) or in the percentage of the preoptic nucleus destroyed by the implanted cannulae ($F<1$, in all cases). A coefficient of correlation was calculated between the percentage of the preoptic nucleus destroyed and the mean mount score for the 5'AMP + Testosterone group, $r=-.38$.

EXPERIMENT IV. EFFECTS OF POA ANDRENERGIC OR CHOLINERGIC MANIPULATION ON MASCULINE SEXUAL BEHAVIOR.

Methods.

Subjects were 18 male rats which had been given two 15 minute screening tests for sexual behavior. Animals which ejaculated on both tests were castrated and given daily injections of testosterone propionate (TP) (150ug) for the duration of the experiment. One week following castration. all subjects were bilaterally implanted in the POA, with double-walled stainless steel cannulae (21 gauge outer, 27 gauge inserts). All animals were then assigned to one of two studies (9 animals/study). In Part A all animals received each of the following treatments via cannula (a different treatment every week) 15 minutes before the start of a test for masculine behavior: 1) Norepinephrine (NE), 2) α-methyl tyrosine (α-MT) and 3) the blank cannulae. The animals in Part B of the study received the following drugs designed to manipulate the cholinergic milieu of the POA: 1) Carbamylcholine (carbachol), a cholinomimetic; 2) Hemicholinium-3 (HC-3) (a synthesis inhibitor of acetylcholine) and 3) the blank cannulae. The chemical in both experiments were administered via the cannulae. A

Figure 14. The locations of maximum cannulae penitration in Experiment III are represented in these cross sectional maps (Pellegrino 1967).

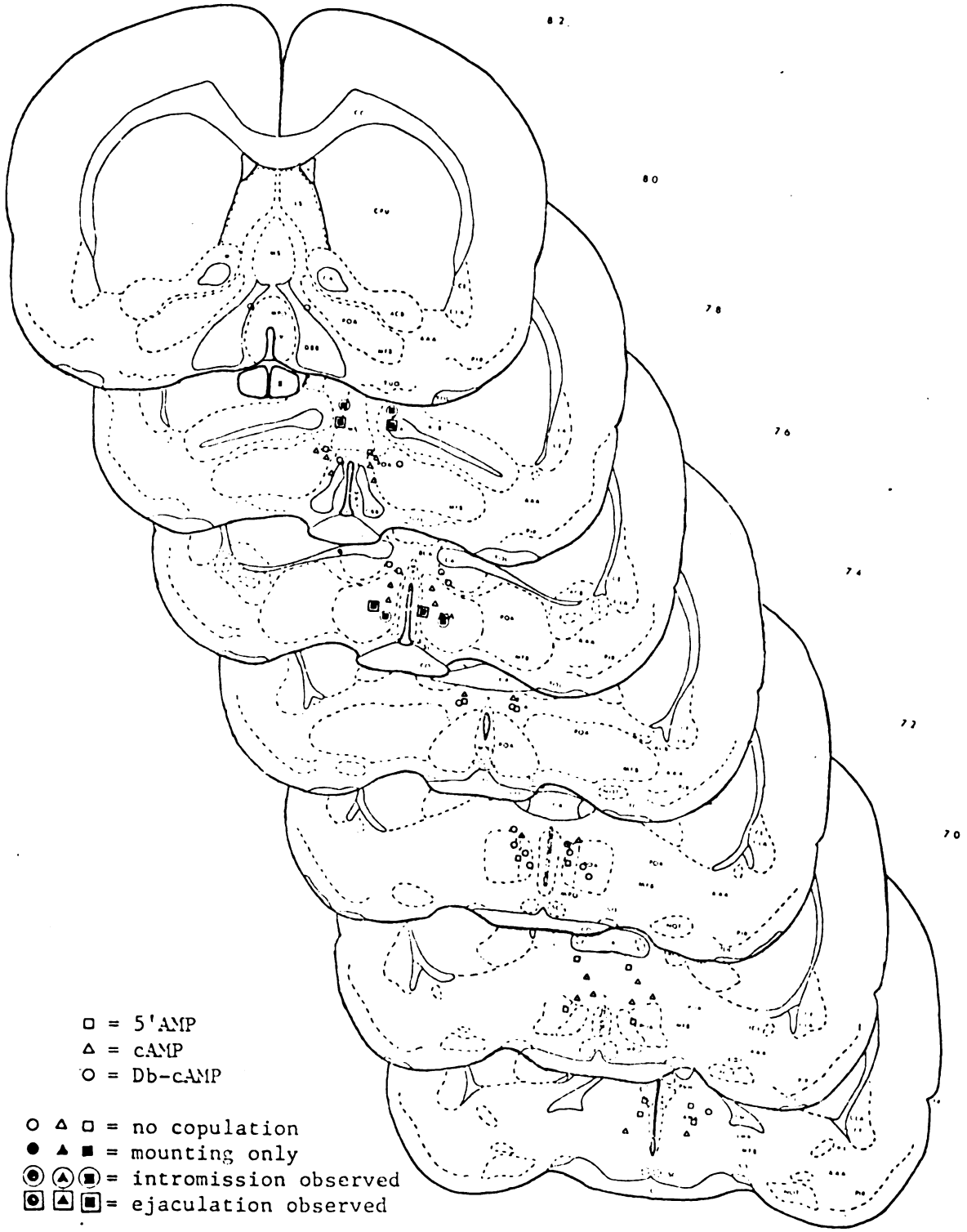
82
80
78
76
74
72
70
□ = 5'AMP
△ = cAMP
○ = Db-cAMP
○ △ □ = no copulation
● ▲ ■ = mounting only
◎ ◬ ▣ = intromission observed
◙ ◭ ▣ = ejaculation observed

Figure 14.

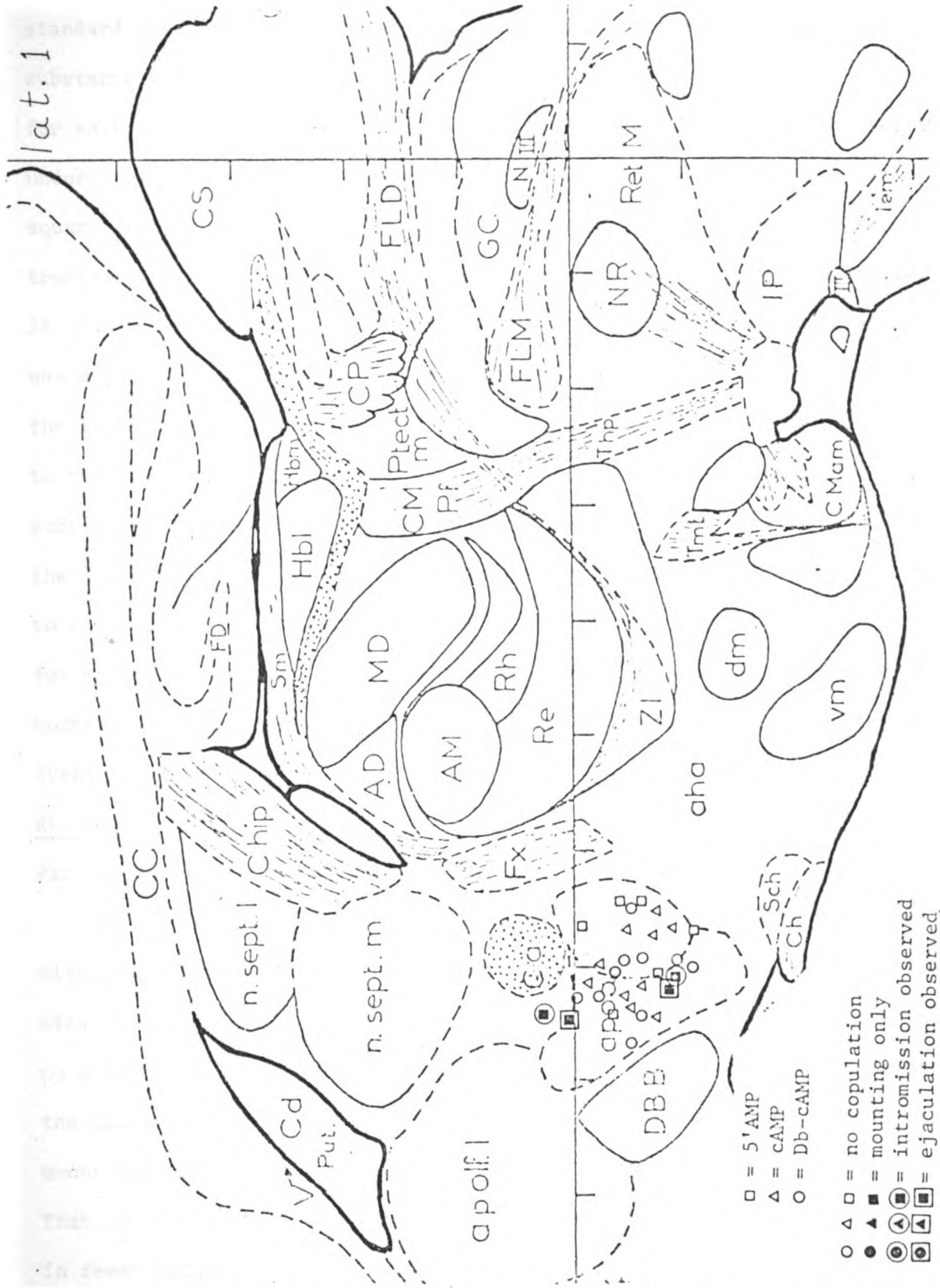

Figure 15. The locations of maximum cannulae penitration in Experiment III are represented in this longitudinal map (Albe-Fessard 1966).

standard dose of 8 taps of the 27 gauge insert, into a thin layer of the substance was used in both experiments (8-10ug). A new insert was used for each chemical and remained in the guide cannulae throughout the testing under that treatment. The experimental design for both studies (a latin square design, Winer 1962) called for each animal to reveive all three treatments during the course of the study. Animals were given an initial 15 minute test for sexual behavior starting 15 minutes after the chemical was applied to the POA. A second 15 minute test was given 4 hours after the initial treatment. The four hour interval from initial treatment to the second test was chosen in order to allow the synthesis inhibitors sufficient time to act. A second administration of the treatment (half the initial dose was given 15 minutes before the start of the 4 hour test to control for the relative speed of action of the various compounds. Tests for masculine sexual behavior were 15 minutes long, and the occurance of mounts, intromissions and ejaculations was recorded on an Esterline-Angus Event Recorder.

Results.

Part A: Adrenergic manipulation:

NE when compared to the sham treatment reduced the number of intromissions and ejaculations in the first test and the number of mounts, intromissions and ejaculations in the second test. The applicaiton of α-MT had no effect on sexual behavior in the first test but significantly decreased the number of ejaculations in test 2. NE increased the latency to first mount and first intromission as well as the time between intromissions. That is to say it slowed down the copulatory performance, resulting in fewer mounts, intromissions and ejaculations being exhibited during the test. Alpha-MT had no effect on the latency measures during either test.

The effects of intracerebral application of norepinephrine, or the synthesis inhibitor, α-methyltyrosine on copulatory behavior are represented in Figure 16 and Table 7. In the 1st test (Test I), given 15 minutes after treatment, neither NE or α-MT significantly affected the mean number of mounts achieved when compared to the sham treatment (blank applicator) ($F<1$ in both treatments). However, NE significantly decreased the mean number of intromissions ($F=8.2>F_{.05,1,6}$) and ejaculations ($F=10.1>F_{.05,1,6}$) achieved during the 15 minutes of Test I. Alpha-MT had no significant effect on intromission frequency ($F=1.3<F_{.05,1,6}$) or ejaculatory frequency ($F<1$). A further analysis of only the first ejaculatory series in Test I (Table 7) revealed that neither NE or α-MT treatment had effects on the mean number of mounts or intromissions. On the other hand, NE treatment increased the mean mount latency (ML) ($F=5.20>F_{.05,1,24}$) in series 1, compared to the sham treatment. There was also a significant increase in the intromission latency of series 1 when the animals were given NE ($F=39.11>F_{.05,1,24}$). There were however, no significant differences between the sham treatment and α-MT during the 1st series in ML or IL. NE but not α-MT, increased the mean inter-intromission-interval (MIII) of series 1 ($F=4.24$[NE], $F<1$ [α-MT]). No significant differences were detected ($p<.05$) between α-MT and sham treatments or between NE and sham treatment in the percentage of animals exhibiting mounts, intromssions or ejaculatory responses in series 1.

In Test II, the 15 minute test given 4 hours after intracerebral treatment, both α-MT as well as NE had significant effects on certain components of masculine sexual behavior. NE but not α-MT significantly decreased the mean number of mounts when compared to the sham treatment ($F=3.98$[NE] and $F=0.86$ [α-MT]). Likewise, NE ($F=9.5>F_{.05,1,24}$) but not α-MT ($F=3.15<$

Figure 16. The effects of intracerebrally administered drugs on parameters of sexual behavior are represented. Two 15 min tests were given under each treatment: Test I (15 min after the initial exposure) Test II (given 4 hrs after the initial exposure). * = significantly different from the sham treatment during that test.

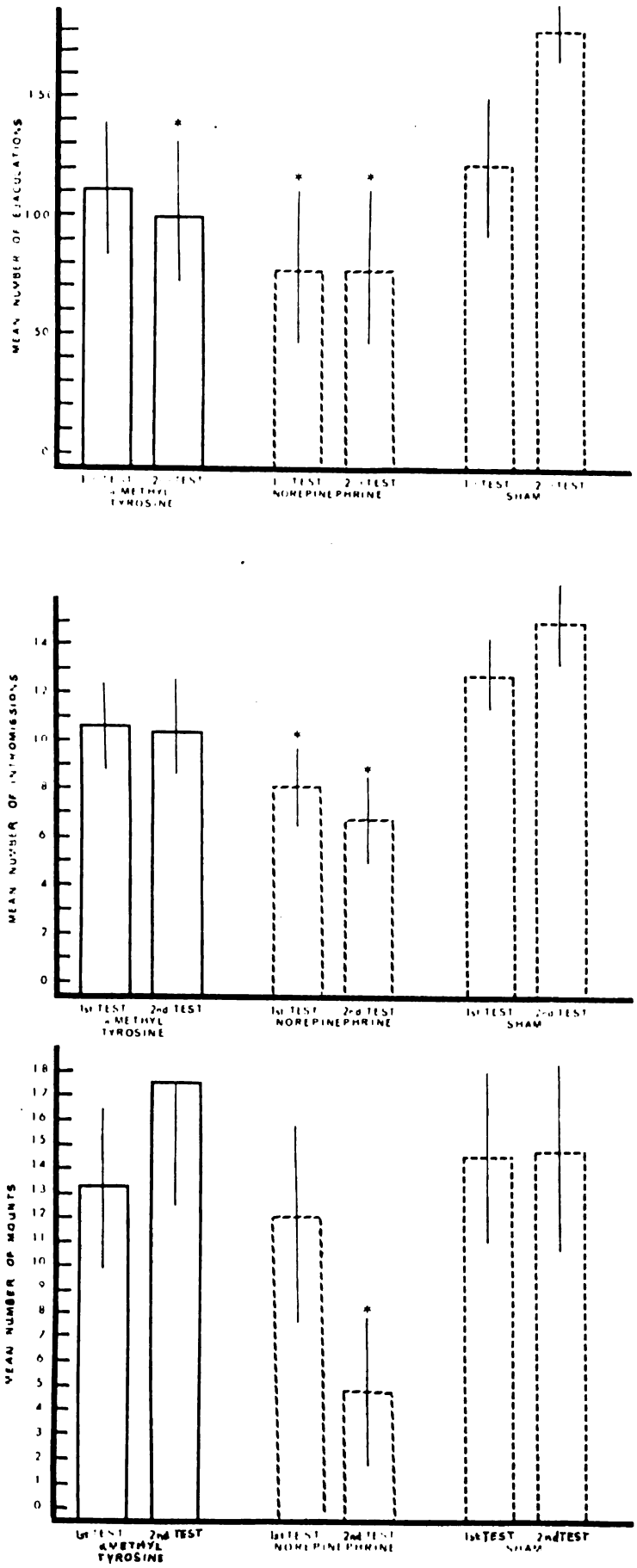
MEAN NUMBER OF EJACULATIONS
150
100
50
0
1st TEST
2nd TEST
α-METHYL TYROSINE
NOREPINEPHRINE
SHAM
MEAN NUMBER OF INTROMISSIONS
14
12
10
8
6
4
2
0
MEAN NUMBER OF MOUNTS
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0

Figure 16

Table 7. Effects of adrenergic manipulation of the POA on parameters of masculine sexual behavior. The following abbreviations are used: MF, mean mount frequency; IF, mean intromission frequency; EF, mean ejaculatory frequency; ML, mount latency, IL, Intromission latency; MIII, mean inter intromission interval. The abbreviations with (-1) behind them refer to parameters in the 1st ejaculatory series. The other abbreviation are for the entire 15 minute test period.

	Test I									Test II								
	Sham			NE			α-MT			Sham			NE			α-MT		
	$\bar{x}$	±	SE	$\bar{x}$	±	SE	$\bar{x}$	±	SE	$\bar{x}$	±	SE	$\bar{x}$	±	SE	$\bar{x}$	±	SE
MF	14.44	±	2.81	11.78	±	3.68	13.13	±	3.34	14.56	±	2.97	4.89	±	2.91	17.33	±	5.04
IF	12.78	±	1.20	7.67	±	2.06	10.56	±	1.55	15.00	±	2.11	6.87	±	2.46	10.33	±	2.17
EF	1.22	±	0.27	0.75	±	0.37	1.11	±	0.27	1.88	±	0.23	0.78	±	0.32	1.00	±	0.29
MF-1	12.33	±	1.89	9.55	±	0.89	9.44	±	1.00	10.56	±	2.52	3.23	±	3.70	10.22	±	2.19
IF-1	10.33	±	2.00	6.11	±	2.10	8.33	±	1.20	17.88	±	1.15	4.89	±	1.85	7.44	±	0.74
ML-1	24.50	±	96.38	309.60	±	190.00	28.55	±	92.30	12.89	±	162.89	403.56	±	227.78	110.89	±	64.89
IL-1	39.05	±	166.50	395.56	±	190.00	182.06	±	23.5	15.78	±	228.96	512.89	±	268.15	205.56	±	39.19
MIII-1	58.21	±	127.58	348.72		162.55	151.60	±	34.56	31.80		229.44	521.57	±	260.33	230.36	±	30.89

$F_{.05,1,24}$) significantly reduced the mean number of intromissions. However, the application of either α-MT significantly decreased the mean number of mounts when compared to the sham treatment (F=3.98 [NE] and 0.86 [α-MT]). Likewise, NE ($F=9.5>F_{.05,1,24}$) but not α-MT ($F=3.15<F_{.05,1,24}$) significantly reduced the mean number of intromissions. However, the application of either α-MT or NE significantly reduced the number of ejaculations achieved in the 15 minute Test II (F=11.67 and $6.17>F_{.05,1,24}$ respectively). Neither α-MT or NE treatment significantly affected the percentage of animals exhibiting mounts or intromissions. However, NE but not α-MT treatment significantly reduced the percentage of animals exhibiting the ejaculatory response in Test II ($X^2=6.92>X^2_{.05,2}$). As in the case of Test I, neither NE or α-MT treatment had significant effects on the mean mount frequency of series 1 in Test II. Likewise, NE but not α-MT treatment significantly increased the ML (F=8.13 [NE]; F<1 [α-MT]) and IL (F=12.59 [NE]; F<1 [α-MT]) in series 1. As in Test I, NE but not α-MT significantly increased the MIII of series 1, Test II (F=12.93 [NE]; F=2.12 [α-MT]).

Part B: Cholinergic manipulation.

The effects of the carbachol, hemicholinium and sham treatments on the number of mounts, intromissions and ejaculatory responses achieved during Test I (given 15 minutes after the initial exposure) and Test II (given 4 hours after the initial exposure) are represented in Figure 17 and Table 8. Application of carbachol abolished all masculine sexual behavior patterns. A total of 3 mounts in Test I (and 1 mounts in Test II) were achieved by the nine animals when treated with carbachol, compared to the sham treatment (104 and 79 in Test I and Test II, respectively). Carbachol effectively inhibited the display of any intromission or ejac-

Figure 17. The effects of intracerebrally administered drugs on the mean number of mounts, intromissions and ejaculations during tests for masculine sexual behavior as represented. * = significantly different from the sham treatment during that test.

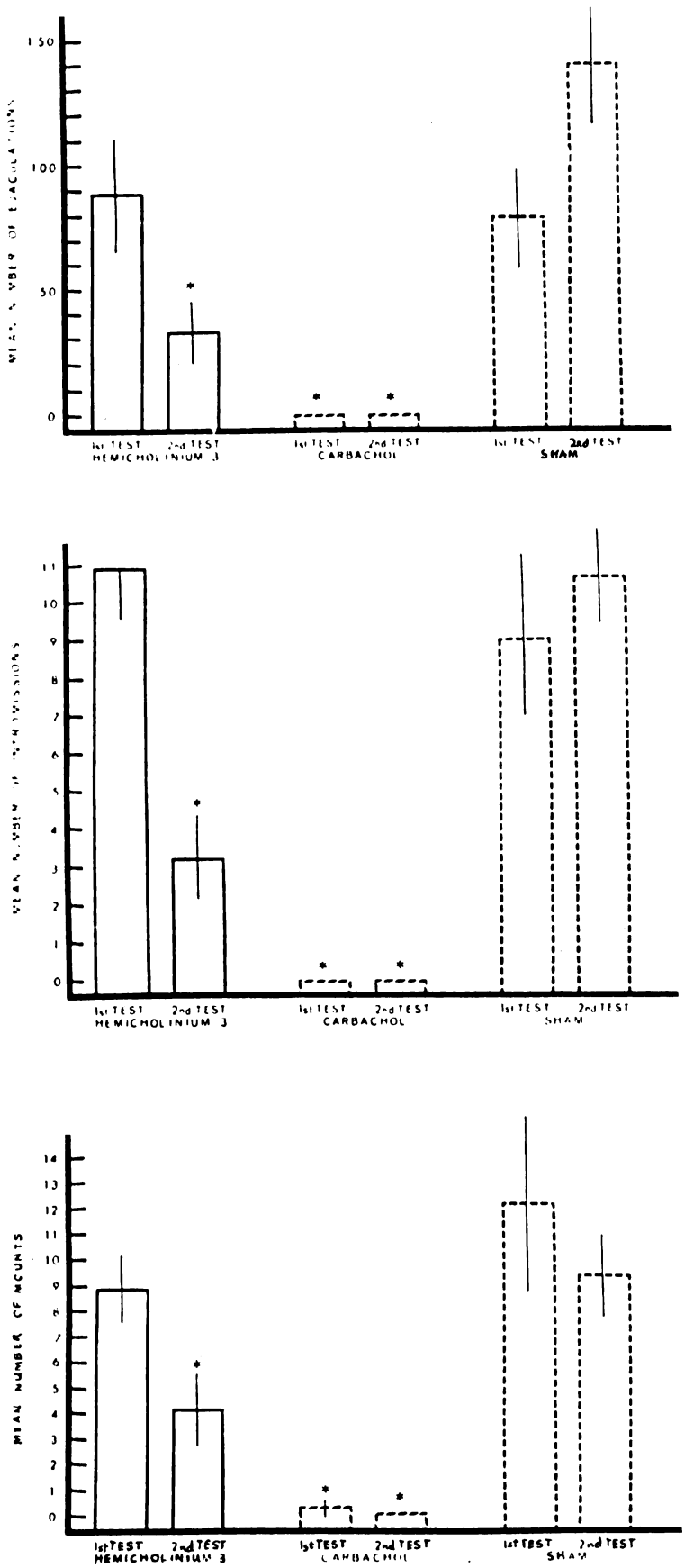
MEAN NUMBER OF EJACULATIONS
150
100
50
0
1st TEST
2nd TEST
HEMICHOLINIUM 3
CARBACHOL
SHAM
MEAN NUMBER OF EMISSIONS
11
10
9
8
7
6
5
4
3
2
1
0
MEAN NUMBER OF COUNTS
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0

Figure 17.

Table 8. Effects of cholinergic manipulation of the POA on parameters of masculine sexual behavior. The following abbreviations are used: MF, mean mount frequency; IF, mean intromission frequency; EF, mean ejaculatory frequency; ML, mount latency; IF, intromission latency; MIII, mean inter intromission interval. The abbreviations with (-1) behind them refer to parameters in the 1st ejaculatory series. The other abbreviations are for the entire 15 minute test period.

	Test I			Test II		
	Sham	Carb	HC-3	Sham	Carb	HC-3
	$\bar{x}$ ± SE	$\bar{x}$ ± SE	$\bar{x}$ ± SE	$\bar{x}$ ± SE	$\bar{x}$ ± SE	$\bar{x}$ ± SE
MF	11.56 ± 4.53	0.33 ± 0.2	8.89 ± 1.24	8.78 ± 1.79	0.1 ± 0.1	4.22 ± 1.50
IF	9.0 ± 2.20	0.00	10.89 ± 1.35	10.67 ± 1.44	0.00	3.11 ± 1.09
EF	0.78 ± 0.28	0.00	0.89 ± 0.24	1.44 ± 0.24	0.00	0.33 ± 0.14
MF-1	11.11 ± 4.4	0.33 ± 0.2	8.66 ± 1.96	6.22 ± 3.0	0.11 ± 0.11	3.22 ± 0.03
IF-1	8.56 ± 2.7	0.00	9.0 ± 3.14	7.89 ± 4.0	0.11 ± 0.11	3.50 ± 0.29
ML-1	58.44 ± 215.52	721.78 ± 447.81	41.67 ± 232.30	54.78 ± 367.93	801.11 ± 379.41	409.25 ± 357.78
IL-1	252.18 ± 163.03	900.00 ± 0.00	95.56 ± 320.60	144.78 ± 377.68	900.00 ± 0.00	522.44 ± 301.78
MIII-1	242.18 ± 161.02	900.00 ± 0.00	67.45 ± 290.1	126.33 ± 402.32	900.00 ± 0.00	559.33 ± 234.56

ulatory responses in Test I and II. Carbachol, however was not totally behaviorally debilitating. A significant number of animals exhibited fighting with the stimulus female ($X^2=8.8>X^2_{.05,2}$) when compared to the sham treatment.

The intracerebral administration of hemicholinium had no significant effect on the occurance of mounts, intromissions or ejaculations in Test I when compared to the sham treatment. However, in Test II when compared to their sham treatment results, the animals under the influence of hemicholinium-3 (HC-3) achieved significantly fewer mounts (F=6.24), intromissions per ejaculation (F=21.01) and ejaculations (F=28.6) (all greater than the comparative $F_{.05,1,24}$). Likewise the percent of animals achieving an ejaculation during Test II was significantly reduced with HC-3 treatment.

An analysis of variance involving the first ejaculatory series of both Test I and Test II reveals a significant increase in ML (F=50.8 [Test I and II, respectively]) when animals were given carbachol compared to the sham treatment. Likewise, carbachol treatment significantly decreased the mean MF and IF of series 1. No significant differences between HC-3 and sham treatment were detected in ML or IL during the 1st series of Test I. However, the HC-3 treatment, when compared to the sham test, significantly prolonged the ML ($F=7.15>F_{.05,1,18}$) and IL ($F=8.2>F_{.05,1,18}$) during Test II. Similarly, the MF (F=5.08) and IF ($F=6.40>F_{.05,1,24}$) of the animals under HC-3 (Test II) was significantly decreased compared to the sham treatment. The increased ML and IL as well as the decreased MF and IF observed during Test II were to a large extent, due to inactivity by animals previously exhibiting a prolonged (over 20 minutes) post ejaculatory interval (PEI). A significantly greater number of these prolonged PEI's

were exhibited during Test I by animals treated with HC-3 when compared to the sham treatment ($X^2=8.87>X^2_{.05,2}$).

Histological results:

The location of the implants from animals in both Part A and B of experiment IV, are exhibited in Figures 18 and 19. The location of the majority of the implants were centralized around the preoptic-anterior hypothalamic continuum. Inspection of Figures 18 and 19 for the anterior-posterior distribution of the implants reveals that they lie fairly well restricted within a range from anterior 6.2 to 7.6. The vertical range for both groups was from 1.1 to 3.3 and lateral from 0.8 to 1.6. The anterior coordinate in this experiment tended to be somewhat more posterior than in the other experiments. This experiment happened to be the first of the four conducted, and corrections were made to move the implants up to the coordinates in the other experiments. The mean location of the implants for the adrenergic and cholinergic groups were determined from corrdinates utilizing Pellegrina et al (1965) Atlas of the Rat Brain: Cholinergic; anterior 7.0, vertical 2.6, lateral 1.3; Adrenergic, anterior 6.9, vertical 2.8 and lateral 1.3.

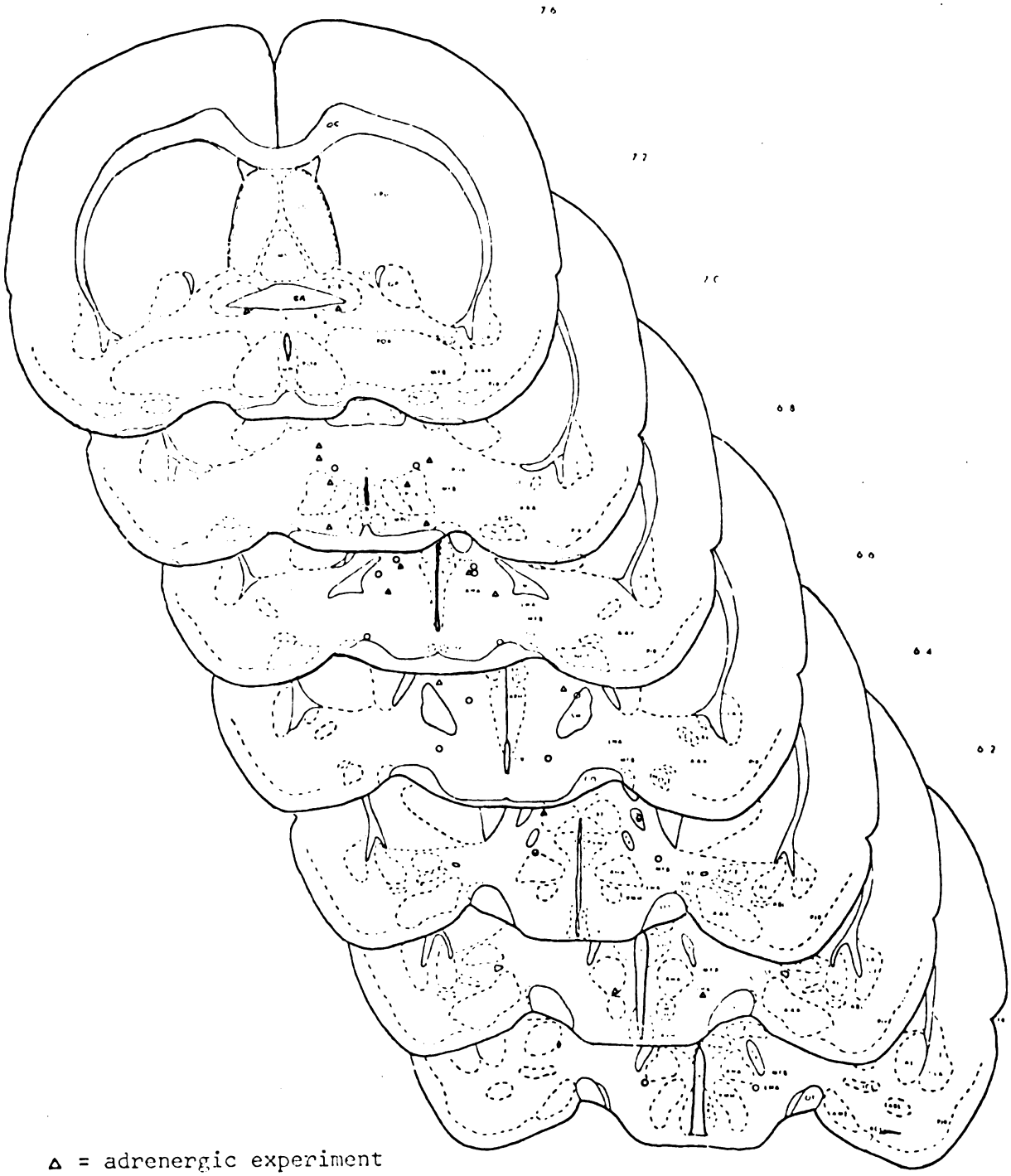

Figure 18. The locations of maximum cannulae penitration in Experiment IV are represented in these cross sectional maps (Pellegrino 1967).

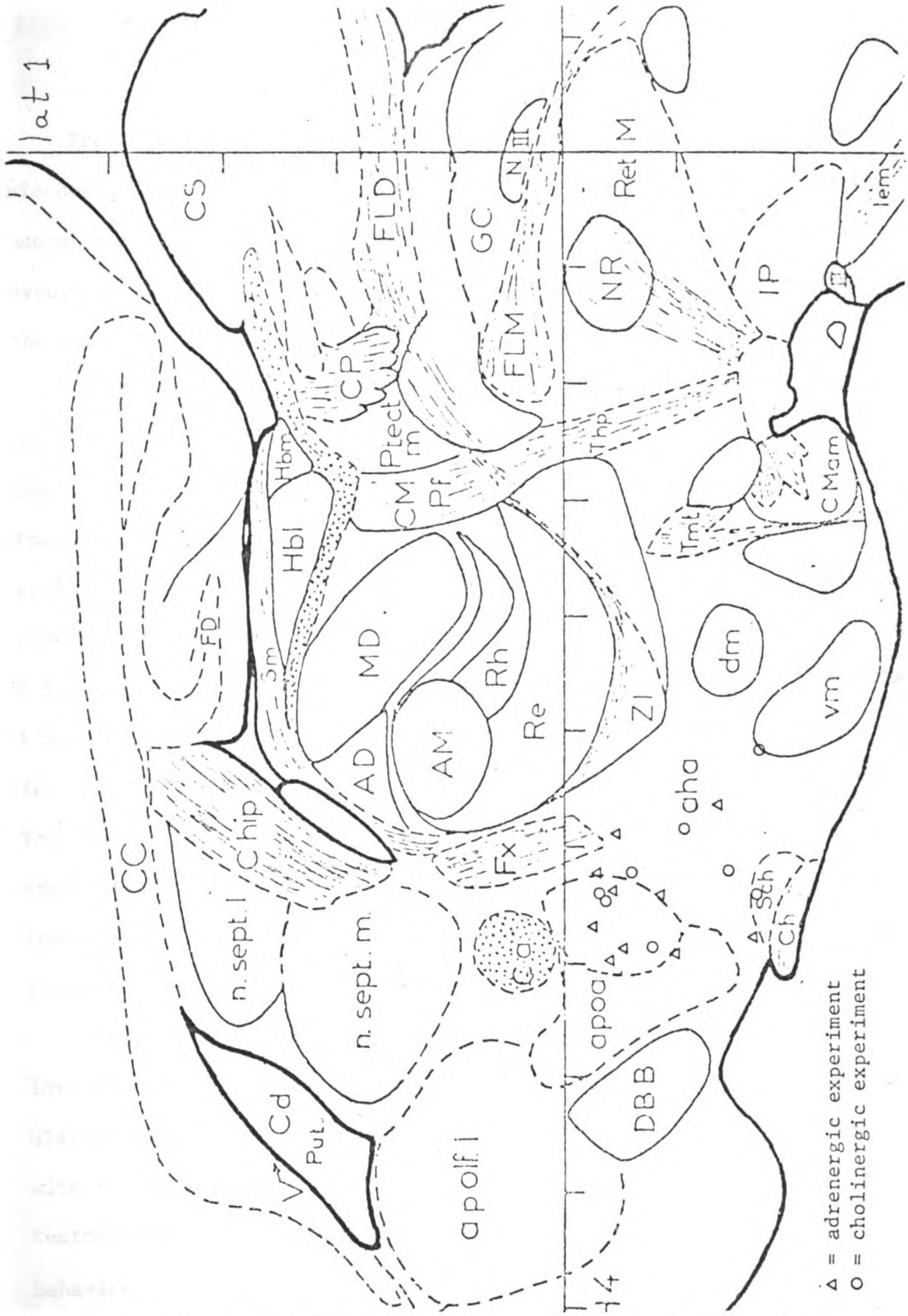

Figure 19. The locations of cannulae placements in Experiment IV are represented in this longitudinal map (Able-Fessard 1966).

DISCUSSION

The results of this study extend the current information on the neurobiochemical control of masculine sexual behavior in three areas: 1) the neurohormonal specificity of behavioral activation, 2) the intracellular events associated with testosterone restoration of mating behavior and 3) the pharmacological control of male copulatory behavior.

Intracerebral application of testosterone or estradiol directly to the POA restored copulatory behavior in long term castrated male rats. Similar implants of testosterone or estradiol in the PHA were less effective than their respective POA comparisons and no more effective than cholesterol (POA) controls, in reinstating copulation. The suggestion that testosterone is converted to estradiol before activating male sexual behavior, was further supported by the finding that application of metapirone, an aromatization inhibitor, to the POA of long term castrated males, blocked the increase in sexual behavior normally associated with testosterone therapy. This inhibition was not a result of the toxic effects of the drug, since administration of estradiol in combination with metapirone resulted in an increase in mounts over the pretreatment tests similar to that observed with testosterone + sucrose treatment.

The infusion of solutions containing cAMP, 5'AMP or Db-cAMP directly into the POA of long term castrates was ineffective in reinstating the copulatory behavior of any of the animals tested. When given in combination with 150 ug of testosterone daily, only the animals treated with 5'AMP + testosterone daily resumed significant amounts of mount and intromission behavior.

Application of drugs to the POA designed to either increase or decrease adrenergic or cholinergic activity, all had similar effects on the gross

measures of sexual behavior, that is. , all decreased some components of sexual behavior. However, the mechanisms by which these changes in behavior were effected appear to be different. In both the cholinergic and adrenergic experiments, the effects of the mimetics were observed in both Test I and II, suggesting an immediate action of the drugs. The inhibitors, on the other hand, had no effects on copulatory measures until the 4 hour test (Test II), suggesting a longer action latency than the mimetics. Likewise, the various treatments affected different copulatory measures (eg. MF, IF, ML and IL).

1) Neural Hormonal Specificity

The findings of the present study support the concept that the POA of male rats is intimately involved in the regulation of male copulation by testosterone. Testosterone implants in the POA facilitated male sexual behavior but implants in the PHA did not. This suggests that the effects of the hormone are specific for the POA. Estradiol was also more effective than cholesterol in stimulating male mating behavior when placed in the POA but not when placed in the PHA. Stimulation of penile morphology by testosterone implants in both the POA and PHA suggests that significant amounts of testosterone entered the systemic cirulation. However, the inability of the PHA implants to effect increases in mating behavior suggests that the levels of testosterone reaching areas of the brain other than the implant site, via plasma circulation or diffusion, were not sufficient to influence behavior. Only when the POA was stimulated by high concentrations of hormone was mating beahvior activated. Thes results indicate that testosterone and/or estradiol effect certain changes in the POA to activate masculine sexual behavior.

Two results in the present study support the concept that testosterone is aromatized to an estrogen in the POA before stimulating masculine sexual behavior. 1) Estradiol implanted in the POA was more effective than POA implants of testosterone in stimulating male copulation. 2) Inhibition of testosterone induced sexual reactivation by infused metapirone. Clinical use of metapirone has shown that prolonged systemic adminsitration of metapirone results in reduced adrenal production of cortisol through its inhibition of adrenal 11 β-hydroxylation. As a result, a compensatory increase in ACTH release follows and the secretion of 11-deoxycortisol is accelerated (Goodman and Gillman 1970). However, the effective blockage by metapirone seen in the present research, does not appear to be the result of this toxic side effect, since when metapirone was given in combination with estradiol the same increase in mounting was observed as with testosterone + sucrose treatment. The suggestion that testosterone is aromatized before activating mating behavior also has support from studies reporting the inability of the reduced androgen DHT, to stimulate male sexual behavior when administered systemically (McDonald et al 1970, Whalen and Luttge 1971) or intrahypothalamically (Hohnston and Davidson 1972). Likewise, systemic administration of estradiol has been shown to be stimulatory to male sexual behavior (Pfaff 1970, Davidson 1966). The concept that the conversion of testosterone to an estrogen occurs in the POA has also received support by the finding that <u>in vitro</u> conversion of androstenedione to estrone occurs in the diencephalon of male rats (Naftolin et al 1972). Thus, for the stimulation of masculine sexual behavior, it appears as though testosterone serves as a prehormone, being carried to the target tissue (the brain) where it is converted to the active configuration, presumably estradiol. This model fits well the established prehormone role testosterone serves in the stimulation of systemic tissues; testosterone being converted to DHT

instead of estradiol in the systemic targets.

2) Intracellular Events Associated With Testosterone Reactivation.

From the results of Experiments II and II information was gained on two possible intracellular events associated with testosterone stimulation of the POA: 1) Aromatization of testosterone to estradiol in the POA and 2) involvement of cAMP in neural-hormonal regulation of masculine sexual behavior. The blockage of testosterone-induced sexual behavior, by administering an aromatization inhibitor (metapirone) adds support to the hypothesis that testosterone is converted in the POA to an estrogen. If the processes involved in the conversion of testosterone to DHT in the seminal vesicle, prostate and epididymus are taken as a model for testosterone in the brain, the conversion process would take place intracellularly. As in the stimulation of androgen-sensitive accessory tissues, testosterone in the POA wouls enter the target cell where it would bind with a cytosol receptor. It would then be metabolized while attached to the receptor, to the active configuration (estradiol) and transferred to the nuclear receptor, which is at least of different molecular weight than the cytosol receptor, although it may be related in part (Jensen, Numata, Brecher and DeSombre 1971). Although the cytosol and and nuclear receptors in the POA along with the corresponding structure of the hormones bound to them have not been isolated as they have been in the case of other tissues (Bruchousky and Wilson 1968), the in vitro conversion of testosterone to estradiol in the diencephalon of rat brains has been demonstrated (Naftolin et al 1972).

The known events in the aromatization process involve the removal of C-19 carbon and the aromatization of the A ring. The removal of the C-19 carbon involves hydroxylation at this site and relies upon a C-19 hydroxylase dependent on $NADP^+$. Thus, the production of the C-19 hydroxylase would

be one of the limiting steps in the stimulation of the nucleus by testosterone. If again, we concider the working hypothesis developed for the stimulation of genital tissues by testosterone via conversion to DHT, the activaty of the rate limiting enzyme in this system, 5α-reductase, is inversly related to the concentration of testosterone (Kniewald, Massa and Martini 1971). Thus, testosterone seems to play a role in regulating the enzymes involved in its conversion to DHT. This may also be the case in the conversion process of testosterone to estradiol, although no experimental data are available to confirm this assumption.

A number of studies have implicated the nucleotide 3'5'cAMP as a rate determining factor in many steroidogenic processes, (ie. corticosterone production in the adrenals, Robinson, Buther and Sutherland 1968 and gonadal steroid synthesis in the testis and ovaries, Sandler and Hall 1966, Connell and Eik-Nes 1968). Specifically, evidence has been presented that cAMP stimulates steroidogenesis by increasing the conversion of cholesterol to pregnenolone (Karaboyas and Koritz 1965). On the other hand, studies have shown that in vitro administration of cAMP inhibits the NAD^+ dependent 5-ene-3β-hydroxysteroid dehydrogenase in adrenal cortex (Koritz, Yun and Ferguson 1968, McCune, Roberts and Young 1970) and ovarian tissues (Sulimovici and Luneufeld). This last enzyme is involved in the conversion of testosterone to androstenedione. Thus, cAMP may be involved in controlling steroidogenic metabolism in a number of different tissues and systems.

The possibility of cAMP being involved in testosterone stimulation of masculine sexual behavior was suggested in an earlier study by Christensen and Clemens (in press). In results of that experiment theophylline potentiated submaximal levels of TP in the stimulation of masculine behavior. One conclusion drawn from these data was that theopylline, in line with its known effects of preventing the degradation of cAMP, stimulated sexual

behavior by preventing the metabolism of cAMP, synthesized during testosterone stimulation. The subsequent build-up in cAMP was thought to have accentuated the TP. Experiment III in the present study ruled out the possibility that cAMP was serving as the sole intracellular mediator for all the action of testosterone, since intra-hypothalamic injection of solutions containing cAMP or Db-cAMP failed to initiate copulation in long term castrated males. An alternative consideration was that intracellular cAMP was involved in the production of substrates (ie. receptor or C-19 hydroxylase) necessary for the incorporation of testosterone into the cell and its eventual movement to the nucleus. However, the data generated from the second half of experiment III imply that the role of cAMP in the neuro-hormonal regulation of sexual behavior is somewhat more complicated. Instead of potentiating low doses of testosterone as the previous study with theophylline suggested, infusion of cAMP or Db-cAMP inhibited the activation of sexual behavior when compared to the 5'AMP treated group. Several hypotheses dealing with the apparent inconsistency between the previous study (Christensen and Clemens in press) and the results of experiment III will now be discused.

Firstly, in addition to the known effects of theophylline in preventing cAMP degradation in many tissues (Greengard and Costa 1970) theophylline has been shown to decrease the content of cAMP in <u>in vitro</u> brain slices (Forn and Krishna 1973, Shimizu, Daily and Creveling 1969, Palmer, Sulser and Robinson 1973). If this were, in fact, the effect of our <u>in vivo</u> administration of theophylline, and its synergism with testosterone was due to a decrease in intracellular cAMP, then these results would collaborate those of experiment II in suggesting that low levels of cAMP potentiate testosterone. In fact, the data of experiment III suggest that high levels of cAMP inhibit the action of testosterone. CyclicAMP has been shown to inhibit

several NAD^+ dependent dehydrogenases, specifically the 17β-hydroxylase dehydrogenase involved in the *in vitro* conversion of testosterone to androstenedione (Sulimovici and Lunenfeld 1972). The possibility therefore exists that infusion of cAMP or Db-cAMP into the POA is preventing the metabolism of testosterone to some effective compound (eg. estradiol), by interfering with the NAD^+ dependent process. Two such NAD^+ dependent processes which may be of importance are 1) the conversion of estradiol to estrone and 2) the conversion of testosterone to androstenedione. In human placental microsomes, androstenedione appears to be the immediate precursor of estrogens rather than testosterone (Menimi and Engel 1967).

A second possible explanation for the variance in the observed results in experiment II is that cAMP and Db-cAMP are duplicating the effects of neurotransmitters. Specifically Rindi, Sciorelli, Poloni and Acanfora (1972) have shown that when applied directly to the lateral hypothalamic area of rats, Db-cAMP but not cAMP mimicked the effect of implanted carbachol, both of which significantly increased food and water intake. Although the suggestion made by Rindi et al. that cAMP acts by releasing acetylcholine at the synaptic cleft, has not been directly proved, it is supported by the finding that Db-cAMP increases miniature end-plate potentials (Goldberg and Singer 1969). The similarity of Db-cAMP (experiment III) and carbachol (experiment IV) in producing decrements in sexual behavior, suggests that Db-cAMP may be having effects similar to carbachol. However, the fact that Db-cAMP in addition to cAMP prevented the resumption of masculine sexual behavior is at odds with the Rindi et al (1972) study where only Db-cAMP and not cAMP duplicated the effects of carbachol. This general hypothesis also assumes there is no mediator role for cAMP in the stimulation of sexual behavior by testosterone. The possibility exists that cAMP is involved in this process, but with the procedure used here its mimicry of carbachol could

be masking this effect.

A third possible explanation of experiment III proposes that cAMP and Db-cAMP are not inhibiting the response to testosterone, but that 5'AMP is potentiating the action of testosterone. Although no testosterone + saline group was run in Experiment III, a comparison of 5'AMP treated group's mount scores with the Testosterone + Sucrose group of Experiment II suggests that this may be the case. After 8 days of testosterone treatment the mean number of mounts in the testosterone + 5'AMP group was 15. Although there was no test on day 8 for the testosterone + sucrose group, tests on days 7 and 10 produced mean mount frequencies of 9 and 10.1 respectively. Although this suggestion may be plaussible only one specific role for intracellular 5'AMP has been identified in any mammalian system (inhibition of precursor incorporation into nucleic acids in adrenal steroidogenesis systems, Tsang and Johnston 1973). The possibility also exists that any three of the presented explanation for the results are acting in combination to produce the observed effects. Further experimentation designed to investigate one or all of the alternatives is needed.

3) Pharmacological Control of Male Sexual Behavior

The data from Experiment IV provides information on the influence of adrenergic and cholinergic systems on the temporal sequence of masculine sexual behavior. Application of either NE or carbachol to the POA of sexually active rats decreased sexual behavior in a test given 15 minutes after exposure to the substance. NE decreased the mean number of intromissions and ejaculations in Test I and II, and likewise decreased the mean number of mounts in Test II but not in Test I. The decreased number of mounts and intromissions was not, however, due to the inability of the male to

copulate, since there was no significant difference in the percent of animals mounting or intromitting in either test. Likewise, there was no difference in the number of mounts or intromissions achieved in the 1st series of Test I or II when the animals were treated with NE compared to the sham treatment. Instead, applicaiton of NE increased the time taken to initiate the 1st mount and intromission, and the time between successive intromissions. It appears that high concentrations of NE in the POA retards the overall temporal sequence of copulation.

When animals were treated with carbachol only a total of 4 mounts was seen in both tests, with no intromissions or ejaculations being achieved. The animals did exhibit interest in the female however, often following her around the cage, but attacking her instead of attempting to mount. Application of carbachol thus appears to interfere with the occurance of copulatory behavior.

The two systhesis inhibitors differed dramatically from the mimetics, in that their effects on behavior were not apparent until Test II (4 hours after the initial exposure), whereas the mimetics had effects on behavior almost immediately (15 minutes after exposure). Neither α-MT or HC-3 influenced the mean number of mounts, intromissions or ejaculations in Test I or the percent of animals exhibiting these responses. However, HC-3 treatment resulted in significant decreases in the means of all these measures and a decrease in the percentage of animals exhibiting mounting or intromitting during Test II. This decrease in all components of sexual behaivor under HC-3 was, to a large extent, a result of a prolonged PEI (over 20 minutes) exhibited by a significant number of the animals. This prolonged PEI occurred either after 1 or 2 ejaculations. Since an average of 0.8 ejaculations occurred during Test I, the PEI usually occurred at the end of Test I or after the first ejaculation of Test II (mean number of

ejaculations in Test II=1.4 for the sham treatment). Only one animal resumed copulation after exhibiting this prolonged PEI and he copulated at a low level. The prolonged PEI appeared very much like satiety, in that the male moved about the cage after a standard period of inactivity, but showed no interest in the female. This pattern of activity would seem to correspond very well with the idea that the depletion of a substance, would terminate the display of copulatory responses. Since HC-3 is known to block the synthesis of acetylcholine and the cholinomimetic (carbachol) both have similar effects on the probability of copulation, even though the temporal and behavioral sequences leading to sexual quiescence are different. One possibility for this paradox is that the application of carbachol hyperpolarized the cholinergic system involved in activating copulation, in effect, creating a chemical lesion. With HC-3 treatment, the initiation and maintenance of copulation is not affected until the acetylcholine stores are depleted by one or two ejaculations, thus preventing the reinitiation of copulation after a PEI.

Alpha-MT treatment significatnly decreased the mean number of ejaculations exhibited in Test II without affecting any other measure of copulatory behavior. If there were a decrease in the number of ejaculations but no change in IL, PEI or the time between intromissions (MIII), one would expect there to be more intromissions under the α-MT treatment than in the sham treatment. However, this was not the case. The variance between individuals probably accounts for this discrepancy (eg. the difference between individuals in Test II MF was significant $p<.055$).

These data on treatment with α-MT are at variance with results reported by Malmnas (1973) who found a significant reduction in the percent of animals mounting and intromitting when given systemic injections of α-MT (150mg/kg).

However, this discrepancy was undoubtedly a dose response since a smaller dose of systemic α-MT (75mg/kg) had no effect on sexual behavior in Malmnas' study. Likewise, no detectable amount of α-MT was released from the cannulae in the present study during the 4 hours of treatment.

Since α-MT inhibits the synthesis of both dopamine and NE (Spector, Sjoerdsma and Undenfriend 1965), the behavioral effect of α-MT treatment connot be delegated to either dopamine or NE systems at this time. Malmnas (1973) however, was able to seperate the behavioral effect of α-MT treatment by selectively blocking NE synthesis or dopamine postsynaptic receptors. He determined that the behavioral deficits seen with α-MT were the result of depleting neurotransmitter substances in dopaminergic systems.

In conslusion, it appears as though high concentrations of NE in the POA significantly increased response latencies for masculine sexual responses. The depletion of catecholaminergic fibers (via α-MT) decreases the occurrence of ejaculation, probably through an effect on dopaminergic systems. Cholinergic systems appear to be involved with whether a response will be shown or not. High concentrations of a cholinomimetic prevent the display of all sexual responses. The depletion of acetylcholine stores however, results in the arresting of copulation.

CONCLUSION

The reinstatement of sexual behavior in long term castrated male rats occurred in the present study when testosterone or estradiol was applied directly to the POA. Estradiol was more effective than testosterone in reactivating masculine sexual behavior, even though a smaller intracerebral dose was used. These data confirm the concepts that 1) estradiol is an effective stimulatory hormone for all components of masculine sexual behavior and 2) the POA is a site for hormonal regulation of copulatory behavior. These data support the suggestion that testosterone may be metabolized to an estrogenic form before acting on the neural substrate to effect the behavioral changes.

This latter hypothesis was tested directly in the present study. The inability of testosterone to raise mounting scores above the preimplant levels in animals that were given intracerebral metapirone, points directly to the necessity of testosteorne aromatization for behavioral stimulation. The ability of the aromatization inhibitor to block the reinstatement of copulation when applied to the POA also suggests that the aromatization occurs in this neural area.

The present study also supports the hypothesis that cAMP is involved in the hormonal regulation of masculine copulatory behavior. Although not entirely clear, the data imply that high levels of cAMP in the preoptic area inhibit the behavioral activation associated with testosterone treatment. The mechanism of this blockage may involve the inhibition of NAD^+ dependent anabolism normally activated by testosterone stimulation.

The results of the present study further demonstrated that high levels of NE in the preoptic-anterior hypothalamic continuum. lengthened

the temporal sequence of copulation. On the other hand, carbachol and hemicholinium treatment resulted in the arresting of sexual behavior. Thus adrenergic systems appear to affect the temporal sequence of copulation, where as the cholinergic regulate the activation of that sequence.

APPENDIX

APPENDIX: METHODS

I. Subject Selection for all Experiments.

a) Subjects:

Adult male Long Evans rats, purchased from a commercial breeder (Charles Rivers, Boston, Mass.) were used in all experiments. The animals were 80-90 days of age at the beginning of testing, and were maintained on food and water ad libitum, in a reversed light-dark cycle of 14 hours light and 10 hours dark, with lights going off at 11:00 A.M. Animals were housed 4-5 in a single 9x14x20 inch stainless steel cage. Once implanted, however, they were transferred to individual 9x9x12 inch stainless steel cages.

b) Testing procedure for masculine sexual behavior:

All tests for masculine sexual behavior were administered to subjects using a standard procedure. Individual male rats were placed in an observation arena 3 minutes before commencement of the test to allow the animal to adapt to the surroundings. At the start of the test, a sexually receptive female was placed in the arena with the male. Occurrence of sexual responses, mounts without intromissions (Mounts), intromissions and ejaculations were recorded on an Esterline-Angus Event Recorder. Unless otherwise noted, sexual behavior tests were continued until one of the following criteria has been met: 1) occurrence of the first intromission following the first ejaculation pattern; 2) 20 minutes has elapsed after the first intromission and no ejaculation

pattern has occurred; or 3) 20 minutes has elapsed after introduction of the stimulus female and no intromission had occurred.

c) Screening for masculine sexual behavior:

Two weeks after arrival in the laboratory, all animals began a series of 3, weekly tests for masculine sexual behavior. Males exhibiting the ejaculatory response in two of the three screening tests were retained for experimental testing. Animals meeting this selection criterion were castrated within a week after completion of the third test. Castration was done under ether anethesia, using a transscrotal approach. Following castration, subjects in experiments I, II and III were maintained in the laboratory for one month without exogenous administration of hormones. At the end of one month, these animals were given weekly tests for sexual behavior until a criterion of 3 tests without an ejaculation had been met by all animals. Once this criterion was achieved, these males were assigned to one of the experimental procedures and subsequently implanted with stainless steel, double-walled cannulae. In experiment IV, immediately after castration, all animals were maintained on 150 ug TP daily for the entire experiment. All males in experiment IV were also bilaterally implanted with double-walled, stainless steel cannulae.

II. Cannulae Implantation Procedure.

a) Cannulae construction:

All animals were bilaterally implanted with double-walled stainless steel cannulae. Each cannulae were made up of a larger outer guide

cannula (21 guage in experiments I and IV, 23 guage in experiments II and III) permanently cemented in postition and an inner hollow removable insert (26 and 27 guage in experiments I and IV, respectively and 30 guage in experiment II and II) which allowed for repeated chemical stimulation. The outer guide cannulae were constructed in the laboratory from 21 or 23 guage stainless steel hypodermic tubing. All guide cannulae used, were made equal in length so as to allow the use of a single applicator insert for the administration of a chemical to all animals in a particular treatment group.

Prior to the implantation, the two guide cannulae used in the bilateral implantation, were fastened together with dental acrylic to insure that the tubes remained parallel and the correct distance apart during the implantation procedure. Each pair of connected guide cannulae were fitted with a pair of hollow inner cannulae or obdurators. These remained in the guide cannulae throughout the experiment. The obdurators were constructed in the laboratory from 26, 27 or 30 guage stainless steel tubing. A small collar of outer guage tubing, about 4 mm in length, was crimped around the top of the obdurator tube to act as a stop, thereby preventing the obdurator from extending more than 1 mm beyond the end of the guide cannulae. A small lenght of polyethalene intramedic tubing, 7-8 mm long, was fitted over both the collar and top part of the guide cannulae, so as to make the stop airtight. The intramedic tubing comes off with the obdurator when the latter is removed.

Chemicals were applied to specific neural loci by utilizing an applicator made of two insert guage tubes. The applicator tubes were soldered together so as to be the same distance apart as all the guide

cannulae used in a particular experiment. Small collars of outer guage tubing were crimped around the applicator tubes to serve as stops, which prevented the applicator from extending 5 mm beyond the tip of all guide cannulae used in that treatment. The chemicals used were applied to the brain in three different manners during the course of this research. In experiment I, crystalline chemicals were loaded into the lumen of the 26 guage applicator by tapping the ends of the tube into a thin layer of the substance. For instance, 10 taps of the applicator into testosterone loaded 15 ug of the hormone into each tube of the applicator. Substances were ejected from this applicator directly to the brain by passing a plunger through the lumen of the 26 guage applicator. The plunger was fashioned out of 26 guage hypodermic needle cleaning wire and was constructed so that the end did not extend more than .25 mm beyond the tip of the applicator, thus expelling the entire chemical pellet from the lumen of the applicator. In experiments II and III the chemical treatments were applied directly to the neural area by infusing a small amount of the chemical in solution through the applicator. A Harvard Apparatus Infusion Pump hooked to an automatic timer was used for the infusion of the solution. Two lines of intramedic tubing leading from two motor driven 2 cc surenges, were connected to the 30 guage tubes of the applicator. The timer and pump were adjusted so that .005 ul of the solution would be expelled from the applicator in 30 seconds. The use of chemical solutions allowed a smaller guage of implant to be used, hence the 30 guage insert in experiment II and II. In addition, it allowed the concentration and PH of the treatment to be more rigidly controlled.

In experiment IV, 27 guage inserts were used as applicators. The

lumen of the applicator was filled with powdered chemical as in experiment I. However, the pellet of chemical was not pushed from the lumen once the applicator was in place. Instead, the substance was allowed to diffuse out of the lumen and into the surrounding tissue. Since this experiment was done before II and III the solution, infusion method was not used, although it is judged to be superior.

b) Chemical application:

In each application of a chemical to an individual animal, both obdurators were removed from the unanethesized implanted animal and the applicators inserted in their place. In experiment I with the applicator in place, the plungers were pushed down, expelling the two pellets, one into each hemisphere of the brain. The applicator was then removed and the obdurator replaced until the next treatment administration. In experiment II and III, with the applicator in place the infusion pump was turned on, expelling .005 ul of solution/cannula into the brain. The applicator was then removed and the obdurator replaced. In experiment IV, the applicator, filled with chemical, was placed into the guide cannulae and left there until the testing under that treatment had finished. The obdurator was then replaced until the next treatment.

c) Implantation technique:

Implantation of the outer guide cannula was done under ether anesthesia with 108 mg of atropine sulfate being injected interperietally 20 minutes before the ether to prevent congestion during the 30 minute operation. A Kopf Stereotaxic instrument was used for implantation. The coordinates used for the placing of cannulae in the desired neural area were taken from two stereotaxic atlases, Abel-Fessard, Stutinsky

and Libouban (1965) and Pellegrino and Cushman (1965). After histological verification of preliminary implants, minor adjustments necessary to correct for strain differences were made to insure proper cannulae placement.

After securing the animal's head in the stereotaxic instrument, the skin, muscle and fascia were cut and pushed aside to expose the skull. At this point, four stainless steel screws were placed in the skull around the area of cannulae insertion. The screws served as an anchor for the dental acrylic, which was used to fasten the implanted cannulae to the skull. Each pair of guide cannulae was lowered with the use of the stereotaxic instrument, through holes drilled equal distance on each side of the midline suture. The guide cannulae had obdurators in them during implantation. After the cannulae were lowered to the correct depth, dental acrylic was applied to and around the screws, cannulae and exposed skull, permanently securing the outer guide cannulae in place. The remaining open wound was closed and the animal placed in an individual cage.

III. Histology

The neural location of cannulae implants was histologically verified after completion of each experiment. Following completion of an experiment, all animals were perfused with saline and formalin solutions, respectively. Animals were then decapitated and all skin removed from the head. The brain was then extracted from the skull and subsequently imbedded in a Paraplast block for sectioning. Brains were cut in a crossection plane, 30 um thick. Sections were mounted and stained, using cresyl violet and methyl blue staining techniques.

Microscopic examination was then made to verify the site of implantation and also to determine the extent of any lesions made during the experiment.

At the time of perfusion, the penises of some animals were also removed. The penises were placed in Bowen's solution for one week, subsequently imbedded in Paraplast and cut into crossections, 25 um thick. The sections were placed on slides and stained with a hematoxylin and eosin procedure. After staining, microscopic examination of the penises were made. The number of penile spines and length of penile papillae were recorded from the dorsal half of the glands penis. The number of penile spines from the dorsal half of the glands were recorded from two different sections of the same penis. Similarly, the length of papillae at the top and both sides of the glands were measured from two different sections. The data from these measurements were used to determine if significant amounts of testosterone are being released from the implant into the systemic circulation.

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