PEPTIDERGIC CONTROL OF MALE SEXUAL BEHAVIOR. EFFECTS OF INTRACEREBRALLY INJECTED SUBSTANCE P AND CHOLECYSTOKININ

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by

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A thesis submitted to the School of Graduate
Studies in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

Department of Psychology
Memorial University of Newfoundland
June 1988

St. John's Newfoundland Canada

ABSTRACT

At this point, nothing is known about how peptidergic afferents/efferents of the medial preoptic-anterior hypothalamic continuum (MPOA-AH) participate in the neural control of male rat sexual behavior. In this dissertation an attempt was made to elucidate the roles of substance P (SP) and cholecystokinin (CCK) (two peptides found within the MPOA-AH) in male reproductive behavior. In Experiments 1 through 4, a variety of parameters of male copulatory behavior were recorded subsequent to intracerebral injections of SP into the MPOA-AH or the dorsal caudate/putamen (CP). Data from these experiments revealed that bilateral cannulae implanted in the MPOA-AH produced a partial lesion which disrupted the pattern of male copulatory behavior which was dependent on a post-operative test interval. Following the establishment of a new testing interval, a second series of experiments were conducted using a similar approach to that of experiments 1-4. In Experiments 8 and 9, an undiluted SP antiserum (0.3 ul or 2 ul) was injected bilaterally into the MPOA-AH. In Experiment 11, three different doses of CCK-8 (sulphated) were bilaterally injected into the MPOA-AH and the effects on male copulatory behavior following these doses were examined. Bilateral injections of 10, 100, and 200 ng/cannula, of acidified SP into the MPOA-AH significantly reduced the latency to initiate copulation, while the two lower doses also reduced ejaculation latencies. In addition, all three doses of SP significantly reduced the number of mounts while the 100 ng dose also

decreased the number of intromissions prior to ejaculation. In contrast to gonadally intact males, MPOA-AH injections of 10 ng of SP in castrated testosterone-deprived animals had no appreciable effect on copulatory behavior. Bilateral injections of 2 ul of a SP antiserum significantly increased mount, intromission, and ejaculations latencies and caused a significant increase in the inter-copulatory interval. In contrast to the MPOA-AH, bilateral injections of SP into the CP had no appreciable effect on male copulatory behavior. Injections of SP into the LV significantly reduced the number of intromissions to ejaculation, while at the same time lengthened both mount latencies and the inter-copulatory interval. CCK-8 injections into the MPOA-AH had no significant effect on any of the parameters of copulatory behavior, although there was a non-significant trend for reduced latencies following the larger doses of CCK-8 compared to saline injected controls. This is the first study to demonstrate that SP, a peptide previously implicated in female sexual behavior, and with a sexually differentiated distribution in the CNS, plays an important role in the neural regulation of male sexual behavior.

ACKNOWLEDGEMENTS

It was Sir Francis Bacon who first pointed out that the mode of pursuit of science for modern scientists ought to be different from the mode of discovery of truth that has been used by the great philosophers. Indeed, Bacon's insistence on a new philosophy of scientific inquiry paved the road for a new scientific approach, which, as a result of the inherent beauty of science, has revealed fresh wonders on almost a daily basis. I am sincerely honored to be part of this endeavour. As Arthur Koestler so poignantly expressed: " We are truly peeping toms at the keyhole of eternity ".

I would like to thank Dr. Graham Skanes for his numerous helpful suggestions and statistical assistance. Also, my thanks go to Dr. Abe Ross, who, continually on short notice, saved my sanity by rescuing me from the great " SPSS " forest, and by being a great squash opponent.

I would also like to thank Dr. Jack Strawbridge, who enabled me to attend some very informative conferences and who also let me experience first hand: " Doctrina sed vim promovet insitam ".

To Dr. Charles Malsbury, who should write a book entitled: " How to be a Great Supervisor ". His support and encouragement throughout every stage of this research was truly appreciated. Above all, thanks Chuck for illustrating to me that in life, nothing below 100 % is a passing grade.

Lastly, one is continually searching for someone to lean on, for someone who, when one is plunged into the black depths of uncertainty during times of adversity, has the strength and commitment to see one through.

Often, the hope of having found that someone is shattered during adversity, and one is rudely awakenened, as from a dream.

Seldom is one truly blessed with someone whose enthusiasm and encouragement add color to even the darkest days. This dissertation is dedicated to Brenda Dornan, that someone, whose love makes difficult things easy.

Additional thanks go to the School of Graduate Studies of Memorial University, for financial support received in the form of a post graduate doctoral fellowship.

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GENERAL INTRODUCTION

In the search for a neurophysiological understanding of rat sexual behavior, experimental interest has focused on two inter-connected physiological systems: the endocrine system and the central nervous system (CNS). Each system has been shown to be of crucial importance for both the initiation and maintenance of female and male sexual behavior. For example, in the male the testes produce male hormones (androgens) which are secreted into the bloodstream, where they interact with different tissues within the body as well as within the CNS, to ensure the adequate functioning of the male copulatory sequence. Similarly, within the ovary secretion of ovarian hormones (estrogen and progesterone) ensures maximum sexual efficiency in the female. Removal of the testes or ovaries results in abolition of the sexual behavior in males and females respectively.

As a result of unprecedented advancements in neuroanatomical techniques employed in brain-behavior research in the past two decades, a considerable amount of information is now available concerning the neural control of male and female sexual behavior. This introduction will deal with the neural basis of male sexual behavior. Primary emphasis will be directed toward the role of the medial preoptic-anterior hypothalamic continuum (MPOA-AH) in male sexual behavior. In addition, the role of neurotransmitters and neuropeptides in the regulation of male sexual behavior in both animals and humans will be reviewed. Prior to this, a description of the copulatory pattern in the male rat will be given.

Below is a schematic representation of the basic features of male copulatory behavior.

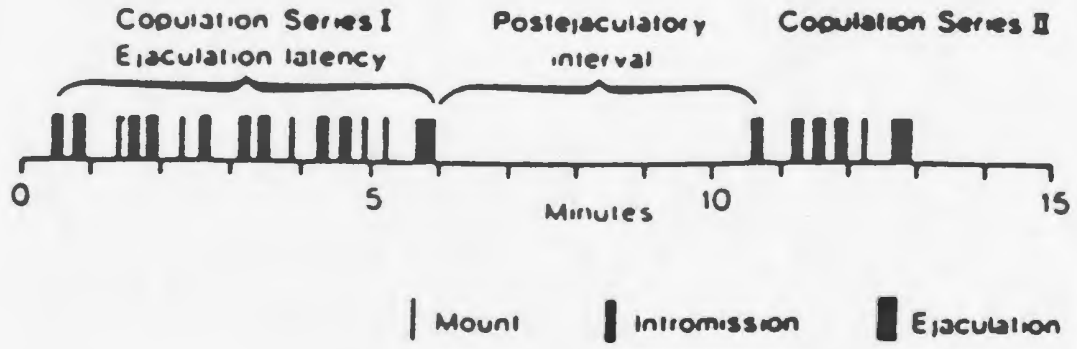


Figure 1 Masculine mating behavior in the rat. Taken from Larsson (1979).

As can be seen, three behaviors, along with their respective latencies, are recorded: 1) mount, this consists of shallow, repetitive pelvic thrusting by the male without penile insertion into the vagina; 2) intromission, a mount with deep pelvic thrusting which results in penile insertion into the vagina, whereupon the male dismounts with a quick backward springing movement; 3) ejaculation, here the mount is terminated by a deep pelvic thrust which is maintained for several seconds with the forepaws extended. As can be seen from the figure, each copulatory pattern consists of repeated mounts and intromissions which eventually culminate in ejaculation. Generally, the initial ejaculation is preceded by 8 to 15 intromissions, whereas second and subsequent ejaculations are preceded by progressively fewer intromissions (3- 7). After the first ejaculation, the male goes through a period of sexual quiescence (about 4 to 8 minutes). This interval

has been termed the " refractory period " or " postejaculatory interval " (PEI). Beach and Holz-Tucker (1949) originally divided the PEI into two components: an " absolute " refractory period during which the male is unresponsive to exogenous stimulation, and a shorter " relative " refractory period, where mounting will take place if the male is given sufficient stimulation (e.g. pinching of the tail). With each successive ejaculation, the PEIs increase. Given sufficient time, however, a male rat may achieve 8 to 12 ejaculations before becoming sexually exhausted.

An interesting component of the male copulatory pattern is its temporal organization. For instance, each intromission lasts about 1/2 second, and there is generally an interval of 30 to 60 seconds between intromissions (inter-copulatory interval, ICI). If the ICI is experimentally manipulated, dramatic effects on the male copulatory sequence result. Hard and Larsson (1970) reported that by prolonging the ICI (enforced-interval), male rats ejaculated after fewer intromissions. However, if this interval is prolonged for too long a period, males may not ejaculate despite repeated mounts and intromissions (Larsson, 1970). What these studies suggest is that the CNS stores the stimulation received during each successive intromission, and that ejaculation is dependent on the culmination of this excitation. Therefore, in the "enforced-interval" paradigm, as the ICIs are progressively increased beyond a certain point, each subsequent intromission is building on less and less excitation.

Besides the above temporal organization of the male rat mating sequence, several circumscribed behavioral patterns characterize the male copulatory sequence. For example, upon presentation of a receptive female, a sexually vigorous male will immediately approach and sniff the female's anogenital region. He will also continually pursue the female, sometimes climbing on her back, or crawling over the body and head (Larsson, 1979).

This section has highlighted some of the major components of the male copulatory pattern. In the next section, evidence in support of MPOA-AH in the regulation of male sexual behavior will be briefly reviewed.

NEURAL CONTROL OF MALE COPULATORY BEHAVIOR

Medial Preoptic Area - Anterior Hypothalamic Continuum (MPOA-AH).

Lesions:

Numerous experimental studies clearly indicate the importance of the MPOA-AH in neural regulation of male copulatory behavior. Large lesions of the MPOA-AH result in the total cessation of male copulation (see figure next page). This has been demonstrated in a variety of species, including the rat (Arendash and Gorski, 1983; Caggiula, Antelman and Zigmond, 1973; Ginton and Merari, 1976; Heimer and Larsson, 1966-67; Singer, 1968; Twiggs, Popolow, and Gerall, 1978), cat (Hart, Haugen and Peterson, 1973), dog (Hart, 1974; Hart and Ladewig, 1979), rhesus monkey (Slimp, Hart and Goy, 1978), and also in several nonmammalian species such as frogs (Schmidt, 1968) and lizards (Wheeler and Crews, 1978).

In a now classic study by Heimer and Larsson (1966-67), the critical areas for male sexual behavior within the MPOA-AH were delineated by making small lesions throughout the continuum. Although lesions within the medial preoptic area (MPOA) suppressed male sexual behavior, Heimer and Larsson concluded that there were no specific locations within the MPOA-AH that were essential for male sexual behavior in the rat.

Recently, however, a number of investigators have revealed the importance of discrete portions of the MPOA-AH. Van de Poll and van Dis (1977) revealed that bilateral destruction of the entire rostral MPOA-AH did not affect male sexual behavior in the rat, whereas destruction of the caudal part of the MPOA-AH completely abolished its expression. Arendash and Gorski (1983) demonstrated

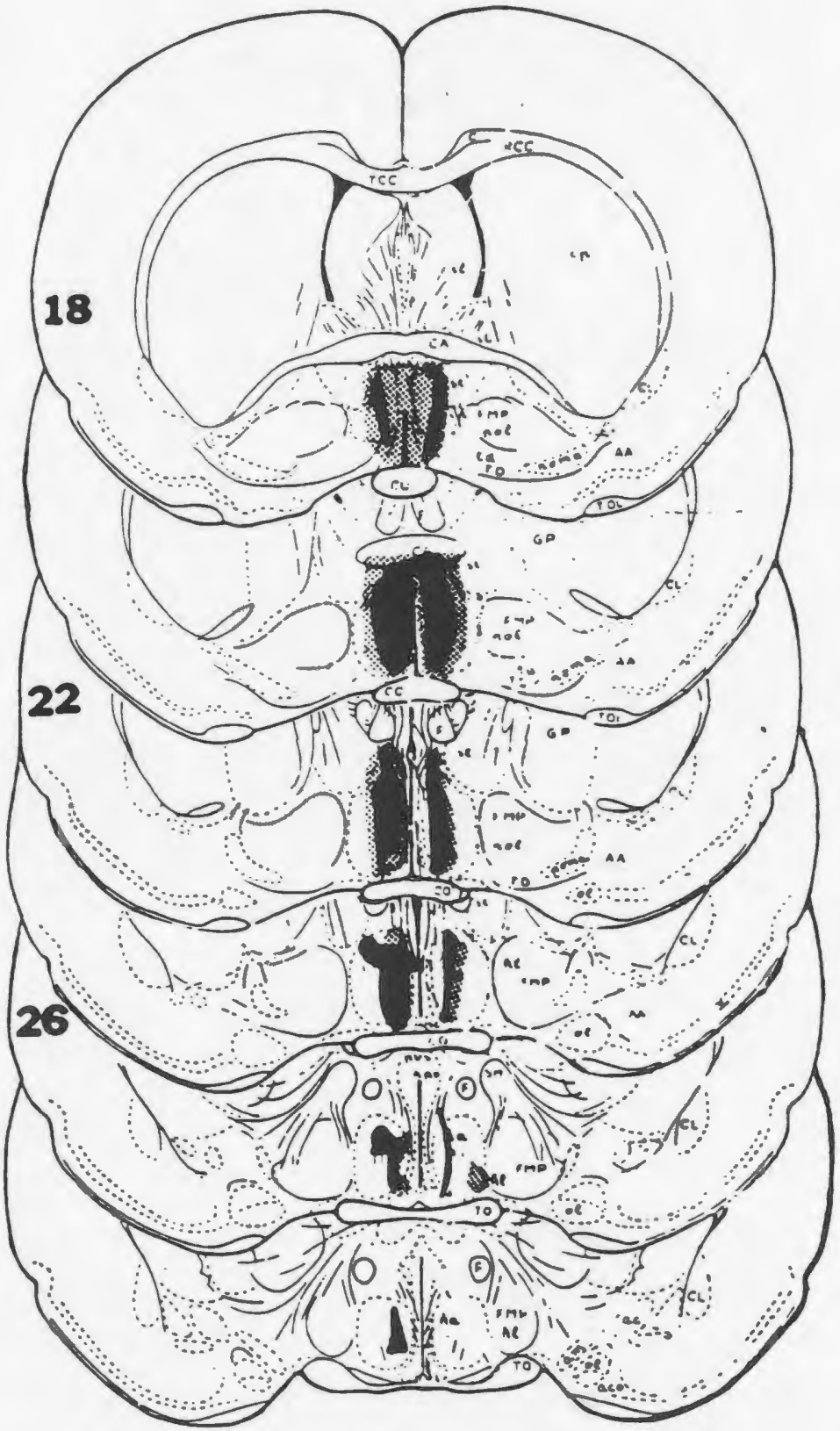


Figure 2 Schematic representation showing extent of MPOA-AH lesions that disrupt male sexual behavior in male rats. Numbers identify the figures in the Konig and Klippel atlas. Taken from Hennessey, Wallen, and Edwards (1986).

that discrete lesions of the dorsal MPOA-AH produced the most dramatic effects on male sexual behavior, while discrete lesions of the ventral MPOA-AH were not as effective. As a result, they concluded that the dorsal MPOA-AH may be differentially more important than other MPOA-AH regions in the neural regulation of male sexual behavior in the rat.

With respect to MPOA-AH lesions, the effects seem to be permanent in that functional recovery of male sexual behavior is not detected for as long as 8 months post lesion (Ginton and Merari, 1976).

In the above studies, non-selective lesioning techniques were employed to destroy MPOA-AH tissue. Therefore, it is not known whether the deficits observed were the result of damage to MPOA-AH neuronal cell bodies or to axons passing through the MPOA-AH (axons of passage). Recently, Hansen, Kohler, Goldstein and Steinbusch (1982) have demonstrated that lesions of the MPOA-AH made with a neurotoxin, ibotenic acid (ibotenic acid produces relatively selective destruction of cell bodies while leaving axons passing through the area intact), were as effective as electrolytic lesions in eliminating male sexual behavior in rats. These data corroborated earlier results with non-selective electrolytic lesions, and further suggest the critical importance of MPOA-AH cell bodies in the neural regulation of male sexual behavior in the rat.

Electrical Stimulation:

The importance of the MPOA-AH in the neural control of male sexual behavior is further strengthened by results obtained with electrical stimulation techniques (Hillarp, Olivercrona and Silfverskiold, 1954; Malsbury, 1971; VanDis and Larsson, 1970). For example, Malsbury (1971) reported that electrical stimulation in the MPOA-AH reduced the number of mounts and intromissions preceding ejaculation. In addition, in two animals, a dramatic decrease in the postejaculatory interval (PEI), along with shortened approach and mount

latencies were observed. In another study by Van Dis and Larsson (1970) they reported an abnormally high incidence of ejaculations following electrical stimulation of the MPOA-AH (one of the rats ejaculated 17 times in only a 30 minute interval). They also recorded drastically shortened PEIs.

Summary

The critical importance of the MPOA-AH in the neural regulation of male sexual behavior has been clearly demonstrated in studies using a variety of experimental approaches. As the review above indicates, bilateral lesions of the MPOA-AH greatly impair or completely abolish male sexual behavior in the rat, while electrical stimulation greatly enhances the behavior. Indeed, as Larsson (1979) states: " the MPOA-AH may be viewed as the origin of the final common pathway for [male] sexual behavior ".

In the following section, evidence for the role of neurotransmitters and neuropeptides in the regulation of male sexual behavior in both animals and humans will be reviewed.

MONOAMINES AND MALE SEXUAL BEHAVIOR

Several lines of experimental evidence indicate that brain monoamines play a significant role in the regulation of male sexual behavior in rat (Ahlenius, Larsson, and Svensson, 1980; Baum and Starr, 1980; Clark, Smith, Stefanick, Arneric, Long, and Davidson, 1982; Hull, Bitran, Pehek, Warner, Band, and Holmes, 1986), cat (Hoyland, Shillito, Vogt, and Tje, 1970), rabbit (Perez-Cruet, Taligamonte, and Gessa, 1971), as well as in humans (Gawin, 1978; Green, 1961; Mitchell and Popkin, 1983; Redmond, Kosten, and Reiser, 1983). In this section, evidence in support of the above will be reviewed.

Dopaminergic Modulation of Male Sexual Behavior

Animal Studies

In the rat, considerable evidence indicates that dopamine (DA) serves a facilitatory role in male sexual behavior. A variety of treatments which decrease DA activity have been reported to impair male copulatory behavior (Baum and Starr, 1980; Malmnas, 1973; McIntosh and Barfield, 1984b), while treatment with drugs which enhance DA activity has produced facilitatory effects (Ahlenius and Larsson, 1984; Hull et al. 1986; Paglietti, Pellegrini-Quarantotti, Meru,and Gessa, 1978).

One of the earlier studies that investigated the role of dopaminergic mechanisms in male rat sexual behavior was done by Butcher, Butcher and Larsson (1969). Following intraperitoneal administration of 3,4,- dihydroxyphenylalanine (L-

DOPA) in sexually experienced males, they observed a significant reduction in intromission frequency (number of intromissions preceding ejaculation), as well as in ejaculation latencies, compared to a saline-injected control group.

In a second study, Paglietti et al.(1978) reported similar results. Following peripheral administration of apomorphine (APO, a DA agonist) or L-DOPA to sexually vigorous gonadally intact males, they observed a significant reduction in both intromission and ejaculation latencies compared to baseline (no treatment) scores. In addition, intraperitoneal administration of L-DOPA significantly reduced the number of intromissions preceding ejaculation, an effect not seen following APO treatment. Pre-treatment with the DA receptor blocker pimozide completely abolished the effects of L-DOPA and APO.

Subsequent investigations using sexually experienced or inexperienced male rats, have reported a facilitation of male copulatory behavior following administration of DA agonists. Indeed, it is generally accepted that DA receptor activation amplifies male copulatory behavior. Agreement as to the site of the DA receptor mediated effect, however, is still to be reached. Two important theories regarding the site of the effect have been proposed. One is based on the assumption that dopamine is an inhibitory transmitter, and suppression of its activity (via autoreceptor stimulation) is how DA agonists facilitate male copulatory behavior (Gower, Berendson, Princen, and Broekkamp, 1984). When activated, these presynaptic autoreceptors via a negative feedback mechanism decrease presynaptic release of DA, consequently reducing endogenous DA at the postsynaptic receptor. The other is in line with the previously mentioned studies, and argues that postsynaptic DA receptor activation is the mediating factor.

Recently, Foreman and Hall (1987) have published a series of experiments which have attempted to clarify the above issue. Their data, along with the previously mentioned studies, strongly support a role for the involvement of increased postsynaptic DA receptor mediated responses as the basis for the facilitation of male copulatory behavior. In the study by Foreman and Hall (1987), activation of

presynaptic DA autoreceptors, or postsynaptic receptors, following subcutaneous administration of different doses of a selective D2 receptor agonist (LY163502), produced dose-related effects on male copulatory behavior. Low doses of LY163502 (0.025-10 ng/kg) significantly reduced the percentage of animals ejaculating during the 30 minute mating test, while intermediate doses produced the opposite effects. Reductions in mount and intromission frequencies, as well as ejaculation latencies, were observed following intermediate doses (25 ng-25 ug/kg). The effects of LY163502 were completely abolished by pre-treatment with the centrally acting dopamine receptor blocker sulpiride, but were not affected by a peripherally acting dopamine antagonist, domperidone. Based on these results, they conclude that low doses of LY163502 impair male copulatory behavior by stimulating central presynaptic DA autoreceptors, whereas the moderate doses enhance copulatory behavior via increased central postsynaptic DA receptor activity.

Clearly, the use of highly selective receptor agonists will provide a useful tool for determining the functional significance of pre and postsynaptic dopamine receptors in the regulation of male sexual behavior. Presently, however, much further systematic research is required before any firm conclusions can be drawn.

A number of studies have revealed that the dopaminergic enhancement of male rat copulatory behavior may be independent of gonadal as well as adrenal hormone influence (Meyerson, Malmnas, and Everitt, 1985; Malmnas, 1977; Ahlenius, 1981). For example, Malmnas (1977) administered APO to castrated rats eight weeks following castration and observed a significant increase in the number of animals that displayed mounting, intromission, and ejaculation compared to a saline-injected control group. In addition, reinstatement of the full copulatory pattern in castrated rats was completely abolished by pre-treatment with pimozide. In that same study, Malmnas tested the possible contribution of adrenal hormones in male copulatory behavior following peripheral injections of APO in a group of castrated/adrenalectomized animals. Here too APO was

effective in reinstating copulatory behavior, although the increase in the number of animals ejaculating did not reach statistical significance.

In the preceding studies, the effects on rat copulatory behavior subsequent to changes of dopaminergic activity were observed following systemic administration of drugs. One major problem with systemically-administered drugs is that it is not clear where the central site of action is, since they affect numerous neural systems. Accordingly, although the MPOA-AH has been demonstrated to play a significant role in male rat sexual behavior, it is not clear from the aforementioned studies if MPOA-AH dopaminergic synapses mediate the effects of the systemically administered drugs. Recently, Hull et al. (1986) have demonstrated the importance of MPOA-AH dopaminergic regulation of copulatory behavior in the male rat. Compared to a saline-injected control group, unilateral injections of APO into the MPOA-AH increased the percentage of mounts with intromission (hit rate), and the number of ejaculations, while they decreased the latency to ejaculate, and the postejaculatory interval during a 30 minute mating test. Injections of APO into the caudate/putamen or the lateral septum had no appreciable effect on copulatory behavior. Interestingly, when injected into the lateral ventricle, APO produced differential effects on some of the parameters of male copulatory behavior. For example, unilateral injections of APO (0.5 ug) significantly increased hit rate, while a lower dose (0.2 ug), actually impaired copulatory behavior, revealed by decreased ejaculatory frequency and an increased inter-intromission interval (i.e. decreased copulatory vigor). The authors suggested that the low dose of APO injected into lateral ventricles was acting on presynaptic DA autoreceptors. They speculated that the subsequent decreased DA activity at post synaptic receptors in the MPOA-AH results in an impairment of copulatory behavior in the rat. Conversely, they suggest that the higher dose of APO was stimulating postsynaptic receptors, which resulted in a facilitation of copulatory behavior. Although an intriguing interpretation, further research is needed before the functional significance of DA autoreceptors for male rat sexual behavior is elucidated.

Human Studies

The significance of dopaminergic mechanisms in human sexuality has been demonstrated by studies which reveal therapeutic or detrimental effects on sexual behavior following administration of drugs that enhance or decrease dopaminergic activity respectively. For example, Bowers, van Woert, and Davis (1971) reported that of nineteen patients that were receiving L-DOPA treatment for Parkinson's disease, seven (37%) reported increased sexual activity during treatment. In fact, hypersexuality has been a reported clinical complication of dopaminergic therapy in Parkinson's disease (Vogel and Schiffter, 1983). Conversely, drugs that decrease dopaminergic activity have been reported to disrupt sexual behavior (Ananth, 1982; Buffum, 1982; Mitchell and Popkin, 1982; Singh, 1963). Indeed, the use of chlorpromazine has been associated with dose-related sexual dysfunctions in men. Mitchell and Popkin (1982) report that in male schizophrenics, chlorpromazine at a dose of 1,200 mg/day resulted in failure to ejaculate, decreased erectile ability, as well as decreased libido. When the dose was lowered to 400 mg/day, libido and erectile ability returned, although ejaculation did not.

In a systematic investigation of dopaminergic mechanisms in male sexual behavior Ghadirian, Chouinard, and Annable (1982) found that, following treatment with a dopamine receptor blocker, fluphenazine, in 55 schizophrenics, 38 percent reported having difficulty in achieving erection, 42 percent in maintaining an erection, and 58 percent in achieving orgasm. Similarly, in a clinical case history Ananth (1982) reported that impotence was induced in a male schizophrenic following treatment with pimozide. That pimozide was directly responsible for the sexual dysfunction and not secondary to the disorder itself, was suggested by the alleviation of the impotence following its discontinuation. Moreover, on two separate occasions following re-instatement of pimozide treatment, his impotence returned.

In summary, it seems clear from the above that dopamine neurons play an important role in the regulation of both animal and human sexual behavior. Drugs that decrease or enhance DA activity impair or facilitate sexual behavior respectively. In addition, in the only study that has investigated the role of DA by using direct injections of APO into the brain, Hull et al. (1984) report that only MPOA-AH, and not caudate/putamen or lateral septum injections of APO, dramatically facilitated copulatory behavior in male rats. As a result, they suggest that the MPOA-AH is an important structure in the dopaminergic regulation of male sexual behavior.

The Role of 5-hydroxytryptamine (5-HT) in Male Sexual Behavior

In contrast to DA, evidence suggests that serotonin (5-HT) serves an inhibitory role in male sexual behavior in animals as well as humans. A variety of treatments which increase 5-HT activity disrupt male sexual behavior (Ahlenius, Larsson, and Svensson, 1980; Hansen, Svensson, Hokfelt, and Everitt, 1983; Malmnas and Meyerson, 1971; Quirk and Einarson, 1982; Svensson and Hansen, 1984; Wilson, Pearson, Hunter, Tuothy, and Payne, 1986), while those which decrease 5-HT activity appear to facilitate sexual behavior (Ahlenius, Heimann, and Larsson, 1979; Dewsbury, 1972; Larsson, Fuxe, Everitt, Holmgren, and Sodersen, 1978; McIntosh and Barfield, 1984a; Sodersten, Larsson, Ahlenius, and Engel, 1976).

Animal Studies

Ahlenius et al. (1980) report that following peripheral administration of a selective 5-HT reuptake blocker, zimelidine, along with a sub-threshold dose of 5-hydroxytryptophan (5-HT precursor) to sexually experienced male rats, they

observed a prolongation of the ejaculation latency and the postejaculatory interval. This effect was completely abolished by pre-treatment with a 5-HT receptor blocker. Further support for an inhibitory role of 5-HT on male sexual behavior comes from studies that have utilized the 5-HT synthesis inhibitor p-chlorophenylalanine (PCPA). In male rats, PCPA has been shown to increase the " intensity " of copulation (increase the number of mounts and intromissions per minute) and to decrease ejaculation latencies (Meyerson, Malmnas, and Everitt, 1985). Furthermore, PCPA treatment has been shown to reinstate the full copulatory pattern in castrated male rats given subthreshold doses of testosterone (Malmnas and Meyerson, 1971). For example, in a study by Sodersten, Larsson, Ahlenius, and Engel (1976), castrated rats were given daily subthreshold doses of testosterone (T) (20 mg/kg) along with PCPA. Following combined treatment with T and PCPA, 80 % of the males ejaculated compared to none in the T alone group. Moreover, pre-treatment with 5-HT completely abolished the facilitatory effect of PCPA.

Despite the evidence supporting an inhibitory role of 5-HT in male sexual behavior, there are some conflicting reports in the literature (Gorzalka and Whalen, 1975; Mendelson and Gorzalka, 1985). In some studies pharmacological treatment which enhanced serotonergic activity was ineffective (Hansen, Svensson, Hokfelt, and Everitt, 1983), or produced contradictory effects on male copulatory behavior (Svensson and Hansen, 1984).

A number of studies have attempted to explain some of these discrepant results. For instance, Larsson (1979), in his review on monoaminergic control of male sexual behavior, describes an experiment where marked strain differences were found in the activating effects of PCPA on the male copulatory pattern. Wistar and Sprague-Dawley rats were castrated at 70 days of age and then treated with PCPA 3 weeks later. On subsequent behavior tests, none of the Sprague-Dawley rats displayed copulatory behavior, whereas 85 % of the Wistar rats ejaculated. Therefore, the inhibitory effects on male copulatory behavior produced by 5-HT

may depend on the strain of rat used. In other words, PCPA may enhance sexual behavior in castrated rats of some strains while being completely ineffective in others.

Another interpretation has been recently postulated by Mendelson and Gorzalka (1985). In that study, peripheral administration of pirenperone and ketanserin (selective 5-HT 2 receptor antagonists) inhibited sexual behavior in male rats compared to the saline- injected controls. This was revealed by dramatic increases in mount, intromission, and ejaculation latencies. They also reported that intraperitoneal injection of a 5-HT 2 receptor agonist (quipazine) in sexually experienced male rats also inhibited sexual behavior. Indeed, the inhibitory effects observed, whether following administration of the antagonist or the agonist, were virtually indistinguishable. Interestingly, while both quipazine and pirenperone when injected alone produced an impairment of copulatory behavior, when administered simultaneously, the inhibitory effects of both drugs were markedly attenuated. They explain the apparent paradox in their results by postulating a dual role (facilitatory and inhibitory) for 5-HT in male sexual behavior. In fact, based on these results, they suggest that experiments should focus on the changes produced in male copulatory behavior following drugs with specific pharmacological effects on 5-HT receptor subtypes. They state that past research investigating the role of 5-HT in male sexual behavior is based on drugs with non-specific effects on 5-HT receptors, which could partly explain the conflicting reports in the literature regarding 5-HT's role in male copulatory behavior.

Recently, Mendelson and Gorzalka (1986) have further explored the possibility of a differential role for 5-HT in male sexual behavior. Based on pharmacological studies which have revealed that the 5-HT 1 class of receptors consists of a further subtype, A and B, and on the recent evidence which has demonstrated differential physiological function of serotonin receptor subtypes, they investigated the role of 5-HT 1 (A) and 5-HT 1 (B) receptors in male copulatory behavior. They report that peripheral administration of 8-OH DPAT, a 5-HT 1 (A) receptor

agonist, significantly reduced ejaculation latencies and the number of intromissions prior to ejaculation. They conclude that activation of both 5-HT 1 (A) or 5-HT 2 receptors facilitate the expression of male copulatory behavior in gonadally intact males, and suggest that the inhibitory effects that have been reported in the literature following increased serotonin activity are probably mediated by a population of serotonin receptors other than those of the 5-HT 1 (A) and 5-HT 2 subtype. Why they conclude that activation of both 5-HT 1 (A) and 5-HT 2 receptors facilitate male sexual behavior, when in their previous study a specific 5-HT 2 agonist impaired male copulatory behavior, is not clear.

Although still an interesting interpretation, in view of a study by Ahlenius and Larsson (1984), the role of 5-HT receptor subtypes in the regulation of male sexual behavior remains unclear. For example, they too reported that, following intraperitoneal administration of 8-OH DPAT in gonadally intact sexually experienced male rats, a significant decrease in intromission frequency and ejaculation latencies was observed compared to control (saline) injections. However, prior treatment with specific 5-HT 1 and 5-HT 2 receptor blockers did not attenuate the 8-OH DPAT-induced facilitation of male copulation in that study. Based on these results, Ahlenius and Larsson (1984) concluded that the 8-OH DPAT-induced facilitation of copulatory behavior observed in their study was not mediated via 5-HT receptors. Indeed, they speculated that the 8-OH DPAT-induced facilitation of male copulatory behavior is probably mediated via catecholamine receptors.

Human Studies

PCPA, used in the treatment of migraine headaches, has been reported to be associated with increased levels of sexual excitation in migraine patients (Sicuteri, Del Bene, and Anselmi, 1975). In that study, male migraine patients were divided into two groups. One group received an oral dose of PCPA plus an intramuscular

injection of a placebo (control group), the second group received an oral dose of PCPA plus an intramuscular injection of testosterone. PCPA plus testosterone treatment significantly increased the number of daily erections compared to the controls. Testosterone alone did not appear to be responsible for the effect as a previous study by Sicuteri (1974) showed that testosterone treatment of migraine sufferers was no better than placebo. This has rather interesting implications. For example, migraine sufferers have been reported to have decreased libido. Moreover, migraines have been associated with increased plasma 5-HT levels. Therefore, it is not inconceivable that in migraine sufferers, chronically high 5-HT levels reduce sexual desire, and that treatment with PCPA by lowering 5-HT levels increases sexual interest by enhancing performance. One problem with this assumption is that frequently it is exceedingly difficult to ascertain whether sexual dysfunctions that occur during an illness are indeed precipitated by changes in neurochemical systems (i.e. 5-HT activity) or represent psychological disturbances that are secondary to the illness. Nonetheless, PCPA treatment in humans produces effects on sexual behavior that are consistent with the animal literature, and as a result, do support a role for 5-HT in the regulation of male sexual behavior.

Further support for 5-HT in male sexual behavior has come from reports of sexual dysfunctions following the use of anti-depressant drugs, some of which are relatively specific 5-HT reuptake blockers (Mitchell and Popkin, 1983). For example, in a recent paper by Jones (1984) he describes a case history of a middle-aged depressed male who experienced ejaculatory inhibition after being treated with Trazodone (a selective inhibitor of 5-HT reuptake). During treatment, the patient denied any problems with his ability to maintain an erection, or pain during sexual intercourse (a frequently reported side effect), but he did complain of an inability to ejaculate on numerous occasions. Three days after the discontinuation of Trazodone, he reported having satisfactory intercourse and had absolutely no difficulty ejaculating.

Summary

In view of the above studies, it can be concluded that 5-HT has an important function in masculine sexual behavior in both the rat and human. The majority of studies are suggestive of an inhibitory function of 5-HT. For example, most of the components of male copulatory behavior are enhanced following decreases in 5-HT activity, whereas inhibitory effects are obtained by increasing 5-HT activity. Recently, however, a number of reports challenge the theory of an inhibitory role for 5-HT in male sexual behavior. Based on observations following treatment with drugs with specific actions on 5-HT receptor subtypes, Mendelson and Gorzalka have revealed differential involvement of 5-HT receptors in the expression of male sexual behavior, and as a result suggest a dual role (facilitatory and inhibitory) for 5-HT in male sexual behavior. As illustrated in the Ahlenius and Larsson (1984) study, however, at present the identity of the neurons which mediate the effect of 8-OH DPAT on male copulatory behavior is unclear.

The Role of Norepinephrine (NE) in Male Sexual Behavior

It is only within the last several years that more attention is being focussed on the role of norepinephrine (NE) in male sexual behavior. Although there is an abundance of studies in the literature on the disruptive effects of anti-hypertensive drugs (almost all of which are anti-adrenergic) on human sexual behavior, ironically, there has been little research investigating NE in rat copulatory behavior. There are, however, numerous studies that clearly suggest a role for NE in the regulation of male sexual behavior in both humans and animals (Ahlenius, Heimann, and Larsson, 1979; Clark, Smith, and Davidson, 1984; 1985; Fernandez-Guasti, Hansen, Archer, and Johnsson, 1986; Hoehn-Saric, Merchant, Keyser, and Smith, 1981; Johnson and Diamond, 1969; Laver, 1974; McIntosh and Barfield, 1984c; Redmond, Kosten, and Reiser, 1983; Stefanick, Smith, Szumowski, and Davidson, 1985; Walker, Gerall, and Kostrzema, 1981).

Animal Studies

Clark et al. (1984) reported that yohimbine hydrochloride, an alpha 2 NE autoreceptor blocker, significantly increased sexual motivation in male rats. In that study, sexually experienced, gonadally intact, male rats were injected intraperitoneally with yohimbine (2 mg per kilogram) or vehicle and, twenty minutes later, were given a local anesthetic in the genital area. The number of mounts during a fifteen minute mating test were then recorded. Sexual motivation was assessed by the number of mounts made by the male in the absence of feedback from the genitals during the fifteen minute mating test with a sexually receptive female. Rats treated with yohimbine displayed significantly more mounts during the fifteen minute test than the vehicle treated controls. Based on these results, they concluded that yohimbine increased arousal via a blockade of alpha 2 autoreceptors which resulted in a potentiation of the postsynaptic action of NE. One difficulty with the use of yohimbine in behavioral studies is that, like most drugs, it has a complex pharmacological profile. For example, not only does it block adrenergic autoreceptors (thereby potentiating NE action), it also blocks 5-HT receptors (Gyermek, 1975). Consequently, the facilitation of male sexual behavior observed in that study could quite conceivably have resulted from decreased 5-HT postsynaptic activity. Indeed, they addressed this issue in their paper by stating that future work is needed with more specific adrenergic drugs in order to ascertain whether the observed enhancement of male copulatory behavior in their study is specific to yohimbine or can be replicated with other noradrenergic agents.

A more recent study by Clark et al. (1985), followed the above approach. Using a variety of drugs which exert specific effects on NE receptors, they explored the role of NE autoreceptors in masculine sexual behavior. They reported that treatment with the anti-hypertensive agent, clonidine (which at low doses

preferentially stimulates presynaptic alpha 2 autoreceptors), completely eliminated ejaculatory behavior in male rats. Moreover, this effect was completely abolished by pretreatment with yohimbine. In that same study, prazosin, an alpha 2 postsynaptic NE receptor blocker, significantly increased the latency to initiate copulation, as well as ejaculation latencies, while having virtually no effect on copulatory activity (i.e. mounts, intromissions). Based on these results and their previous study, they suggest that the NE neurons play a significant role in the regulation of sexual motivation in the rat.

The role of NE in male copulatory behavior has been further clarified by a series of studies by McIntosh and Barfield (1984c). They report that disruption of NE-containing neural pathways in sexually experienced male rats by electrolytic lesions of the locus coeruleus, or peripheral administration of NE synthesis inhibitors, had a profound effect on male copulatory behavior. Following lesions to the locus coeruleus, a significant increase in the post-ejaculatory refractory period compared to the initial baseline score was observed. Interestingly, peripheral administration of diethyldithiocarbamate (DDC), a NE synthesis inhibitor, increased the post-ejaculatory period and the intromission latency, but at the same time reduced the number of intromissions prior to ejaculation compared to saline-injected controls. In addition, DDC treatment significantly increased the ejaculation latency, while having no effect on the percentage of animals ejaculating. Based on these results, they propose that since disruption of NE-containing neural pathways impairs sexual arousal without affecting other parameters of copulatory behavior (e.g. ejaculation), these pathways are critically involved in the control of sexual arousal in the male rat.

Although the McIntosh and Barfield study indicates a functional role for NE in male copulatory behavior, not all studies support this conclusion. For example, Clark (1980) reported that following neurochemical lesions of the dorsal noradrenergic bundle in male rats, no effect was observed on any parameter of copulatory behavior, despite the fact that a significant depletion of brain NE was found.

Human Studies

Clinical reports suggest that decreased adrenergic activity is associated with decreased libido and erectile dysfunction in men (Buffan, 1982). Hoehn-Saric et al. (1981) reported that following clonidine treatment in men suffering from anxiety, during the first week of treatment a substantial number of males reported a decrease in sexual desire and a mild difficulty in obtaining an erection. Conversely, therapeutic effects on ejaculatio praecox (premature ejaculation) have been reported with drugs that block NE receptors (Singh, 1963). Moreover, increased NE activity has been postulated to be responsible for spontaneous ejaculation associated with anxiety in men (Redmond et al. 1983).

Summary

At present, it seems that NE plays a role in male sexual behavior. However, the majority of studies that have investigated noradrenergic systems have used systemically administered drugs. Therefore, these effects may be due to peripheral changes in the sympathetic nervous system. Consequently, further work is needed to evaluate the role of adrenergic systems in male sexual behavior following direct noradrenergic perturbations in the CNS.

ACETYLCHOLINE (ACh) AND MALE SEXUAL BEHAVIOR

Animal Studies

In contrast to studies investigating the role of monoaminergic systems in masculine sexual behavior, there have been comparatively few studies that have examined the role of cholinergic mechanisms. In one of the earlier studies, Soulairac (1963) reported that, following systemic injection of a cholinesterase inhibitor, physostigmine, a prolongation of the ejaculation latency and the postejaculatory interval (PEI) was found. Soulairac and Soulairac (1972) later reported a reduction in ejaculation latencies and in the post ejaculatory interval following administration of the ACh receptor agonist, nicotine.

More recently, Hull, Bitran, Pehek, Warner, Band, and Bazzett (1986) have examined the role of ACh in male sexual behavior following direct injections of a ACh antagonist into the preoptic area of male rats. They report that bilateral injections of scopolamine produced a dose-related impairment of copulatory behavior which was primarily related to disruptions of copulatory mechanisms (i.e. percentage of animals intromitting and ejaculating). In that same investigation, ventricular injections of scopolamine increased intromission latencies, which they interpreted as an impairment of sexual arousal.

Human Studies

There is only one report in the literature of sexual dysfunctions associated with ACh in man (Forsberg, Gustavii, Hojerbaek, and Olsson, 1979). In that study on two male cigarette smokers, nicotine was associated with erectile dysfunction.

This is not surprising since nicotine administration has been reported to decrease blood flow to the penis (Buffum, 1982). Indeed, in the Forsberg study, they reported that penile blood flow was decreased with smoking, and that when smoking was discontinued, blood flow was restored, along with erectile capacity.

THE ROLE OF GAMMA-AMINOBUTYRIC ACID (GABA) IN MALE SEXUAL BEHAVIOR

Animal Studies

Very recently, attention has begun to focus on the significance of GABA in the neural regulation of male sexual behavior. In a study by Fernandez-Guasti, Larsson, and Beyer (1986) unilateral injections into the MPOA-AH of GABA antagonists (picrotoxin or bicuculline) were reported to dramatically shorten ejaculation latencies as well as the postejaculatory interval (PEI) in the experimental animals compared to saline-injected controls. The most dramatic effect was seen on the PEI where all but one animal resumed copulation within one minute after the first ejaculation ! Conversely, compared to a saline injected control group, bilateral injections of a GABA agonist (muscimol) into the MPOA-AH significantly decreased the proportion of animals ejaculating during a 60 minute mating test. An interesting finding was that systemic injections of bicuculline, picrotoxin, or muscimol had no effect on male copulatory behavior. Since a decrease in GABA activity produced its most dramatic effect on the postejaculatory interval, while having little effect on copulatory behaviors (e.g. mount and intromission frequencies), or initiation of copulation (intromission latency), the researchers concluded that the major function of GABA in male copulatory behavior was in determining the interval of sexual inactivity following ejaculation.

In a contemporary study by Qureshi and Sodersten (1986) similar conclusions to that of the Fernandez-Guasti et al. study were reached. They measured the concentrations of amino acids in the CSF in freely moving male rats immediately following ejaculation, and after the PEI. They report that the concentration of GABA increased by more than 1000 % after ejaculation. Since this is a period when sexual behavior is inhibited, they too concluded that GABA may be functionally related to the PEI. Although an interesting conclusion, interpretation of their results is somewhat confounded by the fact that GABA concentrations where also high after the PEI, a period characterized by sexual excitation. Nonetheless, collectively these studies suggest that GABAergic mechanisms are involved in the regulation of male copulatory behavior.

OPIATES AND MALE SEXUAL BEHAVIOR

Considerable research has accumulated on the role of opiates in male sexual behavior. Most studies indicate that opiates play an inhibitory role in both animal (Allen, Renner, and Luine 1985; Band, Warner, Pehek, Bitran, Holmes, and Hull, 1986; Gessa ,Paglietti and Pellegrini-Quarantotti, 1979; Hetta, 1977; Lieblich, Baum, Diamond, Goldbium, Iser, Pick, 1984; Myers and Baum, 1979, 1980; Meyerson and Terenius, 1977; Meyerson, 1981; Sachs, Valcourt, and Flagg, 1981; Szechtman, Simantov, and Hershkowitz, 1979), and human sexual behavior (Cushman, 1972; Deleon and Wexler 1973; Goldstein and Hansteen, 1977; Greenberg, 1984; Mendelson, Ellingboe, Keuhnle, and Mello, 1979, 1980; Miran, Meyer, Mendelson, and Ellingboe, 1980). For example, peripheral administration of morphine has been shown to inhibit copulatory behavior in gonadally intact and castrated male rats in doses which fail to reduce motor activity or social contact (Mumford and Kumar, 1979; Leiblich et al., 1984). In the majority of studies, peripheral injections of low doses of morphine or methadone increase intromission and mount latencies, whereas increasingly higher doses eventually eliminate copulatory behaviors.

Animal Studies

In one of the earlier studies to investigate the role of opiates in male rat sexual behavior, Meyerson and Terenius (1977) reported that, following intraventricular injection of B-endorphin (1 ug) the percentage of male rats mounting sexually receptive females decreased significantly compared to saline injected controls. Moreover, in the males that did initiate mounting, longer latencies were observed. The effect of exogenous B-endorphin on male copulatory behavior was completely blocked by pre-treatment with the opiate antagonist naltrexone.

Subsequently, Meyerson (1981), compared the sexual behavior of male rats following intraventricular injections of either B-endorphin or morphine to a CSF vehicle injected control group. In that study, sexually vigorous male rats were castrated and maintained on weekly injections of testosterone propionate which resulted in about 75 % of the rats mounting receptive females during a 5 minute mating test. Although the lowest dose of B-endorphin (0.25 ug) produced no differences on any parameter of sexual behavior compared to the CSF injected controls, 0.5 ug and 1 ug significantly decreased the number of mounts and intromissions during a 30 minute mating test. Identical doses of morphine had no appreciable effect on sexual activity. The author speculated that the observed differences in copulatory behavior between the B-endorphin and morphine injected groups may have been a reflection of a differential distribution of the two opioids in the brain following intraventricular injections. Another explanation was the possibility that morphine and B-endorphin are working on different opioid receptors.

In a study by McIntosh, Vallano, and Barfield (1980) working with the opiate antagonist naloxone, they reported a facilitation of male copulatory behavior, but suggest an opiate/catecholaminergic interaction. In that study, intraperitoneal

administration of naloxone to sexually experienced male rats 30 minutes before a mating test with a sexually receptive female, caused a significant reduction in mount, intromission, and ejaculation latencies. In addition, they observed a significant decrease in the number of mounts preceding ejaculation. Conversely, intraperitoneal administration of morphine (6 mg /kg) or intraventricular injection of B-endorphin (6 ug) in sexually experienced male rats 30 minutes prior to testing completely eliminated copulatory behavior. In that study, they were also interested in determining whether the effects of opiates were correlated with changes of catecholamine levels in the hypothalamus or corpus striatum. They found that the impairment of copulation observed following intraventricular injections of B-endorphin was significantly correlated with increased NE content in the hypothalamus. In addition, although not significantly different, a trend towards increased striatal DA content was found following intraventricular injections of both B-endorphin and systemic morphine. Based on the above, they suggest that endogenous opiates modulate male copulatory behavior by tonically regulating catecholamine release. Indeed, they postulate that the dramatic facilitation of male copulatory behavior following injection of opiate antagonists results from the ability of these compounds to block the inhibitory effects of endogenous opiates on either DA or NE release.

Similarly, Myers and Baum (1980) examined the effects of different doses (5 or 20 mg/kg) of opiate antagonists on male copulatory behavior in sexually experienced male rats during a 30 minute mating test. They reported that intraperitoneal injections of 20 mg/kg of naloxone or naltrexone, significantly reduced the number of intromissions prior to ejaculation and also reduced ejaculation latencies, compared to a saline-injected control condition. Interestingly, the lower dose of both antagonists significantly shortened both mount and intromission latencies, an effect not seen following the higher dose. Both doses, however, significantly reduced ejaculation latencies. The authors speculated that endogenous opiates may be essential for maintaining a particular pattern of copulatory behavior, that ensures successful impregnation of the female

during a normal mating sequence. This speculation was based on the work of Adler (1969) which revealed that if ejaculation is preceded by too few intromissions, pregnancy does not occur. Why the lower dose of the opiate antagonists preferentially affected arousal mechanisms, and the higher dose, copulatory mechanisms, was not explained.

In a subsequent study, Lieblich et al. (1984) reported that peripheral injections of either naloxone or morphine (5 mg per kilogram) in castrated male rats, significantly reduced the percentage of animals ejaculating in a 60 minute mating test. In gonadally intact males, however, both naloxone and morphine failed to alter copulatory behavior compared to saline-injected controls. The fact that both an opiate antagonist and agonist impaired mating in this study was not explained.

Band et al. (1986) recently examined the role of MPOA-AH opiate systems in male rat copulatory behavior. They reported that injection of naloxone (1 ug) into the MPOA-AH shortened both ejaculation latency and the postejaculatory interval. In addition, injection of 2 ug of morphine significantly decreased the proportion of animals ejaculating during a mating test, and lengthened the postejaculatory interval. These findings strongly suggest that opiates within the MPOA-AH play an inhibitory role in the regulation of masculine sexual behavior.

Human Studies

Heroin has long been known to disrupt male sexual behavior. Among heroin addicts, there is a progressive decline in sexual behavior and libido associated with chronic opiate use. Indeed, in one study, 61 % of heroin addicts reported decreased libido, while 39 % reported suffering from impotence which ceased following detoxification (Cushman, 1972). In addition, the inability to experience orgasm seems to be an inevitable consequence of long term opiate use (Smith, Moser, Wesson, Apter, Buxton, Davidson, Orgel, and Buffum, 1982). Interestingly,

this is in contrast to retrospective accounts of opiate addicts who often equate the drug's effect with heightened sexuality (Mirin et al. 1980).

Consistent with the above, administration of opiate antagonists seems to enhance sexual performance in males. For example, Mendelson et al. (1979a) reported that following oral administration of naloxone to seven healthy volunteers, three of the seven reported recurrent spontaneous penile erections 1-3 hours after taking the naloxone.

Similarly, in a study done with a single subject, the effects on masturbation of weekly injections of increasingly higher doses of intravenous naloxone were examined (Goldstein and Hansteen, 1977). In that study, naloxone had no effect on sexual arousal, penile erection, or ejaculation. In fact, they reported that naloxone produced a dose-dependent trend of increased erection latencies. Closer examination of their data, however, suggests another interpretation. Granted, naloxone did appear to inhibit the ability of the subject to achieve penile erection. However, one wonders if this was secondary to a distraction introduced by the experimenter: the subject was required to press a buzzer every minute to signal that there was no unexpected reaction to the naloxone treatment ! It was not clear from examination of the method whether this precautionary measure was implemented during the saline condition. Indeed, once an erection had been attained following naloxone treatment, there was a reduction of the time required for ejaculation compared to the saline condition.

Not all studies, however, have shown a facilitatory effect of naloxone. Examining the effects of endogenous opiates in human male masturbation, Graber, Blake, Gartner, and Wilson (1984) administered 20 mg of naloxone intravenously to males (n was not reported) following which they were instructed to initiate masturbation and to signal the onset and termination of orgasm. Naloxone produced a significant increase in ejaculation latencies when compared to the saline-injected controls. In that same study they also measured plasma B-endorphin levels in male subjects with intravenous catheters under 4 different

experimental conditions. The first group was asked to masturbate to ejaculation, the second was instructed to masturbate but suppress ejaculation, the third group, to perform masturbation-like movements of the wrist, and the last group to do nothing. None of the conditions produced any significant changes in plasma B-endorphin levels at 10, 20, or 30 minutes, although plasma B-endorphin levels were significantly lower at 60 minutes for all the conditions. Although the authors did not dismiss the role of opiates in male sexual behavior, they encouraged further research be done before any firm conclusions can be made concerning the importance of endogenous opiates in human sexual behavior.

SUMMARY

The above studies clearly demonstrate that monoaminergic, cholinergic, GABAergic, and opiate systems play a role in the neural regulation of male sexual behavior in both animals and humans. Administration of neuropharmacological compounds that change the activity of these respective systems induce dramatic alterations of male sexual behavior. For instance, increasing dopaminergic activity seems to produce a facilitation of sexual behavior, whereas decreasing DA activity disrupts male sexual behavior in both rats and humans. In the rat, these effects seem to be independent of gonadal or adrenal hormone influence. Moreover, with regard to DA, recent studies suggest that the facilitatory effects produced by DA are partly mediated at the level of the MPOA-AH. Injections of drugs that increase activity at DA synapses in the MPOA-AH have enhanced male rat sexual behavior, while injections of the same drugs in other areas of the brain have had no effect (Hull et al. 1986).

With respect to adrenergic and cholinergic systems, both seem to be facilitatory in that increasing or decreasing their activity enhances or impairs copulatory behavior respectively, although further studies are required to reveal their exact role in male sexual behavior.

Both GABAergic and endogenous opiate systems seem to play an inhibitory role in male sexual behavior. Moreover, they appear to produce this inhibitory effect at least partly at the level of the MPOA-AH.

Recently, attention has begun to focus on the role of non-opioid neuropeptides in male sexual behavior. Indeed, the fact that immunohistochemical studies demonstrate that non-opiate peptides are located in brain nuclei implicated in male sexual behavior suggests that they may play a significant role in reproductive behaviors. Therefore, the next section will review this evidence

THE ROLE OF NEUROPEPTIDES IN MALE SEXUAL BEHAVIOR

Although the relative importance of neuropeptides in the regulation/modulation of female sexual behavior is becoming increasingly understood (Bloch, Babcock, Gorski, and Micevych, 1987; Dornan, Malsbury, and Penney, 1987; Harlan, Shivers, and Pfaff, 1983; Sakuma and Pfaff, 1983), at this point, little is known about how peptidergic afferents/efferents of the MPOA-AH or other areas implicated in male sexual behavior (see figure below) participate in the neural control of this behavior.

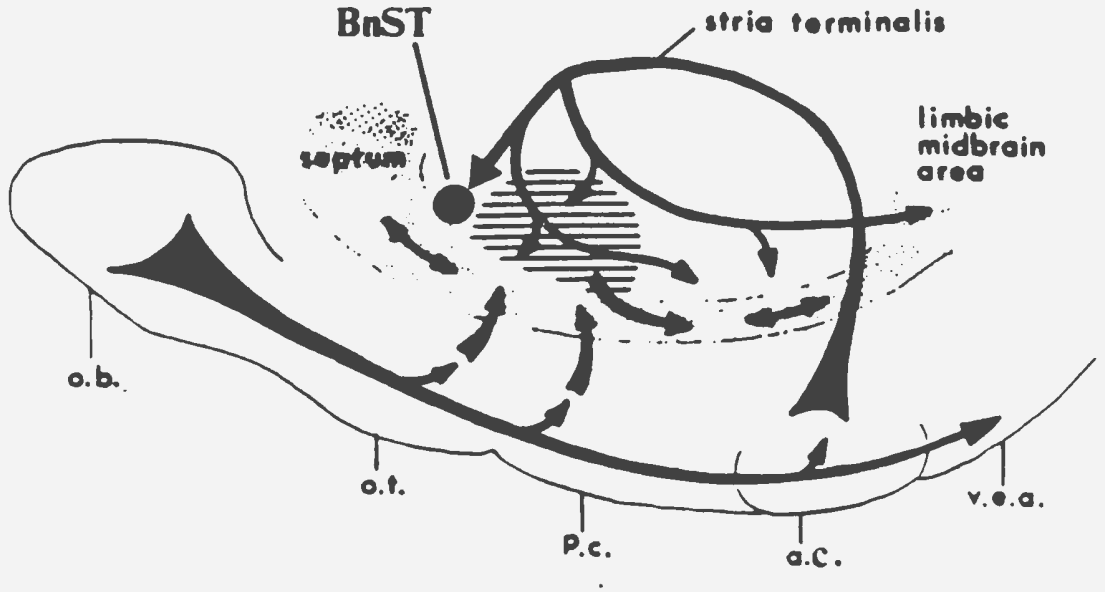


Figure 3 Schematic representation of some limbic and hypothalamic structures implicated in the neural control of male sexual behavior. The MPOA-AH is represented by the hatched area. Another area implicated in male sexual behavior is the Bed nucleus of the Stria Terminalis (BnST) indicated by the black circle. (o.b.) olfactory bulb; (o.t.) olfactory tubercle; (p.c.) prepyriform cortex; (a.c.) amygdaloid complex; (v.e.a.) ventral entorhinal area. Diagram modified from Malsbury and Pfaff (1974).

As illustrated in the Table below, the MPOA-AH contains numerous neuropeptides.

TABLE 1. Indicates some of the neuropeptides found within the MPOA-AH (a).

PEPTIDE	CELLS	FIBERS
Luteinizing Hormone Releasing Hormone (LHRH)	+	+
Cholecystokinin-8 (CCK-8)	+	+
Substance P (SP)	+	+
Neurotensin (NT)	+	+
Neuropeptide Y (NPY)	-	+
Somatostatin (SS)	+	+
Corticotropin- Releasing Factor (CRF)	+	+
Oxytocin (OXY)	-	+
Vasopressin (VAS)	-	+
Enkephalin (ENK)	+	+
Alpha-Melanocyte Stimulating Hormone (A-MSH)	-	+
Thyrotropin-Releasing Hormone (TRH)	+	+
Vasoactive Intestinal Peptide (VIP)	+	+

Despite the accumulating evidence demonstrating that numerous neuropeptides are concentrated in a structure critically involved in the expression of masculine sexual behavior, studies examining the role of non-opioid peptides on male sexual behavior have been conspicuously lacking. Indeed, at present, only the roles of LHRH (Dorsa and Smith, 1980; Moss, Dudley, Foreman, and McCann, 1975; Myers and Baum, 1980), and more recently, neuropeptide Y (Clark, Pushpa,

Kalra, and Kalra, 1985), have been investigated. Moreover, neither peptide has been examined at the level of the MPOA-AH.

Moss et al. (1975) first reported that subcutaneous injections of LHRH (500 ng) in sexually experienced, gonadally intact males, significantly reduced intromission and ejaculation latencies compared to a saline treated control group. In a group of castrated testosterone-deprived males, however, the same dose of LHRH failed to have any effect on copulation.

A similar approach was used by Myers and Baum (1980). In that study, subcutaneous injections of 1 ug of LHRH in gonadally intact and castrated testosterone treated male rats were administered 1.5 hours before a mating test with a sexually receptive female. In the gonadally intact animals, LHRH induced a significant reduction in ejaculation latencies. No other parameter was affected. In the castrated group, however, LHRH, while having no effect on ejaculation latencies, significantly increased the PEI compared to the saline condition.

Clark et al. (1985) reported that injections of neuropeptide Y into the third ventricle of male rats drastically suppressed male copulatory behavior. Although ineffective at the lowest dose, neuropeptide Y virtually eliminated male copulatory behavior in rats that were treated with higher doses (0.02, 0.12, or 0.47 nM). Although the majority of rats initiated copulation following injections of neuropeptide Y into the third ventricle, all sexual activity ceased after one to five mounts. The researchers reported that the three effective doses of neuropeptide Y also induced feeding in a dose-related manner compared to saline-injected controls. Based on these results, they concluded that injections of neuropeptide Y into the third ventricle disrupted sexual behavior by imposing a deficit in sexual motivation. Their basis for this conclusion rested on the observation that most males initiated copulatory behavior, but seemed unable to sustain a sufficient level of sexual arousal to complete the full copulatory sequence. In addition, since injections of neuropeptide Y impaired sexual behavior while at the same time inducing eating behavior, they further suggested that this peptide may be an

endogenous substance which simultaneously inhibits the neural circuitry for male sexual behavior while facilitating the circuits that regulate feeding.

Two other peptides that may play a role in male sexual behavior are substance P (SP) and cholecystokinin (CCK). For example, both SP and CCK concentrations within the CNS have been shown to be dependent on gonadal hormones (Dees and Kozlowski, 1984; Frankfurt, Seigel, Sim and Wuttke, 1986; Micevych, Matt and Go, 1987; Simmerly and Swanson, 1987). Frankfurt et al. (1986) presented data which revealed significantly higher concentrations of CCK in the amygdala and lateral septum, as well as MPOA-AH during the estrous compared to the proestrous part of the estrous cycle in female rats. As a result, they concluded that CCK and SP neuronal systems may play a role in regulating hypothalamo-pituitary function, and more interestingly for this study, in steroid-dependent behaviors. Indeed, in a recent study by Dornan et al. (1987) it was found that intracerebral injections of SP into the midbrain central gray facilitated female sexual behavior in ovariectomized estrogen-treated female rats.

More relevant to male sexual behavior are the recent findings of a sex difference in CCK and SP concentrations in discrete areas of the brain which have been implicated in male sexual behavior (Frankfurt et al., 1985; Micevych et al., 1987). For example, Frankfurt et al. (1985) found significantly higher concentrations of CCK in the MPOA-AH, and higher concentrations of SP in the amygdala in males as compared to females. Moreover, in a recent study by Dees et al. (1984), a decrease in immunostained SP fibers in the medial amygdala was reported following castration of male rats.

A similar effect of castration on CCK concentrations within the CNS has been recently reported by Simerly and Swanson (1987). Indeed, recent immunocytochemical studies from our laboratory have shown that the SP innervation of the medial nucleus of the amygdala and the bed nucleus of the stria terminalis is more extensive in the male than in the female rat (Malsbury and McKay, 1986). What the above studies suggest is that some areas of the brain

which have been implicated in male sexual behavior (see figure 3) contain higher concentrations of both SP and CCK in males than in females. Moreover, it appears that both SP and CCK concentrations within brain loci previously implicated in male sexual behavior are lowered after castration. Therefore, it is conceivable that SP or CCK or both may be involved in the neural control of male sexual behavior.

In summary, the importance of monoamines in the regulation of male and female sexual behavior has been clearly demonstrated. Furthermore, the significance of neuropeptides in female sexual behavior has received extensive experimental investigation. However, at present, only the role of two non-opioid peptides in male sexual behavior have been examined. Accordingly, an attempt was made in this dissertation to elucidate the roles of SP and CCK in male reproductive behavior. The experimental approach involved recording a variety of parameters of male copulatory behavior subsequent to intracerebral injections of different doses of SP and CCK into the MPOA-AH in sexually experienced male rats.

GENERAL METHODS

Animals and Surgery:

Two-hundred and twenty adult Sprague Dawley male rats purchased from Charles River Breeding Farms, St. Constant, Quebec, Canada were used in the following series of experiments. Animals weighed between 350-475 grams at time of surgery. They were housed singly in solid bottom plastic cages (84x48x38 cm) in a controlled environment at 21 C, with a reversed light cycle (lights off 2:00 p.m.). Throughout the experiments food and water was available ad libitum. Animals were handled daily so as to avoid stress during intracerebral injections. Stimulus females of the same strain were ovariectomized under sodium pentobarbital (Somnotol) and implanted with silastic capsules containing estradiol. Four hours before behavioral testing, these females were injected with 1 mg of progesterone subcutaneously to ensure maximum behavioral receptivity.

Each male was given pre-operative screening tests in which it was placed with a fully receptive female on alternate days until one ejaculation was achieved during a 30 minute test. Males were tested on alternate days until they had ejaculated in at least two of these tests. Only males that satisfied this criterion were subsequently used in the study. Twenty-four hours following the second successful pre-operative screening session, each male was anesthetized with Somnotol and received a pair of stereotaxically implanted 22-gauge stainless steel guide cannulae with inner stylets (28 gauge) (Plastic Products, Roanoke, Va) aimed at the MPOA-AH, using the atlas of Paxinos and Watson (1982) as a guide. The actual coordinates, however, were experimentally determined (AP = -0.2; ML = 3.7-4.0; DV, from dura = 8.7-8.9). Another two groups of animals received bilateral cannula placements aimed at the dorsal caudate/putamen or the

lateral ventricles. Coordinates for these areas were also experimentally determined (AP = -0.2; ML = 3.5-3.6; DV = 3.5-3.7; AP -0.2; ML = 1.5; DV = 3.0-3.1; respectively).

Behavioral Testing:

Three days following surgery (four days in experiments 5-11) males were given a post-operative test in which baseline measures were established. Each test lasted a maximum of 30 minutes or until one ejaculation followed by an intromission was achieved. If a male did not have an intromission within the first 15 minutes, the test was terminated and the male re-tested 4 days later. During the baseline testing and subsequent peptide injection tests, the following measures of male copulatory behavior were recorded on an IBM micro-computer using a software program developed by the author for this purpose: 1) number of mounts preceding ejaculation, this included the total number of mounts with pelvic thrusting; 2) number of intromissions preceding ejaculation; 3) mount latency, the time interval between introduction of the female and the first mount; 4) intromission latency, time interval between introduction of the female and first intromission; 5) overall ejaculation latency, time interval between introduction of the female and first ejaculation; 6) adjusted ejaculation latency, latency from first intromission to first ejaculation; 7) post-ejaculatory interval, time from first ejaculation to first ensuing intromission; 8) inter-copulatory interval, mean interval between intromissions, obtained by dividing the overall ejaculation latency by the intromission frequency; 9) contact latency, time interval between introduction of the female and approach with anogenital contact by the male; 10) number of attempts, this included the total number of attempted mounts (the occurrence of which is defined as follows: the male approaches the female, grabs her flanks, but either the female moves away before the male can actually mount her, or he abandons the attempt) before each ejaculation; 11) misdirected mounts, this included the total number of mounts with pelvic thrusting but mounting from the front or side (off target) of the female before each ejaculation; 12) number of

crawls, total number of times the male approaches the female and then crawls over her before each ejaculation. In this study, intromissions were defined as mounts with pelvic thrusting terminated by a rapid springing dismount followed by penile grooming.

All mating tests took place 2 hours into the dark period of the cycle. Males were placed in rectangular test boxes (51L x 38W x 36H cm) containing wood chip bedding (changed after each test day) 10 minutes prior to introduction of the female. This allowed the males a brief period of adaptation. They then were removed and injected with either saline or peptide. Immediately following the injection the males were placed back into the test box where a fully receptive female was introduced.

All post-operative test sessions were video-taped using a RCA VHS Camcorder (model CMR-300). At a later date, the tape of each original test session was analyzed using a video cassette recorder under blind conditions. The procedure for this was as follows: For each session, an assistant located the animal's identification number on the tape. Next, the assistant advanced the tape just past the identification number, whereupon the original observer of the live session was called into the room and recorded the copulatory behavior on the IBM computer in the same manner as with the original session. This time, however, the observer did not know what experimental treatment the animal had received. Inter-observer reliability coefficients on mount and intromission scores were subsequently calculated from the original (live sessions) and blind sessions (taped).

Intracerebral Injections:

Each 28 gauge inner cannula was connected to a 2 microliter syringe by a 12 inch plastic tube. Distilled water was then drawn up the tube until 1 ul of water could be reliably drawn and expelled. Following this, 0.5 ul of air was drawn up the inner cannula, whereupon the inner cannula was placed in the peptide or

saline solution and 1.5 ul of solution was drawn up the inner cannula. During injections, movement of the air bubble ensured that the peptide/saline solution had actually been injected into the brain. Two sets of microsyringes were used to enable consecutive deliveries of the solution into each side of the brain. Solutions were injected manually in a volume of 0.3 ul (each side) over a 60 second period. The inner cannula was left in place for an additional 60 seconds before withdrawal. Aliquots of all peptide and saline solutions were prepared immediately before intracerebral injection.

Histological Analysis:

Upon completion of data acquisition, all animals were anesthetized with an overdose of Somnotol. Brains were subsequently removed and 46 micron sections taken using a cryostat. Sections were then stained using cresyl violet and the location of each cannula placement verified with a microprojector.

Data Analysis:

Inter-observer reliability coefficients on mount and intromissions frequencies between the original test session and the blind session revealed significant positive correlations ($r = 0.92$, $p < 0.001$; $r = 0.96$, $P < 0.001$, respectively). Consequently, scores from the original test sessions were used in the data analysis. The rationale for this is straightforward: judgement errors on any of the measures were more likely to be made on intromission and mount frequencies. Since errors made on these parameters would have a profound effect on other measures (i.e. respective latencies, intercopulatory interval) inter-rater reliability coefficients were done on these parameters. Based on the results of the inter-rater reliability coefficients, it appeared that whether the rater had prior knowledge of the experimental condition did not significantly change the results. Therefore, it was decided that the original test session scores would be used in the data analysis.

For example, the rater had a better viewing perspective during the live sessions. Accordingly, it was felt that these scores would perhaps represent a more accurate reflection of each copulatory session.

Unless otherwise stated, all data were analyzed using an analysis of covariance (covariate = baseline scores) in a repeated measure split-plot design (Edwards, 1985), and post hoc analysis done using t-tests on the adjusted means generated from the analysis of covariance (Winer, 1978).

Experiment 1: The Effects of Intracerebral Injections of SP on Male Copulatory Behavior

There are at present no data to demonstrate whether SP found in the MPOA-AH plays any role in the neural mediation of male sexual behavior in the rat. Nonetheless, SP has been shown to excite neurons in the MPOA-AH after iontophoretic application (Mayer and MacLeod, 1979). In addition, testosterone, which is essential for male sexual behavior, appears to regulate SP concentrations within the CNS (Dees and Kozlowski, 1984). Given this information, it is not inconceivable that SP may be involved in the neural mediation of male sexual behavior in the CNS, and more specifically via an action on MPOA-AH neurons. Therefore, the following experiment was designed to investigate the influence of exogenous SP on various components of male sexual behavior.

Material and Methods

Animals and Surgery:

Thirty adult male Sprague-Dawley rats (375-450) were used. All animals received a pair of stereotaxically implanted guide cannulae aimed at the MPOA-AH.

Behavioral Testing:

In this experiment, when possible, each animal received three post-operative tests. The first was a baseline test which took place 3 days following surgery. Forty-eight hours later males were either injected with peptide or a saline

solution. Four days following the first injection, each animal received a second injection of either peptide or saline, depending on what the animal had been injected with first. All treatments were counterbalanced.

Intracerebral Injections:

Animals were divided into three groups (n=10 each). Two groups received either 10 ng or 100 ng of SP/ cannula (Cambridge Research Biochemical, lot # 02075, Sigma Chemicals, lot # 124F-59202) in a volume of 0.3 ul/cannula into the MPOA-AH, while the other group received acidified saline (same volume). SP was dissolved in a vehicle which contained 0.01 N acetic acid, 0.9 % saline with the pH adjusted to 6.0 using NaOH. This vehicle was also used in the control injections. The rationale for using acidified saline was as follows: i) it helps prevent adsorption to glass, and may make the peptide more stable; ii) Hall and Stewart (1983) compared behavioral effects of SP dissolved in saline or acidified saline injected intraperitoneally in mice. They found consistently stronger behavioral effects when the SP was dissolved in acidified saline.

RESULTS

One major goal in this experiment, and in the series of experiments that follows, was to examine the effects of SP injections in animals with relatively homogeneous cannula placements. As a result, any animal which did not have both cannulae within, immediately dorsal to, or immediately lateral to the MPOA-AH was excluded from the data analysis. Of the 30 animals that began the experiment, two died before completion of the experiment, and two never satisfied criterion at the baseline test session. Histological analysis revealed that of the remaining twenty six-animals, twenty five had both cannula tips within the previously defined boundary of the MPOA-AH; as a result, data analysis was performed on these (25) animals. Figures 4A and 4B illustrate the relative position of the bilateral cannula placements within the MPOA-AH of these animals.

FIGURE 4A

Illustration of bilateral cannula placements in 19 males which received injections of SP into or near the MPOA-AH in experiment 1. Each paired bilateral placement is identified by a common letter. The numbers identify the figure in the Konig and Klippel (1963) atlas from which the figures were redrawn. The figure in the bottom right hand corner is an illustration of the approximate angle of the cannula placements. Abbreviations: ac, anterior commissure; cc, corpus callosum; CL, claustrum; CP, caudate/putamen; F, fornix; LV, lateral ventricle; MAH medial anterior hypothalamus; MPOA, medial preoptic area; oc. optic chiasm; pvm, periventricular nucleus; SM, stria medullaris thalamus; st, stria terminalis.

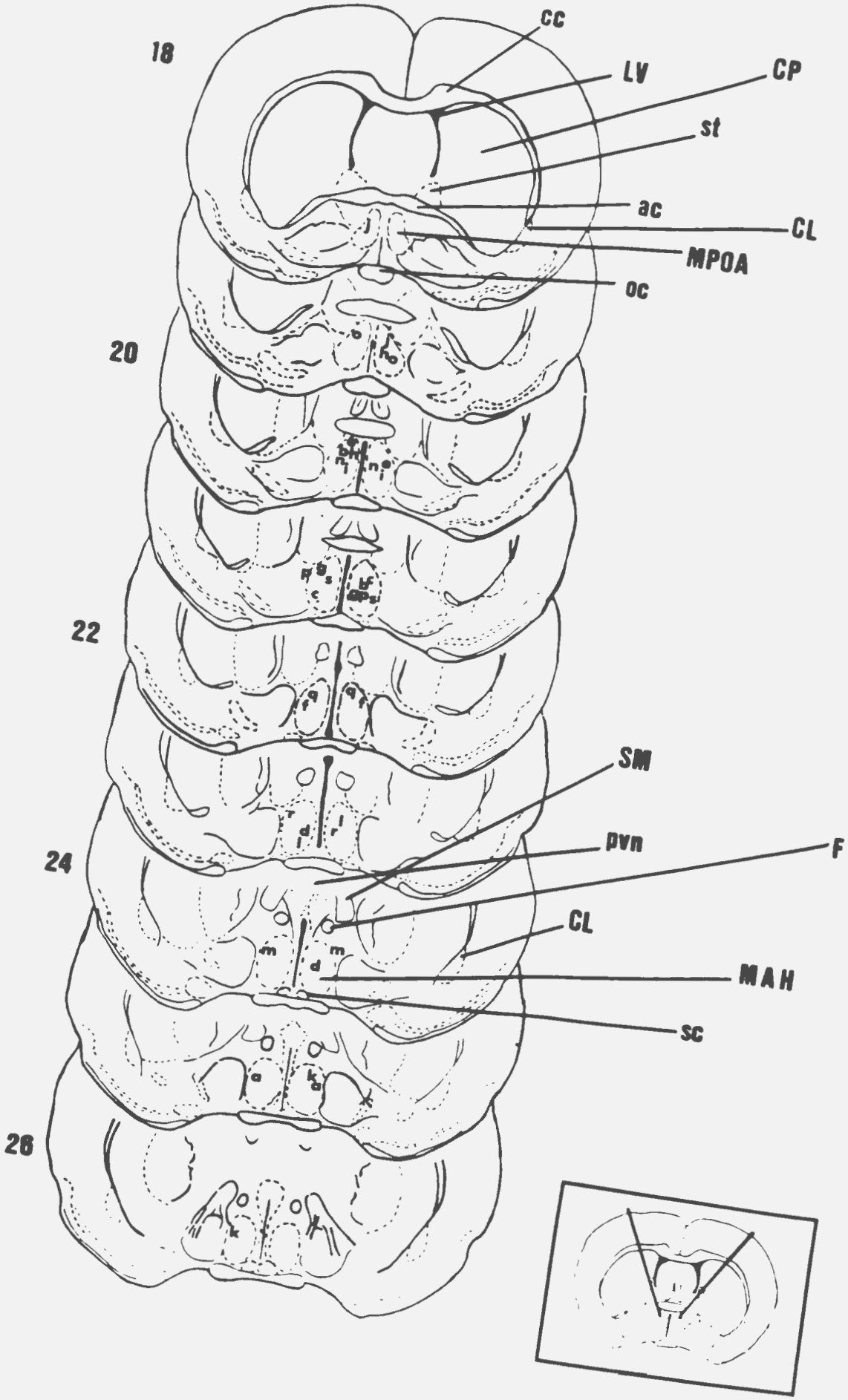
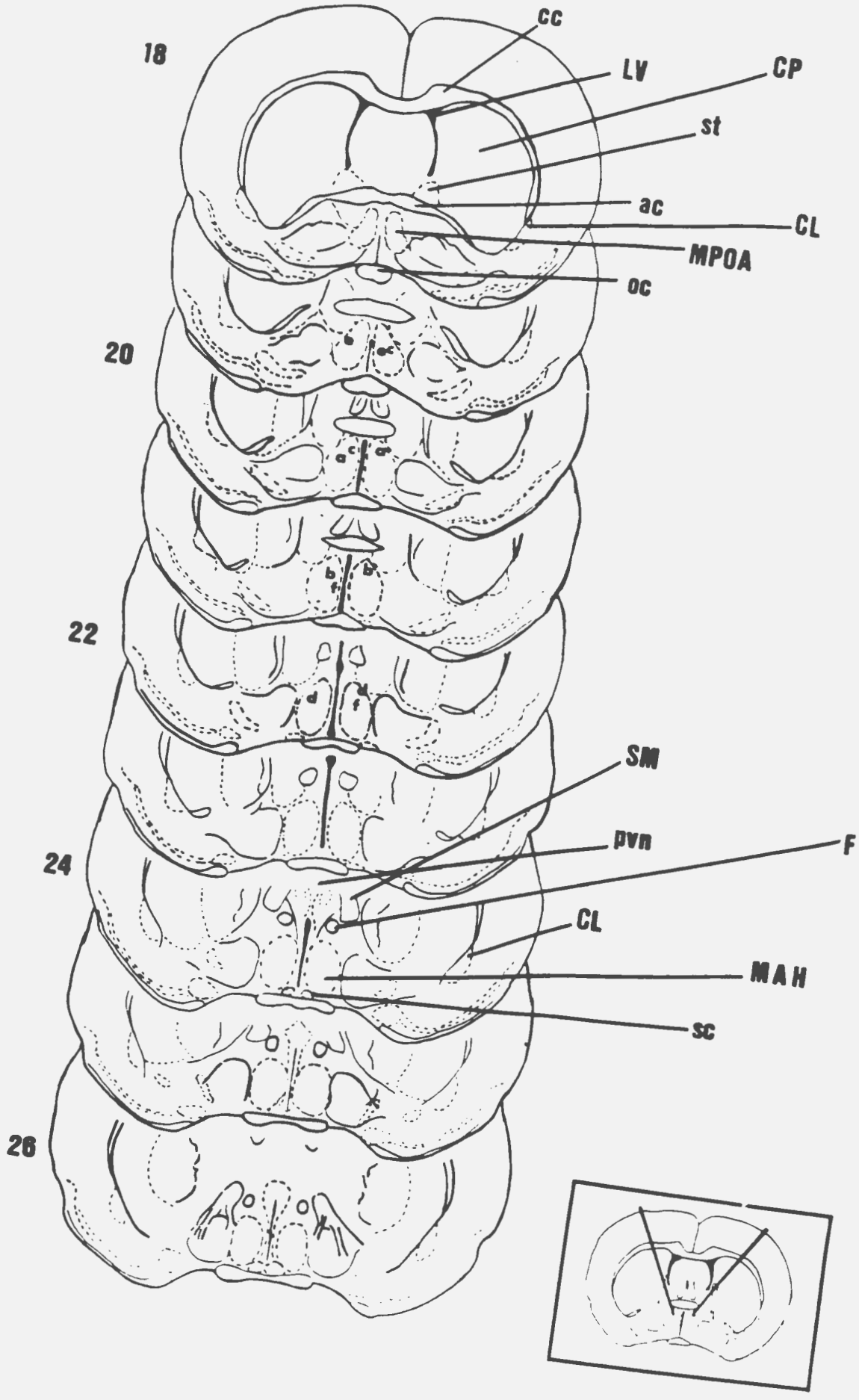

cc
LV
CP
st
ac
CL
MPOA
oc
18
20
22
SM
pvn
F
24
CL
MAH
sc
26

FIGURE 4B

Illustration of cannula placements in 6 males which received bilateral injections of acidified saline in experiment 1. See figure 4A for list of abbreviations.


cc
18
LV
CP
st
ac
CL
MPOA
oc
20
22
SM
pvn
F
24
CL
MAH
sc
26

In order to establish whether the copulatory behavior differed in animals that had received a given dose of peptide on the first or second administration, an analysis of covariance on order was carried out. This analysis revealed that whether an animal received SP on the first or second administration had a dramatic effect on most of the behavioral components recorded. The most striking effect was seen on mount, intromission, and ejaculation latencies ($F = 6.6$, df, 1,7, $P < 0.05$; $F = 14.3$, df, 1,7, $P < 0.01$; $F = 7.5$, df, 1,7, $P < 0.05$, respectively). For example, although ejaculation latencies increased regardless of whether the animals received a SP injection on the first or second injection, the magnitude of this increase differed dramatically according to the order of injection (see figure 5). Consequently, subsequent data analysis was done on the first injection test session only.

As can be seen from tables 2 and 3, intracerebral injections of either 10 ng ($n = 9$) or 100 ng ($n = 10$) of SP, or acidified saline ($n = 6$) into the MPOA-AH dramatically increased most parameters of male copulatory behavior. The magnitude of the increase following SP, however, did not differ significantly from that following saline on any of the measures recorded. For example, following injection of either 100 ng of SP or saline, both ejaculation and intromission latencies increased dramatically; however, the magnitude of the increase was similar for both groups. Consequently, no main effect of session was found ($F = 0.76$, df $= 2,18$, $P > 0.05$; $F = 0.41$, df $= 2,18$, $P > 0.05$). This is also illustrated in figures 6 and 7.

Interestingly, there was a clear though non-significant trend for reduced latencies compared to the saline treated controls (see table 2). However, table 2 also demonstrates that although latencies were lower in the SP compared to the saline groups, fewer animals ejaculated following SP injections.

FIGURE 5

Represents the effects of multiple bilateral injections into the MPOA-AH on the latency to ejaculation in male rats during a 30 minute mating test with a sexually receptive female. Lines correspond to standard error of the mean.

46 (b)

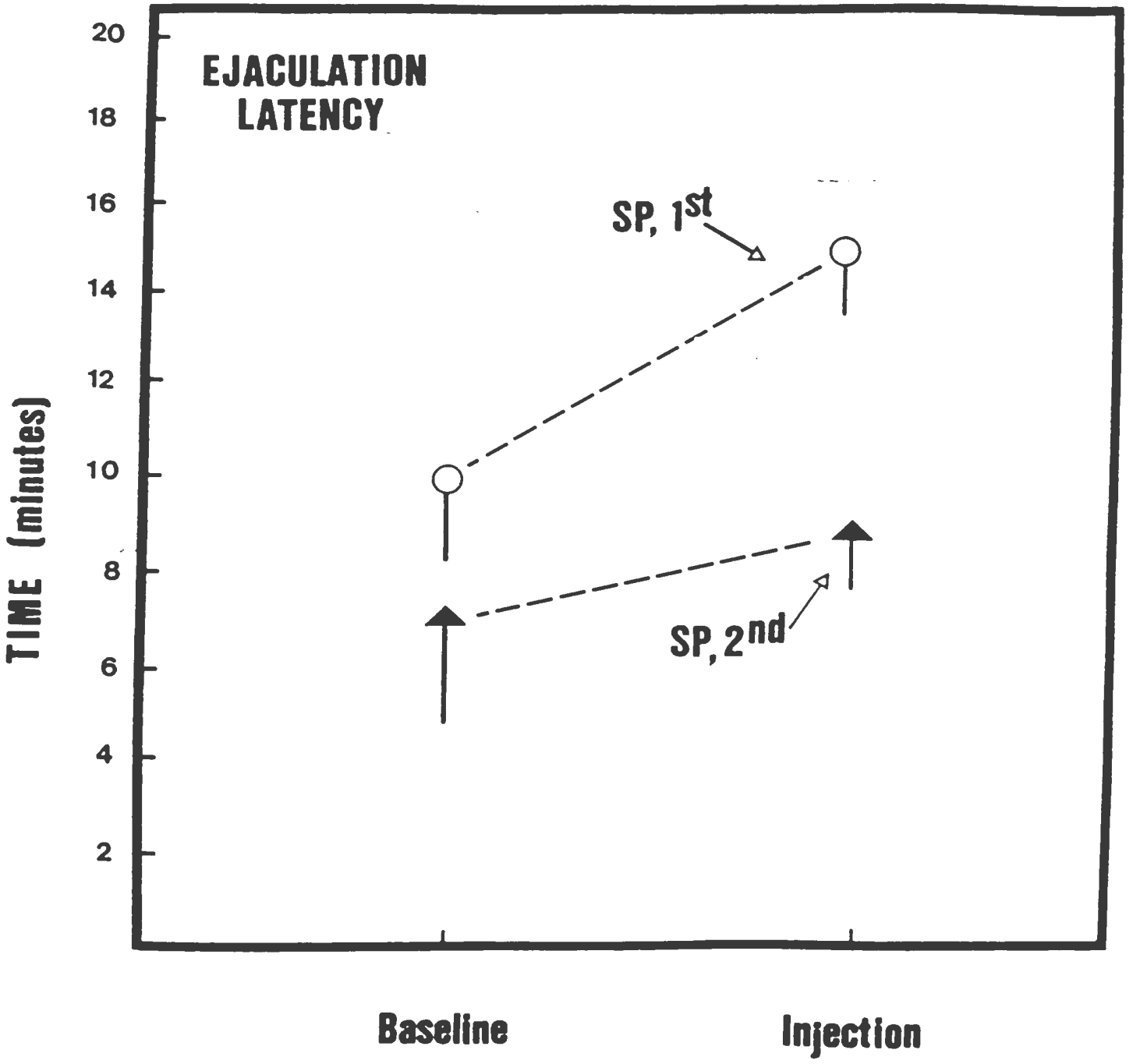

EJACULATION
LATENCY
TIME (minutes)
20
18
16
14
12
10
8
6
4
2
SP, 1st
SP, 2nd
Baseline
Injection

TABLE 2

EXPERIMENT 1 : LATENCIES (in seconds)

Effects of injections of saline or SP into the MPOA-AH on male sexual behavior.

			Substance P	
Measures (a)	Baseline	Saline	10 ng	100 ng
ML				
Experimental	148.7 + 35		387.9 + 115	256.6 + 137
Control	107.2 + 40	405.8 + 109		
IL				
Experimental	119.5 + 30		337.9 + 77	242.0 + 122
Control	58.0 + 16	356.7 + 131		
EL				
Experimental	586.5 + 60		778.4 + 46	837.2 + 180
Control	348.9 + 80	978.9 + 200		
AEL				
Experimental	467.4 + 58		440.1 + 64	594.7 + 136
Control	299.5 + 29	621.7 + 163		
ICI				
Experimental	46.1 + 4		54.7 + 7	52.4 + 10
Control	38.8 + 5	74.5 + 19		
RP				
Experimental	320.5 + 22		341.0 + 26	310.8 + 20
Control	294.6 + 33	325.0 + 37		
% E				
Experimental	100		44	90
Control	100	100		

a) at baseline, all values for the experimental groups are grand means.
All values are given as mean + S.E.M. See text for further details.

TABLE 3

EXPERIMENT 1 : FREQUENCIES

Effects of intracerebral injections of saline or SP into the MPOA-AH on male sexual behavior (a).

			Substance P	
Measures	Baseline	Saline	10ng	100ng
MF				
Experimental	3.2 + .4		4.7 + 1.4	5.4 + 2.0
Control	3.0 + .2	5.5 + 2.0		
IF				
Experimental	13.5 + 1.8		15.0 + 2.3	17.4 + 3.7
Control	11.0 + 2.0	14.5 + 2.3		
ATTEMPTS				
Experimental	1.1 + 0.3		2.5 + 0.9	2.4 + 1.2
Control	1.8 + 0.6	3.3 + 1.1		
CRAWLS				
Experimental	1.4 + 0.1		5.1 + 1.0	2.5 + 0.3
Control	1.1 + 0.4	1.3 + 0.4		
MISDIRECTED				
Experimental	2.1 + 0.2		1.5 + 0.1	2.1 + 0.6
Control	1.4 + 0.7	2.1 + 0.3		

a) at baseline, all values for the experimental groups are grand means.
All values are given as mean + S.E.M. See text for further details.

FIGURE 6

A comparison of the mean ejaculation latency in experiment 1, during baseline and injection tests with two different doses of SP or vehicle. Lines represent standard error of the mean.

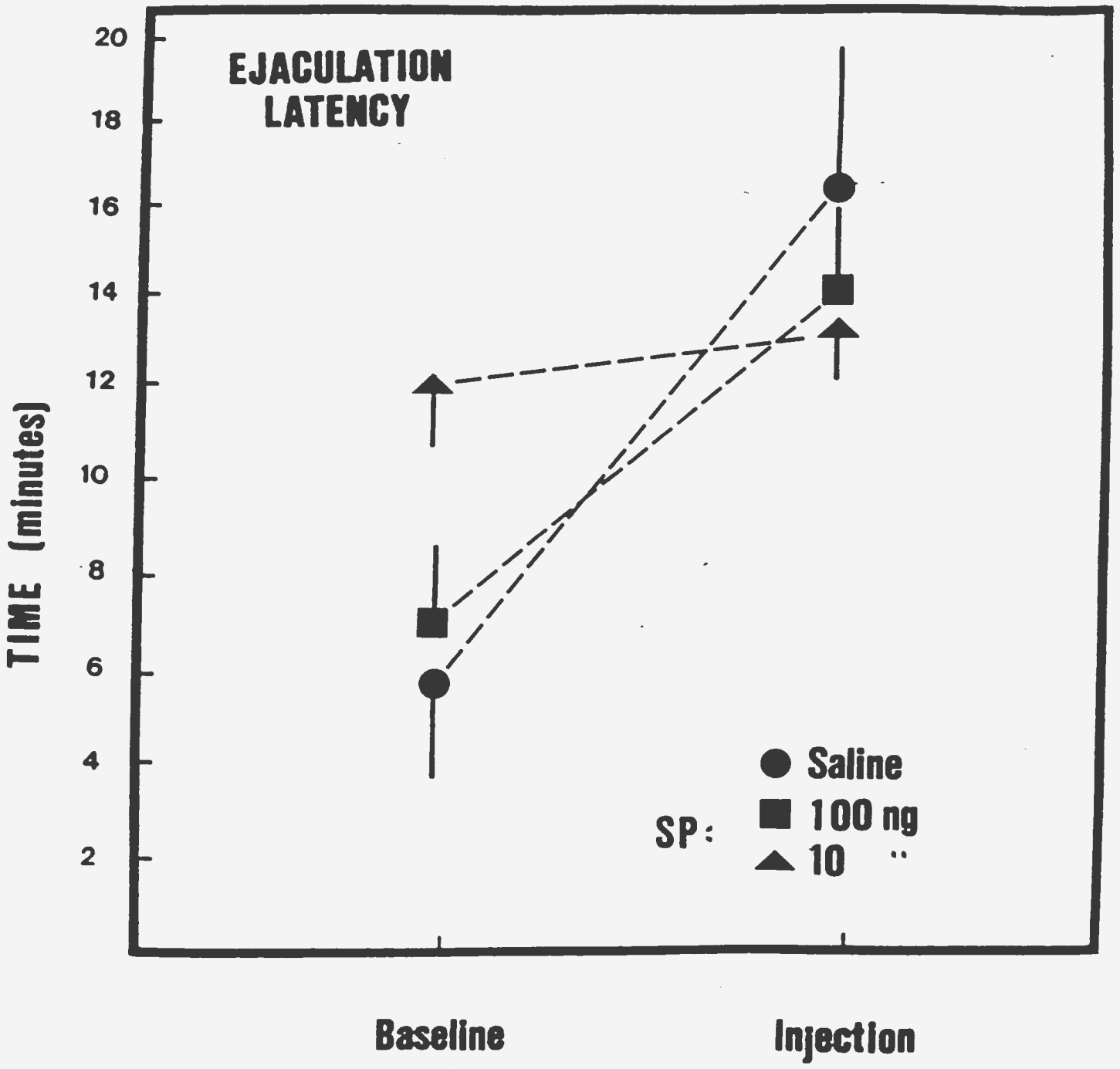
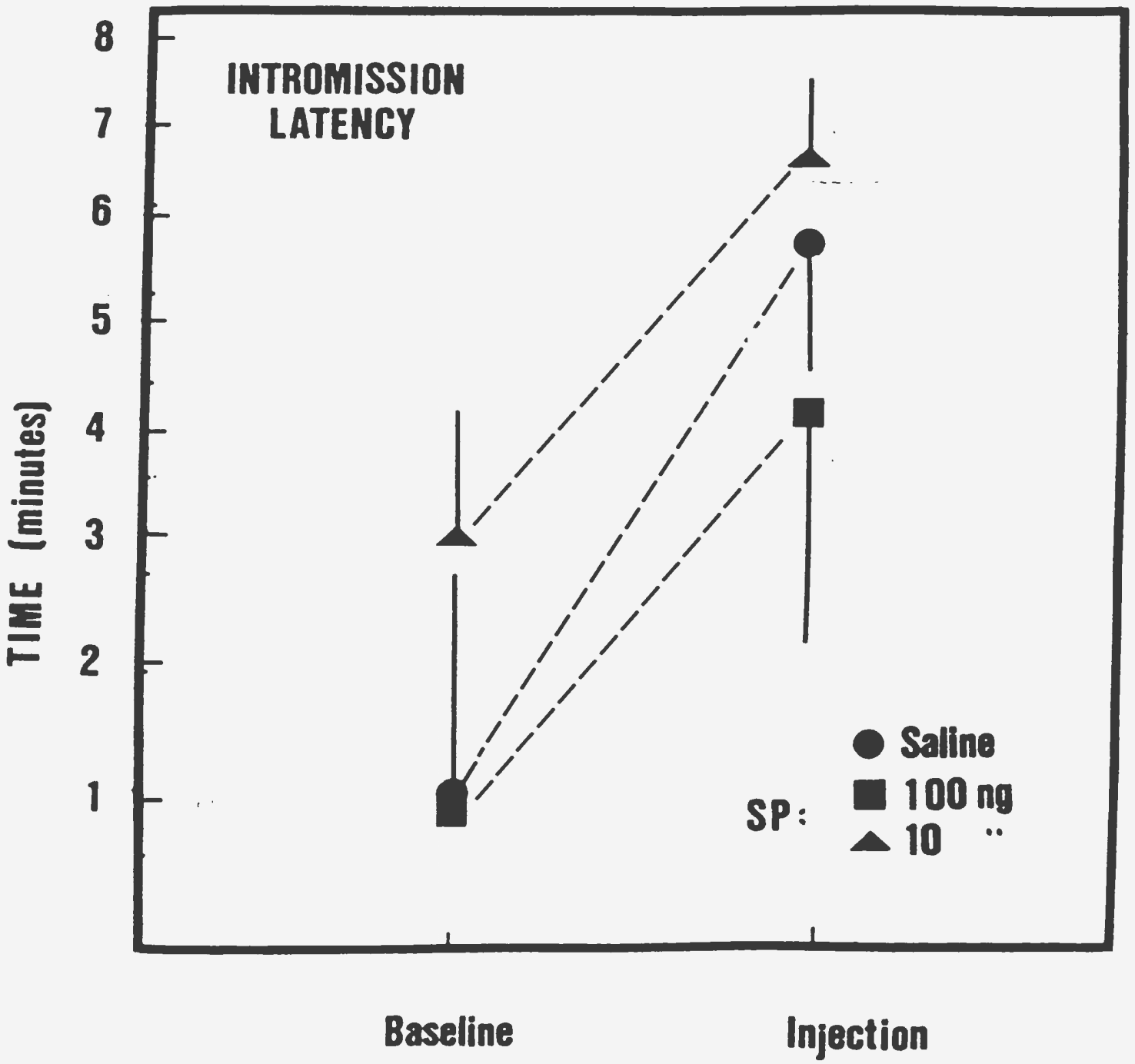

EJACULATION
LATENCY
TIME (minutes)
20
18
16
14
12
10
8
6
4
2
Saline
SP: 100 ng
10 "
Baseline
Injection

FIGURE 7

Effects of two different doses of SP on intromission latencies in experiment 1, during baseline and injection tests with two different doses of SP, or vehicle. Lines represent standard error of the mean.


INTROMISSION
LATENCY
TIME (minutes)
8
7
6
5
4
3
2
1
Saline
100 ng
10 ··
SP:
Baseline
Injection

Experiment 2: The Effects of Surgery on Different Components of Male Sexual Behavior

In Experiment 1, forty-eight hours following an initial baseline test (48 hour test session), scores of most components of male copulatory behavior were dramatically increased following preoptic injections of both saline and SP. However, in animals that received a second injection (4 days following the first), neither SP nor saline produced an increase in scores of a similar magnitude to that seen following the first injection (see figure 5). Therefore, it was not clear whether the increase in scores observed following the first injections resulted from the intracerebral injections per se, a temporary lesion induced by the bilateral cannula placements into the MPOA-AH, the interval between the first and second post operative test, or a combination of these factors. In order to explore these possibilities, measures of male copulatory behavior in a cannula-implanted control group were compared with an untreated control group using testing procedures identical to those in experiment one. If the effects of bilateral cannula placements in the MPOA-AH were disrupting male copulatory behavior during the " 48 hour test session ", then one would expect differences between the cannula-implanted controls and the untreated controls on the " 48 hour test ". If, however, the test interval was a major contributing factor, both the operated and untreated control groups would exhibit increased scores.

Material and Methods

Animals and Surgery:

A new group of twenty adult male Sprague Dawley rats (375-425 grams) was used. The animals were divided into two groups. One group (n = 10) received a

pair of stereotaxically implanted guide cannulae aimed at the MPOA-AH (cannula-implanted); another group served as untreated controls (no surgery).

Behavioral Testing:

Three days following surgery, both groups were given a baseline test of male copulatory behavior. Forty-eight hours later, each male was tested again. None of the males in either group, however, received an intracerebral injection. Data from the baseline test and the forty-eight hour test from the cannula- implanted group were compared to that of the untreated control group.

Results

Of the twenty animals, two died during surgery, one had both cannulae located outside the MPOA-AH, and three failed to satisfy criterion at the post-operative baseline test. Another died before completion of the data acquisition. That left thirteen animals to be included in the data analysis.

Tables 4 and 5 illustrate that, in animals that had bilaterally implanted cannulae in the MPOA-AH ($n = 6$, see figure 8) scores on all parameters of male copulatory behavior increased on the second post- operative test session (although only ML,IL, and EL's were significantly increased). This was in striking contrast to the untreated controls ($n = 7$): their scores were relatively stable (see table 4). The parameters that seemed most affected by surgery were latencies. For example, when compared to the cannula-implanted controls, the untreated controls had significantly shorter mount, intromission, and overall ejaculation latencies ($F = 5.73$, $df = 1,9$, $P < 0.05$; $F = 5.66$, $df = 1,9$, $P < 0.05$; $F = 11.00$, $df = 1,9$, $P < 0.01$, respectively). This is also illustrated in figures 9 through 11. One interesting observation (see table 4) is that in the untreated control group, there appeared to be a prolongation of the refractory period on the

2nd test compared to the cannula-treated controls (although not significantly different, $F = 1.29$, $df = 1,9$, $P > 0.05$)

FIGURE 8

Summarizes bilateral cannula placements into or near the MPOA-AH in 6 males in experiment 2. See figure 4A for a list of abbreviations.

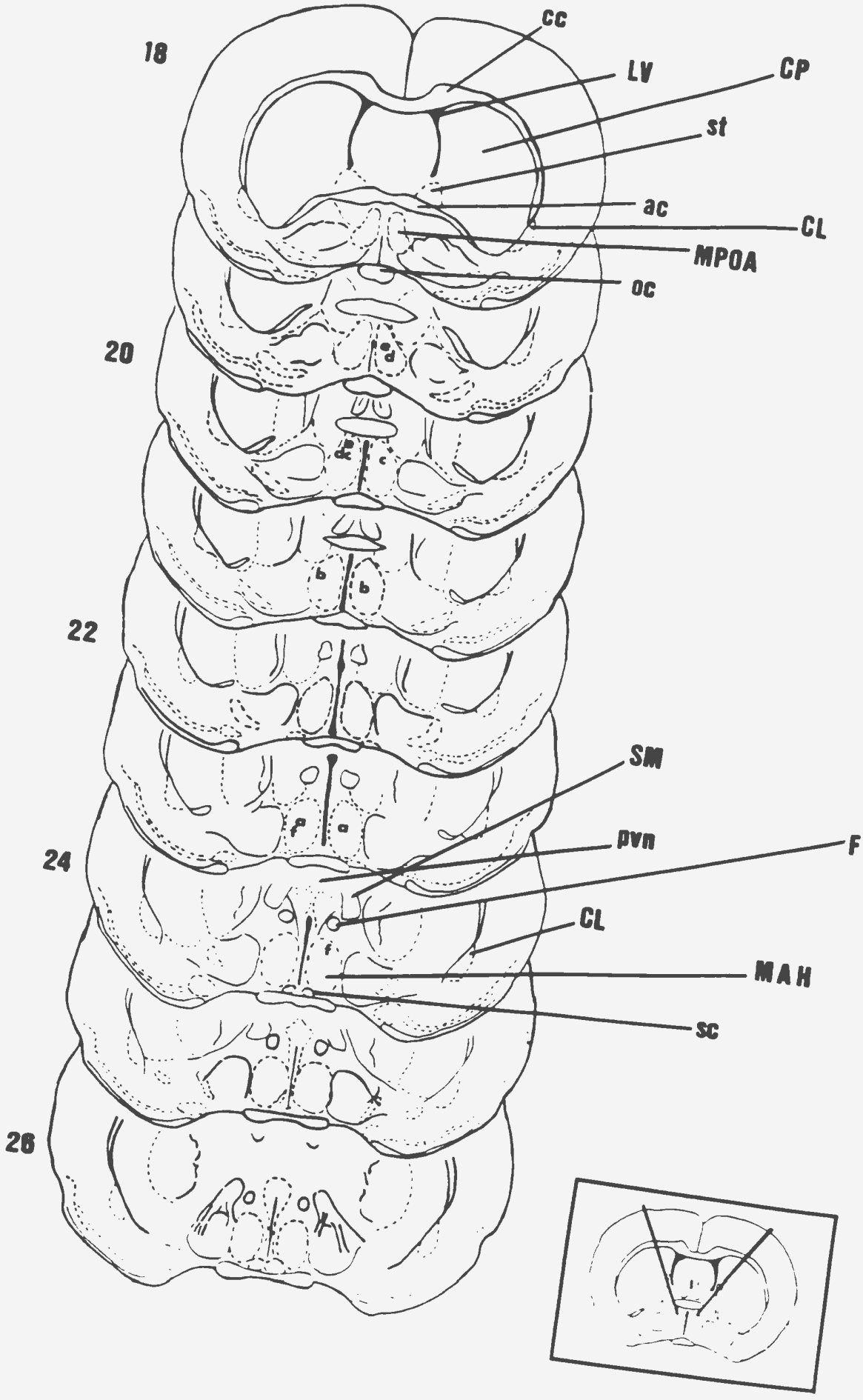

cc
LV
CP
st
ac
CL
MPOA
oc
18
20
22
24
26
SM
pvn
F
CL
MAH
sc

TABLE 4

EXPERIMENT 2 : LATENCIES (in seconds)

Effects of bilateral cannula implants into the MPOA-AH on male sexual behavior.

Measures	Baseline	2nd Test	
ML			
Cannula-implanted	129.9 + 19	391.0 + 125	*
Untreated	141.1 + 20	155.3 + 64	
IL			
Cannula-implanted	70.6 + 13	362.2 + 133	*
Untreated	133.3 + 21	107.7 + 53	
EL			
Cannula-implanted	510.5 + 120	1113.4 + 256	**
Untreated	623.7 + 180	630.2 + 176	
AEL			
Cannula-implanted	439.2 + 116	751.1 + 300	
Untreated	545.6 + 136	465.9 + 135	
ICI			
Cannula-implanted	40.5 + 8	97.6 + 142	
Untreated	48.7 + 14	51.1 + 21	
PEI			
Cannula-implanted	254.2 + 13	291.3 + 42	
Untreated	337.2 + 35	445.2 + 70	
% E			
Cannula-implanted	100	66	
Untreated	100	80	

All values are given as mean + S.E.M. See text for further details.

* = significantly different from untreated, $P < 0.05$
** = significantly different from untreated, $P < 0.01$

TABLE 5

EXPERIMENT 2 : FREQUENCIES

Effects of bilateral cannula implants into the MPOA-AH on male sexual behavior.

Measures	Baseline	2nd Test
MF		
Cannula-implanted	4.2 + 3.9	4.5 + 2.3
Untreated	3.8 + 1.9	4.3 + 1.5
IF		
Cannula-implanted	12.5 + 3.1	13.0 + 7.7
Untreated	12.6 + 2.4	11.0 + 2.2
ATTEMPTS		
Cannula-implanted	1.0 + 0	1.0 + 0.1
Untreated	1.6 + 0.2	1.5 + 0.5
CRAWLS		
Cannula-implanted	1.0 + 0.2	2.0 + 0.1
Untreated	1.1 + 0.2	1.3 + 0.3
MISDIRECTED		
Cannula-implanted	1.0 + 0.3	1.2 + 0.1
Untreated	3.6 + 0.6	2.5 + 0.2

All values are given as mean + S.E.M. See text for further details.

57 (a)

FIGURE 9

A comparison of the mean mount latencies in experiment 2, between bilaterally implanted (no injection) controls (MPOA- AH), and untreated (no surgery) controls displayed over two baseline tests (30 minutes) with a receptive female. Lines represent standard error of the mean. Asterisk indicates significantly different from the cannula-implanted control group.

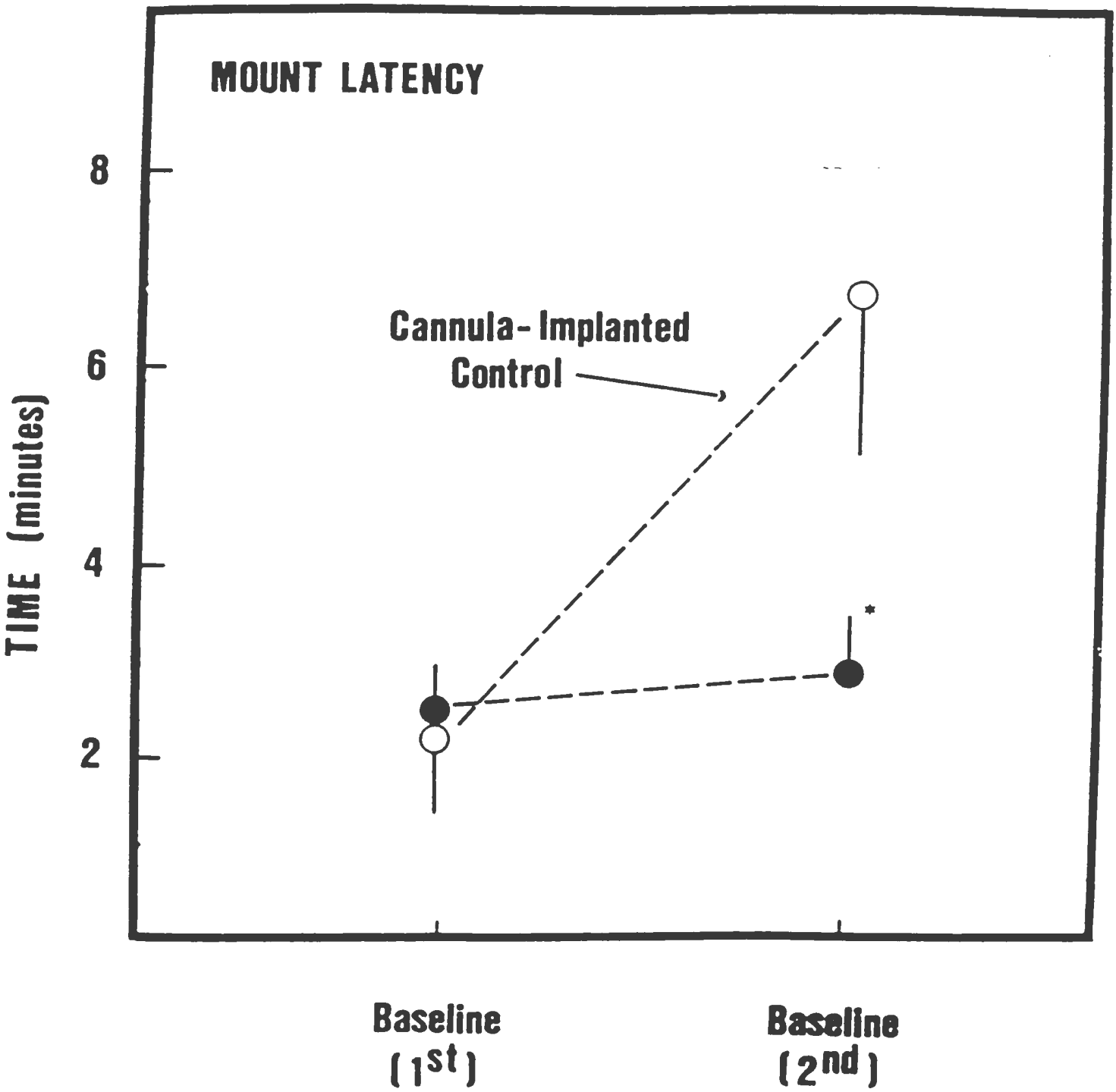

MOUNT LATENCY
TIME (minutes)
8
6
4
2
Cannula-Implanted Control
*
Baseline (1st)
Baseline (2nd)

FIGURE 10

Shows the mean intromission latencies between cannula- implanted and untreated controls displayed over two baseline tests with a sexually receptive female in experiment 2. Asterisk indicates significantly different from controls. Lines correspond to standard error of the mean.

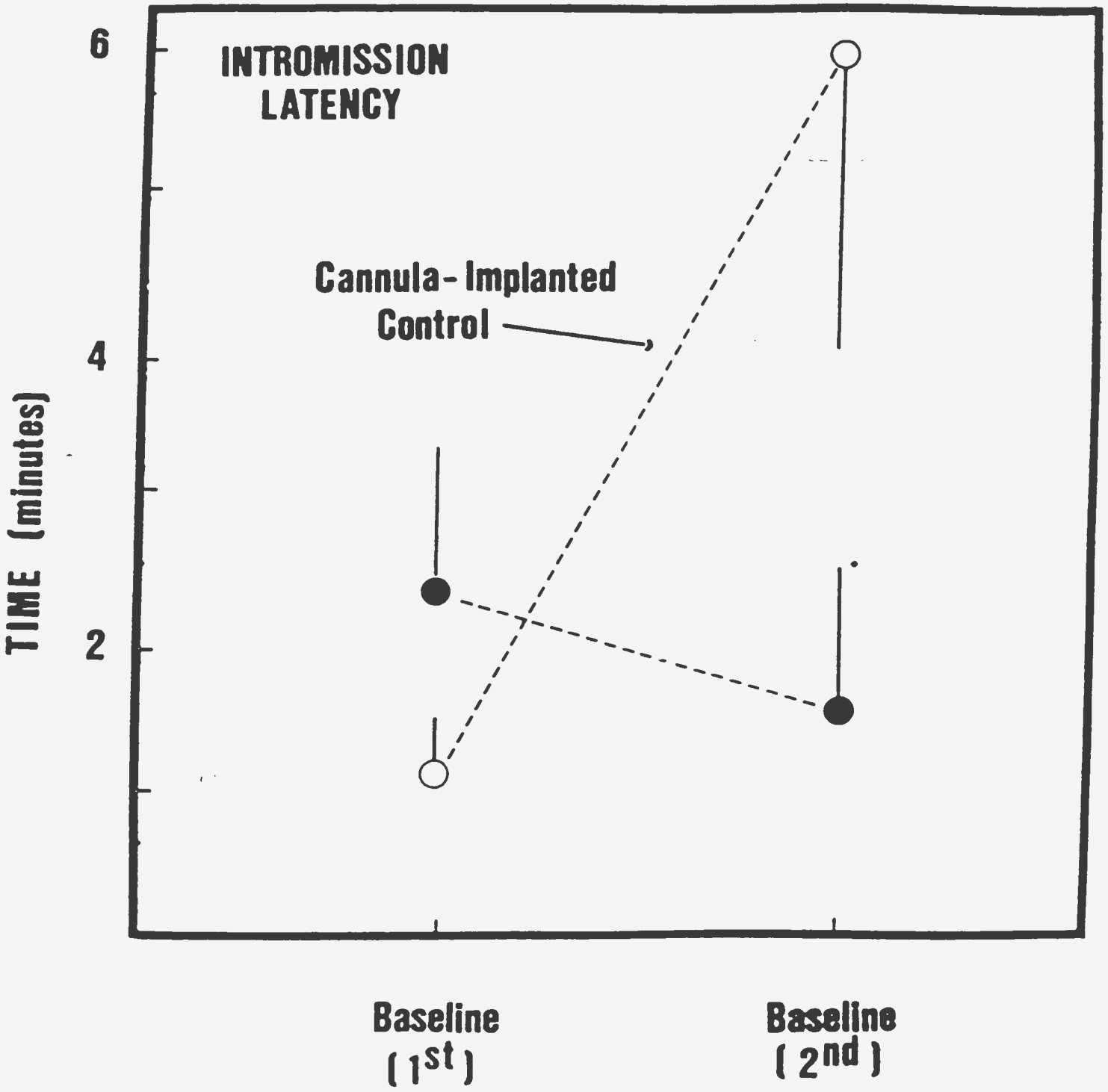
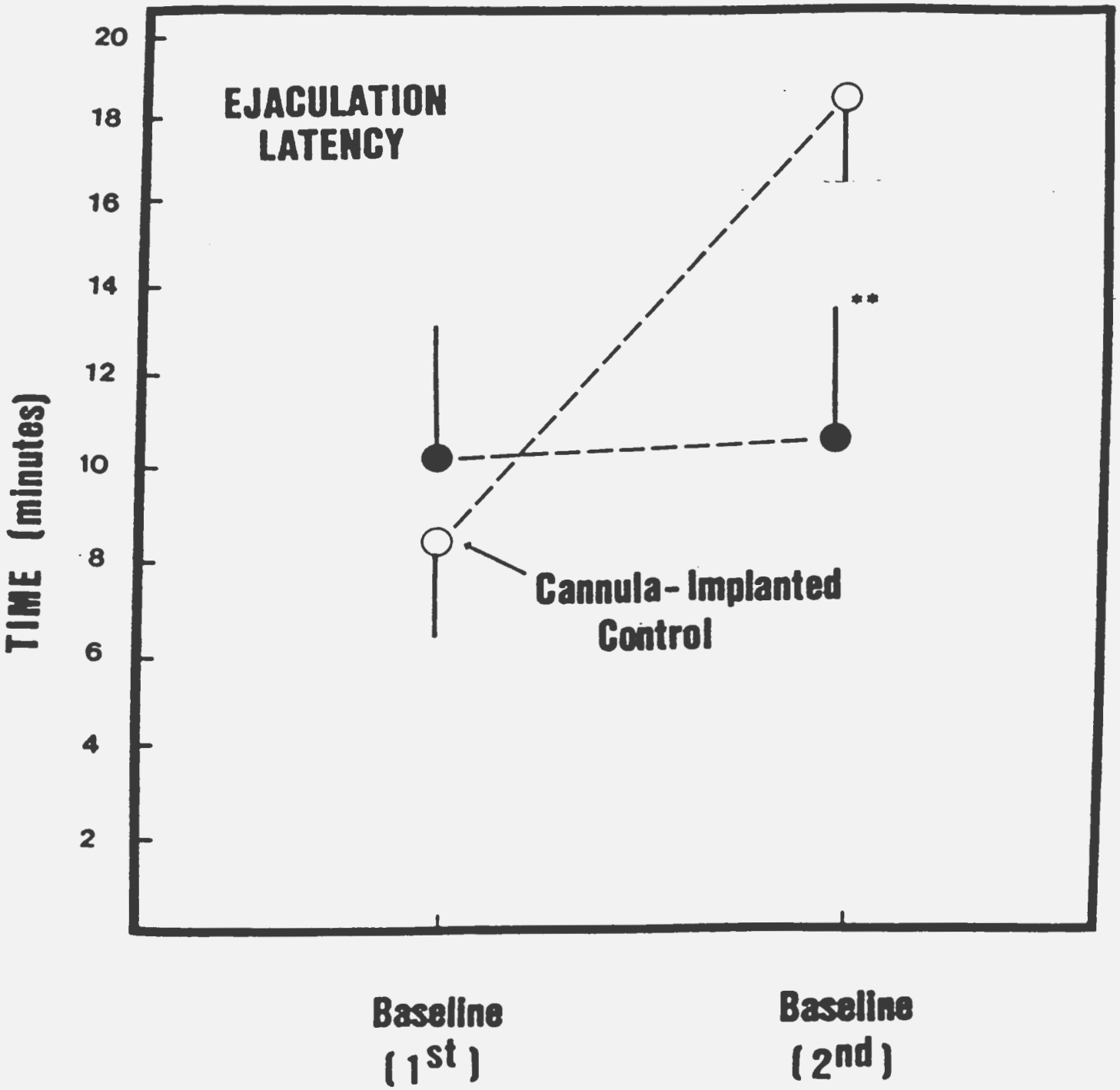

INTROMISSION
LATENCY
Cannula-Implanted
Control
TIME (minutes)
6
4
2
Baseline
(1st)
Baseline
(2nd)

FIGURE 11

Comparison of the effects of bilateral cannula placements in the MPOA in experiment 2, on ejaculation latencies over two baseline test with that of an untreated control group. Asterisks indicate significantly different from cannula-implanted group. Lines indicate standard error of the mean.


EJACULATION LATENCY
TIME (minutes)
20
18
16
14
12
10
8
6
4
2
**
Cannula-Implanted Control
Baseline (1st)
Baseline (2nd)

Experiment 3: The Effects of Injections of SP into the Caudate/Putamen on Male Sexual Behavior

The preceding experiment demonstrates that bilateral cannula placement into the MPOA-AH had detrimental effects on male copulatory behavior 48 hours following an initial post-operative baseline test. Consequently, experiment 3 was designed to explore whether these effects were specific to the MPOA-AH. For example, if stereotaxic surgery itself was affecting male copulatory behavior on the forty-eight hour test, then one would expect similar increases of measures of male copulatory behavior following bilateral cannula implants in another area of the brain. Since the caudate/putamen has been suggested to play a role in male sexual behavior (Caggiula, Shaw, Antelman, and Edwards, 1976; Hull, Bitran, Pehek, Warner, Band, and Holmes, 1986), another group of males were stereotaxically implanted with bilateral cannulae into the caudate/putamen (CP).

In experiment 1, bilateral cannula placement into the MPOA-AH disrupted copulatory behavior regardless of whether the animals had been injected with SP or saline. This was supported by the findings in experiment 2 which revealed that bilateral cannula placement into the MPOA-AH was impairing male copulatory behavior. Accordingly, the purpose of experiment 3 was twofold: firstly, to compare the effects on male copulatory behavior observed in experiment 1 following intracerebral injections of SP into the MPOA-AH to that of another brain site (CP). Secondly, to evaluate whether SP had a functional role in masculine sexual behavior at the level of the CP by examining the effects of SP injections into the caudate/putamen on male copulatory behavior 48 hours following a baseline test.

Material and Methods

Animals and Surgery:

A new group of 10 adult male Sprague Dawley rats (380-440 grams) was included in the experiment. Each animal received a pair of stereotaxically implanted guide cannulae aimed at the dorsal caudate/putamen.

Behavioral Testing:

Beginning three days after stereotaxic surgery, each animal was given a post-operative baseline test. Forty-eight hours later, the males were injected with 10 ng SP/.3 ul bilaterally.

Intracerebral Injections:

Identical to that of Experiment 1.

Results

Of the ten animals that began the experiment, nine had both cannulae located within the dorsal caudate/putamen. However, one died before completion of data acquisition, another was sick on the second test session, and one failed to satisfy criterion at the baseline test. Data from the remaining 6 animals were analyzed. Figure 12 is representative of bilateral cannulae placements in these animals.

As can be seen in tables 6 and 7, an analysis of covariance revealed that, when compared to both the saline and SP (10 ng) injected animals in Experiment 1, the caudate group differed on most parameters of male copulatory behavior. This

FIGURE 12

Is an illustration of a representative bilateral cannula placement into the dorsal caudate/putamen in males which received injection of 10 ng of substance P in experiment 3. The number in the right top corner identifies the figure in the Konig and Klippel (1963) atlas from which the figure was redrawn. Abbreviations: ac, anterior commissure; cc, corpus callosum; CPu, caudate putamen; f, fornix; GP, globus pallidus; ic, internal capsule; MPOA, medial preoptic area; oc, optic chiasm; 3V third ventricle.

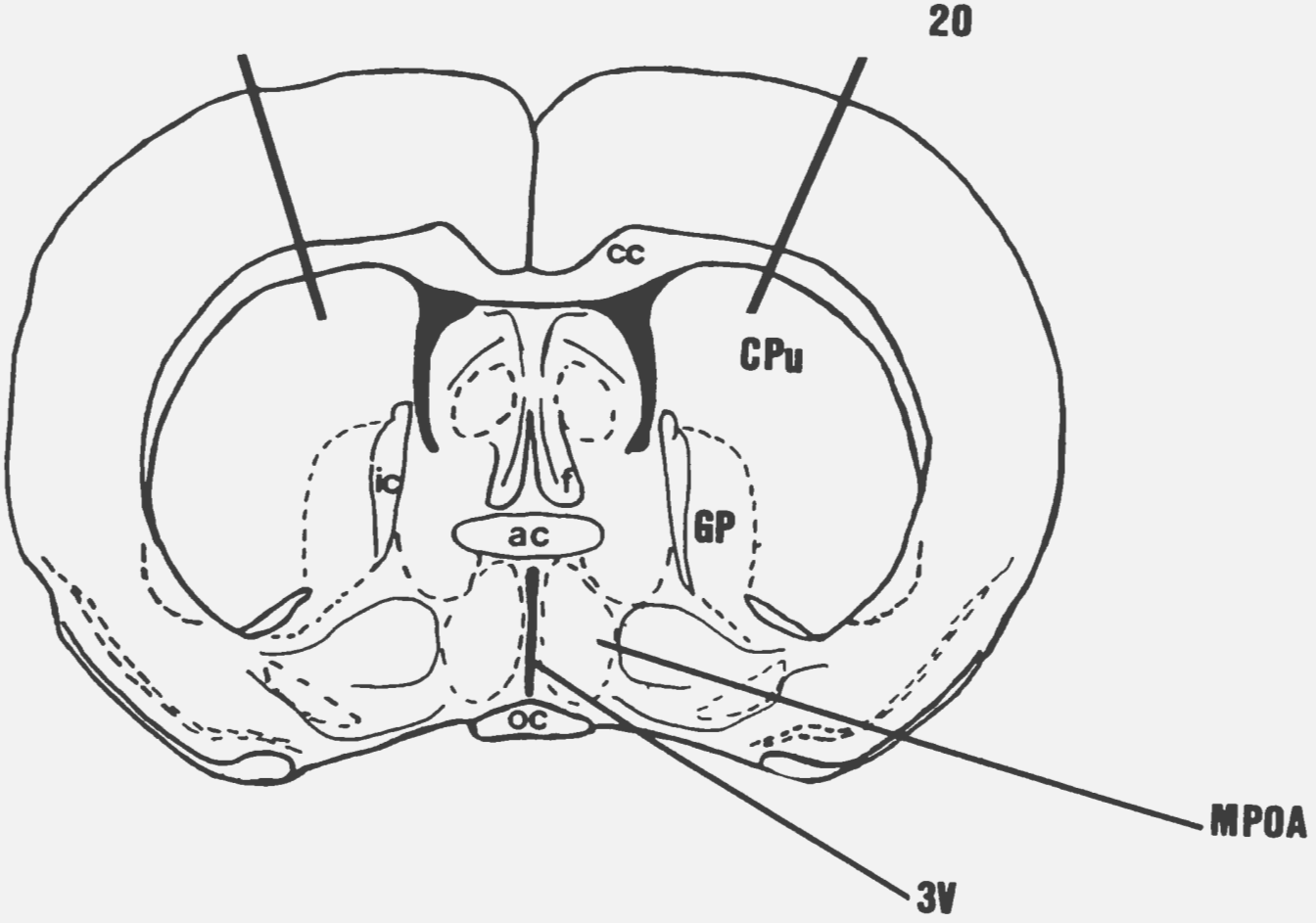

20
cc
CPu
ic
f
GP
ac
oc
MPOA
3V

TABLE 6

EXPERIMENT 3 : LATENCIES (in seconds)

Effects of injections of saline or SP into either the MPOA-AH, or the caudate/putamen on male sexual behavior.

Measures	Baseline	Saline	Substance P (10 ng)	
ML				
MPOA-AH	107.2 + 40	405.8 + 109		
Caudate	149.4 + 16		82.1 + 46	*
MPOA-AH	212.6 + 53		388.3 + 64	
IL				
MPOA-AH	58.0 + 16	356.7 + 131		
Caudate	71.0 + 18		75.7 + 26	**
MPOA-AH	152.2 + 70		338.6 + 115	
EL				
MPOA-AH	348.9 + 80	978.9 + 200		
Caudate	417.8 + 126		506.0 + 50	*
MPOA-AH	586.1 + 45		777.5 + 43	
AEL				
MPOA-AH	229.5 + 29	621.7 + 163		
Caudate	358.5 + 43		506.2 + 55	
MPOA-AH	675.9 + 59		440.1 + 63	
ICI				
MPOA-AH	38.8 + 5	74.7 + 19		
Caudate	45.6 + 6		31.1 + 2.3	
MPOA-AH	47.7 + 6		55.1 + 7	
PEI				
MPOA-AH	294.6 + 33	325.0 +37		
Caudate	290.0 + 15		339.3 + 27	
MPOA-AH	352.6 + 43		340.0 + 36	
% E				
MPOA-AH	100	100		
Caudate	100		100	
MPOA-AH	100		44	

All values are given as mean + S.E.M. See text for further details.

* = $P < 0.05$, caudate vs MPOA-AH injections.
** = $P < 0.01$ caudate vs MPOA-AH injections

TABLE 7

EXPERIMENT 3 : FREQUENCIES

Effects of intracerebral injections of saline or SP into either the MPOA-AH, or the caudate/putamen on male sexual behavior.

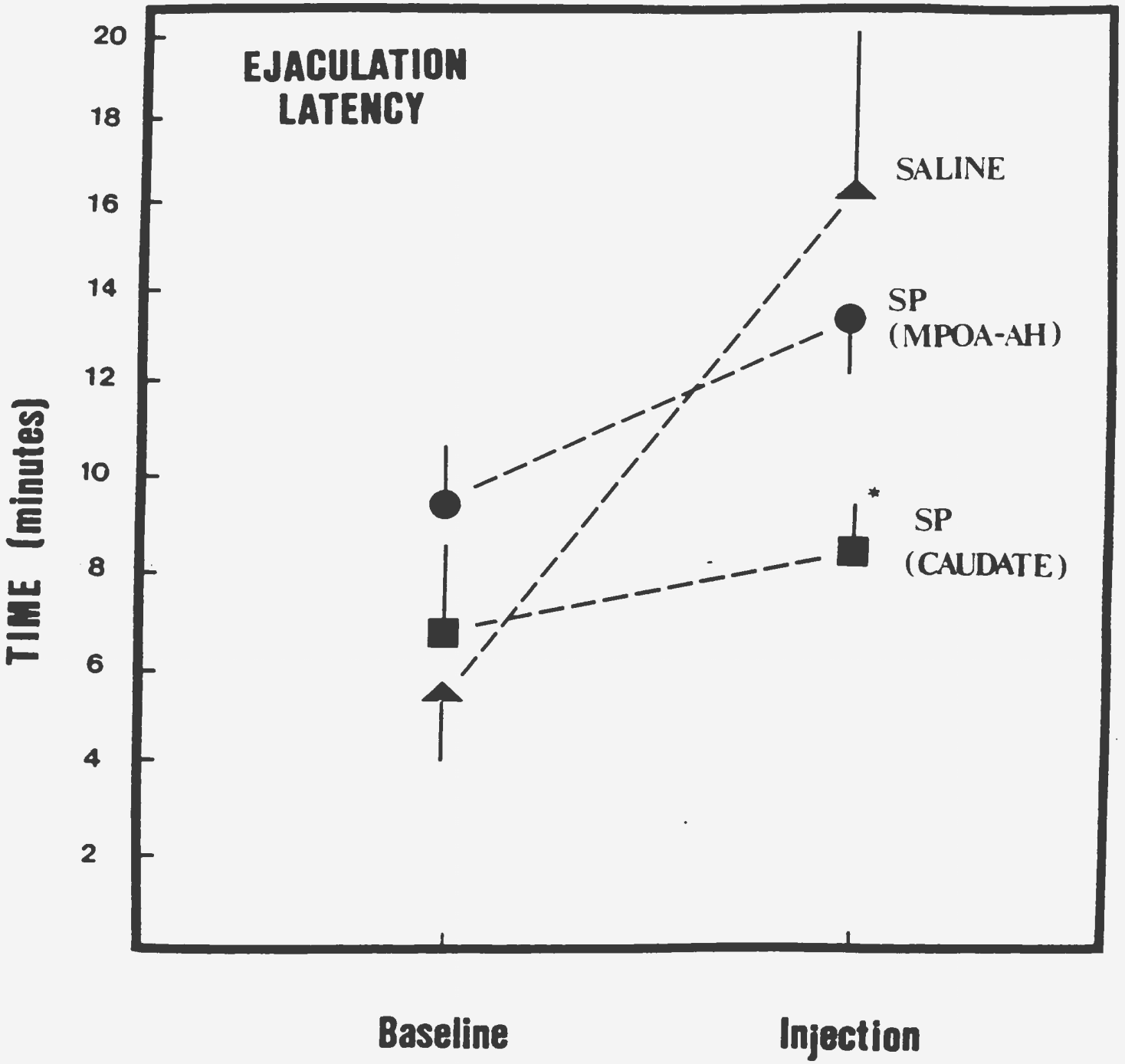
Measures	Baseline	Saline	Substance P (10 ng)
MF			
MPOA-AH	2.6 + .7	5.6 + 3	
Caudate	2.3 + 1		3.6 + 1
MPOA-AH	3.2 + .8		4.7 + 1.2
IF			
MPOA-AH	9.6 + 1.2	14.5 + 3	
Caudate	9.5 + 1.6		16.0 + 5.9
MPOA-AH	18.0 + 2.8		15.0 + 4.6
ATTEMPTS			
MPOA-AH	1.8 + 0.4	3.3 + 0.9	
Caudate	1.6 + 0.1		1.0 + 0.9
MPOA-AH	1.3 + 0.3		2.5 + 0.4
CRAWLS			
MPOA-AH	1.1 + 0.1	1.3 + 0.2	
Caudate	1.0 + 0.3		1.3 + 0.1
MPOA-AH	1.4 + 0.1		1.5 + 0.5
MISDIRECTED			
MPOA-AH	1.0 + 0.1	1.0 + 0.2	
Caudate	1.3 + 0.2		1.1 + 0.3
MPOA-AH	1.6 0.1		1.3 + 0.2

All values are given as mean + S.E.M. See text for further details.

difference was not the result of a SP-induced facilitation following caudate injections, since post hoc analysis using a multiple comparison t-test revealed no significant difference between the first and second test sessions in the SP injected caudate group. Therefore, it appeared that the main effect of condition was primarily the result of an increase in scores on the second test in the MPOA-AH injected groups. This is illustrated in figures 13 and 14. Again, the most dramatic effects were on latencies where mount, intromission, and ejaculation latencies of the Caudate group differed significantly from the other two groups ($F = 4.9$, df, 2,15, $P < 0.05$; $F = 3.9$, df, 2,15, $P < 0.05$, $F = 5.3$, df = 2,15, $P < 0.01$, respectively).

FIGURE 13

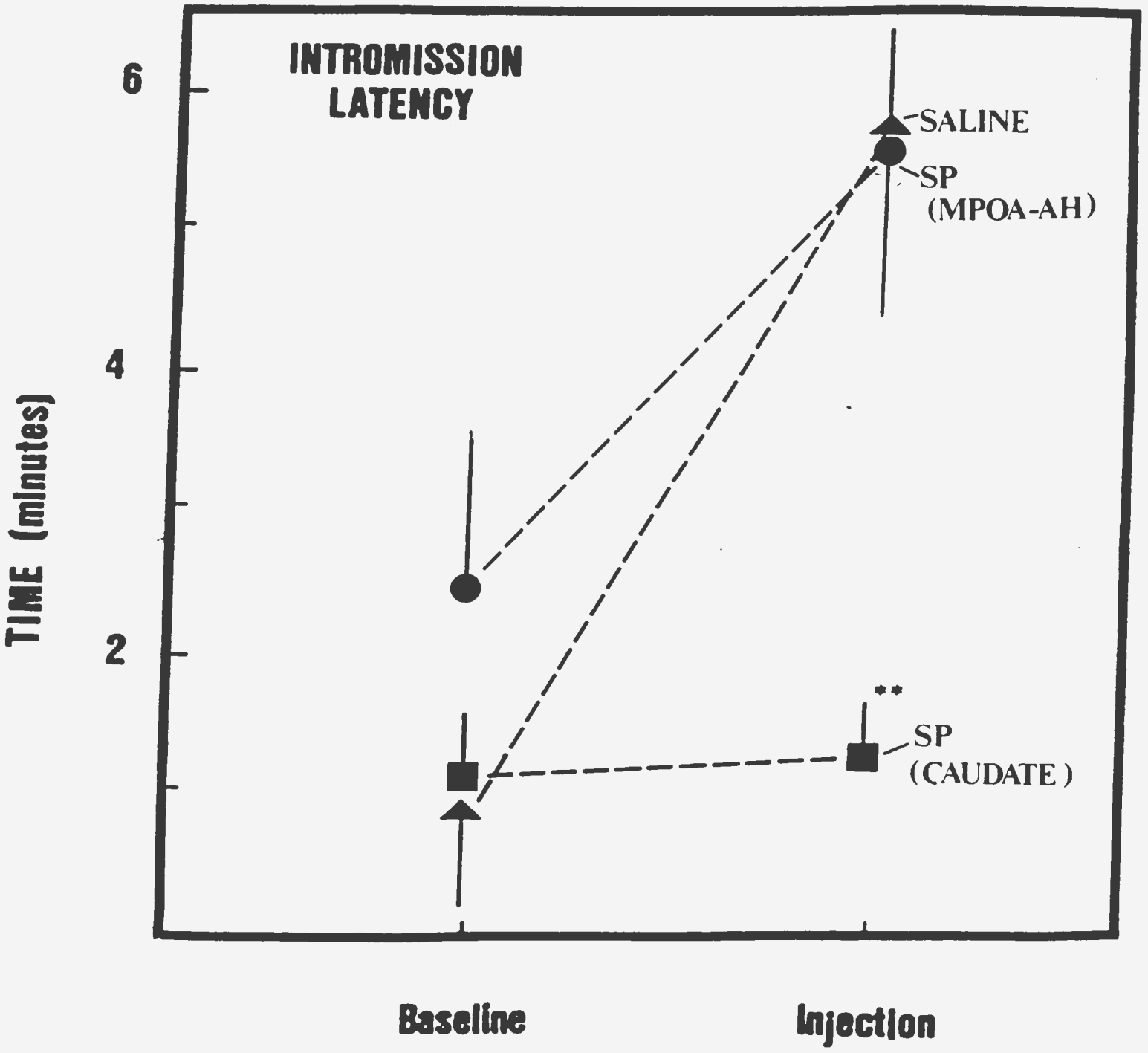
Comparison of mean ejaculation latencies in males in experiment 3, during baseline tests and injection tests of SP or vehicle into the MPOA, or into the caudate/putamen. Asterisk indicates significantly different from MPOA-AH groups. Lines represent standard error of the mean.


EJACULATION
LATENCY
TIME (minutes)
20
18
16
14
12
10
8
6
4
2
SALINE
SP
(MPOA-AH)
SP
(CAUDATE)
*
Baseline
Injection

67 (a)

FIGURE 14

Mean intromission latency between MPOA-AH groups and caudate/putamen group in experiment 3, during baseline and injection tests with SP or vehicle. Asterisks indicate significantly different from the MPOA-AH injected groups.


INTROMISSION
LATENCY
6
4
2
TIME (minutes)
SALINE
SP
(MPOA-AH)
**
SP
(CAUDATE)
Baseline
Injection

Experiment 4: Test Interval

It was clear from the results of Experiment 2 that, in animals with bilateral cannulae within the previous defined boundaries of the MPOA-AH, a second post-operative test 48 hours following an initial baseline test produced dramatic increases in most of the temporal measures of male copulatory behavior. Moreover,it appeared from Experiment 3 that this was specific to the MPOA-AH, in that neither surgery itself nor SP injections into the dorsal caudate/putamen 48 hours after the first post-operative test had any appreciable effect on male copulatory behavior. Thus it appears that the timing of the post-operative tests interacts with the effects of MPOA-AH cannula implants to produce decrements in male copulatory behavior. Consequently, in Experiment 4, different intervals between the first and second post-operative test were assessed in order to determine an interval that would produce relatively stable first and second post-operative scores in animals with MPOA-AH cannulae.

Material and Methods

Animals and Surgery:

A new group of ten adult male Sprague Dawley rats (390-425 grams) began the experiment. Each animal received a pair of bilaterally implanted guide cannulae aimed at the MPOA-AH.

Behavioral Testing:

The following post-operative intervals were assessed in order to establish relatively stable post- operative scores between post-operative tests: A) First test 3

days post-surgery, second test 7 days post- surgery (n = 3); B) first test 4 days post-surgery, second test 8 days post-surgery (n = 3); C) first test 4 days post surgery, second test 9 days post-surgery (n = 4). During this experiment, no intracerebral injections were made.

Results

As can be seen from tables 8 through 13 four days post-surgery (1st test) and 5 days following the 1st testing (9 days post-surgery), produced relatively stable scores on all components of male copulatory behavior. Accordingly, the post-operative interval from condition " C " was used in all subsequent experiments.

TABLE 8

EXPERIMENT 4 : LATENCIES (in seconds)

Post-operative test interval which produced relatively stable measures of male sexual behavior in animals with bilateral cannula implants into the MPOA-AH.

Measures	4 days post surgery	9 days post surgery
ML	125.3 + 38	138.7 + 26
IL	134.2 + 41	104.2 + 60
EL	788.2 + 145	698.6 + 156
AEL	671.2 + 126	590.1 + 186
ICI	51.6 + 3	43.4 + 10
PEI	304.6 + 28	329.8 + 22
% E	100	100

All values are given as mean + S.E.M. See text for further details.

TABLE 9

EXPERIMENT 4 : FREQUENCIES

Post-operative test interval which produced relatively stable measures of male sexual behavior in animals with bilateral cannula implants into the MPOA-AH.

Measures	4 days post surgery	9 days post surgery
MF	5.1 + 1	4.6 + .6
IF	13.2 + .6	11.3 + .2
ATTEMPTS	1.3 + .2	1.0 + .1
CRAWLS	1.1 + .5	1.4 + .3
MISDIRECTED	1.6 + .7	1.3 + .9

All values are given as mean + S.E.M. See text for further details.

TABLE 10

EXPERIMENT 4 : LATENCIES (in seconds)

Post-operative interval that did not produce stable baseline scores of male sexual behavior in animals with bilateral cannulae implanted into the MPOA-AH

Measures	3 days post surgery	7 days post surgery
ML	157.3 + 22	167.9 + 44
IL	111.2 + 49	141.2 + 28
EL	912.5 + 132	951.3 + 200
AEL	801.3 + 100	810.1 + 121
ICI	53.0 + 3	57.3 + 7
PEI	434.1 + 43	400.0 + 16
% E	100	66

All values are expressed as mean + S.E.M. See text for details.

TABLE 11

EXPERIMENT 4 : FREQUENCIES

Post-operative interval that did not produce stable baseline scores of male sexual behavior in animals with bilateral cannulae implanted into the MPOA-AH

Measures	3 days post surgery	7 days post surgery
MF	7.3 + 2	6.9 + 1
IF	15.1 + 2	14.2 + 1
ATTEMPTS	1.2 + .1	1.0 + .2
CRAWLS	1.9 + .1	1.1 + .1
MISDIRECTED	1.5 + .3	2.0 + .5

All values are expressed as mean + S.E.M. See text for details.

TABLE 12

EXPERIMENT 4 : LATENCIES (in seconds)

Post-operative interval that did not produce stable baseline scores of male sexual behavior in animals with bilateral cannulae implanted into the MPOA-AH

Measures	4 days post surgery	8 days post surgery
ML	127.3 + 32	147.9 + 34
IL	140.1 + 59	121.2 + 48
EL	812.5 + 122	901.3 + 212
AEL	640.9 + 111	678.1 + 151
ICI	50.4 + 5	74.1 + 1
PEI	404.1 + 23	378.6 + 26
% E	100	100

All values are expressed as mean + S.E.M. See text for details.

TABLE 13

EXPERIMENT 4 : FREQUENCIES

Post-operative interval that did not produce stable baseline scores of male sexual behavior in animals with bilateral cannulae implanted into the MPOA-AH

Measures	4 days post surgery	8 days post surgery
MF	4.3 + 2	5.6 + 1
IF	16.1 + .1	12.2 + .3
ATTEMPTS	1.0 + .1	1.1 + .1
CRAWLS	1.1 + .1	1.2 + .1
MISDIRECTED	1.3 + .0	1.0 + .1

All values are expressed as mean + S.E.M. See text for details.

DISCUSSION: EXPERIMENTS 1 THROUGH 4

In the preceding experiments, it was revealed that either SP or saline injected bilaterally into the MPOA-AH forty-eight hours after an initial baseline test increased scores on most parameters of male copulatory behavior. This increase, however, seemed to be a result of an interaction between the MPOA-AH cannula implants and the interval between the first and second test session rather than the injections per se. This may have been the result from a temporary lesion induced by the bilateral cannulae into the MPOA-AH, as surgery to the dorsal caudate/putamen had no effect on male copulatory behavior forty-eight hours following the baseline test, nor were any increases observed in the untreated controls in Experiment 2. Indeed, the copulatory behavior observed in some animals during the second post-operative test in Experiment 1 closely resembled the pattern of behavior seen in animals following lesions of the MPOA-AH. For example, these animals would invariably approach the female, sniff the anogenital region, pursue her, have numerous mounts, yet be apparently unable to carry out the full copulatory sequence.

Although both groups demonstrated disrupted male copulatory behavior patterns, there were a number of distinct differences between the saline and the SP-injected groups. Firstly, although scores in both groups increased dramatically on the second post-operative test, in the SP groups, mount, intromission, as well as ejaculation latencies, were lower compared to the saline injected controls. In fact, there was a trend for a dose-related decrease on mount and intromission latencies in the SP groups compared to the saline group on the second post-operative test (see table 2). Secondly, in Experiment 1, a marked increase in the frequency of " crawls " was observed in both SP groups but not in the saline controls (see table 3). For instance, on numerous occasions, males in the SP group would pursue the female, mount and grab the flank region, but instead of an

intromission, would crawl over the female. This is particularly revealing in that this type of behavioral pattern has been referred to as " displacement " , and is believed to reflect a form of sexual frustration (Hansen and Hagetarum, 1984). This type of behavior pattern was rarely observed in the saline injected controls. Given this, it is not inconceivable that in Experiment 1, SP could have been producing a facilitation of male copulatory behavior, but due to the temporary lesion, animals were not able to copulate.

Accordingly, Experiments 5 through 10 were designed to re-evaluate the role of SP on male sexual behavior using the new post-operative test schedule established in Experiment 4.

Experiment 5: The Effects of Three Different Doses of SP on Male Copulatory Behavior

Material and Methods

Animals and Surgery:

Forty adult male Sprague Dawley rats (375-450 grams) were used. All animals received a pair of stereotaxically implanted guide cannulae aimed at the MPOA-AH.

Behavioral Testing:

As per the general methods, with one exception: since it was possible that more animals would have ejaculated in Experiment 1 if the test duration had been longer, the test session was increased to 40 minutes.

Intracerebral Injections:

Animals were divided into four groups. Three groups received either 10 ng, 100 ng, or 200 ng of SP/cannula dissolved in acidified saline in a volume of 0.3 ul into the MPOA-AH, while the remaining group received acidified saline.

Results

Of the 40 animals that began the experiment, two did not complete data

acquisition (sickness, cannula blocked), one had both cannulae located outside the MPOA-AH, and four didn't satisfy criterion at the post-operative baseline test. That left thirty-three animals for data analysis (controls, n = 8; 10 ng SP, n = 8; 100 ng SP, n = 6; 200 ng SP, n = 11). Figure 15A and 15B illustrate the location of the cannula placements within the MPOA-AH of these animals.

Tables 14 and 15 demonstrate that bilateral injections of SP into the MPOA-AH facilitated male copulatory behavior. This was revealed by a significant main effect (followed by post hoc analysis using a multiple comparison t-test) on intromission latencies ($F = 5.78$, df = 3,27, $P < 0.01$), both measures of ejaculation latency ($F = 4.60$, df = 3,27, $P < 0.01$; $F = 3.3$, df = 3,27, $P < 0.05$), and the post-ejaculatory refractory period ($F = 3.2$, df = 3,27, $P < 0.05$). With regard to frequencies, mount frequencies were significantly decreased by bilateral injections of all three doses of SP ($F = 3.48$, df = 3,27, $P < 0.05$). SP injections did not, however, facilitate male copulatory behavior in a linear-related fashion. For example, although 100 ng of SP had the greatest effect on latencies, the 200 ng dose was the least effective (see table 14). This is also illustrated in figures 16 and 17. Again, the most dramatic effect was the shortening of latencies (see table 14).

As can be seen from table 14, a marked decrease in the inter-copulatory interval was also produced by SP injections; initially, however, an analysis of covariance failed to reveal a significant main effect of condition ($F = 1.87$, df = 3,27, $P > 0.05$). In a textbook on statistical design in psychological research, Edwards (1985) discusses the problem of heterogeneity of variance when time is a dependent variable. He suggests that if there are a few extreme measures, heterogeneity of the variance may occur. When this is the case, he advocates transforming the data into their respective reciprocals in order to make the variances more homogeneous. Therefore, a reciprocal transformation was done and the data re-analyzed using an analysis of covariance. After reciprocal transformation of the raw data, a significant main effect on ICI by condition was found ($F = 2.82$, df = 3,27, $P < 0.05$).

FIGURE 15A

Summarizes bilateral cannula placements in 25 males which received injections of three different doses of SP into or near the MPOA-AH in experiment 5. Letters which have a number attached indicate corresponding number of animals with an approximately similar cannulae placement.

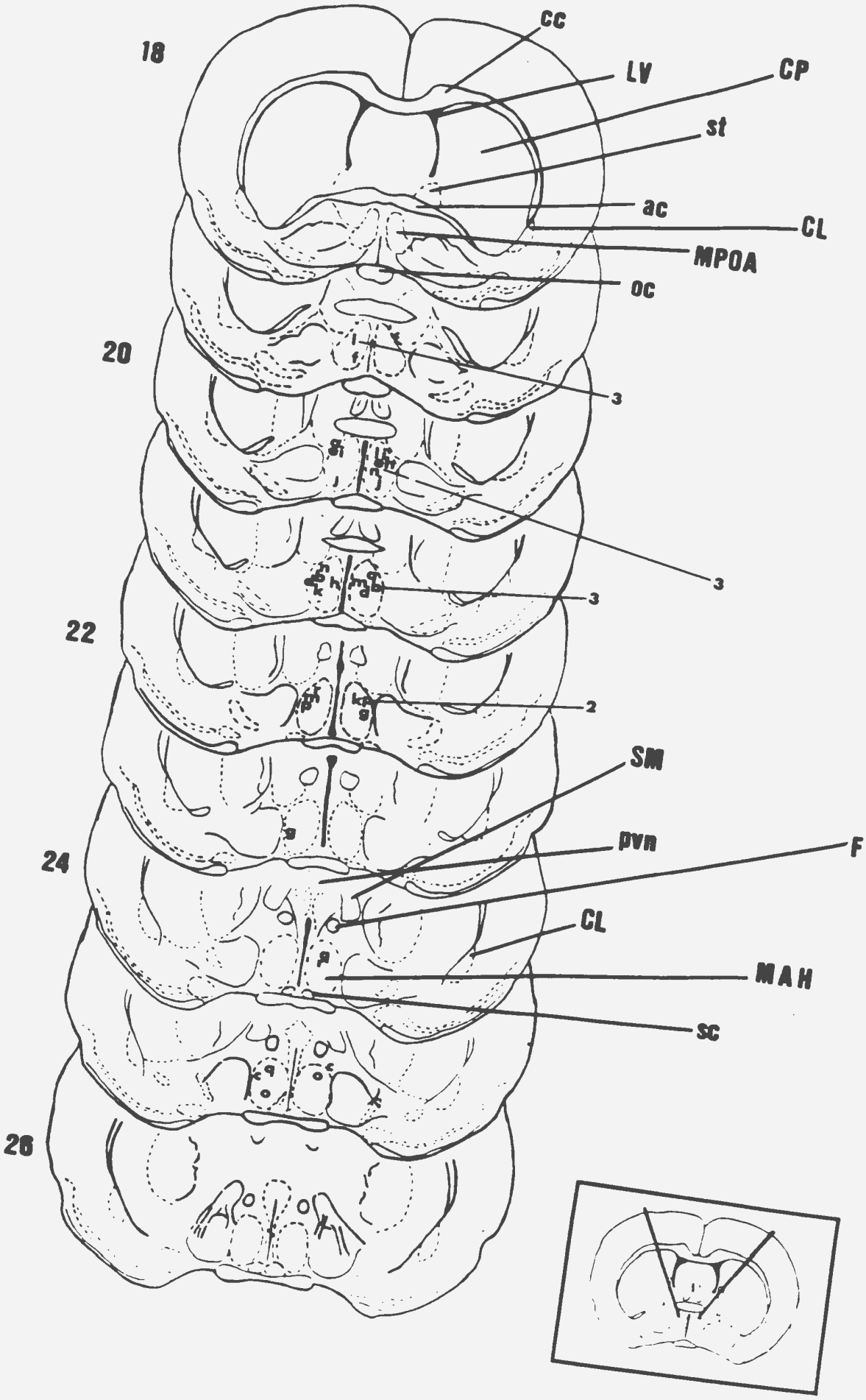

cc
18
LV
CP
st
ac
CL
MPOA
oc
20
3
3
3
22
2
SM
pvn
F
24
CL
MAH
sc
26

FIGURE 15B

Illustration of bilateral cannula placements in 8 animals which received injections of acidified saline in experiment 5.

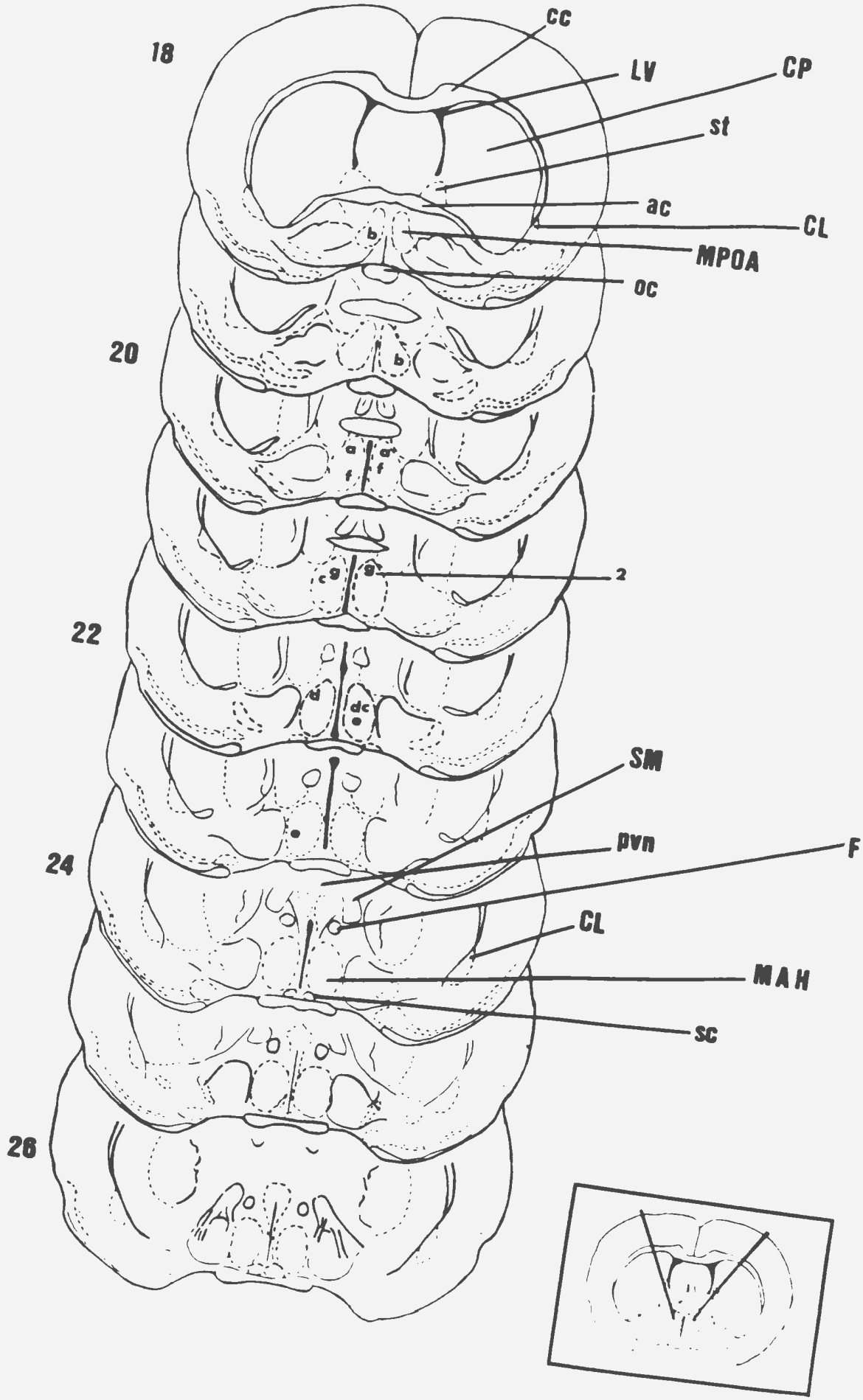

cc
18
LV
CP
st
ac
CL
MPOA
oc
20
2
22
SM
pvn
F
24
CL
MAH
sc
26

TABLE 14

EXPERIMENT 5 : LATENCIES (in seconds)

Effects of injections of saline or SP into the MPOA-AH on male sexual behavior.

			Substance P		
Measures (a)	Baseline	Saline	10 ng	100 ng	200 ng
ML					
Experimental	224.5 + 41		99.5 + 28	67.0 + .6	124.5 + 36
Control	181.6 + 47	147.9 + 23			
IL					
Experimental	168.7 + 40		57.1 + 16**	33.8 + .4**	107.0 + .4**
Control	181.9 + 49	206.3 + 42			
EL					
Experimental	950.9 + 140		438.4 + 82**	267.5 + 110**	630.7 + 130
Control	810.9 + 141	911.9 + 184			
AEL					
Experimental	801.4 + 139		380.8 + 72*	245.8 + 200*	524.2 + 100
Control	629.3 + 106	705.8 + 174			
ICI					
Experimental	57.3 + 6		36.5 + 5*	32.3 + 10*	43.2 + 8
Control	49.2 + 8	55.3 + 5			
PEI					
Experimental	308.3 + 22		294.7 + 14	283.0 + 32*	324.9 + 25
Control	289.4 + 19	347.6 + 20			
% E					
Experimental	100		100	83	100
Control	100	100			

a) at baseline, all values for the experimental group are grand means.
All values are given as mean + S.E.M.

* = $P < 0.05$, significantly different from controls.
** = $P < 0.01$ " " " " "

TABLE 15

EXPERIMENT 5 : FREQUENCIES

Effects of injections of saline or SP into the MPOA-AH on male sexual behavior.

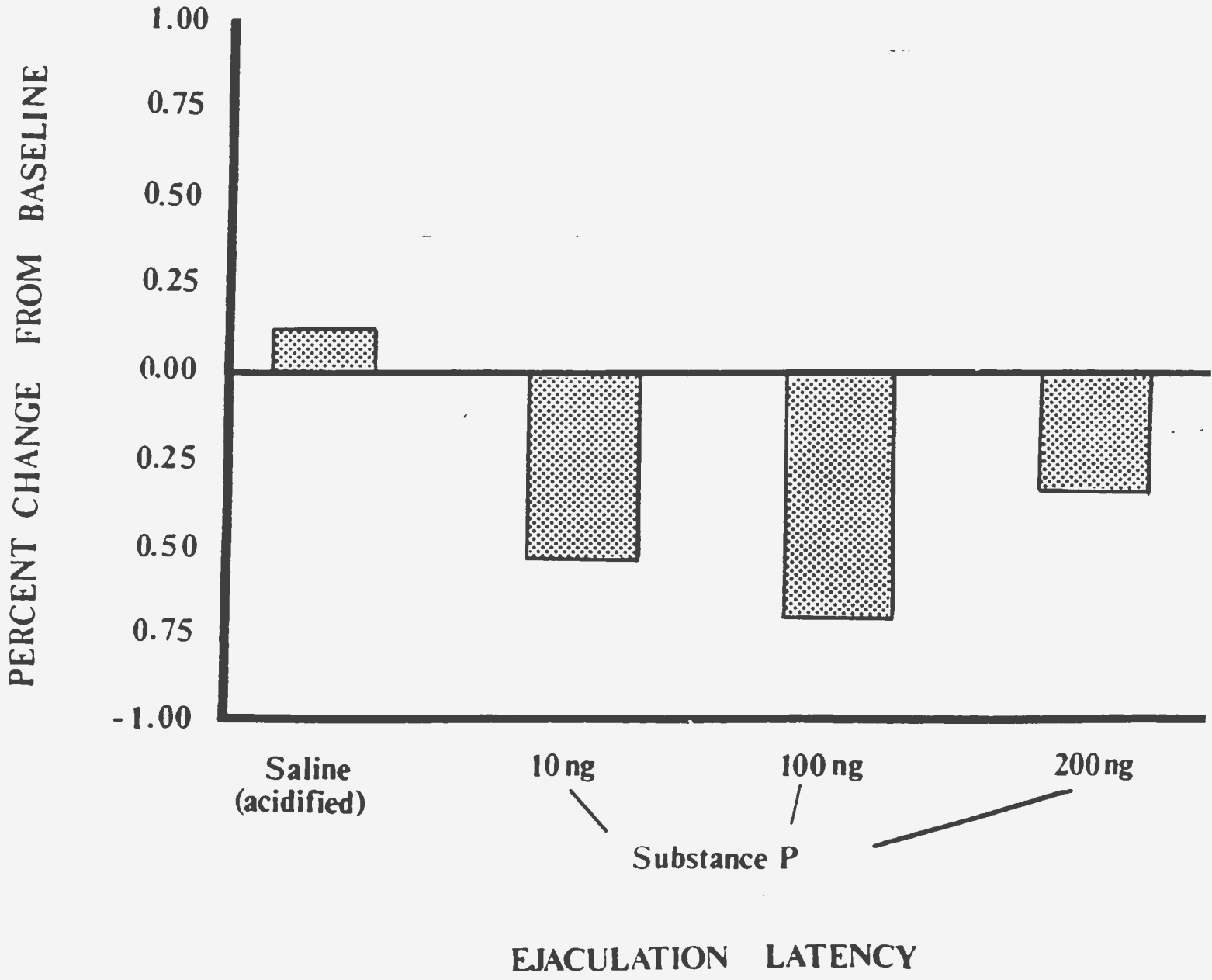
			Substance P		
Measures (a)	Baseline	Saline	10 ng	100 ng	200 ng
MF					
Experimental	6.0 + .3		1.8 + .6 *	2.1 + .7 *	3.5 + .9 *
Control	4.1 + .5	6.1 + 1.3			
IF					
Experimental	17.4 + 2.3		11.5 + 1.3	8.9 + 1.2 *	14.5 + .9
Control	15.5 + 3.1	16.2 + 2.6			
ATTEMPTS					
Experimental	1.1 + 0.1		1.1 + 0.1	1.2 + 0.2	1.3 + .2
Control	1.3 + 0.2	1.2 + 0.3			
CRAWLS					
Experimental	1.0 + 0.1		1.1 + 0.2	1.0 + 0	1.1 + 0.1
Control	1.0 + 0	1.0 + 0.1			
MISDIRECTED					
Experimental	1.0 + 0.1		1.5 + 0.1	1.0 + 0	2.0 + 0.3
Control	1.0 + 0.1	2.0 + 0.2			

a) at baseline, all values for the experimental group are grand means.
All values are given as mean + S.E.M. See text for further details.

* = $P < 0.05$, significantly different from controls.
** = $P < 0.01$ " " " " "

FIGURE 16

Illustrates the proportional change of ejaculation latencies from a baseline test session (no treatment) in animals which received three different doses of SP, or vehicle into the MPOA-AH in experiment 5.

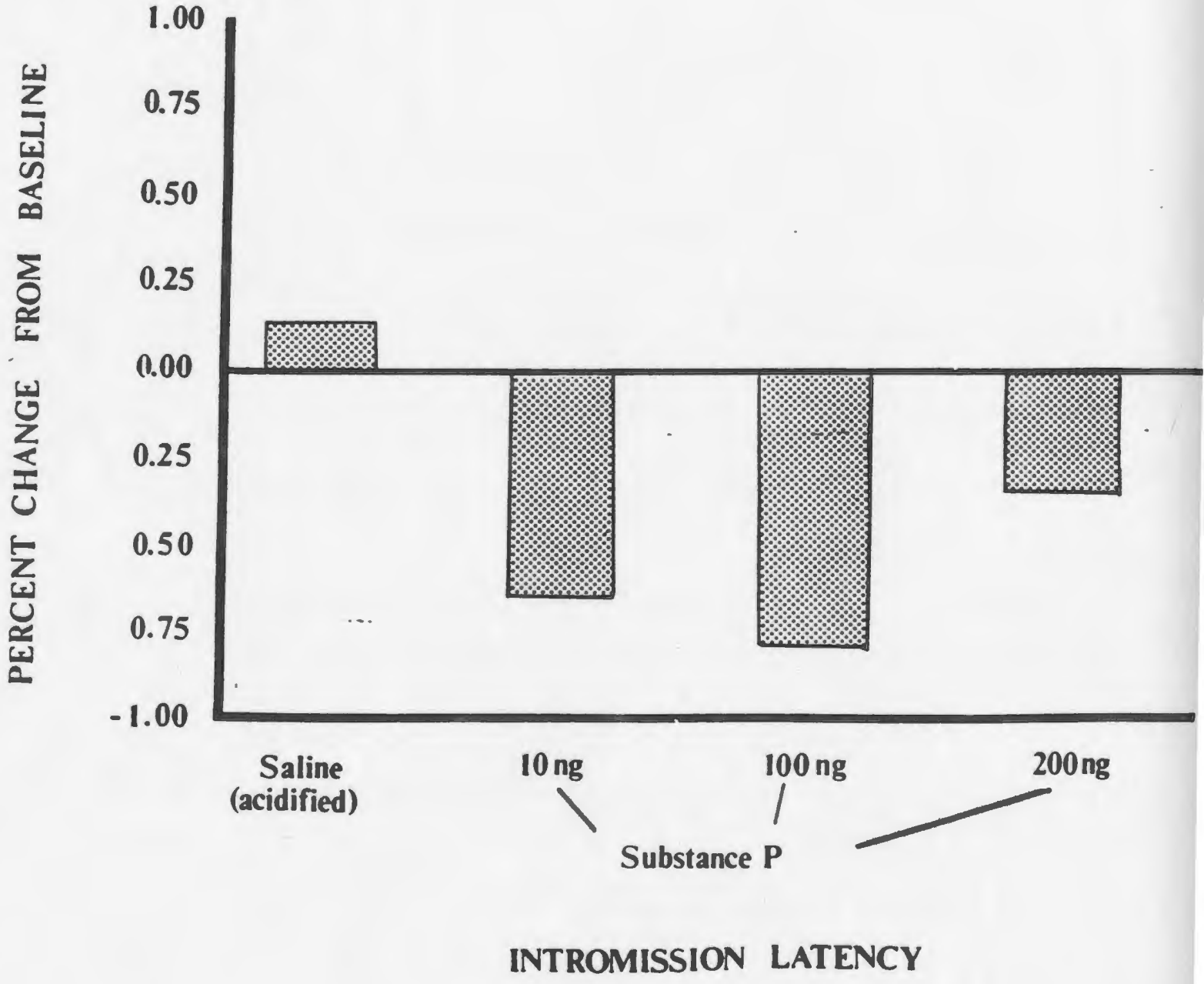

PERCENT CHANGE FROM BASELINE
1.00
0.75
0.50
0.25
0.00
0.25
0.50
0.75
-1.00
Saline (acidified)
10 ng
100 ng
200 ng
Substance P
EJACULATION LATENCY

85 (a)

FIGURE 17

Illustrates the proportional change of intromission latencies from a baseline test (no treatment) in animals which received three different doses of SP, or vehicle

into the MPOA-AH in experiment 5.


PERCENT CHANGE FROM BASELINE
1.00
0.75
0.50
0.25
0.00
0.25
0.50
0.75
-1.00
Saline (acidified)
10 ng
100 ng
200 ng
Substance P
INTROMISSION LATENCY

Experiment 6: Bilateral Injections of SP into the Caudate/Putamen and its Effects on Male Copulatory Behavior

In experiment 3, bilateral injections of 10 ng SP into the Caudate/Putamen had no appreciable effect on male copulatory behavior. It is possible that different results would have been observed in experiment 3 if the interval between the first and second post-operative test had been different. After all, SP injections into the MPOA-AH produced a facilitation of male sexual behavior in the preceding experiment but not in experiment 1. These different outcomes are attributed to the use of longer post-operative test intervals beginning with experiment 5. Therefore, the possible role of SP in the caudate/putamen on male sexual behavior was re-evaluated using the new post-operative schedule established in experiment 4.

Material and Methods

Animals and Surgery:

Ten adult male Sprague Dawley rats (425-485 grams) were used. All animals received a pair of stereotaxically implanted guide cannulae aimed at the dorsal caudate/putamen.

Behavioral Testing and Intracerebral Injections:

Methods were identical to those of Experiment 5. All animals received bilateral injections of 10 ng SP.

Results

One animal did not have both cannulae in the dorsal caudate/putamen (one cannula was embedded in the anterior commissure). Data from the remaining 9 animals were analyzed.

As can be seen from tables 16 and 17, statistical analyses using a paired t-test revealed that bilateral injections of 10 ng of SP failed to significantly alter any parameter of male sexual behavior. For example, in contrast to experiment 5, no effects were seen on mount, intromission, or ejaculation latencies ($t = 1.05$, $df = 8$, $P > 0.05$; $t = 0.95$, $df = 8$, $P > 0.05$; $t = 1.57$, $df = 8$, $P > 0.05$, respectively). In addition,

injections of SP had no appreciable effect on mount or intromission frequencies ($t = 1.35$, $df = 8$, $P > 0.05$; $t = 1.21$, $df = 8$ $P > 0.05$).

TABLE 16

EXPERIMENT 6 : LATENCIES (in seconds)

Effects of injections of 10 ng SP into the dorsal caudate/putamen on male behavior.

Measures	Baseline	Injection Test
ML	87.5 + 10	77.1 + 5
IL	83.0 + 7	73.4 + 6
EL	623.0 + 34	568.1 + 31
AEL	516.5 + 25	493.0 + 45
ICI	40.5 + 3	44.6 + 2
PEI	319.2 + 10	331.1 + 8
% E	100	100

All values are given as mean + S.E.M. See text for further details.

TABLE 17

EXPERIMENT 6 : FREQUENCIES

Effects of injections of 10 ng SP into the dorsal caudate/putamen on male behavior.

Measures	Baseline	Injection Test
MF	5.0 + .2	4.4 + .3
IF	11.5 + .6	10.7 + .7
ATTEMPTS	1.7 + .2	1.3 + .1
CRAWLS	1.6 + .2	1.2 + .1
MISDIRECTED	1.4 + .2	1.3 + .1

All values are given as mean + S.E.M. See text for further details.

Experiment 7: The Effects of Bilateral Injections of SP into the Lateral Ventricles on Male Copulatory Behavior

The results of experiment 5 revealed that bilateral injections of SP into the MPOA-AH facilitated male copulatory behavior. This strongly suggests that SP, acting on MPOA-AH neurons, modulates the male's initiation of copulation, as revealed by dramatic decreases in copulatory latencies. There is, however, another interpretation. The MPOA-AH lies in close contact with the ventricular circulation, therefore it is possible that SP injected into the MPOA-AH may have leaked out into the cerebrospinal fluid and, as a result, produced its effects on another site in close contact with the ventricular circulation. Consequently, experiment 7 was designed to explore this possibility by injecting SP bilaterally into the lateral ventricles. If the effects of SP on male copulatory behavior observed in Experiment 5 were produced at another site via leakage into the ventricular circulation, then similar results would be expected following bilateral injections of SP into the lateral ventricles. Since 10 ng of SP produced the most consistent effects in Experiment 5, this dose was used in the following experiment.

Methods and Materials

Animals and Surgery:

Ten adult male Sprague Dawley rats (380-425 gm) were used. Each animal received a pair of stereotaxically implanted guide cannulae aimed at the lateral ventricles.

Behavioral Testing:

As per general methods section.

Intracerebral injections:

All animals received bilateral injections of 10 ug SP in a volume of 0.3 ul/cannula (Sigma Chemicals, lot # 124F-59203) dissolved in 0.01 N acetic acid, 0.9% saline with the pH adjusted to 6.0.

Results

Of the ten animals that began the study, two had either one or both cannulae located outside the lateral ventricles, and one died during surgery. This left 7 animals that had both cannulae located within the lateral ventricles (see figure 18 for a representative cannula placement). Only data from these 7 animals were analyzed. Data from these animals were compared to the data of the dorsal caudate/putamen injected animals in experiment 6 using an analysis of covariance. As demonstrated in tables 18 and 19, bilateral injections of SP into the lateral ventricles significantly reduced the number of intromissions prior to ejaculation ($F = 15.6$, $df = 1,15$, $P < 0.01$), and at the same time lengthened both mount latencies and the inter-copulatory interval ($F = 10.4$, $df = 1,15$, $P < 0.01$; $F = 5.6$, $df = 1,15$, $P < 0.05$, respectively). No other measures were affected.

92 (a)

FIGURE 18

Represents cannula placements into the lateral ventricles in experiment 7. See legend of figure 11 for a more detailed description.

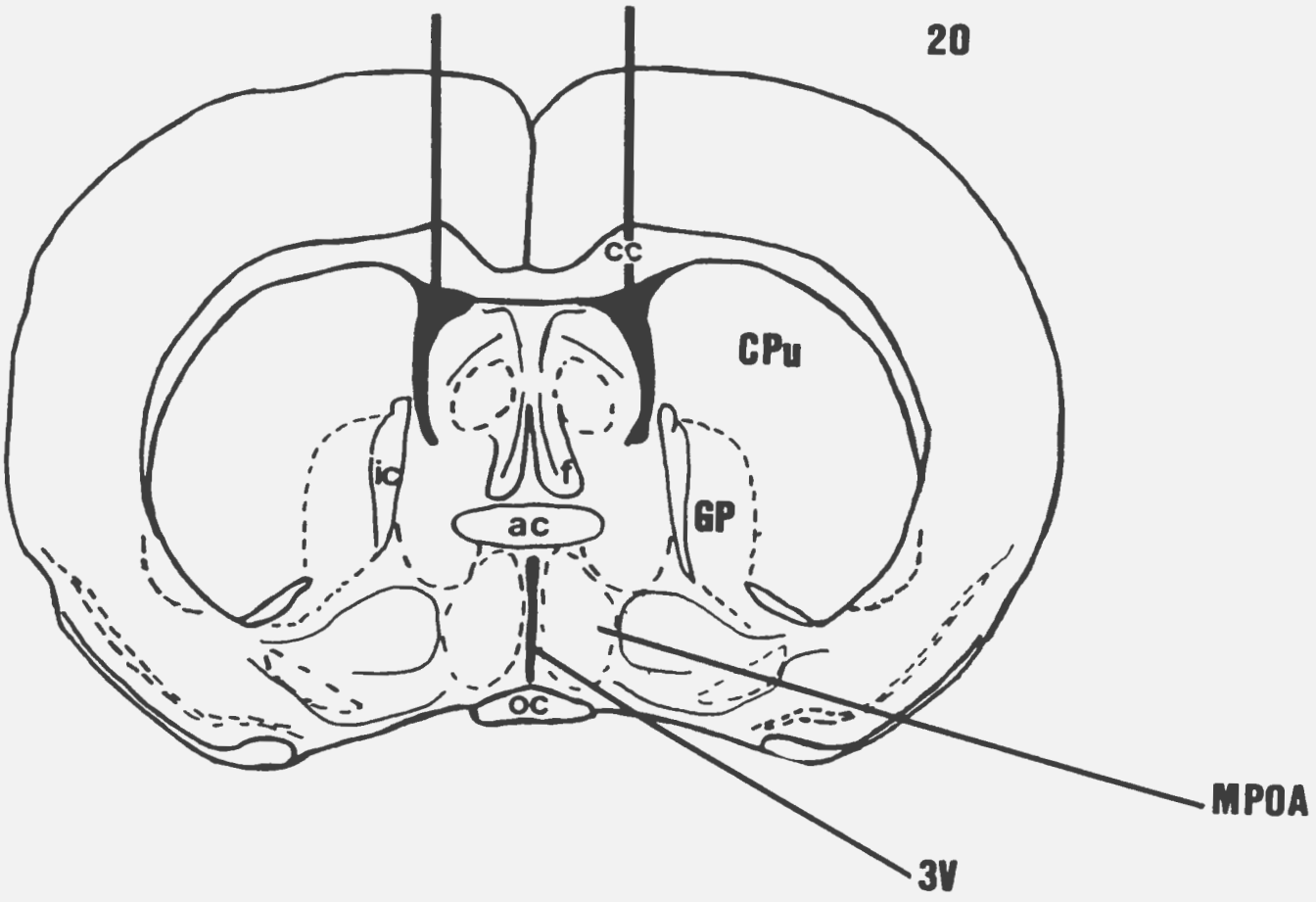

20
cc
CPu
ic
ac
GP
oc
MPOA
3V

TABLE 18

EXPERIMENT 7 : LATENCIES (in seconds)

Effects of injections of SP (10 ng/cannula) into the dorsal caudate/putamen or into the lateral ventricles on male sexual behavior.

Measures	Baseline		Substance P (10 ng)
ML			
Ventricles	194.6 + 49		132.1 + 19 **
Caudate	87.5 + 10	77.1 + 5	
IL			
Ventricles	125.2 + 69		117.7 + 59
Caudate	83.0 + 7	73.4 + 6	
EL			
Ventricles	703.2 + 323		552.1 + 151
Caudate	623 + 34	568.1 + 31	
AEL			
Ventricles	577.2 + 295		444.3 + 133
Caudate	516.5 + 25	493.0 + 45	
ICI			
Ventricles	56.1 + 6		71.9 + 23 *
Caudate	40.5 + 3	44.6 + 2	
PEI			
Ventricles	285.5 + 36		292.1 + 34
Caudate	319.5 + 19	331.1 + 8	
% E			
Ventricles	100		100
Caudate	100	100	

All values are given as mean + S.E.M. See text for further details.

* = P < 0.05, significantly different from caudate group.
** = P < 0.01 " " " " "

TABLE 19

EXPERIMENT 7 : FREQUENCIES

Effects of injections of SP (10 ng/cannula) into the dorsal caudate/putamen or the lateral ventricles on male sexual behavior.

Measures	Baseline	Substance P (10 ng)	
MF			
Ventricles	4.7 + 1.9		4.1 + 3.3
Caudate	5.0 + .2	4.4 + .3	
IF			
Ventricles	14.8 + 5.4		8.1 + 2.5 **
Caudate	11.5 + .6	10.7 + .7	
ATTEMPTS			
Ventricles	1.3 + 0.2		1.7 + 0.1
Caudate	1.7 + 0.2	1.3 + 0.1	
CRAWLS			
Ventricles	1.2 + 0.1		1.3 + 0.1
Caudate	1.6 + 0.2	1.2 + 0.1	
MISDIRECTED			
Ventricles	1.4 + 0.2		1.1 + 0.2
Caudate	1.4 + 0.2	1.3 + 0.1	

All values are given as mean + S.E.M. See text for further details.

** = $P < 0.01$, significantly different from caudate group.

Experiment 8: The Effects of Immunoneutralization of SP on Male Copulatory Behavior

As previously mentioned, both SP-immunoreactive cell bodies, fibers, and SP receptors have been reported in the MPOA-AH in rat brain (Simerly, Gorski, and Swanson, 1986; Mantyh, Hunt, and Maggio, 1984). In addition, as revealed in experiment five, bilateral injections of SP into the MPOA-AH facilitated male copulatory behavior. Collectively, these findings strongly suggest that endogenous SP plays a role in the regulation of male sexual behavior at the level of the MPOA-AH. One way to test this possibility is by using receptor antagonists. For example, if SP is acting via SP receptors, then blocking their activity should attenuate certain parameters of male copulatory behavior measured in the previous experiments. However, while receptor antagonists are commonly used to investigate the role of neuropeptides in a variety of behavioral experiments, with respect to SP, there is currently no specific antagonist for use in the CNS. To overcome this shortcoming, the technique of passive immunoneutralization of SP has been used. Indeed, in a recent study by Dornan, Malsbury, and Penney (1987) the role of endogenous SP in female sexual behavior was explored using a SP antiserum. In that study, a purportedly potent SP antagonist failed to significantly decrease lordosis scores (a measure of female sexual receptivity). A SP antiserum, on the other hand, significantly reduced lordosis scores. Accordingly, in experiment 8, bilateral injections of a SP antiserum into the MPOA-AH were done to explore the role of endogenous SP in male copulatory behavior.

Methods and Results

Animals and Surgery:

Twenty adult male Sprague Dawley rats (350-405 grams) were used. Each animal received a pair of stereotaxically implanted guide cannulae aimed at the MPOA-AH.

Behavioral Testing:

As per general methods, with one exception. In an attempt to use males that were relatively sexually vigorous, all males were given four pre-operative tests.

Intracerebral Injections:

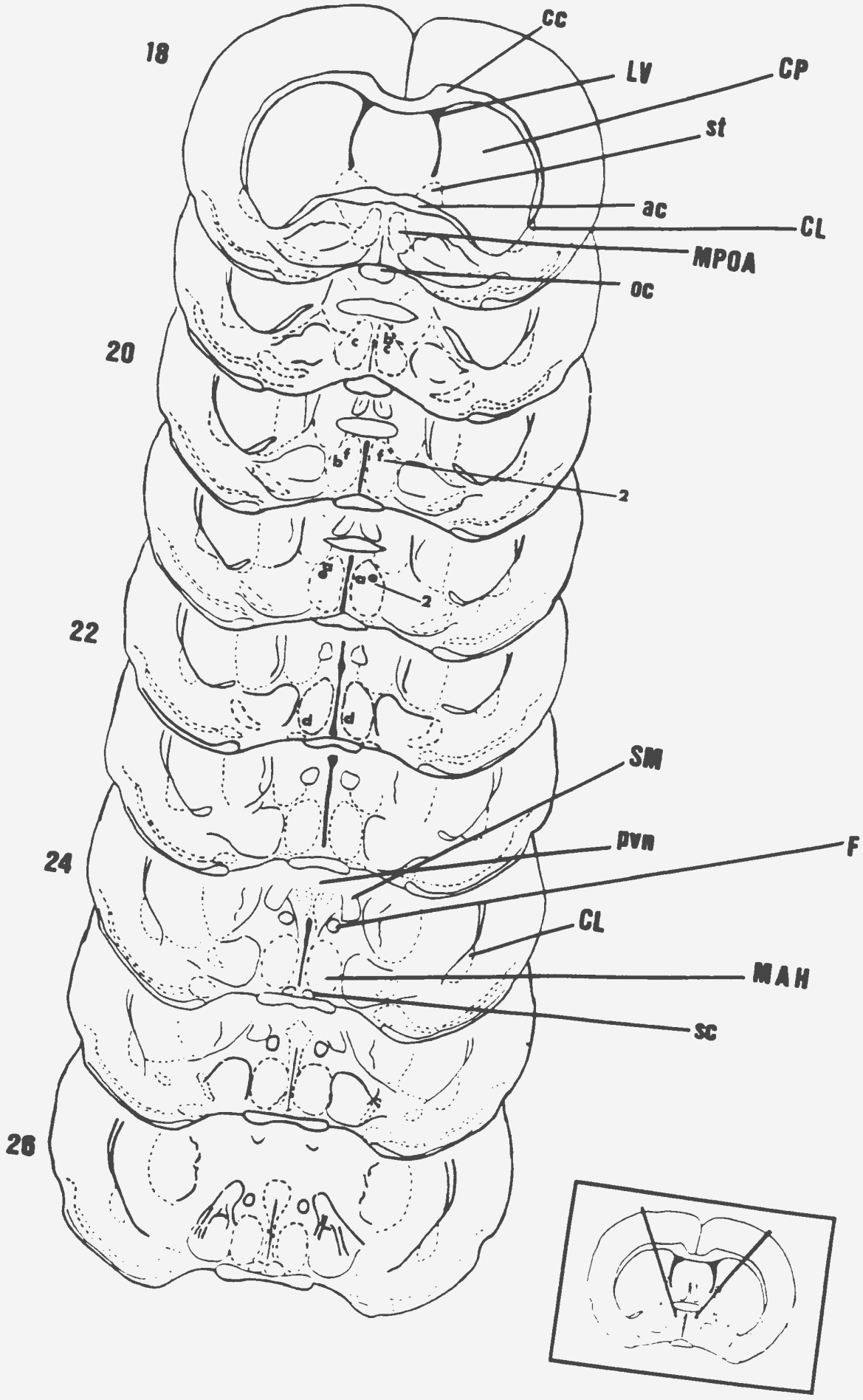
Animals were randomly divided into two groups. One group (n = 10 per group) received bilateral injections of an undiluted SP antiserum (Immuno Nuclear lot # 8605009) in a volume of 0.3 ul/cannula. The other group received bilateral injections of normal rabbit serum (same volume).

Results

Of the twenty animals, four did not satisfy criterion at the first post-operative baseline test, and another could not be injected because the dummy cannula could not be removed. That left 15 animals with both cannulae located within the previously defined boundary of the MPOA-AH (see figure 19A and 19B). Data analysis was performed on these animals. As demonstrated in table 21, bilateral

FIGURE 19A

Bilateral cannula placements in 8 males that received injections of an undiluted substance P antiserum (0.3 microliters) into or near the MPOA-AH in experiment 8.


cc
LV
CP
st
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CL
MPOA
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18
20
22
24
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SM
pvn
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MAH
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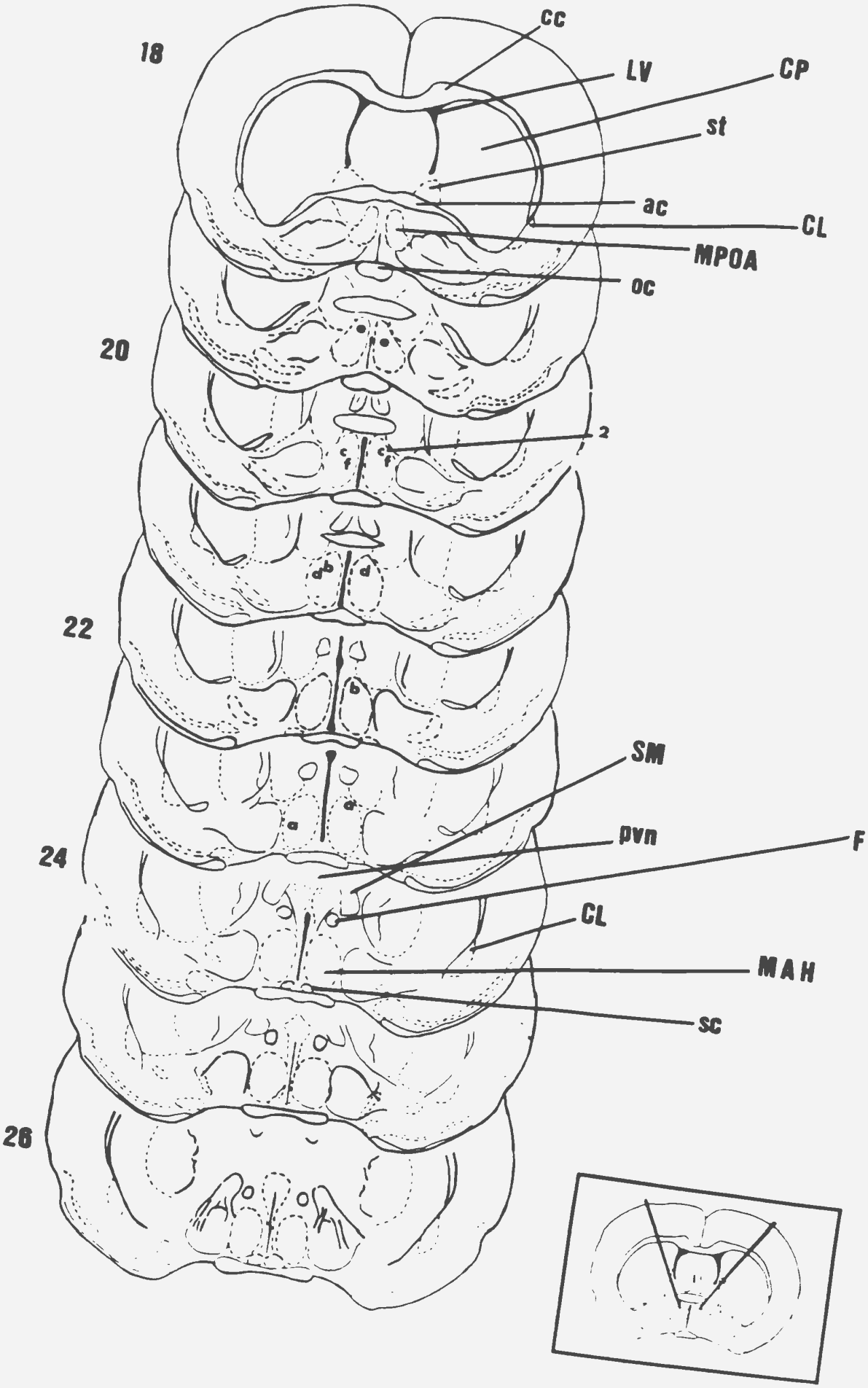
FIGURE 19B

Bilateral cannula placements in 7 males that received injections of normal rabbit serum (NRS) in experiment 8.


18
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24
26
cc
LV
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MPOA
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SM
pvn
F
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MAH
sc

injections of an undiluted SP antiserum ($n = 8$) significantly increased the number of intromissions to ejaculation compared to the normal rabbit serum injected controls ($n = 7$) ($F = 5.2$, df, 1,14, $P < 0.05$). No other parameter was affected (see tables 20 and 21), although, as can be seen from table 20, a trend toward longer latencies is evident in the antiserum group.

TABLE 20

EXPERIMENT 8 : LATENCIES (in seconds)

Effects of injections of normal rabbit serum (NRS) or anti-SP (0.3 ul/cannula) into the MPOA-AH on male sexual behavior.

Measures	Baseline	NRS	Anti-SP
ML			
Experimental	135.0 + 60		141.3 + 60
Control	42.8 + 28	104.3 + 62	
IL			
Experimental	97.1 + 50		119.7 + 65
Control	52.9 + 20	88.3 + 23	
EL			
Experimental	491.4 + 120		743.2 + 225
Control	500.9 + 110	540.5 + 125	
AEL			
Experimental	393.4 + 90		624.3 + 200
Control	448.3 + 132	457.8 + 110	
ICI			
Experimental	49.0 + 16		52.9 + 12
Control	39.0 + 11	89.7 + 32	
PEI			
Experimental	295.6 + 22		369.3 + 32
Control	314.6 + 35	377.1 + 36	
% E			
Experimental	100		100
Control	100		100

All values are given as mean + S.E.M. See text for further details.

TABLE 21

EXPERIMENT 8 : FREQUENCIES

Effects of intracerebral injections of normal rabbit serum (NRS) or anti-SP (0.3 ul/cannula) into the MPOA-AH on male sexual behavior.

Measures	Baseline	NRS	Anti-SP
MF			
Experimental	9.7 + 6.0		5.1 + 2.0
Control	6.0 + 2.0	3.5 + 1.3	
IF			
Experimental	11.0 + 4.0		13.7 + 3.0 *
Control	14.2 + 3.5	7.4 + 1.6	
ATTEMPTS			
Experimental	1.2 + 0.1		1.1 + 0.2
Control	1.0 + 0.2	1.3 + 0.2	
CRAWLS			
Experimental	1.0 + 0.4		1.4 + 0.1
Control	1.2 + 0.1	1.3 + 0.1	
MISDIRECTED			
Experimental	2.4 + 0.4		1.9 + 0.5
Control	2.1 + 0.2	4.6 + 1.2	

All values are given as mean + S.E.M. See text for further details.

* = $P < 0.05$, significantly different from controls.

Experiment 9: The Effects of More Wide Spread Distribution of a SP Antiserum throughout the MPOA-AH on Male Copulatory Behavior

In a recent study by Elliot, Nemeroff, and Kilts (1986), they reported that different volumes of a SP antiserum solution (which contained the same amount of antiserum) injected into the nucleus accumbens produced differential effects on D-amphetamine-induced locomotor activity. In that study, bilateral injections of a SP antiserum in a volume of 0.5 ul had no effect on D-amphetamine-induced locomotor activity, whereas injections of 2 ul containing the same amount of antiserum significantly decreased the locomotor activity. They concluded that the effect of the larger volume probably reflected more wide spread diffusion of the antibody in the nucleus accumbens. Since the size of the nucleus accumbens is relatively similar to that of the MPOA-AH, it is not inconceivable that the lack of effect of the SP antiserum on most parameters of male copulatory behavior observed in Experiment 8 resulted from too small a volume of injection. Therefore, in experiment 9, the effects of a SP antiserum on male copulatory behavior were re-evaluated using a larger volume of antiserum and the identical procedure as that of experiment 8.

Material and Methods

Animals and Surgery:

A new group of 20 adult male Sprague Dawley rats (400-455 grams) was used. Each animal received a pair of stereotaxically implanted guide cannulae aimed at the MPOA-AH.

Behavioral Testing and Intracerebral Injections:

Identical to that of Experiment 8.

Results

Of the 20 animals that began the experiment, 17 had both cannulae located within the MPOA-AH. Two animals, however, failed to satisfy criterion at the post-operative baseline test. Data from the remaining 15 animals were analyzed. Figure 20A and 20B illustrate the rostral-caudal location within the MPOA-AH of the various bilateral cannulae implants in these animals.

As can be seen from tables 22 and 23, intracerebral injection of 2 ul of an undiluted SP anti-serum (Immuno Nuclear, Lot # 8605009) ($n = 8$) impaired copulation compared to the normal rabbit serum (NRS) injected controls ($n = 7$). This was revealed by a significant increase in mount and intromission latencies ($F = 11.9$, $df = 1,13$, $P < .01$; $F = 5.1$, $df = 1,13$, $P < 0.05$, respectively), as well as the intercopulatory interval ($F = 5.2$, $df = 1,13$, $P < 0.05$). Not only did bilateral injections of the antiserum increase the interval to initiate copulation, but they markedly lengthened the ejaculation latency ($F = 9.8$, $df, = 1,13$, $P < 0.01$). In contrast to experiment 8, however, bilateral injections of 2 ul of a SP antiserum had no effect on the number of intromissions necessary to achieve ejaculation.

FIGURE 20A

Bilateral cannula placements in 8 males that received injections of 2 microliters of an undiluted antiserum into or near the MPOA-AH in experiment 9.

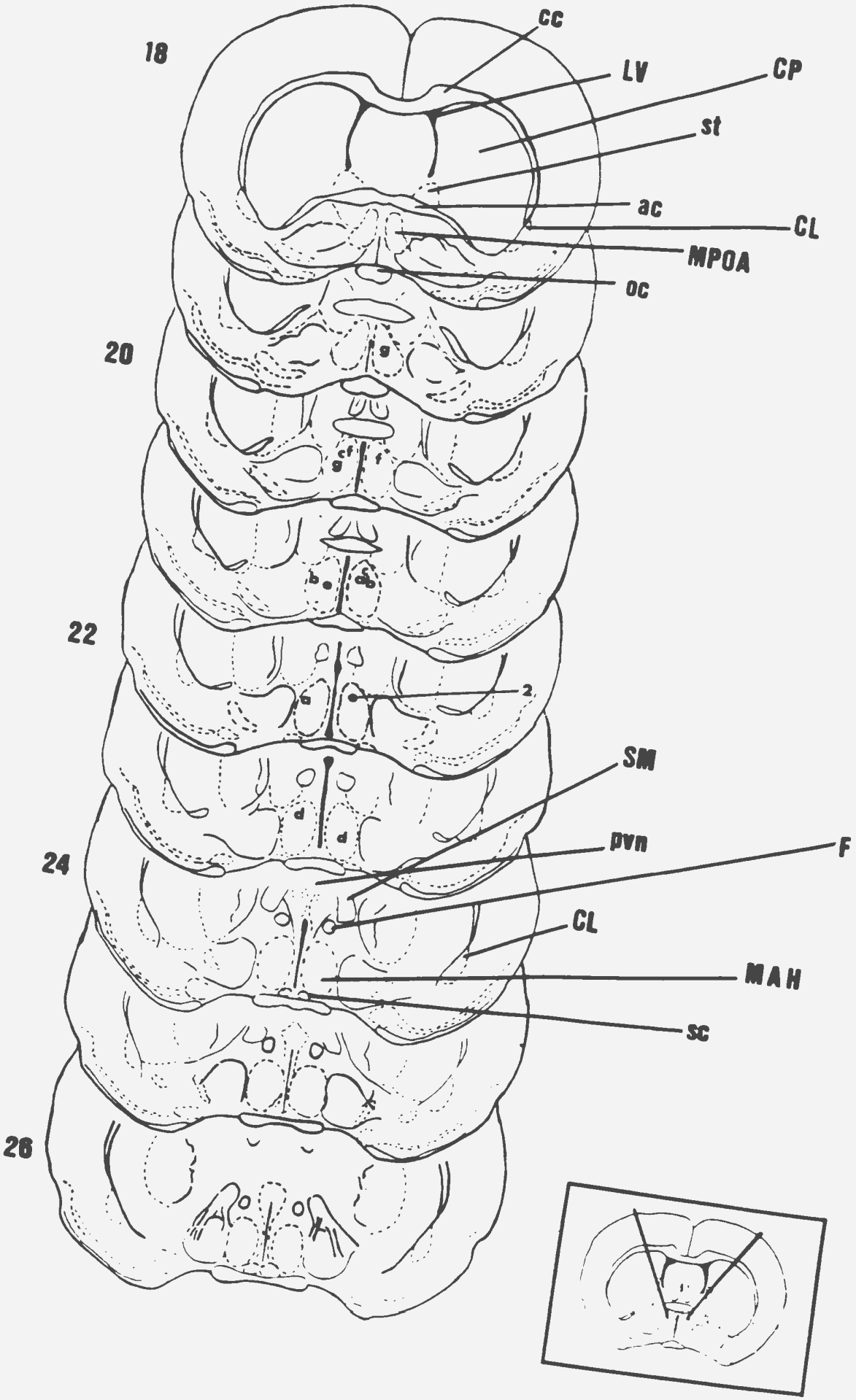

cc
18
LV
CP
st
ac
CL
MPOA
oc
20
22
SM
24
pvn
F
CL
MAH
sc
26

FIGURE 20B

Cannula placements in 7 males that received injections of NRS into the MPOA-AH in experiment 9.

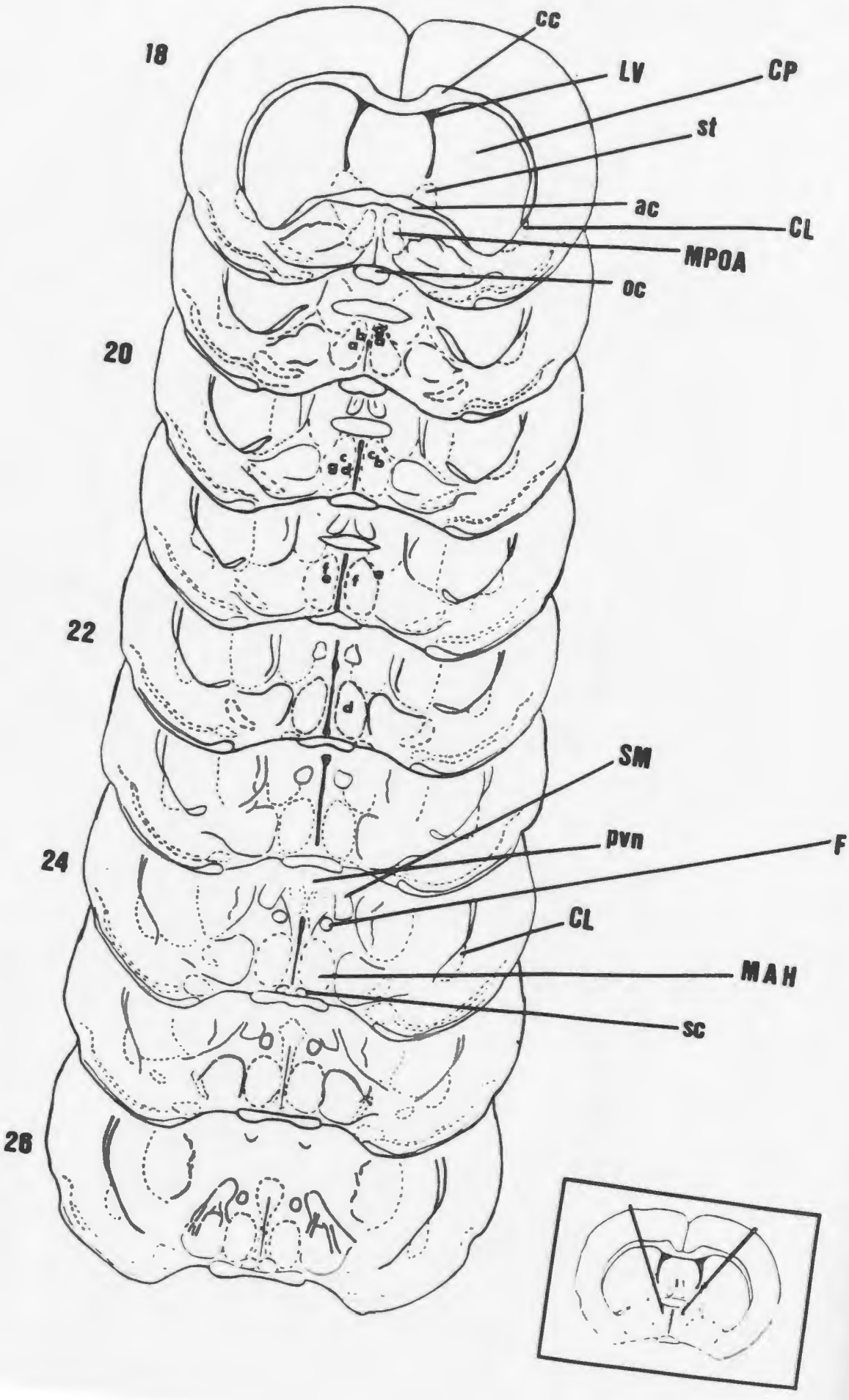

cc
LV
CP
st
ac
CL
MPOA
oc
18
20
22
24
26
SM
pvn
F
CL
MAH
sc

TABLE 22

EXPERIMENT 9 : LATENCIES (in seconds)

Effects of injections of 2 ul of normal rabbit serum (NRS) or anti-SP into the MPOA-AH on male sexual behavior .

Measures	Baseline	NRS	Anti-SP
ML			
Experimental	61.5 + 19		208.3 + 53 **
Control	52.5 + 34	42.5 + 18	
IL			
Experimental	39.5 + 13		70.0 + 13 *
Control	77.4 + 68	42.0 + 16	
EL			
Experimental	533.6 + 155		852.6 + 191 **
Control	430.6 + 161	405.5 + 76	
AEL			
Experimental	494.5 + 90		782.9 + 200 *
Control	352.7 + 97	362.6 + 55	
ICI			
Experimental	51.3 + 8		83.8 + 10 *
Control	42.5 + 12	51.7 + 13	
PEI			
Experimental	326.0 + 18		332.7 + 54
Control	286.0 + 35	377.1 + 36	
% E			
Experimental	100		87
Control	100		100

All values are given as mean + S.E.M. See text for further details.

* = $P < 0.05$, significantly different from controls.
** = $P < 0.01$, " " " "

TABLE 23

EXPERIMENT 9 : FREQUENCIES

Effects of injections of 2 ul of normal rabbit serum (NRS) or anti-SP into the MPOA-AH on male sexual behavior.

Measures	Baseline	NRS	Anti-SP
MF			
Experimental	4.4 + 1.0		5.0 + 1.0
Control	4.8 + 0.9	3.8 + 0.6	
IF			
Experimental	11.1 + 2.3		9.8 + 1.8
Control	9.5 + 1.1	8.0 + 1.0	
ATTEMPTS			
Experimental	1.1 + 0.3		1.1 + 0.1
Control	1.7 + 0.2	1.0 + 0.1	
CRAWLS			
Experimental	1.2 + 0.3		1.1 + 0.1
Control	1.4 + 0.1	.85 + 0.1	
MISDIRECTED			
Experimental	1.4 + 0.1		1.2 + 0.2
Control	1.4 + 0.3	1.2 + 0.1	

All values are given as mean + S.E.M. See text for further details.

Experiment 10: The Effects of Intracerebral Injections of SP on Male Sexual Behavior in Castrated Male Rats.

As previously mentioned in the general introduction, the importance of testosterone for male copulatory behavior has been clearly demonstrated in a wide range of species. For example, in the rat, male copulatory behavior is eventually abolished following castration, and this effect is completely reversed following testosterone treatment. Recently, SP immunoreactivity within certain brain regions has been shown to be dependent on gonadal hormones in both female (Frankfurt et al., 1986; Tsuro, Hisano, Okamura, Tsukamoto, and Daikoku, 1984), and male rats (Dees and Kozlowski, 1984). Indeed, Dees and Kozlowski (1984) reported that castration decreased SP staining in the medial nucleus of the amygdala, an area which has been implicated in male sexual behavior. Based on the above studies, and the results of experiment 5, perhaps the gradual decline in male copulatory behavior following castration is a result of a decline of SP synthesis and hence a reduced availability at synapses in the MPOA-AH. If so, bilateral SP injections into the MPOA-AH might be able to reverse the decline in male sexual behavior that is normally observed following castration.

Material and Methods

Animals and Surgery:

A new group of ten adult male Sprague Dawley rats (450-525 grams) was used. Each male was given two pre-operative screenings. Twenty-four hours following the second successful screening, each animal was anesthetized with Somnotol and bilaterally castrated using a ventral abdominal approach. Three weeks following surgery, all animals received a pair of stereotaxically implanted guide cannula aimed at the MPOA-AH.

Behavioral Testing:

Identical to experiment 5

Intracerebral Injections:

Four days following stereotaxic surgery animals received a baseline test. Five days following the baseline test, animals were injected bilaterally with 10 ng SP/.3 ul per cannula into the MPOA-AH.

Results

Of the ten animals that began the experiment, one died before completion of data acquisition. That left 9 animals which had both cannulae within the previously defined boundary of the MPOA-AH. Figure 21 illustrates these cannulae placements.

As can be seen from table 24, 1 month following castration, sexual performance was greatly reduced compared to pre-castration levels. Subsequent bilateral injections of 10 ng of SP into the MPOA-AH failed to reinstate copulatory behavior in these males.

FIGURE 21

Summarizes bilateral cannula placements in 9 males that received injections of 10 ng of SP into or near the MPOA-AH in experiment 10. See figure 3 for a more detailed explanation.

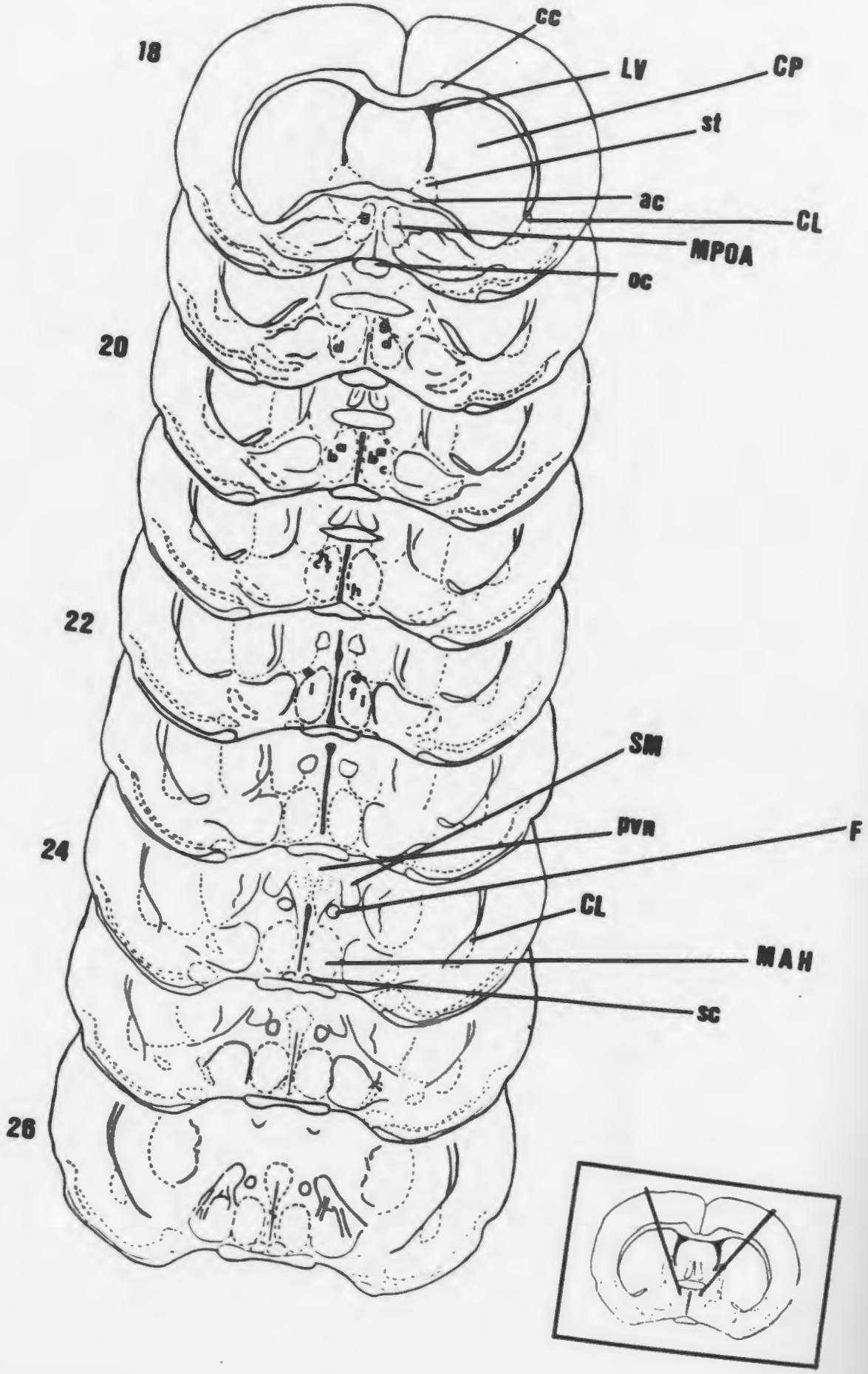

cc
LV
CP
st
ac
CL
MPOA
oc
18
20
22
SM
pvn
F
24
CL
MAH
sc
26

TABLE 24

EXPERIMENT 10

The effects of bilateral injections of SP into the MPOA-AH on sexual behavior in orchidectomized testosterone-deprived male rats.

Number of animals displaying:	Pre-castration (no treatment)	1 month post-castration (no treatment)	SP (10 ng)
MOUNTS	9 / 9	3 / 9	1 / 9
INTROMISSIONS	9 / 9	1 / 9	0 / 9
EJACULATION	9 / 9	1 / 9	0 / 9

Experiment 11: The Effect of Preoptic Injections of CCK-8 on Male Copulatory Behavior

As previously mentioned (general introduction), CCK concentrations within some brain nuclei appear to be sexually differentiated, with males having higher concentrations than females. Also as in the case of SP, CCK concentrations in some brain nuclei seem to be dependent on gonadal hormones (Akesson and Micevych, 1986; Simerly and Swanson, 1987).

Recently, CCK has been found to coexist and interact with the neurotransmitter dopamine (DA). For example, in a study by Kimura, Hashimoto and Kawakami (1983), they reported that implants of CCK into the MPOA-AH facilitated the circadian rise of plasma LH in ovariectomized estrogen-treated female rats. These same authors have recently demonstrated that the CCK-induced facilitation of LH release is mediated by DA (Hashimoto and Kimura, 1986). In that study they reported that injections of CCK into the MPOA-AH enhanced the afternoon rise of LH in female rats and that this enhancement could be completely blocked by pre-treatment with a DA receptor blocker, pimozide. Collectively, the above studies suggest that DA and CCK have a functional interaction at the level of the MPOA-AH. This is of considerable interest since dopaminergic agonists have been shown to facilitate male sexual behavior when injected peripherally (Ahlenius and Larsson, 1984; Clark, Stefanick, Smith and Davidson, 1981), and more recently, when injected into the MPOA-AH (Hull et al., 1986). In light of this, it is possible that CCK may play a role in the neural mediation of male sexual behavior. Therefore, experiment 11 was designed to explored this possibility. Three different doses of CCK were bilaterally injected into the MPOA-AH and the effects of these injections on male sexual behavior examined.

Material and Methods

Animals and Surgery:

Forty adult male Sprague-Dawley rats (385-460 grams) were used. All animals received a pair of stereotaxically implanted guide cannulae aimed at the MPOA-AH.

Behavioral Testing:

Identical to that of Experiment Five.

Intracerebral Injections:

CCK is a peptide consisting of 39 amino acids. Immunochemical studies indicate, however, that in the brain, the octapeptide CCK-8 in sulphated form, and not the longer form (39 amino acid sequence) of CCK has the highest concentrations (Dockray, 1982). As a result, sulphated CCK-8 was used in the present experiment. Animals were divided into four groups (n = 10 each). Three groups received either 10 ng, 100 ng, or 200 ng of CCK-8 sulphated (Cambridge Research Biochemicals, Lot # PA 2048) in a volume of 0.3 ul per cannula into the MPOA-AH. Another group received saline (same volume). CCK-8 was dissolved in physiological saline.

Results

Of the forty animals, five died before completion of data acquisition, five did not satisfy criterion at the baseline test, two had both cannula located outside the MPOA-AH, and one was sick on the second test session, and as a result could not

be tested. That left 27 animals which had both cannulae within, immediately dorsal to, or immediately ventral to the MPOA-AH (see figure 22A and 22B). Only these animals were used for subsequent data analysis.

Tables 25 and 26 demonstrate that bilateral injections of three doses of CCK-8 (10 ng, n = 7; 100 ng, n = 6; 200 ng, n = 7) into the MPOA-AH had no significant effect on any of the behavioral parameters measured when compared

to the saline injected control group (n = 7). For example, in contrast to SP which produced dramatic reductions in latencies, CCK did not seem to have any effect on mount, intromission or ejaculation latencies ($F = 1.8$, df = 3,26, $P > 0.05$; $F = 0.86$, df, 3,26, $P > 0.05$; $F = 2.5$, df, 3,26, $P > 0.05$, respectively). With regard to frequencies, table 20 demonstrates that CCK injections had no appreciable effect on either mount or intromission frequencies ($F = 1.8$, df, 3,26, $P > 0.05$; $F = 0.68$, df, 3,26, $P > 0.05$ respectively). Although no significant effects were observed following bilateral injections of CCK-8, there was a non-significant trend for reduced latencies in the CCK group compared to the saline injected controls which was most noticeable with the higher doses of CCK-8 (see Table 25). This trend is also demonstrated in figures 23 and 24. IADLTS

FIGURE 22A

Summarizes bilateral cannula placements in 20 males that received injections of three different doses of CCK-8 into or near the MPOA-AH in experiment 11.

FIGURE 22B

Bilateral cannula placements in 7 males that received saline injections into the MPOA-AH in experiment 11.

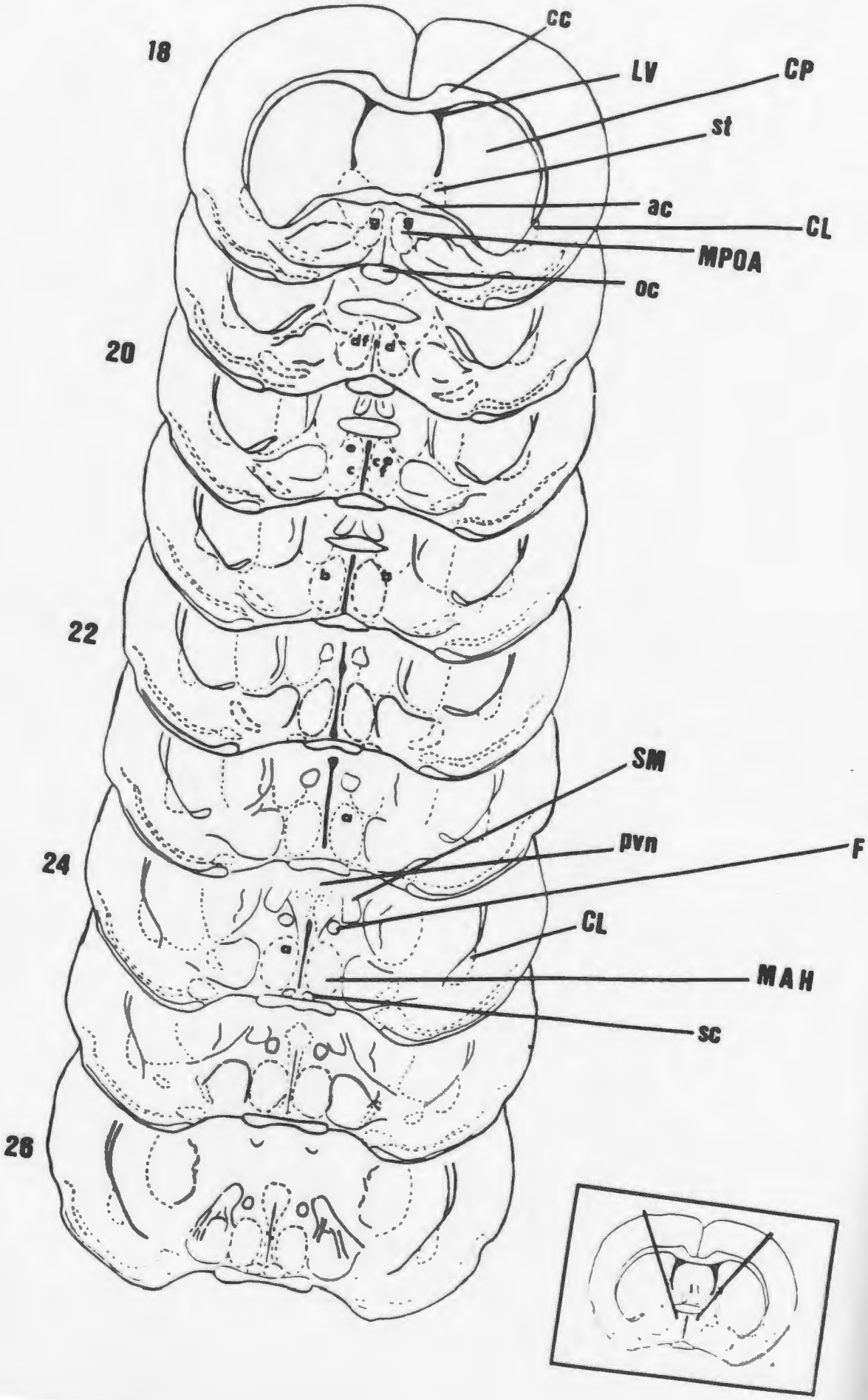

cc
18
LV
CP
st
ac
CL
MPOA
oc
20
22
SM
pvn
F
24
CL
MAH
sc
26

TABLE 25

EXPERIMENT 11 : LATENCIES (in seconds)

Effects of injections of saline or CCK-8 (sulphated) into the MPOA-AH on male sexual behavior.

			CCK-8		
Measures (a)	Baseline	Saline	10 ng	100 ng	200 ng
ML					
Experimental	188.6 + 48		194.0 + 32	145.7 + 40	129.8 + 13
Control	141.1 + 32	190.6 + 35			
IL					
Experimental	183.7 + 48		227.8 + 71	153.5 + 46	136.8 + 14
Control	98.8 + 32	185.6 + 72			
EL					
Experimental	973.2 + 146		826.0 + 235	604.6 + 85	618.5 + 59
Control	753.9 + 126	858.9 + 270			
AEL					
Experimental	789.2 + 129		598.0 + 210	468.0 + 88	495.4 + 56
Control	579.5 + 106	686.5 + 180			
ICI					
Experimental	75.3 + 11		87.3 + 15	53.5 + 8	68.8 + 10
Control	66.1 + 16	90.3 + 20			
PEI					
Experimental	304.4 + 21		370.7 + 21	318.3 + 18	302.3 + 11
Control	284.6 + 27	368.6 + 40			
% E					
Experimental	100		100	100	100
Control	100	100			

a) at baseline, all values for the experimental group are grand means. All values are given as mean + S.E.M. See text for further details.

TABLE 26

EXPERIMENT 11 : FREQUENCIES

Effects of injections of saline or CCK-8 (sulphated) into the MPOA-AH on male sexual behavior.

			CCK-8		
Measures (a)	Baseline	Saline	10 ng	100 ng	200 ng
MF					
Experimental	4.7 + 1.3		3.5 + 1.0	3.1 + 0.8	4.0 + 1.0
Control	3.4 + .4	4.2 + 0.9			
IF					
Experimental	13.0 + 2.0		9.5 + 1.1	12.0 + 1.3	11.2 + .9
Control	11.5 + 1.1	10.7 + 1.5			
ATTEMPTS					
Experimental	1.1 + 0.1		1.2 + 0.2	1.0 + 0.1	1.2 + .2
Control	1.1 + 0.1	1.5 + 0.5			
CRAWLS					
Experimental	1.3 + 0.1		1.2 + 0.4	1.5 + 0.6	1.0 + 0.1
Control	1.1 + 0.5	1.8 + 1.0			
MISDIRECTED					
Experimental	1.8 + 0.3		1.7 + 0.2	1.0 + 0	1.7 + 0.3
Control	1.0 + 0.1	1.5 + 0.3			

a) at baseline, all values for the experimental group are grand means. All values are given as mean + S.E.M. See text for further details.

FIGURE 23

Illustrates the proportional change from a baseline test of ejaculation latencies in animals that received three different doses of CCK-8, or saline into the MPOA-AH in experiment 11.

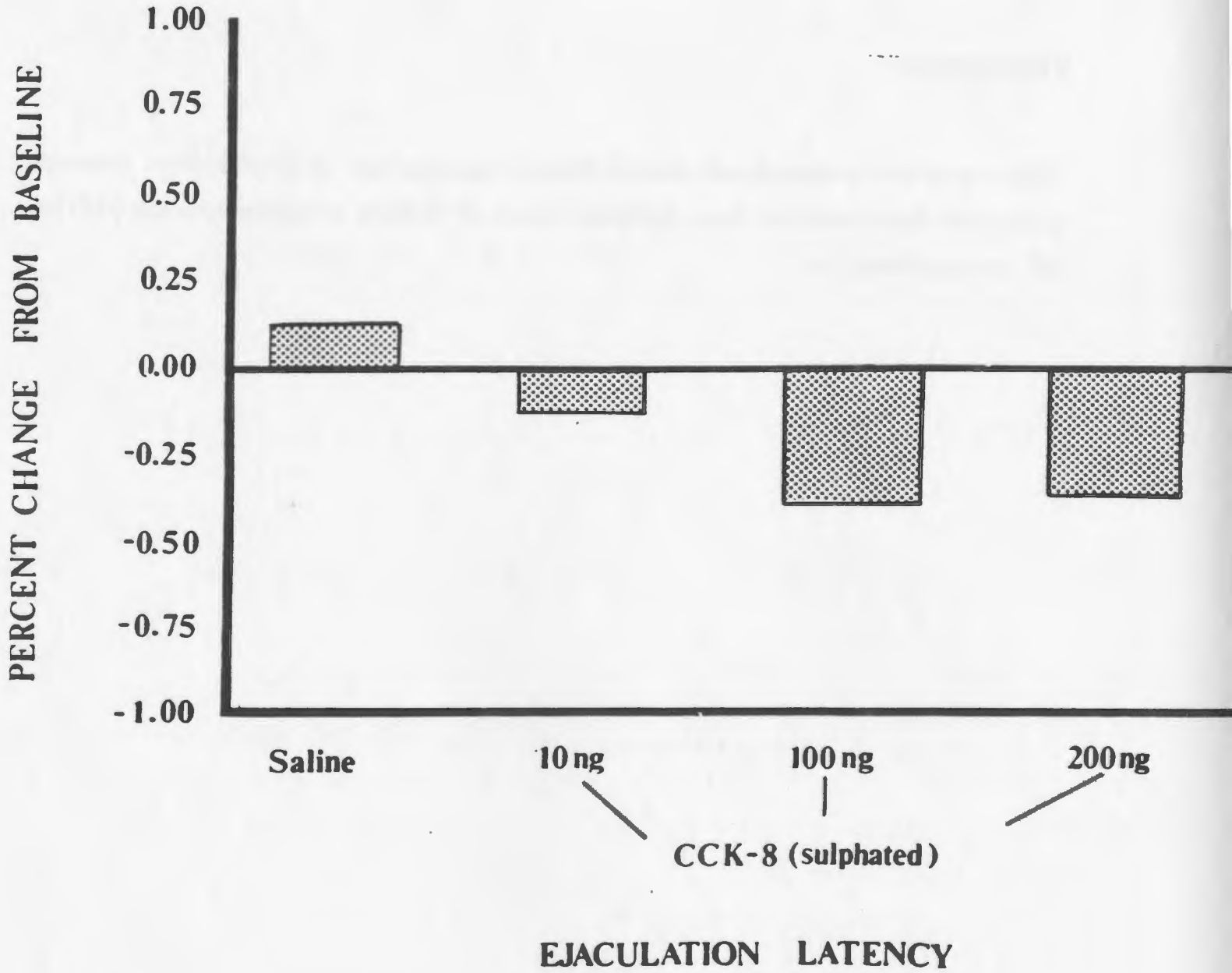
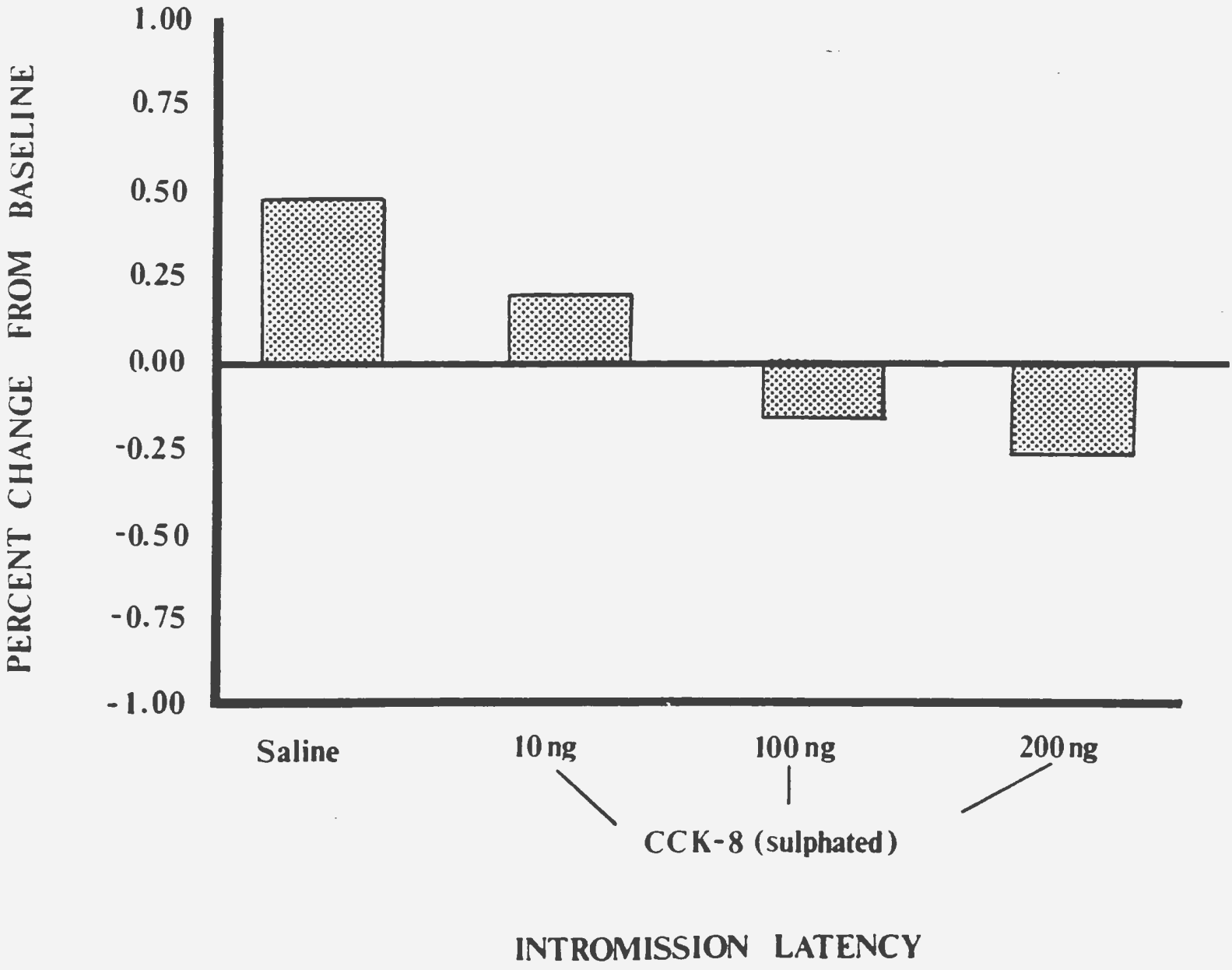

PERCENT CHANGE FROM BASELINE
1.00
0.75
0.50
0.25
0.00
-0.25
-0.50
-0.75
-1.00
Saline
10 ng
100 ng
200 ng
CCK-8 (sulphated)
EJACULATION LATENCY

FIGURE 24

Illustrates the proportional change from a baseline test of intromission latencies in animals that received three different doses of CCK-8, or saline into the MPOA-AH in experiment 11.


PERCENT CHANGE FROM BASELINE
1.00
0.75
0.50
0.25
0.00
-0.25
-0.50
-0.75
-1.00
Saline
10 ng
100 ng
200 ng
CCK-8 (sulphated)
INTROMISSION LATENCY

GENERAL DISCUSSION

The results of the present series of experiments suggest that the SP innervation of the MPOA-AH, and perhaps other structures that lie in close proximity to ventricular circulation, are involved in the facilitation of masculine sexual behavior. This conclusion is partly based on changes that were observed in a variety of components of male sexual behavior subsequent to SP injections into the MPOA-AH (experiment 5) and the LV (experiment 6). In experiment 5, bilateral injections of three different doses of SP into the MPOA-AH produced an immediate facilitation of male copulatory behavior compared to saline injected controls. The most marked effects of SP injections were on latencies, where all three doses significantly shortened the interval to initiate copulation, and 10 and 100 ng, but not 200 ng, of SP also significantly reduced ejaculation latencies. It appeared that not only did SP injections into the MPOA-AH decrease the latencies to initiate copulation, but they also increased the intensity of copulation. This was reflected by an overall decrease in the inter-copulatory interval (ICI) following bilateral injections of all three doses of SP into the MPOA- AH compared to the saline injected controls. Additionally, a reduction of the ejaculatory threshold emerged following injections of 100 ng of SP into the MPOA-AH, as evidenced by a significant decrease in the number of intromissions preceding ejaculation compared to the saline injected controls. Bilateral injections of SP into the MPOA-AH did not, however, facilitate male copulatory behavior in a linear dose-related fashion.

The present study provides the first empirical evidence for a functional role of SP in the regulation of male sexual behavior. This peptide has previously been reported to have a sexually differentiated distribution within the CNS (Frankfurt et al., 1985; Malsbury and McKay, 1986). In addition, concentrations of SP within the CNS are dependent on circulating levels of gonadal hormones (Dees and Kozlowski, 1984).

In an attempt to ascertain whether the facilitation of male copulatory behavior observed in experiment 5 following bilateral injections of SP into the MPOA-AH was specific to the MPOA-AH, 10 ng of SP was bilaterally injected into the dorsal caudate/putamen (CP) in experiments 3 and 6. The results of these experiments demonstrate that SP synapses within the dorsal CP do not seem to be involved in the neural control of male copulatory behavior, as SP injections into this area had no appreciable effect on this behavior.

Contrary to the results obtained in experiments 3 and 6, however, bilateral injections of 10 ng of SP into the lateral ventricles significantly decreased the number of intromissions necessary to achieve ejaculation, while at the same time lengthening mount latencies and the ICI. This suggests several interpretations. Firstly, in addition to the proposed importance of the SP innervation of the MPOA-AH in the expression of male sexual behavior, a site that lies in close proximity to the ventricular circulation may be involved in the mediation of a inhibitory effect of SP on male copulatory behavior. Secondly, a reduction in the number of intromissions preceding ejaculation was observed following intraventricular injections of SP (an effect produced by MPOA-AH injections of SP in experiment 5). Therefore, it is possible that SP injected into the MPOA-AH in experiment 5 may have diffused into the ventricular circulation, and produced some of its effects by acting on the same brain site as that affected by LV injections. However, there were marked differences between the effects of LV and MPOA-AH injections of SP. Firstly, for the most part both mount and intromission latencies were longer in the LV group compared to the controls. Secondly, LV injections of 10 ng of SP significantly reduced the number of intromissions prior to ejaculation. This effect was not observed following MPOA-AH injections of the same dose of SP. Thirdly, MPOA-AH injections significantly reduced the inter-copulatory interval (ICI) compared to the saline injected controls. This was not seen following LV injections of SP. In fact, LV injections of SP produced a significant increase in the ICI (see Table 12). Nonetheless, the fact that both LV and MPOA-AH injections significantly reduced intromission

frequencies still suggests that SP injected into the MPOA-AH may have leaked into the ventricular circulation and produced this effect by acting on the same structure(s) affected by LV injections. It is also possible that the effects of MPOA and LV injections on male copulatory behavior would have been more similar had a higher dose of SP been used in experiment 7. Indeed both the bed nucleus of the stria terminalis and the medial amygdala, two areas known to play a facilitatory role in male sexual behavior, have direct access to the ventricular circulation.

Using the same logic, it is not inconceivable that SP injected into the lateral ventricles may have permeated into the MPOA-AH via the ventricular circulation, and produced its effect on intromission frequency by acting there. It is not known where in the brain the LV injections had their effects. Since bilateral injections of an identical dose of SP into the CP failed to alter any parameter of male sexual behavior, this suggests that SP injected into the lateral ventricles did not produce its effects on male sexual behavior in this area. Unfortunately, the role of SP on male sexual behavior was not examined following injections into other brain sites. Consequently, at this point, it is not known what structure(s) mediated the effects of the ventricular injections of SP.

Therefore, based on the results of experiments 5 through 10, it is suggested that the MPOA-AH, and possibly other structures with direct access to the ventricular circulation, may be important in the SP regulation of male sexual behavior.

Bilateral Cannula Implants into the MPOA-AH Depress Levels of Male Sexual Behavior

As is evident from the data in experiments 1 through 4, bilateral implants into the MPOA-AH were having a detrimental effect on male copulatory behavior as a function of the post-operative test interval. An examination of the baseline level of sexual behavior in experiments 5 through 11, however, revealed that although

scores between testing sessions were relatively stable in MPOA-AH implanted animals using the new post-operative interval established in experiment 4, they were also relatively high. For example, in experiment 5, the mean baseline ejaculation latency in the control group was 810.9 compared to 623.0 in the caudate implanted animals in experiment 6, or 703.2 in the LV implanted animals in experiment 7. Therefore, it appeared that bilateral cannulae in the MPOA-AH produced higher baseline scores compared to non-MPOA-AH implanted animals. Additionally, comparisons of baseline sexual behavior of animals in experiments 5, 8, and 9, all of which had bilateral cannulae in the MPOA-AH reveal differences in baseline scores (e.g., mean baseline ejaculation latencies of the experimental groups: experiment 5, 801.4; experiment 8, 491.4; experiment 9, 533.6). An examination of the distribution of cannulae implants in these animals did not reveal any marked differences. Shorter latencies in experiments 8 and 9 suggest that prior sexual experience could have been a factor. For instance, in both experiments 8 and 9, animals had more preoperative screening tests in an attempt to make them more sexually vigorous. Accordingly, it appears that bilaterally implanted cannula in the MPOA-AH increased baseline copulatory behavior in less sexually vigorous males. Indeed, the ejaculation latencies of animals in experiment 11 (CCK-8 injections) compared to those of animals that underwent the same experimental procedure (i.e., animals in experiment 5) appear similar (e.g. experimental group at baseline in experiment 5, 801.4; in experiment 11, 973.2).

As the above discussion has suggested, it appears that bilateral cannula implants into the MPOA-AH depress baseline copulatory behavior, and that this seems to interact with prior sexual experience. Accordingly, future studies are planned to investigate the effects of SP and CCK-8 injections on male copulatory behavior in unilaterally MPOA-AH implanted animals. In addition, in future studies animals will be screened for baseline levels of sexual behavior, to ascertain whether SP/CCK-8 effects depend on them.

Does SP Influence Male Copulatory Behavior Via Arousal or Copulatory Mechanisms ?

Beach (1956) proposed that, contrary to viewing sexual behavior as a unitary construct, two separate mechanisms were involved in male reproductive behavior: a sexual arousal mechanism and a copulatory mechanism. He suggested that the arousal mechanism mediates the male's initiation of the copulatory sequence, as well as the resumption of copulation following ejaculation. The copulatory mechanism was assumed to maintain copulatory behavior by summating the excitation of each successive intromission until ejaculation occured. Since its original formulation the " two factor " theory of Beach has received considerable attention. As a result, its conceptual framework has been continually revised. Indeed, based on a factor analysis of normative data on sexual behavior in male rats, Sachs (1978) subsequently subdivided the copulatory mechanism into further dimensions, one of which he referred to as a " copulatory rate factor ". This factor contained three components: inter-intromission interval (which I have referred to here as the ICI), ejaculation latency, and the post-ejaculatory interval (PEI). The reason for inclusion of the PEI in the theoretical copulatory mechanism framework was to account for the relative independence of the PEI from mount and intromission latencies (two behavioral components associated with arousal). Sach's stated that rather than adopting a more parsimonious approach to the study of male sexual behavior, at least 4 conceptual mechanisms are need to completely account for male copulatory behavior. I agree with Sach's. Any theory that will ultimately elucidate the neurophysiolgical mechanisms underlying masculine sexual behavior must be comprised of multiple theoretical constructs. The multidimensional approach postulated by Sach's will undoubtedly facilitate our understanding of this complex behavior.

Accordingly, individual parameters of the copulatory pattern that are associated with arousal mechanisms are mount and intromission latencies. Mount and intromission frequencies, along with ejaculation latencies, the ICI, and the PEI, are said to reflect copulatory mechanisms.

Data from experiment 5 support a role for SP being involved in both the arousal and copulatory mechanisms. For example, MPOA-AH injections of 10 ng and 100 ng of SP dramatically reduced the interval to initiate copulation. This was reflected in a significant decrease in the intromission latencies, and a non-significant trend for a reduction in mount latencies. Significant decreases in the inter- copulatory interval, intromission frequencies, and ejaculation latencies suggests that SP also facilitates the copulatory mechanism. Additionally, data obtained in experiment 9 revealed that bilateral injections of 2 ul of a SP antiserum significantly affected parameters of the copulatory sequence which are associated with both arousal and copulatory mechanisms (see Table 18). Collectively, the above results strongly suggest that endogenous SP plays an important role in regulating both the arousal and copulatory mechanisms in male copulatory behavior.

One puzzling result from experiment 5, however, is SP's relative lack of effect on the PEI's. For instance, although MPOA-AH injections 10 and 200 ng of SP reduced PEI's, only the 100 ng dose produced a significant effect when compared to the saline injected controls.

One explanation could be that the PEI is relatively resistant to change in Sprague-Dawley rats. For example, Barfield, Wilson, and McDonald (1975), and Clark, Caggiula, McConnell and Antelman (1975) reported that, following midbrain lesions in sexually experienced male rats, dramatic reductions were seen in the PEI compared to sham operated controls. Furthermore, in a study by Walker, Gerall, and Kostrzewa (1981), they too reported significant reductions in the PEI following midbrain lesions in sexually experienced male rats compared to sham operated controls. The authors noted, however, that the reductions in the PEI's were considerably smaller in their study compared to those in the Barfield et al. and the Clark et al. studies. They suggested that a major factor responsible for the discrepancy among their studies could be strain differences. Barfield et al. used Long-Evans rats, while Walker et al. used Sprague-Dawley rats. Consistent

with this assertion, Foreman and Hall (1987) observed a significant facilitation of male copulatory behavior following peripheral administration of a selective dopaminergic agonist. Although 25, 100, and 250 ng/kg of LY163502 significantly reduced mount, intromission, and ejaculation latencies, no effect was seen on the PEI. This is in contrast to evidence that suggests DA is critically involved in the regulation of the PEI (McIntosh and Barfield, 1984b). Again, Foreman and Hall used Sprague-Dawley rats. Indeed, the magnitude of the decrease of the PEI's in this study (experiment 5) following 10 and 100 ng of SP is almost identical to that seen in the Walker et al. study (18 % versus 17 % reduction, respectively). Accordingly, two possibilities are suggested concerning SP's involvement in determining the length of the PEI. Firstly, SP has only a minor role in the mediation of the PEI. Secondly, an alternative explanation is that SP does play a role in determining the length of the PEI at the level of the MPOA-AH, but this role is relatively difficult to demonstrate in Sprague-Dawley rats.

How Does SP Facilitate Male Rat Sexual Behavior ?

SP and LHRH:

As previously discussed, LHRH administration has been shown to facilitate mating behavior in male rats (Dorsa and Smith, 1980; Dorsa, Smith, and Davidson, 1981; Moss, Dudley, Foreman, and McCann, 1975; Myers and Baum, 1980). Moss et al. (1975) reported that in sexually experienced, gonadally intact Sprague-Dawley male rats, subcutaneous injection of 500 ng of LHRH significantly decreased intromission and ejaculation latencies compared to saline injected controls. More recently, Myers and Baum (1980) have reported similar results. In that study, subcutaneous injections of LHRH (1 ug) 1.5 hr prior to behavioral testing with a sexually receptive female significantly reduced ejaculation latencies in gonadally intact males compared to saline injected controls. In that study, the

effects of LHRH on copulatory behavior were further explored in castrated testosterone- treated males. Interestingly, in this group, LHRH had no appreciable effect on copulatory behavior, other than producing a significant increase in the PEI (an effect not seen in the gonadally intact animals). In light of the preceding discussion, it should be pointed out that, in the Moss et al. (1975) study using Sprague-Dawley rats, LHRH had no effect on the PEI.

The above studies are interesting in view of the recent reports that indicate a role for SP in the regulation of luteinizing hormone (LH) release, presumably through release of LHRH in the median eminence. Vijayan and McCann (1979) reported that when SP was incubated with anterior pituitaries taken from ovariectomized rats, it failed to release LH. When it was injected into the 3rd ventricle, however, it significantly increased plasma LH levels. The authors concluded that this was presumably the result of a SP-induced release of LHRH in the median eminence. This central action of SP on LHRH was further supported in a recent study by Dees, Skelley and Kozlowski (1985). They reported that, in castrated male Sprague-Dawley rats, ventricular injection of a SP antiserum resulted in a significant decrease in serum LH levels compared to the normal rabbit serum (NRS) injected control group. These results provide further (albeit indirect) support for the hypothesis that SP acts centrally to stimulate LH secretion via LHRH.

Perhaps SP releases LHRH from LHRH-containing neurons in the MPOA-AH. If this were the case, it is possible that the SP- induced facilitation of male copulatory behavior observed in experiment 5 could have been mediated via LHRH release within the MPOA-AH. Indeed, immunocytochemical studies have revealed numerous LHRH cell bodies and fibers located within the MPOA-AH (Bennet-Clarke and Joseph, 1982; Ibata, Watanabe, Kinoshita, Kubo, and Sano, 1979; Witkin, Paden, and Silverman, 1982). Moreover, in a recent immunohistochemical study by Hoffman (1985), light-microscopic examination of immunostained SP fibers revealed a close apposition of SP fibers to LHRH cell

bodies in the preoptic area of male mice, suggestive of synaptic associations with these neurons. More recently, Tsuruo, Hisana, Nakanishi, Katoh, and Daikoku (1987) report that electron microscopic examination of SP- immunoreactive nerve terminals in the preoptic area of male rats revealed SP synaptic contacts with LHRH neurons (unpublished data). In light of these recent data, it is possible that bilateral injections of SP into the MPOA-AH result in a release of LHRH and a subsequent enhancement of male copulatory behavior.

SP and Dopamine

A number of " in vitro " and " in vivo " studies have revealed that SP enhances the release of dopamine in the CNS (Garcia-Sevilla, Magnusson, Carlsson, and Folkers, 1983; Michelot, Leviel, Giorguieff-Chesselet, Cheramy, and Glowinski, 1979; Petit and Glowinski, 1986; Silbergeld and Walters, 1979). For example, in a study by Petit and Glowinski (1986), striatal slices taken from adult male Sprague-Dawley rats were continuously superfused with tritiated tyrosine so that the release of newly synthesized radioactively labeled dopamine could be estimated. Forty minutes after beginning the superfusion, SP was added to the superfusion medium and the amount of newly synthesized DA released was compared to the amount released before the addition of SP. They reported that SP applied to striatal slices in this manner markedly increased the release of newly synthesized dopamine. Other studies have shown that SP injected into the substantia nigra causes dopaminergic dependent turning, and stereotyped behavior in rats (Eison, Eison and Iversen, 1982; James and Starr, 1979). Collectively, these studies strongly suggest an interaction between DA and SP neurons in the CNS. As previously mentioned, dopamine has been shown to facilitate male copulatory behavior in both gonadally intact and castrated male rats. Studies have demonstrated a facilitatory effect on male copulatory behavior following peripheral administration of DA agonists, which can be completely abolished by pre-treatment with DA receptor blockers. Moreover, the similarity between the facilitatory effects following SP injections in experiment 5 and those

reported following DA enhancement is particularly interesting in view of the above mentioned interaction between SP and DA. For example, in the Foreman and Hall (1987) study, following subcutaneous administration of a dopamine agonist, reductions in ejaculation latencies were of a similar magnitude to those observed in experiment 5 (46 % versus 53 % respectively).

This evidence is only suggestive. Nonetheless, in view of the studies that support a DA/SP functional interaction in the CNS, and of the similarity of the effects of DA agonists and SP on male copulatory behavior, perhaps exogenous SP injected into the MPOA-AH is facilitating male copulatory behavior via DA release in the MPOA-AH. This would suggest that release of endogenous SP in the MPOA-AH promotes the release of DA. Consistent with this hypothesis, endogenous SP has been recently shown to modulate the release of DA in the nucleus accumbens (Elliott et al., 1986). In that study, injections of a SP antiserum into the nucleus accumbens induced a significant increase in DA concentrations in that area. The authors concluded that this reflected the intracellular accumulation and metabolism (as they also measured DOPAC) of DA which had not undergone a release-reuptake cycle, and they suggested that, at the level of the nucleus accumbens, SP exerts a tonic facilitatory effect on DA release. The above hypothesis is further strengthened by results which demonstrate that simultaneous injections of SP and DA into the nucleus accumbens enhance DA-induced increases in motor activity. Therefore, in view of the above studies, and of the recent report by Hull et al. (1986), which demonstrated a functional role of DA in male copulatory behavior at the level of the MPOA-AH, it is not inconceivable that the SP-induced facilitation of male copulatory behavior was mediated by increased DA release in the MPOA-AH.

Is the Release of Endogenous SP Within the MPOA-AH Important for Male Rat Copulatory Behavior ?

MPOA-AH injections of a SP antiserum were used to explore this possibility in experiments 8 and 9. In experiment 8, injections of an undiluted SP antiserum into the MPOA-AH failed to have a dramatic effect on male copulatory behavior. The only parameter that was affected was intromission frequency, where injections of 0.3 ul of the SP antiserum significantly increased the number of intromissions necessary to achieve ejaculation compared to the normal rabbit serum (NRS) injected controls. In experiment 9, however, bilateral injections of a larger volume (2 ul) of an undiluted SP antiserum into the MPOA-AH produced a dramatic impairment of male copulatory behavior. For instance, compared to the NRS injected control group, injections of 2 ul of the antiserum in sexually experienced male rats significantly lengthened mount, intromission, and ejaculation latencies (see Table 22). It is possible that the failure of the SP antiserum to alter the copulatory behavior in experiment 8, yet have a dramatic effect in experiment 9, is related to the presumably more wide spread distribution of the larger volume of antiserum throughout the MPOA-AH in experiment 9. Indeed, the distribution of SP fibers and receptors within the MPOA- AH is widespread (Ljungdahl, Hokfelt, Nilsson, 1978; Mantyh, Hunt, and Maggio, 1984; Quirion, Shults, Moody, Pert, Chase, O'Donohue, 1983; Simerly, Gorski, Swanson, 1986; Takatsuki, Sakanaka, Takagi, Tohyama, Shiotani, 1983). As a result, it may be difficult to interfere with copulatory behavior with a small volume of SP antiserum. Similarly, small lesions within the MPOA-AH are relatively ineffective in disrupting male copulatory behavior as compared to large lesions (Arendash and Gorski, 1983; Christensen, Nance, Gorski, 1977; Heimer and Larsson, 1966/67). Also, Szechtman, Caggiula, and Wulkan (1978) reported that knife cuts which severed at least 50 % of the medial-lateral connections of the MPOA-AH

had the greatest probability of disrupting male copulatory behavior in sexually experienced male rats. Thus, the data from experiment 9 suggest that the release of endogenous SP in the MPOA-AH is important in the regulation of male copulatory behavior.

How do SP Neurons Participate in the Neural Circuitry Mediating Male Copulatory Behavior ?

It is widely held that, in addition to the MPOA-AH, the medial amygdala (mAMY), and the bed nucleus of the stria terminalis (BnST) are critically important for the expression of male rat sexual behavior (Larsson, 1979). In rats, lesions of the mAMY result in severe impairment of copulatory behavior, characterized by a decrease in the number of animals displaying ejaculation during mating tests. In addition, in some animals when ejaculation does occur, marked prolongation of ejaculation latencies, as well as dramatic increases in the number of intromissions preceding ejaculation have been noted (Beck, Fonberg, and Korczynski, 1982; Harris and Sachs, 1975). Conversely, testosterone or dihydrotestosterone implants into the mAMY have been shown to facilitate male copulatory behavior in both rats and hamsters (Baum, Tobet, Starr, and Bradshaw, 1982; Bergondy, Powers, Matochik, Justice, 1984). Lesions of the BnST impair male copulatory behavior in a similar fashion (Bergondy, and Powers, 1986; Emery and Sachs, 1976; Valcourt and Sachs, 1978).

The MPOA-AH has afferent and efferent connections with the mAMY via the stria terminalis, and via short axonal reciprocal connections, with the BnST (Berk and Finkelstein, 1981; Conrad and Pfaff, 1976; Simmerly and Swanson, 1986; Swanson, 1976; Tanemichi and Murata, 1985). Not surprisingly then, the mAMY--BnST--MPOA-AH circuit (amygdalo-preoptic circuit) has been proposed to be intimately involved in the neural regulation of male copulatory behavior (see Hart and Leedy, 1985 for a review). The identity of the neurotransmitter/peptide that

regulates copulatory behavior within the amygdalo-preoptic circuit in the male rat, however, has not been established. Immunocytochemical studies report large numbers of SP cell bodies and fibers in the MPOA-AH, BnST and in the mAMY (Cuello, Priestley, and Paxinos, 1985; Emson, Jessel, Paxinos, and Cuello, 1978; Ljungdahl, Hokfelt, and Nilsson, 1978; Sakanka, Shioskaka, Takatsuki, Inagaki, Takagi, Senba, Kawai, Matsuzaki, and Tohyama, 1981; Simmerly, Gorski, and Swanson, 1986; Takatsuki, Sakanaka, Takagi, Tohyama, and Shiotani, 1983; Woodhams, Roberts, Polak, and Crow, 1983). Additionally, immunohistochemical studies have revealed that the stria terminalis, which connects the above structures, contains large numbers of SP-immunoreactive fibers (Ben- Ari, LaSalle, and Kanazawa, 1977; Sakanaka et al., 1981). Castration has been shown to significantly reduce the number and intensity of immunostained SP fibers within the mAMY (Dees and Kozlowski, 1984). As previously mentioned, our laboratory has recently reported a sex difference in the pattern of SP- like immunoreactivity within the most caudal part of the medial BnST, with males exhibiting a densely stained capsule surrounding a less dense core, which was barely detectable in female rats.

Based on the above discussion, it appears that most components of male sexual behavior depend on neural impulses within the amygdalo-preoptic circuit. Additionally, the expression of masculine sexual behavior within this circuit is dependent on circulating levels of androgens. In the absence of testosterone, the integrity of the above system is insufficient to maintain copulatory behavior. This has rather interesting implications in view of the previously mentioned immunohistochemical studies of SP within this circuit. In light of these reports, it is possible that SP neurotransmission within this system plays a fundamental role in the expression of copulation in the male rat. Since the mAMY has been implicated in sensorimotor integration (Turner, 1981), perhaps olfactory cues from a sexually receptive female increase the activity of SP containing neurons in the mAMY (the mAMY receives direct innervation from the accessory olfactory bulb, which is itself a recipient of peripheral input from the vomeronasal organ).

In support of the above, neuronal activity in the hypothalamus has been shown to be more responsive to peripheral stimulation following treatment with steroid hormones (Alcaraz,Guzman-Flores, Salas, and Beyer, 1969; Pfaff and McEwen, 1983). Also, Yagi (1967) found neurons in the medial anterior hypothalamus whose activity was raised within minutes following systemic injection of estradiol. Interestingly, mating has been shown to lead to an increase in testosterone levels in the male rat (Graham and Desjardin, 1980), and the mAMY has been shown to contain estrogen and androgen-binding neurons (Pfaff, and Keiner, 1973). Moreover, recently, SP has been reported to be located in hypothalamic neurons which bind estrogen (Akesson, Coquelin, and Micevych 1986). This is particularly compelling in view of the fact that testosterone is converted to estrogen by aromatization (Whalen, Yahr, and Luttge, 1985). Indeed, the mAMY, like the MPOA-AH, and BnST, has been reported to contain high levels of aromatase activity which itself appears to be regulated by circulating levels of steroids (Roselli, Horton and Resko, 1985). Therefore, it is not inconceivable that androgen or estrogen concentrating cells within the mAMY also contain SP. Although only suggestive from the above, it is possible that increased levels of testosterone may enter SP neurons in the mAMY and as a result stimulate SP synthesis. This increased synthesis, along with a heightened sensitivity to peripheral stimulation (i.e. olfactory cues, tactile stimulation from the female, etc.) may enhance SP neuronal responses to excitatory afferents from the olfactory system. Consequently, increased SP synthesis and transport within androphilic or estrophilic neurons in the medial amygdala could lead to increased SP being transported down axons through the ST pathway. This increased SP transport would undoubtedly increase SP postsynaptic activity in the BnST via the stria terminalis SP pathway. The resultant convergence of SP innervation from the medial BnST and from mAMY to the MPOA-AH may be of fundamental importance in the neural regulation of male sexual behavior. Indeed, iontophoretically applied SP has been shown to excite MPOA-AH neurons in the rat (Mayer and Macleod, 1979). Moreover, in the Mayer and Macleod study they further reported that electrical stimulation of the BnST evoked excitations in 60

$^{\sim}_{c}$ of MPOA-AH neurons. More interestingly, of those neurons excited by BnST stimulation, 72 % were excited by SP.

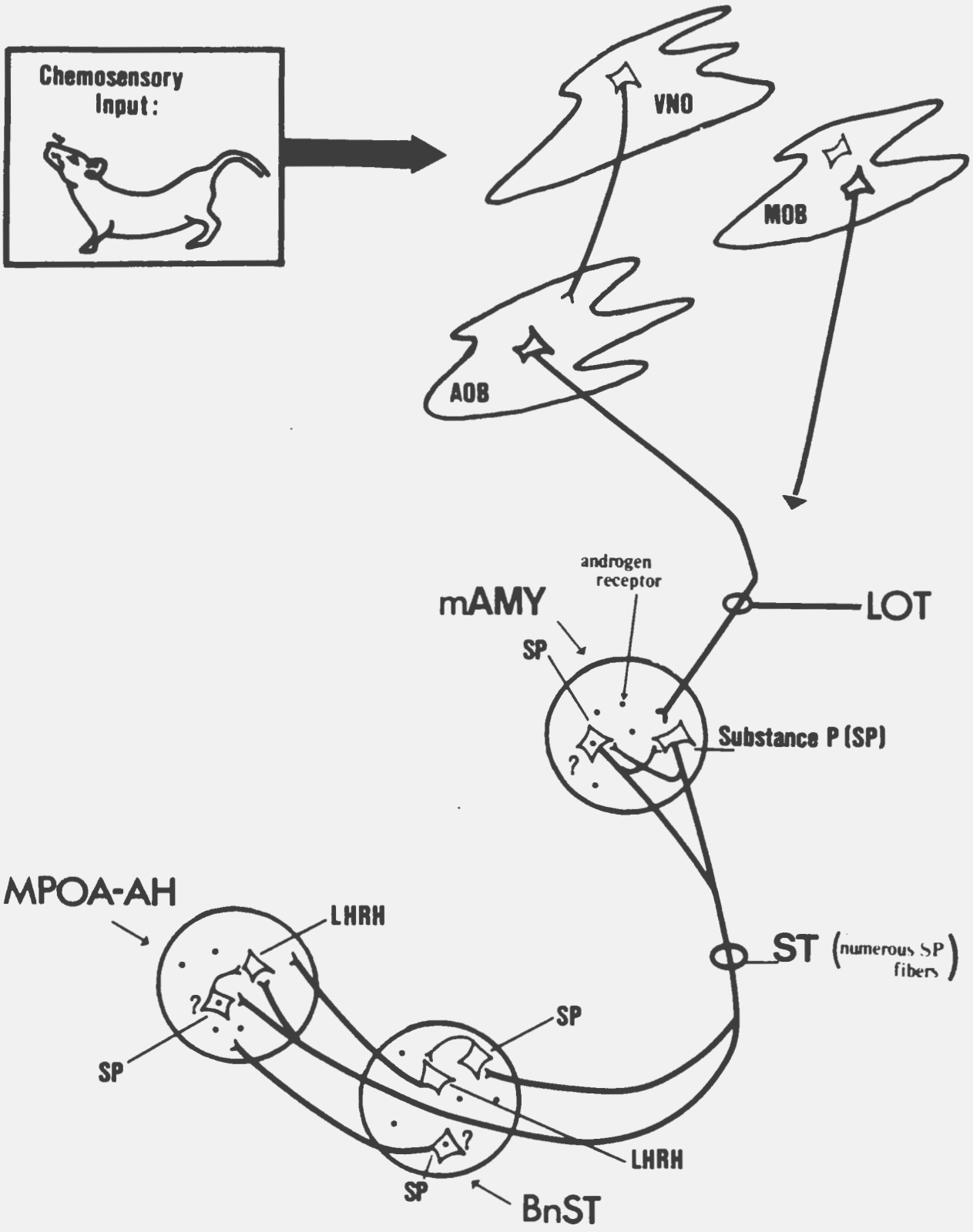
Figure 25 is a schematic representation of the proposed " SP- Sexually-Differentiated Amygdalo-Preoptic Circuit (SP-SDC) " that is postulated to be intimately involved in the neural regulation of male copulatory behavior.

Is Testosterone Required for the SP Activation of Male Copulatory Behavior ?

The results of experiment 10 demonstrate that increased SP activity within the MPOA-AH is incapable of inducing copulatory behavior in castrated rats deprived of testosterone. Bilateral injections of 10 ng of SP into the MPOA-AH failed to increase the number of animals displaying mounting, intromission and ejaculation during a 30 minute mating test with a sexually receptive female. There are a number of explanations that could account for this. Firstly, it is possible that a certain degree of circulating testosterone is necessary in order for SP to facilitate copulatory behavior. Indeed, minimal levels of circulating hormones are required for intracerebral injections to facilitate female sexual behavior (Dornan et al., 1987; Sakuma and Pfaff, 1983; Harlan et al., 1982). Along the same lines, perhaps a particular degree of baseline sexual behavior is needed before localized intracerebral injections of SP can facilitate copulatory behavior. Secondly, in the studies that have revealed a facilitation of male copulatory behavior in the absence of testosterone following enhancement of DA activity, none have used intracerebral injections. Since peripheral administration of pharmacologically active compounds activates multiple brain, as well as peripheral sites, it is possible that increased SP activity within the MPOA-AH alone was insufficient to reinstate copulatory behavior in the castrated, testosterone- deprived males. The results of experiment 10, however, do not support the proposed SP/DA interaction in male sexual behavior, as DA has been shown to facilitate male

FIGURE 25

Illustration of the SP-Sexually Differentiated Amygdalo-Preoptic Circuit (SP-SDC) that is postulated to play a critical role in the neural regulation of male sexual behavior (see text for details). Abbreviations: AOB, accessory olfactory bulb; BnST, bed nucleus stria terminalis; LOT, lateral olfactory tract; mAMY, medial nucleus of the amygdala; MOB, main olfactory bulb; MPOA-AH, medial preoptic area-anterior hypothalamic continuum; ST, stria terminalis; VMO, vomeronasal organ. Question marks indicate the possibility of androgen/estrogen binding cells which also contain substance P (SP).


Chemosensory Input:
VNO
MOB
AOB
androgen receptor
mAMY
LOT
SP
Substance P (SP)
?
MPOA-AH
LHRH
ST (numerous SP fibers)
SP
SP
?
LHRH
SP
BnST

copulatory behavior in the absence of testosterone. The present data, however, are congruous with a postulated interaction of SP and LHRH, as peripheral administration of LHRH failed to reinstate copulatory behavior in castrated male rats (Moss et al., 1975).

How Does Increased SP Activity in the MPOA-AH Mediate Male Sexual Behavior ?

Although the below is highly speculative, a recent report by Blumberg, Mennella and Moltz (1987) may shed some light on how increased SP activity in the MPOA-AH may regulate male sexual behavior. Based on the importance of the MPOA-AH in the expression of male sexual behavior, and on the involvement of the MPOA-AH in the regulation of body temperature, Blumberg et al. explored the possibility of a distinctive temperature profile during copulation in the male rat. They examined this possibility by monitoring both MPOA-AH and deep body temperature in sexually experienced male rats following the introduction of a sexually receptive female through sexual exhaustion. They found that the temperature of the MPOA-AH began to rise within one minute after introduction of the female, and continued until the first ejaculation, whereupon the MPOA-AH temperature began to drop. This specific pattern was evident throughout the testing period. Moreover, following MPOA-AH cooling (which lasted for about 4 minutes) the MPOA-AH began to heat up again. Interestingly, this increase was apparent just prior to the first mount subsequent to the first ejaculation. Changes in body temperature were not as uniform as MPOA-AH changes. The MPOA- AH temperature profile observed during copulation, however, did not seem to be secondary to obvious changes in motor activity that occur during the copulatory sequence. This was tested by running animals on a motor driven exercise wheel and monitoring MPOA-AH temperature. Although the running was sufficiently strenuous to induce physical exhaustion in the animals, the mean MPOA-AH temperature increase following exercise tended to be less than that observed during copulation.

Peripherally, SP is one of the most potent vasodilators known (Chahl and Walker, 1981; Pernow, 1983). In humans, intravenous injection of 1 ng of SP produces an immediate increase in blood flow in the skin and muscle of the forearm (Eklund, Jogestrand, and Pernow, 1977). In a recent study by Dib (1987) using rats, he reported that following intrathecal injection of SP, a significant increase in the amount of time spent bar pressing to obtain a cool wind was observed compared to saline injected controls. Although SP did not have any effect on body temperature, it did stimulate thermoregulatory behavior, which for the rat is one of the major mechanisms of heat dissipation. In fact, Dib (1987) reported that the bar pressing induced by SP in that experiment resembled bar pressing following hypothalamic heating. Moreover, warming of the preoptic area has been shown to increase grooming in rats (Roberts and Mooney, 1974), and grooming has been previously reported to be stimulated by increased SP activity in the CNS (Kelly and Iversen, 1978; 1979). Accordingly, SP may shorten initiation of copulation, as well as the ejaculation latencies, by increasing MPOA-AH temperature. In other words, in view of the Blumberg et al. study, where they reported that the increase in MPOA-AH temperature preceded the first mount following ejaculation, perhaps exogenous SP injected into the MPOA-AH leads to an acute increase in an MPOA-AH generated temperature signal either directly via an interaction with neuronal receptors, or indirectly via increases in MPOA-AH blood flow. Consistent with the increased blood flow hypothesis, Klugman, Lembeck, Markowitz, Mitchell and Rosendorff (1980) report that injections of SP (50 or 500 ng) directly into the hypothalamus in conscious anesthetized rabbits produced a significant increase in hypothalamic blood flow compared to a control group which received injections of a mixture of the amino acids contained in SP. Consequently, the increase in MPOA-AH temperature following exogenous SP might act as a cue to the animals, and thereby shorten the latency to initiate copulatory behavior. Indeed, preoptic area neurons have been shown to increase their firing rate following increases in temperature (Nutik, 1973). Similarly, the marked decrease in ejaculation latencies observed in experiment 5, could be explained by the increase in MPOA-AH temperature if this does, as Blumberg et

al. (1987) suggest, play a role in regulation of ejaculation latencies. One way to test whether SP could alter PEI's, would be to inject SP into the MPOA-AH immediately after ejaculation. For example, if the subsequent increase in MPOA-AH temperature determines the timing of the next copulatory event, then SP should theoretically shorten the PEI. Future studies investigating the thermoregulatory role of SP in the MPOA-AH are needed before any firm conclusions can be drawn.

Is CCK Involved in the Neural Regulation of Male Sexual Behavior ?

Although it was believed that the CCK-8 innervation of the MPOA-AH might play a role in the mediation of male copulatory behavior, in this study, bilateral injections of three different doses of CCK-8 into the MPOA-AH failed to significantly affect any parameter of male copulatory behavior. Numerous CCK-8 immunoreactive cell bodies and fibers are located in the MPOA-AH (Micevych, Park, Akesson and Elde, 1987; Simerly, Gorski, and Swanson, 1986), and CCK- 8 has been shown to be a potent excitant in hypothalamic slice preparations (Pan, Kow, and Pfaff, 1986). Moreover, like SP, CCK concentrations within the CNS have been shown to be dependent on gonadal hormones (Frankfurt, Siegel, Sim, and Wuttke, 1986). Indeed, in a recent study by Simerly and Swanson (1987), they reported that 2 weeks following castration in male rats CCK-8-immunoreactivity was virtually eliminated in the mAMY, BnST, and MPOA-AH. Furthermore, recent reports suggest that concentrations of CCK-like immunoreactivity in the MPOA-AH are higher in male than female rats (Frankfurt, Siegel, Sim, Wuttke, 1986; Micevych et al. 1987). Since CCK-8, like SP, is found in the amygdalo- preoptic cell groups previously implicated in sexual behavior, and since its concentrations are dependent on circulating levels of gonadal hormones, I hypothesized that CCK-8 would be involved in the neural regulation of male sexual behavior. Nevertheless, the present study failed to reveal any effect of MPOA-AH injections of CCK-8.

There are number of possible explanations for this. Firstly, it is possible that CCK regulates male copulatory behavior in another brain site. Although CCK-8 did not have any effect on male copulatory behavior following injections into the MPOA- AH, it may be that different results would have been obtained if CCK-8 would have been injected into another area of the brain which has been implicated in male sexual behavior (i.e. BnST or mAMY). Secondly, although there is some suggestion of a potentiation of DA release by CCK-8 (Crawley, Hommer, and Skirboll, 1985), other data from behavioral as well as electrophysiological studies suggest that DA and CCK-8 may be functionally opposed to each other (Lane, Blaha, and Phillips, 1986; Moroji, Watanabe, Aoki, and Itoh, 1982; Wang and Hu, 1986). Indeed, in a study by Wang and Hu (1986), they report that CCK-8 markedly reduced the inhibitory effects of DA, following simultaneous microiontophoretic application of CCK-8 and DA on spontaneously active or glutamate-activated neurons in the nucleus accumbens. At no time did they detect any evidence of a potentiation. Consistent with the above are the recent findings reported by Lane, Blaha, and Phillips (1986). They report that i.v. administration of CCK-8 produced a dose-related inhibition of DA release in the nucleus accumbens. Moreover, hyperactivity which follows apomorphine injections into the nucleus accumbens can be completely abolished by pretreatment of the nucleus accumbens with CCK-8 (Van Ree, Gaffori, and Wied, 1983). This effect has been recently shown to be mediated by a CCK-8-induced release of endogenous opioids (Kiraly and Van Ree, 1987). Collectively, these studies suggest that CCK-8 may act (in the nucleus accumbens) as a DA-antagonist and that this action is partly mediated by endogenous opioids. Accordingly, a preliminary interpretation would be that the lack of effect of CCK-8 on male copulatory behavior is due to its unique effects on both DA and endogenous opioids. In fact, if CCK-8 acts in the same way in the MPOA-AH, it would be expected to inhibit male copulatory behavior. Thirdly, and certainly more relevant to this study, is the recent report by Babcock, Bloom, Bloch, and Micevych (1986). In that study, peripheral injections of CCK-8 in gonadally intact or castrated male rats had no effect on male copulatory behavior.

Alternatively, the baseline sexual behavior of the males might explain the lack of effect of CCK-8 in the present study. For example, Bloch, Babcock, Gorski, and Micevych (1987) reported differential effects of CCK-8 on female sexual behavior (facilitatory and inhibitory) which was dependent on the initial degree of sexual receptivity. Intraperitoneal administration of CCK-8 in ovariectomized estrogen treated female rats facilitated lordosis only in animals displaying a low level of sexual receptivity. It is possible that bilateral injections of CCK-8 into the MPOA-AH might have had an affect on copulatory behavior in males that were sexually inexperienced or that displayed relatively poor baseline scores. Although again highly speculative, such a hypothesis is not inconceivable in light of the recent reports of the differential effects of opiates on male copulatory behavior which seem to depend on the rat's sexual experience, or baseline copulatory behavior prior to treatment (Gessa et al., 1979; Leiblich et al., 1985; McIntosh et al., 1980).

Finally, it is possible that MPOA-AH neurons involved in male copulatory behavior are not as sensitive to CCK-8 as they are to SP, and as a result may demonstrate a different dose- response profile to CCK-8. For example, perhaps an effect on male copulatory behavior would have been observed in experiment 11 if higher doses of CCK-8 had been used. Indeed there was a non-significant trend of reduced latencies following higher doses of CCK-8. Future studies, employing higher doses of CCK-8 will determine whether the above statement is correct.

Conclusion

In conclusion, this thesis supports the idea that the SP neurons which innervate the MPOA-AH form part of the neuronal circuitry which regulates male rat copulatory behavior. It is postulated that among other pathways that have been shown to be involved in male sexual behavior, the SP-SDC (figure 25) forms part of the neural circuitry which is critically important in the regulation of male

sexual behavior. Furthermore, as a result of the relative lack of effect of both SP and LHRH on the PEI, and the data which reveal SP fibers in close apposition to LHRH neurons in the MPOA-AH, it is postulated that increased SP activity, perhaps along with a SP-induced increase in LHRH activity within this circuit, is of crucial importance for maintaining copulatory behavior in the male rat. Further experiments are required to elucidate the mechanisms by which SP and LHRH mediate male sexual behavior at the level of the MPOA-AH. Additionally, the functional role that SP and/or LHRH play(s) in male copulatory behavior at the level of the mAMY and the BnST requires exploration before the importance of the SP-SDC in male copulatory behavior is clearly established.

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APPENDIX

ANALYSIS OF COVARIANCE TABLES

ORDER EFFECT

SUMMARY OF ANALYSIS OF COVARIANCE

INTROMISSION LATENCY (IL)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
IL	55942.4	1	55942.4	14.3*
EXPLAINED	220913.5	2	110456.7	28.2
RESIDUAL	19538.2	5	3907.6	
TOTAL	240451.8	7	34350.2	

ORDER EFFECT

SUMMARY OF ANALYSIS OF COVARIANCE

MOUNT LATENCY (ML)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
ML	143334.0	1	143334.0	6.5*
EXPLAINED	232899.1	2	116449.5	5.3
RESIDUAL	109017.7	5	21803.5	
TOTAL	341916.8	7	48845.2	

ORDER EFFECT

SUMMARY OF ANALYSIS OF COVARIANCE

EJACULATION LATENCY (EL)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
EL	88958.5	1	88958.5	7.5*
EXPLAINED	214171.5	2	71247.0	9.0
RESIDUAL	49495.5	5	9899.1	
TOTAL	191990.0	7	27427.1	

EXPERIMENT 1

SUMMARY OF ANALYSIS OF COVARIANCE

MOUNT LATENCY (ML)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
ML	93145.8	2	46572.6	0.3
EXPLAINED	95954.2	3	31984.7	0.2
RESIDUAL	2097498.5	15	139833.2.	
TOTAL	2193452.6	18	121858.4	

EXPERIMENT 1

SUMMARY OF ANALYSIS OF COVARIANCE

INTROMISSION LATENCY (IL)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
IL	80244.8	2	40122.4	0.6
EXPLAINED	272556.9	3	90852.3	0.9
RESIDUAL	1452749.6	15	96849.9	
TOTAL	1725306.5	18	95850.4	

EXPERIMENT 1

SUMMARY OF ANALYSIS OF COVARIANCE

EJACULATION LATENCY (EL)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
EL	358739.6	2	179369.8	0.4
EXPLAINED	365903.5	3	121967.8	0.7
RESIDUAL	3526511.5	15	235100.7	
TOTAL	3892415.1	18	216245.3	

EXPERIMENT 1

SUMMARY OF ANALYSIS OF COVARIANCE

ADJUSTED EJACULATION LATENCY (AEL)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
AEL	238155.9	2	119077.6	0.8
EXPLAINED	241401.1	3	80467.0	0.4
RESIDUAL	2048518.5	15	136567.9	
TOTAL	2289919.6	18	127217.7	

EXPERIMENT 1

SUMMARY OF ANALYSIS OF COVARIANCE

POSTEJACULATORY INTERVAL (PEI)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
PEI	219851.9	2	109777.6	0.4
EXPLAINED	41401.1	3	0467.0	0.1
RESIDUAL	2048518.5	15	136567.9	
TOTAL	2289919.6	18	127217.7	

EXPERIMENT 1

SUMMARY OF ANALYSIS OF COVARIANCE

INTERCOPULATORY INTERVAL (ICI)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
ICI	2821.6	2	1410.8	1.7
EXPLAINED	8996.2	3	2998.7	3.7
RESIDUAL	11987.8	15	799.1	
TOTAL	20984.0	18	1165.7	

EXPERIMENT 1

SUMMARY OF ANALYSIS OF COVARIANCE

MOUNT FREQUENCY (MF)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
MF	3.5	2	1.7	0.9
EXPLAINED	18.6	3	6.1	0.9
RESIDUAL	522.0	15	34.8	
TOTAL	540.4	18	30.0	

EXPERIMENT 1

SUMMARY OF ANALYSIS OF COVARIANCE

INTROMISSION FREQUENCY (IF)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
IF	89.7	2	44.8	0.5
EXPLAINED	317.4	3	105.8	0.2
RESIDUAL	966.5	15	64.4	
TOTAL	1284.0	18	71.3	

EXPERIMENT 2

SUMMARY OF ANALYSIS OF COVARIANCE

INTROMISSION LATENCY (IL)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
IL	186414.9	1	186414.9	5.6
EXPLAINED	189838.3	2	94917.1	2.8
RESIDUAL	230758.0	7	32965.4	
TOTAL	420596.4	9	46732.9	

EXPERIMENT 2

SUMMARY OF ANALYSIS OF COVARIANCE

MOUNT LATENCY (ML)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
ML	163779.3	1	163779.3	5.7 *
EXPLAINED	206394.5	2	103197.2	3.6
RESIDUAL	200020.0	7	28574.2	
TOTAL	406414.5	9	45157.2	

EXPERIMENT 2

SUMMARY OF ANALYSIS OF COVARIANCE

EJACULATION LATENCY (EL)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
EL	844866.0	1	844866.0	11.0 *
EXPLAINED	1523841.9	2	761920.9	9.9 *
RESIDUAL	537598.1	7	76799.7	
TOTAL	2061440.1	9	229048.9	

EXPERIMENT 2

SUMMARY OF ANALYSIS OF COVARIANCE

ADJUSTED EJACULATION LATENCY (AEL)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
AEL	318780.8	1	318780.8	2.3
EXPLAINED	661114.6	2	330557.2	0.1
RESIDUAL	1013536.3	9	144790.9	
TOTAL	1674650.9	9	186072.3	

EXPERIMENT 2

SUMMARY OF ANALYSIS OF COVARIANCE

POSTEJACULATORY INTERVAL (PEI)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
PEI	19851.9	1	19851.9	1.2
EXPLAINED	63631.9	2	31815.9	2.0
RESIDUAL	107925.6	7	15417.9	
TOTAL	171557.6	9	19061.9	

EXPERIMENT 2

SUMMARY OF ANALYSIS OF COVARIANCE

INTERCOPULATORY INTERVAL (ICI)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
ICI	56659.8	1	56659.8	1.7
EXPLAINED	60898.0	2	30449.0	0.9
RESIDUAL	229932.3	7	32847.5	
TOTAL	290830.4	9	32314.8	

EXPERIMENT 2

SUMMARY OF ANALYSIS OF COVARIANCE

MOUNT FREQUENCY (MF)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
MF	0.01	1	0.01	0.003
EXPLAINED	3.8	2	1.9	0.5
RESIDUAL	24.5	7	3.5	
TOTAL	28.4	9	3.1	

EXPERIMENT 2

SUMMARY OF ANALYSIS OF COVARIANCE

INTROMISSION FREQUENCY (IF)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
IF	8.1	1	8.1	0.2
EXPLAINED	8.5	2	4.2	0.8
RESIDUAL	206.3	7	29.4	
TOTAL	214.9	9	23.8	

EXPERIMENT 3

SUMMARY OF ANALYSIS OF COVARIANCE

MOUNT FREQUENCY (MF)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
MF	17.4	2	8.7	0.4
EXPLAINED	32.1	3	10.7	0.5
RESIDUAL	243.3	12	20.2	
TOTAL	275.4	15	18.3	

EXPERIMENT 3

SUMMARY OF ANALYSIS OF COVARIANCE

INTROMISSION FREQUENCY (IF)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
IF	17.8	2	8.9	0.3
EXPLAINED	19.8	3	6.6	0.2
RESIDUAL	301.6	12	25.1	
TOTAL	321.4	15	21.4	

EXPERIMENT 3

SUMMARY OF ANALYSIS OF COVARIANCE

INTROMISSION LATENCY (IL)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
IL	289021.7	2	144510.8	3.9*
EXPLAINED	452075.6	3	150691.8	4.1
RESIDUAL	436115.3	12	36342.9	
TOTAL	888191.0	15	59212.7	

EXPERIMENT 3

SUMMARY OF ANALYSIS OF COVARIANCE

MOUNT LATENCY (ML)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
ML	498079.4	2	249039.7	4.9*
EXPLAINED	424535.4	3	141511.7	2.4
RESIDUAL	606557.5	12	50546.7	
TOTAL	1131092.9	15	75406.1	

EXPERIMENT 3

SUMMARY OF ANALYSIS OF COVARIANCE

EJACULATION LATENCY (EL)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
EL	619163.5	2	334581.7	2.6
EXPLAINED	673137.9	3	224379.3	1.7
RESIDUAL	1500071.5	12	125005.9	
TOTAL	2173209.4	15	144880.6	

EXPERIMENT 3

SUMMARY OF ANALYSIS OF COVARIANCE

ADJUSTED EJACULATION LATENCY (AEL)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
AEL	111698.6	2	55849.3	0.7
EXPLAINED	168404.0	3	56134.3	0.7
RESIDUAL	934447.8	12	77870.6	
TOTAL	1102851.9	15	73523.4	

EXPERIMENT 3

SUMMARY OF ANALYSIS OF COVARIANCE

POSTEJACULATORY INTERVAL (PEI)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
PEI	667.7	2	333.8	0.05
EXPLAINED	993.1	3	331.2	0.05
RESIDUAL	71651.9	12	5970.9	
TOTAL	72645.0	18	4843.0	

EXPERIMENT 3

SUMMARY OF ANALYSIS OF COVARIANCE

INTERCOPULATORY INTERVAL (ICI)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
ICI	4821.6	2	2410.8	2.1
EXPLAINED	11996.2	3	3998.7	2.7
RESIDUAL	11987.8	12	799.1	
TOTAL	20984.0	15	1165.7	

EXPERIMENT 5

SUMMARY OF ANALYSIS OF COVARIANCE

MOUNT FREQUENCY (MF)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
MF	82.5	3	27.4	3.4*
EXPLAINED	82.5	4	20.6	2.6
RESIDUAL	212.3	27	7.8	
TOTAL	294.8	31	9.5	

EXPERIMENT 5

SUMMARY OF ANALYSIS OF COVARIANCE

MOUNT LATENCY (ML)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
ML	24029.6	3	8009.8	1.3
EXPLAINED	64100.4	4	16025.1	2.6
RESIDUAL	162829.0	27	6030.7.	
TOTAL	226929.4	31	7320.3	

EXPERIMENT 5

SUMMARY OF ANALYSIS OF COVARIANCE

INTROMISSION LATENCY (IL)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
IL	138625.9	3	46208.6	5.7*
EXPLAINED	244297.9	4	61074.4	7.6
RESIDUAL	215834.5	27	7993.8	
TOTAL	460132.4	31	14842.9	

EXPERIMENT 5

SUMMARY OF ANALYSIS OF COVARIANCE

INTROMISSION FREQUENCY (IF)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
IF	252.5	3	84.1	3.8*
EXPLAINED	266.5	4	66.6	3.0
RESIDUAL	584.6	27	21.6	
TOTAL	851.2	31	27.4	

EXPERIMENT 5

SUMMARY OF ANALYSIS OF COVARIANCE

EJACULATION LATENCY (EL)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
EL	1850287.5	3	616762.5	4.6*
EXPLAINED	2153684.8	4	538421.2	4.1
RESIDUAL	3617735.6	27	133990.2	
TOTAL	5771420.4	31	186174.8	

EXPERIMENT 5

SUMMARY OF ANALYSIS OF COVARIANCE

ADJUSTED EJACULATION LATENCY (AEL)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
AEL	997927.5	3	332642.5	3.3*
EXPLAINED	1140078.9	4	285019.7	2.8
RESIDUAL	2919808.9	27	100733.6	
TOTAL	3859887.8	31	124512.5	

EXPERIMENT 5

SUMMARY OF ANALYSIS OF COVARIANCE

POSTEJACULATORY INTERVAL (PEI)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
PEI	32830.5	3	10943.5	3.2*
EXPLAINED	55318.3	4	13829.5	4.0
RESIDUAL	91466.4	27	3387.6	
TOTAL	146784.8	31	4734.9	

EXPERIMENT 5

SUMMARY OF ANALYSIS OF COVARIANCE (RECIPROCALS)

INTERCOPULATORY INTERVAL (ICI)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
ICI	0.001	3	0.0000	2.8*
EXPLAINED	0.001	4	0.0000	2.1
RESIDUAL	0.004	27	0.0000	
TOTAL	0.005	31	0.0000	

EXPERIMENT 7

SUMMARY OF ANALYSIS OF COVARIANCE

MOUNT LATENCY (ML)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
ML	648.6	1	648.6	0.2
EXPLAINED	3910.5	2	1955.2	0.7
RESIDUAL	30586.8	12	2548.9	
TOTAL	34497.3	14	2464.0	

EXPERIMENT 7

SUMMARY OF ANALYSIS OF COVARIANCE

INTROMISSION LATENCY (IL)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
IL	21273.8	1	21273.8	3.3
EXPLAINED	99692.1	2	49846.0	7.9
RESIDUAL	75521.6	12	6293.5	
TOTAL	175213.7	14	12515.2	

EXPERIMENT 7

SUMMARY OF ANALYSIS OF COVARIANCE

INTERCOPULATORY INTERVAL (ICI)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
ICI	720.0	1	720.0	2.0
EXPLAINED	1403.2	2	701.6	1.9
RESIDUAL	4220.4	12	351.7	
TOTAL	5623.7	14	401.6	

EXPERIMENT 7

SUMMARY OF ANALYSIS OF COVARIANCE

ADJUSTED EJACULATION LATENCY (AEL)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
AEL	238713.0	1	238713.0	1.6
EXPLAINED	282898.5	2	141449.2	0.9
RESIDUAL	1767897.0	12	147324.7	
TOTAL	2050795.6	14	146485.4	

EXPERIMENT 7

SUMMARY OF ANALYSIS OF COVARIANCE

INTROMISSION FREQUENCY (IF)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
IF	240.7	1	240.7	7.1*
EXPLAINED	272.7	2	136.3	4.0
RESIDUAL	402.9	12	33.5	
TOTAL	675.7	14	48.3	

EXPERIMENT 7

SUMMARY OF ANALYSIS OF COVARIANCE

MOUNT FREQUENCY (MF)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
MF	22.1	1	22.1	2.1
EXPLAINED	59.2	2	29.6	2.8
RESIDUAL	124.4	12	10.3	
TOTAL	183.7	14	13.1	

EXPERIMENT 7

SUMMARY OF ANALYSIS OF COVARIANCE

EJACULATION LATENCY (EL)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
EL	406040.2	1	406040.2	2.5
EXPLAINED	584667.6	2	292333.8	1.8
RESIDUAL	1947698.0	12	162308.1	
TOTAL	2532365.7	14	180883.2	

EXPERIMENT 7

SUMMARY OF ANALYSIS OF COVARIANCE

POSTEJACULATORY INTERVAL (PEI)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
PEI	16341.0	1	16341.0	6.9*
EXPLAINED	22828.7	2	11414.3	4.8
RESIDUAL	50911.6	14	2340.2	
TOTAL	50911.6	14	3636.5	

EXPERIMENT 8

SUMMARY OF ANALYSIS OF COVARIANCE

MOUNT LATENCY (ML)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
ML	1372.7	1	1372.7	0.4
EXPLAINED	92012.6	2	46006.3	14.5
RESIDUAL	38071.1	12	3172.5	
TOTAL	130083.7	14	9291.6	

EXPERIMENT 8

SUMMARY OF ANALYSIS OF COVARIANCE

INTROMISSION LATENCY (IL)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
IL	3.2	1	3.2	0.04
EXPLAINED	71974.3	2	35978.1	11.2
RESIDUAL	38279.3	12	3189.9	
TOTAL	110253.6	14	7875.2	

EXPERIMENT 8

SUMMARY OF ANALYSIS OF COVARIANCE

EJACULATION LATENCY (EL)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
EL	169053.1	1	169053.1	1.4
EXPLAINED	1034143.3	2	517071.6	4.4
RESIDUAL	1400861.5	12	116738.4	
TOTAL	2435004.9	14	173928.9	

EXPERIMENT 8

SUMMARY OF ANALYSIS OF COVARIANCE

INTERCOPULATORY INTERVAL (ICI)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
ICI	4379.1	1	4379.1	2.1
EXPLAINED	5369.0	2	2684.5	1.3
RESIDUAL	24250.2	12	2020.8	
TOTAL	29619.3	14	2115.6	

EXPERIMENT 8

SUMMARY OF ANALYSIS OF COVARIANCE

ADJUSTED EJACULATION LATENCY (AEL)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
AEL	144013.9	1	144013.9	1.3
EXPLAINED	321182.5	2	160591.2	1.5
RESIDUAL	1274221.0	12	106185.0	
TOTAL	1595403.5	14	113957.4	

EXPERIMENT 8

SUMMARY OF ANALYSIS OF COVARIANCE

MOUNT FREQUENCY (MF)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
MF	9.4	1	9.4	0.6
EXPLAINED	9.4	2	4.7	0.3
RESIDUAL	176.1	12	14.6	
TOTAL	185.6	14	13.2	

EXPERIMENT 8

SUMMARY OF ANALYSIS OF COVARIANCE

POSTEJACULATORY INTERVAL (PEI)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
PEI	581.1	1	581.1	0.1
EXPLAINED	3024.4	2	1512.3	0.3
RESIDUAL	60356.2	12	5029.6	
TOTAL	63380.9	14	4527.2	

EXPERIMENT 8

SUMMARY OF ANALYSIS OF COVARIANCE

INTROMISSION FREQUENCY (IF)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
IF	149.1	1	149.1	5.2*
EXPLAINED	150.0	2	75.0	2.6
RESIDUAL	342.3	12	28.5	
TOTAL	492.4	14	35.1	

EXPERIMENT 9

SUMMARY OF ANALYSIS OF COVARIANCE

MOUNT LATENCY (ML)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
ML	95521.7	1	95521.7	11.9*
EXPLAINED	96285.0	2	48142.5	6.0
RESIDUAL	88206.2	11	8018.7.	
TOTAL	184491.2	13	14191.6	

EXPERIMENT 9

SUMMARY OF ANALYSIS OF COVARIANCE

INTROMISSION LATENCY (IL)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
IL	3888.6	1	3888.6	5.1*
EXPLAINED	5952.6	2	2796.3	3.9
RESIDUAL	8231.3	11	748.3	
TOTAL	14184.0	13	1091.0	

EXPERIMENT 9

SUMMARY OF ANALYSIS OF COVARIANCE

EJACULATION LATENCY (EL)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
EL	480155.4	1	480155.4	9.8*
EXPLAINED	1343664.1	2	671832.0	13.8
RESIDUAL	535449.8	11	48677.2	
TOTAL	1879114.9	13	144547.2	

EXPERIMENT 9

SUMMARY OF ANALYSIS OF COVARIANCE

ADJUSTED EJACULATION LATENCY (AEL)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
AEL	288108.1	1	288108.1	8.6*
EXPLAINED	1319102.2	2	659551.1	19.1
RESIDUAL	368096.5	11	33463.3	
TOTAL	1687198.8	13	129784.5	

EXPERIMENT 9

SUMMARY OF ANALYSIS OF COVARIANCE

POSTEJACULATORY INTERVAL (PEI)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
PEI	3890.5	1	3890.5	0.5
EXPLAINED	7874.0	2	3937.0	0.3
RESIDUAL	115269.1	11	10479.0	
TOTAL	123143.1	13	9472.5	

EXPERIMENT 9

SUMMARY OF ANALYSIS OF COVARIANCE

INTERCOPULATORY INTERVAL (ICI)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
ICI	2667.1	1	2667.1	5.2*
EXPLAINED	5280.7	2	2640.3	5.1
RESIDUAL	5604.6	11	509.5	
TOTAL	10885.4	13	837.3	

EXPERIMENT 9

SUMMARY OF ANALYSIS OF COVARIANCE

INTROMISSION FREQUENCY (IF)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
IF	5.1	1	5.1	1.7
EXPLAINED	34.8	2	17.4	5.9
RESIDUAL	32.1	11	2.9	
TOTAL	66.9	13	5.1	

EXPERIMENT 9

SUMMARY OF ANALYSIS OF COVARIANCE

MOUNT FREQUENCY (MF)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
MF	5.5	1	5.5	1.8
EXPLAINED	8.6	2	4.3	1.4
RESIDUAL	32.7	11	2.9	
TOTAL	41.4	13	3.1	

EXPERIMENT 11

SUMMARY OF ANALYSIS OF COVARIANCE

MOUNT LATENCY (ML)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
ML	25233.7	3	8411.2	1.8
EXPLAINED	28649.3	4	7162.3	1.5
RESIDUAL	100955.8	22	4588.9	
TOTAL	129605.1	26	4984.8	

EXPERIMENT 11

SUMMARY OF ANALYSIS OF COVARIANCE

INTROMISSION LATENCY (IL)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
IL	34366.3	3	11455.4	0.8
EXPLAINED	44549.1	4	11137.3	0.8
RESIDUAL	291735.4	22	13260.7	
TOTAL	336284.6	26	12934.0	

EXPERIMENT 11

SUMMARY OF ANALYSIS OF COVARIANCE

MOUNT FREQUENCY (MF)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
MF	22.1	3	7.3	1.8
EXPLAINED	26.5	4	6.6	1.6
RESIDUAL	88.0	22	4.0	
TOTAL	114.6	26	4.4	

EXPERIMENT 11

SUMMARY OF ANALYSIS OF COVARIANCE

INTROMISSION FREQUENCY (IF)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
IF	25.6	3	8.5	0.6
EXPLAINED	46.5	4	11.6	0.9
RESIDUAL	272.8	22	12.4	
TOTAL	319.4	26	12.2	

EXPERIMENT 11

SUMMARY OF ANALYSIS OF COVARIANCE

EJACULATION LATENCY (EL)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
EL	767576.9	3	255858.9	2.5
EXPLAINED	1934879.2	4	483719.8	4.7
RESIDUAL	2232693.4	22	101486.0	
TOTAL	4167572.7	26	160291.2	

EXPERIMENT 11

SUMMARY OF ANALYSIS OF COVARIANCE

ADJUSTED EJACULATION LATENCY (AEL)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
AEL	434779.4	3	144926.4	2.1
EXPLAINED	921625.0	4	230406.2	3.3
RESIDUAL	1502753.5	22	68306.9	
TOTAL	2424378.6	26	93245.3	

EXPERIMENT 11

SUMMARY OF ANALYSIS OF COVARIANCE

POSTEJACULATORY INTERVAL (PEI)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
PEI	24183.3	3	8061.1	2.7
EXPLAINED	26066.5	4	6516.6	2.2
RESIDUAL	63810.5	22	2900.4	
TOTAL	89877.1	26	3456.8	

EXPERIMENT 11

SUMMARY OF ANALYSIS OF COVARIANCE

INTERCOPULATORY INTERVAL (ICI)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F
ICI	5833.5	3	1944.5	1.7
EXPLAINED	5898.2	4	1474.5	1.3
RESIDUAL	23832.3	22	1083.2	
TOTAL	29730.6	26	1143.4	