Preoptic Area Stimulation and Male Rat Sexual Behavior

Ph.D. Psychology

THE EFFECTS OF ELECTRICAL STIMULATION OF THE PREOPTIC AREA OF THE HYPOTHALAMUS ON MALE RAT COPULATORY BEHAVIOR

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ABSTRACT

The present study determined the influence of preoptic area stimulation on particular aspects of male rat copulatory behavior. All measures of copulatory activity taken were facilitated by medial (MPO), but not by lateral, preoptic stimulation. The most common change produced by MPO stimulation was a reduction in both the number of mounts and intromissions preceding ejaculation. Short-latency approach and mounting of the female and greatly reduced refractory periods were also seen in two MPO animals. No evidence of a post-stimulation inhibition of behavior resulting from the stimulation itself was seen in these subjects. Most animals in both the medial and lateral groups learned to self-stimulate using long stimulus durations as used in tests of copulatory activity. MPO stimulation which was ineffective in eliciting copulation from a persistent non-copulator facilitated approach to the female once copulation had been established using peripheral stimulation.

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by

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INTRODUCTION

The mating behavior of the adult male rat consists of a relatively complex series of stereotyped actions which are easily quantified by an experienced observer. Elements involved are: recognition of a suitable mate, approach and investigation, mounting, clasping and palpation of the female's sides, pelvic thrusting, penile intromission, and after repeated mounts and intromissions, ejaculation. Following ejaculation there is a brief period (4-8 min.) of sexual inactivity termed the post-ejaculatory interval or refractory period. Large individual differences occur on measures of copulatory activity, but within an individual, provided that tests are given at regular intervals, the behavior pattern remains constant over long periods of time. Because of these characteristics, and because of its obvious importance to the species, male rat copulatory activity seems a particularly appropriate object of study for the neural analysis of behavior. The material presented in the general introduction will be limited to the evidence relevant to such an analysis.

It is recognized that hormonal factors strongly influence this behavior. Data gathered using techniques of experimental endocrinology to manipulate the circulating level of androgens are covered in a recent review by Davidson (1969). Such data provide little information relevant to the identity or physiology of particular neural structures sensitive to androgens and will therefore not be reviewed here.

The Spinal Level

The neural mechanisms necessary for penile erection and ejaculation are organized in the spinal cord below the mid-thoracic level and are capable of functioning in isolation from other levels of the nervous system. In 1897 Spina (cited in Bacq, 1931) noted that when a spinal transection was made between T_{12} and L_1 in the male guinea pig, erection and ejaculation soon followed. Bacq (1931) repeated these experiments and found that within 1 to 7 min. after transection, rhythmic movements of the anogenital region began spontaneously and were followed by a full erection and then ejaculation.

Bacq also found that sympathetic denervation of the genital organs prior to spinal transection prevented ejaculation, but not rhythmic movements of the anogenital region or erection. Erection seems to depend on parasympathetic activation. Sympathetic denervation of the genital organs was also carried out in rats which were later tested for copulatory behavior. Although these animals were unable to successfully impregnate females, their copulatory activity was described as "normal." However, no details were given. Recently Larsson and Swedin (1971), using standardized quantitative measures of mating behavior, confirmed and extended Bacq's findings for the rat. It was concluded that sympathetic smooth muscle innervation of the genital organs was not necessary for normal mating behavior, including the behavioral response associated with ejaculation.

In general agreement with earlier workers using the guinea pig Hart (1968b) has found that not only are the basic genital reflexes of the rat independent of higher levels of the nervous system, but they actually seem to be released from tonic inhibition by spinal transection. Cuts were made between T_6 and T_9 and testing, which consisted of retracting the sheath of the penis while the animal was restrained on its back, was begun 20 days postoperatively. Four reflexes were described; erections, quick flips of the penis, long flips, and violent leg kicking. The relatively complex penile responses were seen more frequently in the spinal animals than in normal males, thus suggesting a release from supraspinal inhibition. The occurance of the responses in clusters suggested a spinal timing mechanism which might be involved in the normal rat's post-ejaculatory refractory period.

These genital reflexes are dependent on androgen for their neonatal organization and later activation (Hart, 1967b, 1968a; Hart & Haugen, 1968). Unlike Hart's (1967a) finding using the spinal dog, ejaculation in response to penile stimulation was never observed in the rat. However, evidence of spontaneous ejaculation was seen, thus the necessary sympathetic mechanism was left intact. Hart speculated that transections at midthoracic levels prevented afferent stimulation from reaching a higher cord level where ejaculation is normally triggered during copulation.

Hypothalamic Control

Beach (1967) has proposed that, for vertebrates in general, the spinal mechanisms mediating copulatory reflexes are under tonic inhibitory control by more anterior levels of the nervous system. Hart's (1968b) work has demonstrated that this is the case for the male rat. However, little is known concerning the location and functioning of the hypothesized inhibitory mechanism.

Large lesions of the medial brainstem at the level of the diencephalic-mesencephalic junction have produced an enhancement of copulatory performance in male rats (Heimer & Larsson, 1964a). In about 50% of the lesioned males, ejaculation occurred after fewer intromissions and with shorter latency postoperatively. The post-ejaculatory refractory period was drastically shortened from the normal 5-6 min. to 1-3 min. As a result, many more ejaculations were observed in a standard 30-min. test session. The lesions included the posterior, medial part of the hypothalamus, the posterior parts of the medial thalamic nuclei, and the rostral portion of the mesencephalic central grey.

One of the structures destroyed by these very large lesions was the posterior mammillary region, which has since been the subject of more specific investigations though the results are conflicting. Soulairac (1963) for example found gonadal atrophy and disruption of copulatory behavior after lesions including the mammillary bodies. Testosterone injections did not succeed in restoring copulatory activity. In contrast, other workers have found little or no change in this behavior after lesions confined to the mammillary region (Giantonio, Lund & Gerall, 1970; Heimer & Larsson, 1964b).

The studies of Lisk (1966a, 1966b, 1969) provide the strongest evidence that the hypothesized inhibitory mechanism involves basal midline structures at the level of the posterior mammillary region and interpeduncular nucleus. However, most of Lisk's data are not based on direct observations of copulatory behavior. An indirect measure of frequency of ejaculation was taken based on daily counts of the number of copulation plugs found below the cages in which single males had free access to continuously receptive females. Using this technique, Lisk found that small lesions in the mammillary region resulted in an increase in the number of copulation plugs formed and in the percentage of days on which plugs were found (Lisk 1966a, 1966b). In a later experiment (Lisk, 1969), lesioned males were observed directly in repeated 10-min. tests with receptive females. More ejaculations occurred per test following lesioning, due mainly to a decrease in latency to ejaculate. In contrast to the results of Heimer and Larsson (1964a) who had made much larger lesions, no decrease was seen in the duration of the refractory period or in the number of intromissions preceding ejaculation. Mammillary-body lesions were also capable of increasing the number of copulation plugs found per day for previously sexually inactive males.

Whereas the inhibition of the mating pattern may depend on posterior hypothalamic areas, the activation of the pattern seems to be controlled by more anterior levels of the hypothalamus although the findings are somewhat contradictory concerning the relative contributions of the lateral or medial preoptic regions in this excitatory mechanism. Fisher (1956) first reported eliciting this behavior in an exaggerated form, using injections of minute amounts of a testosterone solution into the brains of male rats. No quantitative measures of the effects produced were given in this pioneering report. Components of maternal behavior or copulatory behavior were seen depending on the site of the cannula. Fisher suggested that the medial preoptic area (MPO) was the effective locus for activation of maternal behavior and the lateral preoptic (LPO) for male copulatory behavior. However, from photographs of representative brain sections, it appears that the effective cannulae for eliciting copulation were actually on the lateral edge of the MPO (Fisher, 1966). The same exaggerated form of male behavior was seen in a few animals using electrical stimulation of an area described as the "anterior dorsolateral hypothalamus"

(Vaughan & Fisher, 1962). However, no histological verification or even diagrammatic representation of the electrode loci was provided. Recently Madlafousek, Freund, and Grofovà (1970) have demonstrated rather weak facilitatory effects by electrical stimulation of the medial portion of the LPO. Caggiula and Hoebel (1966) and Caggiula (1970), also using electrical stimulation, have shown that facilitation of male copulatory performance can be produced by stimulation of the posterior lateral hypothalamus at points ranging from the level of the ventromedial nucleus to the mammillary bodies.

In summary, the changes attributed to electrical stimulation of the lateral hypothalamic and preoptic medial forebrain bundle (MFB) region have included: stimulation-bound mounting behavior (Caggiula, 1970; Caggiula & Hoebel, 1966; Madlafousek <u>et al</u>., 1970; Vaughan & Fisher, 1962), reduction of the postejaculatory interval (Caggiula, 1970; Caggiula & Hoebel, 1966; Vaughan & Fisher, 1962) and reduction in the number of intromissions preceding ejaculation (Vaughan & Fisher, 1962). Conversely, bilateral lesions of the MFB at the level of the ventromedial nucleus can seriously reduce copulatory behavior in male rats without producing gonadal atrophy (Hitt, Hendricks, Ginsberg & Lewis, 1970). These studies indicate that portions of the lateral preoptic-hypothalamic continuum, from the most anterior to the most posterior extent of the MFB, are involved in the excitatory aspect of copulatory behavior in male rats.

Other studies using lesion and chemical implantation techniques suggest that the MPO plays a more crucial role in the mediation of the male copulatory pattern. Bilateral MPO lesions can abolish this behavior without producing gonadal atrophy (Giantonio _et al_., 1970; Heimer & Larsson, 1966/67; Lisk, 1968; Soulairac, 1963) but see Lott, (1966). Bilateral lesions of the LPO-anterior hypothalamic MFB are relatively ineffective (Giantonio _et al_., 1970; Heimer & Larsson, 1966/67). Male, but not female, mating behavior has also been selectively eliminated by MPO lesions in testosterone-injected female rats (Doerner, Doecke & Hinz, 1969; Singer, 1968). Further substantiation for the importance of the medial area is provided by testosterone implantation studies. Doerner, Doecke and Moustafa (1968) found male mounting behavior could be produced in ovariectomized females by implants in the MPO-anterior hypothalamus. The most effective sites for reactivation of male copulatory behavior after castration are also in the hypothalamic-preoptic region, with the most consistent reactivation resulting from MPO implants (Davidson, 1966; Lisk, 1967).

There are only two reports indicating that electrical stimulation of the MPO can facilitate male mating responses. Roberts, Steinberg and Means (1967) explored both the LPO and MPO in the opossum using a very large number of loci. They were able to elicit separate elements of male sexual behavior such as teeth clicking, or mounting, as well as the complete integrated behavior pattern from the MPO, but not the LPO-MFB region in both males and females. Recently, electrical stimulation of the MPO has been shown to produce a dramatic increase of copulatory activity in rats as well (van Dis & Larsson, 1971). All the facilitatory effects attributed to lateral hypothalamic-MFB stimulation (p. 8) were also seen with MPO stimulation in two animals. In addition, after castration, at a time when interest in the female had completely disappeared, stimulation of one of these males continued to produce approach, mounting, and even a few intromissions, but no ejaculation.

Paxinos and Bindra (in press) have evidence that connections between medial and lateral hypothalamic areas but not connections within the medial hypothalamus are important. Large parasagital knife cuts were used to sever the medio-lateral connections of the hypothalamus, resulting in abolition or impairment of copulatory behavior. In most rats cuts separated all medio-lateral connections from the level of the preoptic area to the mammillary bodies. Coronal cuts restricted between the fornices at levels either anterior or posterior to the ventromedial hypothalamic nuclei did not affect this behavior. This agrees with Rodgers' (1969) demonstration that anteriorly-placed coronal cuts do not disrupt male rat mating behavior.

Evidence has been presented that various hypothalamic areas are involved in the control of male rat copulatory behavior. Basal midline structures at the level of the posterior mammillary region seem to be involved in the inhibition of this behavior, while the MPO-anterior hypothalamic continuum and posterior lateral hypothalamic-MFB region are involved in its activation. How these systems are normally influenced by other brain areas is largely unknown.

Since the presence of testosterone within the central nervous system is important for the expression of male copulatory behavior (Davidson, 1966; Lisk, 1967), studies of the pattern of testosterone uptake in the rat brain using high-resolution auto-radiographic techniques should indicate which neuroanatomical regions might be involved. Several limbic and hypothalamic structures preferentially take up radioactively-labeled testosterone into their cell bodies. In the hypothalamus these areas include: preoptic area, anterior hypothalamus, periventricular nucleus, arcuate and ventromedial nuclei, and extra-hypothalamically they include: olfactory tubercle, septal area, bed nucleus of the stria terminalis, prepiriform cortex, amygdala and hippocampus (Pfaff, 1968; Sar & Stumpf, 1971; Tuohimaa, 1970). However, testosterone is known to influence the expression of other behavior patterns (Moyer, 1968), as well as the functioning of the hypothalamic-pituitary-gonadal axis. Thus, although autoradiographic findings do suggest a role for certain extra-hypothalamic structures in mediating male rat copulatory behavior, their exact behavioral significance remains to be discovered. More direct means of study involving careful observation of various aspects of sexual behavior combined with manipulation (lesions, chemical and electrical stimulation) or observation (EEG, multi-unit, or single cell recording) of neural functioning must be used to determine the functional significance of these structures. Unfortunately, the existing data concerning extra-hypothalamic influences are based almost entirely on the lesion technique.

Sensory Systems

Information about the environment is essential for the initial aspects of the copulatory pattern, the appetitive phase of approach, investigation and recognition of a suitable mate. This sensory information must be processed by the nervous system and on this basis a "decision" must be made to either ignore, continue investigating, or mount the receptive female. The triggering of the mounting pattern probably involves activation of the MPO-anterior hypothalamic continuum as a result of changes in the neural activity of olfactory, cortical, and limbic forebrain areas mediating the initial appetitive aspects of the pattern.

Early studies of the effects of sensory deprivations on male copulatory behavior are reviewed by Beach (1947). In one study both sexually experienced and virgin male rats were deprived of various senses including vision, olfaction and cutaneous sensitivity in the head region (Beach, 1942a). No specific sense modality was necessary for the appearance of mating, although each of the three types of deafferentation increased the proportion of virgin males which failed to copulate. A combined removal of any two sense modalities in inexperienced animals blocked the development of copulatory activity, but did not abolish the response in experienced males. No particular combination of sensory deficits seemed critical. Beach concluded that the initial arousal of copulatory behavior depends not on any single class of sensory input such as odor or vision, but upon a multisensory pattern.

The level of general arousal of the male seems more important than which type of sensory stimulation creates that arousal. This idea is supported by recent studies which show that arousing peripheral stimulation unrelated to that produced by the presence of a receptive female is capable of facilitating certain measures of male copulatory behavior (Barfield & Sachs, 1968; Malsbury & Pajurkova, in preparation) and of eliciting copulation from persistent non-copulators (Expt. 4).

Hård & Larsson (1968) have suggested that although no one sensory modality may be absolutely necessary for the occurrence of mating, a specific stimulus category may still be contributing to sexual responsiveness and the extent of this contribution may vary from one category to another. Beach's (1942a) findings with experienced males agree with such an interpretation, as a marked impairment of copulatory activity followed olfactory bulb lesions while no comparable decrease was found in blinded animals. The irrelevance of visual information for successful copulation in experienced males has been confirmed by Hård and Larsson (1968) and is also supported by Larsson's (1964) report of normal mating behavior in rats with large occipital cortex lesions.

The deficits in copulatory activity after olfactory bulb lesions, first mentioned by Beach (1942a), have since been confirmed in several other experiments (Bermant & Taylor, 1969; Heimer & Larsson, 1967; Larsson, 1969). Bulbectomy in the male golden hamster has recently been reported to eliminate mating behavior completely (Murphy & Schneider, 1970). The effects on male rats are much less severe. Heimer and Larsson (1967) found that after such lesions experienced males were less likely to initiate mounting, and in any given 30-min. session 25-50% of the males failed to ejaculate. When ejaculation did occur, latencies to ejaculate and postejaculatory intervals were prolonged. The number of mounts and intromissions preceding ejaculation was unchanged. Bermant and Taylor (1969) report similar results and also find that the effects of olfactory bulb lesions depend to some extent on the previous sexual experience of the males. Treatment with gonadal or gonadotrophic hormones fails to restore normal levels of copulatory performance after such lesions, thus ruling out the possibility that the deficits are due to disruption of the pituitary-gonadal system (Larsson, 1969). Kaada, Rasmussen and Bruland (1969) studied the effects of lesions in several forebrain areas in a situation in which rats had to cross an electrified grid to approach a rat of the opposite sex. In general agreement with previous studies, olfactory bulb lesions resulted in a loss of sexual motivation as measured by significantly fewer grid crossings for both sexes post-operatively. No disruption of the performance of copulatory responses in animals which do begin mounting has been mentioned in any of the preceding studies.

Investigations of mating behavior after olfactory bulb lesions can be criticized because of the difficulty of interpreting data from bulbectomized animals in terms of a simple anosmia (Bermant & Taylor, 1969; Marks, Remley, Seago & Hastings, 1971; Phillips, 1970). Widespread changes in central neural functioning probably follow such lesions. Alterations of norepinephrine levels after bulbectomy are seen in several brain areas, including brainstem regions not receiving direct olfactory input (Pohorecky, Zigmond, Heimer & Wurtman, 1969; Pohorecky, Larin & Wurtman, 1969). A more suitable method of producing a simple loss of olfactory input in rats has been reported by Alberts & Galef (1971). A dilute zinc sulphate solution was used to bathe the olfactory mucosa, producing a loss of ability to use olfactory cues in a food-motivated task. The effects produced by this method are partially reversible and easier to interpret than those resulting from olfactory bulbectomy. A comparison of the effects of both treatments would be useful in arriving at a more complete picture of olfactory bulb function in copulatory behavior.

The importance of the sensory role of the male genitalia has been demonstrated by several studies. Temporary anesthetization of the penis of the rat by topical application of lidocaine or tetracaine interfers with intromission, but not mounting or thrusting (Adler & Bermant, 1966; Carlsson & Larsson, 1964; Sachs & Barfield, 1970). Erection does not seem to be prevented by this procedure either in the rat (Adler & Bermant, 1966; cf. Carlsson & Larsson, 1964) or the cat (Aronson & Cooper, 1968). Adler and Bermant have concluded that lack of sensation from the penis disrupts the process of precise localization of the vagina which depends on several very rapid shallow thrusts and is normally followed by a single deep penetration or intromission. A disruption of the intromission response attributed to "loss of genital orientation" has also been reported in the male cat as a result of either chemical or surgical desensitization of the penis (Aronson & Cooper, 1968). Sexual arousal was not immediately affected by either method.

Thus stimulation of the penis in both the male rat and cat is necessary for the performance of the intromission response but not for the initial arousal of mounting behavior. However, mounting attempts by the male rat do wane within a relatively short period of time if intromission is impossible (Adler & Bermant, 1966; Carlsson & Larsson, 1964). Similarly, surgical desensitization of the glans penis of the cat produces long term decrements in sexual arousal (Aronson & Cooper, 1968).

Although genital stimulation is important for intromission in rat and cat and is obviously necessary for ejaculation during normal copulation, little information is available concerning the effects of such stimulation on central neural activity. A polyspike EEG pattern has been seen in the posterior hypothalamic-MFB area following mounting, intromission and ejaculation in the male rat, but it is not clear whether this effect is a result of genital stimulation (Ward & Newton, 1970). Chhina, Chakrabarty, Kaur and Anand (1968) have observed an increase in high-voltage low-frequency EEG activity from the supraoptic area and mammillary body region as a result of penile stimulation in the immature male rhesus monkey. These changes were observed only after repeated testosterone injections and were specific to genital stimulation. Although more data are needed to clarify this point, these results suggest that penile stimulation may influence activity in specific hypothalamic areas known to be important for male copulatory behavior.

Neocortex

Beach (1940) was the first to make a detailed study of the influence of the neocortex upon male rat copulatory behavior. Lesions of less than 20% of the cortical surface did not affect the behavior, while lesions including 60%, or more, effectively eliminated it. Testosterone injections did not restore the behavior, and no gonadal abnormalities were seen, indicating that the deficits were not secondary to disruption of the pituitary-gonadal system. No particular area was determined to be crucial for these effects. The size, not the locus of the lesions seemed to be the important factor. Beach concluded that the cerebral cortex of the rat was involved in sexual arousal, maintaining the excitability of subcortical areas.

In subsequent studies certain cortical areas were found to be more important than others. Larsson (1962) found that using relatively small lesions of approximately 12% of the cortical surface, lateral lesions were more effective in eliminating male rat mating behavior than median cortical lesions. The bilateral lateral lesions involved the parieto-temporal area while the median lesions destroyed the medial parts of the frontal, parietal and occipital areas. The behavior of the 4 out of 20 lateral lesioned animals that stopped copulating could not be restored with testosterone injections, and no gonadal abnormalities were seen. In a later study it was found that lesions of the frontal area caused even greater deficits (Larsson, 1964). Eight out of 20 frontally lesioned males stopped copulating postoperatively, while lesions in the posterior cortical areas were ineffective. Kaada _et al_. (1969) found similar localization of cortical lesion effects using their obstruction box technique. Taken together, these studies suggest that the sensorimotor cortex, especially the precentral frontal area, is important for the initiation of male rat copulatory activity. Kaada _et al_. (1969) have suggested that an even smaller region, the lumbosacral (genital) division of the sensorimotor cortex, is crucial for these effects.

Somewhat different effects have been found with regard to the participation of cortical areas in male cat mating behavior (Beach, Zitrin & Jaynes, 1955, 1956; Zitrin, Jaynes & Beach, 1956). Although copulatory behavior was also most affected by frontal cortex lesions, these deficits seemed, in cats, to be due to motor impairment rather than a lack of sexual motivation (Beach _et al_., 1955). In contrast, rats which continue to copulate postoperatively display little or no change in the basic response patterns of mounting and intromission after neocortical lesions. This is true regardless of the size or location of the lesions (Beach, 1940; Kaada _et al_., 1969; Larsson, 1964).

The cortex of the rat seems to be involved more in the arousal of interest in the female and the initiation of mounting attempts rather than in the organization and execution of the responses themselves. Beach (1942b) has suggested that the neocortex may be a locus of intersensory summation and facilitation, its output participating in activating the relevant subcortical mechanisms.

Limbic System

MacLean and Ploog (1962) have noted EEG afterdischarge activity in the dorsal hippocampus accompanying "throbbing" erection after stimulation of the septum and rostral diencephalon in the male squirrel monkey. These observations have suggested that the hippocampal formation might be involved in some aspect of male copulatory behavior (Kim, 1960; Kimble, Rogers & Hendrickson, 1967). Inferences based on this type of evidence are somewhat suspect considering that hippocampal afterdischarge can easily propagate throughout the limbic system. Gol, Kellaway, Shapiro and Hurst (1963) report that sexual activity was never observed following bilateral ablations restricted to the hippocampus in cats, monkeys and baboons. However, no preoperative observations of copulatory behavior are mentioned, so it is difficult to interpret this statement. Studies using ablation of various areas of the hippocampus in the male rat have failed to find dramatic changes in copulatory behavior postoperatively.

Kim (1960) made suction removals of the dorsal hippocampus in sexually active male rats. He found that such ablations increased the frequency of "mounting acts," including mounts with and without intromission, in standard 15-min. sessions. Ablation of the overlying neocortex alone decreased the frequency of "mounting acts." Kim concluded that the hippocampus tends to suppress the sexual mechanism in rats. However, these findings are of limited value as no other measures of the copulatory pattern are given. Kimble _et al_. (1967) also made bilateral suction ablations of the dorsal hippocampus and compared the copulatory behavior of sexually naive hippocampectomized and unoperated male rats. Mounts, intromissions and ejaculations were scored separately, and no differences were found between the groups.

In two subsequent studies (Bermant, Glickman & Davidson, 1968; Dewsbury, Goodman, Salis & Bunnell, 1968) the effects of both dorsal and ventral hippocampal lesions were examined using more complete descriptions of copulatory behavior. Only sexually active males were used in both studies. Bermant _et al_. (1968) suggested that the locus of damage within the hippocampus is an important variable in determining the effect on copulatory activity. Electrolytic lesions involving the ventral hippocampus or both dorsal and ventral hippocampus produced no significant changes in copulatory behavior as compared to sham-operated males. However, dorsal hippocampal lesions alone did result in significant changes. Inter-copulatory and postejaculatory intervals progressively decreased during the second and third postoperative tests, reaching levels which were significantly less than preoperative values. When these data were transformed into a measure of frequency of "mounting acts" they confirmed the results of Kim (1960). Thus the changes produced by dorsal hippocampal damage may be characterized as decreases in the intervals between copulatory events. No changes were seen in the number of mounts or intromissions preceding ejaculation or in the ability to execute copulatory responses. Dewsbury _et al_. (1968) made suction ablations of dorsal hippocampus, dorsal and ventral hippocampus, and neocortex. The only significant effects of total hippocampal ablation were increases in mount latency and intromission latency. Dorsal hippocampal and neocortical lesions failed to produce significant changes in any measure taken. However, there was a consistent trend for dorsally lesioned males to show reduced inter-copulatory and postejaculatory intervals in the direction seen by Bermant _et al_. (1968). Dewsbury _et al_. concluded that differences between their data and that of Bermant et al. may have been of degree rather than kind. Thus it seems that on the basis of data collected using lesion techniques, the hippocampal formation does not play an important part in the mediation of male rat copulatory behavior, except perhaps with respect to the timing of copulatory events.

The septal area is another region of the limbic system which has been implicated in the neural control of male mating behavior. MacLean and Ploog (1962) have called the medial septo-preoptic region one of the "nodal points" in a circuit involved in producing penile erection in the squirrel monkey. The medial preoptic region has since been found to be crucial for male rat copulatory activity. However, large lesions of the septal area have little effect on the behavior of sexually active male rats (Goodman, Bunnell, Dewsbury & Boland, 1969; Heimer & Larsson, 1966/67).

The amygdala has also been studied with regard to a possible controlling function in male mating behavior since the report of "hypersexuality" seen as part of the syndrome following temporal lobe resection in monkeys (Kluver & Bucy, 1939). Male monkeys increased their frequency of copulation and masturbation after complete bilateral lobectomies which included parts of the ventral hippocampus, amygdala and temporal neocortex. The observations of increased sexual activity in monkeys has since been confirmed and the critical region localized to the amygdala (Kling, 1968; Schreiner & Kling, 1956).

Amygdaloid lesions have also been shown to produce changes in the mating behavior of male cats. Lesions confined to the amygdala and piriform cortex produced mounting of other species and inanimate objects as well as "tandem" copulation with other males (Schreiner & Kling, 1953). However similar "abnormal" behaviors have been observed in normal male cats after repeated copulatory experience in a particular testing room and treatment with exogenous hormones (Green, Clemente & DeGroot, 1957). Green _et_ _al_. (1957) did observe changes in copulatory behavior which might be called "hypersexual" only in a rather qualified sense. After lesions in the region of the amygdala, male cats were observed copulating with a variety of partners, including other males, kittens, other species, anesthetized animals, and inanimate objects in an unfamiliar testing room. Untreated males displayed no sexual responses under such circumstances. These authors also found that small bilateral lesions of the piriform cortex were just as effective as combined lesions of the amygdala and cortex in producing this effect. These studies indicate that the amygdala and piriform cortex may be involved in inhibiting the initiation of mating behavior, the removal of this influence through bilateral lesions resulting in an increase in mating in situations where it would not normally be seen. However, no evidence has been reported to indicate that the basic copulatory pattern seen in lesioned males is different from that seen in normal active males. Shealy and Peele (1957) have reported that electrical stimulation of the amygdala in a single male cat produced erection, copulatory movements and ejaculation. It is difficult to interpret these data in terms of a specific role for the amygdala, as this structure is prone to afterdischarge activity which can spread throughout the limbic system.

Unlike monkeys and cats, male rats do not seem to show any increase in copulatory behavior after amygdala lesions. In fact, damage to the amygdala usually results in some decrement in this behavior. Schwartz and Kling (1964) have reported that bilateral amygdala lesions in prepuberal rats resulted in aphagia and adipsia in the majority of the operated animals. The surviving (force-fed) aphagic males did not mate, but this failure was attributed to the debilitation produced by the aphagia. Operated non-aphagic males showed normal copulatory activity. Bermant *et al*. (1968) found that bilateral amygdala lesions produced a large but transient decrease in the rate of copulatory responding, but no changes were noted in number of mounts or intromissions preceding ejaculation. In this study sexually active males were given three postoperative tests spaced seven days apart and begun 13-15 days after surgery. Large increases in inter-copulatory and postejaculatory intervals were seen during the first postoperative test, but these changes disappeared by test three. Giantonio *et al*. (1970) also found decrements in copulatory activity in sexually active male rats postoperatively. Their data suggest that specific areas within the amygdala are responsible for this effect. No changes occurred following bilateral lesions of lateral basolateral amygdala, anterior amygdala, or piriform cortex, while lesions of the stria terminalis and to a lesser extent, basomedial-corticomedial amygdala, produced an increase in latency to ejaculate and a slight decrease in number of ejaculations to exhaustion. In contrast to the results of Bermant *et al*. these changes seemed to be relatively permanent, as they did not disappear over the three post-operative tests given at two-week intervals. In agreement with studies using direct measures of copulatory behavior, Kaada _et_ _al_. (1968) found that bilateral amygdala lesions resulted in a decrease in sexual motivation as measured with their obstruction box method. Interestingly, they also report that small lesions of the overlying piriform cortex resulted in an increase in the number of grid crossings to reach an animal of the opposite sex. Although Green _et_ _al_. (1957) have proposed this for the cat, this is the only report suggesting that the piriform cortex may be involved in the inhibition of sexual responsiveness in the rat.

Bilateral destruction of portions of the amygdala in the sexually active male rat results in decrements less severe, but similar to, those following olfactory bulbectomy. This is not too surprising considering the close anatomical connections which link the olfactory bulbs with the amygdala (Powell, Cowan & Raisman, 1965) and those linking the amygdala with the MPO-anterior hypothalamic continuum (Heimer & Nauta, 1969; Leonard & Scott, 1971). Lesions of the amygdala, like bulbectomy, may produce increases in inter-copulatory and post ejaculatory intervals resulting in longer latencies to ejaculate and decreases in the number of ejaculations to exhaustion. Olfactory bulbectomy and neocortical lesions sometimes produce more severe deficits in that some lesioned males consistently fail to initiate mounting (cortical lesions-Larsson, 1962, 1964) or initiate mounting irregularly during postoperative testing (bulbectomy-Bermant & Taylor, 1969; Heimer & Larsson, 1967; Larsson, 1969).

Thus the olfactory bulbs, neocortex and amygdala participate in the excitatory aspect of male copulatory behavior, probably by influencing the activity of the MPO-anterior hypothalamic continuum. On the other hand, lesions of the dorsal hippocampus sometimes produce a decrease in inter-copulatory and post ejaculatory intervals, suggesting that this structure may be involved in the hypothesized inhibitory system. The hippocampus does have strong connections through the fornix to the mammillary bodies, an area suggested as part of the inhibitory mechanism. None of these extra-hypothalamic structures seems to have any influence on the number of mounts or intromissions preceding ejaculation or on the performance of the consummatory responses of mounting and intromission. The influence they do exert on the copulatory pattern is quite weak as compared to that demonstrated for hypothalamic areas. This reinforces the idea that their influence is normally exerted via modulation of activity in the crucial hypothalamic mechanisms. Seen in this way, the hypothalamus is the region of convergence for olfactory, neocortical and limbic activity relevant to male copulatory behavior.

THE PRESENT INVESTIGATION

The present study was prompted by somewhat contradictory findings concerning the function of the LPO-MPO regions of the hypothalamus in male rat mating behavior. It employs electrical stimulation of the MPO-anterior hypothalamic continuum and LPO-MFB region in an attempt to localize the neural elements involved more accurately and make a finer analysis of the behavioral changes produced by the stimulation than has previously been attempted. A sensitive measure of the effects of stimulating a particular locus can be made by comparing the behavior of the same animal on control and stimulation tests as was done in Expt. 1. Expt. 2 is an investigation of possible inhibitory aftereffects of stimulation. Reward properties of the same electrodes are studied by the self-stimulation method in Expt. 3. The effect of MPO stimulation on the behavior of persistent non-copulators is examined in Expt. 4.

Experiment 1

Effects of Preoptic Area Stimulation on Copulatory Activity

Method

Subjects. Data were obtained from twenty-three male, Royal Victoria Hospital strain, hooded rats (Canadian Breeding Laboratories, Montreal). Rats were housed in groups of two to four in 12 x 14 x 6 3/4 in. plastic cages with free access to Purina rat chow and water. Lights were on in the animal room from 7 A.M. until 9 P.M., and testing was conducted from 3 to 6 P.M. Animals were tested for copulatory activity in four test sessions prior to electrode implantation. Each male was placed in the testing chamber and two minutes later a receptive female was introduced. The session lasted until the male had achieved an ejaculation or until 30 minutes had elapsed without an intromission. Animals that had ejaculated during at least one of these preliminary tests were considered sexually active and retained for implantation.

Females used in mating tests were brought into receptivity with a schedule of subcutaneous hormone injections consisting of 0.1 mg. of estradiol dipropionate (Diovocylin, Ciba) 3 days prior to the test day, an additional 0.1 mg. 1 day before the test, and 1.0 mg. of progesterone (Lutocylin, Ciba) approximately 4 hours before the test session. The hormones were in sesame oil solution with 0.5% chlorbutanol as preservative. Before each experimental session the females were tested for behavioral receptivity with a sexually vigorous nonexperimental male which was allowed to intromit once or twice. Only those females obviously receptive, with vigorous lordosis responses, no attempts to fight off the male, and no vocalizations upon intromission were used.

Surgery. Subjects weighed 250-400 gms. at the time of surgery. They were given atropine sulfate and fifteen minutes later anesthetized with sodium pentobarbital (Nembutal) supplemented by chloral hydrate. Either unilateral or bilateral, 0.01 in. diameter monopolar stainless steel electrodes, insulated except for the tip cross-section were implanted stereotaxically in each animal aimed for the medial or lateral preoptic area or bed nucleus of the stria terminalis. With the skull surface between bregma and lambda held horizontal, MPO electrodes were implanted from 0.2 mm anterior to 0.5 mm posterior to bregma, from 0.5 to 0.7 mm lateral to the midline, and from 6.7 to 7.2 mm below the cortical surface. LPO electrodes were implanted either 0.2 mm anterior or on line with bregma, either 1.7 or 1.8 mm lateral to the midline, and 7.0 mm below the cortical surface. Bed nucleus electrodes were implanted on line with bregma, 1.5 mm lateral to the midline, and 5.5 mm below the cortical surface. Two jeweler's screws approximately 1.5 mm anterior to lambda and 3.0 mm. on either side of the midline were wired together to serve as an indifferent electrode for the monopolar stimulation.

Apparatus. The testing chamber was a 9½ x 14 x 13½ in. wooden box, with a one-way vision plexiglass front and wire mesh floor, illuminated by an overhead bulb. The room was darkened so that the animals could not see out of the box. Mounts with thrusts, intromissions, and ejaculations were recorded along with an automatic record of the stimulation schedule on an event recorder (Esterline-Angus).

The electrical stimulus was a 30 sec. train of biphasic square waves at 100 pulse-pairs per second, produced by a Grass S8 stimulator and SIU5 stimulus isolation units. Pulses in each biphasic pair were 0.2 msec. in duration and separated by 0.1 msec. The first pulse in each pair produced a cathodal stimulating current at the electrode tip. An approximation to a constant current source was obtained by placing a 200 KΩ resistor in series with the electrodes.

The wave form was monitored and peak current was determined by measuring the voltage drop across either a 500 or 250 Ohm resistor in series with the electrode on a Telequipment type D43 oscilloscope. A tape timer (Gerbrands) programmed the 30 sec. on, 30 sec. off pattern of stimulation.

Preliminary screening. One week after the operation electrodes were screened for behavioral effects in the absence of a female. The object was to find a single electrode and current level for each animal which might facilitate male copulatory activity. Screening procedure in the test box involved initial stimulation at 50μA for the 30 sec. train with increases, depending on the behavior observed, up to a maximum of 200μA. Current levels resulting in behavior related to reward such as increases in locomotor exploration and sniffing, or related to male sexual activity such as partial penile erection and emission, were selected to be used in a second test either the same or next day with a receptive female. If escape behavior or "wet dog" shaking, implying the possibility of localized after-discharges, was seen at 100μA or less, the subject was not tested further.

Screening was then conducted with a receptive female present. By making adjustments above and below the value arrived at in the initial test, an optimal current level for facilitating mounting behavior was found. Often no current level would facilitate mounting. In those cases, the lowest level obviously producing a behavioral change, such as an increase in locomotor exploration or grooming, was chosen. Most animals were tested on the next day in the same manner and a final current level arrived at to be used in all further tests. In a few cases this level was readjusted after one or two copulatory tests. In subjects with two electrodes, only the most effective was tested after the initial screening procedure.

Copulatory tests. Tests of copulatory activity were conducted every two days for each animal. Stimulation (S) days alternated with non-stimulation control (C) days. The only exceptions to this were two subjects (11 & 12) that were tested every day, stimulation days also being alternated with non-stimulation days. Usually 3 or 4 sessions were conducted under each condition for each animal.

A test session was started by placing the male alone in the observation box for two minutes. A receptive female was then introduced and observation and timing of the behavior began. A session lasted until the male had achieved one ejaculation and then resumed mounting, ending the refractory period. If no intromissions took place within the first 15 min. or if 15 min. elapsed between intromissions, the session was ended. The procedure was identical for S and C tests except that on stimulation days brain stimulation was applied on a 30 sec. on, 30 sec. off schedule. Leads were connected to the electrodes during every test session and background noise from the programming equipment was also held constant.

Mounts, intromissions and ejaculations were distinguished on the basis of behavioral criteria. A "mount" was recorded only if the animal approached from the rear, mounted, clasped and palpated the female's sides, and showed some pelvic thrusting. An "intromission" consisted of the same behavior followed by rapid springing back off of the female and genital grooming. An "ejaculation" was easily distinguished by the prolonged motionless clasping of the female following the final thrust or by the "frozen" posture of the male over the female. This clasp or frozen posture usually lasted several seconds. The "duration of the ejaculatory series" was defined as the time between the first mount after the female was introduced and the ejaculation. The "refractory period" was defined as the time between the first ejaculation and the next mount.

Histology. After the subjects had completed all Experiments in which they were to participate, histology was performed as follows. Animals were sacrificed with Nembutal and were then perfused through the heart with physiological saline followed by a saline and 10% formalin solution. Brains were removed and after soaking in the formalin solution for at least a week followed by two days in 20% ethanol, 40μ frozen sections were cut through the site of the electrode tracks. The coronal sections were alternately mounted for staining with either cresyl violet for cell bodies or hematoxylin (Weil Method) for fibers.

Results

The changes in copulatory activity resulting from stimulation are presented separately for each animal in Table 1 A & B. A negative percentage change indicates a reduction in the mean for any particular measure on S tests as compared to C tests, a positive percentage change indicating an increase. Thus negative changes represent a facilitation of copulatory performance as a result of preoptic area stimulation. The "% mounts during S" was calculated on the basis of the number of mounts occurring during the "on" versus "off" periods during S tests only.

The mean measures of copulatory activity used to calculate the percentage changes were based on data from either three or four ejaculatory series under each condition for each animal with the exception of numbers 11, 16, and 17. The means for these animals were calculated from fewer sessions because of failures in meeting the criteria for completing an ejaculatory series. Numbers 11 and 17 were quite sluggish on non-stimulation days. Number 11 completed an ejaculatory series in only 2 out of 5 control tests and in 4 out of 6 stimulation tests. Number 17 completed an ejaculatory series in only 2 out of 6 control tests, and in 4 out of 4 stimulation tests. Number 16 began showing interfering motor behaviors and finally a seizure after the first stimulation test. Thus, there were data from only one stimulation session but from 4 control sessions for this animal.

The Table is divided into "Medial" (1A) and "Lateral" (1B) sections on the basis of the anatomical locus of each animal's electrode tip. All animals in the "Medial" section had electrodes in the medial preoptic-far anterior hypothalamic continuum except for numbers 34, 39, and 43 which were closer

TABLE 1 A

Percentage Change in Copulatory Activity as a Result of Stimulation and Median SS Score for Animals with Medial Electrode Placements

Rat No.	Current (in μA)	Duration of Ejac. Series	Refract. Period	Mounts only to 1st Ejac.	Intro.'s to 1st Ejac.	Percent Mounts During S	SS Score (Expt. 3)
11	150	-51.0*	-25.0	-63.3	-67.3**	74	39
12	150	-59.1**	-78.7**	+23.7	-57.9**	84	43
13	120	-58.3*	-32.7**	-80.0*	-44.8*	35	24
14	160	-45.6*	-17.8	-50.0	-44.4**	37	27
15	160	+14.1	-12.5	- 6.7	+ 8.9	46	22
16	160	-90.5*	-85.1**	-92.5	-84.8**	80	--
17	160	-85.6**	-44.4	-62.5	-78.7**	65	--
34	90	-40.3*	-16.4	-66.7**	-37.5**	45	13
38	100	-89.2**	-87.0**	-64.0*	-63.8**	54	35
39	80	-20.0	-14.6*	0.0	-31.2*	23	31
43	80	+14.3	- 7.5**	+ 5.9	-38.8**	7	9
Column Mean		-46.5	-38.3	-41.5	-49.1	50.0	27.0

* $p \leq 0.05$; ** $p \leq 0.01$; One-tailed t tests.

TABLE 1 B

Percentage Change in Copulatory Activity as a Result of Stimulation and Median SS Score for Animals with Lateral Electrode Placements

Rat No.	Current (in μA)	Duration of Ejac. Series	Refract. Period	Mounts only to 1st Ejac.	Intro.'s to 1st Ejac.	Percent Mounts During S	SS Score (Expt.3)
25!	100	+91.7	-13.2	-100.0	+37.7	22	42#
28	100	+21.0	-10.8	- 20.0	+ 8.7	94	54
29	80	-21.4	-27.1	+125.0	-32.0*	57	46
30	80	-40.7*	-19.0	+ 4.2	-10.3	22	42
36	130	+15.5	+ 1.6	+133.3*	+ 5.7	41	36
40	90	-19.4*	-21.8	- 11.3	+ 5.7	47	21
41	90	+46.9	+ 4.0	- 20.0	-18.2	4	37
42	90	-18.4	- 6.7	- 11.1	0.0	31	25
Column Mean		- 2.4	-11.4	+ 28.6	- 5.8	42.3	37.3

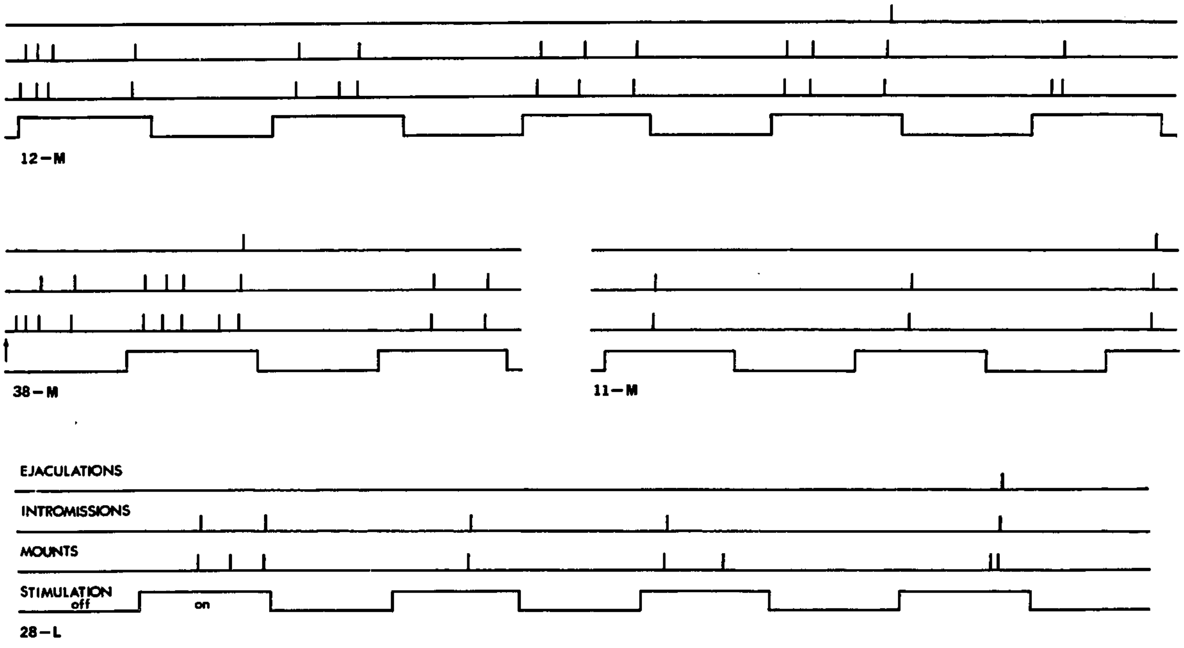
* $p \leq 0.05$; ** $p \leq 0.01$; One-tailed t test. # Motor seizure during SS. ! Electrode in bed nucleus of stria terminalis. Data not included in group analyses.

to the Diagonal Band of Broca. All animals in the "Lateral" section had electrodes in the lateral preoptic-far anterior hypothalamic MFB except for number 25 whose placement was in the bed nucleus of the stria terminalis. The locus of the electrode tip for each animal can be seen in Fig. 1.

In order to obtain a more objective criterion for the effectiveness of particular stimulation loci, the mean scores under the S condition were compared to those under the C condition for each measure of copulatory activity within each animal. T tests for independent samples were done in this manner and the results are presented in Table 1 A & B. All tests were one-tailed. As can be seen from Table 1, MPO stimulation was generally much more effective for facilitating copulatory behavior than was LPO-MFB stimulation. Standard current levels were also generally higher in this group, but this cannot account for the difference since the same criterion of maximally facilitating approach and mounting behavior towards a female was used to select current levels for all animals. It must be emphasized that current levels in Table 1 do not represent threshold values, but optimally effective values. MPO facilitation can be demonstrated at lower current levels as is shown in rat 38, one of the most effective electrodes tested at 100μA. The overall difference in current levels between medial and lateral placements resulted from differences in behavior due to stimulation. The current could be raised much higher in the medial animals without producing obvious behavioral effects. When lateral electrode stimulation was raised above 120μA in the initial screening tests with females, it usually produced such a high level of locomotor activity that it interfered with interest in the female. Most medial animals never showed this great increase in locomotor exploration in the absence of a female, but would most often show orienting reactions with some locomotion or increases in grooming during stimulation. However, with the most effective medial electrodes, such as numbers 12 and 38, when a female was present these behaviors would be replaced by rapid pursuit and mounting.

Fig. 1 displays the anatomical locus and the more striking stimulation effects for each animal's electrode. Only three effects of stimulation are displayed; the "stimulation-bound" nature of the behavior, the reduction of number of intromissions to ejaculation, and the shortening of the refractory period. "Stimulation-bound" copulation was

Fig. 1. Location and stimulation effects for electrode placements of Experiment 1. Sections 1-9 are redrawn from König and Klippel, (1963), sections 17b-25b. Abbreviations: AA, anterior amygdaloid area: AC, anterior commissure; ah, anterior hypothalamus: CC, corpus callosum: cp, caudate-putamen: DB, diagonal band of Broca: F, fornix: GP, globus pallidus: lh, lateral hypothalamus: LOT, lateral olfactory tract: lp, lateral preoptic area: MFB, medial forebrain bundle: mp, medial preoptic area: OC, optic chiasm: OT, olfactory tubercle: poma, magnocellular preoptic nucleus: sl, lateral septal area: SM, stria medullaris: ST, stria terminalis: st, bed nucleus of stria terminalis.


12—M
38—M
11—M
EJACULATIONS
INTROMISSIONS
MOUNTS
STIMULATION
off
on
28—L

defined in terms of the "mean percent mounts during stimulation" seen for each animal in Table 1. Any animal having a mean score of over 70% on this measure was shown in Fig. 1 as a stimulation-bound copulator. Reduction of number of intromissions to ejaculation and shortening of the refractory period were only shown in Fig. 1 if they occurred with $p \leq 0.01$. The presence of motor seizures during any of the test sessions of Expt. 1 was also indicated along with "wet dog" shaking and escape.

Discussion

From examining Table 1 A & B and Fig. 1, it is clear that all measures of copulatory activity taken can be facilitated by preoptic area stimulation and that this facilitation is almost exclusively confined to the MPO-anterior hypothalamic stimulation. This localization of effective points agrees very well with that found in the opossum by Roberts _et al_. (1967) and also confirms the importance of the MPO for copulatory behavior previously demonstrated with lesion and chemical implantation techniques in the rat.

The MPO and LPO-MFB regions are intimately related anatomically. Golgi studies have shown a reciprocal interchange of axon collaterals between MPO and LPO-MFB neurons

(Millhouse, 1969), while dendrites of some of the MPO neurons lie in the LPO-MFB region and dendrites of some LPO-MFB neurons lie in the MPO area (Matano, Sakai, & Ban, 1969; Millhouse, 1969). Thus it would not be surprising for manipulation of the functioning of one area to affect the other.

However, despite this intimate anatomical relation, functional differences in the control of male mating behavior have been demonstrated using the technique of electrical stimulation in the present study and that of Roberts _et al_. (1967). Two possible explanations exist. One is that the two areas are roughly equipotential with regard to this particular behavior, but that the LPO has a lower threshold for motor and other distracting effects. The other is a true functional differentiation of the two areas with regard to male copulatory activity. Some support for the former comes from the observation noted in the Results section that locomotor behavior was noticed at a much lower level of stimulation in LPO-MFB animals. However, greater support for the latter comes from the fact that weak facilitatory effects of MPO, but not LPO stimulation could be demonstrated at these low stimulation levels. The final stimulation levels shown in Table 1 are the optimally effective and not the threshold values for facilitation of male copulatory activity. The lesion data also strongly support a true functional differentiation between the two areas (Giantonio *et al.*, 1970; Heimer & Larsson, 1966/67).

The most common change produced by "medial" stimulation was a reduction in both the number of mounts and intromissions preceding an ejaculation. This was seen in 10 out of the 11 "medial" animals but only very weakly in 1 out of the 7 LPO-MFB animals. The effect in its most extreme form is illustrated by number 17 which in one session ejaculated after only 2 intromissions, 42 sec. after introduction of the female ($C\bar{X} = 15.0$ intromissions, $S\bar{X} = 3.2$).

These changes have not been described with posterior hypothalamic stimulation (Caggiula, 1970; Caggiula & Hoebel, 1966). However, they did appear in the Vaughan and Fisher (1962) study and with MPO stimulation in the recent work of van Dis and Larsson (1971). A study using control measures of each animal's behavior is needed to demonstrate whether or not this occurs with posterior hypothalamic stimulation. The present data point out that, in looking for this effect, all animals should be tested, not only those showing obvious stimulation-bound copulation in preliminary tests. Stimulation which reduces the number of mounts and intromissions necessary for ejaculation often does not produce short-latency approach and mounting of the female.

Mounts, intromissions and ejaculations seen on stimulation test days never differed in appearance from those seen on control days for any animal. Also, sperm plugs were often seen in the female after stimulation-facilitated ejaculations, indicating that the behavioral indices of ejaculation were accompanied by emissions. In tests with subject number 12, the mean refractory period after control session ejaculations was found to be identical to that following stimulation-facilitated ejaculations ($\overline{X}$ = 4.7 min.) when stimulation was terminated immediately after ejaculation on two S tests. This suggests that the time course of recovery of sexual arousal is also normal after a stimulation-facilitated ejaculation.

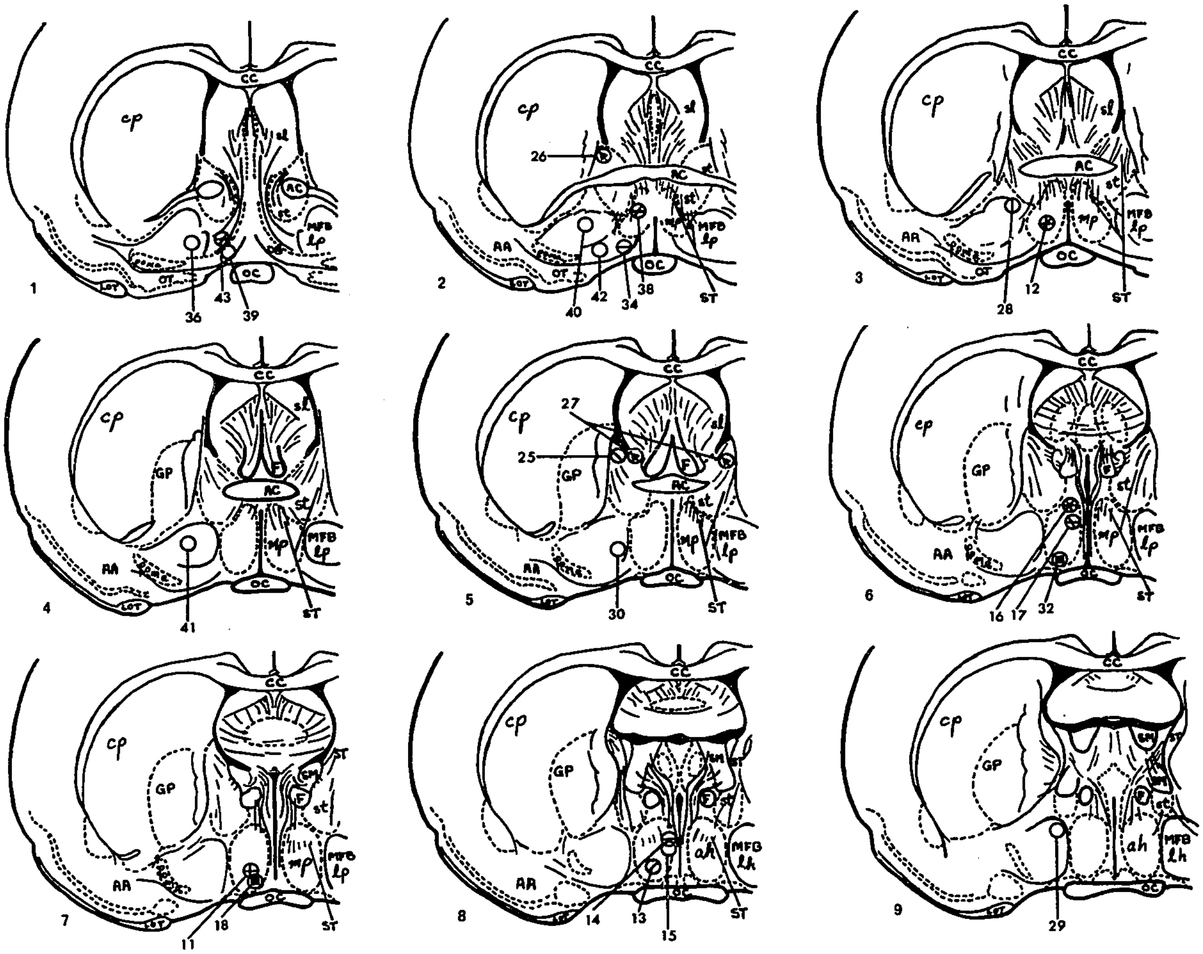
A reduction in the duration of the ejaculatory series due to stimulation can be attributed largely to the reduction in number of intromissions necessary for ejaculation and not to an increase in copulatory frequency. With fewer intromissions necessary, time to the ejaculation was reduced.

This was not always the case however, especially for number 43. Although this animal took many less mounts and intromissions to achieve an ejaculation, it required about the same amount of time as on control days. This was because approach to the female was actually inhibited during stimulation while the animal would rear and generally explore the box. It mounted the female a total of 4 times during stimulation in all 4 stimulation test sessions, yet its number of mounts and intromissions to ejaculation was significantly reduced. As most mounts occurred during off intervals between stimulations, this suggests that the effect of facilitating ejaculation may have outlasted the stimulation itself.

The short-latency approach and mounting of the female in response to stimulation, seen even in the first minute of the post ejaculatory interval (Caggiula, 1970; van Dis & Larsson, 1971; Vaughan & Fisher, 1962) was also seen very dramatically in 2 animals, numbers 12 and 38. Sample records from numbers 11, 12, 28, and 38 are shown in Fig. 2. The lack of grooming behavior after an intromission and the constant penile erections during stimulation observed in the Vaughan and Fisher study were not seen. Partial penile erections were sometimes observed, but these were more often seen at stimulation offset.

On the basis of percent mounts during stimulation, numbers 11, 12, and 28 were classified as stimulation-bound copulators. However, numbers 11 and 28 showed neither a consistently short-latency approach and mounting of the female in response to stimulation, nor a significant reduction in the duration of the refractory period. In contrast, subjects 12 and 38 displayed intense facilitation of copulatory behavior and would immediately approach and mount the female. Their refractory periods were also reduced to a mean of 1.0 and 0.7 min. respectively. Despite this facilitation, number 38 was not classified as a stimulation-bound copulator, because of the particular test procedure used and the small number of intromissions necessary for ejaculation on S test days. The electrodes in these two animals were located in the same region of the anterior portion of the medial preoptic area, very close to the area in which van Dis and Larsson (1971) obtained their positive effects.

Fig. 2 Copulation under the control of preoptic area stimulation. Sample records from stimulation test days are shown for numbers 12, 38, 11 and 28. The arrow at the start of number 38's record denotes the introduction of the female at the beginning of the session. The entire ejaculatory series, including refractory period, is shown for this animal. For the others, only the last few minutes of the ejaculatory series are shown. Each stimulation off or on period lasts 30 sec.



○ = ELECTRODE TIP LOCUS ↖ = WET-DOG SHAKING ╲ = MOTOR SEIZURE ● = ESCAPE

— = INTROMISSIONS TO EJACULATION REDUCED / = REFRACTORY PERIOD SHORTENED | = STIMULATION — BOUND COPULATION

Experiment 2

Does Stimulation Which Facilitates Copulatory Activity Have Inhibitory Aftereffects?

In the studies of Vaughan and Fisher (1962), Caggiula and Hoebel (1966), Caggiula (1970) and in several animals of this study there was a remarkable lack of mounting during interstimulation intervals. It is worth pointing out that in previous studies this stimulation-bound nature of the behavior was one of the most important criteria for identifying effective electrodes.

The rapid appearance of mounting at stimulation onset and the abrupt cessation of interest in the female at stimulation offset suggested the presence of a poststimulation inhibitory process to Caggiula (1970). He noted that when stimulation ended, males would often reorient away from the female even if in the process of mounting. This was also seen quite dramatically in animal number 12 of the present study. Caggiula also noted that termination of stimulation was often followed by some exploratory activity, much grooming, and occasional eating, thus indicating an inhibition of copulation but not of other behaviors.

What is the mechanism determining this dramatic lack of mounting during stimulation-off periods? It seemed possible that some aspect of the performance of the response could have negative feedback properties and thus selectively inhibit mounting when the artificial facilitation was removed. This possibility was investigated with respect to stimulation-bound eating and drinking by Cox, Kakolewski and Valenstein (1969). Poststimulation inhibition of consummatory behavior had already been observed by Valenstein and his colleagues (Valenstein, Cox & Kakolewski, 1969). They noted instances of animals displaying stimulation-bound eating, often to the point of choking, that seldom drank during the interstimulation interval. Stimulation-bound eaters were also seen pushing food away during the off periods. In order to decide if some aspect of the response itself produced the behavioral inhibition, Cox _et_ _al_. conducted an experiment in which the possibility of making consummatory responses was eliminated during stimulation. Animals deprived of food or water or both were presented with food, water or both only during the interstimulation interval. Under these conditions poststimulation inhibition was still seen in 9 of the 11 animals who had exhibited stimulation-bound eating or drinking. Thus it seems that some aftereffect of the electrical stimulation alone was sufficient to inhibit eating or drinking in the case of electrodes capable of eliciting those behaviors. I decided to see if the same conclusion would hold for electrodes facilitating copulation in the present study.

Method

Four subjects, numbers 12, 28, 29, and 38 were used in this test of poststimulation inhibition. Stimulation elicited short-latency mounting and greatly reduced refractory periods in animals 12 and 38, and while number 28 remained unaffected on these measures, he was included because 94 percent of his mounts occurred during stimulation. Although no dramatic change in his copulatory behavior was seen during Experiment 1, number 29 was included because it appeared as if there were a general inhibition of activity in the interstimulation intervals.

Experiment 2 was not begun until these animals had completed Experiment 1. Procedure was identical to that already described except that on both S and C test days the receptive female was placed in the box for 30 sec. and then removed for 30 sec. This cycle was repeated until the male had achieved one ejaculation and ended his refractory period. On S days the female was present only during the interstimulation intervals. There is reason to believe that putting in and taking out the female might facilitate male copulatory behavior in itself; thus it was essential to take measures of the male's behavior on C days using the same procedure. The same stimulus parameters used in Experiment 1 were used in these tests. Number 12 was tested every day with S days alternating with C days. Numbers 28, 29, and 38 were tested only every other day with S and C sessions also alternating.

Results and Discussion

The results of this experiment are shown in Table 2. The mean measures of copulatory activity used to calculate the percentage changes were based on two ejaculatory series under each condition for each animal with the exception of rat 38 which was tested three times under each. Number 12 completed an ejaculatory series in only one of the control tests. Testing was terminated during the second control session when it failed to ejaculate after 30 minutes. Data collected up to that point were used. Statistical analysis of the data was done in the same manner as in Experiment 1.

Perhaps the most interesting result in Table 2 is the reduction of mounts and intromissions preceding ejaculation for animals 12 and 38, despite the lack of opportunity to

TABLE 2

Post-Stimulation Inhibition?

Percentage Change in Copulatory Activity on Stimulation Days for Each Animal

Experiment 2

Rat No. and Tip Locus	Percent Mounts During S#	Duration of Ejac. Series	Refract. Period	Mounts only to 1st Ejac.	Intro.'s to 1st Ejac.	Mounting Frequency
12-M	84	-61.1	-13.0	-74.5	-42.2	+ 5.4
38-M	54	-42.1	+20.0	-53.5	-45.1*	+ 4.2
28-L	94	-30.8	+25.4*	-53.6	-36.0	-15.0
29-L	57	+42.9**	+12.2	-25.0	-15.4	-42.5

Data from Experiment 1

* $p \leq 0.05$; ** $p \leq 0.01$; One-tailed t tests.

mount females during stimulation. This is conclusive evidence that the facilitative effect of MPO stimulation on ejaculation outlasts the stimulation. These subjects displayed a similar number of intromissions before ejaculation on S test days in both Experiments 1 and 2, therefore it seems that MPO stimulation facilitates ejaculation regardless of whether intromissions occur during or after stimulation.

Table 2 also contains a measure of frequency of mounting, included because the duration of the ejaculatory series is confounded by stimulation effects on the number of intromissions preceding ejaculation. The frequency measure was calculated by taking the mean of the number of mounts (with and without intromission) per minute for each animal under each test condition. This measure was almost identical under S and C conditions for numbers 12 and 38, indicating that there was no post-stimulation inhibitory effect on mounting of the female for these two most effective MPO electrode placements. The lack of change in the refractory periods also supports this conclusion. Stimulation-bound copulatory behavior may sometimes result from a true facilitation of approach to the female during stimulation rather than a post-stimulation inhibitory mechanism as proposed by Cox _et al_.

(1969) for MFB electrodes producing stimulation-bound eating and drinking.

For numbers 28 and 29, with LPO-MFB electrode placements, there is some support for the idea of a post-stimulation inhibitory effect on mounting. The refractory period of number 28 was lengthened by the stimulation, and the duration of the ejaculatory series was prolonged in number 29 as a result of a depression in the frequency of mounting on S test days. Perhaps the high degree of stimulation-bound behavior seen in number 28 was partly due to an inhibition of mounting during stimulation-off periods rather than a dramatic facilitation during on periods. Thus a high percentage of mounts during stimulation might result from activation of different mechanisms at different electrode placements.

Experiment 3

Self-Stimulation

Glickman and Schiff (1967) have pointed out that species-specific approach behavior is almost always correlated with self-stimulation (SS) behavior. That is, an electrode that will elicit feeding, drinking or copulatory behavior with long durations of stimulation (20 sec. to several minutes) will also elicit self-stimulation behavior using the customary short trains (½ sec. or less). Their theory states that this is really the essence of SS, that reinforcement is synonymous with the activation of neural circuits which facilitate species-specific approach behaviors.

Caggiula (1970) reported that all of his posterior hypothalamic animals which showed stimulation-bound copulation using 3 min. stimulation trains also learned to self-stimulate at comparable current levels using 0.5 sec. trains. Madlafousek _et_ _al_. (1970) also found that all of their LPO animals learned to self-stimulate in a situation where they could regulate the duration of stimulation themselves. However, Vaughan and Fisher (1962), who used 5 minute stimulation trains to produce stimulation-bound copulation from the LPO area, mention that their animals appeared to be in an "unusually intense" drive state which they believed may have included aversive components.

Robinson and Mishkin (1966) found that MPO stimulation in an M. mulatta monkey which produced penile erection and projectile ejaculation yielded mixed motivational effects. The animal would self-stimulate with a 2-sec. train at low rates, but in bursts of pressing (8-10 presses) separated by rests of 2 to 5 min. Using this temporal pattern the animal would produce his own ejaculation. In an escape test, using 15-sec. trains of stimulation, he rapidly learned to escape with an average latency of 2.3 seconds. Thus, although experimenter-applied 15-sec. trains were quite effective for eliciting ejaculation, the monkey would escape from stimulation after only a few seconds if given the opportunity.

To provide a simple test of whether the stimulation used in Experiment 1 was rewarding in itself, the same stimulus parameters were used in tests for SS behavior. This procedure provides information directly relevant to the Glickman and Schiff theory.

Method

All subjects completing Experiment 1 were tested for SS with the exception of rats 16 and 17 who began exhibiting motor seizures. SS testing began as soon as each subject completed Experiment 1 except for rats 12, 28, 29, and 38 which were tested for post-stimulation inhibitory effects (Expt. 2) before SS. Testing was conducted in an identical box to that used in Experiments 1 and 2 except that a 2½ x 1½ in. plexiglass lever was mounted in the center of the short side (9½ in.) of the box 1½ in. above a plexiglass floor which replaced the mesh floor used previously. A depression of the lever delivered a 30-sec. train of square-wave stimulation identical to that used for each animal in Experiments 1 and 2. The only exception to the procedure of testing with identical currents was rat 38 whose current was raised 20μA for SS testing. (It was found that for rat 38, the current level used in Experiment 1 had become less effective for facilitating mounting after the procedure of Experiment 2. Raising the current produced a behavioral effect closer to that seen before, but still did not facilitate mounting as much as in Experiment 1.) If an animal pressed the lever again during an already initiated 30-sec. train, it had no effect on the stimulation and was not recorded. Only the number of stimulation trains successfully initiated was counted as the daily SS score. Each animal was tested in one 30 min. session each day for 5 consecutive days. No shaping of the lever-press response was required because of the duration of the sessions and the animals' familiarity with the box.

Results

The first two days in the 5-day series of tests were not considered in data analysis because it was assumed that the animals were learning to lever-press during these sessions. The individual animal's final SS score was taken as the median score of the last three SS sessions. With stimulation trains lasting 30 sec., the maximum rate of train delivery is two per min., making a possible total of 60 for each 30 min. session. Thus the maximum possible median SS score was also 60. The median SS score for each animal is presented in Table 1. Looking at the table, it can be seen that most animals in both the medial and lateral groups would lever-press for the same stimulus parameters as used in Experiment 1.

Behavioral Observations During SS

Contrary to the findings of Caggiula (1970) and Herberg (1963) self-stimulation was never accompanied by ejaculation of sperm plugs. Perhaps rapid repetition of short trains of stimulation is necessary to produce this phenomenon. Self-stimulation in the present study was accompanied by some dribbling from the penis and also "partial erections" in several animals. A "partial erection" consisted of the penis protruding slightly but quite noticeably, from its sheath. Partial erections and penile twitches associated with "marking" of the SS lever were seen in 4 out of the 7 LPO animals and in 1 of the 9 MPO subjects. Marking of the lever most consistently occurred at the initiation of a stimulation train, but was also seen during trains. The animal would walk over the lever, lifting one of its hind legs and bringing the penis in contact with the lever. During this stereotyped pattern a clear fluid was left on the lever. A similar pattern of behavior (in the absence of brain stimulation) has recently been described by Pottier and Baran (1971). Partial erections and penile twitching without marking of the lever, but associated with dribbling of a whiter, more viscous fluid, were seen in 4 out of the 9 medial animals. This occurred most often during stimulation-off periods. Genital grooming was rarely associated with these emissions. Thus it seems that partial erections with marking of the lever were more associated with LPO stimulation, while partial erections with dribbling of possibly seminal fluid were associated with MPO stimulation.

LPO animals generally showed more locomotor activity than MPO animals in response to stimulation. Rat 28, the only lateral subject that performed a very high percentage of his mounts during stimulation, also had the highest SS rate of any animal in the study. This animal displayed a very consistent carrying behavior completely tied to the stimulation. After the initiation of each lever press, it began searching the box until it found a bolus to carry in its jaws. Sometimes it would manage to pick up two or three during a 30 sec. train and carry them until the stimulation offset when it would immediately drop them, approach the lever, and initiate the cycle again. Purina lab chow pellets were placed in the box after formal SS testing was completed, and the same carrying behavior was displayed with the pellets. Chewing of boli or pellets was never seen during or after stimulation. The most consistent behavior seen in rats 12 and 38, the two most effective MPO animals, was an increase in face grooming in response to stimulation.

Data Analysis

The MFB courses through the LPO but not the MPO. This fiber bundle has often been associated with high rates of

SS. To see if there was any difference between the lateral and medial groups on SS rate, a Mann-Whitney U test was performed on the scores. No significant difference was found, ($U=17$, $p > .10$, two-tailed) however, current levels were not equated in the two groups.

If the Glickman and Schiff hypothesis that positive reinforcement is synonymous with the activation of neural circuits facilitating species-specific approach behaviors is correct, stimulation at the same current levels and duration which facilitates pursuit and mounting of the female should elicit SS behavior. This is seen to be the case in Table 1. According to the same hypothesis, assuming the areas stimulated to be specifically involved in the mediation of the copulatory approach pattern rather than other approach behaviors such as eating and drinking, we would expect there to be a positive correlation between SS rate and degree of stimulation-bound copulation. In order to test this, the animals in both the medial and lateral groups were pooled and ranked in terms of percent mounts during stimulation and separately in terms of median SS score. A Spearman rank correlation coefficient was then calculated, and a significant positive correlation was obtained, ($r_s = .426$, $p < .05$, one-tailed). The same correlation coefficient calculated for the medial group alone showed a greater degree of correlation between mounting and SS rate, ($r_s = .700$, $p < .05$, one-tailed). There was no significant correlation for the lateral group alone, ($r_s = .357$, $p > .05$, one-tailed).

In order to see if there is a correlation between facilitation of ejaculation and SS behavior, the animals in the medial group were ranked separately with regard to percentage reduction of intromissions to ejaculation and SS score. A Spearman rank correlation coefficient was calculated, and a significant positive correlation was obtained ($r_s = .667$, $p < .05$, one-tailed). There was no significant correlation between these measures for the LPO animals ($r_s = .152$, $p > .05$, one-tailed).

Discussion

Experiment 3 showed that most animals in both medial and lateral preoptic groups would learn to self-stimulate using the same stimulation parameters as were used for copulatory behavior testing in Experiment 1. In fact, a positive correlation was found between the percentage mounts during stimulation and SS rate, which was significant for the medial preoptic group. This correlation supports the Glickman and Schiff hypothesis at least with regard to one particular hypothalamic area and one specific approach pattern. A significant positive correlation was also found between percentage reduction of intromissions to ejaculation and SS rate for the medial but not the lateral group. Thus SS rate, stimulation-bound copulation, and degree of facilitation of ejaculation are positively correlated in MPO, but not LPO animals. The lack of correlation in the case of LPO animals may be due to variable degrees of facilitation of other motivated behaviors by the stimulation.

Experiment 4

Effects of Preoptic Area and Peripheral Stimulation in Sexually Inactive Male Rats

Large individual differences occur in degree of sexual responsiveness of male laboratory rats. In any group there are a certain number of healthy males who persistently fail to mate when placed with receptive females (Whalen, Beach & Kuehn, 1961). Various hypotheses have been advanced to account for the lack of responsiveness in these animals.

It is well known that testosterone is necessary for the maintenance of male rat mating behavior, and it has been proposed that a direct relationship exists between copulatory performance and androgen level. Although some evidence exists in favor of this viewpoint (Beach & Holz-Tucker, 1949; Beach & Fowler, 1959), non-hormonal factors seem to play a more important role in accounting for individual variations in sexual responsiveness (Larsson, 1966; Whalen, *et al.*, 1961). If the lack of responsiveness in males which persistently fail to mate is not directly related to testosterone level, perhaps it is due to a deficit in the central nervous system mechanisms which are normally influenced by hormone level in the expression of male copulatory behavior. Whalen, *et al.* (1961) have suggested that persistent non-copulators may be less "sensitive" to the hormone. "It is conceivable that central nervous mechanisms which are normally influenced by androgen are refractory in the non-copulating male."

Another line of evidence suggests that a lack of sufficient arousal in response to introduction of the female may underlie the behavioral deficit in non-copulators. Pottier and Baran (1971) have found that non-copulators display a general behavioral syndrome characterized by relative inactivity when compared to copulators in a variety of test situations. These males seem to be deficient in their responses to normally arousing stimuli. Experimental manipulations which might be expected to increase the general level of arousal have been successful in facilitating copulatory performance in active males (Barfield & Sachs, 1968; Hård & Larsson, 1969; Larsson, 1957, 1963), and Caggiula and Eibergen (1969) have recently shown that peripheral stimulation in the form of tail-shock is capable of eliciting copulatory responses from "non-copulators."

However, the studies of Whalen <u>et</u> <u>al</u>. (1961) and Caggiula and Eibergen (1969) can be criticized for their definition of "non-copulators." In both studies lack of copulation with a receptive female in 5 15-min. sessions classified males as "non-copulators." In this laboratory it has been observed that males which do not copulate after 5 or 6 half-hour test sessions may become quite vigorous and regular copulators if testing continues. This tendency for the cumulative percentage of copulators to increase over repeated testing is apparent in the Whalen _et al_. study in which 4 of the 5 untreated "non-copulators" initiated sexual responding during sessions 6 through 8.

The purpose of the present experiment was to determine if electrical stimulation of the MPO, previously shown to be effective in facilitating copulatory behavior in sexually active males (Expt. 1), would elicit sexual responses in persistent non-copulators. In order to be more certain of a persistent lack of copulatory activity, males were tested in 20 half-hour test sessions over a period of several months prior to electrode implantation.

Method

Selection of non-copulators. Five sexually inactive, male hooded rats were selected out of a group of 60 animals. Subjects were tested for copulatory activity, as described in the Method section of Experiment 1, in 20 half-hour sessions, most of which occurred at 2-day intervals. Unlike the procedure used in Expt.'s 1 - 3, these animals were kept on a modified light-dark cycle with the lights off from noon to midnight. Testing was conducted from approximately four to eight P.M. The five males selected (less than 10% of the larger group) were the only ones that showed no copulatory responding during any of the 20 test sessions. After selection, these males were housed together in groups of 2 and 3 in large plastic cages.

MPO stimulation. Several weeks after the last selection test, bilateral, monopolar electrodes were implanted in each male aimed at the MPO as described in the Method section of Expt. 1. Subjects weighed 405-520 gms. at the time of surgery. Starting one week post-operation 3 more half-hour sessions were conducted without stimulation. All post-operative testing was conducted at two-day intervals. On the first session one animal (number 3) made one mount, but did not initiate copulatory responding in the following 2 tests. The animals were then screened for effects of MPO stimulation on copulatory activity in the presence of a receptive female in the manner previously described in the Method section of Expt. 1. Both electrodes were tested in each animal in two separate sessions to determine suitable current levels. Copulatory activity was not seen in response to stimulation of any of the 10 electrodes. In order to see if sexual responding might develop over repeated stimulation tests, at least 8 15-min. sessions were conducted with each animal. A "high" and "low" current value for each electrode were chosen on the basis of the screening tests, and two tests were conducted at each current level for each electrode placement. Only one current value and one electrode placement were tested in each session using the 30 sec. on, 30 sec. off pattern of stimulation previously described. None of the 5 males initiated copulatory responding during these stimulation tests.

Tail-pinch tests. I then decided to see if tail-pinch (TP) would elicit copulatory activity in these animals. Testing was carried out in the standard manner except that if no mounts occurred by 15 min., tail-pinching was begun. A single TP was applied using a large toothed forceps once every 60 sec. until the first intromission occurred or to a maximum of 10 pinches. Pinches were usually strong enough to evoke squealing and jumping. A total of 5 TP sessions were conducted for each animal.

At the end of the TP sessions, all animals were given an additional "screening" session in order to see if previously ineffective MPO stimulation would now facilitate copulatory activity. Both electrodes in each animal were tested, and a single optimally effective electrode and current level was selected for each of the 3 animals which had initiated copulatory activity as a result of TP. These 3 animals were then given S and C tests to assess stimulation effectiveness in the manner described in Expt. 1, followed by tests of possible post-stimulation inhibitory effects as described in Expt. 2.

Further efforts to elicit copulation. The 2 animals which had not begun copulating during the TP tests were then given a variety of treatments in an effort to elicit copulation. Amphetamine has been effective in facilitating copulatory performance at low to moderate doses, from 0.5 to 2.0 mg/kg, (Bignami, 1966; Leavitt, 1969; Soulairac, 1963). A cursory examination of the effects of D-amphetamine sulfate was therefore made at 1 and 2 mg/kg doses. Intraperitoneal injections were given 15 min. prior to testing. One test was given to each animal at each dose level. The 2 mg/kg dose was given 2 days after the stimulation screening session following the last TP test. The 1 mg/kg dose was given 10 days later. In the intervening period 3 15-min. sessions were conducted in which the receptive females were changed after the first 10 min. of the session. None of these attempts at arousing the males were successful in eliciting copulatory activity. In fact, the amphetamine injections seemed to decrease interest in the females, producing mainly rearing throughout the 15 min. sessions.

Following the method of Barfield and Sachs (1968) safety pins were then placed under the skin of the back dorsal to the flanks and approximately 4 cm. apart in order to see if back-shock would prove more effective than TP for eliciting copulation from these 2 animals. This method has been effective in facilitating certain aspects of performance in sexually active males in this laboratory (Malsbury & Pajurkova, in preparation). Five days after safety pin implantation each animal was tested with a receptive female. When no mounting occurred by 10 min., 0.5 sec., 60 Hz A.C. shocks were delivered approximately once every min. for another 10 min. The shock level was adjusted between 0.9 and 4.4 MA with a 200 K ohm resistor in series with the animals. Shock was found effective in eliciting squealing and jumping but not copulation.

Two tests for each animal were then conducted to see if the presence of an active male would elicit copulation. This method has succeeded in facilitating copulatory performance in sluggish males (Larsson, 1957). Each non-copulator was presented with a receptive female for 10 min. and then an active male was dropped into the observation box. Testing was continued for 10 more min. in which time the active males achieved at least one ejaculation. These tests were also ineffective in eliciting copulation.

Testosterone propionate injections were then begun. Two mg. of the hormone suspended in sesame oil were injected subcutaneously each day throughout the series of tests. This was a dose of approximately 4 mg/kg body weight. Testing was begun after the 2nd injection. Five tests were conducted on alternate days in the standard manner for each male. No copulatory responses were seen.

Results and Discussion

TP tests. Sexual responses were not elicited from the 5 persistent non-copulators during the repeated MPO stimulation tests. However, the subsequent TP tests were effective in eliciting copulatory activity from 3 of the 5. These results confirm and extend the observations of Caggiula and Eibergen (1969) that peripheral stimulation is often capable of eliciting copulatory behavior from previously inactive males. On the basis of the TP tests I have concluded that the primary deficit in many non-copulators is a lack of sufficient arousal in response to introduction of the receptive female. Several aspects of the present data support such a conclusion.

During sessions in which TP was used to elicit the first intromission, subjects would continue copulating to ejaculation without further stimulation. This occurred in 5 out of 6 instances. Thus the deficiency seems to lie in the mechanism which produces the initial intromission response, the "sexual arousal" mechanism of Beach and Jordan (1956), and not in the copulatory-ejaculatory performance mechanism.

Once an animal had achieved intromission in the first 15 min. of a test session without TP, it continued this behavior on every subsequent test session including the MPO stimulation and control series. The development of this behavior can be seen in Table 3. These results indicate that adequate arousal in response to the stimulus female alone can be conditioned by previous trials in which copulation

TABLE 3

Development of Copulatory Behavior in Sexually Inactive Males During Tail-Pinch Tests.

Latency to Ejaculate is Given
to the Nearest Second for Each Rat.

Rat No.	Session 1	2	3	4	5
1	X*	34:07*	33:36*	17:13	16:41
2	62:06*	42:49*	42:17	30:14	29:04
3	X*	31:04*	I*	8:05	43:05
4	X*	X*	X*	X*	X*
5	X*	X*	X*	X*	X*

* Tail-pinch was used. I Intromission, but no ejaculation.

X No intromission.

has been elicited by artificial stimulation (i.e. TP).

It should be pointed out that the types of arousing stimulation employed in this study are not capable of eliciting copulation in all sexually inactive males. Caggiula and Eibergen (1969) also failed to elicit copulation in 2 of their 6 non-copulators using tail-shock. In the present study 2 of the 5 persistent non-copulators failed to initiate a single mount in 49 separate opportunities in which a variety of treatments were employed. It remains to be seen whether the difference between animals which can and cannot be converted into copulators by arousing stimulation is one of degree or of type.

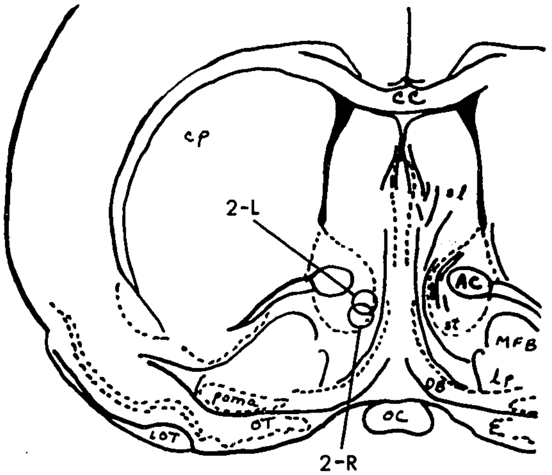
MPO stimulation. The bilateral electrode loci for all five persistent non-copulators are seen in Fig. 3. All electrodes are in the general area of the MPO. Those of number 2 are slightly anterior to the MPO as diagrammed in Konig and Klippel (1963) and are located amongst fibers of the stria terminalis as they continue caudally entering the MPO from the ventromedial edge of the bed nucleus of the stria terminalis. Electrode locus 2-R was effective for facilitating sexual arousal, and this suggests that the stria terminalis may play an excitatory role in controlling the activity of MPO cells.

The nature of the facilitating effects of MPO stimulation is further elucidated by the series of S and C tests conducted on the three animals which started copulating as a result of TP. The results are presented in Table 4. The mean measures of copulatory activity used to calculate the percentage changes were based on four ejaculatory series under each condition for each animal. Statistical analysis was done in the same manner as in Expt.'s 1 and 2. One of the males, number 2, was a stimulation-bound copulator as defined by the criterion of a mean of at least 70% mounts occurring during stimulation. Another male, number 3, came close to this criterion with a mean of 68% and on one particular S session had 94% of his mounts during stimulation. The number of intromissions preceding ejaculation was reduced in two of the animals. The refractory period was also reduced in number 2.

Tests for post-stimulation inhibition of copulatory behavior were conducted for numbers 2 and 3, as these animals showed high percentages of their mounts during stimulation. Four tests were conducted under each condition (S and C) for each animal as described in Expt. 2. A dramatic inhibition of interest in the female was seen in number 3 as an aftereffect of stimulation. This animal completed an ejaculatory series according to the criteria described in Expt. 1 on only one of the four S test sessions but on all four C sessions. During the one S session where ejaculation did occur, the postejaculatory refractory period was greater than 26 min., at which time testing was stopped. In contrast, its longest refractory period of the four C sessions was 7.0 minutes. Due to the failures in completing ejaculatory series in this animal the percentage changes in copulatory activity were not calculated. The behavior of number 3 during post-stimulation inhibition testing and the data presented for the standard tests in Table 4 lead me to conclude that stimulation in this male was truly facilitatory only in the sense of reducing the number of intromissions to ejaculation. The high percentage (68%) of mounts during stimulation can be explained by a depression of mounting during the off periods rather than a facilitation during the on periods.

The percentage changes in copulatory activity as a result of stimulation during the post-stimulation inhibition tests are presented in Table 5 for number 2. No evidence

Fig. 3. Locations of the bilaterally placed electrodes in the five non-copulators of Expt. 4. L or R denote the placement as being in either the left or right hemisphere. Sections are redrawn from König and Klippel, (1963). For abbreviations see Fig. 1.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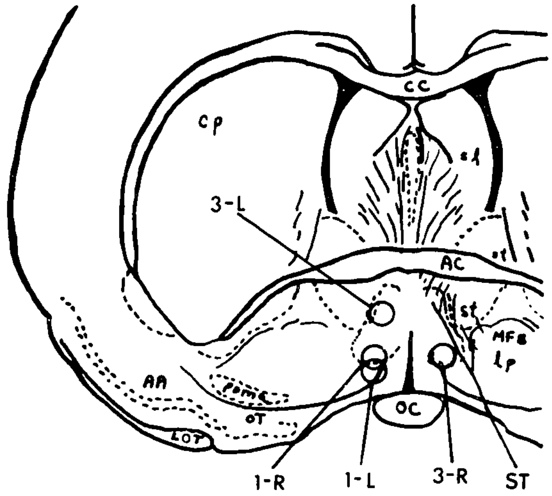
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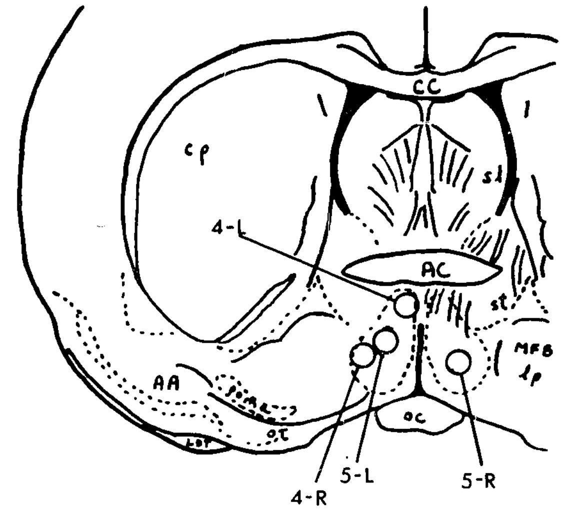
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5-R

TABLE 4

Percentage Change in Copulatory Activity on Stimulation Days for Active Animals of Experiment 4

Rat No.	Current (in μA)	Duration of Ejac. Series	Refract. Period	Mounts only to 1st Ejac.	Intro.'s to 1st Ejac.	Percent Mounts During S
1	L-80	-36.9*	- 7.2	-52.0**	-46.4**	34
2	R-90	-29.6	-25.5	-23.5	+ 6.1	77
3	L-90	+56.3	+86.8	+45.4	-37.4**	68

* $p \leq 0.05$; ** $p \leq 0.01$; One-tailed t tests.

TABLE 5

Post-Stimulation Inhibition Tests

Percentage Change in Copulatory Activity on Stimulation Days

Experiment 4

Rat No.	Current (in μA)	Duration of Ejac. Series	Refract. Period	Mounts only to 1st Ejac.	Intro.'s to 1st Ejac.	Mounting Frequency
2	R-90	-21.2	-19.1*	-38.9	-16.7	-4.0

* $p \leq 0.05$; ** $p \leq 0.01$; One-tailed t tests.

of inhibition is seen for number 2, thus his high mean percentage of mounts during stimulation (77%) seen in the standard tests cannot be attributed to an inhibitory aftereffect of the stimulation. Thus the data from numbers 2 and 3 in this Expt. support the conclusion made in Expt. 2 that stimulation-bound behavior may result from activation of different mechanisms at different electrode placements.

Although more data are needed to clarify the issue, the fact that stimulation which was capable of facilitating copulatory performance in number 2 would not elicit this behavior before the TP tests may contribute to the understanding of the effects of hypothalamic stimulation on consummatory behavior in general. Several other recent experiments demonstrate that the effect of hypothalamic stimulation is clearly influenced by the non-stimulation level of performance shown by the individual animals in the test situation (Valenstein, 1969).

Vergnes and Karli (1969) have demonstrated that stimulation of the posterior lateral hypothalamus in "non-killer" rats fails to elicit mouse-killing. However, stimulation of the same area consistently facilitates the response in rats which will kill mice spontaneously in the test situation.

Similarly, Panksepp (1971) found that stimulation-bound quiet-biting attack was more easily obtained from rats that exhibited spontaneous mouse-killing than from "non-killers." Panksepp concluded that, "The ease of obtaining quiet-biting attack by stimulation of the hypothalamus thus seems to interact with the endogenous disposition of rats to kill mice" (p. 321).

Madlafousek _et_ _al_. (1970) have data to support this conclusion for copulatory behavior as well. Males were divided into a group of vigorous copulators and a group of "seeming non-copulators," animals which would not copulate unless presented with maximally receptive females. Lateral preoptic stimulation produced less clear facilitation and more clear inhibition in the "non-copulators."

Studies such as the above cannot avoid the possibility that electrode placements are not equivalent in the two groups being compared. The present experiment overcomes this difficulty by demonstrating that ineffective stimulation can become facilitatory by changing the disposition of the subjects in the test situation. Unlike the "switching" experiments of Valenstein and his colleagues (Valenstein _et_ _al_., 1969) this change in stimulation effectiveness comes about as a result of training sessions which do not involve hypothalamic stimulation. These results clearly confirm the idea that the "natural" tendencies of the subjects in the particular test situation must be taken into account when assessing the effects of hypothalamic stimulation. It seems that it is much easier to facilitate species-typical behaviors in individual animals already displaying them than it is to elicit them for the first time.

GENERAL DISCUSSION

Beach and Jordan (1956) proposed that two at least partially independent neurophysiological mechanisms are involved in male rat sexual behavior: an arousal mechanism which mediates the increase of sexual excitement leading to copulation, and a copulatory-ejaculatory mechanism which mediates the behavioral components of mounting, intromitting and ejaculation. The state of the arousal mechanism was held to be indicated by the latency to the first intromission after the female is introduced, and by the refractory period after ejaculation. The state of the copulatory mechanism was held to be indicated by the number of intromissions necessary to achieve ejaculation and by the duration of the ejaculatory series. This theoretical framework has since been elaborated and modified by Beach and his co-workers (Beach, 1956; Beach & Whalen, 1959). Other authors have pointed out its weaknesses and suggested their own revisions of the same theme (Cherney & Bermant, 1970; McGill, 1965). Certain aspects of the present data support the idea of separate neural mechanisms for the initiation of copulatory behavior (arousal mechanism) and ejaculation (ejaculatory mechanism) and indicate that these two mechanisms show some degree of separation at the hypothalamic level.

Roberts _et_ _al_. (1967) found that the MPO was the only one of a great number of hypothalamic regions tested in which electrical stimulation produced penile erection in the opossum. MacLean and Ploog (1962) have called the medial septo-preoptic region one of the "nodal points" in a brain circuit involved in producing penile erection in the squirrel monkey, and this is also the only area from which a normal projectile ejaculation preceded by erection has been elicited in the monkey (Robinson & Mishkin, 1966). It seems that in several species MPO activity is involved in facilitation of genital reflexes in the male.

MPO stimulation in the present study was found to greatly reduce the number of intromissions to ejaculation and in some cases, in the absence of a female, produced partial penile erections and dribbling from the penis. The release of genital fluid containing sperm, not accompanied by erection, has been associated with electrical stimulation of the MPO as well as many other areas of the brain in restrained male rats (van Dis & Larsson, 1970). However, the relation of seminal fluid release seen in a restrained male rat to the mating behavior of the animal is obscure, as sperm have also been found in fluid released by the male rat during the "marking" of inanimate objects (Pottier & Baran, 1971). The methods used in the present study were necessary to demonstrate the facilitation of the ejaculatory response by MPO stimulation during actual copulatory behavior.

The present study along with that of Roberts et al. (1967) and van Dis and Larsson (1971) demonstrate that sexual arousal may also be facilitated by MPO stimulation. Such stimulation produced short-latency approach and mounting and greatly reduced refractory periods in two animals of the present study as well as dramatically reducing the number of intromissions to ejaculation. However, there were several medial animals which showed a significant facilitation of the ejaculatory mechanism without showing obvious signs of increased sexual arousal during stimulation (See Table 1). Thus in some cases MPO stimulation is able to facilitate the ejaculatory mechanism independently of the sexual arousal mechanism.

Experiment 2 has shown that facilitation of the ejaculatory mechanism outlasts the stimulation itself and is seen regardless of whether the animal intromits during or after stimulation. This suggests some kind of long-term summation of neural excitability finally resulting in ejaculation. Facilitation of sexual arousal seems to be tied much more closely to stimulation. There was no indication of a change in mounting frequency or refractory period duration as an aftereffect of stimulation in the two MPO animals of Expt. 2, once again indicating the presence of separate neural mechanisms for ejaculation and sexual arousal.

The exploration of both the LPO-MFB and MPO regions in the present study has also helped clear the confusion over the locus of facilitatory stimulation points in the far anterior hypothalamus (see Introduction). Although these two areas are intimately related anatomically (Matano, et al. 1969; Millhouse, 1969), functional differences can be demonstrated using the techniques of lesioning or electrical stimulation. In agreement with work done on the opossum (Roberts et al., 1967) MPO, but not LPO, stimulation was found to facilitate various aspects of male copulatory behavior.

Although bilateral LPO-MFB lesions have had relatively little effect in comparison with MPO lesions (Giantonio et al., 1970; Heimer and Larsson, 1966/67), more posterior MFB lesions, including the parafornical region, have been shown to signifi-

summation of neural excitability finally resulting in ejaculation. Facilitation of sexual arousal seems to be tied much more closely to stimulation. There was no indication of a change in mounting frequency or refractory period duration as an aftereffect of stimulation in the two MPO animals of Expt. 2, once again indicating the presence of separate neural mechanisms for ejaculation and sexual arousal.

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Although bilateral LPO-MFB lesions have had relatively little effect in comparison with MPO lesions (Giantonio et al., 1970; Heimer and Larsson, 1966/67), more posterior MFB lesions, including the parafornical region, have been shown to significantly reduce male rat mating behavior (Hitt et al., 1971). The parafornical, medial MFB is the area where Caggiula (1970) most consistently obtained positive stimulation effects. Mating responses elicited by MPO stimulation in the male opossum can be abolished or attenuated by ipsilateral posterior MFB lesions (Bergquist, 1970). These studies suggest that most of the LPO-MFB does not contain fibers involved in male mating behavior; however the more posterior, medial aspect of the lateral hypothalamus-MFB does seem to be part of a necessary circuit, probably excited by MPO stimulation via medio-lateral hypothalamic connections (Paxinos & Bindra, in press). Activation of these descending pathways in the medial part of the MFB probably results in facilitation of brainstem and spinal cord systems where mounting, intromission and ejaculation are organized. From the evidence collected by Beach (1967), it seems that this eventual facilitation may occur through disinhibition at a lower brainstem level.

Activation of the MPO is probably greatly influenced by the presence of testosterone. The preoptic region, along with other hypothalamic and limbic areas, preferentially takes up radioactively-labeled testosterone into its cell bodies (Pfaff, 1968; Sar & Stumpf, 1971). The implantation studies of Davidson (1966) and Lisk (1967) point to a selective effect of testosterone on MPO neurons as far as facilitation of copulatory behavior is concerned. They found the most consistent reactivation of male copulatory behavior after castration resulted from implants in or near the MPO. Although little is known about specific inputs to this area, perhaps MPO neurons are activated by stimuli resulting from the introduction of a receptive female, the level of such activation being influenced by the presence of testosterone. Thus the MPO may be an integrating area where peripheral stimuli and hormonal levels interact to trigger the male copulatory pattern.

Activation of the MPO does not seem to be the only condition necessary for triggering this behavior. Repeated electrical stimulation of the area in persistent non-copulators does not elicit copulation (Expt. 4). However, once copulation had been elicited by the use of tail-pinch, stimulation in one of the animals was effective in facilitating measures of sexual arousal. I would like to suggest that activation of the MPO is a necessary but not sufficient condition for the initiation of male copulatory behavior. Evidently changes in the activity of other brain areas are also necessary for the initiation of this behavior and can be brought about by a sudden increase in somatic stimulation (Caggiula & Eibergen, 1969; Expt. 4).

The importance of olfactory, cortical and limbic forebrain areas in mediating the initial appetitive aspects of the pattern has been mentioned in the Introduction. Several lesion studies show that some degree of sexual arousal is still present even after MPO lesions have completely eliminated intromissions and ejaculation. Perhaps the important extra-hypothalamic areas are able to maintain arousal by exciting the MFB in the absence of the MPO. Giantonio _et_ _al_. (1970) report that postoperatively, males showed sniffing and pursuit behavior and even palpation of the female's sides, but never any pelvic thrusting. They state that the animals were aroused, but incapable of consummatory responding. Heimer and Larsson (1966/67) describe almost identical behavior for their lesioned males. Soulairac (1963) reported that in the first postoperative test, intromissions became rarer and were replaced by mounts and thrusts without penile intromission. Over 10 to 15 days all interest in the female disappeared. This successive disappearance of arousal may have based on learning that successful copulatory responses were no longer possible. These reports indicate that with loss of MPO function, males can still be behaviorally aroused by the introduction of the female, but this arousal no longer results in successful copulatory attempts.

The primary role of the MPO in male copulatory behavior seems to be that of participating in the control of genital responses including intromission and ejaculation. Stimulation of this area in sexually experienced males will sometimes dramatically facilitate appetitive aspects of the pattern as well (arousal mechanism). However, stimulation of the MPO in persistent non-copulators will not elicit copulation, and some degree of sexual arousal is present in experienced males after bilateral lesions of this area. This leads me to conclude that the facilitation of sexual arousal by electrical stimulation of the MPO may depend on prior conditioning whereby genital sensations and non-specific arousal become associated with the appetitive responses of pursuit and mounting during the development of copulatory behavior in the normal male. Once this has occurred, a sudden increase in genital sensations and non-specific arousal as a result of electrical stimulation of the MPO might initiate approach and mounting. Viewed in this way, the sensations produced by MPO stimulation in persistent non-copulators are meaningless until the full copulatory pattern can be established.

SUMMARY

The present study was prompted by somewhat contradictory findings concerning the function of the lateral preoptic-medial forebrain bundle region (LPO) and medial preoptic-anterior hypothalamic continuum (MPO) in male rat copulatory behavior. It employed electrical stimulation of these areas in an attempt to localize the neural elements involved more accurately and make a finer analysis of the behavioral changes produced by the stimulation than has previously been attempted.

A sensitive measure of the effects of stimulating a particular locus can be made by comparing the behavior of the same animal on repeated control and stimulation tests as was done in Expt. 1. All measures of copulatory activity taken were facilitated by medial, but not by lateral, preoptic stimulation. The most common change produced by MPO stimulation was a reduction in both the number of mounts and intromissions preceding ejaculation. Short-latency approach and mounting of the female and greatly reduced refractory periods were also seen in two MPO animals.

Expt. 2 was conducted to determine if the high degree of stimulation-bound mounting behavior seen in some animals

could be due to an inhibitory aftereffect of the stimulation alone. Repeated stimulation and control tests were given in which the female was present only during the interstimulation intervals. No evidence of a post-stimulation inhibition of copulatory behavior was seen for the animals with the two most effective MPO electrode placements. However evidence for a post-stimulation inhibitory effect on mounting was seen in other animals in this Expt. and in additional animals during Expt. 4. Thus a high percentage of mounts during stimulation may result from activation of different mechanisms at different electrode placements.

Reward properties of the electrodes were studied by the self-stimulation method in Expt. 3. Most animals in both the medial and lateral groups learned to self-stimulate (SS) using the same 30-sec. trains of stimulation as used in earlier tests of copulatory activity. SS rate, stimulation-bound copulation and degree of facilitation of ejaculation were positively correlated in MPO, but not in LPO animals. These results were discussed in terms of the Glickman and Schiff (1967) hypothesis concerning the nature of reinforcement.

The effect of MPO stimulation on the behavior of 5 persistent non-copulators was examined in Expt. 4. Repeated

MPO stimulation tests failed to elicit sexual responses in any of these animals. However, subsequent tests using strong peripheral stimulation (tail-pinch) were effective in eliciting copulatory activity from 3 of the 5. The previously ineffective MPO stimulation did produce a true facilitation of mounting behavior in one of these 3 following tail-pinch sessions. These data support the contention that hypothalamic stimulation is much more effective in facilitating species-typical behaviors in animals already displaying them than it is in eliciting them for the first time.

Beach and Jordan (1956) proposed that two separate mechanisms are involved in male rat sexual behavior: an arousal mechanism which mediates the increase of sexual excitement leading to copulation, and a copulatory-ejaculatory mechanism which mediates the behavioral components of mounting, intromitting and ejaculation. The fact that several MPO animals showed a significant facilitation of ejaculation without showing signs of increased sexual arousal during stimulation supports the idea of independent arousal and ejaculatory mechanisms and indicates that these may be separated at the hypothalamic level.

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