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Research Papers

MPOA and BNST lesions in male Syrian hamsters: differential effects on copulatory and chemoinvestigatory behaviors

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Electrolytic lesions were made in the medial preoptic area (MPOA) and bed nucleus of the stria terminalis (BNST) to evaluate their participation in the neural regulation of copulatory and chemoinvestigatory behaviors in male hamsters. Damage to either the MPOA or the BNST caused severe deficits in copulatory performance in a subset of the animals in each group. In the MPOA group all males displaying severe deficits had lesions which included a small central region of the caudal MPOA. In the BNST group, animals with severe copulatory deficits all had large lesions which covered most of both the medial and lateral parts of the nucleus. In contrast, MPOA and BNST lesions differentially affected chemoinvestigatory behaviors. MPOA lesions did not affect any of the males' anogenital investigation rates or attraction to female odors, even though some of these hamsters had stopped mating completely. Males with BNST lesions, on the other hand, all displayed significant reductions in their chemoinvestigatory responding even though the majority of them continued to mate normally. We suggest that the MPOA and BNST may in part regulate male sexual behavior by differentially responding to 'attractant' and 'mounting' substances within female hamster vaginal secretion.

INTRODUCTION

The medial preoptic area (MPOA) is an important substrate for the regulation of male reproductive behavior. Lesions of the MPOA eliminate or severely impair mating behavior in a wide variety of vertebrate species^{2,13,20,23,29,31–34,66,72}. Conversely, male sexual behavior can be facilitated by either electrical or hormonal stimulation of this limbic forebrain region^{12,47,50,52}. The function of the MPOA in neural regulation of male hamster reproductive behavior has been of particular

interest to our laboratory because chemosensory pathways which are critical for the initiation of mating behavior in this species project directly to the MPOA^{40,51,53,60}.

Bilateral removal of the main and accessory olfactory bulbs, which eliminates all input from peripheral olfactory and vomeronasal receptors, abolishes male hamster copulation^{18,54,74}. The cortical and medial nuclei of the amygdala are major target areas for the second order chemosensory neurons in the main and accessory olfactory bulbs, respectively^{15,60}. Lehman et al.⁴⁶

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demonstrated that lesions of the medial nucleus of the amygdala (M) abolish male hamster copulatory behavior and severely reduce chemosensory investigation of females and their odors. Lesions of the cortical nuclei of the amygdala do not have comparable effects^{44,46}. These behaviors are androgen-dependent^{25,48,56,57} and M contains androgen-concentrating neurons¹⁷. Additionally, the electrophysiological characteristics of cells in this area of the amygdala can be influenced by testosterone^{38,39} and by olfactory cues⁵³. These findings support the hypothesis that the medial nucleus could be a substrate responsive to both hormonal and chemosensory information critical for the expression of male hamster sexual behavior.

Anatomical studies in hamsters have demonstrated that caudal M projects via the stria terminalis (ST) to both the MPOA and the bed nucleus of the stria terminalis (BNST)⁴⁰. Subsequent experiments showed that the BNST, but not the MPOA, also receives an input from rostral M via a non-strial, ventral pathway (VP)⁴⁵. In order to test whether direct strial projections from M to MPOA were necessary for male hamster copulatory behavior, we evaluated the effects of bilateral lesions or knife cuts of the ST⁴³. Although this treatment produced temporal alterations in the copulatory sequence, it did not mimic the effects of lesioning the medial nucleus of the amygdala. However, combined knife cuts of both the ST and the VP abolished copulation and severely reduced anogenital investigation of females⁴². Because in hamsters, efferents of M in the VP project to the BNST and not to the MPOA, these results suggested an important role for the BNST in mediating male sexual behavior.

The BNST, as well as M and MPOA, has connections through which it might influence processing of a variety of internal and external signals regulating social behavior^{68,71}. It also contains androgen-concentrating neurons¹⁷, and links the limbic forebrain with mid- and hindbrain aminergic systems that modulate many behaviors²¹. Specifically with respect to sexual behavior, lesions of the BNST in male rats can significantly alter normal patterns of copulatory responding^{19,59,70}. Conversely, androgen im-

plants in the vicinity of the BNST may facilitate this behavior⁴. In hamsters, the BNST may mediate the arousal and consummation of mating behavior in part by relaying specific chemosensory information from M to MPOA. Evidence in rat^{11,55,62,68} and preliminary data in hamster indicate that BNST is anatomically linked to MPOA.

In this paper we evaluate the behavioral effects of lesioning areas within the MPOA and the BNST which receive chemosensory afferents from M. Our results demonstrate that such lesions can produce robust deficits in both copulatory and chemoinvestigatory behaviors, but that the effects on these two components of sexual behavior can be remarkably dissociated.

MATERIALS AND METHODS

General

Animals

Adult male Syrian hamsters weighing approximately 120 g were obtained from Harlan-Sprague-Dawley (Madison, WI), and were housed in groups of 3 under a LD 14:10 illumination cycle (lights off at noon). Food and water were available ad libitum. Prior to experimental use all males were provided sexual experience with receptive females and non-copulators were eliminated from further use. Animals were then castrated and implanted s.c. with 20 mm Silastic capsules (i.d. = 1.98 mm, o.d. = 3.18 mm) filled with crystalline testosterone (T). This procedure provided all subjects with chronic physiological levels of T (ref. 10); it controlled for any effects that subsequent lesions might have on systemic levels of this hormone.

Behavioral tests

A. Copulatory behavior (CB) tests were conducted in Plexiglas boxes (45 × 30 × 38 cm) 1–5 h into the dark portion of the L:D cycle. Males were given 10–15 min to adapt to the box, after which the test began, defined by the introduction of a receptive female. Test females were ovariectomized and implanted s.c. with 20-mm Silastic capsules filled with 25 mg estradiol/ml

sesame oil and were injected s.c. with 500 μg progesterone 2–4 h prior to use. CB tests lasted for 10 min or until the male had ejaculated twice. Mount, intromission and ejaculation latencies and frequencies were recorded. The latencies were defined as follows: (1) to first copulatory act (LFCA) – beginning of test to first mount or intromission; (2) to first intromission (IL) – beginning of test to first intromission; and (3) to first ejaculation (EL) – first intromission to first ejaculation. The postejaculatory interval (PEI) was defined as the interval between the first ejaculation and the next mount or intromission.

B. Chemoinvestigatory behaviors were quantified in two ways. During CB tests the frequency and duration of sniffing and/or licking the female's head, body, and anogenital (AG) regions were recorded. Investigatory behavior was scored if the male's nose was judged to be within 0.75 cm of the respective body region. Durations were converted to rates by expressing the time investigating each body region as seconds per minute of test. Rate measures were used because test durations varied among animals depending upon how long they took to complete the CB test.

Separate tests which measured the interaction of male hamsters with female hamster vaginal secretions (FHVS) in the absence of any other female derived cues were also conducted using the same Plexiglas boxes employed for the CB tests. Following a 5-min adaptation period within the test chamber, the experimental male was briefly covered with a small box while a target area on one wall (6 cm above the floor) was swabbed with FHVS. Vaginal secretions were freshly collected by inserting a small cotton-tipped applicator into the vagina of a receptive female brought into behavioral estrus in the same manner as the females used for CB tests. The control area on the wall opposite the FHVS was swabbed with a clean applicator. FHVS tests lasted for 2 min, and began when the male was uncovered. Behaviors scored were latency to, and duration of, sniffing and/or licking the FHVS or control target areas. The total time spent investigating the FHVS target was considered the FHVS Investigation Score.

Histological analyses

Upon completion of all behavioral testing, animals were perfused through the left ventricle with 0.9% saline followed by 10% formal saline. Brains were stored in formal saline for 1 week, then transferred to 30% sucrose in formal saline until sectioning. Frozen sections were cut at 80 μm in the coronal plane, and alternate sections through the rostrocaudal extent of the MPOA and BNST were stained with Cresyl violet. Lesion damage was assessed by microscopical analysis of all stained sections. Areas of cell loss and accompanying gliosis were drawn onto standard sections of hamster brain prepared in our laboratory.

Statistical analyses

The differences between prelesion and post-lesion behavioral performance were evaluated by within-groups *t*-tests using the Vanderbilt MINITAB computational facility; between-groups *t*-tests were used to compare subgroups of animals with differing behavioral deficits on post-lesion tests. All comparisons described below as significant had 2-tail probabilities equal to or less than 0.05.

Specific procedures

Experiment 1. MPOA lesions

Forty-three males (castrated, with T replacement) were tested to establish baseline levels of copulatory and chemoinvestigatory performance. Based on their scores, animals were divided into MPOA lesion ($n = 37$) and SHAM lesion ($n = 6$) groups such that the mean scores for each behavior were as equivalent as possible between groups.

Bilateral electrolytic lesions were placed in the MPOA using stereotaxic procedures with bregma and lambda in a horizontal plane. Lesion coordinates were 1.1 mm anterior to bregma, 0.4 mm lateral to the mid-sagittal suture, and 8.0 mm below dura at the lesion site. An anodal constant current of 0.6 mA was passed for 30 s through a tungsten electrode (diameter = 0.38 mm), insulated except for 0.5 mm at the tip. SHAM lesions were made in the same manner, except that the

electrode was lowered to a point 1.0–1.5 mm above the experimental coordinates and no current was passed.

Following a one-week recovery period, subjects were given two CB tests and a subset of animals was given two FHVS tests. CB and FHVS tests occurred 2 and 4, and 3 and 5 weeks, respectively, following the placement of MPOA lesions.

Experiment 2. BNST lesions

Thirty-five hamsters were pretested and, based on their behavioral results, were assigned to BNST lesion ($n = 28$) or SHAM lesion ($n = 7$) groups such that copulatory and chemoinvestigatory measures were as equivalent as possible between groups. Bilateral electrolytic lesions were placed in BNST using stereotaxic procedures as described above. Lesion coordinates were 1.1 mm anterior to bregma, 1.2–1.3 mm lateral to the mid-sagittal suture, and 6.7 mm below dura at the lesion site. An anodal constant current of 0.7–0.75 mA was passed for 30 s through a stainless steel electrode (diameter = 0.3 mm),

insulated except for 0.5 mm at the tip. SHAM lesions were made in the same manner, except that the electrode was lowered to a point 1.0–1.5 mm above the experimental coordinates and no current was passed.

Following a one-week recovery period, subjects were given a single CB test each week for the next 4 weeks. One week later an FHVS test was conducted.

RESULTS

Experiment 1

MPOA lesions had distinct effects on both the initiation and completion of copulation although they did not alter the males' investigation of female odors. Behavioral effects ranged from total abolition of all mating, to little if any copulatory impairment. For analysis of these results we first classified animals according to the nature of their ejaculatory deficits on the two postlesion tests. Males were categorized as having SEVERE, PARTIAL, or NO ejaculatory deficits if they had

TABLE 1

Copulatory performance of MPOA-lesioned male hamsters

Numbers in parentheses indicate a different n for that test. * Pre values significantly different from post; ** Posttest comparisons with SHAM group significant. LFCA, latency to first copulatory act (s); IL, latency to first intromission (s); EL, latency to first ejaculation (s); I/E, intromissions to first ejaculation (s); III, inter-intromission interval (s); PEI, postejaculatory interval (s); all values are means \pm S.E.M.

		Ejaculatory deficit			
		SEVERE ($n = 8$)	PARTIAL ($n = 12$)	NO ($n = 17$)	SHAM ($n = 6$)
LFCA	Pre	54.0 \pm 5.8	52.4 \pm 6.6*	47.3 \pm 4.4*	41.3 \pm 8.9
	Post	233.1 \pm 78.3	146.2 \pm 21.5**	82.3 \pm 10.3**	38.9 \pm 3.8
IL	Pre	99.8 \pm 15.9	83.4 \pm 10.9*	80.4 \pm 10.0	74.3 \pm 14.0
	Post	–	157.2 \pm 27.6**	119.0 \pm 17.9**	64.4 \pm 12.7
EL	Pre	122.9 \pm 15.9	128.0 \pm 19.0	126.2 \pm 11.7	133.4 \pm 33.2
	Post	–	174.9 \pm 19.0**	115.7 \pm 9.3**	84.5 \pm 9.6
I/E	Pre	13.7 \pm 1.4	11.1 \pm 1.3	12.9 \pm 1.0*	10.6 \pm 0.9
	Post	–	8.3 \pm 1.4	7.7 \pm 0.9	11.3 \pm 1.4
III	Pre	9.3 \pm 0.6	12.2 \pm 1.5*	10.2 \pm 0.8*	12.3 \pm 2.7
	Post	–	25.1 \pm 4.4**	16.9 \pm 1.5**	7.5 \pm 0.4
PEI	Pre	34.4 \pm 2.0	32.4 \pm 1.6*	32.4 \pm 2.0*	35.8 \pm 4.0
	Post	–	50.4 \pm 2.9**	50.1 \pm 2.0**	33.8 \pm 2.4

either (1) no ejaculations on either test (SEV, $n = 8$), (2) fewer than two ejaculations on at least one test (PAR, $n = 12$), or (3) two ejaculations for both tests (NO, $n = 17$). Although this categorization was based on ejaculatory deficits, the 8 males in the SEV group showed essentially no copulation at all. Three of these animals mounted but did not intromit during test 1. On test 2, all 8 males failed to mount. Males in the PAR and NO categories had behavioral impairments that were less pronounced; SHAM-lesioned hamsters continued to mate normally. These findings are summarized in Table I which compares results among all 4 groups of animals for 6 behavioral measures, reflecting both the initiation and completion of copulation.

Analysis of variance indicated that the animals comprising the 3 postlesion behavioral deficit groups did not differ in sexual performance on prelesion tests. SHAM lesions did not significantly affect any of these behavioral parameters. In contrast, males in the PAR and NO categories were slower to initiate copulation; both groups showed significantly elevated LFCA's and IL's. The time required to achieve ejaculation (EL) however, once intromissions had begun, was not affected in the same way. Although none of the prelesion–postlesion EL comparisons was significant, postlesion EL's did fall considerably among the SHAM-lesioned males, but they did not do so among males in the other 2 groups. Partly because of this, postlesion EL's among the PAR, NO and SHAM groups were ordered linearly, and they were all significantly different from each other (Table I).

The elevation of postlesion EL's among PAR males was accompanied by a sizeable, but not significant, decrease in the number of intromissions preceding ejaculation (I/E). These factors combined to produce a significant increase in their mean inter-intromission intervals (III). Animals with NO ejaculatory deficits showed changes in the same direction for the I/E and III measures, but for this group both effects were significant (Table I). The time required to reinitiate copulation after the first ejaculation (PEI) was affected similarly to most of the other behavioral parameters. Specifically, the PAR and NO

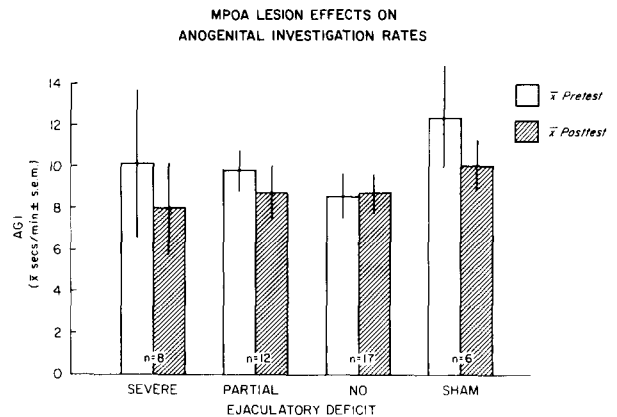


Fig. 1. Mean pre- and posttest (pre- and postlesion) anogenital investigation rates among MPOA-lesioned males during copulatory behavior tests. None of the pretest–posttest differences was significant.

groups showed significant postlesion elevation in PEI compared to prelesion values, whereas the SHAM group did not. This resulted in significant postlesion differences in PEI's between SHAM animals and animals in each of the other 2 groups, but the latter did not differ from each other. These results clearly demonstrate that lesions of the MPOA can result in impairments of copulatory behavior even when the ejaculatory response is not eliminated.

In contrast to the obvious effects that MPOA lesions had on copulation, they did not diminish the males' chemoinvestigation of female odor. Postlesion anogenital investigation (AGI) rates were not significantly lower than prelesion AGI rates among any of the 4 groups (Fig. 1). There was however, a tendency of lesioned males to show an increased rate of total investigation (due to increased investigation of the head and body regions). Additionally, there were no significant differences in postlesion FHVS Investigation Scores among the subset of males in the 3 ejaculatory deficit groups that were tested in this fashion (data not shown).

Histological analysis of the brains revealed that the lesions varied considerably in their size and bilateral symmetry. By visual inspection of the standardized drawings there was no obvious correlation between lesion size or location and the type of behavioral deficit it produced. Con-

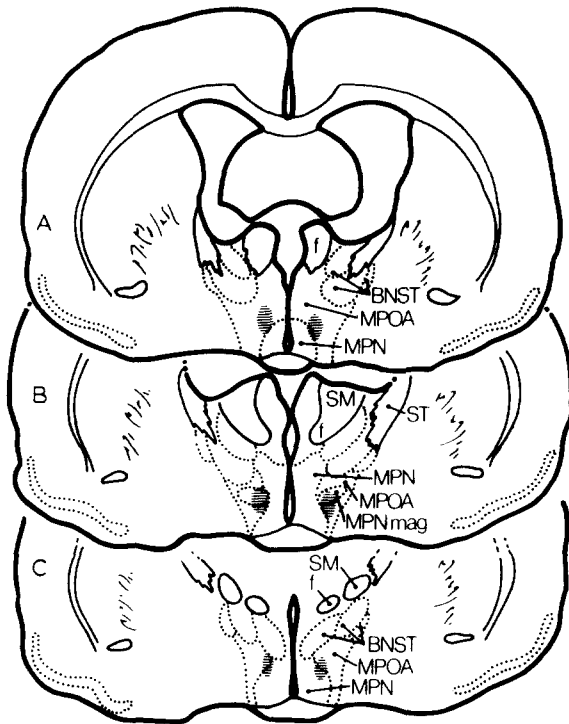


Fig. 2. Schematic drawing of coronal sections through the medial preoptic area (MPOA). The region of common lesion damage among the 8 hamsters with severe copulatory impairments is depicted by the striped areas. BNST, bed nucleus of the stria terminalis; f, fornix; MPN, medial preoptic nucleus; MPN mag, magnocellular MPN; MPOA, medial preoptic area; SM, stria medullaris; ST, stria terminalis.

sequently, we performed an additional analysis on the brains from animals whose lesions had abolished all copulation. We first traced the lesions from the 8 SEV males onto acetate. By overlying these tracings for each of the standard sections on which damage was present, we determined what part of the MPOA was lesioned in common among all of these animals. A region of common overlap was found in the middle-to-caudal portions of the MPOA (Fig. 2; striped area). It consisted of a reasonably small, ovoid-shaped area that was nearly symmetrically displaced from the midline by approximately 0.5 mm. This subregion of the MPOA fell on the lateral border of the mid-rostrocaudal part of the medial preoptic nucleus (MPN). In the middle of this area (Fig. 2B) the common lesion area damaged a group of large cells which extends laterally

from the MPN into the lateral part of the MPOA. We have named this the magnocellular MPN.

This analysis of lesion overlap among the SEV males suggested that damage to a relatively small part of the MPOA might be sufficient to abolish essentially all copulatory behavior. This also suggested that partial or no ejaculatory deficits had been observed in animals in which this critical region was not completely destroyed. We assessed this possibility by estimating for each male in the PAR and NO groups, what proportion of this MPOA region had been damaged. Both sides of the brain were evaluated separately at each of the 3 rostral-caudal levels shown in Fig. 2. We ranked on a scale of 0–3 the extent to which the existing lesion for any given animal impinged upon the area of common overlap we found among the 8 SEV males. In none of the PAR or NO hamsters was more than 25% of this area damaged bilaterally. Additionally, on average, the PAR males had more of this area lesioned than did the NO males, thus correlating with the severity of their copulatory deficit.

Experiment 2

BNST lesion damage varied considerably in size and configuration. Sixteen of 28 animals had extremely small, and in many cases asymmetric lesions, making their evaluation difficult. For this reason they were eliminated from further analysis for this report. However, these 16 males did exhibit a variety of deficits that closely paralleled the profile of effects we observed in the remaining 12 animals. The 12 males reported here had larger lesions, all of which damaged some portion of the BNST; a subset of these hamsters had lesions which affected their copulatory behavior (Table II). In contrast, all of the males with BNST lesions displayed significantly impaired chemoinvestigatory behavior with receptive females and with female-derived odors.

For behavioral analyses, we first categorized lesioned males by distinguishing between SEVERE (SEV) and NOT SEVERE (NOT SEV) deficits in ejaculatory behavior, using 2 rather than 3 categories because of the reduced numbers of animals in this experiment. In the SEV group one animal intromitted and ejaculated

TABLE II

Copulatory performance of BNST lesioned male hamsters

Numbers in parentheses indicate a different *n* for that test. * Posttest comparisons with SHAM group significant. LFCA, latency to first copulatory act (s); IL, latency to first intromission (s); EL, latency to first ejaculation (s); I/E, intromissions to first ejaculation (s); PEI, postejaculatory interval (s); all values are means \pm S.E.M.

		<i>Ejaculatory deficit</i>		
		<i>SEVERE</i> (<i>n</i> = 4)	<i>NOT SEVERE</i> (<i>n</i> = 8)	<i>SHAM</i> (<i>n</i> = 7)
LFCA	Pre	46.7 \pm 7.4	63.6 \pm 16.8	72.1 \pm 21.1
	Post 1	112.2 \pm 99.0 (2)	31.2 \pm 5.9	38.6 \pm 5.8
	Post 4	-	38.1 \pm 9.4	46.5 \pm 4.7
IL	Pre	50.9 \pm 9.0	79.4 \pm 20.0	93.4 \pm 29.3
	Post 1	-	73.4 \pm 35.9	79.6 \pm 16.1
	Post 4	-	94.2 \pm 55.0	68.2 \pm 7.0
EL	Pre	95.8 \pm 23.2	125.6 \pm 16.3	127.2 \pm 13.4
	Post 1	-	156.8 \pm 18.3* (6)	102.4 \pm 14.1
	Post 4	-	90.1 \pm 6.7	122.9 \pm 24.0
I/E	Pre	11.3 \pm 2.1	13.5 \pm 1.4	12.9 \pm 1.5
	Post 1	-	11.2 \pm 1.4 (6)	10.0 \pm 0.6
	Post 4	-	10.0 \pm 0.4	10.9 \pm 0.8
PEI	Pre	34.0 \pm 3.4	35.0 \pm 1.5	41.5 \pm 2.9
	Post 1	-	30.1 \pm 2.8 (6)	31.4 \pm 3.1
	Post 4	-	30.1 \pm 1.6	32.5 \pm 1.9

on a single test, and two mounted on the first through third tests. None of these males displayed any component of the mating sequence on the fourth test. The 8 males in the NOT SEV group for the most part displayed normal copulatory behavior. These hamsters mounted and intromitted on every test following their lesions and ejaculated twice on at least 3 of these 4 tests; 2 animals did not ejaculate on test 1 and another male had only one ejaculation on test 4. Table II depicts LFCA, IL, EL, I/E and PEI measures derived from tests 1 and 4, with males grouped according to their ejaculatory deficit. Comparisons between the NOT SEV and the SHAM animals indicated there were no significant differences for LFCA, IL or PEI on either test 1 or 4. However, on test 1, ejaculation latencies were significantly longer among the 6 of 8 NOT SEV males that did ejaculate on this test (Table II).

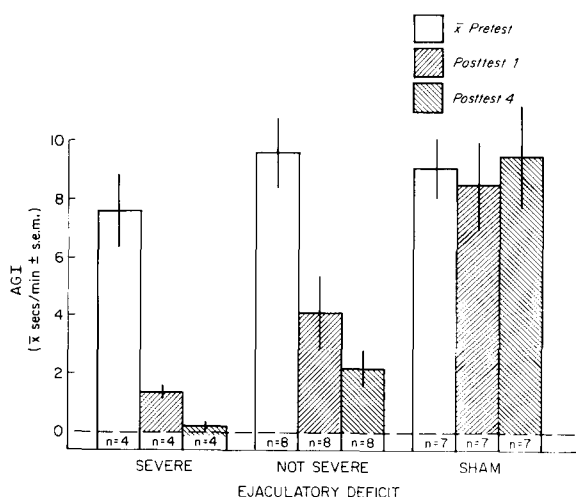


Fig. 3. Mean pre- and posttest (pre- and postlesion) anogenital investigation rates (AGI) among BNST-lesioned males during copulatory behavior tests. Both posttest 1 and posttest 4 AGI rates were significantly lower than pretest rates for hamsters in the SEVERE and NOT SEVERE ejaculatory deficit categories.

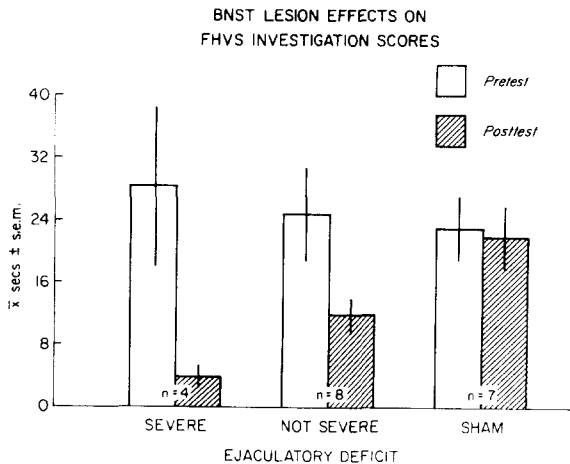
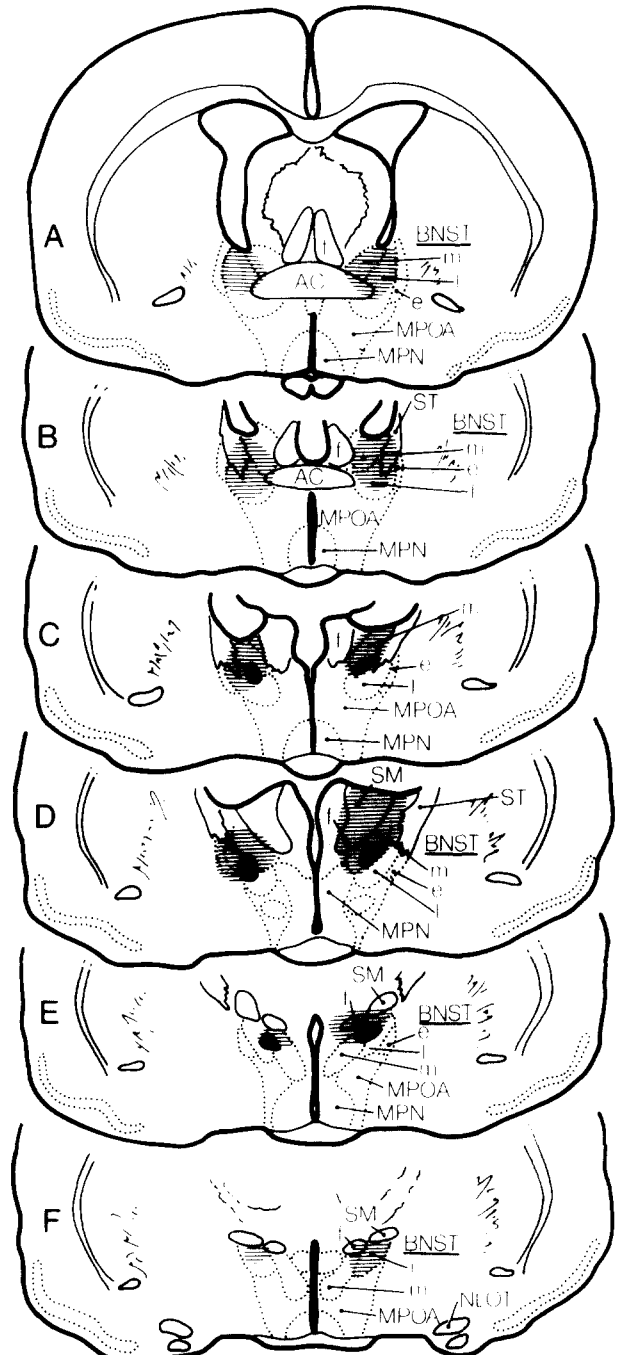


Fig. 4. Mean pre- and posttest (pre- and postlesion) FHVS Investigation Scores. Pretest–posttest differences were significant for SEVERE and NOT SEVERE subjects.

Figs. 3 and 4 illustrate the profound chemo-investigatory deficits that occurred among all lesioned males, independent of whether an ejaculatory deficit was or was not also present. AGI rates (Fig. 3) decreased significantly on postlesion tests 1 and 4, compared to pretest values, for males in both the NOT SEV and the SEV ejaculatory deficit categories ($P < 0.01$), but did not do so among the SHAM-lesioned males. FHVS Investigation Scores (Fig. 4) showed essentially the same effect. This measure also declined significantly among all lesioned males, independent of the accompanying ejaculatory deficit ($P < 0.05$). Interestingly, for both measures of chemoinvestigation, males in the SEV groups were noticeably more impaired than were the NOT SEV subjects (Figs. 3 and 4). These chemoinvestigatory deficits were also present on postlesion tests 2 and 3 but are not shown.

Fig. 5. Schematic drawings of coronal sections through the bed nucleus of the stria terminalis (BNST). The region of common lesion damage among the 4 males with severe copulatory impairments is depicted by the striped areas. The region of common damage among the 12 hamsters with significant chemoinvestigatory impairments is depicted by the filled black areas. AC, anterior commissure; BNSTm, l, and e: medial, lateral and external divisions of the BNST; f, fornix; MPN, medial preoptic nucleus; MPOA, medial preoptic area; NLOT, nucleus of the lateral olfactory tract; SM, stria medullaris; ST, stria terminalis.

Histological analysis of lesioned brains indicated that animals displaying SEV ejaculatory deficits had large lesions encompassing most of the medial division of the BNST (BNSTm), part of its lateral division (BNSTl) and fibers within



the stria terminalis. Some damage to the fornix and stria medullaris was also apparent. In contrast, those animals without ejaculatory deficits had small lesions, typically along the border between the BNSTm and BNSTl, yet these same males had obvious impairments in chemoinvestigatory behavior. In order to learn whether destruction of any particular subregion of the BNST was associated with the behavioral deficits displayed, we determined the area that was lesioned in common among the brains from animals in similar behavior deficit categories, as we had done for MPOA-lesioned hamsters. Males with BNST lesions showing SEV ejaculatory deficits had an area of lesion overlap that in the rostral BNST included most of both its medial and lateral subdivisions (Fig. 5, striped area, levels A, B). Caudally the area of overlap involved BNSTm more than BNSTl. A similar analysis was performed with respect to chemoinvestigatory deficits; we placed males in a SEVERE category if their AGI rates were below a 95% confidence interval constructed around the mean of the SHAM animals' performance on this behavioral measure. All lesioned males could be placed into this category. Among these animals, lesion analysis revealed a small area of common overlap along the BNSTm/BNSTl border (Fig. 5C, D, E, blackened area), destruction of which would presumably be sufficient to produce significant decreases in the measures of chemoinvestigation that we obtained.

DISCUSSION

MPOA and BNST lesions had dramatically different effects on copulatory and chemoinvestigatory behaviors. MPOA lesions abolished all copulation in a subset of males, but both in these animals and in those in which copulation was less affected, MPOA lesions had no effects on the olfactory investigation of receptive females or their odors. BNST lesions also caused severe copulatory deficits in some males, but in contrast to the effects of MPOA lesions, all hamsters with damage to the BNST showed significant deficits in chemoinvestigatory behaviors, uncorrelated with copulatory performance. These behavioral

effects were independent of changes in the brain's regulation of gonadal function because all experimental males were castrated and received exogenous hormone treatment to facilitate their sexual behavior. Our findings may have important implications concerning the role of chemoinvestigatory behaviors in arousing male hamsters to mate. Below, this issue is evaluated after discussing pertinent features of the two experiments separately.

MPOA lesions, in some animals, eliminated all copulation (SEV), while in others, the ejaculatory response was maintained either consistently (NO) or inconsistently (PAR) on postlesion tests (Table I). We found that the area of lesion overlap among the 8 males in which all components of the copulatory response were abolished (SEV) was located in a relatively small and bilaterally symmetrical part of the caudal MPOA (Fig. 2). For heuristic purposes we have termed this area the 'critical' zone. Although we concluded from this lesion analysis that destruction of the critical zone might be sufficient to cause severe copulatory impairments, we cannot exclude the possibility that damage to a smaller region within it would be just as effective. Lesion analyses among the PAR and NO males indicated that no more than 25% of the critical zone was damaged bilaterally in any of these animals. Additionally, the extent of damage that did occur was related to the magnitude of their ejaculatory deficit. The fact that males in these two behavioral categories for the most part continued to ejaculate is consistent with the hypothesis that destruction of this critical zone, or some part of it, is sufficient to eliminate all mating behavior in male hamsters. This region also appears very similar to the site depicted by Eskes²⁰ to illustrate an MPOA lesion which caused a severe copulatory deficit in male hamsters, although her lesions were made for other reasons.

Although MPOA lesions could affect sexual behavior by influencing a number of different mechanisms, interference with the processing of chemosensory information is a likely possibility. Anatomical studies in hamster have demonstrated that the critical zone we identified lies within the MPOA projection field of stria termi-

nalis efferents specifically from the medial nucleus of the amygdala⁴⁰. It is important to note that the copulatory impairments demonstrated by the PAR and NO males reported here were in many respects similar to those produced in males with lesions of the ST⁴³. That is, the capacity to ejaculate was not affected, but the temporal patterning of the copulatory behaviors preceding ejaculation was altered (Table I). Clearly, MPOA lesions affected a variety of behavioral components during the first ejaculatory series among males in which the ejaculatory response was not abolished. Because the lesions of these animals were within the ST terminal field of the MPOA, it seems reasonable to postulate that their behavioral deficits resulted in part from disconnecting olfactory and vomeronasal system information from integrative sites within the MPOA.

It is interesting that deficits in the arousal of copulation were not reflected in deficits in the lesioned males' AGI rates that were obtained during CB tests (Fig. 1). In other experiments using different species, it was also the case that lesion-induced copulatory impairments occurred in the absence of effects on other behaviors normally associated with sexual arousal^{5,28,31,69}. One interpretation of our results is that the lesioned hamsters, by means of their investigatory behavior, were still accessing the chemosensory cues that initiate copulation, but that cells in the MPOA that normally respond to these cues were absent, non-functional, or disconnected from sensory inputs.

All BNST-lesioned males, on the other hand, showed significant decreases in both AGI rates and FHVS Investigation Scores whether or not an accompanying copulatory deficit was present (Figs. 3 and 4). Given that chemosensory input is critical for stimulating male hamsters to mate, how can these measures be so impaired without concomitant effects on sexual performance? It is first useful to consider what is known concerning the chemical constituents of FHVS and their function in male hamster sexual performance. Dimethyl disulfide (DMDS), a volatile constituent of hamster vaginal secretions, can attract males, but does not stimulate them to mount^{49,64}. On the other hand, a high molecular weight, non-

volatile, fraction of vaginal secretions facilitates mounting, and must be physically contacted for this to occur⁶⁵. Based on the results presented here, and on our earlier work concerning the role of testosterone metabolites⁵⁷, we favor the view that our chemoinvestigatory measures primarily reflect a male's attraction to a female or her odors. This suggests that the presence of DMDS (or related volatile compounds), plus an intact neural substrate to process this input, are necessary for males to display normal AGI rates and FHVS Investigation Scores, but that these chemosensory behaviors may be ancillary to the primary function that this mechanism is intended to serve.

If male and female hamsters were tested in very large and semi-natural enclosures, rather than in small boxes, a male's attractant response could be important in the process by which it locates and subsequently comes into contact with a potential mate. If this function were impaired by CNS lesions for example, copulatory deficits could result indirectly from the male's increased difficulty or inability to locate females. However, even when a female is found and contacted, either via an intact 'attractant' mechanism or by the male's being placed in close proximity to the female, the initiation of copulation would still be dependent on a 'mounting' mechanism that responds to high molecular weight constituents in FHVS. Once a mating sequence had begun, its completion might be less dependent on continued chemosensory input. This was demonstrated by Devor and Murphy¹⁶, who observed that acute blockade of chemosensory inputs had no effect on the continuation of copulatory behavior, providing the male hamster was not interrupted for more than 2 min. Another possibility is that the requirements for such stimulation might be met by the low rates of AGI we have observed, or by other means of making periodic contact with relevant vaginal products (e.g. males' self-genital grooming).

MPOA and BNST lesions may have differentially affected these postulated attractant and mounting mechanisms. That is, animals whose deficits were categorized as severe after either an MPOA or BNST lesion may have failed to initiate

copulation because of an impaired processing of relevant non-volatile odors, that is, an impaired mounting mechanism. The attractant function of chemosensory stimulation could have remained intact following MPOA lesions (Fig. 1), but have been seriously jeopardized among all males with BNST lesions (Figs. 3,4). We would predict that had we used a drastically different testing situation which necessitated that experimental males first detect and seek out receptive females, MPOA lesioned males would have had little difficulty in this regard, but that the 8 SEV animals still would not have copulated after the females had been found. In contrast, the chemoinvestigatory deficits shown by all BNST-lesioned males suggest that these animals all would have experienced difficulty finding the female, even though most of them would have copulated normally if this initial task had not been imposed.

If our interpretation of the differential effects of MPOA and BNST lesions has merit, does this suggest that the neural pathways carrying attractant and mounting information from vaginal odor cues may be anatomically separate? Our earlier findings concerning the participation of olfactory and vomeronasal systems in stimulating male hamsters to mate⁷⁵, and other results as well, have prompted speculation that these two receptor systems could play different roles in mediating the male's response to conspecific odors³⁶. The present results at a minimum suggest that the attractant function, as reflected in our chemoinvestigatory measures, involves pathways from peripheral receptors, via the olfactory bulbs and M, to some parts of the BNST. Relevant projections that presumably leave the BNST are unknown, but they almost certainly do not involve a connection with the MPOA. The mounting function also depends on inputs from nasal chemoreceptors to BNST, but may well include an additional projection to MPOA.

In this view, attractant functions would be served by inputs to BNST via M, utilizing both the ST and VP. The zone of lesion overlap we found among all BNST-lesioned males with significant chemoinvestigatory deficits (Fig. 5) presumably includes the BNST region involved in this function. The initiation of mounting, though,

appears to require additional chemosensory processing. We suggest that BNST cells receiving mounting information from M in turn project to the critical zone of the MPOA (Fig. 2), and that this area also receives chemosensory input directly from M via the ST. Males with BNST lesions that abolished copulation most likely had sustained damage both to the ST projection to MPOA and to the origin of the BNST innervation of MPOA. The remaining BNST lesions may have failed to affect copulation because they did not impinge on both these sources of inputs to the MPOA. Based on an entirely different experimental approach, Kendrick³⁷ has suggested that two sources of neural inputs from the cortico-medial amygdala may converge on single neurons within the MPOA; such an hypothesis helps to account for the electrophysiological response properties of these cells to amygdala stimulation.

Although we have presented our lesion effects in male hamsters primarily in terms of disrupted chemosensory processing, this limbic system damage could affect behavioral regulation in many other ways. Anatomical, physiological and neurochemical aspects of MPOA or BNST organization that might relate to their neuroendocrine control of reproduction have been described²¹. Most likely, the amygdala, BNST, and MPOA all are affected by various neural signals that may act independently, or may also become integrated with chemosensory stimulation in order that normal male-female sociosexual interactions occur. Our lesions may have affected such processes. For example, both the MPOA and BNST contain androgen-concentrating neurons¹⁷, and testosterone implants into this region of the hypothalamus can facilitate hamster copulatory behavior⁴⁷. Destruction of these androgen-concentrating neurons might be critical for generating the behavioral impairments we observed. MPOA and BNST lesions might also have damaged efferent pathways that regulate copulatory behavior and specifically spinal reflexes^{7-9,22,27,30,69,73}. It is informative to relate our findings to those of Szechtman et al.⁶⁹ who produced bilateral knife-cuts, either dorsal or sagittal to the rat MPOA. They found that these two treatments resulted in quite different be-

havioral impairments, and concluded that sagittal efferents from the MPOA were essential to the initiation of mating, while dorsal afferents to the MPOA were more involved in the continued execution of the copulatory response. This dichotomy of behavioral function may be useful, but it is undoubtedly oversimplified. For example, it is clear that their sagittal knife-cuts affected both III and I/E measures, as well as those reflecting the initiation of copulation. However, what is most important for our purposes is that the specific effects on III and I/E that they found were identical to what we observed in MPOA-lesioned male hamsters categorized as having partial or no ejaculatory deficits. Perhaps these effects resulted primarily from our interference with critical output pathways that originate in the MPOA.

The question of whether electrolytic lesion effects can be attributed primarily to damage of cells, or to disruption of fibers of passage, has been frequently raised. It is known in rats that neurotoxic lesions of the MPOA which spare fibers of passage can impair male sexual behavior as effectively as can electrolytic lesions²⁹. Thus it is not likely in the present experiment that disruption of fibers passing through the MPOA was necessary to produce the copulatory deficits we observed. Rather, disruption of input-output relationships within the MPOA represents a more likely source of behavioral deficits. However, as discussed above, damage to the ST fibers coursing through the BNST could easily have contributed to the results after BNST lesions.

Finally, it is of interest to relate the data presented here to recent work concerning the sexually dimorphic characteristics of MPOA, BNST, and other brain regions in a number of different species^{3,6,14,24,26,35,58,61-63,67}. Although most effort has been placed on accurately describing the morphological features that are dimorphic, some attention has been paid to their function. Lesions reportedly restricted to the sexually dimorphic nucleus of the rat preoptic area (SDN-POA) do not affect male sexual behavior², but a recent demonstration indicates that the size of the SDN-POA is positively correlated with the vigor of male rat sexual performance¹. Destruction of the apparently homol-

ogous area in gerbils does have some effects on male copulation and other sociosexual behaviors¹³. Recent analyses of the rat SDN-POA have emphasized that it is part of the medial preoptic nucleus (MPN) within the MPOA, and that the density of serotonin input to its central and lateral components is different between males and females. Additionally, the MPN appears to receive its most prominent input from the 'encapsulated part' of the BNST⁶², which appears to be homologous to the hamster BNSTm.

In the course of our anatomical investigations we have identified the MPN in male hamster brains, but we have not studied the female nuclear pattern in relation to the male. Our observations on the male agree fully with those of Bleier et al. in terms of cellular configuration⁶. We disagree only on the terminology for structures in the caudal half of their 'sexually dimorphic nuclear complex', which they consider to be the anterior hypothalamus, but which we have identified as a continuation of the MPN within the MPOA. In addition, by studying serial coronal sections we have determined that a separate group of very large cells, which extends laterally from the caudal MPN is not an extension of the BNST, as suggested by Bleier et al., nor is it continuous with the more rostral parastrial nucleus of Simerly et al.^{62,63}. We have named this cell group the magnocellular MPN (see MPN mag, Fig. 2B).

The area which we have identified as being lesioned in all males with severe ejaculatory deficits involved only the lateral part of the MPN at its mid-rostrocaudal levels, and the MPN mag (Fig. 2). We cannot, however, exclude the possibility that damage to MPN alone is the most relevant factor in producing these copulatory impairments. In future work it clearly will be of interest to determine the specific anatomic connections between M, MPN, and the BNSTm.

We do not wish to imply by our analyses that the only way the MPOA and BNST might regulate hamster copulatory behavior is by their response to chemosensory inputs. Clearly the complex organization of these two neural regions suggests that our interpretation of these lesion results is just one of many possibilities. We have however provided a useful basis for further under-

standing of how the chemosensory inputs that are required by male hamsters to initiate their sexual interactions with females might influence these important regions of the limbic forebrain.

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