BEHAVIORAL EFFECTS OF MAMMILLOTHALAMIC TRACTOTOMY IN CATS[1]

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THE MAMMILLOTHALAMIC TRACTS are distinctive features of the mammalian diencephalon. In the phylogenetic scale of mammals these structures maintain, or even increase, their relative anatomical prominence. It is well established that the tracts consist of efferent fibers from the mammillary bodies projecting to the anterior thalamic nuclei of the thalamus. Recent work has shown conclusively for the cat that each lateral mammillary nucleus projects bilaterally to the anterodorsal nuclei, and fibers from the medial mammillary nuclei project ipsilaterally to the anteromedial and anteroventral thalamic nuclei. Collaterals or primary fibers also appear to leave the tracts diffusely to terminate in the ventromedial thalamic nuclei (5).

In contrast with the well-described anatomy of the mammillothalamic system is the paucity of consistent and unequivocal information regarding its functional significance. The mammillothalamic tracts comprise one of the major links in the limbic system circuit hypothesized by Papez to subserve emotional reactions (13). From a survey of the relevant literature, Akert (1) is of the opinion that, when care is taken to preclude spread of current to neighboring hypothalamic structures, stimulation of the mammillary bodies in awake cats through chronically implanted electrodes is without behavioral consequence. More recently, MacLean and Ploog (9) reported that electrical stimulation of the mammillary bodies of squirrel monkeys produces penile erection in the awake monkey, and stimulation is also effective when the animals are anesthetized but higher intensity of stimulation is required. It is frequently reported in secondary sources that lesions in the mammillary bodies in monkeys are associated with behavioral somnolence, but perusal of the series of original papers by Ranson and his associates (see 16) shows that somnolence was a consequence of lesions in the region of the posterior hypothalamus. In those cases in which the lesions were restricted to the mammillary bodies or mammillothalamic tracts, no disturbance of alertness or general emotionality was observed.

In the clinical literature, disturbance of recent memory in man (Kor-

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sakoff's syndrome) has been related to pathology in the mammillary bodies. The association is not compelling, however, since lesions often extend diffusely into other neural structures and exceptions have been reported (10). It is of interest that confusion in temporal orientation and disturbances of recent memories similar to some behavioral aspects of Korsakoff's syndrome have been observed in humans after lesions in various parts of the forebrain limbic system, namely, after bilateral hippocampal lesions (17), after sectioning the fornix (20), and after ablation of the cingulate cortex (22). Related findings from animal studies have not yielded consistent results, in part attributable to the fact that we have not identified, unequivocally, behavioral functions in animals which are neurophysiologically homologous with "short-term memory" in humans. Orbach, Milner, and Rasmussen tested retention and relearning on a variety of behavioral tasks with monkeys after amygdala-hippocampal ablation (12). Disturbances of retention and relearning were observed on a number of tests, but not on a delayed response test which, a priori, should be very sensitive to any disturbance of short-term memory ability. Pribram, Wilson, and Connors (15) found no loss of postoperative retention of alternation behavior in monkeys following ablation of the cingulate cortex or the hippocampus. Recently, Ploog and MacLean (14) investigated behavioral changes in squirrel monkeys following ablation of the mammillary bodies. Their monkeys were not somnolent and they showed no significant postoperative loss of retention of a preoperatively learned differential conditioned avoidance response. In contrast with the above studies severe deficiency in learning discrimination problems based on successive comparison of stimuli after amygdala-hippocampal resection was found in monkeys by Stepien, Cordeau, and Rasmussen (19). Limbic lesions produced behavioral deficits in tasks involving both vision and hearing. Modality-specific loss was observed after ablation of auditory or visual neocortical areas.

Other than a preliminary report of some of the results in the present paper (21) one experimental report has come to our attention in which behavioral evaluation of animals was made following discrete lesions which sever the mammillothalamic tracts (MThTs). Dahl, Ingram, and Knott (2) recently reported that a variety of diencephalic lesions in cats failed to disturb retention of preoperatively learned conditioned avoidance responses. Of special relevance to the present paper are their results from two cats which sustained complete mammillothalamic tractotomies and two other cats in which the mammillary bodies were completely ablated. Neither lesion interfered with retention. The present paper describes experiments in which behavioral changes in cats after bilateral mammillothalamic tractotomy were evaluated with three testing procedures. The results are in partial disagreement with the findings of Dahl *et al.* (2).

PROCEDURES

Subjects. Mongrel, female, adult cats weighing 4.5 lbs. or more were used as subjects.

Surgical procedures. The aim of the surgery was to sever the MThTs bilaterally with minimal damage to other structures in the brain. Most of the cats sustained lesions produced by irradiation with stereotaxically guided beams of focused ultrasound according to techniques developed by W. J. Fry and his associates in this laboratory. Detailed descriptions of the equipment and procedures have been presented elsewhere (3). Sufficient for present purposes is a brief description of surgical procedures as they affect the cats. Under sodium pentobarbital anesthesia, the cats were inserted into a specially designed stereotaxic head holder, and with aseptic precautions the dorsal convexity of the skull from the floor of the frontal sinus to the tentorium and well down the sides was rongeured off. Care was taken to avoid tearing the dura mater and to minimize bruising of the cortex and bleeding. The wide opening in bone is required to admit the cone of ultrasound into the brain deeply enough for the point of focus to reach the mammillothalamic tracts. A shallow pan with a flanged opening in the bottom was fitted over the cat's head, and the incised scalp (single midsagittal incision) was pulled up over the flange and drawn snug by a wire tourniquet to make a watertight seal. At completion of the operation the dorsal surface of the brain lay exposed in the bottom of the pan which was then filled with sterile, degassed (boiled) mammalian Ringer's solution at body temperature. The liquid medium is necessary to conduct the ultrasound from the transducer to the brain. The Ringer's solution was maintained at constant temperature by heat-exchange coils mounted on the wall of the pan. The ultrasonic transducer was then lowered into the Ringer's solution and positioned to place the focus of the ultrasonic beam at the desired point for the array to be irradiated. In a few cats lesions were produced by irradiating with a single pulse of ultrasound (duration about 2 sec.) at a frequency of 1 megacycle/sec. for each mammillothalamic tract. Most of the lesions, however, were produced with focused ultrasound at a frequency of 4 megacycles/sec. Five to seven brief exposures (0.50–0.55 sec.) were made on each side with the adjacent positions of the focus spaced 0.2 mm. apart laterally and aimed at the mammillothalamic tracts. The ultrasonic level was set (based on previous calibration) so that at the site of focus a small, oval-shaped, selective lesion with a long axis of 1–2 mm. and a minor axis of 0.25–0.50 mm. would be produced in a homogeneous region of white matter.

After completing the array of irradiations the Ringer's solution was drawn off and the pan removed. A cranioplast cap, molded into the shape of a cat's skull, was placed over the brain. The cap rested on bone both in front and back so it did not press on the brain, and it extended down over the site of the bony defect on the sides. It was held in place by suturing the temporal muscles together over the top of the cap. The scalp was closed with silk sutures. For 3 days following surgery the animals were given prophylactic antibiotic treatment.

In other cats the lesions were produced by the more conventional method of d.-c. electrolytic fulguration through a stereotaxically guided stainless steel electrode, 0.15 mm. in diameter and insulated except for about 0.5 mm. at the tip. The lesions were produced by passing 3.0-mA. anodal current through the electrode for 20 sec. The return (cathodal) lead was attached to the metal head holder. The lesioning method employed for each cat will be indicated in the tables of RESULTS.

When conventional positioning methods were used, attempts to cut the tracts on both sides with small lesions were not generally successful because of individual variations in the location of the target sites relative to standard Horsley-Clarke coordinates. In a later series of cats the accuracy of placement of the lesions was improved by determining the coordinate references from roentgenograms of the cats' skulls so that bony landmarks nearer to the target sites, rather than the usual skull landmarks, could be used. This procedure is described in detail by Fry *et al.*, appendix A (5).

Specifying the coordinates of the target site with reference to bony structures more intimately related to the brain than those used in standard stereotaxic procedures resulted in considerable reduction in errors of placement due to individual variations from cat to cat in the location of given loci in the brain, but within the limitations of our attempts to keep the lesions small the problem was not entirely solved. In a few animals in these experiments, the coordinates of the target sites were determined with reference to intrabrain structures visualized by contrast ventriculography accomplished by injecting radiopaque material (Conray, Mallinckrodt Chemical Works) into the ventricles. In the few cats in which this procedure was used, the accuracy of placement of the lesions was quite gratifying. Procedures for use of ventriculography for determining stereotaxic coordinates in the cat brain have been described elsewhere (4).

Behavioral tests. Effects of mammillothalamic tractotomy in three behavioral situations were evaluated. In the first experiment, cats were trained to criterion in avoidance conditioning in a double-grill box prior to surgery. Following surgery and 2 weeks or more postoperative recovery, the animals were tested for retention of the avoidance response. They were then retrained to the same criterion of performance. In the second experiment a similar paradigm was followed, except that the conditioning task involved preoperative learning of a visual form-discrimination habit under conditions of food reward with postoperative relearning trials as the test of retention. The third experiment tested for effects of the lesions on postoperative acquisition of the avoidance response, i.e., the animals were first operated, and after postoperative recovery they were then trained. More detailed descriptions of apparatus and behavioral methods will be presented in connection with the account of each experiment.

Anatomical controls. After completion of behavioral tests the cats were sacrificed under deep sodium pentobarbitol anesthesia by pericardial perfusion with physiological saline followed by 10% formalin in saline. The brains were removed from the skulls and, after further hardening in formalin, were prepared for histological study. Most of the brains were prepared by the frozen method in which 50 μ thick sections were cut in the frontal plane and every fifth section was stained for myelin by the Weil method. Fewer of the brains were embedded in paraffin, cut at 10 μ and every twentieth section stained for myelin. Sections from some of the brains were stained for cells with cresyl violet. The brains of all animals were examined microscopically for evaluation of the damage to the mammillothalamic tracts and neighboring structures. Photomicrographs of the lesions are shown in the RESULTS sections.

EXPERIMENT 1. RETENTION AND RELEARNING OF CONDITIONED AVOIDANCE RESPONSES

Apparatus and Procedure

The cats were trained in avoidance conditioning in a double-grill box in which the floor of each compartment measured 12×18 in. A guillotine door 18 in. long served as a separator between compartments. The threshold of the door extended 4.5 in. above the floor of the box which consisted of 3/8-in. diameter stainless steel rods spaced 1.5 in. center to center. One side of the box was made of glass so the cats could be observed. The experimenter observed the animals through a one-way-vision mirror. The conditioned stimuli (CS) consisted of switching off the light in the compartment in which the cat sat and simultaneously switching it on in the other compartment while, at the same time, the door separating the two compartments was raised by remote control. The unconditioned stimulus (US) was electric shock applied through a grid scrambler to the cat's feet through the grid bars. The CS-US interval was 10 sec. and was automatically timed. If the cat responded within the 10-sec. CS-US interval, it was not shocked and the door was lowered behind it.

Most experimenters who train cats in avoidance conditioning use a "shaping" technique in which the punishing shock is applied judiciously by hand. For example, if the animal is in the process of responding at the termination of the CS-US interval, shock is withheld so it will not be punished for walking across the bars. If this precaution is not taken, many cats fail to learn to avoid the shock because they develop maladaptive response patterns such as "freezing" in bizarre postures or rolling over on their backs. However, if shaping procedures are used, the cat's performance is confounded with the skill of the experimenter. As several different experimenters were to be involved in training the cats, it was deemed desirable to proscribe a standard training procedure, free from experimenter bias, which would provide a gradual increase in the stringency of conditions for the cat and allow time for adaptation to the disruptive effects of shock in order to minimize the likelihood of development of maladaptive responses and to maximize the likelihood of adaptive learning. To this end a schedule for gradually increasing the demands made on the animals in adapting them to the conditioning procedure was developed. For the first 8 days the cats received only five trials per day with 5-min. intertrial intervals. The first day 5 sec. of forced shock were given, i.e., the door between compartments was not raised until 5 sec. after the onset of shock. The shock level was quite low (2 mA.) barely enough to cause the animals to "dance" about. They did not howl or defecate. Over the next 3 days, opening of the door was moved forward in time until on the 5th day the door was raised at the same time the lights shifted, i.e., 10 sec. prior to the onset of shock. Through the 8th day, shock levels gradually increased and intertrial intervals decreased. On the 9th day, the number of trials was increased to 10/day and remained there through the 12th day while intertrial intervals decreased from day to day and shock level increased. On the 13th day of training, final training conditions were achieved: 20 trials/day, intertrial interval of 1 min., and a shock level of 4 mA. Training continued under these conditions until the animals reached a criterional performance of 3 consecutive days in which no more than one shock was received on any one day. Performance scores consist of the number of trials to criterion (including criterional trials) and the number of shocks received (not including the 20 forced shocks given during the first 4 days of training). After the animals achieved the learning criterion, they were scheduled for surgery.

Two weeks after surgery the animals were lively and normal in general cage behavior. Most of the cats were tested for retention of the avoidance habit on the 16th day after surgery. A few had longer postoperative recovery periods, but none was tested sooner than 15 days after surgery. The retention test consisted of placing the animal in the double-grill box and giving 20 presentations of the CS (switching the lights and opening the door between compartments) at the rate of once a minute. If the animal crossed to the opposite compartment, the door was lowered behind it. If, after 20 sec. (twice the CS-US interval employed during training trials) the animal had not responded the door was lowered and the trial terminated. The performance score is the number of responses with latencies less than 20 sec. out of 20 trials. No shocks were administered during the retention trials. On the next day regular training conditions were reinstated and the animals were again trained to the same level of performance that had been required preoperatively, namely, 3 consecutive days of 20 trials each in which no more than one shock was received on each day.

Twenty-six cats were used in this experiment. Four cats died postoperatively. One cat was eliminated from the experiment when it was discovered that the lesions invaded the optic tracts. Another cat was not tested postoperatively because it had a persistent wound infection, and one cat was eliminated because of an unavoidable delay of 76 days between completion of training and surgery. The data reported in this study are based on 19 cats.

Results

After the lesions had been evaluated from the histological material, the cats were divided into two groups. One group of nine cats that sustained only partial or no damage to the MThTs comprise the operated control group. The experimental group (ten cats) consists of those cats that had sustained essentially complete, bilateral transection of the MThTs. Performance scores in the double-grill box are shown in Table 1 for each cat in each group. The "E" or "U" behind each cat's identification number indicates which type of lesion was produced: electrolytic or ultrasonic. The two columns to the right of the identification numbers show the number of trials and shocks needed for the animals to reach criterion in original, preoperative conditioning. The proscribed training schedule required 120 trials as the minimum number of trials in which a cat could reach criterion. The number of shocks received is the more sensitive measure of performance. The control group, *A*, with medians of 140 trials (range, 120–300) and 24 shocks (range, 9–89), does not differ significantly from the experimental group, *B*, with medians of 200 trials (range, 120–260) and 35 shocks (range, 14–54), by two-tailed Mann-Whitney U-test (18). The next column, labeled "postoperative retention responses," shows the number of responses in 20 trials with latencies less than 20 sec. that each cat made on the retention test under extinction conditions, i.e., with no shocks. Median score for the control group is 20 (range, 12–20). In contrast, the median score for the experimental group is 1 response (range, 0–18). There is one exception to the generally poor performance of this group on the retention test; *cat 576* made 18 responses. Notwithstanding the exceptional performance of this one animal the two groups differ significantly in retention scores ($P < .002$).

On the day following the retention test, original training conditions were re-established (CS-US interval of 10 sec. and US of 4-mA. shock for responses

Table 1. Postoperative retention and relearning of conditioned avoidance responses

Cat. No.	Preoperative Learning		Postoperative Retention	Relearning	
	Trials	Shocks	Responses	Trials	Shocks
A. Control group: cats with partial or no lesions in MThTs					
508, E	160	31	20	60	3
514, E	180	32	20	60	1
526, U	240	24	20	60	3
529, U	140	9	20	120	3
531, U	120	19	17	100	5
547, U	120	10	20	160	10
548, U	300	89	19	60	2
553, U	120	19	19	60	2
578, U	120	31	12	100	9
Medians	140	24	20	60	3
B. Experimental group: cats with bilateral section of MThTs					
513, E	200	40	1	260	44
517, E	200	53	2	90	21
527, U	200	54	9	120	9
574, U	160	34	10	120	16
575, U	160	34	0	120	12
576, U	240	18	18	80	4
577, U	200	15	1	80	5
579, U	260	46	0	340	81
585, U	120	14	1	80	8
586, U	120	36	1	200	27
Medians	200	35	1	120	14
P values	> .10	> .10	< .002	< .08	< .01

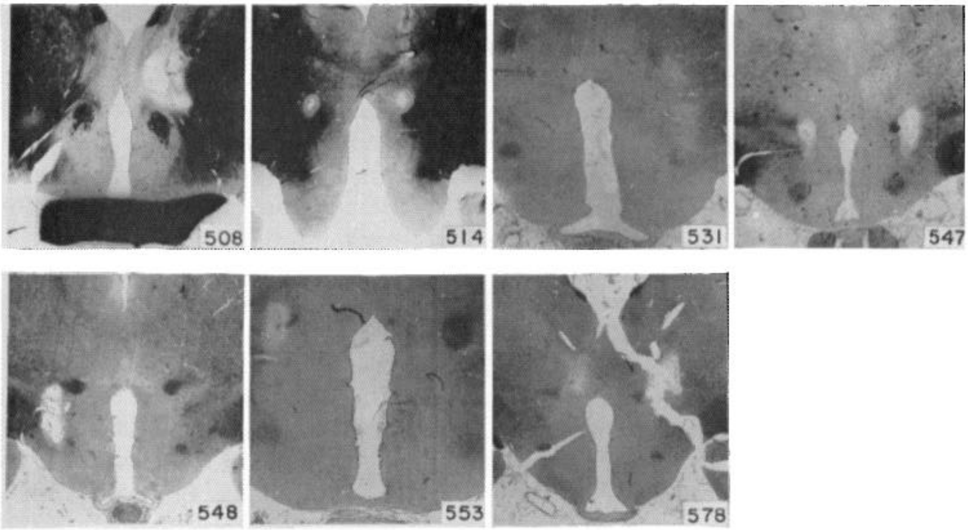
P values derived from two-tailed Mann-Whitney U test.

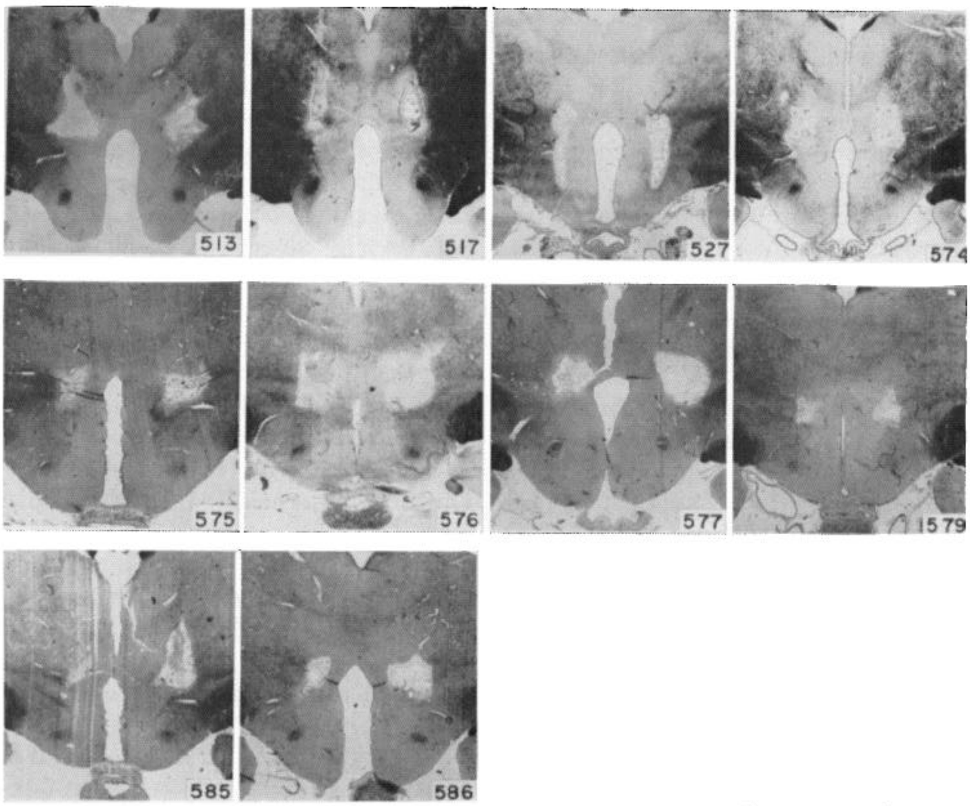
of latency greater than 10 sec.) and continued at 20 trials/day until criterional level of performance was rereached. The minimum possible number of trials required for criterional performance was 60. The last two columns in Table 1 present number of trials and shocks required for relearning. The control group medians are 60 trials (range, 60–160) and three shocks (range, 1–10) to rereach criterion. The experimental group performed less well than the control group. Medians are 120 trials (range, 80–340) and 14 shocks (range, 4–81). The difference between groups is significant for shocks by two-tailed U-test ($P < .01$). The difference between groups in number of trials to criterion approaches significance ($P < .08$). Most of the experimental cats showed positive savings in reconditioning but two cats, *513* and *579*, actually required more trials and shocks to relearn than they had required in original preoperative training.

Photomicrographs of brain sections showing the largest extent of the lesions are presented in Fig. 1. Two cats (*526* and *529*) were sham-operated controls which underwent completely the extensive surgery required for the ultrasonic procedure, but they were not irradiated. They sustained no histologically visible brain damage so no photomicrographs of these brains are shown. Both of these sham-operated control cats showed 100% retention of the conditioned avoidance response. The top seven pictures demonstrate the lesions in brains of the control cats. Three of these (*508*, *531*, and *548*) had unilateral lesions in the lateral region of the hypothalamus or anterior thalamus, but the lesions did not involve the MThTs. All three of these cats retained the avoidance response well, making retention scores of 20, 19, and 19. The remaining four animals of the control group sustained some damage to the MThTs that failed of complete bilateral transection. *Cat 547* had the most severe damage to the MThTs. The tract is completely severed on one side and about one-third cut on the other. This animal showed perfect retention. *Cat 578* had the poorest performance, making only 12 retention responses. Its lesions encroached on the tracts rather severely but did not cut them completely. The illustration of the lesions in *cat 553* (shown at higher magnification than the other pictures) is noteworthy because of the tiny, selective lesion within MThT on the right side. Only one animal (*578*) of the control group deviated from the otherwise uniformly high (85% or better) retention scores. The lesions in the MThTs of its brain are no more extensive than lesions in MThTs of other cats that had good retention, e.g., *cat 547*. However, because of the small number of cases involved, we are not able to draw any conclusions regarding effects of partial damage to the MThTs on retention of conditioned avoidance responses.

The experimental group comprises those animals in which mammillothalamic tractotomy was essentially complete on both sides. The lesions are illustrated by the bottom ten photomicrographs in Fig. 1. The peculiar shape of the lesions in brain of *cat 576* should be noted. It derives from the fact that ultrasonic lesions are usually quite thin in the rostrocaudal direction, and slight distortions of the tissue during preparation for cutting sometimes pull the sheet of damaged tissue out of plane so that no one section contains the total area of the lesion in the plane of the microtome cut. It is obvious that the MThTs are both completely severed, but this animal displayed an exceptionally high level of performance (90%) on the retention test.

Also of special interest is *cat 579*, in which the lesions approximate the ideal of small lesions that sever the tracts with very little damage to surrounding gray matter. This cat had the most seriously disturbed behavior of the experimental group. It made no responses on the retention test and required almost twice as many shocks to relearn to criterion as it did in original





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learning. The next most severely affected cat (*527*) took four more shocks to relearn postoperatively than it required in original learning. It sustained larger lesions than the other cats in this group. The lesions not only severed the MThTs but extended ventrally to interrupt the fornix on both sides, and the lesions also invaded the intervening gray matter of the hypothalamus. The remaining cats sustained lesions of a variety of sizes but in each the lesion had severed the MThTs. Damage in the region of the ventromedial thalamic nuclei and in the dorsal hypothalamus was generally quite small. No specific anatomical structures except the MThTs were consistently and severely damaged in this group of brains.

The performance of *cat 576* is clearly exceptional compared with that of the other cats in the experimental group. Its normal retention score is not attributable to a shorter lapse of time than with the other cats between completion of training and retention testing. The time between the last day of criterional performance and the retention test was 16 days for this cat which is the median time for the experimental group (range, 15–26 days). The control group, which maintained good retention of the conditioned avoidance response, had a longer median time of 27 days between the end of training and the retention test (range, 18–53 days), so the lapse of time between training and testing does not appear to be a significant factor in interpreting these data. The fact that one cat with bilateral mammillothalamic tractotomy was able to perform at a high level on the retention test, and the negative findings of Dahl, Ingram, and Knott (2) (based on a similar behavioral test of two cats with comparable lesions) indicate that loss of retention of conditioned avoidance responses after bilateral mammillothalamic tractotomy is not obligatory. A highly significant number of tractotomized cats do, however, show a marked behavioral deficit. Others factors, as yet unidentified, must be critical in determining whether or not a given mammillothalamic tractotomized cat will show a loss of retention of avoidance conditioning.

EXPERIMENT 2. POSTOPERATIVE RELEARNING OF A VISUAL FORM-DISCRIMINATION HABIT

The purpose of this experiment was to determine if mammilllothalamic tractotomy affects the retention of a difficult discriminative conditioned response which was reinforced with food reward.

Apparatus

Training and testing were carried out in a modified Wisconsin general test apparatus (6) consisting of a restraining cage, a stimulus tray (Klüver form board), and opaque and

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FIG. 1. Photomicrographs of sections showing the largest extent of the lesions (Experiment 1: postoperative retention of conditioned avoidance responses). The top two rows of pictures illustrate lesions which did not sever the mammillothalamic tracts (control group). The bottom three rows show lesions which severed the tracts (experimental group).

one-way-vision screens. The restraining cage was 24 in. wide, 18 in. deep, and 12 in. high. It had opaque walls except for the front which was made of 1/4-in. diameter steel rods aligned vertically and spaced 1.5 in. center to center. Immediately outside the cage in front of the bars a guillotine door could be lowered which prevented the cat from seeing the stimulus tray or the experimenter. The tray, which was level with the floor of the cage, moved on runners and contained two food wells with sloping sides located 12 in. apart and 4 in. from the edge adjacent to the cage. The tray could be pushed to a position immediately proximal to the front grill of the cage so the cat could reach one food well at a time with its forepaws, or the tray could be withdrawn out of reach. Another guillotine door consisting of a half-silvered mirror, through which the experimenter could observe the cat, was located 12 in. from the front of the cage. Each animal learned two discrimination problems. The discriminative stimuli for the preliminary problem were black and white plywood disks 1/4 in. thick and 4 in. high. The disks stood vertically on small bases that covered the food wells. Training on the black-white discrimination problem allowed the animals to learn to work efficiently in the apparatus. The stimulus objects used for the main discrimination problem consisted of two forms, a T and an upside-down Y, cut from 1/8 in. thick aluminum. The forms were 4 in. high with 1 in. wide stems and bars. These objects also stood vertically on small bases which covered the food wells.

Procedure

The cats were placed on a reduced food regimen and adapted to eating in the apparatus. When the cats became accustomed to the apparatus, they were taught to rake in bits of meat (liver, stew beef, or beef heart ca. 1 cc. in size) from the top of the tray, then from out of the food wells, and finally to displace objects covering the food wells in order to secure pieces of meat. In formal training trials the opaque door was closed while the experimenter baited the food well and placed the correct stimulus object over it. The incorrect stimulus object was placed over the empty well. He then lowered the one-way-vision screen, raised the opaque door, waited 2 sec. to allow the cat to inspect the stimuli, and then pushed the tray forward so the cat could reach the stimuli. If the cat displaced the correct object it was allowed to retrieve the reward. If it displaced the wrong object the tray was withdrawn out of reach before the animal could respond to the other stimulus, i.e., noncorrection procedure was used. Half the cats were given preliminary training with the black disk as the correct stimulus and half with the white disk as the correct stimulus.

Training on the black-white problem was not carried out to a high level of correct discrimination. As soon as the animals were working efficiently and rapidly and making about 75% correct responses they were shifted to the form-discrimination problem. During training on the form-discrimination problem the cats received a minimum of 20 trials/day and often as many as 80 trials. Half the cats were trained with the T as the positive stimulus and half with the upside-down Y as positive. The correct stimulus was varied from right to left following a predetermined irregular order, except that whenever a cat showed any sign of developing a position preference the correct stimulus was kept on the side opposite its preference until the response shifted. All animals were trained to a stringent criterion of no more than one error in each of three consecutive blocks of 20 trials.

As soon as the cats reached criterion they were scheduled for surgery. Most cats underwent surgery within a week after reaching criterion on the form problem, but some were delayed as long as a month. Following surgery the cats were allowed a minimum of 2 weeks postoperative recovery before behavioral testing was resumed. No animal waited more than 3 weeks postoperatively before undergoing behavioral testing.

Thirty-two cats started the experiment, but data are reported for only 22 animals. Three cats were dropped from the experiment preoperatively because they failed to reach criterion in 1,000 trials; four animals died postoperatively; one animal was eliminated from the experiment because the lesions invaded the optic chiasma; and two cats were not tested postoperatively because of severe postoperative wound infections. Data from only those cats that completed behavioral testing in apparent good health and whose lesions did not involve the optic system are included in the results.

Results

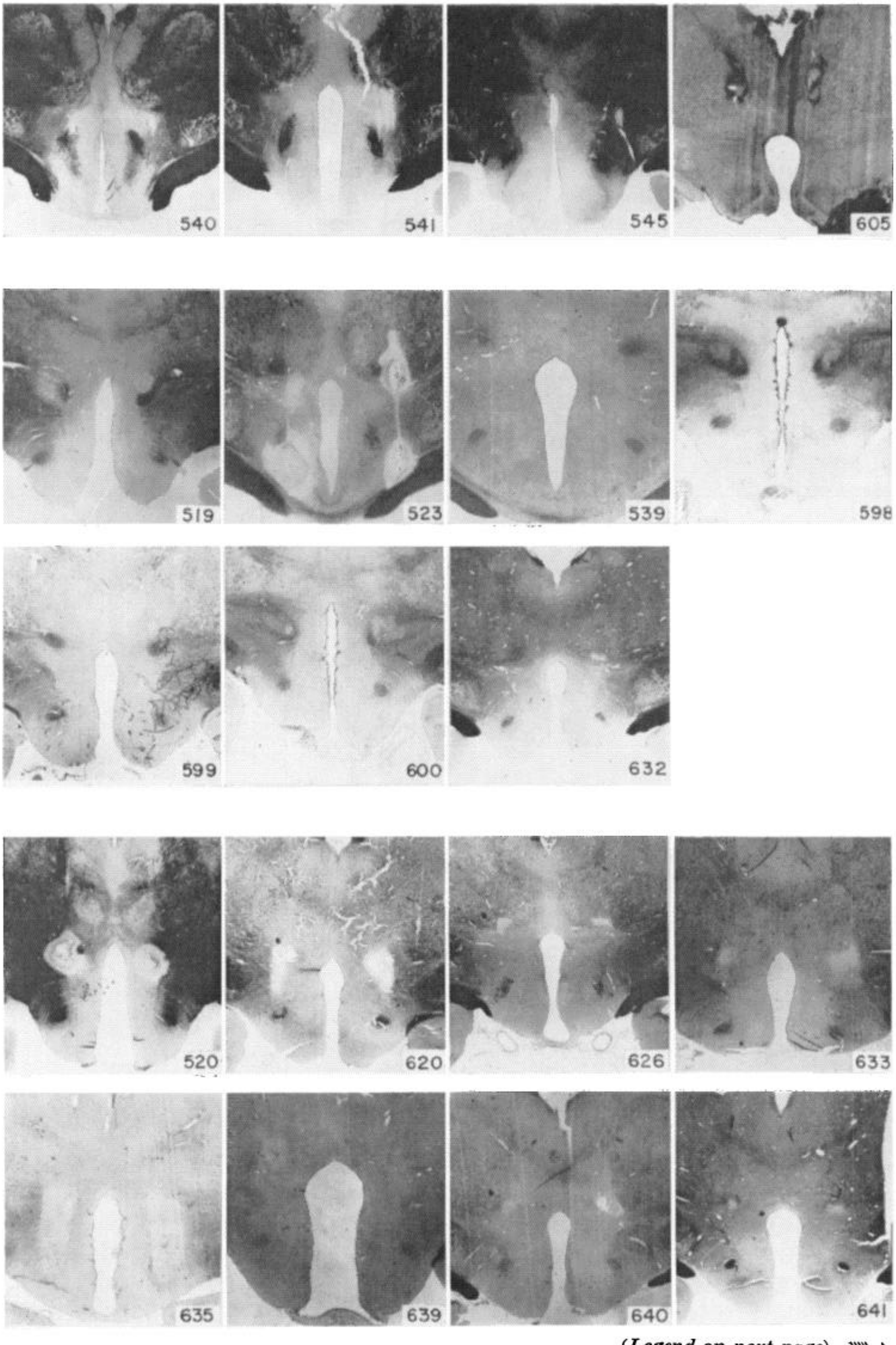
On the basis of histological evaluation of the lesions the cats were divided into three groups. Scores for seven cats that sustained no damage to the MThTs are shown in Table 2*A*. The first two columns to the right of the identifying numbers show the original learning scores (trials and errors to criterion), the next two columns show postoperative relearning scores, and in the last column, the percentage of savings is shown (difference between error

Table 2. Postoperative relearning of visual form discrimination

Cat. No.	Preoperative Learning		Postoperative Relearning		Percentage of Savings
	Trials	Errors	Trials	Errors	
A. Control group: cats with no lesions in MThTs					
518, E	320	91	140	7	92
540, U	460	157	100	7	95
541, U	460	102	80	4	94
545, U	393	154	120	9	98
546, U	300	72	120	7	90
602, U	540	157	60	1	99
695, U	200	31	60	3	90
Medians	393	102	100	7	94
B. Control group: cats with partial damage to MThTs					
519, E	640	221	60	0	100
523, E	580	145	60	3	98
539, U	340	100	356	42	58
598, U	200	39	80	5	87
599, U	636	236	180	26	89
600, U	446	112	60	2	98
632, U	640	156	80	1	99
Medians	580	145	80	3	98
C. Experimental group: cats with bilateral section of MThTs					
520, E	700	180	60	1	99
620, U	808	237	112	6	97
626, U	151	36	60	1	97
633, U	356	122	120	6	95
635, U	360	111	120	17	85
639, U	268	84	96	4	95
640, U	526	96	140	10	89
641, U	380	98	112	9	91
Medians	370	105	112	6	95

None of the group differences is statistically significant by H-test.

scores in original learning and in relearning divided by errors in original learning ×100). Table 2*B* shows comparable scores of seven cats in which the MThTs were damaged to some extent on one or both sides; and part *C* shows the scores of eight cats in which the MThTs were completely severed on both sides. Glancing down the last two columns to the right shows that almost all the animals rereached criterion postoperatively with few errors and considerable percentage of savings. *Cat 539,* with 42 errors in relearning (58%



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savings), had the worst memory score. The Kruskal-Wallis H-test (18) shows that there are no statistically significant differences among these groups.

Illustrations of the lesions in brains of cats in group *A* are shown in the top row of photomicrographs in Fig. 2. Three sham-operated control animals in group *A* (*518*, *546*, and *602*) had no lesions so no photomicrographs are shown for these brains. Two brains had unilateral lesions, one (*545*) in the lateral hypothalamus and one (*541*) in the anterior hypothalamus. Two more had bilateral lesions lying far forward: *cat 540* shows very faint lesions just dorsal to the fornix in the anterior hypothalamus and *605* (cell stain) has lesions lying mostly within the medioventral thalamic nucleus on one side and in the medial margin of the ventroanterior nucleus on the other. All the cats in this group displayed a high level of postoperative performance.

All brains in group *B* contained some damage to the MThTs, although in some cases the lesions are very small. The lesions are illustrated in the middle group of seven photomicrographs in Fig. 2. Of interest are the brains of two animals with the worst performance (*539*, 42 errors and *599*, 26 errors). They have the smallest lesions which appear as tiny light spots within the MThT on one side in each case. The cause of the poor performance of these two cats cannot be attributed to the lesions. *Cat 523* which had large hypothalamic lesions and *cat 600* in which the MThTs were very nearly severed both showed very good postoperative memory of the form-discrimination habit.

The bottom group of eight photomicrographs in Fig. 2 illustrates the lesions in the brains of group *C*. All cats sustained essentially complete bilateral mammillothalamic tractotomy. In one cat (*635*) not only were the MThTs severed, but the lesions also extended ventrally to interrupt the fornix on both sides. That cat exhibited the poorest postoperative performance (17 errors) in this group. Lesions in five of the cats (*626*, *633*, *639*, *640*, and *641*) are noteworthy in that they are all very small, and they sever the tracts without extending into the surrounding gray matter to any extent.

It seems justified to conclude that damage to the MThTs does not have a significant effect on the memory of a preoperatively learned form-discrimination habit. Thus, the behavioral functions which were altered by mammillothalamic tractotomy to cause the loss in postoperative retention of the double-grill box avoidance habit cannot be attributed to a general memory deficit since comparable lesions have no effect on postoperative memory for a form-discrimination habit learned under positive reinforcement.

FIG. 2. Photomicrographs of sections showing largest extent of lesions (Experiment 2: postoperative retention of a visual discrimination habit). The top row of pictures show lesions in brains of group *A* (see Table 2) in which the lesions did not invade the mammillothalamic tracts. The two middle rows of pictures illustrate lesions in group *B* (Table 2) in which the lesions invaded the tracts but did not completely sever them. The bottom two rows of pictures show lesions which completely severed the mammillothalamic tracts bilaterally (group *C*, Table 2).

EXPERIMENT 3. POSTOPERATIVE ACQUISITION OF CONDITIONED AVOIDANCE RESPONSES

This experiment investigated the effects of mammillothalamic tractotomy on postoperative acquisition of avoidance conditioning.

Procedure

Twenty-three cats were used in this experiment. There were no deaths, and no cats had to be eliminated from the experiment. Eight of the cats had been used as subjects in Experiment 2. After they had reached criterion performance postoperatively on the visual-discrimination problem, training was started in the double-grill box. Fifteen additional cats were also operated, and following at least 2 weeks of postoperative recovery they were started in avoidance training. Apparatus and training procedure in this experiment were the same as in Experiment 1. After the cats reached the criterion performance level of 3 consecutive days of 20 trials each in which no more than one shock was taken each day, they were sacrificed and the lesions evaluated. The control group of 12 cats includes all cats in which the lesions failed to sever completely both MThTs. The experimental group of 11 cats contains all cases in which the MThTs were essentially interrupted on both sides.

Results

The behavioral scores for the control group are shown in Table 3*A*. The median trials to criterion for the control group is 120 (range, 120–440) and the median number of shocks is 17 (range, 6–74). These scores do not differ significantly (U-test) from the preoperative conditioning scores of the 19 animals in Experiment 1: median trials, 160 (range, 120–300), median shocks, 31 (range, 9–89). This finding provides a control for nonspecific effects of the extensive surgical procedures required to implement the ultra-

Table 3. Postoperative conditioned avoidance response acquisition

A. Control group: cats with partial or no damage to MThTs			*B. Experimental group: cats with essentially total bilateral section of MThTs*		
Cat No.	Postoperative Learning		Cat No.	Postoperative Learning	
	Trials	Shocks		Trials	Shocks
556, U	550	58	*620*, U	140	14
559, U	120	6	*626*, U	500	91*
561, U	160	31	*633*, U	120	9
632, U	240	14	*635*, U	720	202*
723, U	240	74	*639*, U	600	143*
725, U	120	11	*640*, U	120	21
740, U	120	10	*641*, U	140	30
822, E	120	10	*726*, U	220	22
823, E	120	19	*754*, E	420	135*
825, E	120	11	*987*, U	120	13
1054, U	220	46	*1025*, U	160	17
1063, U	120	26			
Medians	120	17	Medians	160	22

* Animals that failed to reach criterion.

sonic method for producing lesions. Operated control cats do as well in avoidance conditioning as unoperated animals.

The control group sustained a variety of lesions, none of which produced complete bilateral mammillothalamic tractotomy. The lesions in the brain of *cat 632* are shown in Fig. 2. This cat was used in the visual-discrimination experiment. No lesions were found in the brain of *cat 561* so no photomicrograph for this brain is shown. The lesions in the brains of the remainder of this group are illustrated by the top ten photomicrographs in Fig. 3. Lesions in two of the brains (*cats 823* and *825*) effected unilateral mammillothalamic tractotomy. In five brains (*cats, 556, 559, 725, 740*, and *822*) minimal damage to the MThTs was produced. (Note the tiny spot lesion in the MThT on the right side in *cat 723*.) Two brains (*cats 1054* and *1063*) show more severe bilateral damage to MThTs. Postoperative conditioning scores exhibit no apparent relation with variations in lesions.

The results for the 11 cats with bilateral mammillothalamic tractotomies (shown in Table 3*B*) are equivocal. Seven of the animals had learning scores falling well within the range of scores of the control cats, while four cats performed very poorly and failed to reach criterion. In each case training was stopped when a cat reached criterion or when it seemed clear that a cat was not improving in performance or was actually retrogressing, i.e., the number of shocks per training session was gradually increasing. Animals that failed to reach criterion tended to develop fixated and rigid response patterns of tonic immobility in which they crouched in bizarre postures and either did not move until the CS-US interval terminated and shock came on (when they usually made a quick escape response), or they responded so slowly or started so late in the 10-sec. CS-US interval that they frequently received shocks. This nonadjustive behavior tended to persist for many trials.

Of the four cats that failed to achieve criterion, three had been used as subjects in Experiment 2. Lesions in the brains of these cats (*626*, *635*, and *639*) are illustrated in Fig. 2. Two of these cats (*626* and *639*) had very small lesions restricted almost completely to the MThTs. *Cat 635* had larger lesions which extended into the hypothalamus below MThTs and also severed the fornix on both sides. A photomicrograph showing the lesions in the brain of the fourth cat that did not learn (*754*) appears in the bottom group of pictures in Fig. 3. Both MThTs were cut by small electrolytic lesions. The rest of the cats in the experimental group all performed within the normal range, and their lesions were small. Lesions in the brains of *cats 620*, *633*, *640*, and *641* are illustrated in Fig. 2. The lesions in the brains of *cats 726*, *987*, and *1025* are shown in Fig. 3.

There is certainly no obligatory deficit in the acquisition of avoidance responses following bilateral mammillothalamic tractotomy. The finding that most of the tractotomized cats learned with normal facility but that 4 of the 11 animals failed to learn to criterion suggests, but does not prove, that the lesions can produce a behavioral deficit in avoidance conditioning, probably in interaction with unknown variables in the training conditions or in preoperative temperament of the cat. Nevertheless the group difference between control and tractotomized cats in avoidance conditioning is not statistically significant (U-test).

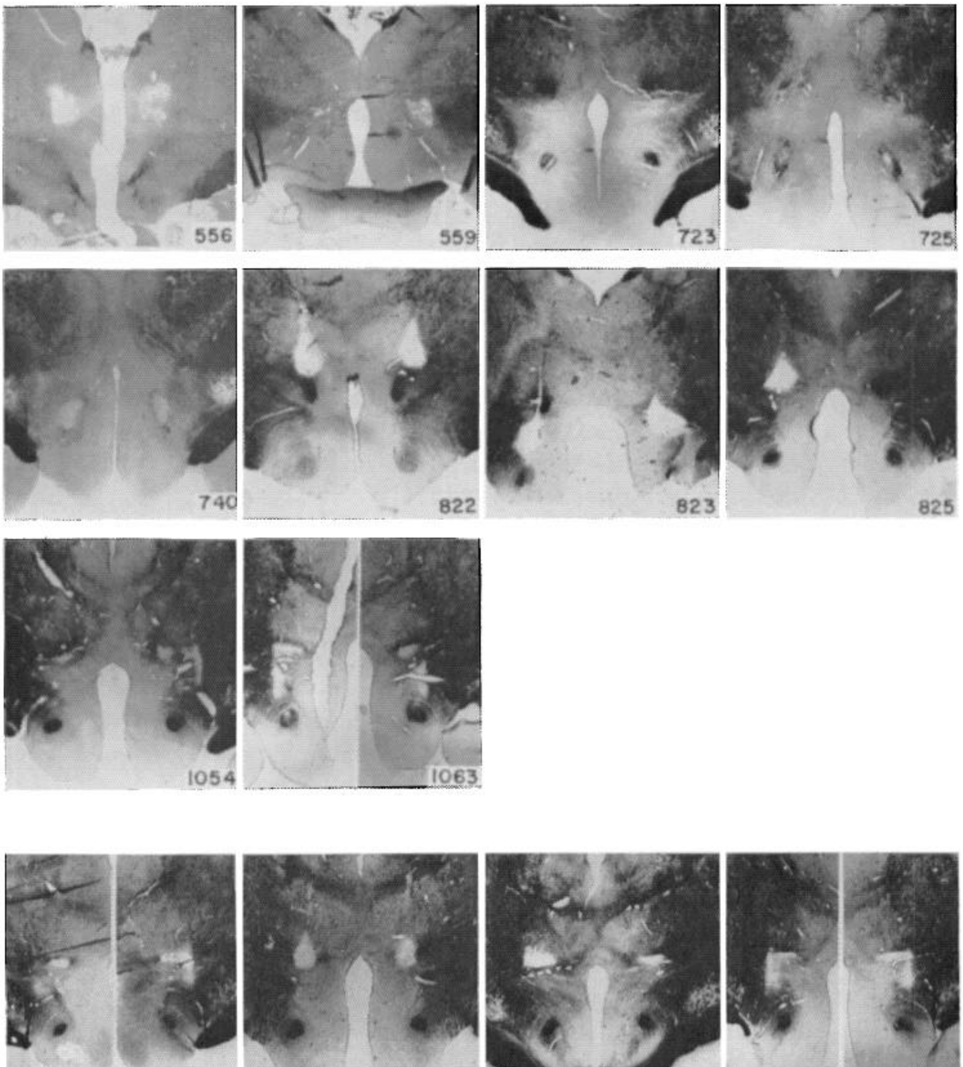

FIG. 3. Photomicrographs showing largest extent of lesions (Experiment 3: postoperative avoidance conditioning). The top three rows of pictures show lesions in the control group in which the mammillothalamic tracts were not severed. The bottom row of pictures illustrate lesions in the experimental group with complete bilateral mammillothalamic tractotomy. The thin line dividing some of the pictures in the middle indicates that two different sections were photographed in order to show the lesion at its maximum size.

Discussion

The findings of the experiments reported here are that bilateral mammillothalamic tractotomy in cats results in a significant impairment in retention of conditioned avoidance responses in the double-grill box. Bilateral interruption of the tracts has no effect on postoperative retention of visual form discriminations learned preoperatively under positive (reward) reinforcement. And finally, although group differences were not statistically significant, 4 of 11 cats with bilateral mammillothalamic tractotomy failed to acquire criterional level of avoidance responding in the double-grill box postoperatively, suggesting that the lesions can result in impaired performance in some cats. The results of the experiments clearly demonstrate that the mammillothalamic tracts are not without function which is measurably reflected in behavior.

The significance of these results regarding the functional role of the mammillothalamic tracts and their associated structures in behavior is not clear. Certain negative conclusions can be drawn. The observed deficit in postoperative retention of avoidance responding does not reflect some general impairment of neural mechanisms of memory because bilaterally mammillothalamic-tractotomized cats showed no loss in retention of a difficult visual form-discrimination habit. Observation of the general behavior of the cats yielded no evidence that mammillothalamic tractotomy impaired alertness or motor coordination.

Mowrer's theory of avoidance conditioning hypothesizes two factors: *1*) a classical (pavlovian) conditioning of "fear" to the CS through its paired presentations with the US (pain), and *2*) an instrumental response which is reinforced by reduction of the fear when the animal escapes from the conditioned aversiveness of the CS (11). This theory, in conjunction with Papez's theory that the mammillothalamic tracts comprise part of a forebrain circuit subserving emotional reactions (13), might suggest that the tractotomized cats show a deficit in retention in the double-grill box because they have lost their fear and thus are no longer motivated to respond. This does not seem to be the case. Cats undergoing aversive conditioning characteristically develop generalized fear of the experimenter, the testing room and the testing apparatus. When the experimenter approaches the animal's cage, it withdraws to the back, often hisses when it is picked up, and resists being removed from its home cage and placed in the testing apparatus. In the grill box the cat displays the usual autonomic and somatic signs of fearfulness. We did not observe any diminution of these behavioral characteristics in the cats after surgery. The cats that exhibited marked deficits in performance still displayed behavioral signs of intense fearfulness. On the other hand, cats trained on the visual-discrimination problem usually became very friendly and approached the experiment eagerly. After surgery no change was noted in this friendliness and eagerness to get to the testing apparatus where they would be fed. Any changes in the emotional behavior of the cats following bilateral mammillothalamic tractotomy were not obviously apparent in their cage behavior or in reactions to handling.

In view of the observations that humans may suffer disturbances of the temporal organization of experience or recent memories after lesions in some structures of the limbic system, it might be hypothesized that the cats, after bilateral mammillothalamic tractotomy, have a similar deficit, and that the shuttling response required in the double-grill box is peculiarly susceptible to disturbance of this capacity. However, if such were the case, one would expect to find a more consistent deficit in the tractotomized cats than was found in postoperative acquisition of the avoidance response (Experiment 3).

Another hypothesis is suggested by observations of what the tractotomized cats were doing instead of responding in the double-grill box during the retention test. Characteristically, the cats that failed to make avoidance responses to the CS showed a strong "freeze" response. They assumed a tense, crouched posture of tonic immobility, a response pattern that appears to be homologous with the "conditioned emotional response" described in the rat by Hunt and Brady (7). It would be plausible to suppose that bilateral interruption of the mammillothalamic tracts, by disturbing the normal balance of facilitory or inhibitory influences on downstream centers, produces an enhanced predisposition in the cat to freeze as a defensive response to overwhelmingly fearful stimuli. This innate defensive response is incompatible with the more adaptive response of fleeing to the opposite compartment in the double-grill box when the CS is presented. During retention testing, given under extinction conditions (no shocks), tractotomized cats tend to remain in this rigid, immobile posture, thus making none or few avoidance responses. When punishment is reintroduced during retraining, the cats are forced by the shocks to make escape responses and eventually they reach criterion level of avoidance responding, but only after receiving significantly more shocks than control cats. During postoperative avoidance acquisition the effect of bilateral mammillothalamic tractotomy becomes apparent only in interaction with other unknown factors such as misadventures during training (e.g., at a critical point in learning, the cat's foot slips and it is delayed in making its escape, and as a consequence receives an unusuallv long period of punishing shocks) or variations in preoperative temperament of the cat, e.g., boldness or timidity. Bilateral mammillothalamic tractotomy merely increases the likelihood that the innate response of freezing will become prepotent. The visual-discrimination problem, learned under positive reward, does not provide occasion for cats to develop the defensive freeze response, so the tractotomy has no effect on their performance.

A growing body of evidence suggests that the limbic system subserves the more primitive behavioral patterns significant for survival of the species and the individual (8). It seems likely that the deficit in postoperative performance of conditioned avoidance responding described in this report derives from lesion-induced alterations in neural mechanisms underlying species-specific behavioral dispositions. The behavioral loss is not believed to depend on disturbance of neural mechanisms subserving associative or storage aspects of memory.

Summary

1. The effects of bilateral mammillothalamic tractotomy on behavior of cats were evaluated with three behavioral tests. Lesions were produced by stereotaxically positioned, focused ultrasound and by conventional d.-c. electrolytic fulguration.

2. Cats that sustained complete bilateral transection of the mammillothalamic tracts showed a marked deficit in retention and relearning of preoperatively learned avoidance responses compared with the performance of sham-operated control cats or cats in which the lesions failed to interrupt the tracts completely.

3. No disturbance in the postoperative retention of a positively reinforced visual-discrimination response was found after bilateral mammillothalamic tractotomy.

4. Bilaterally tractotomized cats as a group did not differ to a statistically significant degree from control cats in postoperative acquisition of conditioned avoidance responding; however, scores from the 11 tractotomized animals appear to fall into two distinct sets. Seven of the tractotomized cats learned rapidly with conditioning scores well within the range of scores of control cats. On the other hand, four of the experimental cats were severely impaired and failed to achieve criterional level of avoidance conditioning. The strikingly deviant performance of this latter set of four cats indicates that mammillothalamic tractotomy affects acquisition of avoidance conditioning in some cats.

5. It is concluded that the postoperative loss of retention and impaired relearning of conditioned avoidance responses is not attributable to disturbance of neural mechanisms subserving associative or storage aspects of memory because no disturbance of retention of the positively rewarded visual-discrimination habit was found. From qualitative observations of the cats' behavior it is hypothesized that the loss of retention of the avoidance habit (Experiment 1) derives from lesion-induced enhancement of an innate defensive response of "freezing" as a reaction to the conditioned stimuli which is incompatible with the avoidance response.

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