Approach-avoidance, Stress Response, and Body Temperature of Dogs Following Removal of the Mamillary Bodies

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유두체를 떼어버린 개의 접근-회피반응, 스트레스에 대한 반응 및 체온 변동

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유두체의 기능을 탐색하기 위하여 외과 수술에 의하여 유두체를 떼어버린 개를 마련하고 수술전에서부터 수술후에 걸쳐 다음의 세가지 실험을 실시 하여 그 성적을 비교하였다.

첫째 실험에서는 먹이 먹는 개의 혀에 전기 충격을 가한다음 이어서 동물이 다시 먹이 그릇에 접근하기까지의 시간을 측정하여 동물이 무서움를 타는정도를 짐작하였으며, 둘째 실험에서는 높은 소리(12,000 cps, 100 db)를 한 시간 동안 동물에게 들려 스트레스로 삼고 혈액 호산구 수의 변동을 추적하여 스트레스에 대한 반응의 지표로 삼았다. 셋째 실험에서는 아침과 저녁 일정한 시간에 항문 온도를 반복 측정하였다.

실험 결과는 다음과 같다.

1. 유두체 제거후의 동물이 유두체 제거전 보다 무서움를 더 혹은 덜 탄다는 증거는 없었다(제 1표).
2. 유두체가 제거된 후에도 스트레스에 대한 반응은 유두체 제거전과 같은 정도로 현저히 나타나나 스트레스에서의 회복과정은 유두체 제거전에 비하여 유두체 제거후에 유의하게 지연되었다(제 1도).
3. 유두체가 제거된 동물의 체온은 유두체 제거전에 비하여 떨어지는데 그 정도는 미약하나 통계적으로 유의한 차이를 나타내었다(제 2표).

A variety of functions such as memory (Gamper, 1928), sexual activity (MacLean & Ploog, 1962), stress mechanism (De Groot & Harris, 1950), food intake (Maire & Patton, 1956), and general activity (Ranson, 1939) have been assigned to the mamillary bodies. The fact betrays our ignorance of the role played by this hypothalamic structure in the integrative activity of the central nervous system.

In a previous work the laboratory was unable to affirm any deficit in memory function following surgical removal of the mamillary bodies in dogs (Kim, Chang & Chu, 1967). As a continuation of this series of work, the present study deals with tests in approach-aviodance and stress situation, and also with measurement of body temperature using the same mamillary body-removed dogs.

GENERAL METHOD

Subjects

Eight pointer dogs, 2～3 year of age, were the subjects of this study. Each subject was maintained in an individual cage.

Surgical Technique

Details of technique utilized in surgical removal of the mamillary bodies was described in the previous communication (Kim, Chang, & Chu,

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1967), but it was briefly as follows. The subjects were anesthetized with intravenous pentobarbital sodium(30 mg/kg). With the right side of the head up, a skin incision was made aseptically between the rostral midline region of the head and the right zygomatic area, followed by splitting of the temporal group of muscles. Care was taken to preserve the facial nerve. With the mouth wide open, thus lowering the coronoid process of the mandible below the level of the zygomatic arch, most of the right temporal region of the skull, including a part of the zygomatic arch, was removed. In this manner the base of the skull was easily approached. The head was tilted in order to elevate the base of the brain, facilitating exposure of the mamillary bodies. The arachnoid was incised in the area of the mamillary bodies. The mamillary bodies were pricked with a spinal needle, then removed using an 18 gauge needle as a suction tip. The dura was closed and the wound was sutured in layers. Postoperative experiments were carried out 3 weeks after surgery.

Histology

After termination of the allocated experiment, each subject was sacrificed with deep pentobarbital sodium anesthesia and the brain perfused with saline followed by 10% formalin. A cube of brain tissue containing a mamillary lesion was embedded in celloidin. Coronal sections were cut at 20~30μ. Every fifth section was stained with cresyl violet and luxol fast blue, after Klüver and Barrera (1953).

Experiment 1. Approach-avoidance Resonse

The mamillary body is a link of Papez's circuit comprising the hypothalamus, the anterior thalamic nuclei, the gyrus cinguli, the hippocampus, and their interconnections (Papez, 1937). Papez hypothesizes that this group of structures represents the anatomic basis of emotion. After reviewing the literature he concluded that a tumor involving the mamillary body, the fasciculus of Vicq d'Azyr or the anterior thalamic nuclei produces loss of "emotive dynamics." Thomas et al. (1963) found that cats that failed to acquire conditioned avoidance following mamillothalamic tractotomy showed a strong "freeze" response, assuming a tense, crouched posture of tonic immobility.

In the course of stimulation experiment of the limbic structures, the senior author had an opportunity to stimulate the mamillary bodies of freely moving cats through permanently implanted bipolar electrodes (Kim, 1963). With stimulation intensity over 0.6 ma, the animals became alert, sniffed, searched the environment visually, and showed pupillary dilatation. Rage responses were never observed by stimulation of the mamillary body in sharp contrast with the furor produced with stimulation of the neighbouring portions of the hypothalamus. One female cat showed a response resembling fear when she received very strong stimulation(biphasic square wave pulses of 2 ma peak-to-peak intensity and 2 msec. duration for 10 sec.).

In the present experiment we attempted to discover whether or not a mamillary mechanism exists which facilitates or inhibits the fear response. We examined the possibility by testing the subjects in the approach-avoidance situation.

METHOD

Subjects

Six dogs including two females (Lucy & Mary) were tested, two of them (Mary & Cemy) through the pre- and postoperative periods. Two other subjects (Lucy & Kepy) were tested exclusively in the preoperative period and the remaining two subjects (Paul & Jong) only in the postoperative period.

Apparatus

A wire-mesh cage (150×80×90 cm) provided with a window (10×18 cm) and a floor covered with galvanized iron sheet was used. A metal

Table 1. Pre- and postoperative response latency (sec.) of postpunishment trial in approach-avoidance situation

Session with postpunishment trial	Subjects					
	Cemy	Mary	Kepy	Lucy	Jong	Paul
Preoperative period						
1	54	24	54	240	—[b]	—
2	36	3	54	310	—	—
3	57	14	36	300	—	—
4	18	300	38	∞	—	—
5	14	∞[a]	23	∞	—	—
6	8	∞	12	∞	—	—
7	6	∞	11	∞	—	—
8	9	∞	8	∞	—	—
Postoperative period						
1	32	∞	—	—	5	1
2	30	∞	—	—	120	60
3	16	∞	—	—	110	50
4	14	∞	—	—	90	36
5	7	∞	—	—	54	18
6	6	∞	—	—	36	15
7	7	∞	—	—	13	36
8	7	∞	—	—	12	11

[a]— : The subject did not respond in 15 minutes time limit.
[b]— : The subject did not receive test.

food tray was placed on a shelf beneath the window. Wire connections were made so that when the subject standing on the galvanized iron sheet touched the food tray, an electric circuit was closed causing a mild electric shock from a 2 volt, 60 cycle alternating current source. The experimenter could open or close the circuit unnoticed by the subject.

Procedure

Each subject was tested in the morning after a 12-hour fast. With the subject in the cage, the experimenter placed a piece of bait (dried fish) in the food tray and pushed it through the window close to the subject, allowing the subject to take the bait undisturbed. This procedure was carried out 17∼18 times per day. Generally the electric circuit remained open, but approximately once in every 60 bait presentations it was closed in an unpredictable fashion so that the subject unexpectedly received an electric shock while taking the bait. Immediately after a punishing trial the food tray with new bait was pushed close to the subject. The placing of a new bait in the presence of the subject was necessary because animals would not take the bait in the postpunishment trial unless it was replaced. The time lapse between the electric shock and the first postpunishment intake of a bait was termed "response latency" and served as an index of intensity of fear. A postpunishment trial was terminated within 15 minutes whether or not the subject responded.

All the sudjects were pretrained in the cage for about 10 sessions without shock and were thoroughly adapted to the situation so that they responded immediately to the bait upon presentation. The pretraining sessions were followed by the preoperative test sessions. Surgery was then performed with a 3-week rest followed by the postoperative trial sessions. Eight punishing trial sessions were conducted both pre- and postoperatively.

RESULTS

As shown in Table 1, there was no substantial difference between the pre- and postoperative performances. After experiencing 4 punishing trials in the preoperative testing sessions, dog Mary would not respond to the incentive in the remaining postpunishment trials and not at all in the postoperative period. Dog Lucy ceased to respond after 3 punishing trials in the preoperative period. Dog Cemy always responded. The response latency of this subject was actually shortened postoperatively ($p=.05$, Wilcoxon). Comparison of the performances of dogs Cemy and Kepy in the preoperative period with those of dogs Jong and Paul in the postoperative period showed no significant difference in the response latencies.

The measurement of response latency revealed two different tendencies. In 4 males (Cemy, Kepy, Jong, & Paul), the response latency tended to become short as the punishing trial was repeated. However, the remaining two females (Mary & Lucy)ceased to respond in the postpunishment trial as the punishing trials accumulated.

Experiment 2. Response to Sound Stress

Through stimulation and lesion experiments by De Groot and Harris (1950), and subsequently by Porter (1953), it has been reported that the tuberal and mamillary regions of the hypothalamus have important connections with the stress mechanism. The exact hypothalamic site which controls the stress mechanism, however, is still not positively defined. Is the mamillary bodies the seat of stress mechanism or at least a part of it? An answer to this question was sought by comparing the pre- and postoperative responses of subjects to sound stress.

The present experiment was also prompted by our observation (Kim & Kim, 1961) that a hippocampal lesion retarded recovery from the stress response in rats. Since the hippocampus projects a large number of its efferent fibers through the fornix to the mamillary bodies, it was felt desirable to see whether the hippocampus exerts its influence upon the stress mechanism through the mamillary bodies.

METHOD

Subjects

Three dogs that had unterwent the approach-avoidance test were subjected to this experiment. One subject (Cemy) was used through the whole experimental period, another (Kepy) only in the preoperative period, and the remaining one (Paul) in the postoperative period.

Apparatus

A wooden box(105×85×65 cm) with a tweeter (high frequency speaker) 100cm above the top was prepared, so that the subject in the box was exposed to a stressful sound of 12, 000 cps at 100 db. sensation level through an oscillator.

Procedure

Each animal was subjected to repeated venepuncture without anesthesia and was thoroughly adapted to the situation until the blood sampling could be done with minimal stress. In the main experiment blood eosinophil cell counts, using the method described by Thorn et al. (1948), were carried out at 9 : 00 A.M. for 3 days before a stress session. On the 4th day, the suject was exposed to the sound stress for one hour. Eosinophiles were counted immediately before stress, and also 0, 2, 4, and 6 hours after termination of stress, and thereafter only once a day at 9 : 00 A.M. The exposure to stress was carried out once every 4th day until the subject received 6 exposures both in the pre- and postoperative periods. The preliminary experiment demonstrated that the blood eosinophil count regained the control value in 3 days. Only a limited number of subjects was available because of the difficulty in drawing blood samples without inflicting significant stress in unanesthetized animals.

RESULTS

In Figure 1, the mean eosinophil counts in 6 stress sessions were expressed against the time post stress. As indicated in the figure, the pre- and postoperative eosinophil counts decreased to the same extent until 2 hours after termination of the stress. However, counts after 4 and 6 hours showed that the recovery process was significantly retarded following removal of the mamillary bodies ($p=.05$, C.R.).

Experiment 3. Body Temperature

The posterior hypothalamus is the general region in which mechanisms of heat generation and conservation are located. However, neither lesion nor stimulation experiments seem to have defined the exact location of the latter mechanism (Ström, 1960). It is equally obscure whether the mamillary body, which is a structure in the posterior hypothalamus, participates in the regulation of body heat. Therefore, we tried to determine if isolated removal of the mamillary bodies

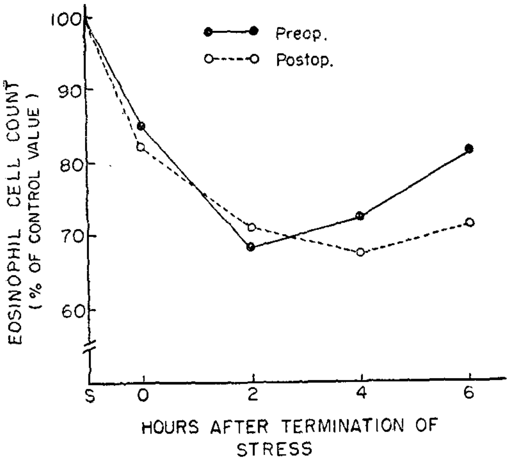

Fig. 1 Pre- and postoperative eosinophil cell counts (percentage of control value) following a stressful sound (12,000 cycle, 100 db) which was administered for one hour between S and O on the abscissa.

Table 2. Mean rectal temperatures(°C±S.D.) before and after removal of mamillary bodies

Subjects	Preoperative period		Postoperative period	
	N. of Measurement	Mean Temperature	N. of Measurement	Mean Temperature
Mamillary body removed				
Cemy	20	38.4 ±0.18°	20	38.2 ±0.14°
Domy	20	38.3 ±0.25°	20	38.1 ±0.23°
Jong	20	38.6 ±0.25°	20	38.2 ±0.26°
Paul	20	38.3 ±0.23°	20	38.1 ±0.27°
Total	80	38.4 ±0.36°	80	38.15 ±0.33°
Normal control				
Malk	20	38.2 ±0.12°	20	38.2 ±0.19°

would produce change in body temperature.

METHOD

Subjects

Five dogs(Cemy, Domy, Jong, Paul, and Malk) were the subjects. Among them Malk served as unoperated control animal.

Procedure

A clinical thermometer with decimal centigrade scale was used. Four centimeters of the tip of the thermometer was introduced into the anus and the body temperature was read 1 minute later. The measurement was carried out twice a day at 8 : 00 and 20 : 00. This measurement was repeated 20 times (10 days) each in the pre- and postoperative periods.

RESULTS

As shown in Table 2, the rectal temperature of the experimental subjects dropped slightly, but significantly following surgery ($p = .05$, Mann-Whitney U). The body temperature of the control animal did not change significantly throughout the experiment.

Histological Findings

Macroscopic examination of the fixed brains revealed in some animals slight superficial damage on the inferior operated temporal lobe cortex. The hypophysis was never damaged.

Representative sections through the brain lesions of the subjects are shown schematically in Figure 2. To indicate the location of the mamillary bodies, the unsuccessful brain lesion of dog Abes is also shown in the figure. Except in one case (Domy), the rostral margin of the brain lesions of all experimental subjects was located at premamillary region, producing damage of varying size to the premamillary nuclei. In the brain of dog Domy, the lesion extended to the posterior margin of the tuberal region where a slight tissue defect arose from the left wall of the 3rd ventricle and directed dorsolaterally between the left fornix bundle and the mamillothalamic tract without seriously damaging the ventromedial and dorsomedial hypothalamic nuclei.

In two animals (Cemy & Jong), the lesions in the mamillary region were too extensive in the

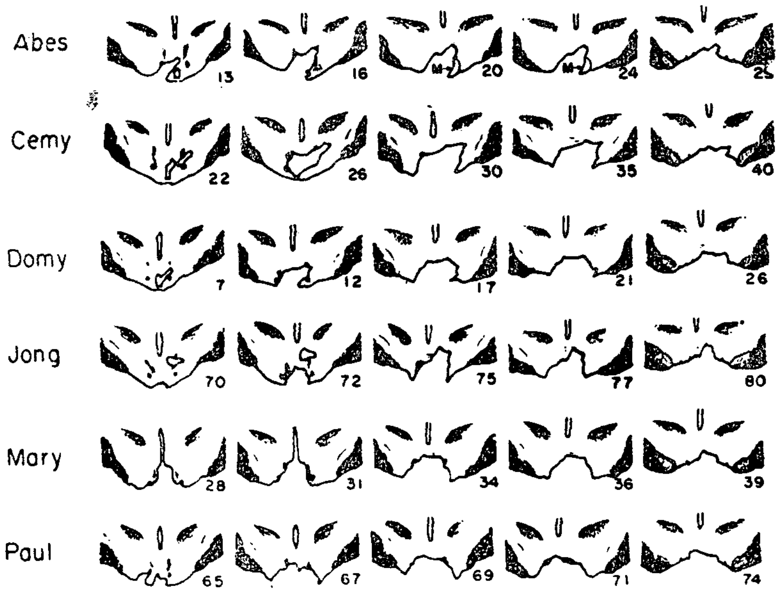

Fig. 2. Lesions in the brains of animals in which surgical removal of the mamillary bodies was performed. Beginning from the left, the first two drawings represent lesions in the premamillary region, and the third and fourth show lesions in the mamillary region, while the last drawing on the right represents a lesion in the retromamillary region. M=mamillary body.

levodorsal direction, perhaps due to technical difficulties in securing appropriate visual field during the surgical approach. In these subjects the medial forebrain bundle on the left side was partly or completely severd. The zona incerta was also damaged on this side. In the remaining subjects the lesion did not extend much beyond the left and dorsal boundary of the mamillary bodies. The right boundary of the lesion coincided rather exactly with that of the mamillary bodies in all brains. In no operated experimental subjects was the mamillary tissue identifiable, except that a tiny tissue fragment with a few nerve cells of the medial mamillary nuclei remained at the dorsal margin in dog Paul, and at the right dorsolateral margin in dog Jong.

Caudally the lesion dwindled rapidly in the retromamillary region, though in a few cases it deviated slightly to the left. In the brain of dog Jong, the lesion extended caudalward for some distance but did not reach the level of interpeduncular nucleus. The subthalamic structures were never encroached upon.

DISCUSSION

In the approach-avoidance situation in the present study, a female dog (Mary) that would not approach the food tray after receiving a few punishing preoperative trials showed the same reluctance to approach following surgery. On the other hand, dog Cemy, that always responded to the postpunishment trial preoperatively, did so in the postoperative period. Also the response latencies of our subjects revealed no significant difference between the pre- and postoperative performances. Thus the results demonstrate no indication of increased or reduced fear following removal of the mamillary bodies. Was the fear response elicited by the senior author (1963) in a cat upon a strong electric stimulus to the mamillary bodies caused by current spread?

Akert and Andy (1955) found no change in emotional expression following removal of the mamillary bodies in cats. Krieckhaus (1964) interpretes the freezing of his mamillothalamic tract-damaged cats under the two-way avoidance situation as the result of accumulating anxiety engendered by being forced to solve a difficult problem rather than a cause of reduced avoidance response following the mamillothalamic tract injury. On the other hand, a reduced emotional response has been noted following damage to the mamillary bodies by Ranson (1939), and placidity of affect following lesions of the mamillothalamic tracts by Meyer and Hunter (1952). It seems premature to draw any conclusion as to whether the mamillary body participate in emotional activity.

The approach-avoidance response may also be regarded as an index of inhibitory capacity. Again we do not find any postoperative change in the response of our subjects in the light of this interpretation.

During the early hours after stress, pre- and postoperative stress responses of our subjects were of similar magnitude. Recovery from the postoperative stress response was found to be significantly retarded, however, compared with the preoperative response in the later hours following stress. These findings may be interpreted in two ways. First, it may suggest that the mamillary bodies do not significantly participate in the production of stress response itself, but may be chiefly concerned with the recovery process from stress. Earlier we found retarded recovery from chronic surgical stress in rats following damage to the hippocampus. The similarity of the two findings suggests itself to us, though the comparison may be premature. Our finding of normal postoperative stress responce may also be interpreted to indicate that the mamillary bodies as well as other central nervous system structures (e.g. tuberal region of the hypothalamus) participate in the stress mechanism on the asusmption that the stress response is normal as long as a part, at least, of the stress mechanism remains undamaged.

De Groot and Harris (1950) produced a bilateral electrolytic lesion which completely destoyed the mamillary bodies without surrounding injury in their rabbit No. 280. A lymphopenic response was not observed in this animal following emotional stress. This finding is at variance with ours, whether we explain our findings in the light of the first or the second interpretation cited above. At present we do not have an explanation of the discrepancy between the two findings.

All of our subjects had slightly, but significantly subnormal body temperatures following removal of the mamillary bodies. Ranson's monkey No. 15 showed no disturbance in temperature regulation following removal of the mamillary bodies. His observation is too limited and the change in body temperature of our subjects is too slight to compare.

Summing up the results of the present study, we found no definite change in fear response, a normal stress response with significantly retarded recovery, and a slight but significant reduction in body temperature following removal of the mamillary bodies. However, further works seem desirable before we can accurately define the role of the mamillary bodies in the above functions, as our study is limited in scope and also as the possibility cannot be denied that incidental damage to other structures may have affected our results.

SUMMARY

As a continuation of a series of work on the physiology of the mamillary bodies, 3 experiments were carried out using 8 pointer dogs subjected to surgical removal of this hypothalamic

structure by subtemporal approach.

In the first experiment, animals were tested pre- and postoperatively in approach-avoidance situation. Food served as incentive, electric shock to the tongue as punishment, and response latency of postpunishment trial as an index of fear. The second experiment dealt with pre- and postoperative tests in stress situation. A high frequency sound (12,000 cycle, 100 db sound for 1 hour) was regarded as a stressor, and decrease in blood eosinophil cell count as an index of response to the stress. Pre- and postoperative measurement of rectal temperature was carried out in the third experiment, using a clinical thermometer with decimal centigrade scale.

The results obtained were as follows:

1. Tests in approach-avoidance situation showed no indication of increased or decreased fear response following removal of the mamillary bodies.

2. Postoperative stress response was as marked as that of preoperative period, but the recovery from the stress was significantly retarded after surgery.

3. The body temperature dropped slightly, but significantly following damage to the mamillary bodies.

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