

## Effect of Damage to the Amygdala on Stress Mechanism in Rats

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As the view is widely held that limbic structures may be concerned with emotional life<sup>1)</sup> and as the amygdaloid nucleus is intimately connected with the hypothalamus through fiber tracts such as the stria terminalis, it seems natural to suspect a possible influence of the amygdala upon the stress mechanism which is known to be integrated in the hypothalamus. Although there are already a few reports on this matter with controversial results<sup>2-5)</sup>, the present study is planned to test the matter anew.

### Materials and Methods

Twenty-six male Sprague-Dawley rats weighing around 300 gm. were used. They were divided into two equal groups and in one group the amygdaloid nuclei on both sides were destroyed electrolytically under Nembutal anesthesia (amygdala-damaged group). The electrode employed for the electrolysis was a straight piano wire insulated except at the tip. Utilizing de Groot's coordination<sup>6)</sup>, the electrode was implanted stereotaxically to the site of A5.8, L4.3, H2.4, and a direct current of 3mA. intensity was applied for 30 seconds between this electrode as anode and a subcutaneous needle electrode as cathode. In the other group, all procedures including placement of electrode in the amygdala on both sides were carried out as in the amygdala-damaged group, except that current was not passed. This group served as control.

Seventy-two hours after the electrode placement, every animal was subjected to laparotomy under ether and the left adrenal gland was excised. Usually this procedure required no more than 5 minutes, and was regarded as a stress. The abdominal cavity

was then closed with a few stitches and the animal was kept in a quiet place for four hours before it was sacrificed by rapid decapitation and the right adrenal gland was removed.

The ascorbic acid content of left and right adrenal glands was measured separately and the difference in the amount (mg/100 gm. sample) was regarded as an index of reaction to the stressful stimuli. Each adrenal gland was weighed wet, ground with sand in 5% metaphosphoric acid solution, and the ascorbic acid content of the extract was measured colorimetrically with the Evelyn photoelectric colorimeter utilizing the reduction of 2,6-dichlorophenolindophenol sodium.

All animals were deprived of water and food from the time of electrode placement on to the end of the experiment. These measures were taken in consideration of the preliminary observation that all amygdala-damaged rats stopped eating and drinking.

After completion of the experiment the brains were fixed in 10% formaldehyde and embedded in celloidin. Coronal sections were cut at 20 $\mu$  thickness, and every tenth section was stained with luxol fast blue and cresylechtviolet following Klüver method. Microscopic examination of the brains with amygdaloid damage (Fig. 1) revealed that the lesions on both sides were round or oval in shape, mostly with the diameter of about three millimeters. Usually the lesion extended from around A5.0 to A7.8 approaching pyriform cortex laterally, reaching globus pallidus and part of optic tract medially, and somewhat encroaching upon the striatum dorsally at the level of the rhinal fissure, but frequently it did not reach the orbital surface of the brain. Among the amyg-

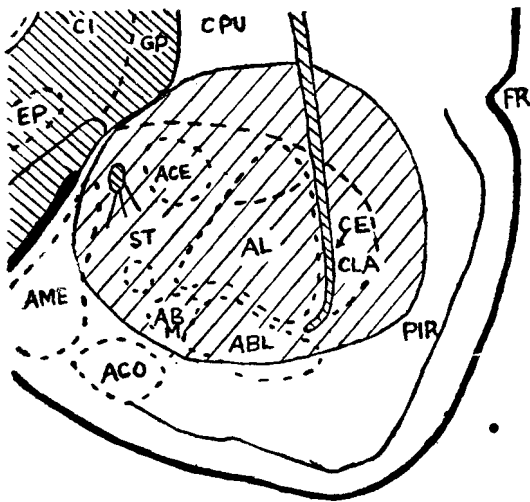


Fig. 1. Structures most commonly involved in the electrolytic lesion (shaded area in the center). ABL, Nucleus amygdaloideus basalis pars lateralis; ABM, Nucleus amygdaloideus basalis pars medialis; ACE, Nucleus amygdaloideus centralis; ACO, Nucleus amygdaloideus corticalis; AL, Nucleus amygdaloideus lateralis; AME, Nucleus amygdaloideus medialis; CE, Capsula externa; CLA, Clastrum; CPU, Nucleus caudatus/Putamen; EP, Nucleus entopeduncularis; FR, Fissura rhinalis; GP, Globus pallidus; PIR, Cortex piriformis; ST, Stria terminalis

dalar nuclear masses commonly involved in the lesion were Nuc. amygdaloideus lateralis, Nuc. amygdaloideus centralis, and Nuc. amygdaloideus intercalatus. At least part of the Area amygdaloidea anterior, Nuc. amygdaloideus basalis, and Nuc. amygdaloideus medialis were involved in the damage, but the Nuc. amygdaloideus corticalis and neighbouring part of Nuc. amygdaloideus medialis frequently escaped damage. There were structures other than the amygdaloid nuclear complex mostly involved in the damage. They were clastru mand part of striatum. Sometimes the lateral border of the pyriform cortex was also found damaged. Sections of brains of control rats showed slight damage along the needle tract which usually coursed through the striatum. Slight lesion along the needle tract was also noticed in the amygdala-damaged brains.

### Results

The results are summarized in Table 1. Both amygdala-damaged group and control group reacted to the stress with marked depletion of adrenal

ascorbic acid content. But the decrease in adrenal ascorbic acid content following stress was less pronounced in the amygdala-damaged group than in the control animals. According to the t test, the difference in adrenal ascorbic acid decrease between amygdala-damaged and control group was significant at 2% level.

Following lesion, all amygdala-damaged rats stopped eating and drinking. In addition, they became very quiet and inactive in contrast to the control animals, which began to move about and search for food and water within 24 hours following electrode placement. The final mean body weight of the amygdala-damaged rats and control animals were 72.2% and 75.3%, respectively, of those (the mean body weight) at the beginning of the experiment.

Table 1. Decrease in Adrenal Ascorbic Acid Content

Group	N	Decrease in ascorbic acid (mg/100 gm. sample)	S.D.
Amygdala-damaged	13	216.2	65.71
Control	13	302.5	83.75

t test(amygdala-damaged vs control):  $t=2.796$   $p<.02$

### Discussion

The result of the present experiment that the decrease in adrenal ascorbic acid content following stress was less pronounced in amygdala-damaged rats than in control animals seems to indicate that normally the amygdaloid nucleus exerts chiefly facilitatory influence upon stress mechanism. The result, however, does not tell whether there is no inhibitory mechanism in this nucleus.

Mason<sup>2)</sup> worked with monkey and found significant increase in plasma corticosteroid content following stimulation of amygdaloid nucleus. Martin et al.<sup>3)</sup> on the other hand, reported that removal of the amygdaloid nuclei in cats resulted in a marked increase in adrenal cortical secretion associated with a reduction in general activity and resistance to emotional stimuli. There is still another report by Kling et al.<sup>4)</sup> who obtained comparable adrenal ascorbic acid depletions following operative stress (laparotomy) from normals and amygdaloidectomized cats with varying removals of hippocampus, cingulate gyrus, posterior septum,

fornix, and anterior midline thalamic nuclei. Yamada and Greer<sup>5)</sup> also obtained similar result. They noted an increased adrenal weight in rats with electrolytic lesion of the amygdala. While they felt that the increased adrenal size was probably a non-specific result of the experimental procedure, they thought the adrenal hypertrophy was apparently related to the increased adrenocortical secretion since thymic involution was observed in all lesioned animals. Therefore they concluded that the amygdala was not essential for the increased ACTH release in the rat.

It is not always clear what is the cause of discrepancies among these data. It may be that the amygdala contains both facilitatory and inhibitory sites to the hypothalamic stress mechanism, and individual workers have studied chiefly one or the other site of the amygdala. Another possibility is that individual workers studied under different conditions. Unfortunately the detail of the experimental procedure Martin et al. adopted is not available, but Kling et al., for example, worked on animals with extensive limbic lesions involving hippocampus. The hippocampus appears, according to the work of the author, to be chiefly inhibitory to the stress mechanism.<sup>7,8)</sup> Besides, they inflicted operative stress to animals under pentobarbital anesthesia. The anesthesia may have obscured the difference in adrenal ascorbic acid depletion between operated animals and normal controls. Anesthetic dose of pentobarbital is said to decrease the resting level of blood ACTH significantly.<sup>9)</sup> The results of present experiment fully confirm the notion of Yamada and Greer that the amygdala is not essential for stress mechanism in rats, because marked depletion of adrenal ascorbic acid content is also seen in amygdala-damaged rats in the present experiment (Table 1). But the depletion is apparently less pronounced compared with that of control rats.

### Summary

Twenty-six Sprague-Dawley male rats weighing around 300 gm. were divided into two equal groups, and in one group electrolytic lesion was produced under Nembutal in the amygdaloid nuclei on both sides (amygdala-damaged group), while the other group which underwent exactly the same procedure

except passage of current served as control.

Seventy-two hours after the electrode placement, every rat was subjected to left adrenalectomy under ether and this procedure was regarded as a stress. Four hours after stress the animal was sacrificed by rapid decapitation and the ascorbic acid content of each adrenal gland was measured colorimetrically.

Both amygdala-damaged and control group reacted to the stress with marked depletion of adrenal ascorbic acid content. But the decrease in adrenal ascorbic acid content following stress was less pronounced in amygdala-damaged rats than in control animals. The difference was significant at 2% level.

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### 국 문 초 록

### Amygdaloid nucleus 가 파괴된 흰쥐의 stress 에 대한 반응

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Amygdaloid nucleus 가 파괴된 흰쥐와 대조흰쥐들에게 stress 를 준 다음 부신 ascorbic acid 함유량이 변화하는 모습을 비교관찰 하였다.

26마리의 Sprague-Dawley 종 흰쥐수컷을 두 군으로 나누어 한 군에서는 Nembutal 마취하에 Streotaxic method 를 써서 양쪽 amygdaloid nucleus 에 electrolysis 를 일으켰으며 (amygdala 파괴군), 나머지 한 군에서는 양쪽 amygdaloid nucleus 에 전극을 넣기까지의 모든 과정은 amygdala 파괴군에서와 똑같이 시행하였으나 전류만은 흘리지 않았다(대조군). 이후 실험이 끝날때까지 두 군은 모두 먹이와 물이 금지되었다.

Amygdaloid nucleus 에 전극을 넣은지 72시간될때 ether 마취하에 동물을 개복하고 부신을 적출하는 것으로써 stress 로 삼았다. Stress 를 가한지 4시간될때 오른쪽 부신을 마지 떼었으며 왼쪽과 오른쪽 부신의 ascorbic acid 함유량을 비교하여 stress 에 대한 반응정도를 짐작 하였다.

Stress 에 의하여 amygdala 파괴군과 대조군에서 모두 부신 ascorbic acid 함유량이 감소되었는데 amygdala 파괴군에서는 감소정도가 대조군에서보다 적었다. 이 차이는 2% 수준에서 유의하였다.

이상의 결과로 미루어 amygdaloid nucleus 는 stress 에 관련된 뇌하수체-부신피질계의 활동에 촉진성 영향을 끼칠 것으로 추측하였다.

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