Effect of Hippocampal Ablation on Chronic Stress in Rats

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In the course of search for the function of the hippocampus the author studied the effect of hippocampal ablation on chronic stress in rats in succession to the observation that the hippocampus appeared inhibitory to the pituitary-adrenocortical activity in acute stress situation.[1] The possibility of hippocampal influence upon stress mechanism has been contemplated because the hippocampus sends a massive fiber bundle fornix to the hypothalamus, which is generally recognized to play an important role in the integration of reactions to stressful stimuli.[2,3]

Material and Method

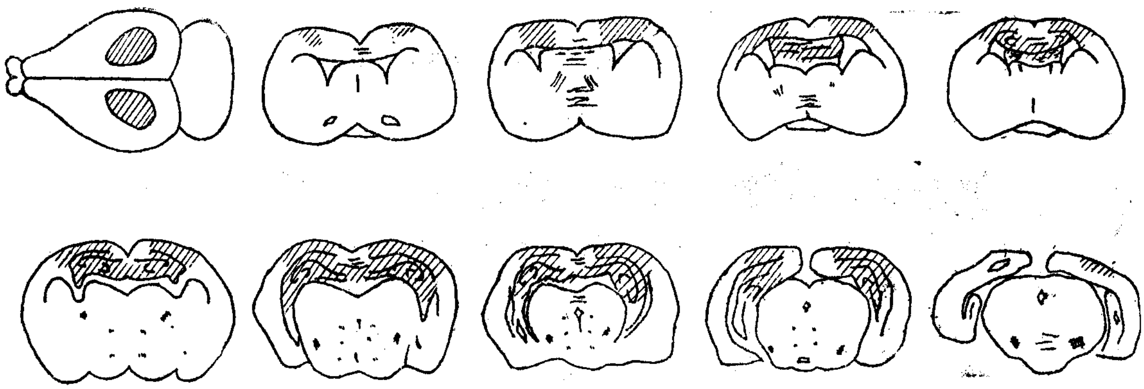
Sixty-eight male albino rats weighing 280 to 400 gm. were used. They were divided into three groups and subjected to hippocampal ablation, neocortical ablation, or kept intact as normal control.

The operative procedures were as follows. After removing most of the parietal bone on both sides excepting the part along the sagittal sinus, an oval-shaped portion of neocortex on the dorsal surface of the brain overlying the hippocamups was sucked out, and then an attempt was made to remove as much as possible of the hippocampus by suction. Special care was taken to remove the dorsal portion of the hippocampus and to interrupt the fimbria and hippocampal commissure without damaging the overlying mid-line cortical tissues. This preparation served as the hippocampus-ablated rat. A second group of rats was prepared in which only the portion of neocortex over the hippocampus was ablated. This was the neocortex-ablated rat.

More than three weeks after the brain ablation, all rats were subjected to left adrenalectomy under Nembutal around eight in the morning and this procedure was regarded as the beginning of chronic stress. Chronic stressful stimuli were continued by inflicting skin incisions of five centimeter in length. Under ether anesthesia four successive skin incisions were made: the first one on the right flank, the second on the left back, the third on the right back, and the last over the vertebra. The wound was immediately closed with several cotton sutures and 60,000 units of procaine penicillin was given intramuscularly.

Each of the three groups of rats were divided into three subgroups according to the stress procedures. These three subgroups were treated differently only in respect to the time of inflicting the stress and of sacrificing. One subgroup received daily skin incisions beginning on the day following left adrenalectomy; another received incisions on alternate days beginning on the second day following adrenalectomy. The third subgroup received incisions on alternate days like the second subgroup but were sacrificed at 72 hours after the final incision instead of at 48 hours as occurred in the first and second subgroups. The rats were sacrificed by rapid decapitation and the right adrenals were removed.

The ascorbic acid content of left and right adrenal gland was measured separately and the difference in the amount (mg. per 100 gm. sample) was regarded as an index of reaction to the stressful stimulus. 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.



<Fig. 1> **Brain tissues involved in the hippocampal ablation. Ablated brain tissues are schematically represented in shade.**

Examination of the brain of the hippocampus-ablated animals after completion of the experiment showed that practically all of the dorsal portion of the hippocampus between the mid-line cortical tissue and the thalamus extending from the level of the anterior commissure to the level of the posterior commissure had been successfully removed, while the posteroventral portion of the same structure caudal to the posterior commissure remained largely intact.

Results

The results are summarized in Table 1. In general, the adrenal ascorbic acid content increased following chronic stress. The increase was most marked in neocortex-ablated rats and least in hippocampus-ablated animals, while in the control animals the value was intermediate but almost the same as that of neocortex-ablated rats. In addition, the increase was somewhat more marked when the stressful stimulus was applied every other day than when it was applied every day, and much more pronounced when the right adrenal ascorbic acid content was measured 72 hours after the last stress than when it was measured 48 hours after.

<Table 1> **Adrenal ascorbic acid increase (mg. per 100 gm. sample) following chronic stress.**

Stress application, and hours sacrificed after last stress	Hippocampus-abl. Mean	S.D.	Neocortex-ablated Mean	S.D.	Normal control Mean	S.D.	Total Mean	S.D.
Every day, 48 hours	45.5 n=8	—	75.3 n=8	—	49.9 n=8	—	57.8 N=24	55.51
Every other day, 48 hours	53.5 n=8	—	108.3 n=8	—	101.8 n=8	—	87.9 N=24	73.92
Every other day, 72 hours	99.0 n=7	—	177.2 n=7	—	169.7 n=6	—	147.7 N=20	52.31
Total	**64.6 N=23**	**66.87**	**117.8 N=23**	**69.44**	**101.4 N=23**	**68.54**	**N=68**	

<Table 2> **Analysis of variance of the data from the chronic stress experiment.**

Source of variance	Σx^2	d.f.	σ^2	F-ratio	P
Among major groups (M) (ablations & control)	34,175	2	17,087	4.67	.05>P>.01
Among subgroups (S) (stress procedures)	91.602	2	45,801	12.53	<.01
Interaction	9,993	4	2,498		
Residual	215,668	59	3,655		
Total	**351,438**	**67**			

Analysis of variance of the results showed that the differences among the three major groups i.e. hippocampus-ablated, neocortex-ablated, and normal control groups were significant at 5% level(F-ratio =4.67), the differences among the subgroups according to stress procedures were significant at 1% level(F-ratio=12.53), while their interaction was without significance (Table 2).

The differences in adrenal ascorbic acid increase among the three major groups and among the subgroups were further analyzed by the t-test. According to this analysis, the difference was not significant between rats stressed every day and stressed every other day($t=1.61$, d.f.=47), but the difference was significant between rats sacrificed 48 hours and sacrificed 72 hours after the last stress($t=3.05$, d.f.=43). The difference between normal control and neocortex-ablated group($t=0.78$, d.f.=44) and between normal control and hippocampus-ablated group($t=1.78$, d.f.=44) were not significant, but the difference between neocortex-ablated and hippocampus-ablated groups was significant at 5% level($t=2.59$, d.f.=45).

Usually the adrenal weight increased following chronic stress, but no significant differences in adrenal weight increment could be ascertained among the three major groups, excepting that among the subgroups the least increase in adrenal weight was found in the group where the stress was applied every other day, and the right adrenalectomy was carried out at 72 hours following the last stress.

Discussion

Long[4] and others reported that the adrenal ascorbic acid content increased under chronic stress in Selye's stage of resistance[5]. While the general tendency was confirmed in this study, the present result also showed that the increase in adrenal ascorbic acid content was most marked in neocortex-ablated rats and least in rats whose hippocampus was ablated through neocortex, and was more pronounced when the right adrenal ascorbic acid content was measured 72 hours after the last stress than when it was measured earlier.

The inference from the results of the present study may be that the hippocampus is exerting sustained inhibitory influence upon the pituitary-adrenocortical mechanism. The mode of action of the hippocampus in stress situation is like a rebound or delayed reaction, being most pronounced late following application of chronic stress. Thus the hippocampus appears to act as a break to the pituitary-adrenocortical system protecting it from prolonged excessive activity and facilitating reparative processes in the same system.

Recently Mason[6] stimulated the hippocampus in monkeys and found marked decrease in plasma 17-OH-CS level 24 to 48 hours after stimulation. While his experiment was not particularly chronic in nature, the result seems to agree well with that of the present study.

Summary

Rats whose hippocampus was ablated through neocortex (hippocampus-ablated rats), rats in which only the portion of neocortex over the hippocampus was ablated (neocortex-ablated rats), and normal control rats were prepared. Their reaction to the chronic stress of repeated skin incisions was measured by comparing the adrenal ascorbic acid content before and after the stress. The results are as follows.

The adrenal ascorbic acid content generally increased following chronic stress in all preparations. The increment was least in hippocampus-ablated rats, and most pronounced in neocortex-ablated animals, while the value was intermediate in control rats.

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국 문 초 록

Hippocampus를 떼어버린 흰 쥐의 만성 stress에 대한 반응

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Hippocampus를 떼어버린 흰 쥐, neocortex를 떼어버린 흰 쥐 및 정상 흰 쥐들에게 만성 stress를 가하여 부신 ascorbic acid 함유량이 변화하는 모습을 비교 관찰하였다.

68마리의 흰 쥐 수컷들을 세 군으로 나누어, 한 군에서는 neocortex를 거쳐서 hippocampus를 떼고 (hippocampus 제거군), 다른 한 군에서는 hippocampus를 덮는 neocortex 부분만을 떼었다(neocortex 제거군). 세째 군은 정상 대조군이다.

수술후 3주일 이상되기를 기다려, 아침 여덟시 경에 쥐를 Nembutal 마취하에 개복하고 왼쪽 부신을 떼어 내는 것으로써 만성 stress의 시초로 삼었다. 이후 네번에 걸쳐 날마다, 또는 하루 건너 한번 씩 옆구리 내지 등허리의 살갗을 5cm의 길이가 되게 절개한 후 곧 다시 봉합하는 것을 되풀이하여 만성 stress를 계속하였다. 마지막 stress에서 48시간 또는 72시간 후에 오른쪽 부신을 떼었으며 왼쪽과 오른쪽 부신의 ascorbic acid 함유량을 비교하여 stress에 대한 반응정도를 짐작하였다.

만성 stress에 의하여 부신 ascorbic acid의 함유량은 일반적으로 많아졌는데 그 중에서도 neocortex 제거군이 제일 현저한 증가를 보여주었고, hippocampus 제거군에서 증가도가 제일 미약하였으며 정상군은 그 중간수준에 있었다. 또 마지막 stress에서 72시간 후에 오른쪽 부신을 뗀 군이 48시간 후에 뗀 군보다 더 현저한 ascorbic acid의 증가를 보여주었다.

이상의 결과로 미루어 hippocampus는 stress에 관련된 뇌하수체—부신피질계의 활동에 제지성 영향을 끼칠 것으로 추측하였다.

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