expansion was studied in young harbor seals. Negative pressure breathing did not increase rate of urine flow. Expansion of plasma volume with seal plasma or with 1% gelatin in saline caused a solute diuresis with increase in glomerular filtration rate similar to the changes seen in the post-prandial diuresis of the seal. It is suggested that the post-prandial diuresis of the seal is mediated *via* a volume receptor.

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Adrenocortical Response to Stress in Rats with Lesions in Hippocampus and Amygdala.* (26832)

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A continually mounting volume of research, summarized regularly(1-3), has explored mechanisms of neural regulation of ACTH secretion. The most intense interest is centered about isolation and characterization of the ACTH-regulating neurohumor(4-7) and identification of the hypothalamic area(s) in the region of the median eminence which represent the terminal link(s) between the central nervous system and the adenohypophysis(8-11). More recently attention has been directed also to other regions of the brain which may have direct connections with the final hypothalamic effector sites or which may influence indirectly the amount of ACTH neurohumor produced there. Among these areas are the cerebral cortex(12,13), limbic system(14,15), brain stem and reticular formation(12,16-18). The present report describes alterations in basal levels of plasma free corticoids (PFC) and in the pattern of PFC response to stress occurring in rats with lesions of hippocampus or amygdala.

Materials and methods. Bilateral electrolytic lesions (3 ma, 30 sec) were placed by stereotaxic means in hippocampus, amygdala and habenular complex of adult male rats. The insulated stainless steel needle used for lesioning was bared approximately 1 mm at its tip for placement of lesions in amygdala and habenula; for hippocampus, the needle tip was bared 1.5-2 mm. Using a stereotaxic instrument with reference planes adjusted to the atlas of de Groot(19), the anterior vertical and lateral coordinates used for lesioning amygdala, hippocampus and habenula were (+5.4, −2.8, ± 4.5), (+3.0, −2.5, ± 5.0) and (+3.4, +0.8, ±0.5) respectively. Two months after lesioning, I^{131} uptake was measured by counting the external neck activity 18-24 hrs after intraperitoneal administration of 5 μc carrier-free NaI^{131}; a region of the hind limb was counted as a measure of the non-thyroidal neck activity. After one additional month, peripheral PFC levels were determined. Basal PFC levels and PFC response to 30 min and 4 hrs of continuous physical immobilization (paws bound with adhesive tape) were examined in both control and lesioned animals. Animals were sacrificed by decapitation, blood collected in heparinized beakers and PFC determined fluorometrically according to Guillemin *et al.*

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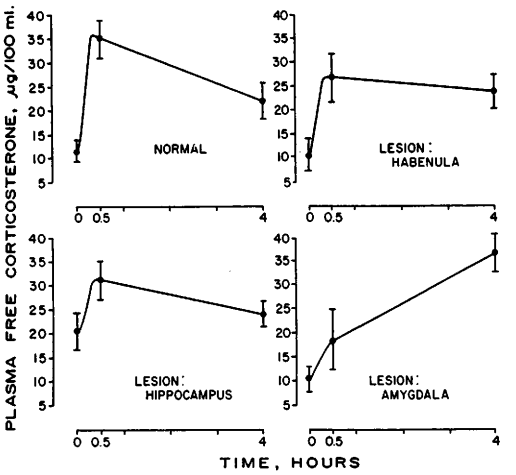

FIG. 1. Plasma free corticosterone response to immobilization stress in rats with lesions in habenula, hippocampus, amygdala.

(20). Brains of all lesioned animals were fixed in 10% formalin and position of lesions determined by reconstruction from 75 μ frozen sections. Sections of thyroid, adrenal and testis of control and lesioned animals were prepared also for histological examination.

Results. Thyroid and testis. Two months after lesioning, thyroid I^{131} uptake of all experimental groups was not significantly different from that of control animals (Table I); also, thyroid weight and histological appearance were not changed when examined at time of sacrifice 3 months after lesioning. No impairment of pituitary gonadotropic activity was evident as judged by testicular weight or histology.

Adrenal. Resting PFC levels in normal rats were 11.8 $\pm$ 2.1 μg/100 ml; immobilization stress evoked a 200% increase (35.1 μg/100 ml) in PFC at 30 min which declined to 22.4 μg/100 ml after 4 hrs of continuous immobilization (Table I; Fig. 1). PFC values of similar magnitude have been reported to occur in the rat after such stressors as ether anesthesia, painful stimuli(21), formalin injection(22) and electroshock(23). In animals with lesions in the habenular complex, the mean PFC response after 30 min of stress was not as great as in normal animals, nor was the decline after 4 hrs of stress to the same level as normal; at both time intervals, however, PFC values were not statistically different from normal. After electrolytic destruction of relatively large areas of hippocampus, resting PFC levels of 20.6 $\pm$ 3.8 μg/100 ml were significantly higher than normal; in response to stress of immobilization, PFC levels after 30 min and 4 hrs were in the same range as in non-lesioned animals. Bilateral destruction in the amygdaloid nuclei did not alter resting PFC levels (Table I); the pattern of response to immobilization stress, however, was notably different from normal or hippocampal-lesioned animals. Only moderately elevated and not statistically different PFC levels were observed after 30 min and this rose to 37.0 $\pm$ 3.8 μg/100 ml after 4 hrs (Fig. 1).

Three months after placement of these relatively large lesions in hippocampus and amygdala, reconstruction of the exact extent of the lesions was difficult because of collapse of the nervous tissue around the lesion site, involvement and destruction of the lateral ventricular walls and distortion of the normal contour of the temporal lobe. In Fig. 2 the area common to the lesions placed in hippocampus and amygdala is presented as reconstructed from the brains of 6 animals of each

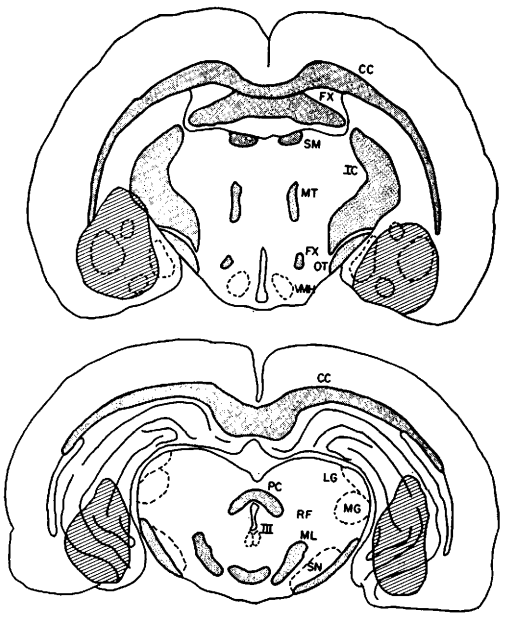

FIG. 2. Localization of lesions in amygdala (upper section) and hippocampus (lower section) which produced alterations in basal PFC levels and in PFC response to immobilization stress.

TABLE 1. Endocrine Function and Response to Stress in Rats with Lesions in the Limbic System.

Group	No.	Testis, g	Thyroid, mg	I^{131} uptake, % A.D.	Adrenal, mg	P.F.C., μg/100 ml
Non-lesioned						
a. Non-stressed	9	1.60 ± .71	16.7 ± 1.9	9.18 ± .36	23.9 ± 3.1	11.8 ± 2.1
b. Stressed, .5 hr	12	1.70 ± .14	15.9 ± 2.0		25.2 ± 2.3	35.1 ± 4.0
c. " ,4 "	12	1.66 ± .10	15.9 ± 2.0		23.8 ± .7	22.4 ± 3.8
Lesioned: Hippocampus						
a. Non-stressed	10	1.68 ± .08	17.3 ± 3.2	10.09 ± 1.27	21.2 ± 2.3	20.6 ± 3.8
b. Stressed, .5 hr	7	1.74 ± .10	14.8 ± 2.4		24.6 ± 2.0	31.2 ± 4.1
c. " ,4 "	9	1.65 ± .09	15.8 ± 1.6		24.0 ± 2.5	24.2 ± 2.5
Lesioned: Amygdala						
a. Non-stressed	10	1.67 ± .17	16.5 ± 2.2	9.74 ± 1.22	23.8 ± 3.4	10.6 ± 2.6
b. Stressed, .5 hr	8	1.67 ± .10	14.3 ± 3.2		23.7 ± 2.5	18.7 ± 6.2
c. " ,4 "	6	1.55 ± .08	14.5 ± 1.9		26.7 ± 5.1	37.0 ± 3.8
Lesioned: Habenula						
a. Non-stressed	7	—	16.5 ± 1.9	8.75 ± .72	25.2 ± 2.7	10.8 ± 3.3
b. Stressed, .5 hr	9	1.70 ± .07	15.6 ± 2.0		24.5 ± 1.2	27.0 ± 5.1
c. " ,4 "	7	1.52 ± .28	14.8 ± 2.0		25.2 ± 1.2	24.6 ± 3.6

All values represent mean ± stand. dev.
I^{131} uptakes were determined on all animals of each group 1 mo after lesioning.

group sacrificed 12-25 days after lesioning. No attempt was made using these preparations to define more closely the components of hippocampus or amygdala involved in the adrenocortical responses observed. Experiments are in progress using progressively smaller lesions in the amygdaloid nuclei to identify the region which exerts this modulating influence upon ACTH secretion.

Discussion. Although the role of afferent nervous impulses in ACTH secretion, including those from the limbic system, has been reviewed(24,2), the amount of experimental evidence is not great. Direct physiological and anatomical evidence for such a role(Mason, 14, 15; Nauta 25,26) is the observation that stimulation of the amygdaloid nuclei in the conscious, unrestrained monkey leads to elevated 17-hydroxycorticoid levels. Additional work by these investigators indicated that the adrenocortical response evoked by stimulation of amygdala or of hypothalamus may be inhibited by prior excitation of hippocampus. Porter(27) observed a similar inhibitory effect of hippocampal stimulation, using eosinophil response as an index of adrenocortical activation. In the dog and cat, Martin *et al.*(28) found elevated adrenocortical function after bilateral amygdalectomy; and Yamada and Greer(29) noted some increase in relative adrenal gland weights of rats 1-15 days after massive lesioning of the amygdaloid nuclei (but including hippocampus and lateral hypothalamus also), and suggested that this adrenal response was due either to non-specific stress or to destruction of a brain region which normally exerts some inhibitory influence upon ACTH secretion. In view of the short interval between lesioning and examination of the glands, the former possibility seems more likely to be correct.

Results of the present work add to the evidence that hippocampus and amygdala have a modulating influence upon the pituitary-adrenal axis, and suggest separate roles for these 2 components of the limbic system. The amygdaloid complex (or some portion thereof) is apparently required for acute, rapid activation of the hypothalamo-hypophyseal system; release of ACTH and adrenocortical response are not necessarily prevented, but are delayed following destruction of the amygdaloid nuclei. After 4 hrs of continuous immobilization, however, PFC levels were comparable to those attained by non-lesioned animals after 30 min or less of immobilization. On the other hand, chronically high resting PFC levels (without significant changes in adrenal weight) observed in rats

with lesions of hippocampus indicate that this brain area normally contributes a suppressive influence to the milieu of afferent stimuli which reach the hypothalamus and maintain the basal or resting ACTH secretion.

Neural mechanisms which interplay upon the final source of ACTH-regulating neurohumor are both excitatory and inhibitory in nature; further experiments are necessary to determine the manner in which these neural influences are integrated under normal and stressful conditions into a resultant signal at the terminal cells responsible for the ACTH neurohumor.

Summary. Bilateral electrolytic lesions of hippocampus, amygdala or habenula of the rat did not produce notable changes in pituitary gonadotropic or thyrotropic activity as judged by testicular or thyroid weight and histology and I^{131} uptake. In response to immobolization stress, PFC levels of normal rats increased 200% after 30 min; after placement of lesions in amygdala, the response to stress was markedly delayed, but eventually reached a 200% increase after 4 hrs. Lesions of hippocampus did not alter the temporal pattern of response to stress; resting or basal PFC levels were, however, significantly higher than normal.

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