Effect of Amygdaloid Lesions on Pituitary-Adrenal Axis in the Deermouse.*† (31375)

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(Introduced by M. X. Zarrow)
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The role of the hypothalamus in the regulation of pituitary hormonal secretion is well documented. However, knowledge of regulation of hormonal secretion by the amygdaloid complex is limited(1-5), although extensive research has been conducted on the effects of lesions and electrical stimulation of the amygdala on a number of behavioral phenomena (6-14).

This work deals with the effects of lesions in the medial amygdaloid group on pituitary and plasma ACTH, and adrenal and plasma corticosterone levels in the male deermouse (*Peromyscus maniculatus bairdii*).

Materials and methods. Animals and treatment. Adult male deermice (*Peromyscus maniculatus bairdii*), weighing 15 to 19 g each, were anesthetized intraperitoneally with sodium pentobarbital and oriented in a modified rat stereotaxic instrument. Bilateral lesions were produced by electrocoagulation using a High Frequency Hyfrecator discharging 1.5 mA of current 7 seconds through a monopolar varnish-coated stainless steel electrode in the medial group of the amygdaloid complex according to the stereotaxic atlas for this species(15). A large stainless steel bar, inserted in the rectum, served as the indifferent electrode.

All animals were placed 2 per cage for at least a week prior to treatment. Following the operation, the animals were placed in their respective cages and not disturbed until sacrificed.

Three groups of 10 animals each were sacrificed at 0 (no lesion), 12 hours and 1, 3, 6, and 16 days following the operation. In addition, one group of 10 animals was sham-operated and sacrificed 3 days post-treatment. Animals were sacrificed by decapitation within 25 seconds after entering the cage. Blood was collected in heparinized tubes, and the plasma was obtained by centrifugation at 1000 $\times$ *g* for 15 min. Pituitaries and adrenal glands were removed, weighed to the nearest 0.1 mg and immediately frozen for later analyses.

Position of lesions was confirmed by histological examination of formalin-fixed brains stained with Cresyl Violet Blue.

Corticosterone determination. Total plasma and adrenal corticosterone levels were determined fluorometrically according to the methods of Peron and Dorfman(16) and of Moncloa, Peron and Dorfman(17).

Adrenocorticotropin assay. Hypophysectomized male rats (Holtzman strain) weighing between 90 to 110 g each were obtained from Hormone Assay Laboratories, Chicago, for the adrenocorticotropin assay. Seven days were allowed to elapse between the operation and the beginning of pre-treatment. Animals, at 40 days of age, were pretreated on day 0 with 2 units of ACTH and 4 units on days 4 and 6. On day 7, they were injected intravenously *via* the tail vein with pituitary homogenate (1.0 mg/0.1 ml) or plasma (0.4 ml) and sacrificed 30 minutes later. Two animals each were injected for every pituitary homogenate or plasma sample for a total of 72 hypophysectomized rats. In addition, 4 animals each were injected with the following: saline, 5, 10, 20 and 40 milliunits (mU) of ACTH. Blood was collected and plasma corticosterone levels were determined fluorometrically by the method previously stated. Values were initially expressed in micrograms of corticosterone per 100 ml of plasma and transformed to milliunits of ACTH using a standard (log) curve derived from the following equation: $\log Y = 1.566 + 0.638 (\log X - 1.150)$, where Y is expected micrograms of corticosterone and X is milliunits of ACTH.

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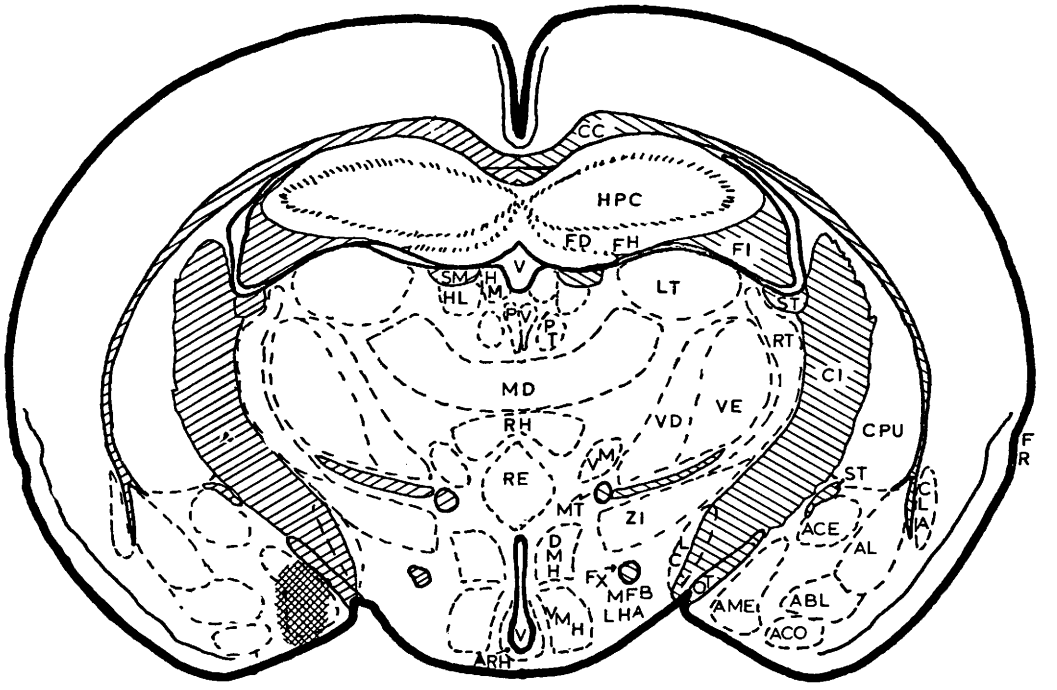


FIG. 1. Diagrammatic representation of the brain of *P. m. bairdii*. Meshed area on left hemisphere represents location of lesions. Right hemisphere represents location of various nuclei: ABL, basolateral amygdaloid nucleus; AME, medial amygdaloid nucleus; ACE, central amygdaloid nucleus; ACO, cortical amygdaloid nucleus; AL, lateral amygdaloid nucleus.

Results. Localization of lesions was confirmed through histologic examination and found to be in the medial amygdaloid complex (Fig. 1).

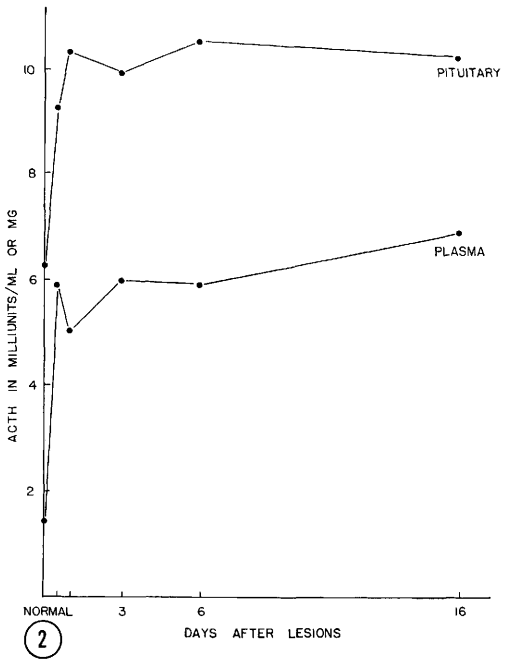
Within 12 hours after lesions were placed in the medial amygdaloid group, pituitary ACTH content increased significantly ($p<.01$) from a normal level of 6.36 to a level of 9.30 mU/mg (46% increase) (Fig. 2). Essentially, thereafter the pituitary content of ACTH, of animals with lesions, remained unchanged for the 16 days of the experiment. The plasma level of ACTH, however, rose by 309% in the treated animals from a control level of 1.45 to 5.93 mU/ml at 12 hours. Although the plasma level of ACTH rose to 6.97 mU/ml at 16 days after lesions were placed in the medial amygdaloid complex, it remained essentially unchanged between 12 hours and 16 days having reached a significantly ($p<.001$) higher level than normal.

Adrenal corticosterone content rose significantly ($p<.01$) from 0.93 to 2.33 μg/100 mg 12 hours after lesions were made (Fig. 3). The adrenal corticosterone content remained essentially level throughout the 16 days of the experiment.

The free plasma corticosterone level of control *bairdii* was 7.98 μg/100 ml. However, within 12 hours after establishment of lesions in the medial amygdaloid group, the corticosterone level rose significantly ($p<.01$) to 33 μg/100 ml. Following that initial increase, it declined to 24.8 μg/100 ml at 3 days posttreatment. Between day 3 and 16, however, the free plasma corticosterone remained essentially unchanged, and at a significantly ($p<.01$) higher level than control *P. m. bairdii*. Sham-operation did not result in any substantial changes.

Discussion. The results indicate that bilateral lesions in the medial amygdaloid complex are followed by significant increases in plasma ACTH levels. Simultaneously with the plasma increases, pituitary ACTH content also increases, reflecting a pituitary ACTH synthesis increase.

The increased synthesis and circulatory



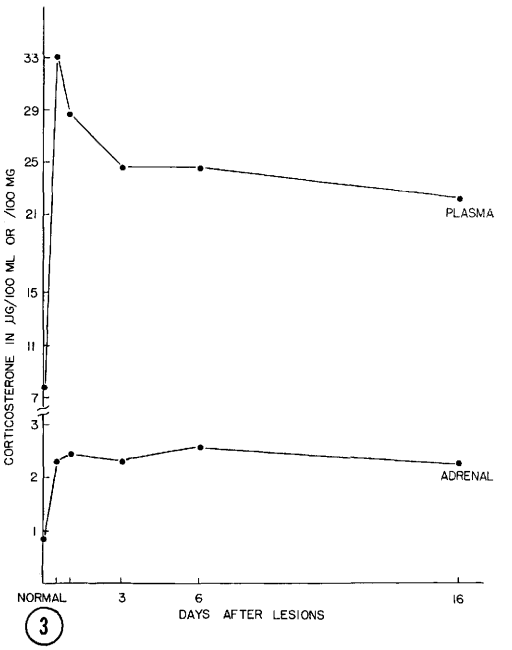


FIG. 2. Pituitary and plasma ACTH levels (in mU/mg or mU/ml) in normal male deermice and in those with lesions in the medial amygdaloid complex for 12 hours and 1, 3, 6, and 16 days.

FIG. 3. Adrenal and plasma corticosterone (in µg/100 ml or µg/100 mg) in normal, without lesions, male deermice and at 12 hours and 1, 3, 6 and 16 days after lesions were placed in the medial amygdaloid complex.

levels of ACTH are best reflected in increased production of adrenal corticosterone, which is accompanied by significant increases in free plasma corticosterone. Thus, the increases in adrenal and plasma corticosterone reflect clearly adrenal activation as a result of increased circulatory levels of ACTH.

The results, together with previous work (2-4), clearly indicate possible existence of a separate inhibitory center to regulate the pituitary-adrenal axis, although the possibility also exists that injury from lesions produces a focal point of irritation which may be the starting point of potentials that spread to other areas of the brain, and mainly to the hypothalamus(19). In our opinion, however, it seems unlikely that such an irritation would produce sustained stimulation over 16 days. It is interesting to note that we have found similar inhibitory regulation by the basolateral amygdaloid group of pituitary and plasma luteinizing hormone(18). The manner in which this type of possible inhibition is mediated needs further elucidation.

Summary. The effects of lesions in the medial amygdaloid complex in the deermouse on the pituitary-adrenal axis was investigated. Within 12 hours after lesions, plasma and pituitary ACTH increased significantly and remained significantly higher than control, unlesioned animals. The increases of ACTH also led to significant increases of plasma and adrenal corticosterone. It was concluded that the medial amygdaloid group possibly may exert an inhibitory effect on the pituitary-adrenal axis in the deermouse.

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Effects of Hydrobiotites on Sr^{89} Absorption and Deposition in Chicks.* (31376)

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The invasion of food products by radioactive contaminants constitutes a potential health hazard; consequently, the efforts of many laboratories have been directed toward a practical means of preventing or limiting the absorption and deposition of various radionuclides. Reports have appeared in the literature concerning numerous means of limiting absorption of radionuclides from the intestinal tract(1,2,3). Based on the assumption that if the absorption of the radionuclides (radiostrontium in particular) from the gastrointestinal tract can be limited, then the radionuclide must be effectively bound in the intestinal tract and subsequently excreted.

The use of cation-binding agents, such as Amberlite IRC-50, has been shown to be partially effective in increasing the amount of radionuclide excreted(4). Vermiculite (a cation-binding agent) has been investigated in this respect and has met with varying degrees of success(4,5).

In this investigation, a highly refined vermiculite (verxite) produced by thermal exfoliation of hydrobiotite was included in the chicks' diet to determine if absorption from the gastrointestinal tract and subsequent deposition of Sr^{89} in the bones could be prevented.

Material and methods. Forty-day-old male chicks were randomly divided into 8 groups of 5 chicks each and fed a standard broiler starter diet (Texas A&M University, Poultry Research Center) supplemented with 0.25% and 1.00% commercial vermiculite (verxite) granules and flakes both untreated and acid activated with 25% (w/w) H_3PO_4. The birds were placed on the above diets 3 hours before administering the initial doses of Sr^{89}. Each chick was given 0.86 μc of Sr^{89} orally, twice daily, for 5 days, so that each bird received a total of 8.6 μc Sr^{89}. In addition one group of 5 birds was given Sr^{89} and fed a diet containing no verxite, and a final group of 5 birds served as a control and received neither Sr^{89} nor verxite.

At the end of the 5-day experimental period all birds were killed and the right and left tibiotarsus taken for analysis of Sr^{89} content by the method of Ansari *et al*(6). Total feces were collected once daily and analyzed for Sr^{89} content by the above method. All calculations were made by computers using the program of Ansari *et al*(6). To facilitate

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