thoroughly with distilled water, lyophilized immediately, and kept at −20°C until use. The lyophilized mycelia were ground to a fine powder in a chilled mortar before the addition of buffer. Previously described procedures were followed from then on for the preparation of a homogenate, the application of homogenate to the starch gel column, the electrophoretic separation of the components of dehydrogenases, and finally the direct visualization of the loci of dehydrogenases on gel column (*4*). The only modification made was the use of malate at one-tenth of the concentration previously used to reduce excess precipitation of formazan from neo-tetrazolium in the incubation media. The solution for the direct visualization of 6-phosphogluconate dehydrogenase activity in the starch gel has not been described previously. The substrate concentration used was 0.002*M* and the other constituents were the same as those for the detection of glucose-6-phosphate dehydrogenase. To assure the identity of various bands of formazan, the homogenate prepared with strain 5297 was used as the standard to which the positions of the bands from other strains were always referred.

The results of the electrophoretic fractionation of the dehydrogenases are summarized in Fig. 1. Lactate dehydrogenase and α-glycerophosphate dehydrogenase were not detected. Visual estimation of the color of the formazan precipitate which appears at the loci of specific dehydrogenase activity does not give reliable quantitation of enzyme concentration. However, there seem to be strain differences in the relative intensities of various bands. Otherwise, strain or culture media do not affect the number or location of the bands for each dehydrogenase. Since there is a concentration limit below which activity is not detectable, the observed number of components for each dehydrogenase represents the minimum number present. In spite of the care taken in preserving enzyme activity, it must be assumed that labile components of dehydrogenases, even if present in the organism in sufficient quantities, might also have escaped detection.

It is clear that the heterogeneity of specific dehydrogenases can occur in relatively simple organisms as well as in the mammalian tissues. Results from studies on yeast lactate dehydrogenase support this observation (*6*). The fact that similar patterns of dehydrogenases were observed with different strains and media indicates that there is intrinsic diversity which is not influenced by the external factors tried. One might speculate that this diversity rises as a result of subcellular differentiation or due to limited randomization during the synthesis from subunits of enzyme proteins. Furthermore, this diversity very likely was evolved before the apparent strain difference occurred and was controlled by genes other than those responsible for the characteristics that set these strains apart (*7*).

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7. This work was supported in part by an institutional research grant to the University of Michigan from the American Cancer Society and a grant from the University of Michigan Cancer Research Institute.

3 November 1961

Amygdaloid Suppression of Hypothalamically Elicited Attack Behavior

Abstract. Electrodes were implanted in the hypothalamus and in the amygdala of adult cats. Electrical stimulation of the amygdala suppressed attack behavior elicited by the hypothalamic stimulation.

Much experimental evidence implicates the amygdala in the control of emotional behavior (*1*). The present study presents direct evidence that the amygdala modulates the emotional activity of the hypothalamus, supplementing data from ablation (*2*) and unit-activity (*3*) studies, and supporting speculations based on anatomical and theoretical grounds (*4*).

Bipolar electrodes were implanted aseptically in the hypothalamus and in the amygdala of ten cats. Preoperative tests had shown that these cats would not normally attack rats, which were used as attack objects during experimental sessions. Two other cats, which did attack rats normally, were eliminated from the study. The hypothalamic electrodes were aimed at a region known to produce directed attack (*5*, *6*), which served in this experiment as the model of emotional behavior.

Nine of the ten operated cats, when stimulated in the hypothalamus, savagely attacked a rat placed in the cat's cage. The attack ceased immediately when the stimulation was turned off. The electrical stimulation was a train of 62.5 pulse/sec, 2-msec biphasic square waves lasting usually only until the attacking cat touched the rat with tooth or claw. However, in some trials stimulation lasted from 10 to 25 seconds regardless of the cat's behavior; during such trials, several of the cats killed a rat virtually each time before the hypothalamic stimulation was terminated. Between trials, none of the cats appeared to pay any attention to the rats.

The threshold current for eliciting the attack response was generally about 0.30 ma. There was a tendency for this threshold to increase slightly with repeated stimulations.

As reported previously (*6*), two forms of hypothalamically elicited attack were observed: a stalking form characterized by biting and the relative absence of concomitant autonomic signs, and a more "rage-like" form characterized by repeated striking with the paw and a full complement of autonomic signs such as piloerection and salivation. The stalking attack was elicited by electrodes slightly lateral to those eliciting the "rage-like" form. However, amygdaloid stimulation did not seem to act differently on these two forms of attack.

The amygdaloid electrodes, either bilaterally or unilaterally, were connected to a second stimulator identical to that used to stimulate the hypothalamus. For the simultaneous stimulation of amygdala and hypothalamus which was used in this experiment, the pulses from the two stimulators were interlocked so that a 2-msec pulse in one region was followed 6 msec later by a similar pulse in the other region. Temporal separation of the pulses is necessary to avoid stimulating intervening structures.

In five of the nine cats tested, when amygdaloid stimulation was added to the hypothalamic, either the attack occurred after a longer period of stimulation or, on many trials, it did not occur at all. The effective amygdaloid suppressing current, both unilateral and bilateral, was generally 0.15 to 0.25 ma. Histological examinations of the brains of these five animals at the end of the

Table 1. Mean attack latency for hypothalamic stimulation and mean increase when amygdaloid stimulation is added to hypothalamic stimulation. Mean increases in attack latency were computed by summing over all levels of amygdaloid stimulation for all of these cats except 606. Since this cat showed a tendency to attack faster with high-level amygdaloid stimulation, its mean was computed including levels of amygdaloid stimulation up to 0.30 ma only.

Cat	N*	Mean attack latency (sec)	Mean ± S.D.$_M$ increase in attack latency (sec)	P†	P_B‡
515	48	7.48	+2.32 ± 0.11	83	48
519	22	8.10	+3.46 ± 0.54	91	41
606	36	5.33	+3.58 ± 0.54	94	28
623	54	8.98	+7.10 ± 0.62	93	57
701	14	8.29	+3.49 ± 1.37	64	0

*Number of trial pairs on which means are based. †Percentage of trial pairs on which amygdaloid stimulation added to the hypothalamic resulted either in increased attack latency or in no attack at all. ‡Percentage of trial pairs on which attack did not occur during the combined hypothalamic and amygdaloid stimulation. Such trials were included in the computation of the mean increase in attack latency as though attack actually occurred at time of stimulus termination.

experiment showed that the electrodes most effective in suppressing the hypothalamically elicited attack were located in the lateral nucleus of the amygdala, near the border of the magnocellular portion of the basal nucleus of the amygdala. Electrodes a millimeter or so distant from this location were ineffective in suppressing hypothalamically elicited attack.

Of the other four cats that showed the hypothalamic attack response, one died before sufficient data were collected; one subsequently began to attack rats without electrical stimulation; one showed no effect at all of the "amygdaloid" stimulation; and one attacked slightly faster on the average when amygdaloid stimulation was added to the hypothalamic. However, the "amygdala" electrodes of the cat for whom simultaneous stimulation did not affect the attack were found to be in the optic tract and the posterior hippocampus, missing the amygdala altogether. Those of the cat that attacked slightly faster with "amygdaloid" stimulation were found to be in the border region of the putamen and nucleus intercalatus of the amygdala, at some distance from the site of the electrodes of the five cats whose hypothalamically elicited attack responses were suppressed by the addition of amygdaloid stimulation.

Table 1 gives the results for the five cats that showed the suppression effect. The mean increases in attack latency shown in Table 1 are conservative estimates of the inhibitory effect of the amygdaloid stimulation, since when no attack occurred during a trial with the combined stimulation, the stimulation was turned off after a predetermined length of time and the trial was subsequently included in the computation of the mean as though attack had actually occurred at the time of stimulus termination.

All the mean increases in attack latency in Table 1, except for that of cat 701, are significantly different from zero at $P < .001$ level of confidence. This marked and consistent increase in attack latency could not have been due to any general motor effect of amygdaloid stimulation: for all cats, the time from the onset of stimulation to the first occurrence of a translational movement was compared for the simultaneous amygdaloid and hypothalamic stimulation trials versus the trials with hypothalamic stimulation alone. Of the five cats whose data are given in Table 1, two showed no effect of the dual stimulation on their movement latencies, two were only slightly, but significantly, slowed in their initial movement following the dual stimulation, and one cat actually had a slightly, but significantly, shorter movement latency with the dual stimulation.

Cat 606 which, like the other cats in this study, normally attacked mice, was tested on several occasions with a mouse in its cage. Amygdaloid stimulation also blocked this "naturally elicited" attack response. In addition, the cat that came to attack rats without hypothalamic stimulation was blocked in its attack by amygdaloid stimulation. Its amygdala electrodes were located similarly to those of the five cats that showed a suppression effect of amygdaloid stimulation on hypothalamically elicited attack. It is also of interest to note that cat 606, while inhibited in its hypothalamically elicited attack at low levels of bilateral amygdaloid stimulation, continued to attack at the same latency, or even attacked slightly faster, if the amygdaloid current was increased to 0.40 ma.

In contrast to simultaneous stimulation, stimulation of the amygdala alone without hypothalamic stimulation at the low current levels used in this experiment did not elicit any fixed behavior. During amygdaloid stimulation, the cats seemed alert, sometimes sniffed about the cage, and were as responsive to handling and to petting as they were in the absence of electrical stimulation.

Whereas neither the hypothalamic nor the amygdaloid stimulation alone produced electrical afterdischarges, that is, intense, rhythmic electrical activity continuing after the termination of the electrical stimulus, in some of the cats the simultaneous stimulation tended to do so. Because of this tendency amygdaloid blocking of the attack response occurred without afterdischarges only when the hypothalamic stimulation was just moderately above threshold value. Since afterdischarges lead to spread of excitation to areas other than those originally stimulated, behavioral effects observed during afterdischarges cannot be attributed to the circumscribed region stimulated. Hence, we discarded all behavioral data recorded during stimulations leading to afterdischarges.

Histological verification of the electrode placements permits us to conclude that the suppressing effect arises from a rather circumscribed region of the amygdala. Furthermore, the facilitation effects observed in two cats appear to have been due possibly to spread of stimulation to the putamen.

Thus, it appears clear from our data that a portion of the amygdala exerts an inhibitory influence on hypothalamically elicited aggressive behavior (*7*).

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7. This work was supported by a grant from the National Science Foundation. We thank Mildred Groves for her aid in preparing brain sections.

24 October 1961