5-Hydroxytryptophan Decarboxylase in Rat Brain: Effect of Hypothalamic Lesions

Abstract. *Section of the medial forebrain bundle within the lateral hypothalamus of the rat reduces the serotonin content and the 5-hydroxytryptophan decarboxylase activity in the brain. The integrity of this fiber tract appears necessary for the maintenance of normal concentrations of the amine and enzyme in the telencephalon.*

Significant decreases in the serotonin content of the rat brain are produced by bilateral lesions sectioning the medial forebrain bundle within the lateral hypothalamus or ablating areas known to contribute fibers to this pathway (*1*). Unilateral lesions affecting the medial forebrain bundle cause a decrease in the amine only in the ipsilateral half of the brain containing the lesion, a finding consistent with the anatomic distribution of the fiber tract. In addition, the time course over which the serotonin decreases after such lesions have been made correlates well with the time course for degeneration of the fibers sectioned (*2, 3*). Since the decrease in the amount of amine is dependent on section of the medial forebrain bundle and occurs simultaneously with the appearance of degenerating fibers in the tract, it was suggested that the reduction in amine is a consequence of section and degeneration of fibers within the bundle which normally produces serotonin (*1–3*). If this is the case, destruction of these fibers might be expected to result in a decrease in the amount of 5-hydroxytryptophan (5-HTP) decarboxylase, the enzyme necessary for the synthesis of serotonin. The experiments described here demonstrate that a decrease in activity of this enzyme does occur in rat brain after lateral hypothalamic lesions that section the fibers of the medial forebrain bundle have been made.

Bilateral medial or lateral hypothalamic lesions were produced in 85- to 90-day-old male albino rats as previously described (*1*). In addition, sham operations were performed on a group of control rats. The animals were housed two to a cage and given free access to food and water. Thirty to 60 days after operation the animals were decapitated and their brains removed for analysis. Previous experiments have shown that there is no change in serotonin concentrations in brain during this interval.

Two separate experiments were conducted. Prior to analysis for serotonin or enzyme activity in the first experiment, the brain was cut in a coronal plane and the area of the lesion photographed. Examination of the photographs allowed verification of the lesion placement. The concentration of either serotonin or 5-HTP decarboxylase and malic dehydrogenase was determined in whole brain. Serotonin was analyzed by the spectrofluorometric method of Bogdanski *et al.* (*4*); the quantities of reagents specified by Udenfriend *et al.* were used (*5*). The concentration of 5-HTP decarboxylase was determined by a modification of the method of Lovenberg *et al.* (*6*) with 1.8×10^{-4} *M* pyridoxal 5-phosphate and tris buffer at *p*H 8.0. Malic dehydrogenase activity was determined by the method of Mehler and co-workers (*7*), and the amount of activity present served as a control for possible nonspecific effects of the lesions on cellular enzymatic activity.

In the second experiment the brains were dissected into two separate pieces. The first piece, containing only telencephalic structures, was analyzed for serotonin or 5-HTP decarboxylase as in the first experiment. The second piece, containing diencephalon and brainstem, was fixed in formalin and prepared for histologic study. The medial and lateral hypothalamic lesions from this group were identical in size and location to those reported previously (*1*). No data are included from animals in which the lesions were not properly placed. The behavioral effects of these lesions has already been discussed in detail (*1*).

The results of these experiments are presented in Table 1. Lateral hypothalamic lesions transecting the medial forebrain bundle produced a 17-percent decrease in 5-HTP decarboxylase activity as measured in whole brain, and a 33-percent decrease in serotonin concentration. The latter finding is in close agreement with the results of our previous studies (*1, 2*). Medial hypothalamic lesions not involving the fibers of the medial forebrain bundle did not affect the concentration of either 5-HTP decarboxylase or serotonin. Neither lesion affected malic dehydrogenase activity. We reported recently that the effect of lesions of the medial forebrain bundle on serotonin and norepinephrine concentrations is restricted to telencephalic structures in the cat (*8*). This is also true in the rat, and the effects of these lesions on telencephalic serotonin and 5-HTP decarboxylase are presented in the second part of Table 1. Lateral hypothalamic lesions caused a 44-percent decrease in telencephalic 5-HTP decarboxylase and a 72-percent decrease in serotonin, in comparison with sham-operated controls. Medial hypothalamic lesions had no signficant effect.

Thus, a lesion producing a decrease

Table 1 The effect of medial (sparing the medial forebrain bundle) and lateral (sectioning the medial forebrain bundle) hypothalamic lesions on serotonin, 5-hydroxytryptophan (5-HTP) decarboxylase, and malic dehydrogenase in rat brain (*p*-values were obtained by the use of a two-tailed *t*-test).

Group	Serotonin				5- HTP decarboxylase				Malic dehydrogenase	
	n	Amt. μg/g	Change from control (%)	*p*	*n*	Activity (μmole)*	Change from control (%)	*p*	Activity (10^2 μmole)†	Change from control (%)
					Whole Brain					
Control	4	0.54			7	0.41			173	+2
Medial hypothalamic lesion	4	0.50	−7		6	0.42	+2		176	−6
Lateral hypothalamic lesion	2	0.36	−33	<.02	5	0.34	−17	<.01	162	
					Telencephalon					
Control	8	0.58			6	0.41				
Medial hypothalamic lesion	5	0.54	−7		6	0.40	−2			
Lateral hypothalamic lesion	6	0.16	−72	<.002	6	0.23	−44	<.002		

* Micromoles of serotonin formed per gram wet weight per hour. † Micromoles of NADH oxidized per gram wet weight per hour.

in concentration of serotonin in the brain, presumably as a result of section and degeneration of fibers which normally produce serotonin, also reduces the amount of enzyme available for the synthesis of the amine. Whether the decrease in amine concentration is a result of a reduced synthesis secondary to the loss of 5-HTP decarboxylase activity cannot be stated with certainty, since it is not possible to correlate decreases in enzyme activity as measured in vitro with synthesis of serotonin in vivo. For example, Brodie *et al.* (*9*) have shown that doses of N-(3-hydroxybenzyl)-N-methyl hydrazine, which produce an apparent 75-percent inhibition of 5-HTP decarboxylase as measured in vitro, do not affect the biosynthesis of brain serotonin in vivo. The demonstration by Grahame-Smith (*10*) of the enzymatic hydroxylation of tryptophan in brain homogenates must also be considered. A determination of the effect of lesions of the medial forebrain bundle on this first step in the biosynthesis of serotonin would be helpful in deciding whether such lesions lower the concentrations of serotonin by reducing one or both of the enzymatic activities necessary for the synthesis of the amine. Despite these considerations, it is interesting that the percentage decrease in 5-HTP decarboxylase activity is only about half the percentage reduction in amine concentration. This quantitative difference in percentage change of amine and enzyme suggests that part of the decrease in amine concentration may be secondary to some non-enzymatic mechanism such as loss of binding sites due to degeneration of the fiber tract. The failure of either lesion to alter malic dehydrogenase activity indicates that the change in 5-HTP decarboxylase is not a reflection of some non-specific effect of the lesion on cellular enzymatic activity.

The similarity of the effect of sectioning the medial forebrain bundle on the concentration of serotonin in the brain to the effect of sectioning autonomic nerves on tissue catecholamines and ganglionic acetylcholine has been discussed (*1*, *2*). In addition, Hebb and Waites (*11*) demonstrated that the loss of capacity of the superior cervical ganglion to synthesize acetylcholine after section of the cervical sympathetic trunk (*12*) was due to a decrease in choline acetylase activity in the ganglion. Similar effects on dihydroxyphenylalanine decarboxylase in spinal cord and sympathetic nerve have been presented by Anden *et al.* (*13*). Thus, our findings demonstrating a decrease in both serotonin and 5-HTP decarboxylase after section of the medial forebrain bundle are consistent with the suggestion that the bundle contains serotonin-producing fibers. We have recently shown, however, that the decrease in amine occurs both in areas directly innervated by the medial forebrain bundle and in areas related to it only through polysynaptic connections (*8*). The reduction in amine content of these latter areas indicates that the lesion can produce transynaptic neurochemical effects in neurons outside the medial forebrain bundle. The results, therefore, not only demonstrate that the integrity of the medial forebrain bundle is necessary for the maintenance of normal serotonin content and 5-HTP decarboxylase activity in the rat telencephalon, but, in addition, support the view that the brain contains serotonin-producing fibers either within the medial forebrain bundle or under its influence.

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Duplication of Evoked Potential Waveform by Curve of Probability of Firing of a Single Cell

Abstract. *Computer compilation of the probability of firing of a single cell in cat cortex following a physiological sensory stimulus (somatic or light flash) indicates that the frequency distribution of the firing of a single cell closely corresponds to the average waveform of the evoked potential recorded from the same microelectrode. This high correlation holds for both positive and negative and early and late components of the evoked response.*

The relation between evoked potentials and the electrical activity of single cells in brain is still not well understood, and sustained interest and a considerable amount of data have resulted in a number of contrasting points of view (*1*). Most interpretations of this relation have been based on single and multiple superimposed oscilloscope traces, but such small variable samples can yield only impressions of the pattern of single-cell firing and make agreement regarding the relation to the evoked potential difficult to reach. Although the use of nonphysiological (electrical) stimulation to produce synchronously evoked potentials provides better control of the stimulus and response and reduces the variability of the relationship, the probabilistic nature of the relation between the evoked potential and the single cell, which is characteristic of physiologically evoked responses, makes interpretation of oscilloscope data difficult.

Despite frequent disagreement as to which wave components of an evoked potential are attributable to which level of the cortex or cellular mechanism, it is generally agreed that different neural elements sequentially activated relate to the sequential appearance of various components of the waveform of the electrically evoked potential. By means of a computer we have attempted to clarify the relation between the probability of firing of single cortical cells and the components of the waveform of the evoked potential recorded from the same microelectrode. In this way, quantitative data from a large number of single oscilloscope sweeps could be stored in the computer and summed while the original time relations were maintained.

If the suggested relation of individ-