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Septal Tryptophan-5-Hydroxylase: Divergent Response to Raphe Lesions and Parachlorophenylalanine

Abstract. *A soluble form of tryptophan-5-hydroxylase activity was found to be present in areas rich in serotonergic terminals (colliculi, hippocampus, septal area, and remaining telencephalon) as well as in brainstem, an area rich in cell bodies. The enzymatic activity in all brain regions, except the septal area, was inhibited to varying degrees following administration of parachlorophenylalanine. Destruction of the raphe nuclei in the midbrain led to a large and comparable decrease in both serotonin content and tryptophan hydroxylase activity of the hippocampus. In contrast, these lesions did not significantly affect the enzymatic activity of the septal area although the serotonin content was decreased by 72 percent. These findings suggest that the major portion of the tryptophan hydroxylase activity of the septal area is uniquely different from that found in other telencephalic areas in that it is not localized in serotonergic nerve terminals nor is it inhibited by parachlorophenylalanine.*

It has been demonstrated that the serotonin content of the telencephalon is exclusively localized in axons whose cell bodies lie primarily in the raphe nuclei of the brainstem (*1*). Destruction of the cell bodies in the raphe nuclei or of the fibers of passage in the medial forebrain bundle leads to a parallel loss of serotonin fluorescent terminals, synaptosomal uptake of serotonin, serotonin content, and activity of the rate-limiting enzyme, tryptophan hydroxylase (*2*). It has therefore been concluded that serotonin and tryptophan hydroxylase are uniquely localized in telencephalon within the axon terminals of serotonergic neurons.

Injection of parachlorophenylalanine (*p*-CP) produces an inhibition of tryptophan hydroxylase activity in brain (*3*). Recently, however, it has been reported that *p*-CP does not inhibit the tryptophan hydroxylase activity of the septal area (*4*). This finding suggests a unique characteristic of the septal enzyme which might reflect a difference in its compartmentalization. The activity of septal tryptophan hydroxylase has not been measured following brain lesions. We have therefore conducted the appropriate experiments utilizing brain lesions and *p*-CP. Our results suggest that the bulk of the soluble tryptophan hydroxylase activity of the septal area differs from that found in other telencephalic areas in that it is not inhibited by *p*-CP and is not localized within serotonergic nerve terminals.

Male albino rats (Holtzman), 70 to 80 days of age, were housed two per cage and maintained on Purina Lab Chow and tap water. In the first experiment animals were injected intraperitoneally with a suspension of DL-*p*-CP (300 mg/kg) or an equivalent volume (3 ml/kg) of vehicle (*5*).

Table 1. Effect of *p*-CP (300 mg/kg) on soluble tryptophan-5-hydroxylase activity.

Brain region	Enzyme activity (nmole/mg/hr)*	Change from control (%)
Brainstem		
Control	5.98 ± 0.49 (9)	
p-CP	1.09 ± 0.29 (3)	−82†
Colliculus		
Control	4.46 ± 1.10 (9)	
p-CP	2.01 ± 0.68 (3)	−55†
Hippocampus		
Control	0.56 ± 0.12 (6)	
p-CP	0.32 ± 0.01 (5)	−43†
Telencephalon		
Control	1.22 ± 0.13 (6)	
p-CP	0.12 ± 0.01 (6)	−90†
Septal area		
Control	1.55 ± 0.23 (12)	
p-CP	1.27 ± 0.32 (8)	−18

* Values are given as means ± S.E.M. The number of determinations is shown in parentheses. † $P < .05$; *t*-test, two tailed.

Animals were decapitated 3½ days later, and the brains were removed and dissected on ice. The following brain regions were obtained: brainstem, including midbrain and pons but excluding cerebellum; colliculus, including both superior and inferior colliculi; hippocampus (both hippocampi were combined); septal area, the region lying between the lateral ventricles, and bounded by the corpus callosum dorsally, the anterior commissure ventrally, the genu of the corpus callosum rostrally, and the columns of the fornix caudally; and telencephalon, all of the telencephalon except for the septal area and hippocampi. Soluble tryptophan-5-hydroxylase activity was measured by the method of Gál and Patterson (*6*) which utilizes 2-amino-4-hydroxy-6-methyltetrahydropterin as the cofactor.

All brain regions exhibited the presence of a soluble tryptophan hydroxylase (Table 1). Enzymatic activity was highest in both the brainstem, an area rich in serotonergic cell bodies, and in the colliculus, an area rich in their terminals. Telencephalic areas contained lower levels of enzyme activity. Injection of *p*-CP led to a large and equivalent inhibition of enzymatic activity in both telencephalon and brainstem, and a smaller although significant inhibition in hippocampus and colliculus. In contrast, there was no inhibition of tryptophan hydroxylase activity in the septal area. The absence of enzyme inhibition in the septal area might be due to an insufficient quantity of drug reaching this brain region. We therefore injected a group of animals with DL-[2-^{14}C]-*p*-CP (90 μc/kg) with a specific activity of 3.27 μc/mg (*7*). Five animals were decapitated 1 day and seven 3 days after injection, and the radioactivity within each brain region was determined by liquid scintillation counting (*8*). The brainstem, colliculus, and hippocampus demonstrated essentially the same amount of radioactivity at 1 day after injection, with a mean ± S.E.M. of 26,337 ± 1,521 disintegrations per minute (DPM) per gram. This decreased to 10,356 ± 426 DPM/g by 3 days. The septal area did not differ from other brain regions, demonstrating 27,974 and 9,872 DPM/g at 1 and 3 days, respectively, after injection.

Knapp and Mandell (*4*) have suggested that there are two forms of tryptophan hydroxylase in brain. Homogenates of various brain regions in 0.35*M* sucrose yielded a soluble and a

Table 2. Effect of lesions in the raphe nuclei (RN) on tryptophan-5-hydroxylase activity and serotonin (5-HT) content of brain.

Brain region	Enzyme activity (nmole/mg/hr)*	Change from control (%)	5-HT content (nmole/g)*	Change from control (%)
Septal area				
Control	0.99 ± 0.09 (5)		9.89 ± 2.04 (4)	
RN lesion	0.80 ± 0.05 (5)	−19	2.78 ± 0.45 (4)	−72†
Hippocampus				
Control	1.07 ± 0.14 (6)		2.73 ± 0.40 (6)	
RN lesion	0.39 ± 0.06 (6)	−64†	0.85 ± 0.28 (4)	−69†

* Values are given as means ± S.E.M. The numbers in parentheses are the numbers of animals.
† Significant difference ($P < .02$, t-test) between control and lesioned rats.

particulate fraction of divergent cofactorial dependence. The soluble enzyme was reported to predominate in areas rich in serotonergic cell bodies, while the particulate was associated with areas rich in nerve terminals. Furthermore, these investigators suggested that the noncompetitive inhibition of tryptophan hydroxylase by *p*-CP occurred in cell bodies only. The lack of inhibition of septal tryptophan hydroxylase by 3 days after administration of *p*-CP was therefore attributed to the particulate form of the enzyme, and to the time required for the transport of the inhibited enzyme from the cell bodies in the raphe nuclei to the axon terminals in the septal area (*4*). In our studies septal tryptophan hydroxylase was completely solubilized by homogenization in 0.05*M* tris-acetate buffer, *p*H 7.5, yet even this soluble form was completely unaffected by administration of *p*-CP. Since we did observe inhibition of the soluble enzyme in other regions rich in serotonergic terminals (colliculi, hippocampus, and remaining telencephalon), the absence of inhibition in the septal area cannot be attributed to either the particulate localization of the enzyme or to its presence in cell bodies as compared to axon terminals. The lack of inhibition of septal tryptophan hydroxylase by *p*-CP can also not be attributed to the time required for the transport of the inhibited enzyme from the raphe cell bodies to the terminals within the septal area, unless this axoplasmic flow is for some reason considerably slower than that to hippocampus and remaining telencephalon. This suggests some unique property of the septal tryptophan hydroxylase. Experiment 2 examined the possibility that this unique property might be related to a difference in the localization of this enzyme within the cellular elements of the septal area. We therefore examined the effect of raphe lesions and the consequent loss of serotonergic terminals in the septal area and hippocampus on tryptophan hydroxylase activity.

Lesions in the dorsal and medial raphe nuclei of the midbrain were produced under ether anesthesia by passage of a cathodal current through a stereotaxically placed Nichrome wire electrode. The electrode, insulated except at the cross section of the tip, was angled 10° from the vertical plane. Two midline lesions were produced in each animal in order to destroy both the dorsal and medial raphe nuclei. Both lesions were placed 1.5 mm anterior to lambda (*9*). For the dorsal raphe the electrode was lowered 6.7 mm below the surface of the skull from a point 1.2 mm lateral to the midline, and the lesion was produced by passage of a 2-ma current for 20 seconds. The electrode was then retracted and lowered 8.6 mm below the surface of the skull at a point 1.5 mm lateral to the midline, and the medial raphe nuclei were destroyed by passage of a 2-ma current for 25 seconds. Surgical controls were treated in the same manner except that the electrode was not lowered into the brain. Ten days after surgery animals were decapitated and tryptophan hydroxylase activity was determined in the septal area and hippocampus. The serotonin content of these brain regions was determined in a separate group of lesioned and control animals, by a modified butanol extraction method (*10*) utilizing the *o*-phthaldehyde condensation technique (*11*). Separate animals were perfused under pentobarbital anesthesia with buffered formalin, and frozen sections through the lesion were stained by the Kluver-Barrera method (*12*). Examination of the histological material revealed complete destruction of the dorsal and medial raphe nuclei.

The raphe lesions produced a large and equivalent depletion of serotonin content in both the septal area and hippocampus (Table 2). The tryptophan hydroxylase activity of hippocampus was depleted to the same extent, a finding that is in agreement with Kuhar *et al.* (*2*). However, there was no significant effect of the raphe lesion on the enzymatic activity of the septal area. The total destruction of the raphe nuclei observed in histological sections and the large (72 percent) depletion of serotonin content in the septal area indicate that the serotonergic axon terminals had been substantially eliminated. The small (19 percent) and nonsignificant decrease in tryptophan hydroxylase within the septal area suggests that the bulk of this enzyme is not localized within serotonergic terminals in this region of the brain.

The septal area as defined in this study contains both the medial and lateral septal nuclei as well as a number of fibers of passage and nonserotonergic axon terminals. It is possible, therefore, that the soluble tryptophan hydroxylase activity measured in this study exists in one of these neural elements. On the other hand, one cannot exclude the possibility of a nonneural localization in glial elements.

Taken together the results of these experiments indicate that septal tryptophan hydroxylase is uniquely different from that found in other areas of brain in that the bulk of the enzyme is not inhibited by *p*-CP and is not localized to the serotonergic raphe system. These findings have several implications: (i) the tryptophan hydroxylase activity in the septal area, that is not inhibited by administration of *p*-CP, may represent an isoenzyme; (ii) the presence of tryptophan hydroxylase activity within a brain region does not imply the presence of serotonergic neurons; (iii) there may be other discrete regions of brain that also contain a tryptophan hydroxylase activity having the same properties as that found within the septal area; and (iv) some of the divergent behavioral and pharmacological effects obtained with brain lesions or injections of substrates, and drugs, may reflect the differences in the response of the serotonergic and nonserotonergic tryptophan hydroxylase.

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Operant Control of Occipital Theta Rhythm Affects Performance in a Radar Monitoring Task

Abstract. *Detection efficiency of human observers deteriorates rapidly in monotonous monitoring tasks; this effect (the vigilance decrement) has been associated with increased theta band activity in the electroencephalogram. Suppression of theta activity by operant methods enhances monitoring efficiency, whereas theta augmentation further degrades task performance. These results demonstrate a lawful relationship between operantly regulated cortical activity and behavior in man.*

The detection efficiency of human observers deteriorates rapidly in a variety of monotonous monitoring tasks (*1*). This vigilance decrement is usually attributed to a decline of the level of central nervous system arousal. Thus, physiological signs of arousal might be expected to covary with detection efficiency; but, among the electroencephalographic (EEG) indicators, only the abundance of occipital activity in the theta frequency band (3 to 7 hertz) appears to be a reliable correlate of vigilance behavior (*2*). Since the relative abundance of activity in the various frequency bands of the EEG may be modified by operant conditioning techniques (*3*), we hypothesized that learned regulation of theta band activity would affect detection behavior in a prolonged monitoring task. Specifically, regulated suppression of theta activity should maintain more efficient performance, whereas regulated theta augmentation should result in a greater than normal decrement in performance of a monitoring task.

Nineteen experimentally naive undergraduate students were recruited through an advertisement in a university newspaper to serve as paid subjects. They were told nothing of the nature of the EEG patterns to be reinforced; the effects of expectation on subsequent performance were thereby minimized (*4*).

Monitoring performance was assessed by using a complex and realistic radar simulator. The subject's display was a cathode-ray tube 23 cm in diameter presenting the image of a conventional radar plan position indicator. The sweep line rotated at 10 rev/min, painting continuous video noise and occasional single targets. Targets (3 mm by 2 deg at a signal-to-noise ratio of 4 db) were presented at preselected times at random combinations of range and azimuth. Targets were repainted on successive sweeps until detected by the subject. The subject was required to both depress a microswitch indicating that a target was detected and to verbally identify the range and azimuth of the signal. The numbers of sweeps before detection constituted the basic behavioral data.

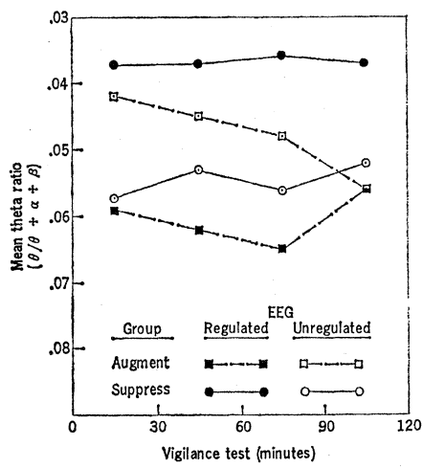

Fig. 1. Mean theta ratio as a function of time in the vigilance test for the theta augment group and the theta suppress group with regulated and unregulated EEG. The ordinate scale is inverted; θ, the number of waves in the theta frequency band (3 to 7 hertz); α, the number of waves in the alpha band (8 to 12 hertz); and β, the number of waves in the beta band (13 to 30 hertz).

In the initial session, each subject was trained to perform the monitoring task. After being shown sample targets, he performed a series of 5-minute training trials in which eight targets were presented at unpredictable intervals and locations on the display. In the first trial, knowledge of results was provided after every response of the subject. In subsequent trials this information was given only after the completion of the 5-minute period. Training continued until the subject performed in a stable and efficient manner for three consecutive trials. After a short rest period, the subject began his preliminary monitoring sequence: a 5-minute initial test with eight targets; a 120-minute vigilance test, with five targets in each successive 15-minute period; and a 5-minute final test of eight signals. The initial and final tests were employed to estimate detection efficiency under alerted conditions. The vigilance test constituted a continuous period of monitoring, in which the usual vigilance decrement was expected to occur. The preliminary sequence was included the first day to permit adaptation to any arousing aspects of the test environment and to familiarize the subject with the task he was later to perform under critical experimental conditions.

Subjects were assigned to one of two experimental groups. The theta suppress group was trained to reduce the probability of theta frequency activity in the EEG, while the theta augment group was trained under the opposite reinforcement contingency, which controlled for nonspecific effects of the training procedure. Since we were primarily interested in the predicted facilitatory effects of theta frequency suppression on performance, the theta suppress group was larger ($N = 12$) than the theta augment group ($N = 7$).