Serotonin: Release in the Forebrain by Stimulation of Midbrain Raphé

Abstract. *Electrical stimulation of the midbrain raphé, an area in which neuronal perikarya containing serotonin are aggregated, produces an increase in 5-hydroxyindoleacetic acid and a decrease in serotonin in the forebrain. These changes indicate that serotonin in the brain can be released via a specific neural pathway, namely, the system of axons projecting into the forebrain from serotonin-containing neurons in the midbrain raphé.*

We report evidence that electrical stimulation of the raphé region of the midbrain results in a neurally mediated release of serotonin (5-hydroxytryptamine) within the brain. Although serotonin in the brain is contained within a specific neuronal system (*1*) and certain neurons are responsive to its microelectrophoretic application (*2*), a neurotransmitter function has not been clearly established for this biogenic amine in the central nervous system. Attempts to detect a release of serotonin in the spinal cord in response to electrical stimulation of the intact brain stem or of the proximal stumps of isolated cords show a change in the concentration of serotonin in the cord and a leakage of released serotonin into the bathing medium when monoamine oxidase is inhibited (*3*). However, in these studies stimulation was not confined to an area consisting primarily of neurons containing serotonin, and the concentration of 5-hydroxyindoleacetic acid, the principle metabolite of serotonin, was not measured.

In the rat brain, the largest collection of neuronal perikarya containing serotonin is located in the raphé area of the midbrain; the axons of this system project into various regions of the forebrain (*4*). A lesion in the vicinity of these perikarya (*5*) or their axons in the rostral midbrain (*6*) results in a depletion of serotonin in the brain without affecting the concentration of norepinephrine. Since most of the serotonin-containing perikarya are clustered together in a region (that is, midbrain) that is anatomically removed from the axonal projections, this system would seem ideally suited for testing the possibility that release of serotonin is mediated by a specific neural pathway. Moreover, it should be possible to stimulate a significant proportion of the serotonin-containing neurons with a single electrode.

A neurally mediated release of serotonin would be expected to result secondarily in an increased tissue concentration of 5-hydroxyindoleacetic acid since released amine would potentially have greater access to monoamine oxidase. In addition, some reduction in the overall concentration of serotonin might also be expected if ongoing synthesis did not entirely keep pace with the demands of increased utilization. Accordingly, in our study concentrations of serotonin and 5-hydroxyindoleacetic acid were measured in the whole brain and forebrain of rats after electrical stimulation of the midbrain raphé region.

Rats (250 to 300 g, Charles River males) were lightly anesthetized with chloral hydrate and placed in a Kopf stereotaxic instrument. A concentric bipolar electrode (tip separation 1.5 mm) was inserted through a hole drilled in the skull into the raphé region of the caudal midbrain. Coordinates according to the rat brain atlas of König and Klippel (*7*) were: A (frontal plane), 350 μ; L (sagittal plane), 0; H (horizontal plane), −2.6. The stimuli were biphasic pulses (10 cycle/sec, 2 msec in duration, 6 volts) applied for 30 minutes or 1 hour from a Grass S4 stimulator. Control rats were treated similarly, an electrode being implanted without passage of any current. Five other control rats were identically stimulated for 1 hour in a site lateral to the raphé region (A, 350 μ; L, 1.4; H, −0.6). After stimulation, the rats were immediately decapitated, and their brains were dissected out and frozen prior to chemical assay. The forebrain was removed from the brainstem by a section passing between the rostral border of the superior colliculi and the caudal border of the hypothalamus. In separate animals, histological examination after placement of electrodes in the midbrain raphé revealed that the electrode tracts extended into the central gray and raphé region at the level of the inferior colliculus and that they were within 0.25 mm of the midline.

The concentrations of serotonin and 5-hydroxyindoleacetic acid were determined in duplicate from the same brain (*8*) for both substances (*9*). Briefly individual whole brains or forebrains were homogenized with four volumes of 0.1*N* HCl, and proteins were precipitated by the separate addition of 1 ml of 10 percent $ZnSO_4$ and 0.5 ml of 1*N* NaOH per 900 mg of brain. 5-Hydroxyindoleacetic acid was then extracted into ethyl ether from the protein-free supernatant adjusted to a *p*H of 1.0 to 1.5. The 5-hydroxyindoleacetic acid was then returned to phosphate buffer (0.25*M*, *p*H 7.0). Serotonin in

Table 1. Effect of electrical stimulation of midbrain regions on indole concentrations in whole brain. Stimulation was carried out for 1 hour. In the controls, electrodes were implanted and kept in place for 1 hour, but no stimulation was given. The significance of differences between control and stimulated levels was calculated using Student's *t*-test. S.D., standard deviation; N.S., not significant.

Animals (No.)	Serotonin			5-Hydroxyindoleacetic acid		
	ng/g ± S.D.	Change (%)	*P*	ng/g ± S.D.	Change (%)	*P*
			Control			
8	529 ± 89			320 ± 28		
			Midbrain raphé			
8	404 ± 93	−23.7	< .025	455 ± 83	+ 42.3	< .001
			Lateral midbrain			
5	582 ± 45	+10	N.S.	321 ± 95	0	N.S.

Table 2. Effect of electrical stimulation of the midbrain raphé area on indole concentrations in forebrain. The significance of differences between control and stimulated levels was calculated using Student's *t*-test. S.D., standard deviation.

Animals (No.)	Serotonin			5-Hydroxyindoleacetic acid		
	ng/g ± S.D.	Change (%)	*P*	ng/g ± S.D.	Change (%)	*P*
			Control			
8	460 ± 63			276 ± 34		
			Midbrain raphé			
8	380 ± 61	−17.4	< .025	454 ± 94	+64.5	<.001

turn was removed from the remaining supernatant by extraction into *n*-butanol at a *p*H of 9.5 to 9.8 and then returned to 0.1*N* HCl by the addition of *n*-heptane to a portion of the butanol extract. The concentrations of both indoles were estimated fluorimetrically according to the methods of Bogdanski *et al.* (*9*). The wavelengths for activation and fluorescence for both indoles were respectively 295 and 540 mμ. We performed duplicate assays of each brain by using two equal portions of the same initial protein-free supernatant (3.0 to 3.5 ml or 450 to 525 mg of brain per milliliter). Duplicate values were totaled, and the final values were calculated as nanograms of free base or acid per gram of brain.

Stimulation of the midbrain raphé region for 1 hour induced a 23 percent decrease in the concentration of serotonin and a 42 percent increase in the concentration of 5-hydroxyindoleacetic acid in whole brain (Table 1). Similar changes were found after a 30-minute period of stimulation. In contrast, stimulation of the lateral midbrain did not produce a significant change in the concentration of either indole. Since some of the changes following stimulation of the midbrain raphé could be caused by local effects, the forebrain alone was assayed in a separate group of animals. The pattern of change in indole concentrations was identical to that of whole brain; there was a large increase in the concentration of 5-hydroxyindoleacetic acid and a concomitant decrease in that of serotonin (Table 2).

The increase in 5-hydroxyindoleacetic acid and the accompanying decrease in serotonin indicate a release and subsequent oxidative deamination of serotonin. Since these changes were evident in the forebrain as well as in the brain as a whole, it would appear that stimulation of serotonin neurons in the midbrain leads to a release of serotonin from the axon terminals of these neurons which project into the forebrain. This conclusion is supported by the fact that after stimulation of the lateral midbrain, an area virtually devoid of neurons containing serotonin, no increase in 5-hydroxyindoleacetic acid or decrease in serotonin was found.

Lesions in the lateral hypothalamus which disrupt the medial forebrain bundle produce a decrease in the concentration of serotonin in the brain (*10*). The decrease of serotonin begins approximately 2 to 3 days after the lesion is placed, and, if the lesion is unilateral, the change is confined to the side with the lesion (*11*). There is some controversy over whether this change in serotonin concentration involves a polysynaptic pathway (*12*) or results from the degeneration of direct axonal projections from neurons in the midbrain raphé (*4*). In any event, we find that 2 weeks after a unilateral lesion of the medial forebrain bundle is made the usual changes in indole concentrations within the forebrain after stimulation of the midbrain raphé are prevented on the side with the lesion but not the control side.

Although the changes in indole concentrations found after midbrain stimulation are most directly explained in terms of a neurally mediated release of serotonin, other influences on biosynthesis or biodegradation may be involved in the observed changes. The actual cellular site (for example, limiting membrane, vesicles, synapse) of release cannot be ascertained on the basis of the data obtained. Exogenous (tritiated) serotonin injected into the cerebral ventricles is taken up by certain nerve endings in areas rich in endogenous serotonin (*13*). Electron-microscopic autoradiography has revealed that the tritiated serotonin is localized in relation to synaptic vesicles within these endings. Tritiated monoamines are also taken up by brain slices (*14*). Electrical stimulation of the slices facilitates release of the exogenous amine into the medium. It seems likely that the endogenous amines would also be released by such stimulation. In any case, our approach provides a means for investigating the process of release of endogenous serotonin mediated by a specific neural pathway of the intact brain.

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Rapid Eye Movement Sleep Deprivation: A Central-Neural Change during Wakefulness

Abstract. *Three adult cats were deprived of rapid eye movement sleep for six separate periods of up to 32 days. Animals were allowed normal amounts of sleeping time during which rapid eye movement sleep was interrupted, whenever it occurred, by human observers who continually monitored the animals and their electrocortical activity. Cortical responses evoked by pairs of acoustic clicks were recorded during wakefulness. Recovery functions derived from these data were facilitated during periods of deprivation of rapid eye movement sleep and returned to base-line values when animals were allowed normal amounts of this sleep phase. This change was noted repeatedly within, as well as between, subjects. It did not occur during control periods when non–rapid eye movement sleep was interrupted on identical schedules, nor did it occur when the cats were deprived of all sleep for 22 hours a day for 5 days.*

Current evidence demonstrates that normal sleep is composed of two general and consistently occurring stages, and that at least one (rapid eye movement or REM phase) is actively main-