Light-Induced Changes in Pineal Hydroxyindole-O-Methyltransferase: Abolition by Lateral Hypothalamic Lesions

Abstract. *The activity of hydroxyindole-O-methyltransferase, the melatonin-forming enzyme in the pineal gland, is several times greater in rats kept in continuous darkness than in those kept in continuous light. Lesions transecting the medial forebrain bundle in the lateral hypothalamus suppress these differences in enzyme activity and abolish light-induced changes in pineal weight. These findings indicate that the medial forebrain bundle may participate in the control of this enzymatic response to environmental lighting.*

Several enzymes (*1*) and the concentrations of serotonin (*2*) and noradrenaline (*3*) in the pineal gland are affected by environmental lighting. One of these enzymes, hydroxyindole-*O*-methyltransferase (HIOMT) is highly localized in the mammalian pineal gland and has an activity three to five times higher in the pineal glands of rats kept in continuous darkness than in those of rats kept in continuous light (*1*). This effect of light on HIOMT activity is abolished after the animals have been blinded by bilateral enucleation and after the superior cervical ganglia have been removed bilaterally (*4*). These observations indicate that the pineal gland receives information about environmental lighting from the retina via the brain and sympathetic nerves. The central pathways transmitting this information are not understood.

The sympathetic nerves innervating the rat pineal gland are unusual in that they contain both serotonin and noradrenaline (*5*). These monoamines are also found in brain, and it has been shown that the maintenance of normal concentrations of both serotonin and noradrenaline in brain is dependent upon the integrity of the medial forebrain bundle in the lateral hypothalamus (*6*). Destruction of the medial forebrain bundle significantly decreases the amount of serotonin and noradrenaline in the brain (*6*). The loss of monoamines produced by such lesions takes place throughout the telencephalon, both in areas directly innervated by the medial forebrain bundle and in areas distant to the tract and its direct connections (*7*). The effects of lesions of the medial forebrain bundle in these areas distant to the tract are mediated across at least one, and probably several, synapses. The existence of these transsynaptic or "indirect" effects of lesions of the medial forebrain bundle on telencephalic monoaminergic neurons suggested that the medial forebrain bundle might be important in the control of other distant monoaminergic systems, such as the sympathetic nerves of the pineal. We now describe the abolition of the effects of environmental lighting on the melatonin-forming enzyme, HIOMT, in the rat pineal gland by lesions of the medial forebrain bundle.

Bilateral electrolytic lesions were placed stereotaxically to transect the medial forebrain bundle in the lateral hypothalamus of Holtzman–Sprague-Dawley male rats, aged 80 to 86 days. These animals and a group of controls that underwent a sham operation were prepared as described (*6*). Approximately 30 days after operation, the animals with lesions of the medial forebrain bundle and the controls were divided into two groups. One group was placed in continuous light and the other in continuous darkness. The animals were kept at a uniform temperature of 25°C. After 7 days, the rats were killed in the morning, and their pineals were removed and examined for HIOMT activity (*8*). The brains from the group having the lesion of the medial forebrain bundle were fixed in formalin and prepared for histological examination.

Table 1. Abolition of light-induced changes in enzymatic synthesis of melatonin (HIOMT) in pineal gland by lesions of the medial forebrain bundle. Individual pineal glands from groups of rats (six to eight) kept continuously in darkness or in light for 7 days were assayed for hydroxyindole-*O*-methyltransferase activity. The results are expressed as means plus or minus standard error of the mean. The *P* values were obtained by a *t*-test for differences between the groups. The differences in pineal-body weights and in melatonin formed between the groups with the lesions are not statistically significant.

Treatment	Pineal weight (mg)	Melatonin formed ($\mu\mu$ mole hr^{-1})	
		Per whole gland	Per milligram of gland
Sham operation			
Light	1.58±0.12	6.5±1.5	4.1±3.4
Dark	1.98±0.09*	41.2±6.0†	20.7±2.6†
Medial forebrain bundle lesion			
Light	1.24±0.12	14.8±3.3	12.0±2.9
Dark	1.32±0.06	22.4±4.4	16.8±3.7

* $P < .05$. † $P < .001$.

In the animals that received the sham operation and were kept in the dark for 7 days there was the expected rise in weight of the pineal body, accompanied by a nearly fivefold increase in HIOMT activity (Table 1). In animals with lesions of the medial forebrain bundle, however, there was no significant difference with respect to either weight of the pineal body or HIOMT activity between the group kept in constant light and that kept in constant darkness. Thus the lesion of the medial forebrain bundle blocked the usual effect of altered environmental lighting on pineal weight and HIOMT activity.

Histological examination of the lesions of the medial forebrain bundle showed that they did not differ significantly from similar lesions described earlier (*6*). Primarily the lesions (Fig. 1) destroyed the medial forebrain bundle in the lateral hypothalamus at the level of the caudal half of the ventromedial hypothalamic nucleus. Occasional lesions extended into medial hypothalamus, zona incerta, subthalamic nucleus, and the cerebral peduncle; but this was unrelated to the effect of the lesion in abolishing the HIOMT response to light. That is, extension into adjacent structures did not alter the effect of the lesion, whereas involvement of the medial forebrain bundle was essential to obtain the effect. In no instance was there significant damage to the primary optic tracts, and all lesions were placed caudal to the optic chiasm.

In addition to the diurnal cycling of pineal HIOMT activity there are 24-hour rhythms in the serotonin (*2*) and noradrenaline (*3*) content of the rat pineal. The serotonin rhythm persists in darkness and in animals blinded by bilateral enucleation (*9*); but it can be

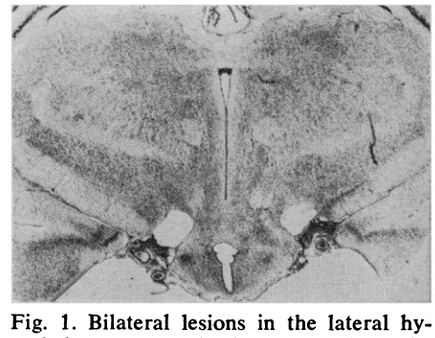

Fig. 1. Bilateral lesions in the lateral hypothalamus completely transecting the medial forebrain bundle. The lesions are centered in the lateral hypothalamus and extend very little beyond its borders. Frozen section, cresyl violet stain, magnified 22 times.

abolished by a single period of 4 hours of light. Thus, this rhythm is endogenous but influenced by changes in environmental lighting. The noradrenaline rhythm, on the other hand, is like the HIOMT rhythm; it is exogenously controlled and suppressed both by continuous darkness or continuous light (*10*). Both serotonin (*11*) and noradrenaline (*12*) rhythms may be obliterated by sectioning the medial forebrain bundle.

These effects of lesions of the medial forebrain bundle on pineal monoamine rhythms and the elimination of the light-induced decrease in pineal HIOMT implies that this central neural pathway has a part in the control of certain pineal functions. Since no components of the medial forebrain bundle project caudally beyond the midbrain tegmentum, its control on distant biochemical events in the pineal must be mediated through a polysynaptic neuronal system which eventually terminates on the cervical sympathetic nerves. This is of particular interest in that the effects of lesions of the medial forebrain bundle on serotonin and noradrenaline (*7*) and on the enzyme, aromatic L-amino acid decarboxylase (*13*) in the telencephalon, are also mediated, in large part, transsynaptically. The medial forebrain bundle appears, therefore, to have a controlling influence on both peripheral and central monoaminergic neurons.

It had been suggested that brain tracts which mediate the effects of light on the pineal gland might be traced by observing the effects of their interruption on responses of HIOMT (*4*). Our studies provide evidence that one central pathway involved in the transmission of information from the retina to the pineal is the medial forebrain bundle.

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Subacute Sclerosing Leukoencephalitis: Ultrastructure of Intranuclear and Intracytoplasmic Inclusions

Abstract. *Intranuclear inclusions found in glial cells of two cases of subacute sclerosing leukoencephalitis have been examined in the electron microscope. The inclusions are composed of filamentous tubules 170 to 230 angstroms in diameter, which bear a general resemblance to the intranuclear structures observed in cells infected with measles virus. This finding provides further suggestive evidence that a virus may be involved in the pathogenesis of subacute sclerosing leukoencephalitis.*

Subacute sclerosing leukoencephalitis (SSLE) is a slowly evolving disorder of the human central nervous system. Pathological changes are prominent in the hemispheral white matter. Characteristic microscopic changes include perivascular infiltration by plasma cells and lymphocytes, hypertrophy of astrocytes, microglial proliferation, and the destruction of myelinated axons (*1*). Intranuclear and intracytoplasmic inclusion bodies similar to those seen in known viral infections are usually present at some stage of the disease (*2*). Under the light microscope, the intranuclear inclusions appear as homogeneous eosinophilic masses which occupy a central position and displace the nuclear chromatin to the periphery (*2*).

Because of these pathological features, SSLE has long been suspected to be a disease of viral etiology. Although efforts to isolate an infectious agent by inoculating SSLE material into animals and tissue cultures have been unsuccessful (*3*), a viral etiology for the disease has received recent support from the electron microscopic observation of intracytoplasmic spherical virus-like particles in the brains of patients with SSLE (*3, 4*). These particles measure 600 to 800 Å in diameter and contain a dense central osmiophilic core approximately 400 Å in diameter, surrounded by an outer envelope. Although the morphology of these particles does not permit their definite identification with one of the major groups of animal viruses, their appearance recalls that of certain enveloped, lipid-containing viruses such as arboviruses (*5*), myxoviruses (*6*), and murine and avian tumor viruses (*7*).

In an attempt to obtain more information about the development of the virus-like particles, the ultrastructure of the intranuclear inclusions in two clinically and pathologically typical cases of SSLE was examined with the electron microscope.

Tissue from frontal cortex and white matter, cerebellar white matter, and pons was obtained from brain slices (fixed with formalin), in one case, and, from a cerebral biopsy specimen, in the other case. The specimens were fixed in osmium tetroxide, dehydrated in ethanol, and embedded in Epon and Araldite. Thin sections were cut with a Porter-Blum microtome, mounted on bare copper grids, and stained with lead acetate and uranyl acetate. Hitachi HS7 and Siemens Elmiskop I electron microscopes were used in the examination.

In both cases, the intranuclear inclusions were seen in glial cells and were composed of interwoven filamentous structures that filled and replaced most of the nucleus (Fig. 1). At higher resolution, these filamentous structures consist of fine tubules 170 to 230 Å in diameter (Fig. 2). A tubular structure is suggested by a circular hollow profile in cross section (Fig. 2) and a triple density (dark-light-dark) in longitudinal section. They measure