pooled in the control group. Table 1 gives the mean time taken to reach criterion for the four experimental groups. Natural taildrops (group B) and surgically divided tails (group C) of planarians that had been habituated to a criterion level were allowed to regenerate for 10 days. Both these groups gave results differing significantly from group A. Regenerated offspring (groups B, C) of fully habituated planarians showed a significantly lower level of responsiveness to light in the first 2 days of testing and reached the habituation criterion more quickly than the initially naive controls (group A).

The pooled cannibal habituation group ($N = 6$) were fed two fully habituated planarians every fourth day for 20 days. These were shared by the group and all were observed to be eating at each feeding. Then the course of their habituation to light was compared with controls ($N = 6$) fed in the same manner with planarians naive to light but otherwise handled in the same way as the habituated planarians that were eaten. The cannibal habituation group (D) showed fewer responses to light over the first 50 trials and reached criterion significantly sooner than their controls (Table 1). Five out of the six planarians in group D reached criterion before the first of their matched controls had done so.

The gradual quantitative change of response leading to the loss of responses (called habituation) persists, at least in part, from day to day. It is slowly reversible. Ten planarians that showed 28 percent (±10) responses in their first 50 trials, were then fully habituated (zero responses in 50 consecutive trials). They still showed a significant reduction in responsiveness to light after 3 and 7 weeks without intervening testing, namely: at 3 weeks postcriterion, 12 percent (±8) responses ($P < .01$), and at 7 weeks, 16 percent (±7) ($P < .01$).

Habituation to light occurs exclusive of changes of stimulus or qualitative changes of response. The latter is shown by the fact that planarians habituated to light in the normal position still responded when presented with a light stimulus differing both qualitatively and quantitatively from that to which they were habituated (*10*).

The results for day 1 (Fig. 1, inset) demonstrate that for one planarian on a given day the frequency of response to a single light trial is related to the position of that trial in the day's sequence of 25 light presentations. This, together with the electrophysiological findings of Behrens (*11*), suggests that adaptation of the photoreceptors occurs. Responsiveness of planarians on day 16 is greatly reduced by habituation, but still shows evidence of adaptation, as graphically shown by Fig. 1, inset.

There is evidence (*6, 12*) that habituation in mammals is a central phenomenon with overall inhibitory effect which utilizes pathways and is dependent upon synaptic mechanisms common to learning and conditioning. The suggestion (*6, 12*) that habituation is a fundamental physiological mechanism of adaptation is borne out by the ability of planarians to habituate. Past (*6, 12*) and present results distinguish habituation from adaptation of sensory receptors.

A possible chemical basis for inheritance and transfer of conditioning in planarians has been suggested (*5*). The present results are of interest because habituation is an extinction of response or a learned failure to respond. Though quite distinct from conditioning, it shows very similar transfer by cannibalism. Such habituation in planarians may differ sufficiently from other learning situations to require a molecular coding different from that for conditioning, to be transferred in the manner described (*13*).

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Electroencephalographic Desynchronization during Deep Sleep after Destruction of Midbrain-Limbic Pathways in the Cat

Abstract. *Lesions which interrupt the ascending limb of the midbrain-limbic circuit in the cat at different levels, or which even destroy it completely, do not prevent electroencephalographic desynchronization at the beginning of periods of deep sleep, nor do they affect the maintenance of desynchronization throughout the sleep episodes. The pontine mechanisms responsible for these electroencephalographic patterns can apparently exert their influence through ascending pathways other than those directly impinging on the hypothalamus and the limbic system.*

A behavioral pattern, characterized by postural atonia with sudden loss of neck muscle tone and rapid jerks of eyeballs and body muscles, and paradoxically associated with an electroencephalographic change from synchronization to the desynchronized rhythms generally considered typical of arousal, has recently been described as deep sleep in mammals (*1*). The neural mechanisms which induce these episodes appear to be located in the pons (*1, 2*), but little is known about their ascending connections. However, these connections have been tentatively identified (*1*) with the ascending limb of the so-called "midbrain-limbic circuit," which connects the caudal and rostral midbrain tegmentum with hypothalamic and limbic structures (*3*). Since evidence on this topic is scanty and since the functional significance of the midbrain limbic pathways is uncertain (*4*), we undertook experiments to clarify the issue (*5*).

Screw-electrodes were implanted in the skull of cats for monitoring electrical activity from the right and left hemispheres, and in both orbits for recording eye movements. The electromyogram of the neck was obtained through two needles permanently inserted into the paravertebral cervical musculature. The animals were placed in a soundproof, electrically isolated, lighted cage and were observed through a large glass window. Graphic recordings were made on a multichannel inkwriter (*6*). After a sufficient number of deep sleep episodes in the intact animals had been observed, various types of midbrain lesions were made

electrolytically on anesthetized cats with steel electrodes which had been stereotactically oriented. The needles were placed either at a 90° or a 45° caudal inclination (depending on the type of lesion to be made) over the interaural-zygomatic plane of the head. The effect of these lesions on the electroencephalographic and behavioral patterns of sleep was studied during the survival period (up to 14 days). The extent of the lesions was ascertained in serial histological sections stained alternately by the Nissl and Weil methods.

The ascending limb of the midbrain-limbic circuit is composed of two main fiber systems, which are schematically represented in the sagittal drawings on the left of Fig. 1: (i) the ascending component of Schütz's dorsal longitudinal fasciculus originates in Gudden's dorsal tegmental nucleus and receives further contributions throughout the length of the periaqueductal grey substance, branches out to the periventricular and posterior hypothalamus and to the intralaminar thalamus; (ii) the system of the corpus mamillare peduncle, which originates in both the dorsal and ventral tegmental nuclei (possibly also in the nucleus centralis superior), and in the ventral tegmental area of Tsai, impinges on the corpora mamillaria, the lateral hypothalamus, the preoptic region, and the medial septal nuclei where it connects with neurons directed to the hippocampus and the amygdaloid complex.

Ascending conduction along the midbrain-limbic circuit has been interfered with by means of three types of lesions shown in Fig. 1. In the first group of animals, a lesion in the rostroventral portions of the midbrain tegmentum led to interruption of both peduncles of the corpus mamillare, the rostral pole of the interpeduncular nucleus, and the ventral tegmental area of Tsai, thus blocking the rostral outflow of the corpus mamillare peduncle system (*A*). In the second group, a lesion in the dorsal part of the caudal midbrain tegmentum destroyed the posterior pole of the central mesencephalic grey and both the dorsal and ventral tegmental nuclei of Gudden, thus interfering with the very origin of both the Schütz's and the corpus mamillare peduncle systems (*B*). Finally, in the third group very large lesions involved practically all the midbrain structures with limbic connections—the caudal pole of the central grey, dorsal and ventral tegmental nuclei, nucleus centralis superior, interpeduncular nucleus, peduncles of the corpus mamillare, and at least part of the ventral tegmental area of Tsai (*C*).

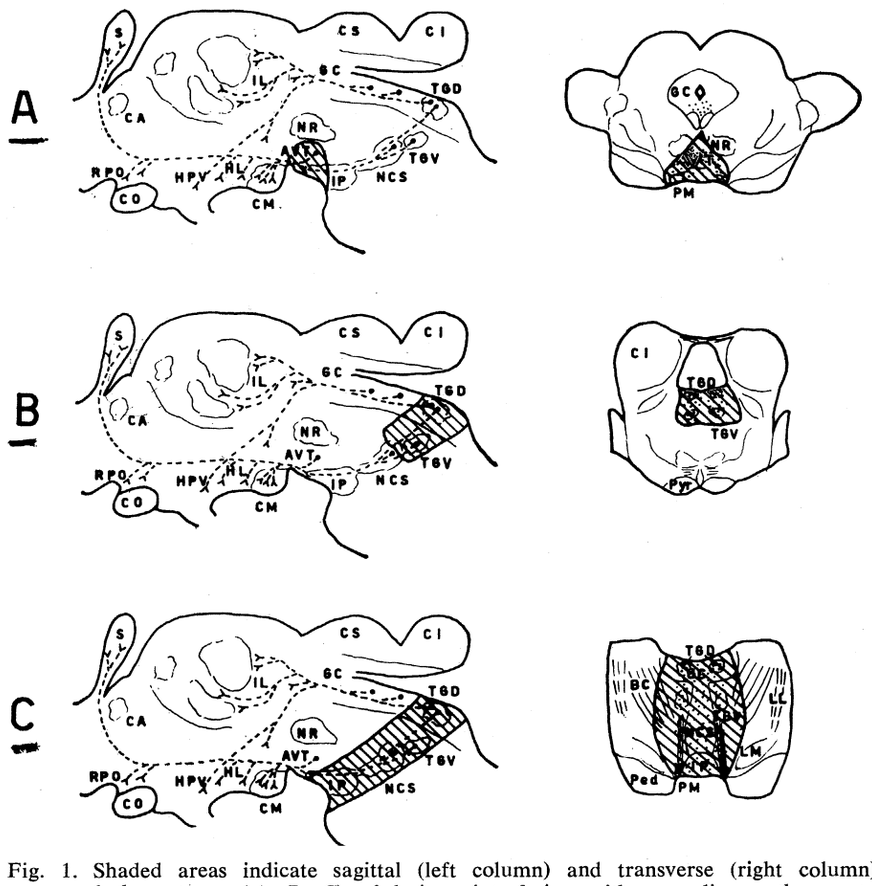

Fig. 1. Shaded areas indicate sagittal (left column) and transverse (right column) extent of three types (*A*, *B*, *C*) of lesions interfering with ascending pathways to hypothalamic and limbic structures. On the left the sagittal course of these connections is shown by dashed lines. Dots in the drawings on the right indicate areas of origin or transit (or both) of these pathways. Drawings on the right are frontal sections through the maximal transverse extent of the lesion. In *A* and *B* these sections are cut parallel to the frontal plane of the stereotactic instrument, whereas in *C* the frontal section is cut with a 45° (instead of a 90°) inclination over the horizontal plane. *AVT*, area tegmentalis ventralis; *BC*, brachium conjunctivum; *CA*, anterior commissure; *CI*, inferior colliculus; *CM*, corpora mamillaria; *CO*, optic chiasma; *CS*, superior colliculus; *GC*, central grey; *HL*, lateral hypothalamus; *HPV*, periventricular hypothalamus; *IL*, intralaminar thalamus; *IP*, interpeduncular nucleus; *LL*, lateral lemniscus; *LM*, medial lemniscus; *NCS*, nucleus centralis superior; *NR*, red nucleus; *Ped*, pes pedunculi; *PM*, corpus mamillare peduncle; *Pyr*, pyramid; *RPO*, preoptic region; *S*, septum; *TGD*, dorsal tegmental nucleus; *TGV*, ventral tegmental nucleus.

The results obtained for all three categories of lesions were remarkably similar. As expected, the behavior of the animals during periods of deep sleep was substantially unchanged, except for some postural disturbance induced by large tegmental lesions, and except for the frequent disappearance of the eyeball jerks; in all cases the sudden loss of muscle tone, especially in the neck, and the bursts of clonic movements of the mimic musculature were clearly apparent. Surprisingly enough, even the largest lesions, amounting to a complete interruption of all components of the ascending midbrain-limbic pathways (Fig. 1-*C*), did not interfere with the appearance of desynchronized electroencephalographic patterns at the beginning of an episode of deep sleep, or with the maintenance of such patterns during the several minutes of its duration. Electroencephalographic desynchronization could also be observed when the animals had been aroused or when they were awake.

The ascending limb of the midbrain-limbic circuit and the corpus mamillare peduncle system, in particular, are not indispensable either for the appearance or the maintenance of the electroencephalographic desynchronization accompanying deep sleep. Although these pathways may well be

involved in inducing the hippocampal rather than the neocortical rhythms of deep sleep, the electroencephalographic patterns characterizing this stage of sleep can hardly result from activity limited to the specific pontolimbic connections which we investigated. Whether the conduction system from the rhomboencephalic pacemaker of deep sleep to the more rostral mechanisms regulating cortical activity is restricted to small regions of the brain stem, other than those projecting to the rhinencephalon, or is widely scattered through the midbrain, cannot be determined as yet, but the latter hypothesis is supported by the persistence of desynchronized sleep patterns after such large lesions as those shown in Fig. 1*C*.

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Algal Virus: Isolation

Abstract. *Freshwater blue-green algae of the genera* Lyngbya, Plectonema, *and* Phormidium *are susceptible to a virus recently isolated from a waste-stabilization pond. Electron micrographs of a partially purified preparation show that the viral particle has an icosahedral structure about 66 mμ in diameter.*

Rapid decomposition of algae is often observed under circumstances that suggest the presence of a factor not generally associated with degeneration. From studies on the phytoplankton populations of shallow lakes, Tucker (*1*) reported that inhibitory agents of unspecified origin were responsible for fluctuations in the development of these organisms. Krauss (*2*) suggested that the solution to many of these unresolved incidences might be explained by the presence of viral agents.

In view of the ubiquitous nature and distribution of viruses, it seems unlikely that the biological characteristics of the algal cell are such that the entire group would be immune to viral infections. With this in mind, emphasis was placed on determining the susceptibility of blue-green algae to these agents. Screening for the virus was carried out with samples collected from environments having a dense and somewhat unstable algal population. The present report describes the isolation of a virus-like agent which, to the authors' knowledge, is the first definite evidence that freshwater algae (*3*) may be infected by such agents.

The agent was isolated from samples collected at a waste-stabilization pond used exclusively for the treatment of domestic sewage. The pond, approximately 4 acres in size, is located in southeastern Indiana and supports a considerable variety of algal forms. Composite samples taken from the pond were added to algae-enriched media, incubated for one week, and then centrifuged. The supernatant was treated with chloroform (*4*), and the final preparation was screened for viral activity. Lysis of one of the blue-green algal test organisms, *Plectonema boryanum* IU 594 (*5*), in liquid medium and demonstration of plaque development in solid medium indicated the presence of a virus-like agent in the assayed sample. Examination of the lytic agent showed that it could readily be passed through an ultrafine sintered-glass filter and that exposure to high temperatures (90° to 100°C) completely inactivated it. Bacteria-free filtrates recovered from lysed cultures of *P. boryanum* have been transferred through 35 subculturings, the final subculture being equivalent to a 10^{-70} dilution of the original material. Additions of small numbers of this agent to growing algal cultures resulted in total lysis of the algae and a 5- to 6-log increase in the titer of the agent. It is, therefore, evident that the agent possesses those characteristics that are generally ascribed to a virus.

To insure the purity of the viral strain, the virus was picked and replated twice from single plaques selected at random. The isolated strain was examined for its capacity to lyse algae, actinomycetes, and bacteria. Of the 78 organisms tested, the virus lysed 11 filamentous algal strains belonging to the class Myxophyceae. Included in these forms were members of three genera—*Lyngbya, Plectonema,* and *Phormidium*. The first letters in the names of these three genera have been used to classify the blue-green algal (BGA) virus as strain LPP-1. The algal strains from the Indiana University (IU) culture collection which are susceptible to the BGA virus are *Phormidium luridum* var. *olivace* IU 426, *Phormidium* species IU 485, *Phormidium fareolarum* IU 427, *Plectonema boryanum* IU 581, 594, 597, and 790, *Plectonema calothricoides* IU 598, *Plectonema notatum* IU 482, *Lyngbya* species IU 487 and 488.

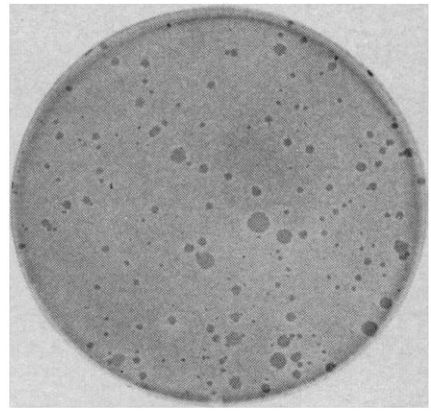

Fig. 1. Plaques of BGA virus strain LPP-1 on *Plectonema boryanum* culture plate.

The viral agent was propagated under static conditions with *Plectonema boryanum* IU 594 as the host and a modified Chu No. 10 broth (*6*) as the medium. Cultures were incubated at 20°C under a light intensity of 160 to 180 lu/ft^2. After an incubation period of 2 to 3 days, the titer was generally about 10^8 viral particles per milliliter of broth. On solid medium the virus and the host cells were incubated 3 to 4 days before plaque counts were made. Additional incubation of the virus often resulted in lysis of the entire culture plate. Figure 1 shows relatively clear plaques with a well-defined border that is somewhat frayed by the filamentous nature of the host cells. Plaques produced by this strain differ considerably in size and vary from less than 0.1 mm to more than 8 mm in diameter.

Because of the symbiotic and tenacious relationships between bacteria and blue-green algae, it is not inconceivable that a bacteriophage could indirectly inhibit algal development. Algal degeneration under such circumstances would undoubtedly be concomitant with a nutritional deficiency, but microscopic examination of unialgal cultures showed that the algal cells are