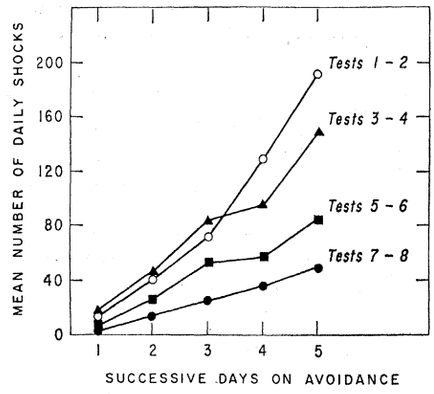

Fig. 1. Mean number of shocks per day on successive prolonged avoidance tests.

successive tests, as well as the "days × tests" interaction, were statistically significant at well beyond the .01 level. During tests 7 and 8 subjects were doing as well on the fourth day of continuous work as they had originally done on the second day (tests 1 and 2). The largest improvements occurred on the fourth and fifth days of the tests (*4*).

Even though on tests 1 to 3 individual monkeys showed marked differences in their ability to maintain performance over the 120 hours, every monkey clearly exhibited an adjustment-to-fatigue similar to that of the averaged results in Fig. 1. During tests 7 and 8 all monkeys were doing well throughout the entire 5 days; the individual differences among the monkeys were much smaller than they had been originally.

Since the monkeys were excellent avoidance performers prior to the first prolonged sessions (and no monkey received more than 20 shocks on the first day of test 1), the effect shown in Fig. 1 was certainly not due to simple acquisition of the lever response. What, then, was the mechanism through which this improvement took place? Do these results illustrate "learning" in the usual sense? Our monkeys may have learned to adjust to the situation by a change in work methods, either through the elimination of unnecessary, tiring movements or by the acquisition of more adaptive responses.

For example, we observed one monkey pressing the lever with different hands, and another monkey closing his eyes between lever presses. We did not, however, notice such techniques in all the subjects. Another possibility is that the monkeys actually learned to "sleep" and lever-press simultaneously (*5*), a phenomenon that has already been examined in humans (*6*). We are currently obtaining continuous electroencephalographic and photographic records of our subjects to determine whether the usual objective indices of sleep are modified during the course of repeated prolonged tests.

It is possible that this adjustment-to-fatigue may be correlated with some physiological or biochemical adaptation, caused by changes in the normal sleeping cycles of the monkeys or by the stressful nature of the task. If the improvement were an indirect effect of such adaptation, it might not be appropriate to apply the term "learning" to the adjustment described here.

Further analysis of adjustment-to-fatigue may prove of value in practical problems which involve prolonged monitoring, such as might occur in industry, communications, and space flight (*7*).

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4. Later, two of the monkeys were also tested in 8- or 10-day sessions of continuous avoidance. During a total of five such tests neither subject reached the cutoff criterion of 250 daily shocks. One subject received no more than 80 shocks on any day of these extra-long tests.
5. The data from another well-trained monkey that had to make a single lever press to one of two randomly-presented visual stimuli to prevent shock (responses to the second stimulus were punished) suggest that learning to sleep-and-press cannot wholly explain the improvement reported here. This monkey had to keep his eyes open to observe stimuli and still showed an improvement with repeated tests.
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Electroencephalogram in the Permanently Isolated Forebrain of the Cat

Abstract. The cerebral cortex of cats with chronic or permanent mesencephalic transections of the brain stem showed both high-voltage, slow-wave synchronized electroencephalographic patterns and low-voltage, fast-wave desynchronized rhythms when studied postoperatively. The alternation of sleep and wakefulness electrical activity in the permanently isolated forebrain is contrasted with the observations of Bremer in the acute "cerveau isolé" preparation.

Since the work of Bremer in the "cerveau isolé" preparation (*1*), it has been generally accepted that the cat forebrain with a high transection of the brain stem exhibits a fixed EEG pattern of high-voltage, slow-wave activity similar to that found in one of the stages of sleep in the normal cat. In recent years this stage has been called deep sleep by Dement (*2*) and slow sleep (sommeil lente) by Jouvet *et al.* (*3*). Bremer's observations have been confirmed recently (*3, 4*) in cats with rostropontine and mesencephalic transection. All of these studies have been performed, however, in experimental preparations with survival times of only 3 to 9 days after transection of the brain stem. Only Batsel (*5*) has studied, so far, long-term preparations; his observations, in dogs, are similar to those reported here in the cat.

In addition to the dramatic physiological and behavioral changes seen in high decerebrate cats, one is also impressed by the clear-cut alternation between sleep and wakefulness in these animals.

The explanation of such striking change probably rests in the restablishment of homeostasis and in the lessening of shock-like factors in the long-term preparation. Similarly, the same mechanisms could determine the permanent "slow sleep" pattern of the short-term "cerveau isolé" preparation, and conversely, a long-term isolated forebrain could exhibit both high-frequency, low-voltage waking patterns, and slow, high-voltage sleep rhythms.

Woods and Bard have emphasized the importance of the time factor in neuroendocrine experiments performed in animals with extensive lesions in the nervous system (*6*). Adametz (*7*) has also shown the importance of what he calls "shock factor" (diaschisis) in the behavioral and neurological results of acute experimental procedures.

To explore the problems of sleep-

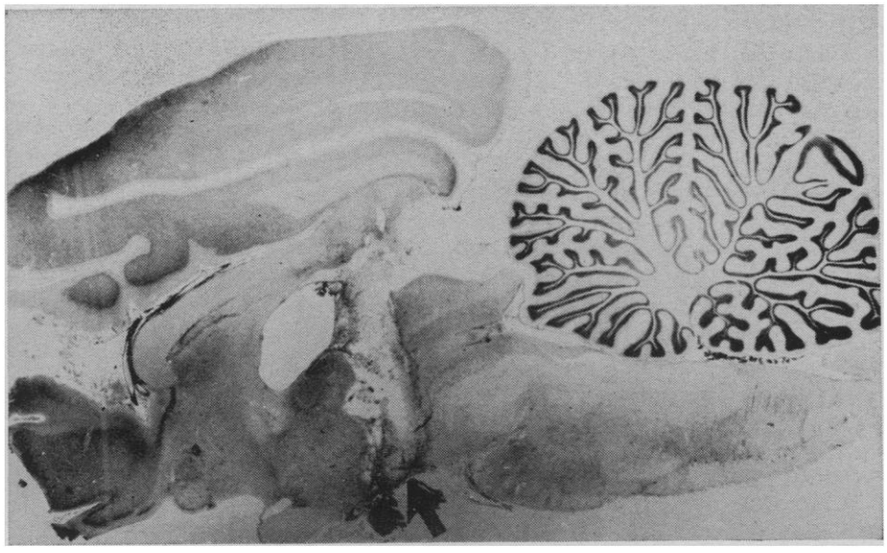

Fig. 1. Thionin stained saggital section of the brain of cat No. 6 shows complete transection (arrow) of the brain stem at mesencephalic (precollicular) level.

wakefulness alternation in decerebrate cats our experiments were carried out as follows.

Bipolar electrodes were implanted aseptically in the primary auditory cortex or the somatosensory cortex, or in both structures, in cats under barbiturate anaesthesia. A Sheatz type multilead electrode holder was used to fix the electrodes firmly to the skull. After recovery from the operation, the cats were observed for alternation between sleep and wakefulness. Electroencephalographic activity, rectal temperature, respiration rate, electrocardiograms, and electromyograms (*8*) were recorded.

After several definitive recordings had been obtained, the brain stem of each cat was transected by aspirating a thin wedge of nervous tissue from between the rostral border of the anterior colliculi and the caudal borders of the pulvinar and medial geniculate bodies. This section, extending just behind the mammillary bodies, resulted in a highly decerebrate preparation. The techniques (*9*) developed to overcome the difficulties in postoperative care of decerebrate cats enabled us to maintain the preparations for a minimum of 23 days and a maximum of 63 days.

Postoperative recordings were begun 2 or 3 days after the operation and repeated every 5 to 7 days thereafter. A total of 35 hours of recording before the brain stem transection and recordings for 75 hours after the operation were compiled for five cats. Four brains were studied histologically to verify the characteristics of the transections and the placements of the electrode tips. In three cases the transection was shown to be complete (a representative specimen is shown in Fig. 1), and in the fourth brain a small unilateral tectal remnant was found.

During approximately the first 10 days after transection, the EEG cortical activity was represented by high-voltage, slow waves of the coma or slow sleep pattern. Later on, long-lasting periods of desynchronized EEG rhythms appeared which were indistinguishable from the EEG pattern of the awake normal animal. The two patterns alternated (Fig. 2). Both patterns seemed to be correlated with behavioral wakefulness and sleep, but more observations are needed to be certain about this important point. It is known that long-term decerebrate cats often exhibit catatonic or cataplectic-like states which are difficult to define either as sleep or wakefulness.

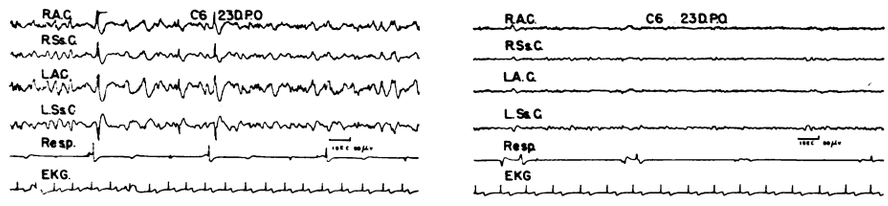

Fig. 2. Electrocorticogram of cat No. 6, 23 days after the transection of the brain stem as shown in Fig. 1. The tracing at the left shows a synchronized "asleep" EEG pattern, and the one at the right shows a desynchronized, "awake" rhythm. *R*, right; *L*, left; *A.C.*, primary auditory cortex; *Ss C.*, primary somatosensory cortex; *Resp.*, respiration; *EKG.*, electrocardiogram.

The appearance of fast EEG rhythms in the isolated forebrain coincided with neurological improvement of the animal. By the 10th or 15th day after transection most cats could walk spontaneously and spent much of the time in a sitting or standing position. Their autonomic condition also changed, since in two cats, heart arrhythmia occurred during the first 10 or 15 postoperative days and then vanished thereafter.

These findings indicate that in a long-term isolated forebrain, EEG patterns present during sleep and during the waking state of a normal cat can be exhibited alternately contrary to Bremer's classic "cerveau isolé."

There is evidence that certain forebrain and diencephalic structures influence the electrocorticogram of the sleep and wakefulness states (*10*). There are no clear reasons why those structures should not be able to promote alternation between sleep and wakefulness when they remain as a part of a long-term isolated forebrain. One could hypothesize that the surgical trauma is responsible for an initial temporary period of depression of these structures. Whether only this factor or others are implicated in the genesis of fast EEG cortical rhythms in long-term forebrain preparations, remains to be studied. Preliminary observations on the action of metrazol and adrenalin on the isolated forebrain, reveal that a supersensitivity phenomenon of the Cannon-Rosenblueth type (*11*), should also be considered. It could be postulated then that a brain stem reticular formation mechanism could promote neurohumoral changes that are able to "awake" a sensitized forebrain. Additional studies of the humoral influences on the chronic isolated forebrain and of the correlation between cortical and brain stem EEG activity are urgently needed (*12*).

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Biosynthesis of Infectious Tobacco Mosaic Virus Ribonucleic Acid in a Cell-Free Medium

Abstract. When cell-free preparations containing large molecules and subcellular bodies extracted from tobacco-leaf cells infected with tobacco mosaic virus were incubated with $MgCl_2$ and the four ribonucleoside-5′-triphosphates, the level of infectivity increased about threefold in 30 minutes. The mechanism synthesizing the new infectivity was believed to be located within isolated nuclei. Ultrasonic rupture of the nuclear membranes appeared to increase the rate of synthesis.

Evidence for the existence of naturally occurring infectious ribonucleic acid (RNA) of tobacco mosaic virus in infected plant cells has been reported by several virologists (*1*). Data presented by Engler and Schramm (*2*) suggested that this is the first-formed infectious stage of the virus. Recent success in the biosynthesis of RNA from its nucleoside-5′-triphosphates (*3*) suggested to us that similar techniques might be applied to the synthesis of infectious viral RNA (*4*).

We assumed that a synthesizing mechanism might be isolated from infected plant cells which would be capable of combining adenosine triphosphate, guanosine triphosphate, uridine triphosphate, and cytidine triphosphate in the proper linear sequence to form infectious viral RNA.

We knew that the presence of ribonuclease in the synthesizing system could destroy the newly formed viral RNA, and we assumed that any synthesizing mechanism isolated from infected cells would be as large as, or larger than, the RNA being formed.

Gel filtration techniques in which 2-percent particulate agar gels were used, were devised for isolating the synthesizing mechanism in a ribonuclease-free state. (It had been determined that the pores of 2-percent agar excluded tobacco mosaic virus RNA while admitting smaller molecules.) Agar gel particles prepared by spraying hot agar through cold atmospheres or by chopping solidified agar were sized by passage through 24-mesh screens and retention on 48-mesh screens. Slurries of the agar particles were allowed to settle in chromatographic tubes to give columns 40 to 60 cm high, which were then conditioned by passage of isotonic sucrose buffered at *p*H 7.2 (0.25 mole of sucrose, 0.003 mole of $CaCl_2$, and 0.006 mole of tris).

Unstable tobacco mosaic virus RNA, phenol-prepared, was used to inoculate the leaves of Turkish or Havana 38 tobacco so that there would be no external carry-over of inoculum that might complicate interpretation of the experimental results. After the desired infection time had elapsed (14 hours or longer), the leaves were placed in a plier-type juice extractor and pressed so that the extracted sap fell directly on the top of the gel columns. Approximately 40 g of leaf tissue were extracted in each experiment. The applied sap was driven through the columns by the application of additional sucrose buffer. The downward migration of the green chloroplasts served as a visual indicator of the movement of the large molecules and subcellular bodies. Collection of the effluent was begun just prior to the elution of the chloroplasts and was continued in most experiments until 100 to 150 ml had been collected.

Extensive studies with the light microscope showed that the effluent contained chloroplasts, starch grains, nuclei, and many smaller particles, but no structures as large as cells were seen. It was noted that a green zone always remained at the top of the gel column in each experiment. When samples from these zones were studied, single cells and groups of cells were observed. It was also noted that our 2-percent agar columns would not pass a single-celled alga considerably smaller than tobacco leaf cells. All of these observations suggested that the gel columns were filtering out intact cells to give only cell-free extracts.

The movement of a brown-colored zone down the column indicated the migration rate of the smaller molecules. Collection of effluent was always stopped before this zone was eluted.

Low-speed centrifuging (4000*g* for 15 minutes) was used to concentrate the synthesizing mechanism in the effluent while discarding most of the free tobacco mosaic virus and viral RNA. The green-colored pellets were usually resuspended in 4 to 6 ml of sucrose buffer containing 0.003 mole of K_2HPO_4 and 0.01 mole of $MgCl_2$. The suspension was then divided into two equal parts, of which one, the control solution, received no further treatment and the other, the chemically treated solution, received 1-mg amounts of the four ribonucleoside-5′-triphosphates. The tubes were usually incubated side by side for 1 hour, samples being taken from each tube at 10-minute intervals and inoculated on opposite halves of 24 to 36 pinto bean leaves for comparative assay of levels of infectivity. Counts of the necrotic local lesions produced indicated the effectiveness of the induced biosynthesis.

The first experiment, conducted on 12 February 1962, suggested that synthesis of viral RNA was occurring in the chemically treated solution. The results of this experiment are presented in Table 1. Assay showed the level of virus in the chemically treated solution to be 184 percent higher than the level in the control solution. Numerous subsequent experiments, with slight modifications of the technique described, were completed, with similar results. Usually, no differences could be found at zero time, slight synthesis was evident at 10 minutes and more at 20 minutes, and synthesis was maximum after 30 minutes of incubation. Amino acids and penicillin G were added in some experiments and did not appear to influence the rate of synthesis.

In one experiment virus protein and C^{14}-labeled adenosine triphosphate were mixed with the virus-synthesizing extract before it was divided into control and treated fractions. The three other nucleotides and $MgCl_2$ were added to the treated solution. After incubation for 30 minutes at 25°C the synthesizing mechanisms were removed by centrifuging and resuspended in a second