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-

tained by an internal biological system, which, in its complexity, may rival the process necessary for the maintenance of wakefulness (*1*). A logical approach to the understanding of the nature and function of REM sleep is to determine the effects of its curtailment upon the organism's biological patterns. It is well established that a compensatory increase in the REM phase occurs following experimentally induced periods of deprivation and, further, that the amount of increase and its time course are related to both amount of loss and duration of time over which the loss was incurred (*2*). Although this finding holds true not only for man but also for rabbit (*3*), cat (*4*), monkey (*5*), and rat (*6*), it sheds little light on the basic problem of function because of its circularity. Presence of a compensatory process does, however, demonstrate the plasticity of this particular sleep state and, by inference, its importance.

If normal amounts of REM sleep are necessary to the organism, a demonstration that changes occur during wakefulness as a result of selective REM deprivation should offer valuable insights into its function. However, no completely reliable behavioral or physiological alterations have yet been reported in studies performed on either animal or human subjects, and it appears that the problems of adequate control have not yet been solved. In order to demonstrate central-neural changes (other than those taking place within the process of REM sleep itself), we felt that we needed a neurophysiological parameter that (i) could be evaluated over relatively long periods; (ii) was adequately stable or whose instability could be determined by controls; and (iii) was sufficiently sensitive to reflect alterations correlated with interference in a fundamental biological pattern. The cat was chosen as the experimental animal, and the auditory recovery function (*7*) was used as the dependent variable.

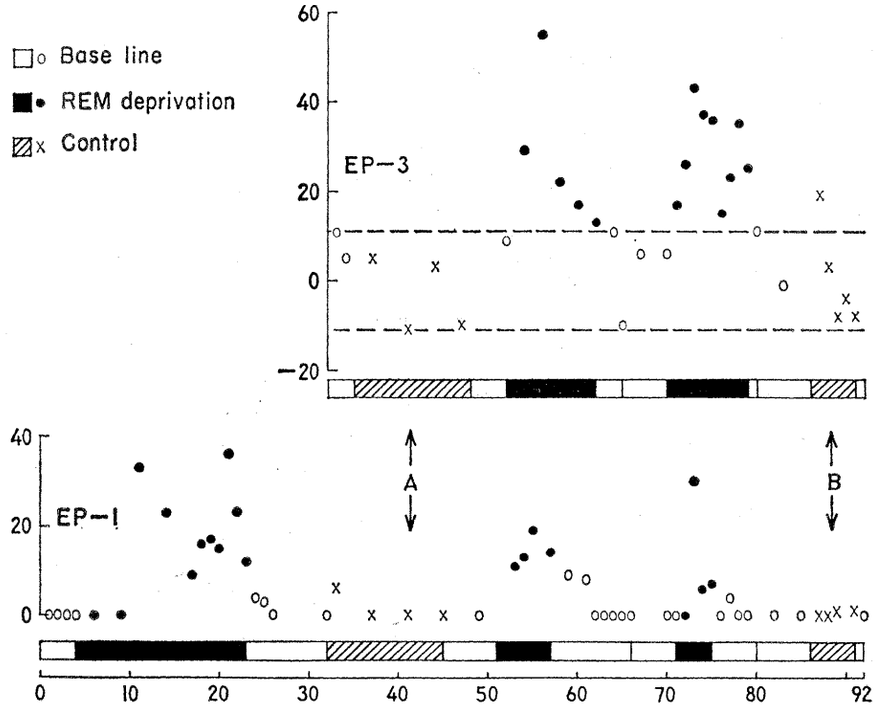

Fig. 1. Percentage change from base-line level of auditory cortical recovery for paired clicks separated by 25 msec (cat EP-1, lower graph) and 12.5 msec (cat EP-3, upper graph). The two cats were tested simultaneously in this experiment. Time, in days, is on the abscissa; percentage change, on the ordinate. Total test days for the entire experiment for each cat are indicated (horizontal numerical scale), as well as the time of occurrence and duration of the several manipulations (horizontal pattern bar). Two breaks totaling 28 days interrupt the continuity of the experiment and are indicated by two thin vertical lines on the pattern bar at day 63–64 and day 80. (*A*), Period of control arousals for each cat; (*B*), period of sleep deprivation (22 hours per day) for each cat. Lack of a true "range" of change values for cat EP-1 during base-line and control periods is due to the fact that, at the click separation illustrated, the evoked response to click No. 2 was either not detectable or of extremely low amplitude.

Adult cats, anesthetized with Nembutal, had stainless steel bipolar electrodes chronically implanted subdurally on the anterior ectosylvian (auditory projection area A1) and lateral gyri, with the use of aseptic procedures. Electrodes were terminated in a subminiature connector that was affixed to the skull over the frontal sinus with dental acrylic. Muscle and cutaneous layers were sutured, and the animals were allowed 1 week for postoperative recovery. Electromyographic (EMG) potentials were led from the posterior neck muscles through stainless steel Michel clips.

Cats were maintained on a rigid sleep-wakefulness regimen throughout the experiments. For approximately 15 hours each day, wakefulness was enforced by means of a slowly moving treadmill (*8*). The animals soon adapted to this device and would walk to the front of their aisle, sit down, and ride toward the back. The belt speed was 1 m/min, which allowed maximum periods of immobility of 20 seconds. The EEG was sampled while the cats were on the treadmill and, although there were many periods of EEG slowing which indicate drowsiness, frank non-REM sleep with full-blown slow waves and spindles was never seen. Thus, all time on the treadmill was considered awake time. For exactly 8 hours every day the animals were allowed to sleep while continuous polygraphic recordings were taken. During this period, animals were housed in sound-treated chambers, were given access to water, and were free to move about; they were given routine care and food when they were removed from the treadmill during the wakeful period.

Polygraph records were scored for the amount of time in the slow-wave (non-REM) phase, in the REM phase, and during wakefulness. Criteria for non-REM sleep were slow waves and spindles in the electroencephalogram (EEG), together with tonic EMG discharge; for REM sleep, EEG activation associated with complete EMG suppression; and for wakefulness, EEG activation in the presence of tonic EMG discharge. Selective deprivation of REM sleep was accomplished by awakening the cat at the onset of each REM period; this procedure continued for a varying number of consecutive days, after which the cats were again allowed 8 hours of undisturbed sleep per day (*9*).

Table 1. Mean daily sleep times and number of arousals during various experimental conditions. TST, total sleep time; NREM, total non-rapid eye movement (slow wave) sleep time; REM, total rapid eye movement sleep time; BREM, mean daily base-line REM time; and REM-DEP, REM deprivation. All values tabulated from daily 8-hour recording sessions.

Cat	Condition	Days (No.)	TST	NREM	REM	REM/ TST	REM/ BREM	Awakenings (No.)
EP-1	Base line	4	7:04	5:04	2:00	0.283		
EP-3	Base line	4	6:10	4:46	1:24	.227		
EP-1	1st REM-DEP	19	5:59	5:38	0:21	.058	0.175	146
EP-1	1st REM-DEP	1st 13	5:59	5:40	0:19	.053	.158	134
EP-1	Control	13	5:55	4:22	1:33	.262	.775	132
EP-3	Control	13	5:33	4:16	1:17	.231	.917	154
EP-3	Control	1st 10	5:38	4:23	1:15	.222	.893	133
EP-3	1st REM-DEP	10	5:44	5:36	0:08	.023	.095	86
EP-3	2nd REM-DEP	9	5:13	4:56	0:17	.054	.202	163
EP-1	Sleep loss	5	2:13	1:27	0:46	.346	.383	
EP-3	Sleep loss	5	2:14	1:22	0:52	.388	.619	

Cortical auditory recovery functions were measured intermittently throughout the entire experiment. The animal, restrained in a tight, cloth-packed box with only its head exposed, was studied during the first hour after the daily 8-hour period for recording sleep. The box was placed in a standard location inside a sound-treated testing chamber, where pairs of acoustic clicks of equal intensity were delivered through a 6-inch (15-cm) loudspeaker mounted 11 inches directly above the cat's head. Clicks were determined to be approximately 72 db above human threshold by comparing, across the loudspeaker terminals, the voltages necessary to produce both "just-audible" clicks and the clicks used in our experiments. Twenty pairs of clicks (one pair per second) at a desired interstimulus interval were presented, and the evoked electrocortical activity was amplified, led to an averaging computer (Mnemotron Corp. CAT 400A), and written out by a strip-chart recorder for measurement and analysis. In all instances, the base-line-to-peak voltages of the initial deflection of the evoked cortical response No. 2 to response No. 1 was expressed as a percentage and plotted as a function of the interval (in milliseconds) between members of the click-pair.

We used three cats in these experiments. Because of the extraordinary amount of around-the-clock effort anticipated for a successful study of this nature and in order to assess the feasibility of the total procedure, we studied only one cat in the first run. This animal was recorded over a 12-day base-line period and then during a single period of REM sleep deprivation that lasted 32 days, after which it was allowed an undisturbed sleep recovery period of 10 days. Cortical auditory recovery functions were assessed at 5-day intervals throughout the duration of the experiment and showed a marked, consistent, and reversible facilitation correlated with base-line, REM sleep deprivation, and recovery periods. This facilitation occurred only at shorter click-pair intervals, namely, 25, 50, and 100 msec; no changes in recovery function were seen at click-pair intervals of 150, 175, 200, 250, or 300 msec—intervals in which recovery was at or near 100 percent during the base-line period.

The other two cats were more rigorously studied, and it is data from them that are completely documented in this report. In addition to REM sleep deprivation, a control for possible stress of arousals and partial loss of sleep was instituted in which all conditions were the same as those for test animals except that cats were awakened during non-REM sleep, and REM periods were not interrupted. Animals were tested simultaneously with non-REM and REM arousal periods counter-balanced as a check for order effects.

Measurements were made at least once every 4 days; however, at times when more critical resolution was desirable, recovery functions were determined once every 24 hours. Facilitation of the auditory recovery function at shorter click-pair intervals occurred during REM deprivation in both cats. This effect was not seen during the period of control arousals. In order to insure that extreme loss of non-REM sleep would not similarly affect the recovery function, an additional experiment (control for loss of sleep) was performed in which the two cats were kept completely awake 22 hours a day by the constant attendance of the experimenters. Each time the slightest suggestion of drowsiness occurred, the cats were alerted by whatever stimulation was necessary. Long-term recovery functions were derived at the shortest click-pair intervals. The findings given in this report concern the shortest click-pair interval at which cortical recovery could be assessed for each of the two cats, namely, 25 msec for cat EP-1 and 12.5 msec for cat EP-3.

Figures 1 and 2 illustrate the changes in cortical auditory recovery function from base-line levels as a function of the various sleep manipulations over the 120 days during which the two cats were studied. Table 1 presents the breakdown of type of sleep for each of the animals during each major condition of the experiment. As in the original cat of the series, cortical re-

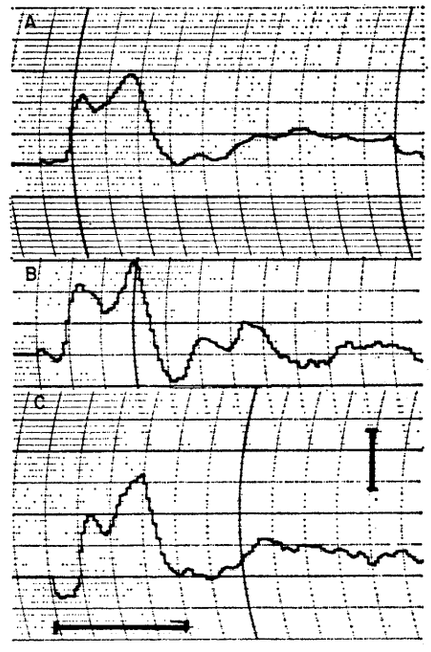

Fig. 2. Cortical responses evoked by a pair of clicks separated by 25 msec. Records from cat EP-1 encompassing the first of three periods of REM deprivation. (A) Base line; 3 days prior to REM deprivation. (B) REM deprivation; after 17 days of REM deprivation. (C) Recovery; after 3 recovery days. Note the virtual absence of cortical response to the second click in both upper and lower records and the well-developed cortical response to the second click in the middle record. The calibration is 25 msec and 100 μv.

covery was found facilitated at the shorter click-pair intervals without exception during all periods of selective REM sleep deprivation for both animals. This facilitation was reversible as shown by the return to base-line levels during periods of undisturbed sleep and, moreover, it was not seen during control periods which sought to evaluate the effects of (i) stress and (ii) total sleep loss upon the auditory recovery function. In all three cats (after six periods of REM deprivation) the percentage of REM on the first recovery day (8 hours) ranged from 48.4 (after 4 days) to 67.7 (after 30 days).

Table 1 shows the near-perfection of control arousal periods. For cat EP-1, the mean daily sleep times and number of arousals for the first 13 days of REM deprivation were identical to mean values obtained for the 13-day control period. Daily REM time during the control period was somewhat reduced below the base-line level, but in cat EP-3 the reduction was less than 10 percent. Since the control period occurred first in EP-3, the number of arousals was estimated and in this case proved to be higher than the number required during the subsequent deprivation period, although total sleep times were again equal. However, the higher number of control arousals was additional evidence that the awakenings per se were not implicated, and during the second period of REM deprivation the number of arousals was more nearly equal to that of the control period.

Many studies of REM deprivation have been conducted by placing the animal in a situation in which REM sleep cannot occur throughout the entire 24-hour day. Under these circumstances, animals will show a marked rebound in REM sleep when allowed to sleep normally, but a basic drawback in interpreting this result is that there is no adequate control for the stress placed upon the test animal. We feel that the only method that allows adequate control of the stress variable is the one that we have used (the reason it is not used more commonly is that continually watching the emerging EEG and EMG tracings for the onset of a REM period places an inordinate demand upon the experimenters). Since awakenings during REM sleep deprivation mount into the hundreds during an 8-hour session and since the deprivation extends for many consecutive days, it is little wonder that the present method enjoys no popularity. Nonetheless, if an effect is to be attributed specifically to the loss of REM sleep, such loss should be the only significant experimental variable.

In this study, then, a sensitive measure of central nervous system function was followed through periods of selective REM deprivation in cats. All measurements were taken during the waking state and showed no change during base-line periods or periods of control arousals. Although the precise significance of the alterations that occurred is uncertain, it is possible to conclude that a change in central-neural function is assessable during the waking state as a result of loss of REM sleep. The particular change noted in this study could be due to changes in auditory processing as far peripherally as the cochlea (*10*). It is certainly possible that a subtle effect upon auditory perception had occurred in these animals, but such a premise will require additional experiments to substantiate (*11*).

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Perceived Number and Evoked Cortical Potentials

Abstract. *Evoked cortical potentials and the number of flashes perceived were compared when subjects were presented with short trains of flashes under conditions where each presented flash could not be counted individually, but the train of flashes appeared to be flickering (1 to 14 flashes at 33.3 flashes per second). The rate at which each successive perceived flash was added appeared to correspond with the rate at which the successive components of the evoked response pattern were added. The temporal nature of this pattern was similar for both single flashes and trains of flashes. The results suggest that the onset of stimulation triggers a process which has a marked effect on both the cortical and perceptual response to subsequent stimulation.*

The perceived number of flashes has been compared to the actual number of flashes presented (*1–4*). In these studies, short trains of flashes (0 to 1000 msec) were presented at a rapid rate (20 to 50 flash/sec) under conditions where the flashes appeared to be flickering but where each presented flash could not be counted individually. The flashes were somehow grouped into perceptual units of approximately 100 msec, the perceived number of flashes being much less than the actual number of flashes presented. For example, when 14 flashes were presented at 30 flash/sec (a flash-train duration of 430 msec) subjects most frequently reported seeing four flashes, a perceived flash being added for approximately each 100 msec of stimulation (*1, 2*).

In working with averaged cortical potentials evoked by stimulus conditions similar to those used in the above experiments, we noted (i) that the temporal nature of visually evoked cortical potentials appeared to be related to the number of flashes perceived, and the occurrence of each successive perceived flash appeared to correspond with the occurrence of the successive components of the evoked response pattern, and (ii) that the temporal characteristics of cortical responses evoked by trains of flashes appeared to be similar to those evoked by single flashes. These observations suggest that "the onset of stimulation in some way initiates a process (or processes) which can have a marked influence on the perceptual response to any succeeding