Sleep: Suppression of Rapid Eye Movement Phase in the Cat after Electroconvulsive Shock

Abstract. *Electroconvulsive shock, administered for 5 to 7 days, reduced the daily rapid eye movement sleep time of seven cats to as little as 28 percent of base line levels. After day 4, eye movements during periods of cortical activation without tonic electromyographic activity were greatly reduced. Although partially deprived of rapid eye movements for as long as 7 days, the cats showed no compensatory rise in rapid eye movement time during the recovery period, but controls equally deprived gave significant rebounds. Rapid eye movement time of anesthetized cats was not affected by current that usually produces convulsions; it was lowered in animals convulsed with metrazol, but the same dosage of this drug, administered so as to avoid convulsions, had little effect. It appears that some aspect of the convulsion is responsible for lowering the rapid eye movement time.*

Recent studies have demonstrated that rapid eye movement (REM) sleep, in contrast to non-REM or slow-wave sleep, is a period of intense activity of the central nervous system (*1*). Electroencephalographic (EEG) activation and the occurrence of rapid eye movements are readily observable, as is suppression of tonic electromyographic (EMG) potentials. Microelectrode recordings on unanesthetized, unrestrained animals have shown that REM sleep is accompanied by a marked increase in spontaneous unit discharge in the visual cortex (*2*), mesencephalic reticular formation (*3*), and lateral geniculate nucleus (*4*). REM sleep is also associated with increases in cortical blood flow (*5*) and elevation of brain temperature (*6*). In humans, this stage of sleep is closely identified with dreaming (*7*).

While studying the effect of deprivation of REM sleep on convulsive threshold in the rat (*8*), we observed that rats that were convulsed tended to spend less time in the REM phase than their nonconvulsed controls; we speculated that the intense activity of the convulsion could in some manner substitute for or interfere with REM sleep. Our present study was designed to explore this hypothesis.

In a preliminary experiment, two adult cats were convulsed twice each day for five consecutive days in the manner described below. REM sleep time was reduced 28 to 62 percent below base line levels on convulsion days. In addition, no compensatory rise was apparent on the recovery days, which followed immediately, while substantial rebounds were observed in two control cats deprived by interrupting REM periods for the same length of time. Since two convulsions each day produced such a striking effect, further observations were confined to cats receiving only one electroconvulsive shock per day in order to minimize the possible deleterious effects of convulsions.

Five adult cats (three male, two female) were implanted under general anesthesia with subdermal electrodes for recording of EEG and electrooculographic (EOG) potentials. EMG was obtained through Michel clips placed high on the neck. When these animals had recovered from surgery, three were allowed up to 6 hours of sleep per day and two were allowed 8 hours. REM sleep was prevented at all other times by placing the animals on a treadmill. After several days of this schedule, EEG, EMG, and EOG recordings of the sleep period were begun. Total amounts of sleep time and REM time were noted for a base line period of 4 to 13 days. After the base lines were obtained, animals were convulsed by application of current (60 cy/sec; intensity, 12 ma; duration, 1 second) between the ears. Gauze pads saturated with electrode jelly were applied to the ears prior to stimulation to assure good electrical contact while minimizing abrasion. Animals were grounded through a Michel clip in the neck.

Electroconvulsive shock was administered once each day at the beginning of the sleep period for 6 consecutive days to four of the cats, and for 7 consecutive days to the fifth cat. Recordings were begun at the termination of the convulsions, which were generally maximal, tonic-clonic seizures lasting 35 to 45 seconds. Following the final convulsion day, three animals were recorded for a recovery period of 5 days and the remaining two for 7 and 9 recovery days.

Base line recordings of the convulsed group gave a mean REM sleep time of 120 minutes per day (range, 81 to 158), with a mean daily total sleep time of 328 minutes (268 to 401). By contrast, mean REM time for the convulsion days was 80 minutes (36 to 110), with a mean total sleep time of 325 minutes (258 to 373). The difference in REM time between base line and convulsion days was highly significant ($t = 8.7$, $P < .001$; two-tailed t-test for correlated means). Besides the drop in REM time, a remarkable fall in the frequency of eye movements during REM periods became evident by the 4th day of convulsions. The mean number of eye movements fell from approximately 20 to 4 per minute during the first 2 minutes of each REM period in four of the five convulsed animals. This is a relative rather than an absolute measure of the frequency because of the variability in registration of individual eye movements. Tracking eye movements in the waking animal did not appear to be altered. In most animals eye-movement frequencies during REM periods had returned to base line levels by recovery day 5.

Mean REM sleep time during the 5-day recovery period for the convulsed group was 109 minutes per day (28 to 192), slightly below but not significantly different from base line values ($t = 1.35$, $P > .10$). The two experimental animals that were recorded for 7 and 9 recovery days showed no substantial increase in REM time, that is, they did not have a "delayed rebound."

Further tests were made on three animals to determine whether the REM deprivation that accrued during the convulsion period was sufficient to produce a rebound in REM time. One of the animals had been convulsed previously and served as his own control; the other two were paired with the convulsed animals nearest to their base line REM time. After at least 5 consecutive base line days had been recorded, partial deprivation was begun. REM sleep time was then reduced by the same percentage below base line as that of the convulsed animals on convulsion days. After their daily quota of REM sleep, these animals were awakened at the onset of each subsequent episode of REM for the remainder of the sleep period.

There were no awakenings from non-REM sleep since prior investigations have shown that these awakenings do not affect overall REM time, nor do they produce a deprivation-compensation effect (*9*). After partial

Table 1. Mean time of awake, non-REM sleep, and REM periods on base line, convulsion, and recovery days for five convulsed cats. Ranges are in parentheses.

Animal No.	Mean time (minutes) of the three periods on indicated days								
	Base line			Convulsion			Recovery (days 1 and 2)		
	Awake	Non-REM	REM	Awake	Non-REM	REM	Awake	Non-REM	REM
1	38 (28–52)	162 (157–191)	123 (105–154)	48 (30–61)	237 (221–261)	81 (51–103)	24 (17–30)	253 (224–282)	74 (70–87)
2	46 (26–73)	184 (137–220)	108 (92–143)	35 (15–55)	251 (210–276)	59 (36–73)	30 (21–38)	213 (208–218)	118 (109–127)
3	87 (53–118)	163 (118–215)	131 (103–158)	106 (68–136)	166 (127–188)	92 (70–110)	84 (61–107)	161 (136–185)	132 (131–133)
4	106 (48–174)	260 (194–235)	108 (81–139)	114 (77–144)	291 (257–317)	82 (73–94)	95 (90–99)	273 (269–277)	108 (104–110)
5	73 (30–104)	272 (242–313)	129 (111–149)	110 (75–150)	280 (235–327)	84 (59–105)	136 (119–153)	257 (235–276)	84 (82–85)

deprivation, the cats were recorded for 3 to 5 recovery days. The partial deprivation group gave a mean base line REM time of 99 minutes per day (65 to 143), with a mean daily total sleep time of 295 minutes (292 to 369). REM sleep time was reduced to a mean of 66 minutes (50 to 81) during the period of partial deprivation with a mean total sleep time of 232 minutes (111 to 395). Eye-movement frequencies did not change in these animals. Mean REM sleep times for the first 3 recovery days were 141 (139 to 143), 137 (122 to 151), and 102 minutes (81 to 115). Rapid eye movement sleep time for the partially deprived controls was significantly greater on recovery days 1 and 2 than their mean base line REM time ($t = 8.6$; $P < .001$). It was also significantly greater than that obtained from the corresponding recovery days from the convulsed group ($t = 4.4$; $P < .01$). Recovery day 3 was not significantly different from base line.

There was also a control for the effect of the electric shock itself. Two additional animals were placed on an 8-hour sleep schedule and spent the remaining time on the treadmill. Sleep time was recorded for 10 consecutive base line days. On the 11th day each received two subconvulsive shocks 3 minutes apart. Sleep was then recorded for 8 hours. On day 12 they were both lightly anesthetized with ether prior to recording, and a 1-second electroshock was administered to one of the cats in exactly the same manner as that described for the convulsed group. Their sleep was recorded again for an 8-hour period. On the following day both were etherized and only the second cat received a 3-second shock prior to recording sleep. Several days later, one of these animals was given a convulsive dose of metrazol, 30 mg (8 mg/kg), administered intravenously. The second animal received the same intravenous dose, injected very slowly over a 3-minute interval to avoid provoking seizure. Sleep of both animals was recorded for an 8-hour period after injection. These animals showed no change in REM sleep time after ether alone, two subconvulsive shocks, or shock while under ether anesthesia. Increasing the duration of the stimulus had no effect on REM time.

Administration of metrazol in convulsive doses was followed by a lowering of REM sleep time to 57 percent of base line values, while the nonconvulsive dose resulted in a reduction to 82 percent of base line levels on the day of administration. Decrease in REM sleep in the latter case was due mainly to prolonged wakefulness at the beginning of the sleep period. The proportion of this animal's total sleep time spent in REM was 30 percent, a figure well within the normal base line range. However, the convulsed animal spent only 19 percent of total sleep time in REM sleep, a value comparable to those obtained following electrically induced convulsions.

Results confirm the hypothesis that electroconvulsive shock suppresses REM sleep. More surprising was the finding that when REM sleep was inhibited after electroconvulsion, compensatory REM rebounds did not occur. This result is remarkable because rebounds in REM time have occurred without exception after total REM deprivation by awakenings at the onset of each REM period (*1, 7, 9*). In our study, rebounds were seen after partial deprivation caused by awakenings. In humans, partial REM deprivation by awakenings will also produce a REM compensation effect (*10*). Compensatory rebounds have been seen after reduction of REM by the administration of dexedrine (*11*), barbiturates (*12*), and alcohol (*13*).

Results of a concurrent study of sleep in a group of patients receiving electroconvulsive therapy are in general

Table 2. Mean time of awake, non-REM sleep, and REM periods on base line, partial deprivation, and recovery days for three partially deprived cats. Ranges are in parentheses.

Animal No.	Mean time (minutes) of the three periods on indicated days								
	Base line			Partial deprivation			Recovery		
	Awake	Non-REM	REM	Awake	Non-REM	REM	Awake	Non-REM	REM
2	46 (26–73)	184 (137–220)	108 (92–143)	82 (57–115)	122 (54–205)	58 (50–70)	57 (54–60)	175 (155–195)	145 (139–151)
6	200 (150–284)	132 (53–247)	92 (65–112)	143 (62–239)	129 (101–138)	68 (54–81)	95 (58–133)	111 (103–118)	137 (134–139)
7	105 (53–146)	273 (228–315)	96 (67–113)	177 (158–197)	263 (194–297)	73 (61–80)	157 (83–239)	212 (162–252)	132 (122–143)

agreement with our data. These patients have shown reduction in REM sleep time, drop in the frequency of eye movements, and absence of compensatory rebound after shock (*14*).

Electrical damage to brain tissue may be ruled out as a possible explanation of these results since shock administered to anesthetized animals, as well as repeated subconvulsive shock to unanesthetized animals, had no effect on REM sleep time. A convulsion induced by intravenous injection of metrazol, however, resulted in a lowering of REM time to levels comparable to those obtained with electroconvulsive shock, which suggests that some property of the convulsion itself leads to reduction of REM sleep time without producing the usual REM deprivation effect of compensatory rebounds. There is growing evidence that REM sleep is biochemically mediated (*15–19*). If electrically induced brain damage is ruled out, it is plausible that the intense activity accompanying the seizures in some manner altered the brain levels of substances, or their precursors, that induce REM sleep.

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Hemoglobin Polymorphism in Macaca nemestrina

Abstract. *Four hemoglobin phenotypes have been noted in the pigtailed monkey* (Macaca nemestrina). *Pedigree studies suggest a simple codominant Mendelian explanation for inheritance of three of these phenotypes, including one electrophoretically identical with human type A. The fourth type occurred in only one animal in this series.*

An unusual hemoglobin polymorphism has been observed during an electrophoretic study of biochemical variants in primates. A number of distinct patterns were inscribed by hemoglobins from pigtailed monkeys (*Macaca nemestrina*). No intraspecific variation has been reported for this species to date.

In all, 75 *Macaca nemestrina* were tested. A number of these represented families of a wild-caught male and female and an offspring born at the Regional Primate Research Center at the University of Washington, but the majority of the experimental subjects had been captured in the wild. The latter animals had been obtained in a number of shipments presumably containing animals from several local populations (*1*).

Hemoglobin was analyzed by vertical starch-gel electrophoresis on a continuous buffer system at *p*H 8.6 with tris–ethylenediaminetetraacetic acid (EDTA) –borate stock solution. The concentration of this buffer was 0.9*M* tris, 0.5*M* boric acid and 0.2*M* EDTA (*2*). The percentage of alkaline resistance was measured by the method of Betke *et al.* (*3*).

Four hemoglobin phenotypes were noted on the basis of electrophoretic mobility and staining intensity (Fig. 1). The first type, a band of the identical mobility as human A, was observed in 12 of the 75 animals. The second type, characteristically faster than the normal human A type, occurred in 43 monkeys. Two bands of equal staining intensity and of mobilities suggesting the presence of both of the first two types characterize a third phenotype. The third type was found in 19 monkeys. The fourth type, a possible variant of the third, has a two-component system of the same speed as the third but of differing staining intensity. The third type displayed an equal distribution of hemoglobin between the two bands. In the fourth type, the ratio was 75 to 25 percent in the intensity of the staining. These unusual proportions appeared consistently in four determinations made on two fresh samples obtained several days apart. The observed gene frequencies at this locus differ significantly from the expected. This difference is probably due to sampling error. No A_2 component—the minor hemoglobin fraction characteristic of man and higher primates—has been found in macaques tested to date.

The percentage of alkaline-resistant hemoglobin ranged from 0 to 1.8, but there was no significant relationship between this characteristic and any of the four electrophoretically determined types. The percentage range of alkaline-resistant hemoglobin in this study confirmed the findings of Tuttle *et al.* (*4*).

Pedigree studies suggested a simple codominant Mendelian explanation for the inheritance of the first three of these phenotypes. For example, when a male with the first phenotype was bred with a female of that phenotype, their offspring was of that type. When the same male was bred with a female of the second phenotype, their offspring was of the third phenotype (which, as noted, appeared to indicate presence of the first two types of hemoglobin in equal quantities).

No pedigree data are available for the fourth type, as only one animal

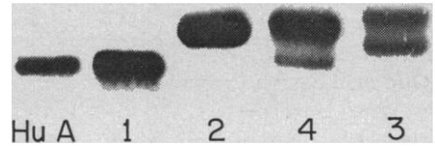

Fig. 1. Four hemoglobin types in *Macaca nemestrina* compared to human A, designated Hu A; other terminology devised for this paper. (Composite of three starch gels.)