References and Notes

1. W. K. Noell, V. S. Walker, B. S. Kang, S. Berman, *Invest. Ophthalmol.* **5**, 450 (1966); W. K. Noell, H. Themann, B. S. Kang, V. S. Walker, *Fed. Proc.* **25**, 329 (1966); R. A. Gorn and T. Kuwabara, *Arch. Ophthalmol.* **77**, 115 (1967); W. K. Noell, in *The Clinical Value of Electroretinography*, H. N. Burian, Ed. (Karger, New York, 1968), p. 290; T. Kuwabara and R. A. Gorn, *Arch. Ophthalmol.* **79**, 69 (1968).
2. W. K. Noell, M. C. Delmelle, R. Albrecht, *Science*, this issue.
3. J. T. Dingle and J. A. Lucy, *Biol. Rev.* **40**, 422 (1965); M. R. Daniel, J. T. Dingle, I. M. Sharman, T. Moore, *J. Biochem.* **98**, 476 (1966); O. A. Roels, in *The Vitamins*, W. H. Sebrell Jr. and R. S. Harris, Eds. (Academic Press, New York, 1967), vol. **1**, p. 193; D. Glick and W. J. Kerr, *Exp. Cell Res.* **53**, 129 (1968).
4. In the preceding paper (*2*), illumination during the cyclic light was obtained by 100-watt incandescent light instead of 40-watt. Rhodopsin levels of A— after 10 weeks on the diet (Figs. 2 and 3, this paper), therefore, are not as low as in figure 3 of (*2*).
5. The A— animals used for measuring the loss of retinol in strong light were selected for minimum light damage on the basis of their ERG's 24 hours after exposure. Measurements of retinol at the end of exposure were also made but gave less reliable information than rhodopsin regeneration because criteria for light damage could not be applied. Damage itself (as in A+) was associated with the rapid loss of retinol because of PE deterioration. Hence, measurements of retinol as well as of rhodopsin during and shortly after exposure were inconclusive if used for comparing A+ and A—. Experimental analysis had to be restricted to relating different initial conditions and different types of exposure to the magnitude of the damage.
6. The postulated "cell adaptation" is clearly different from the "dark" or "light-adaptation" measured in visual physiology. As used here, adaptation relates to cellular activities in a general way including metabolism, catabolism, electrolyte balance, acid-base balance, membrane permeability, and the like.
7. It must be understood that this reaction to light is only one of several mechanisms by which strong light can damage the visual cells. Another one, for instance, is by thermal injury as in eclipse and laser burns [B. S. Fine and W. J. Geeraetz, *Acta Ophthalmol.* **43**, 684 (1965)]. Vitamin A deficiency also may produce visual cell death but only after many months (6 to 8) as most recently shown [H. A. Hansson (*Virchow's Arch. Abt. B Zellpathol.* **4**, 368 (1970)]. Light exposure is essential to render the deficiency effective upon the retina (*2*). Hence, while one type of damage by light can be prevented by "cell adaptation" to light or by the retinol depletion occurring in vitamin A deficiency from daily exposure to weak light, weak light itself initiates changes that make vitamin A deficiency detrimental to the visual cells if the condition is maintained for a considerable length of time. In Hansson's experiments the animals were exposed to 50 lux for 10 hours daily. Concerning the "cell adaptation" to light, it is an intriguing fact that light "stimulation" of vertebrate photoreceptors produces hyperpolarization, not depolarization, as in axon and nerve cell. Photoreceptor membrane conductance is highest in darkness; light reduces it in a graded manner [B. Baylor and M. G. F. Fuortes, *J. Physiol.* **207**, 77 (1970)]. Similarly, oxygen consumption of retina decreases with illumination [I. Hanawa and W. K. Noell, *Physiologist* **5**, 153 (1962); W. Sickel, in *Clinical Electroetinography*, H. M. Burian and J. H. Jacobson, Eds. (Pergamon, New York, 1966), p. 115].
8. W. K. Noell, *N.Y. Acad. Sci.* **74**, 337 (1958); *Amer. J. Ophthalmol.* **48**, 347 (1959); *J. Opt. Soc. Amer.* **53**, 36 (1963); in *Biochemistry of the Retina*, C. N. Graymore, Ed. (Academic Press, New York, 1965), p. 51; E. Koenig, *J. Cell Biol.* **34**, 365 (1967).
9. D. S. Faber, thesis, State University of New York, Buffalo (1968).
10. B. Heiss and W. K. Noell, unpublished data.

11. Supported by NIH grant EY-00297 and the Buffalo Eye Bank and Research Foundation. We thank Dr. B. S. Kang and R. Schlosser for assistance during the initial part of this study.

10 September 1970

Retrograde Amnesia: Electroconvulsive Shock Effects after Termination of Rapid Eye Movement Sleep Deprivation

Abstract. *Mice that were deprived of rapid eye movement sleep for 2 days immediately after one-trial training in an inhibitory avoidance task and were given an electroconvulsive shock after deprivation displayed retrograde amnesia on a retention test given 24 hours later. Electroconvulsive shock produced no amnesia in comparable groups of animals that were not deprived of rapid eye movement sleep.*

It is well documented that susceptibility of a memory trace to disruption by an electroconvulsive shock (ECS) decreases as the interval between the learning trial and the application of the amnesic treatment is increased. Generally, little or no amnesia is obtained if the ECS is given several hours or longer after training (*1*). This evidence supports the general hypothesis that memory trace consolidation processes are time-dependent.

Evidence from a recent pilot study (*2*) suggests that, if ECS is given immediately after several days of rapid eye movement (REM) sleep deprivation (REMD), the memory trace of an event experienced just before the REMD may be disrupted. Our report examines this finding further. Separate groups of mice were subjected to continual REMD for the 48 hours after their training in a one-trial inhibitory avoidance (passive avoidance, or PA) task. They were then administered an ECS at one of several intervals after termination of the REMD. On a 24-hour retention test, the mice displayed a retrograde amnesia gradient that varied with the interval between termination of REMD and administration of ECS.

We used as subjects 226 naive Swiss Webster mice that were 50 to 60 days old (*3*). The training apparatus was an alleyway (2.6 by 19.8 cm) leading to an open field (16.0 by 16.0 cm), with a grid floor mounted 0.6 cm above the base. The grid was constructed of 2.4-mm stainless steel rods, 7 mm apart, covering the entire floor of the enclosure. The apparatus was connected to a shock scrambler. All animals were given one training trial and one retention test trial. On each trial, the animal was placed in an alleyway, and the latency of its entrance into an open field (all four legs in the field) was recorded to the nearest 1/100 second. On the first trial, a scrambled shock of 500 msec at 0.6 ma was delivered to the floor of the open field as a gate behind the mouse was closed. On the retention test trial no shock was given; latency of entering the open field was recorded as in the training trial, with a maximum latency of 30 seconds allowed. In order to control for diurnal rhythm effects, care was taken to train and test each animal at the same time of day (within a few minutes). Since small groups of animals were run each day, control for age differences was maintained by starting the animals in the experiments when they were no younger than 50 days and no older than 60 days. Control for seasonal differences was maintained by assigning animals at random to each of the groups through the course of the experiment. REMD was produced by placing each mouse on a Plexiglas pedestal 3 cm in diameter, approximately 4 cm above the floor of the cage in which the animals were maintained. The floor was covered with water 3 cm deep. Food and water were always available (Fig. 1). Fishbein (*4*) has described this technique as it is used with mice. The method of deprivation takes advantage of the fact that during REM sleep there is a generalized inhibition of spinal motor activity (*5*). Since this inhibitory process appears to take place only during the REM periods, postural tonus persists during the non-REM (NREM) synchronized, high voltage, slow wave sleep. This means that the mice can sleep while crouching or sitting on the small pedestals. However, when the mice begin to enter the REM phase and postural tonus of the whole body, particularly of the muscles of the head and neck, diminishes, they either awaken briefly or fall

into the water. In either case, REM sleep is interrupted before it becomes fully developed. The advantage of this procedure is that many animals can be deprived of REM sleep simultaneously.

Confirmation of REMD was evident by observation and by 24-hour electroencephalographic (EEG) and electromyographic (EMG) monitoring of several mice with permanently implanted electrodes. Initially, 2 to 5 days of continuous base line recordings were obtained from three animals housed in Plexiglas cages with wood shavings. The animals were maintained throughout the recording session in a 12-hour, light to dark cycle (7 a.m. to 7 p.m.). The base line recordings revealed three behavioral states that could be easily characterized by the EEG and EMG criteria: the awake state included a desynchronized EEG accompanied by tonic EMG activity; the NREM sleep state comprised high voltage, synchronized, slow waves in the EEG and a decrease in EMG activity, relative to the awake state; the REM state contained low voltage, fast, desynchronized EEG in the presence of periodic body twitches, flaccid sleeping posture, twitching of the whiskers and ears, irregular breathing, and relative EMG suppression. Continuous EEG and EMG recordings were then obtained from these animals for the 3 to 5 days they were on the 3-cm pedestals for REMD. Total sleep time for these mice was reduced only 11 percent from the base line data; on the other hand, total REM time was reduced more than 80 percent [base line REM time = 10.1 percent of total sleep time; standard error of the mean (S.E.M.) = 0.9 percent; pedestal REM time = 1.9 percent of total sleep time; S.E.M. = 0.6 percent].

It is important to point out that mice living on pedestals in water are quite active. They are constantly climbing, hanging, and playing about the wire mesh top of the cage. We have systematically observed the behavior in an open field of separate groups of REMD mice ($N = 16$ per group) for as long as 5 to 7 days and have found no change in activity when compared with control animals not REMD.

To avoid the confounding effects of adaptation to the pedestals immediately following the first trial, all mice, when they were 35 to 40 days old, were put on the pedestals for 3 days (72 continuous hours of REMD). At 50 days, they were assigned at random to groups.

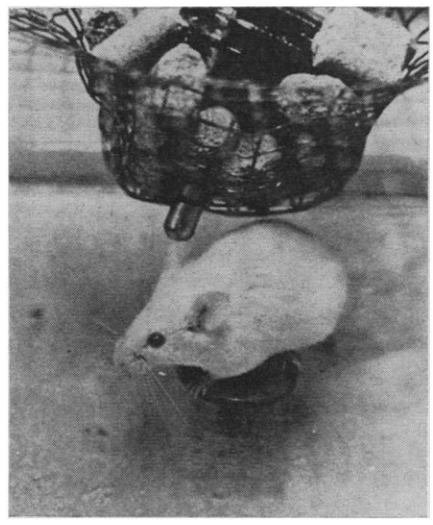

Fig. 1. Mouse placed (for REMD) on a Plexiglas pedestal 3 cm in diameter, approximately 4 cm high. Water 3 cm deep covers the floor of the cage. Food and water are freely available to the subjects.

Mice that had undergone REMD and ECS (REMD + ECS) were continuously REMD for 48 hours after training in the PA task; then, at varying intervals after termination of REMD, the animals were put under light ether anesthesia and administered a transcorneal ECS (800 msec at 8 ma). The ether anesthesia, by preventing the tonic convulsion, eliminates the fatalities generally caused by ECS alone, but, in the concentration we used, it does not produce amnesia (*6*). The control groups (control + ECS) were isolated for 48 continuous hours in a cage with wood shavings after training in the PA task. They were anesthetized and given ECS at the same intervals of time as the experimental animals. Other control groups (REMD + sham ECS, control + sham ECS) were treated identically as the REMD + ECS and control + ECS groups, except that after being anesthetized they were given only a sham ECS in order to determine the effect of the ether anesthesia treatment.

Each of the groups (REMD + ECS, control + ECS) was divided into six subgroups ($N = 16$ animals per group). Each subgroup received the ether anesthesia and ECS at a different time: 5 minutes, 30 minutes, 1 hour, 3 hours, 6 hours, or 12 hours after termination of REMD. The REMD + sham ECS and the control + sham ECS groups were anesthetized and given the sham ECS at the 5-minute interval. The next day, 24 hours after termination of REMD, all mice were given a retention test. It should be noted that REMD and ECS treatments were given after

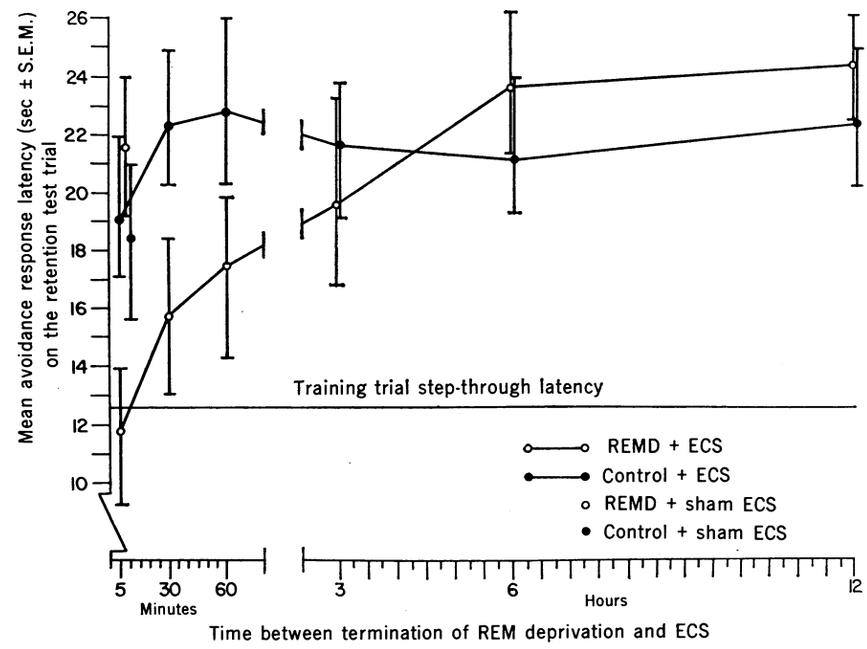

Fig. 2. Retrograde amnesic effects of ECS on REM-deprived and control mice. Inhibitory avoidance retention test latencies: mice were (i) trained, (ii) deprived of REM for 2 days (on controls), (iii) treated with ECS at one of several intervals after termination of deprivation, and (iv) tested for retention 24 hours later. Each point is based on 16 animals.

the initial learning experience and that the retention test was administered after the acute effects of REMD and ECS had disappeared.

On the training trial there were no differences among the groups in latency of entrance into the open field (*7*). Mice that received ECS 5 minutes, 30 minutes, or 1 hour after being removed from the REMD situation displayed marked retention deficits when compared with the groups of mice that were administered ECS without having had REMD (overall analysis: $F = 8.54$; d.f. $= 1/90$; $P < .01$; comparisons between pairs: $P < .05$; $P < .05$; $P < .10$, respectively). Mice that were administered ECS 3, 6, or 12 hours after removal from the REMD situation displayed no memory loss ($F = 0.50$; d.f. $= 1/90$; not significant). The anesthetized controls showed normal retention (REMD + sham ECS versus control + sham ECS, not significant; REMD + sham ECS versus REMD + ECS, $P < .01$; control + sham ECS versus control + ECS, not significant) (see Fig. 2).

Computations for trend analysis were performed on the REMD + ECS group. The linear component was highly significant ($F = 15.52$; d.f. $= 1/90$; $P < .01$), suggesting that the retrograde amnesia was graded linearly and was related to the interval between removal of the animals from the REMD situation and administration of the ECS.

Evidence from numerous studies supports the view that memory processes are susceptible to interfering agents only for a period of several hours after training. The present finding suggests that the memory trace of a previously learned experience can remain susceptible to disruption several days after training if the animals are REMD during the interval. The precise neurobiological bases for the maintained susceptibility are unclear. It has been shown, however, that several factors which may influence memory consolidation and storage can be altered as a consequence of REMD: among them, increased norepinephrine turnover in the telencephalon and diencephalon (*8*); decreased acetylcholine content of the telencephalon (*9*); decreased potassium content in the blood and brain (*10*); and decreased glycogen content in the subcortex and caudal brain stem (*11*). These changes may cause increased neural excitability, which Cohen and Dement (*12*) observed in the form of decreased thresholds to ECS in REMD rats and prolongation of the tonic phase of ECS-produced convulsion in REMD mice. Thus, ECS may not affect REMD mice in the same way it affects the various controls. This suggests the alternative interpretation that REMD may have rendered the ECS a more effective amnesic agent. Nevertheless, biochemical evidence based on work with rats suggests that endogenous neurochemicals, which are important for maintaining long-term memory, could be synthesized and used during the REM sleep of mice. Our experiment, therefore, provides further evidence (*2, 4*) that processes which occur during sleep, and most predominantly during the REM sleep state, influence the mechanisms which underlie the storage of long-term memory.

WILLIAM FISHBEIN
Department of Psychology,
The City College of the City
University of New York,
New York 10031

JAMES L. MCGAUGH
JEFFREY R. SWARZ
Department of Psychobiology,
School of Biological Sciences,
University of California,
Irvine 92664

References and Notes

1. For review of these experiments → S. D. Glickman, *Psychol. Bull.* **58**, 218 (1961); A. J. Deutsch, *Annu. Rev. Physiol.* **24**, 259 (1962); J. L. McGaugh, *Science* **153**, 1351 (1966).
2. W. Fishbein, *Physiol. Behav.*, in press.
3. The mice were obtained from Simonsen Laboratories, Gilroy, Calif.
4. W. Fishbein, *Commun. Behav. Biol.* **5**, 171 (1970).
5. R. Hodes and W. Dement, *Electroencephalogr. Clin. Neurophysiol.* **17**, 517 (1964); O. Pompeiano, in *Aspects Anotomo-Fonctionels de la Physiologie du Sommeil*, M. Jouvet, Ed. (Centre National de la Recherche Scientifique, Lyon, France, 1965), p. 309.
6. The anesthetic procedures were the same as those used by J. L. McGaugh and H. P. Alpern, *Science* **152**, 665 (1966). The animals were placed in a 500-ml jar that contained cotton saturated with 4 ml of diethyl ether and were removed approximately 30 seconds later. The anesthesia caused complete ataxia for about 1 minute. The ECS was administered immediately after the animals were taken out of the jar.
7. Analysis of variance was used for overall analyses. Mann-Whitney U tests were used for all comparisons between pairs of groups.
8. J. F. Pujol, J. Mouret, M. Jouvet, J. Glowinski, *Science* **159**, 112 (1968).
→ M. B. Bowers, E. L. Hartmann, D. X. Freedman, *ibid.* **153**, 1416 (1966).
10. L. Heiner, Y. Godin, J. Mark, P. Mandel, *J. Neurochem.* **15**, 150 (1968).
11. V. Karadzic and B. Mrsulja, *ibid.* **16**, 29 (1969).
12. H. B. Cohen and W. C. Dement, *Science* **150**, 1318 (1965); *Psychophysiology* **4**, 381 (1968).
13. Supported by research grant MH 12526 to J.L.M. During the course of this study, W.F. was an NIMH postdoctoral research fellow 1-FO2-MH-41, 172-02 at the University of California, Irvine.

19 November 1970

Cycloheximide: Its Effects on Activity Are Dissociable from Its Effects on Memory

Abstract. *Cycloheximide, when injected subcutaneously or intracerebrally, produces changes in the activity level of mice. Isocycloheximide, injected intracerebrally, produces identical effects on activity, but it does not produce inhibition of cerebral protein synthesis or amnesia. Amphetamine, in doses that can antagonize the amnesic action of cycloheximide, does not antagonize the effect of cycloheximide on activity. Effects of cycloheximide on activity do not appear to be responsible for its amnesic action.*

Protein synthesis inhibitors have been used extensively in an attempt to determine the role of cerebral protein synthesis in memory storage. Two classes of drugs, puromycin and the glutarimides, have been used for this work. Both groups of drugs impair long-term memory storage, but differences were found between them (*1*). Most notably, it was found that puromycin, in addition to inhibiting cerebral protein synthesis, produces disturbances in cerebral electrical activity, but that cycloheximide does not (*2*). It was therefore concluded that, of the available drugs, the glutarimide derivatives were the drugs of choice for such studies, although it was recognized that they, too, might have side effects that could contribute to their amnesic properties (*3*).

In the course of continued studies on the effects of cycloheximide on learning and memory, we and Squire *et al.* (*4*) found that cycloheximide, injected either subcutaneously or intracerebrally, produced alterations in the activity of mice. This finding raised the possibility that the amnesic effect of cycloheximide might be due to its effect on activity rather than to its capability of inhibiting cerebral protein synthesis specifically required for memory storage. This possibility seemed particularly worthy of attention since amphetamine, which can produce increased activity,