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.

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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,

did, under certain conditions, antagonize the cycloheximide effect on memory (*5*).

In addition to describing alterations in the activity of mice that had been injected subcutaneously or intracerebrally with cycloheximide (in the same doses used in previous memory studies), we now demonstrate (i) that isocycloheximide, which has no effect on memory or cerebral protein synthesis, produces similar activity changes; and (ii) that the antagonism by amphetamine of the cycloheximide effect on memory is not accompanied by an antagonism of its effect on activity. Although the amnesic properties of cycloheximide may be related to its effects on activity, our experiments dissociate these two effects and support the contention that the amnesic effect of cycloheximide is due to inhibition of the cerebral protein synthesis required for long-term memory storage.

Cycloheximide (120 mg/kg), administered subcutaneously (Fig. 1) or intracerebrally (Fig. 2), has significant effects on the activity of mice (*6*). For two groups of mice, we measured activity continuously for 3 hours after subcutaneous injection of cycloheximide or saline; for separate groups, we measured activity for one of several 10-minute intervals during the 24 hours after subcutaneous injection. In both cases, cycloheximide produced initial hyperactivity. Squire *et al.* (*4*) found that this initial hyperactivity was apparent within 3 minutes after injection of the drug. At about 30 to 40 minutes after injection, activity returned to normal. Thereafter, mice showed a marked decrease in activity. The decrease was more apparent in the mice that were observed continuously (Fig. 1B) than in the mice that were observed at one 10-minute interval (Fig. 1A). Apparently, the depressive effects of cycloheximide were antagonized until the mice became accustomed to the chamber. This may explain our earlier failure to detect changes in the activity of mice that we observed casually for short periods in an open chamber: indeed, the mice in the present study appeared normal upon casual observation.

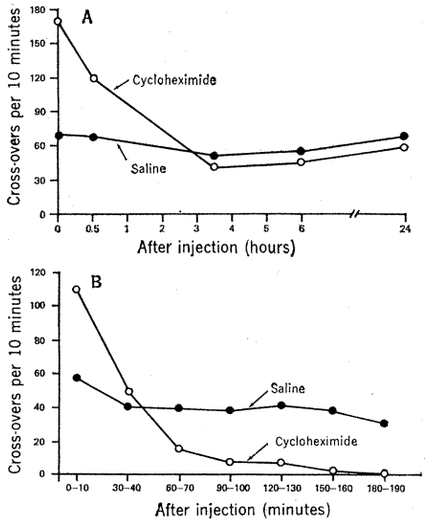

Fig. 1. The effects of cycloheximide (120 mg/kg) or saline on gross locomotor activity of mice that were monitored for 10 minutes at one of the indicated intervals after subcutaneous injection (A) or continuously for 3 hours and 10 minutes after injection (B). In B, 10-minute samples were selected at regular intervals from the continuous record. The two groups monitored continuously are significantly different at all times, except at the 30- to 40-minute interval ($P < .05$, Mann-Whitney U test). When measurements were made for only one 10-minute interval, only the cycloheximide-induced hyperactivity was significantly different from control values ($P < .05$, Mann-Whitney U test).

When injected subcutaneously, cycloheximide must be given in large doses in order to produce amnesia or to affect activity. Since intracerebral injections require much smaller doses to produce amnesia (*3*), we studied the effects on activity of intracerebral administration of cycloheximide. We did not measure activity until 1 hour after intracerebral injection, in order that the animals might recover from the ether anesthesia and the acute trauma of the injections. Upon intracerebral administration of 200 μg of cycloheximide [a dose that inhibits more than 90 percent of cerebral protein synthesis for several hours and that has marked amnesic effects (*3*)], a significant decrease in activity was observed from 1 to 4 hours after injection (Fig. 2).

For studies of memory, we usually train mice 4 hours after an intracerebral injection to allow for diffusion of the drug in the brain. Since decreased activity levels would be expected at the time of training, we considered that this effect of cycloheximide on activity might somehow be related to its amnesic effect. We investigated this possibility by using a small amount of isocycloheximide, a stereoisomer of cycloheximide, which has no significant effect on cerebral protein synthesis (*7*). Mice were injected intracerebrally with 200 μg of cycloheximide or isocycloheximide. Isocycloheximide produced depression in activity from 1 to 4 hours after injection. This depression did not differ significantly from depression produced by cycloheximide (Fig. 2). However, in contrast with cycloheximide, isocycloheximide did not have any amnesic effect. Mice treated with isocycloheximide and trained in a light-dark discrimination 4 hours after intracerebral injection (*8*) showed retention 3 days later that was indistinguishable from retention exhibited by saline-treated mice. Saline-treated animals had 65 percent savings ($N=14$); isocycloheximide-injected animals had 64 percent savings ($N = 13$); but cycloheximide-injected animals had 13 percent savings ($N = 15$). Thus intracerebrally injected isocycloheximide, which depresses activity as cycloheximide does but does not markedly inhibit cerebral protein synthesis (*1, 7*), has no amnesic effect. Depression of activity is apparently unrelated either to amnesic action or to inhibition of cerebral protein synthesis.

Since injections of amphetamine (1 mg/kg) administered 3 hours after the completion of training antagonize the amnesic effect of cycloheximide admin-

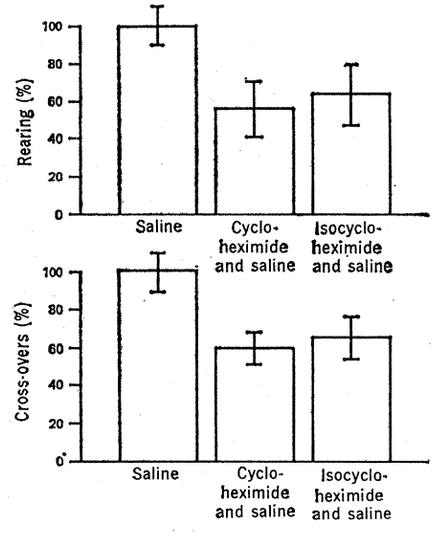

Fig. 2. The effects of intracerebral administration of saline, cycloheximide, or isocycloheximide on two measures of activity: rearing (top) and cross-overs (bottom). A 15-μl dose of isotonic saline, with or without 100 μg of cycloheximide or isocycloheximide, was injected intracerebrally into each temporal region (1 mm rostral to the caudal sutures, 1 mm lateral to the sagittal suture, and 4 mm below the skull surface). Activity was measured continuously between 1 and 4 hours after injection. Both cycloheximide and isocycloheximide significantly reduced motor activity when compared to saline controls ($P < .05$, Mann-Whitney U test).

istered subcutaneously 30 minutes before training (*5*), we sought to determine whether such injections of amphetamine also antagonize the effects of cycloheximide on activity. Mice were first injected with cycloheximide; 3 hours and 45 minutes later, they were injected subcutaneously with amphetamine (1 mg/kg) or saline. This interval between drug injections is the same one employed in memory studies, where amphetamine, but not saline, was found to antagonize the effect of cycloheximide. Activity was measured continuously during the 1st hour after amphetamine or saline injection. Mice given cycloheximide and saline ($N = 8$) exhibited marked inhibition of activity during this period; their activity was significantly less than that of mice given only saline (Fig. 1) before activity ($P < .01$). Amphetamine ($N = 8$) did not antagonize the depression of activity. Thus, the effect of amphetamine on memory in cycloheximide-treated mice (*5*) is not correlated with a measurable increase in activity.

We conclude from these studies (i) that cycloheximide affects activity by acting on the brain, (ii) that this action is unrelated to its inhibition of protein synthesis, and (iii) that these effects of cycloheximide on activity do not appear to be responsible for its amnesic action. It is possible, of course, that cycloheximide has some other property, unrelated to inhibition of cerebral protein synthesis, that is responsible for its amnesic effect.

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7. A 12-mg sample of isocycloheximide was provided by Francis Johnson, Dow Chemical Company, Wayland, Mass. The small amount of the drug limited our study to effects of intracerebral injections. In accordance with previous observations (*1*), we determined that this sample had a negligible inhibitory effect (12 percent) on cerebral protein synthesis.
8. For training, a mouse was placed in the stem of the T maze; after 5 seconds, shock (0.4 ma) was applied through the grid floor. The shock was terminated when the mouse entered the dark limb of the maze. The mouse was removed 5 seconds later. Training was continued with an intertrial interval of 30 seconds and a criterion of five out of six correct responses. Savings was calculated as described by S. H. Barondes and H. D. Cohen [*Science* **151**, 594 (1966)]. Mice that required more trials to reach criterion on retest than on original learning were scored as having zero savings. The savings of mice given cycloheximide were significantly less than the savings of mice given saline ($P < .02$) or isocycloheximide ($P < .02$).
9. Supported by PHS grant MH-18282.

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Models for the Transport of DDT: Verification Analysis

In their article Harrison *et al.* (*1*) presented interesting models for the transport of DDT (*2*) through several trophic levels. Their models essentially represent a series of mass transfer equations in a homogeneous spatial medium. Harrison *et al.* claim that the models they propose are "sufficiently descriptive to yield the general nature of population response, and to make possible the prediction that a significant variation of a predator population would cause upsets throughout the entire system, some of which might be of sufficient magnitude to create 'out-of-control' conditions." However, nowhere in the article does there appear a verification analysis of the models proposed which would lend even minimum support to such a contention.

A verification analysis should compare the analytical model to observed data from the real world. Such an analysis is basic to a systems analysis of any phenomenon. The presentation of a model without some indication of how well the model does in "independent" test predictions represents only part of the systems analysis. One must always be prepared to demonstrate the utility of the model for decision-making. For this reason it is necessary to go substantially beyond mere structuring of the analytical model. Numerous changes in the model may be necessitated by such a verification analysis. Certainly this step must be carried out before any claim is made about the ability of the model for prediction purposes.

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Activity Change as the Cause of Apparent Aversiveness during Prolonged Hypothalamic Stimulation

Mendelson (*1*) reported that rats in the presence of relevant goal objects (for example, food for a stimulus-bound eater) while receiving rewarding hypothalamic stimulation turn off the stimulation less frequently than rats do when there are no relevant goal objects. The rats could switch the stimulation ON by moving into one half of a shuttle box and switch it OFF by running into the other half. Mendelson states that the phenomenon is due to a suppression of the normally aversive effects of long lasting stimulation by consummatory behavior. He then proposed that the aversive effects of the brain stimulation (*2, 3*) are due to the excessive arousal of drive, and that the opportunity to engage in consummatory behavior reduces the drive and makes the stimulation less aversive. However, a much simpler hypothesis can account for his result. During hypothalamic stimulation, the rat shows a high level of activity such as sniffing, searching, and moving, but if the relevant goal object is supplied during stimulation, such activity is reduced because the rat becomes engaged in eating, drinking, or gnawing (*4*). This simple "activity" hypothesis can account for Mendelson's finding that the rat would prefer a longer duration of brain stimulation with a goal object than without one. Such a sim-