The human cell function or functions which restrict Moloney leukemia virus synthesis do not appear to be transferred to nonfused 3T3 cells, since a high percentage of 3T3 cells in these cultures support virus synthesis. We have also been unable to induce resistance in 3T3 cultures by treatment of the cells with extracts of WI-38 cells produced by sonic disruption. These results indicate that resistance to murine leukemia virus is dominant, and that the restriction can be conferred on permissive cells by fusion with nonpermissive cells.

We have attempted to determine the step in virus replication which is restricted by fusion with nonpermissive cells at intervals after the initiation of infection in 3T3 cells. However, two factors complicate such experiments. (i) Once detectable virus protein has been synthesized, it is not possible to determine by our methods if further synthesis is inhibited; (ii) the fusion process itself tends to delay virus synthesis in permissive cells, presumably because of the effects of chilling and other manipulations on normal cell function. When WI-38 cells were fused 2, 8, and 24 hours after the ^{3}H-labeled 3T3 cells were infected, 4.2, 6.6, and 9.2 percent, respectively, of the polykaryons were positive for viral protein.

Hybrids of human and mouse cells have been produced, but human chromosomes are rapidly lost during multiplication of the hybrid cells (*18*). Five clones of human/mouse hybrids (HEL-C, KLE-J, KEH-9, KEJ-4, KEH-2), produced by fusion between KL-strain human cells and 3T3-4E mouse cells (*5, 19*) were tested for their ability to support virus replication. These clones possessed between 10 and 16 metacentric chromosomes, an indication that fewer than half of the human chromosomes were retained by these hybrids. All five hybrid clones were permissive for Moloney leukemia virus synthesis; by 48 hours after infection, up to 20 percent of the cells in these cultures were infected. These results indicate that the nonpermissive state of the human cells may be due to a function, specified by one or more chromosomes, which is dominant in the heterokaryons. The dominant state implies a restrictive control over virus expression and supports the speculation that cells synthesize a "repressor" which inhibits virus expression (*1*). The nature of the cellular control observed in our studies is not apparent but, since viral protein synthesis could not be detected in nonpermissive cells or in heterokaryons, it appears that the control process may involve a function which is an early event in the virus replication cycle. Since some human cells with an apparently normal karyotype can support leukemia virus replication (*3*), host range variation or host-induced modification (*20*) of the virus may overcome the restriction.

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Memory Traces: Experimental Separation by Cycloheximide and Electroconvulsive Shock

Abstract. *Mice given cycloheximide or saline were trained with a single trial. Electroconvulsive shock was administered to both groups at various times after training. Cycloheximide led to memory that decayed with time. Cycloheximide plus electroconvulsive shock produced complete amnesia at times when neither treatment alone produced amnesia. Only two types of processes appear to support memory storage in our study.*

Many studies have focused on the delineation of hypothetical processes underlying memory storage in experimental animals. Two classes of memory processes are usually cited (*1*). A short-term process is proposed to begin at training and decay as the interval between training and testing lengthens (*2*). A long-term process is proposed to strengthen with the passage of time after training and is believed dependent on some aspect of the short-term process (*3, 4*). Additional memory storage processes have been hypothesized as well (*5*).

We now report evidence for the existence of only two processes supporting memory storage. The long-term process appears to depend on protein synthesis, as suggested previously (*4*). The short-term process evidently does not rely on protein synthesis. When effective disruptive treatments of both long-term and short-term memory processes are combined, complete amnesia results. The amnesia is permanent and is not attributable to retrieval deficits but to deficits in actual memory storage processes. The cerebral protein synthesis inhibition produced by electroconvulsive shock (ECS) (*6*) appears to be unrelated to ECS-produced amnesic effects.

A heterogeneous strain of mice (230 males and 230 females, 60 to 80 days old) was used. The mice were housed ten to a cage, with mice from different experimental groups represented in

equal numbers. Male and female mice were equally distributed in all experimental groups. Training occurred in a single trial on an inhibitory avoidance apparatus (*7*). Each mouse was individually placed on a small metal platform (2.5 by 7 cm) that extended horizontally from beneath a hole (3 cm in diameter) in the vertical wall of a darkened box. As the mouse stepped from the highly illuminated platform (150-watt bulb located 25 cm above platform), through the hole and into the box, it received a brief footshock (constant 5 ma, 60 hz). Latency of the step-through response was recorded to the nearest second, and the mice were allowed 5 seconds in the box prior to removal. The ECS was given transcorneally (15 ma, active tube regulated d-c; 0.4-second duration), and the mice were artificially respired when required. All mice given ECS exhibited immediate, full tonic convulsions and were returned to home cages within 60 seconds after ECS treatment. All mice also received intraperitoneal injections of saline (0.9 percent NaCl) (10^{-2} ml per gram of body weight) or cycloheximide (160 mg/kg, dissolved in saline) 30 minutes prior to training.

Testing consisted of placing the mice individually on the small platform and recording the step-through response latency. The time elapsing between training and testing for different experimental groups was 60, 90, 120, 180, or 240 minutes, corresponding to training to ECS intervals of 15 seconds, or 30, 60, 120, and 180 minutes. A constant period of 60 minutes always elapsed between ECS (or scheduled time of ECS for mice not actually receiving ECS) and testing. Mice that failed to step through within 30 seconds were removed from the apparatus and a complete avoidance was recorded.

Four experimental groups ($n = 80$ in each group) represent the effects of our treatment conditions on learning and memory. One half of all saline- or cycloheximide-treated mice received ECS. Group 1 received saline, the training trial, but no ECS; group 2, saline, the training trial, and ECS; group 3, cycloheximide, the training trial, but no ECS; and group 4, cycloheximide, the training trial, and ECS. Individual subgroups ($n = 16$) were tested at the five different training to ECS intervals and corresponding training to test intervals. The treatments produced different alterations in memory depending on when the ECS and test were administered (Fig. 1). Table 1 summarizes some of the statistical comparisons (*8*).

Table 1. Major statistical comparisons made for experimental and control groups. Nonsignificant levels are presented as $P > .05$. All tests were two-tailed, nonparametric (*8*); Kruskal-Wallis, K-W; Mann-Whitney U, U; Wilcoxon, W.

Comparisons	Test	P
Memory storage		
Saline, no ECS	K-W	$> .05$
Cycloheximide, ECS	K-W	$> .05$
Saline, ECS	K-W	$< .05$
Train to ECS interval		
0.25 to 30 minutes	U	$< .02$
30 to 60 minutes	U	$> .05$
Cycloheximide, no ECS	K-W	$< .005$
Train to test interval		
60 to 90 minutes	U	$> .05$
90 to 180 minutes	U	$< .02$
Learning		
Saline, no ECS	W	$< .001$
Cycloheximide, ECS	W	$> .05$
Permanence	W	$< .001$
Performance	K-W	$> .05$
Retrieval	K-W	$> .05$

Regardless of when the saline-treated mice that received no ECS were tested, all of the mice showed similar high levels of retention as indicated by uniformly ($P > .05$) long step-through latencies. In contrast, all subgroups of the cycloheximide-treated mice that received ECS showed little retention as indicated by uniformly ($P > .05$) short step-through latencies. In any single comparisons, the mice in a subgroup receiving saline but no ECS always showed significantly ($P < .005$) better retention than the mice in a subgroup receiving cycloheximide and ECS. Notably, no mice in the cycloheximide and ECS subgroups exhibited response latencies that differed ($P > .05$) from original untrained response latencies, whereas mice in the saline but no ECS subgroups always exhibited significantly ($P < .001$) increased latencies after training. Both the groups treated with saline and ECS and the groups treated with cycloheximide without ECS differed significantly ($P < .05$ and $P < .005$, respectively), depending on the time when the test or ECS was administered. Individual comparisons indicated that the saline- and ECS–treated mice with a training to ECS interval of 15 seconds had significantly ($P < .02$) shorter response latencies than mice with longer training to ECS intervals. The response latencies of these mice did not differ ($P > .05$) from those of untrained mice. Although mice in the subgroup that received ECS 30 minutes after training generally showed shorter response latencies than mice with longer training to ECS intervals, these differences were not significant ($P > .05$). The subgroups receiving cycloheximide without ECS differed as a consequence of the time when tests were administered. The mice tested 60 or 90 minutes after training exhibited long response latencies not significantly ($P > .05$) different from any saline but no ECS subgroups. The mice receiving cycloheximide but no ECS, when tested 180 or 240 minutes after training, showed brief response latencies not different ($P > .05$) from the response latencies of untrained mice. Finally, the mice in the subgroup tested at 120 minutes after training exhibited response latencies of intermediate duration, suggesting neither the complete retention indicated in 60- and 90-minutes tests nor the complete amnesia indicated in 180- and 240-minutes test.

These results show that control animals receiving only saline learn and remember the task during our test periods. The mice receiving cycloheximide also appear capable of learning, but with delayed tests the retention of these animals drops to levels not distinguishable from untrained mice. If ECS is administered to saline mice quickly enough after training, subsequent retention is low. As the delay between training and ECS is lengthened, subsequent retention approaches levels indistinguishable from retention shown by mice that received saline but no ECS. The mice treated with cycloheximide and then ECS show no retention, regardless of the interval between training and ECS.

In addition to the four experimental groups, we also studied the following control groups.

1) *Permanence.* Since the maximum training to test interval used above was 240 minutes, it was possible that the observed effects were not permanent. Such possibilities have been suggested in regard to ECS (*9*) and several protein synthesis inhibitors (*10*), although permanence has been established both for ECS effects in the present task (*11*) and several protein synthesis inhibitors in different tasks (*4*). The permanence of our treatment effects was established by testing again the surviving mice (*12*) 72 hours after the initial 60-minute test. Not only did the amnesic effects persist, but all animals, regardless of prior treatment, showed shorter step-through latencies ($P < .001$) than on the 60-minute test. Thus, in the present task amnesia, whether obtained with ECS or cycloheximide treatments, persisted for at least 3 days.

2) *Performance.* Several control groups were tested concurrently with experimental groups to guard against performance changes produced by the treatments with saline, cycloheximide, and ECS. Groups of ten mice each were given standard saline or cycloheximide injections followed by ECS treatments coincident in time with the 15-second or 30-, 60-, 120-, and 180-minute training to ECS intervals of the experimental groups. None of these control groups, however, received training. Sixty minutes after the ECS the groups received an initial training trial. The response latencies on this trial, regardless of treatment, were indistinguishable ($P > .05$) from latencies of untreated animals. The saline, cycloheximide, and ECS treatments could not account, therefore, for the response changes on the basis of direct performance changes.

3) *Retrieval.* The controls noted above do not eliminate the possibility that our treatments simply alter the ability of mice to retrieve information from memory. In this case, the mice might have stored information but simply been unable to make that memory available to demonstrate retention during testing. To assess this possibility mice were trained on the inhibitory avoidance task. Twenty-four hours later four groups of ten mice each were submitted to the standard treatment conditions (saline or cycloheximide and ECS or no ECS) without further training. One hour after these treatments and 24 hours after original training all mice were tested. No differences in response latencies occurred ($P > .05$) and all mice exhibited uniformly good retention primarily with criterion level avoidances. Clearly, the treatment conditions possessed no detrimental effects on retrieval of information once that information is successfully stored.

We interpret the results as supportive of a dual-trace hypothesis of memory storage. In the presence of cycloheximide a single training trial initiates a memory trace that deteriorates within about 3 hours. Although cycloheximide inhibits 96 percent (*13*) of cerebral protein synthesis, the mice learn and remember the single trial task for brief periods. If ECS treatments are superimposed on cycloheximide treatments, complete amnesia follows. We interpret this finding as evidence for ECS disruption of the short-term memory that is resistant to cycloheximide treatments. When cycloheximide-sensitive, long-term memory is blocked, the effects of ECS on short-term memory can be demonstrated for much longer training to ECS intervals than usually observed (*2*). Thus, the amnesic ineffectiveness of training to ECS intervals exceeding 30 minutes in saline-treated mice is interpreted as the consequence of memories quickly being transferred into ECS-resistant long-term memory. Our findings do not support multitrace hypotheses of memory storage (*5*). It remains possible, however, that our treatments simply mask additional memory processes.

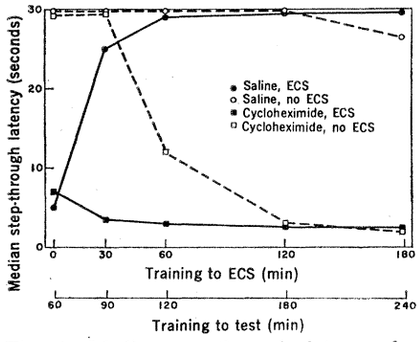

Fig. 1. Median step-through latency for the four experimental groups. Each point represents median test latency for 16 mice. Injections of saline or cycloheximide were given 30 minutes prior to training. The ECS treatment was administered either 15 seconds or 30, 60, 120, or 180 minutes after training. All mice, regardless of whether they actually received ECS, were tested 60 minutes after the time of ECS administration.

Recently ECS has been shown to briefly inhibit protein synthesis (*6*) and to produce polysome disaggregation (*14*). Puromycin and cycloheximide also inhibit protein synthesis (*4*) and produce polysome disaggregation (*15*). However, cycloheximide has been shown to protect brain polysomes from further disaggregation by ECS, whereas puromycin does not (*15*). By contrast, cycloheximide appears to enhance neural effects of ECS usually associated with amnesia (*16*). Our study indicates that the cycloheximide protection against further polysome disaggregation by ECS does not concurrently diminish the amnesic effects of ECS. In the presence of cycloheximide, the amnesic effects of ECS are more pronounced than in saline control animals. These results are in agreement with the cycloheximide plus ECS effects on neural activity. Polysomal disaggregation and the inhibition of protein synthesis produced by ECS, therefore, do not appear adequate to explain ECS-produced amnesic effects. The ECS effects on neural activity, perhaps a transient electrical bias of some type, seem more able to explain the amnesic effects of ECS treatments.

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