Table 1. Maximum pressures exerted by intact and deafferented monkeys; means of maximum responses on the best 2 of 4 days.

Monkey		Pressure (g/cm^2)
No.	Weight (kg)	
	Intact	
61	2.0	378
62	3.4	463
63	4.5	475
64	6.4	435
	Deafferented	
71	3.9	380
72	5.9	505

its responding limb severely before these data could be obtained.)

The values in Table 1 are means of the maximum responses on the two best days. It is clear that the grasp in a deafferented limb is as powerful as the grasp in an intact limb; indeed, the animal with the strongest grasp measured was deafferented. These preliminary results thus seem to indicate that the strength of a muscle is not contingent on its afferent innervation.

The responses of the deafferented monkeys appeared no more abrupt or ballistic than those of the normal monkeys, especially during the "maximum determination" period, when deliberate, effortful movements were frequently observed in both groups. Similarly, the oscilloscope traces revealed no gross differences in the temporal or pressure characteristics of the responses of normal and deafferented limbs. Moreover, it was found that, at the end of training, deafferents "tracked" as efficiently as normals; that is, when the prescribed pressure setting was low or changed suddenly, deafferents did not "overshoot" or miss to any greater extent than did normals. This finding is probably explainable in terms of effective use by deafferents of buzzer termination as a source of indirect, but immediate, information concerning the performance of a successful response.

After determination of maximum pressure, interlimb transfer of the response was studied for 1 day. The usual treatment of the limbs was reversed: the manipulandum was taped into the left (rather than the right) hand, and the right (rather than the left) arm was tied to one of the vertical supports of apparatus. The deafferented subjects were allowed to view their limbs for several minutes before the session began. (Animal 72 was unavailable because of previous incapacitation of its left hand.) On the first five trials shock was not presented and the buzzer could not be terminated; the usual procedure, with electric shock, was reintroduced for the remaining 15 trials.

The results were unexpected. None of the intact animals displayed transfer on the initial five trials. Even after receiving punishment, only two of the subjects exhibited responses of the left hand and these movements were minimal in amplitude. In each instance, however, and on every trial, conditioned responses were made inappropriately with the right hand; the animals continued to grasp nonexistent manipulanda. In contrast, both deafferented subjects transferred immediately. On the initial "transfer" trials, animal 71 achieved 50 percent of the maximum pressure response given by the trained hand; with electric shock, the magnitude of the response was increased to within 20 g/cm^2 of its previous maximum. This finding suggests that movements of one limb normally have, at least in part, an inhibitory or interferent effect on movements of the contralateral paired extremity. Deafferentation of both forelimbs abolishes this mutual inhibitory or interferent influence in monkeys and thus facilitates interlimb transfer of certain types of conditioned responses.

In general, these results tend to confirm and extend our previous findings concerning the range of conditioned movement that is possible in a deafferented limb. It is clear that even movements of the distal musculature can be learned and performed in the absence of somatic afferent feedback.

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Puromycin Effect on Successive Phases of Memory Storage

Abstract. *Mice injected bitemporally with puromycin 5 hours before training learned to escape or to avoid shock by choosing the correct limb of a Y-maze. When retested 15 minutes after training they had normal retention. In the ensuing 2¾ hours the animals injected with puromycin, unlike the controls, showed a progressive decrease of savings to less than 7 percent.*

Memory storage is generally believed to progress through two phases—a "short-term" or "labile phase" and a "long-term" or "stable phase." That the "short-term" phase may be mediated by a reversible molecular change (for example, a configurational change in a protein at the synapse) whereas the "long-term" phase would probably be mediated by a self-replicating biosynthetic process (for example, synthesis of a protein at the synapse) has been suggested (*1*). We now report experiments to test this hypothesis and we believe the results are consistent with it.

Injections of puromycin into both temporal regions of the brain inhibit more than 80 percent of protein synthesis in this zone for from several hours to more than half a day after injection (*2*). Furthermore if mice are trained to solve a Y-maze and are then injected intracerebrally with puromycin from 1 to 3 days after training they appear to have forgotten the solution to the maze when tested 3 days thereafter (*3*). The foregoing experiments suggest that protein synthesis in the temporal region is required for maintenance of a memory within the period studied. They provide no information, however, on the time, during or after training, when the puromycin-sensitive process first becomes necessary for memory storage. We now report our attempts to answer this question.

Male Swiss albino mice (30 to 40 g, Charles River Breeding Co.) were lightly anesthetized with pentobarbital (40 mg/kg) and, when necessary, with small amounts of ether and mounted in a stereotaxic instrument. Their scalps were incised and reflected and a hole was made in each "temporal" site (*3*). Ten microliters of a freshly prepared solution containing 90 μg of puromycin dihydrochloride (titrated to *p*H 6 with NaOH) was injected at each temporal site at a depth of 2.5 mm from the

outer surface of the skull, perpendicular to its horizontal axis. The animals were awake within several hours of this procedure. Five hours after injection, a time at which protein synthesis in the temporal zone has already been inhibited more than 80 percent for several hours (*2*), the mice were trained to choose the left limb of a Y-maze to escape or avoid shock (Fig. 1; *4*). The animals were trained to a criterion of nine out of ten correct responses.

Puromycin-injected animals learned the maze in an average of 17.7 trials. They did not differ significantly from controls that were injected with 0.06*M* NaCl, which approximates the NaCl concentration in the puromycin solution. Memory was evaluated by retraining the animals at one of a number of time intervals after initial learning and by comparing the number of trials to reach criterion on retraining with the number of trials to reach criterion in initial learning. The percentage of savings is $[(I - 9) - (R - 9)]/(I - 9) \times 100$, where I is the number of trials required to reach criterion in initial learning and R is the number of trials required to reach criterion on retraining. When puromycin-treated animals were evaluated 15 minutes after they had finished learning the task, they had savings percentages indistinguishable from those of controls injected with 0.06*M* NaCl. In the ensuing few hours the puromycin-injected animals showed a progressive loss of savings and, 3 hours after learning, their savings was less than 7 percent (Fig. 1).

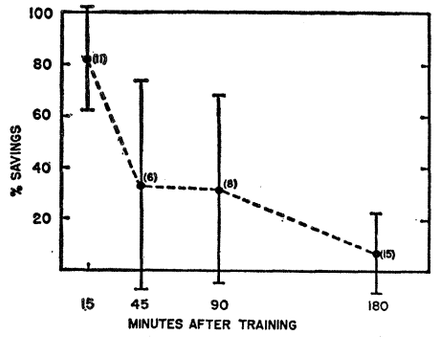

Fig. 1. Percentage savings after training. All animals were injected with puromycin at both temporal sites. Five hours later they were trained to choose the left limb of a Y-maze. The floor of the maze was a stainless steel grid and the left limb was insulated with colorless cellulose nitrate. Shock (approximately 0.5 ma) was administered 5 seconds after the animal was placed in the starting limb. A trial was considered correct if the animal did not enter the incorrect limb before reaching the correct limb. A trial was completed when the mouse entered the correct limb. The animal was allowed to remain on the safe area for 10 seconds before being returned to his home cage where he remained for about 1 minute before the next trial was begun. The animals learned in 17.7 ± 6.5 trials (mean ± S.D.). Savings was determined at the indicated times after completion of initial training. The results are mean ± S.D., and the number of animals in each group is shown in parentheses. The group tested at 15 minutes differs significantly from that at 3 hours ($P < .001$, t-test) and also from that at 45 minutes ($P < .05$).

In contrast to the loss of memory in the treated mice, injection of 0.06*M* NaCl at the temporal sites did not interfere with savings 3 hours after training (Table 1). This suggests that the impairment of memory is not due to some nonspecific effect of the temporal injections. Furthermore, injection of identical amounts of puromycin at "frontal" sites (*3*) does not interfere with memory storage (Table 1), an argument against the phenomenon being due to a nonspecific toxic effect of intracerebral puromycin. It was also established that animals trained 8 hours after bitemporal puromycin injections learned normally and showed normal savings when tested 15 minutes after training (Table 1). Thus, at a time when animals have forgotten what they learned 3 hours before, their capacity for learning and "short-term" memory is retained.

These experiments are consistent with the hypothesis that there is an initial phase of memory storage which is independent of protein synthesis in the temporal lobe and that this overlaps a second phase which is dependent on protein synthesis in this region of the brain. The effects of puromycin on memory in the goldfish (*5*) may also be interpreted in this way. Nevertheless puromycin may be exerting its effect on memory storage by some mechanism other than inhibition of protein synthesis. We have found (*4*) that injections of actinomycin D which inhibit cerebral RNA synthesis 94 to 96 percent do not interfere with retention of the solution to a Y-maze within 4 hours after training. This suggests that, if protein synthesis is indeed required for the second phase of memory storage, such protein synthesis is directed by a stable messenger RNA which was synthesized before and independent of acquisition.

Table 1. Learning and savings in control groups. Animals received bilateral injections of 10 μl of puromycin solution or 0.06*M* NaCl at frontal (F) or temporal (T) sites (*3*). The results are mean ± S.D., and the number in each group is shown in parentheses.

Site	Period (hr)		Initial trials to criterion	Savings (%)
	Training*	Tested†		
			Sodium chloride	
T	5	3	17.0±3.1(8)	91.2± 9.1
			Puromycin solution	
F	5	3	17.1±5.7(9)	86.7±20.4
T	8	¼	17.7±4.0(8)	89.4±14.5

* Hours after injection. † Hours after training.

It is customary to consider that memory storage has two phases—"short-term" and "long-term." Our experiments and those of Flexner *et al.* (*3*), when considered together, suggest that there are at least three phases of memory storage in the shock-motivated maze learning which they and we studied. There appears to be an initial phase, uninfluenced by puromycin, which extends for a number of minutes after learning; a second phase, inhibited by temporal injections of puromycin, which may extend for several days; and a third phase, beyond these, which can be interfered with only by more diffuse intracerebral injections of puromycin. It is therefore more appropriate to consider memory storage a triphasic or multiphasic process rather than a biphasic one.

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