Deafferentation in Monkeys: Effect on Conditioned Grasp Response

Abstract. *A preliminary technique was developed for conditioning grasp response in monkeys, for use in studying the effect of damage to the central nervous system on skilled movement. That subjects were able to learn this response with a deafferented hand, in the absence of vision, indicated that purposive movements of the distal musculature are less under the control of peripheral feedback than had been generally believed.*

Work in our laboratory during the past 8 years (*1*) has demonstrated that somatic sensation is not necessary for the performance of voluntary movement, contrary to a prevalent view based on a classic study by Mott and Sherrington (*2*). In initial experiments we showed that a single deafferented extremity of a primate could participate in purposive movements under certain restrictive conditions: for example, animals naive at operation were able to learn to avoid electric shock by flexing an unseen deafferented forelimb in response to a buzzer.

Forearm flexion, however, is admittedly a crude movement. It remained possible that tactile and proprioceptive feedback are necessary for the performance of finer, more complex movements of the distal musculature. In attempting to explore this possibility we encountered the difficulty that most of the conditioned-response techniques available today for testing neurological deficits entail crude movements of a whole limb or of an entire body. The lack of a satisfactory technique for studying skilled movement in primates, under controlled and quantifiable conditions, led us to develop a method for conditioning a grasp response.

A shaping procedure was employed in an avoidance-conditioning situation and, after much trial and error with different manipulanda and different methods of immobilizing the responding arm, a technique was standardized with four intact, adolescent, male rhesus monkeys. In the final stage they were required to grasp a fluid-filled polyvinyl cylinder at the onset of a buzzer in order to avoid shock.

The animals were first placed in a restraining chair, which was then fitted into a complementary apparatus contained in a small sound-insulated chamber into which a masking white noise was introduced. The collar of the restraining chair abutted against a horizontal piece of plywood so as to obstruct an animal's view of its limbs; thus vision could not help to guide the movements of a deafferented hand. The manipulandum, a drip chamber from a blood-administration set, with the plastic filter removed, was taped firmly into an animal's right hand; it was filled with water and connected to a precision transducer which provided an output voltage linearly proportional to the input pressure. Pressure applied to the manipulandum was communicated through the fluid medium to the transducer, which in turn activated circuitry that terminated the buzzer and ended the trial if the exerted pressure exceeded a predetermined value. The output of the transducer was also displayed on an oscilloscope; thus were enabled observation and measurement of some of the pressure characteristics of the response (*3*).

Initially the responding arm was left completely free so that pressure could be exerted on the manipulandum, not only by a grasp, but also by banging or pressing it against the sides and floor of the apparatus. Only after this had been learned did we require the subjects to develop a specific grasp response by securing the right forearm in a holder in such a way that the hand could not be brought into contact with a rigid surface. As a result the only movement capable of producing pressure on the cylinder was a grasp. The arm holder could be adjusted with respect to height, length, angle of elevation, and angle of lateral rotation. The optimal set of conditions for responding had to be determined for each animal, since it tended to differ somewhat between individuals.

On experimental days the animals were given 13 trials, with an intertrial interval varying randomly from 30 to 60 seconds. The first three trials of the series were considered ranging trials. The conditioned stimulus was presented for 10 seconds and, in the absence of the prescribed response, an electric shock of 3 ma was delivered to the left ear for the final 5 seconds of the trial.

All subjects displayed a consistent grasp response from the very outset. In a previous unsuccessful attempt here to condition a grasp response, the responding arm was immobilized in a holder from the beginning. The critical part of the present successful procedure thus seems to be the initial "shaping" period, when the arm is left free; the probable reason is that learning in this situation entails the performance of movements that are opposite in direction to the unconditioned response to electric shock, which is dorsiflexion and fanning of the fingers. This view is supported by the fact that our subjects either avoided or "took" shock; they rarely terminated shock, in sharp contrast with other avoidance situations.

We are now attempting to further refine the grasp technique. The task now being developed requires that subjects maintain pressure within a certain "pressure window" (that is, between a minimum and a maximum) for a prescribed time, rather than merely exert pressure above a specified minimum.

In a first application of the preliminary technique, three naive animals were subjected to deafferentation of both forelimbs by bilateral intradural section of dorsal roots C2 to T3. Several months later the animals were placed in the conditioning situation and the shaping procedure was initiated. For the first several days the restraining chair was pulled a short distance away from the rest of the apparatus before the beginning of the training session, and the monkeys were allowed to see briefly the manipulandum after it had been taped into their hands. It is emphasized that none of the animals could see their limbs during conditioning; nor had they had preoperative training. Yet all three monkeys were able to learn the grasp response. (Comparison of normal and deafferented monkeys with respect to speed of acquisition is difficult because of the frequent changes in procedure that were necessary while the technique was being developed.) When the responding had stabilized, we tried to determine the maximum pressure that each animal could exert; a modified method of limits was employed, using ascending series only. The animals were run in this fashion for four consecutive days. (One deafferented subject injured

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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References and Notes

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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