Effect of Deafferentation on a Conditioned Avoidance Response

Abstract. To determine whether peripheral afferent pathways could be bypassed in the performance of purposive movement, a group of monkeys was trained to avoid shock by flexing a forelimb in response to an auditory stimulus. The dorsal roots of the responding limb were then sectioned. Postoperatively it was found that the affected limb could be reconditioned.

In 1898 Mott and Sherrington (*1*) first reported on the severe impairment of movement which occurs after sectioning the sensory roots innervating the limbs of monkeys. The deficit they described was even greater than the one reported after motor cortex ablations. They concluded that the peripheral afferents, particularly the cutaneous fibres, were necessary for the execution of "the highest level movements."

More recently Twitchell (*2*) investigated in more detail the exact nature of the movement deficit following limb deafferentation. After complete sectioning of dorsal roots C3 through T3, the only movements the animals could perform were flexion and extension of the proximal limb musculature associated with the tonic neck reflex. Unless the tonic neck reflex was abolished by sectioning dorsal roots C1, C2, and C3 bilaterally, these residual movements could be adapted for purposeful, if limited, use, under sufficiently high motivation and only with the accompaniment of vision. He concluded that the role of the peripheral sensory system was to direct the cortical components of movement.

Evidence from conditioning studies, however, indicates that it should be possible to direct limb movements through purely central processes. A recent study by Beck and Doty (*3*) described the conditioning of a flexion response while the responding limb was temporarily paralyzed by crushing its ventral roots. The flaccid paralysis resulting from this procedure should have eliminated the possibility of any "feedback" of sensory impulses during the conditioning procedure, yet, after regeneration of the nerves supplying the limb, the animals gave conditioned flexion responses to a buzzer. This seemed to indicate that the entire conditioning process involved purely central mechanisms.

If this interpretation is correct, then the conditioning procedure should enable central factors to direct the actual performance of conditioned responses. The preliminary study described here (*4*) was designed to test this assumption. This was done by observing the performance of a conditioned response in monkeys after total deafferentation of the responding limb.

Five rhesus monkeys were trained to avoid shock by flexing a limb in response to a buzzer. A large opaque collar, 25.5 by 14.5 in., attached to the chair in which the animals were seated, precluded the use of any visual cues in the learning or performance of the response. Two electrodes used to deliver shock were fastened to the left forelimb, while an elastic tied to the right forelimb was attached at the free end to a microswitch. Upon the flexion of the right forelimb, the elastic pulled the microswitch lever, breaking the circuit and ending the trial. The conditioned stimulus was a buzzer which sounded for 3 seconds prior to the onset of shock. If the animal did not respond, the buzzer and shock continued for 1½ seconds together, unless the trial was terminated by a response during this interval. Thus, a response during the first 3 seconds would result in avoidance of shock, while a response during the next second and a half would result in termination of shock. Two animals were trained at a shock intensity of from 1 to 2 ma, while three were trained at intensities of from 4 to 6 ma.

Table 1. Postoperative data for first and last conditioning sessions. Percentages are calculated on the basis of ratio of avoidance responses to total number of trials per day.

Animal	Shock intensity (ma)	Avoidance responses (%)	
		First day	Last day
1	4–6	29	100
2*	1–2	0	40
3	4–6	0	100
4	1–2	0	80
5†	4–6	100	100

* Monkey No. 2 never fully recovered and died 3 weeks after surgery.
† Monkey No. 5 had 30 percent of the fibers of dorsal root C8 intact.

After the animals had performed to a criterion of 13 avoidance responses in 15 trials on each of two consecutive days, the responding limb of each was deafferented, from dorsal roots C3 through T2. On completion of the experiment, the operative field was reexamined under magnification. One animal in the high-shock group was found to have a few intact C8 fibres. Another animal in the high-shock group was found to have retained a single aberrant fibre, from the caudal region of C2 running into the rostral area of C3. The three remaining animals had complete dorsal root sections running from C3 to T2 inclusive. The first postoperative testing was started after the animal had fully recovered from the procedure, usually within from 5 to 7 days.

The results are presented in Table 1 and Fig. 1. All five monkeys were reconditioned to avoid shock by making a flexion response without the use of

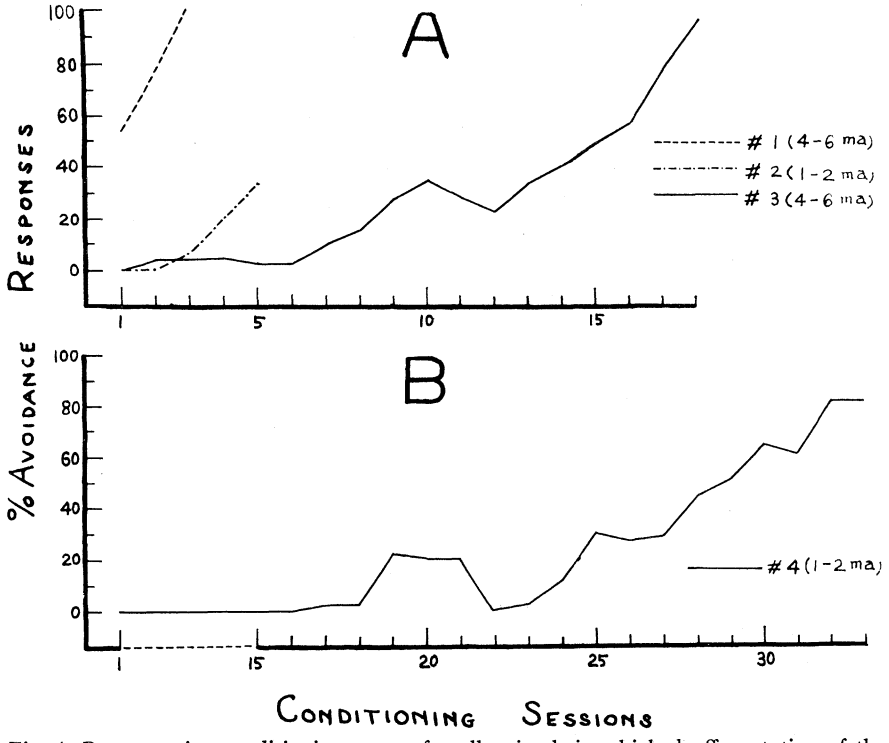

Fig. 1. Postoperative conditioning curves for all animals in which deafferentation of the limb was complete. The curves represent smoothed data obtained by the method of running averages of three (*5*). In *A*, monkey No. 2 died after reaching a 40-percent avoidance response level; in *B*, the data for the first 15 days were condensed.

vision. From these preliminary results it appeared that shock intensity (as measured in milliamperes) was not the determining factor in the postoperative performance. In the free situation the animals behaved exactly as described by both Sherrington and Twitchell. While the monkey was running, the limb was held in a semiflexed position, hand and fingers hanging loosely. The deafferented limb was not used for climbing, and occasional attempts to use the limb for defense always ended in failure. In the conditioning situation, however, the flexion responses were fairly consistent and occurred without obviously associated head and neck movements.

The questions of whether these responses were centrally directed or whether the animals learned to make use of cues provided by intact afferents in other parts of the body are presently being investigated.

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References and Notes

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4. This study was supported by a grant from the Fund for Neurobiology.
5. A running average of 3 is obtained by averaging a particular day's data with the data of the preceding and succeeding days. Thus, each point on the graph represents an average for 3 days.

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Restoration of Tryptophan Synthetase Activity in Escherichia coli by Suppressor Mutations

Immunological studies with mutants of *Neurospora crassa* defective in the ability to form the enzyme tryptophan synthetase (TSase) (indole + L serine → L-tryptophan) have shown that certain of these strains form large amounts of a protein, designated CRM, which is immunologically similar to TSase (*1*). One mutant, td_1, allelic by genetic criteria with the other mutant strains (*2*), was found to lack CRM (*1*). On the basis of these observations it was tentatively concluded that CRM represents an altered form of TSase and that one gene controls the formation of TSase and CRM (*3*, *4*).

Extensive mutational studies carried out with strain td_1 have shown that this strain does yield tryptophan-independent cultures and that these cultures invariably result from reversions at the td locus rather than suppressor mutations (mutations of different genes reversing the effect of the primary mutation) (*5*). Furthermore, other tests demonstrated that td_1 does not respond to suppressor genes which affect one or more of other mutants lacking TSase (*2*). Since lack of suppressibility appeared to be associated with inability to form CRM in this strain, the possibility was considered that only strains capable of forming a slightly altered TSase are capable of responding to suppressor genes (*3*).

Mutants of *Escherichia coli* lacking TSase also fall into two categories with respect to CRM formation; one group forms a protein which is immunologically similar to TSase, while the other group does not (*6*). The latter group appears to be comparable to the td_1 type in *Neurospora*. In an effort to examine more thoroughly the question of whether mutants lacking CRM are suppressible, one *Escherichia coli* stock which lacks CRM, strain T-41, was selected for further study (*7*). Cell suspensions of this strain were irradiated with ultraviolet light and plated on a medium lacking tryptophan in a search for suppressor-type mutations. Many small and large trytophan-independent colonies appeared on the plates after 3 days of incubation; several of each type were picked and purified by streaking on a medium lacking tryptophan. Three small- and three large-colony types were selected and examined further in accumulation tests. The three small-colony types accumulated indoleglycerol as does T-41, the mutant they were derived from, while the three large-colony types did not accumulate detectable amounts of any compound related to an intermediate in the tryptophan pathway.

The six selected cultures were also examined in transduction tests (*8*) to determine whether suppression or reversion was responsible for their tryptophan independence. Phage grown on each of the strains listed was used to transduce a cysteine-requiring stock (the cysteine and T-41 genes are closely linked) to cysteine independence, and the treated cells were plated on a medium containing tryptophan. The resulting colonies were then tested for tryptophan dependence. If typtophan-dependent colonies were obtained, the original stock must have carried both the T-41 mutant gene and a suppressor gene. If no mutants were recovered, the original stock probably was a revertant. The three small-colony types yielded typical T-41-like trytophan-dependent colonies in these tests, indicating that suppression was responsible for their ability to grow in the absence of tryptophan. The three large-colony types did not yield mutants, and thus they appear to represent reversions at the T-41 mutant locus. The presence of suppressor genes in the presumed suppressed stocks was confirmed by transducing the suppressor genes from these stocks into strain T-41.

Table 1. Characteristics of the various strains examined.

Strain	Type	Accumulation	Specific activity of TSase	TSase neutralized by 0.02 ml of antiserum
T41	Mutant	Indoleglycerol	0	—
T41-R2	Suppressed mutant	Indoleglycerol	1.5	1.08
T41-R3	Suppressed mutant	Indoleglycerol	1.5	1.38
T41-R4	Suppressed mutant	Indoleglycerol	1.0	1.28
T41-R6	Revertant	None	2.7	1.41
T41-R7	Revertant	None	3.3	1.3
T41-R8	Revertant	None	3.0	1.15
K-12	Wild type	None	2.8	1.24

Enzyme and immunological studies were performed with the six cultures and are summarized in Table 1. It can be seen that extracts of all six strains exhibit TSase activity, while the strain they were derived from, T-41, does not. It can also be seen that the TSase formed by the six cultures is normal in the sense that approximately equivalent amounts are neutralized by TSase antiserum. The three suppressed mutants appear to form somewhat less TSase than the revertants and the wild-type strain.

These findings indicate that suppressor mutations can restore the ability to form an enzymatically and antigenically active protein to a mutant which lacks CRM.

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