Transfer Following Operant Conditioning in the Curarized Dog

Abstract. *Dogs were trained to press a pedal to avoid shock. They were then operantly reinforced for making or for refraining from making a series of electromyographic responses while almost completely curarized. Tests after recovery from curarization showed that operant conditioning under curare influenced the original pedal-press response.*

Curare-like drugs have been employed to control skeletal responding in many types of conditioning experiments. Black, Carlson, and Solomon (*1*), Black and Lang (*2*), Black (*3*), and Church and LoLordo *et al.* (*4*) used curare to prevent skeletal responding in research on classical (*5*) heart-rate conditioning. Trowill (*6*) and Di Cara (*7*) used it for the same purpose in research on the operant conditioning of heart rate. Black (*8*), Solomon and Turner (*9*), Leaf (*10*), and Overmier (*11*) studied the effects of prior classical conditioning in curarized subjects on subsequent operant responding in the normal state. In experiments such as these, the assumption has been made that operant reinforcement of skeletal activity does not take place under curare. There are, however, a number of ways in which operant reinforcement that influences skeletal behavior might occur in curarized subjects. If curarization is incomplete, vestigial skeletal responses might be operantly reinforced. Even if curarization is complete, central correlates of overt responses might be reinforced by operant procedures. In each of these cases, the effects of operant reinforcement under curare could transfer to overt skeletal behavior in the normal state. There is, however, so little evidence on operant reinforcement in curarized subjects and its transfer, that one does not know whether this hypothesis is plausible or not. The experiment described here was carried out in order to provide such evidence.

Our first aim was to demonstrate that almost completely curarized subjects could be operantly trained either to increase the rate of or to refrain from making electromyographic responses (*12*). Our second and main goal was to determine the effects of such operant training on subsequent skeletal behavior in the normal state.

In the first phase of the experiment, operant conditioning procedures were employed to train dogs to avoid an intense shock. The avoidance response was pressing a pedal with the left foreleg in the presence of a conditioned stimulus (CS). The CS was a 75-db white noise. A potentiometer measured vertical leg movements. Electromyographic responses were recorded from two locations on the left forelimb by the use of Grass needle electrodes. One location was over the deltoid muscle and the other was over the extensor carpi radialis and the extensor digitorum muscles.

On each trial, if the dog pressed the pedal during the CS-shock interval (15 seconds), the CS was terminated and shock avoided. If the dog failed to press during the CS-shock interval, a series of 4- to 10-ma shocks of one-tenth second duration were presented at 5-second intervals until a pedal-press was made. Fifty trials a day were given. Each dog was trained to a criterion of 20 consecutive avoidances, and then given 50 additional training trials on the next day.

Twenty-four hours later, each dog was given ten more training trials, and then the second phase of the experiment began. The dogs were curarized with doses of *d*-tubocurarine chloride (*13*) which varied from 7.8 to 30.0 mg, with a mean of 14.7 mg. Curare injections and artificial respiration were continued during the course of the experiment. The dogs were maintained at a level of curarization such that electromyographic responding in the left foreleg occurred, but little or no leg movement was present. Of those trials under curare on which electromyographic activity was observed, the median amplitude of vertical movement was 0 mm and the range was 0 to 50 mm. The scores were clustered at the lower end of the distributions; for example, 95 percent of the observations were less than 10 mm. The effects of curare were further assessed by comparing maximum electromyographic responses and vertical leg movements on the last conditioning trial in the normal state and on the first conditioning trial under curare. (Measurements were taken before the presentation of shock, if shock occurred on a given trial.) The median of the maximum leg movements was 185 mm in the normal state and 2.5 mm under curare; the median of the maximum electromyographic responses was 2.59 mv in the normal state and 0.17 mv under curare.

The treatments under curare were designed to operantly reinforce a high rate of electromyographic activity in one group of dogs (group R) and a zero rate of such activity in a second group of dogs (group NR). The nine dogs in group R were reinforced for making a series of seven electromyographic responses to the CS. On each trial, a series of seven electromyographic responses during a 7-second CS-shock interval resulted in CS termination and avoidance of shock. If the dog failed to make seven electromyographic responses during the CS-shock interval, a series of shocks was presented. The shocks were programmed 7 seconds apart and were delayed for 7 seconds by an electromyographic re-

Table 1. Results for the dogs trained to make electromyographic responses under curare (group R) and for the dogs trained to refrain from electromyographic activity under curare (group NR).

Item	Median number		Mann-Whitney U test
	Group R	Group NR	
Original avoidance training in the normal state			
Shock trials to criterion	7.5	7.5	P ($U \leq 3.0$) = 0.880
Training in the curarized state			
Trials to criterion	24.0	23.0	P ($U \leq 20$) = 0.800
Shock trials to criterion	4.5	8.0	P ($U \leq 17$) = 0.130
Shock per shock trial	2.4	4.6	P ($U \leq 5$) = 0.002
Total shocks (median)	12.0	51.5	P ($U \leq 5$) = 0.002
Test in the normal state			
Pedal-presses during first 10 seconds of the CS	10.0	3.0	P ($U \leq 3.5$) = 0.001
Pedal-presses on trial No. 1	35.5	4.0	P ($U \leq 10.0$) = 0.010
Trials to five consecutive failures to respond to the CS	50+	17.0	P ($U \leq 14.5$) = 0.037

sponse. The series of shocks and the CS were terminated by the seventh electromyographic response. Eight other dogs, in group NR, were reinforced for a period of 7 seconds *without* an electromyographic response to the CS. On each trial, 7 seconds of electromyographic inactivity resulted in CS termination and avoidance of shock. If an electromyographic response occurred in the presence of the CS, it was followed by a brief shock. The CS and shocks could be terminated only by 7 seconds of muscular inactivity. Dogs in both groups were trained until they met a criterion of 20 consecutive trials without a shock, or until 130 trials had passed.

In the third and final phase of the experiment, I tested the transfer from training under curare to pedal-pressing in the normal state. On this test, which took place 2 days after recovery from curarization, each dog was given a series of 50 extinction trials without shock. On each extinction trial, the CS terminated only after 10 seconds had passed without a pedal press. This special extinction procedure was employed in order to reduce the amount of time required for between-group differences to emerge. In previous work, I found that the response was extremely difficult to extinguish when we employed the usual extinction procedures in which the pedal-press produced CS termination. Also, this special extinction procedure permitted us to obtain measures of pedal-pressing before the data were contaminated by the reinforcing effects of CS termination.

The results are shown in Table 1. The number of shock trials before the acquisition criterion was reached is an index of speed of conditioning of the pedal-press response. There was no significant difference between the two groups on this measure.

During the curare phase of the experiment, 15 of the 17 dogs that were trained met the criterion of 20 consecutive trials without a shock. The two dogs that did not meet the criterion were in group R. (One reached 19 trials without a shock, and the other reached four; the latter dog was discarded from the experiment.) These results indicate that operant procedures can be used to alter the probability of electromyographic responses under curare.

There was no difference between the groups in number of trials required to meet the training criterion under curare.

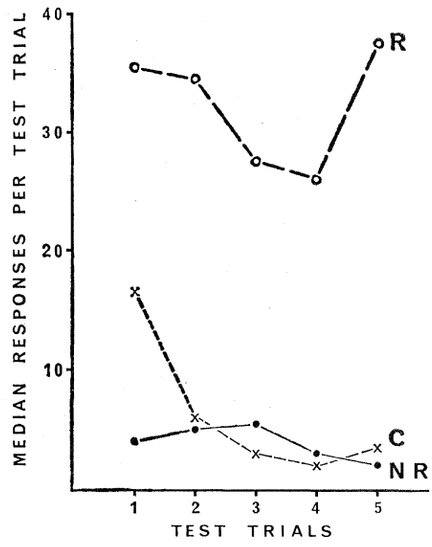

Fig. 1. Pedal-presses for each of the first five test trials for dogs trained to make electromyographic responses (R), dogs trained to refrain from electromyographic responding (NR), and control dogs (C).

However, the dogs trained to make an electromyographic response required significantly fewer shocks per trial and significantly fewer total shocks to reach the criterion. It took less shock to train the dogs to make a series of electromyographic responses than to train them to refrain from electromyographic activity under curare.

On the test after recovery from curarization, the dogs that were trained to make electromyographic responses under curare displayed significantly greater rates of pedal-pressing than the dogs that were trained to refrain from electromyographic activity on all the measures shown in Table 1. Reinforcing electromyographic activity under curare led to a high level of pedal-pressing in the normal state, and reinforcing electromyographic inactivity led to a low level of pedal-pressing in the normal state.

There is some question concerning the interpretation of these test data because of the differential exposure to shock under curare. One might argue, for example, that more occurrences of shock under curare or more pairings of CS and shock under curare result in fewer pedal-presses in the normal state. These possibilities were ruled out by four control groups which received different numbers of classical pairings of CS and shock under curare: 0, 4, 12, or 36 pairings. (In addition, all control dogs received 20 presentations of the CS alone in order to equate them with the experimental dogs, since the latter received 20 presentations of CS alone on the criterion trials under curare.) There was no significant difference among these control groups on any of the test measures after curarization.

In Fig. 1 the R, NR, and control groups are compared. (Data for the control groups were combined for this comparison, since there were no differences among them.) Data are shown for the median number of pedal-presses per trial for the first five test trials. The operant conditioning procedures under curare that increased electromyographic responding produced a significant increase in pedal-pressing relative to the control-group dogs on all five test trials. The operant procedures under curare that decreased electromyographic responding produced a significant decrease in pedal-pressing relative to the control-group dogs on the first test trial. Thus, transfer from operant conditioning under curare is different from that produced by classical conditioning procedures.

The present results lead one to question the curare procedure as a control for operant reinforcement that is related to overt skeletal responding. For example, in experiments on the operant conditioning of heart rate under curare, it may very well be that electromyographic responses were actually conditioned, and that these led to reflexive changes in heart rate. Similarly, demonstrations of how prior classical conditioning in curarized subjects affects subsequent operant behavior in the normal state may in fact be demonstrations of the effects of prior operant conditioning. This is not to say that all the results of training procedures in curarized subjects can be accounted for by operant conditioning; rather, the point is simply that relevant operant conditioning may be occurring inadvertently during experiments on classical conditioning.

These conclusions, of course, hold only for subjects that are not completely curarized. Would not the completely curarized preparation avoid the difficulties described above? While the present experiment provides no direct answer to this question, the data from the operant conditioning of refraining from electromyographic activity are relevant. Two major sources of reinforcement were employed: (i) avoidance of shock and CS termination following a period of no electromyograph-

ic activity, and (ii) the occurrence of shock following electromyographic activity. It seems reasonable to assume that at least the former type of reinforcement could occur in completely curarized subjects. If this is so, then the completely curarized preparation would not avoid the difficulties described above. Before we can decide on the adequacy of the completely curarized preparation unequivocally, however, we must determine how much electromyographic activity, if any, is necessary for operant conditioning and transfer to occur. It may be that no electromyographic activity is necessary, and that the transfer could be mediated by the reinforcement of central nervous system events associated with movement (*14*). If this is the case, then the operant reinforcement of motoneuron electrical activity in completely curarized dogs should show the same type of transfer as was shown by the dogs in this experiment.

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References and Notes

1. A. H. Black, N. J. Carlson, R. L. Solomon, *Psychol. Monog.* **76**, 29 (1962).
2. A. H. Black and W. H. Lang, *Psychol. Rev.* **71**, 80 (1964).
3. A. H. Black, in *Classical Conditioning*, W. Prokasy, Ed. (Appleton-Century-Crofts, New York, 1965), p. 20.
4. R. M. Church V. LoLordo, J. B. Overmier, R. L. Solomon, L. H. Turner, *J. Comp. Physiol Psychol.* **62**, 1 (1966).
5. In this report classical conditioning refers to conditioning that depends on the pairing of two stimuli—the conditioned stimulus and unconditioned stimulus. Operant conditioning refers to conditioning that depends on the pairing of a response and a reinforcing stimulus.
6. J. A. Trowill, paper presented at the meetings of the Eastern Psychological Association, New York, 1966.
7. L. V. Di Cara, paper presented at the meetings of the Eastern Psychological Association, New York, 1966.
8. A. H. Black, *J. Comp. Physiol. Psychol.* **51**, 519 (1958).
9. R. L. Solomon and L. H. Turner, *Psychol. Rev.* **69**, 202 (1962).
10. R. C. Leaf, *J. Comp. Physiol. Psychol.* **58**, 446 (1964).
11. J. B. Overmier, *ibid.* **62**, 15 (1966).
12. R. F. Hefferline, B. Keenan, and R. A. Harford [*Science* **130**, 1338 (1959)] have demonstrated that human subjects could be operantly trained to make extremely small electromyographic responses. It should be pointed out also that training a subject to refrain from electromyographic activity is not identical with training a subject to "hold still" in the normal state. The latter could involve a cessation of electromyographic activity as in relaxation, or a high level of electromyographic activity as in isometric contractions where movement does not occur because one set of muscles is pitted against another.
13. The *d*-tubocurarine chloride was provided by E. R. Squibb and Sons of Canada Ltd.
14. While the central nervous system events that might be involved cannot be specified, it does seem that feedback from the pedal-press is not a crucial factor, since Gorska and Jankowska [*Acta. Biol. Exp. Polish Acad. Sci.* **21**, 219 (1961)] and Taub, Bacon, and Berman [*J. Comp. Physiol. Psychol.* **59**, 275 (1965)] have demonstrated operant conditioning after deafferentation. The present results are consistent with theirs in that operant conditioning in curarized dogs had a clear-cut effect on subsequent pedal-pressing even though the afferent feedback was very different under curare from that occurring during pedal-pressing.
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Reversible Aggregation of α-Gliadin to Fibrils

Abstract. *Acetic-acid (0.01 molar) extracts of wheat flour contain fibrils of α-gliadin which are about 80 angstroms thick and up to several thousand angstroms long. These fibrils dissociate to globular protein subunits at very low ionic strength and low* p*H. The fibrils can be reformed by increasing the* p*H to 5.1 and the ionic strength to about 0.005.*

Bernardin, Kasarda, and Mecham (*1*) observed that the wheat protein α-gliadin aggregated specifically in aqueous solutions under certain conditions of *p*H and ionic strength, and they devised a procedure, based on the aggregation reaction, to separate it from other wheat proteins. We have studied the form of these aggregates by electron microscopy, and we find they are long threads with a fairly uniform thickness of about 80 Å (Fig. 1, A and B). These aggregates are formed from subunits that have a molecular weight of 49,000 or less (*1*). Their appearance in the electron micrographs, combined with the observation that such aggregates can be dispersed and reformed reversibly by varying conditions of *p*H and ionic strength, indicate their similarity to such proteins as actin (*2*) and insulin (*3*), which are known to undergo a reversible transformation from globular to fibrous form (G-F transformation). The formation of threads or fibrils does not necessarily mean that the globular form of the protein subunit involved is unfolded. Electron micrographs of actin (*2*) show fibrils of approximately spherical subunits linked into chains. Until recently (*4*), no plant protein was known to undergo a similar reversible transformation. The phenomenon may be more general, however, and simply may have been overlooked, as gliadin mixtures have been studied by analytical ultracentrifugation since 1935 (*5*) without recognition of the ability of α-gliadin to form specific aggregates.

A 0.01*M*-acetic-acid extract of wheat flour contains in solution gliadins, glutenin, albumins, globulins, carbohydrates, and lipids in addition to salts and low-molecular-weight organic molecules. For electron microscopy, specimens of such a crude extract were sprayed onto grids and shadow-cast with uranium. Micrographs showed the presence of fine fibrils, some with lengths up to 5000 Å, imbedded in a film of low-molecular-weight material. The apparent thickness of these fibrils was about 50 Å, but this estimate is undoubtedly low since the film partially obscured their contours.

Centrifugation of the crude extract at 133,000*g* (average) for 2 hours sedimented a clear, gelatinous pellet. Electron micrographs of the supernatant solution showed a film of low-molecular-weight material, but almost no fibrils, with the exception of a relatively few short segments. The pellet was dissolved in 0.017*M* aluminum lactate, *p*H 3.1, a solvent known to dissociate gliadin proteins to aggregates with particle weights less than 50,000 (*6*); the solution was then analyzed by electrophoresis on polyacrylamide gel. The pellet consisted almost entirely of α-gliadin.

We tried to purify the pellet by repeated sedimentation. When the material was resuspended to the original volume either in the 0.01*M* acetic acid used for the initial extract (but now buffered only by α-gliadin as opposed to the many other substances contained in the flour extract) or in 0.001*M* HCl, it could not be sedimented by the usual centrifugation. The absence of fibrils in the solutions was confirmed by electron microscopy. By contrast, about 50 percent of the redissolved α-gliadin could be sedimented when the solvent was an ammonium-acetate buffer of 0.006 ionic strength, *p*H 5.1.

Polyacrylamide-gel electrophoresis of twice-sedimented α-gliadin aggregates subsequently dissolved in aluminum lactate buffer showed the α-gliadin to be free of contaminating low-molecu-