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Classical Conditioning with Auditory Discrimination of the Eye Blink in Decerebrate Cats

Abstract. *Cats were subjected to complete lower brainstem transection, and were then tested for learning ability according to a classical conditioning paradigm. An auditory stimulus was systematically paired with a brief shock to the eyelid. Within a few weeks after the operation, the decerebrate cats could learn the conditioned response with a tone frequency discrimination and then a discrimination reversal. Our results support the notion that the brainstem reticular formation can support a conditioned response which is behaviorally similar to that obtained in the intact animal.*

The problems encountered in investigating the neurophysiology of learning are due not only to the complexity of the nervous system but also to the difficulty in defining "learning" broadly enough for the definition to be behaviorally meaningful yet sufficiently constrained so that it can be analyzed (*1*). Our current approach to this problem is to delimit the "minimal" mammalian brain capable of sustaining a conditioned motor response with formal properties similar to those in intact animals. By reducing the volume and circuitry of the neural tissue which may participate in a conditioned response, the task of determining the processes involved in learned associations should become simpler.

The classically conditioned eye-blink response was selected as the standard learning task because it has been widely studied and there are considerable normative data available on the behavioral (electromyogram, EMG) response elicited (*2*). Moreover, neurophysiological analysis of this response should be simpler than that of other somatic conditioned responses, for example, leg flexion, which are complicated by postural reflexes and feedback mechanisms. In the intact cat, a conditioned eye-blink response can be established by systematically pairing a neutral acoustic conditioned stimulus (CS), which does not produce a response (*3*), with an unconditioned shock stimulus (US), which produces an unconditioned blink response (UR). After repeated pairings, the CS begins to evoke a conditioned blink response (CR).

The conditioned eye-blink response has several properties which provide important definitional guidelines for determining the validity of conditioning in our experimental preparations. From CS onset the conditioned eye-blink response has a variable latency with an absolute minimum of 100 to 125 msec (*2*), in contrast to shorter, fixed latency, acoustic reflex responses (*4*). The CR also reflects the temporal relationship of the CS and US, so that the maximum amplitude of the CR tends to occur toward the end of the CS-US interval (*2*). In a discrimination paradigm, the blink response can be conditioned selectively to a particular stimulus dimension, for example, tone frequency, by pairing one frequency (the CS+) with the US and not reinforcing the second frequency (the CS−). Finally, when the CS and US are presented randomly without temporal association, the CS does not elicit the blink response. We, as well as others (*2*), have utilized these features to define the conditioned eye-blink response that we have chosen to investigate.

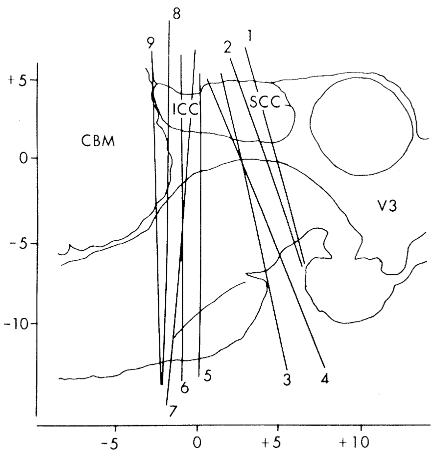

Fig. 1. Mid-saggital brainstem plane showing levels of transection of cats included in this study. The numbers correspond to those in Table 1.

In a previous study, the conditioned eye-blink response was shown to develop essentially normally in the absence of both cerebral hemispheres (*5*). Because components of the remaining diencephalon in these preparations showed retrograde changes, we hypothesized that the lower brainstem alone might be sufficient to support this simple form of learning. Here we report data from a subsequent study of eye-blink conditioning in the decerebrate cat.

The brains of adult cats were transected at the mesencephalic or rostropontine levels, under sterile conditions (*6*). The left occipital lobe was elevated and the underlying brainstem was exposed; subsequent aspiration with a fine probe, which could be visually guided except at the extreme upper right aspect of the brainstem, usually resulted in a complete transection. The general extent of the lesion could be observed directly during surgery, and was in all cases (except one) verified postmortem by means of both gross and histological examinations of the brains. The extent of the decerebration was determined by projecting Nissl-stained sections through the area of the lesion onto relevant brain atlas sections. Typically, all ascending and descending fiber tracts (lemnisci and peduncles), as well as the central tegmentum, were entirely sectioned so that the brainstem was completely separated from the forebrain (Table 1). Figure 1 shows the planes of section in the decerebrate cats enumerated in Table 1. Because we found no systematic relation between the plane of section and the behavioral results, we considered all the animals as a single group.

The general postoperative care of these decerebrate animals (*6*) was particularly focused upon proper temperature control, because all the animals were poikilothermic. By means of a thermister implanted in the cranium or in the rectum, an infrared heater or a cooling fan was automatically controlled so as to maintain body temperature at 37° ± 0.5°C. In spite of these precautions, accidental overheating caused the deaths of several of our animals; no ill effects were ever encountered by cooling, even when body temperature dropped as low as 32°C. In contrast to hemispherectomized animals (*5*), these animals were never able to feed themselves and were maintained on a liquefied diet of canned cat food, water, and vitamins given by intubation two to three times daily. Urine density and volume were normal with this diet and weight remained relatively constant up to 3 or 4 months at which time a general weight loss often began which was difficult to reverse. The animals were bathed and groomed daily because it was particularly important to keep the skin in good condition and prevent the development of abrasions resulting from the constant pressure of lying on one side.

The general postoperative behavior of these animals was similar to that previously described for pontile and low mesencephalic decerebrate cats (7). The corneal blink reflex, as well as intense decerebrate spasticity in all limbs, was present as soon as the anesthetic effects had worn off. During the second to fourth week, spasticity diminished and acoustic reflexes (that is, eye blink, startle, orientation to sounds) as well as head-righting reflexes reappeared. Between the fourth and sixth weeks, body righting reappeared and often resulted in a maintained sitting posture; scratch reflexes, biting reflexes, and forward progression could all be induced. During this same period, the conditioned eyeblink responses were often observed for the first time.

As early as the second week after the operation, intermittent training commenced with 50 to 100 trials per day given at 20- to 60-second intervals to test the ability of the cats to be conditioned. The animal was restrained in a bag and placed in a sound-attenuated training chamber. A conditioning trial consisted of the presentation of a tone or white noise (500 msec, 80 db with respect to a pressure of 0.0002 μbar at the external acoustic meatus), followed immediately by a shock to the eyelid (1 msec, single pulse) delivered subcutaneously through bipolar needle electrodes. The EMG responses from the orbicularis oculi muscle were recorded bipolarly. A conditioned response was defined, for scoring purposes, as a sustained burst of EMG activity which began 100 msec or more after CS onset but prior to the US.

Seven out of the ten decerebrate cats studied gradually developed responses to the CS which were similar in latency and form to the conditioned responses of normal cats (Table 1). In most cases the percentage of CR's per daily session was low when compared to normal cats; however, overall percentages did not accurately reflect the capabilities of the animals. Indeed, within a session there were often blocks of 20 to 50 trials during which high rates of responding (70 to 90 percent) were observed; these alternated

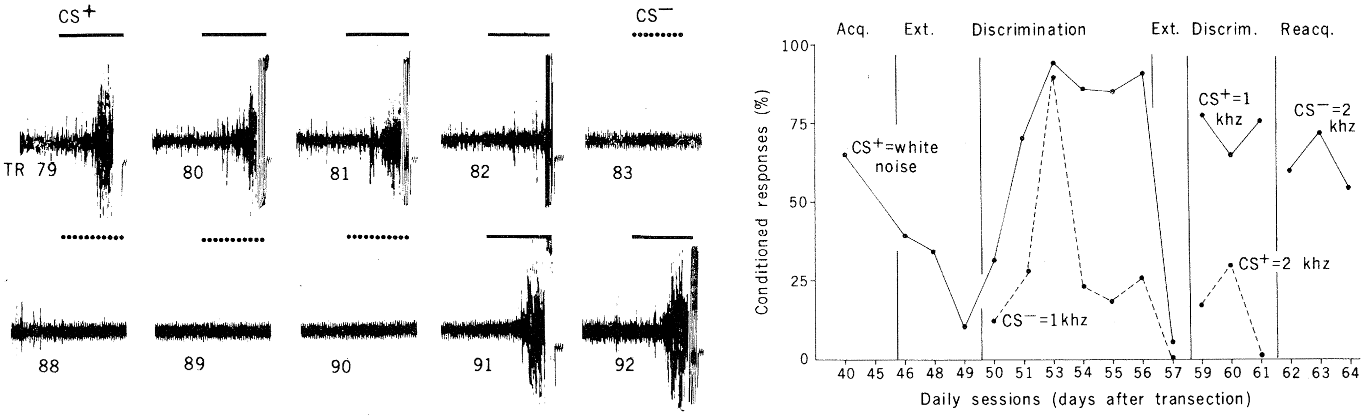

Fig. 2. Conditioned discrimination in a decerebrate cat. (Left) Representative EMG traces from a discrimination session (day 55). The CS duration is 500 msec. (Right) Summary of the training of one animal (Tigresa). Each data point represents the number of conditioned responses in 100 trials during acquisition days or 50 trials on discrimination days (expressed as percentages). After several training sessions in which white noise was used as the CS+, conditioned responding reached a moderately reliable level (day 40); the animals were trained to extinction and were then reconditioned with a discrimination paradigm of white noise as the CS+ and a 1-khz tone as the CS− (day 50). Good discrimination was attained after 6 days and the response was again extinguished. Frequency discrimination was reestablished by using the previous CS− as the reinforced stimulus.

Table 1. Results obtained with decerebrate cats. Survival indicates the time survived after transection. The first CR's indicate the first week after transection in which a CR was obtained. Levels of transection refer to those shown in Fig. 1. The training procedures included simple conditioning (C), frequency discrimination (FD), discrimination reversal (DR), pseudoconditioning control (PC), and extinction (E); under these headings, + indicates that the normally expected result was obtained (that is, the subject stopped responding after extinction training), while 0 indicates a failure to obtain the appropriate result (that is, conditioning did not occur). Blanks indicate that either no data were collected or that insufficient data were available for a judgment to be made.

Animal	Survival (weeks)	First CR's (weeks)	Best session (% CR's)	Training procedures					Level of transection		Remnant tissue connections*
				C	FD	DR	PC	E	Dorsal	Ventral	
Trueno	18		0	0					A3	A6	Fragment, lateral to right S.C.
Overa	8	5	50	+					A2	A6	Fragment, extreme right side
Wrat	12	2	53	+	+†				A0	A5	Fragment, extreme right side
Demian	22	4	65	+	+‡	+	0		A1	A4	Dorsal cap right S.C.
Sheila	3	2	30	+					A0	A0	Dorsal cap right S.C.
Tigresa	11	4	84	+	+§	+		+	P1	P1	
Schwartz	9	6	52	+					P0.5	P1.5	
Rosshalde	12	6	60	+	+‖		+		P2	P2	
Chrono	12		0	0					P3	P2	
Kilgor	6	5	30								Brain lost

*In Overa and Wrat a 1-mm rim of intact tissue at the lateral extreme of the right brainstem may have been functional. In the other cases, any remnant tissue connections were small, histologically abnormal, and questionably functional (S.C. = superior colliculus). †The CS+ was white noise, and the CS− a 500 hz tone. ‡ The CS+ was a 1-khz tone, and the CS− was white noise and there was a subsequent reversal of the FD pair. § Discriminations were formed to the following CS+/CS− pairs: white noise/1-khz tone; 1-khz tone/2-khz tone; 2-khz tone/1-khz tone. ‖The CS+ was a 2-khz tone and the CS− was a 1-khz tone.

with trial blocks during which there was little or no activity (*8*).

When a decerebrate subject reached a relatively constant performance level, the conditioned response was further tested by means of discrimination procedures with a CS+ reinforced by the US and a CS− not reinforced. The sound intensities of the CS+ and CS− were equal and their relative frequencies were balanced across animals. The effectiveness of the two stimuli was further controlled by a discrimination reversal procedure (Table 1, Fig. 2). Successful discrimination was taken as further evidence that the responses elicited by the CS were due to associative CS-US coupling rather than to other nonspecific effects of the shock stimulus (*9*). Finally, some animals were tested with a pseudoconditioning control procedure in which tones were presented at the same intervals as those used during conditioning, but shocks were given intermittently with no systematic temporal relationship to the CS. During pseudoconditioning, previously conditioned responses were extinguished within one session in one animal (Rosshalde) and diminished but did not completely disappear in another (Tigresa). In a third animal the conditioned response did not become extinguished in 200 trials, although a conditioned discrimination and discrimination reversals had previously been established (Demian) (*10*). In one animal (Tigresa) extinction was tested in the absence of the US during two sessions. The response to the CS diminished in magnitude within these two sessions but did not decrease in frequency (*11*).

The decerebrate cats often showed conditioned responses on the initial trials of a session following a period of conditioning and they continued to show conditioned responses (although diminishing in amplitude) over 2 days of extinction. Furthermore, during discrimination reversal, the previously conditioned CS+ retained a relatively greater effectiveness for several training days. These results indicate that the prior learning seemed to be "remembered" and suggest that the isolated brainstem is capable of providing a form of long-term memory storage which codes the frequency characteristics and temporal relationships of the CS and US as incorporated in the conditioned response.

Previous attempts to demonstrate eyeblink conditioning in permanently decerebrate animals were also successful (*7, 12*). In each of two decerebrate cats, one with an upper pontine transection and another with a midmesencephalic transection, conditioned eye-blink responses are anecdotally reported after pairings of a 3-second tone with an air puff to the eye (*7*). Under more controlled conditions, eye-blink conditioning to a 800-msec tone paired with an air puff to the eye was demonstrated in a series of decerebrate rats with midmesencephalic transections (*12*). As in the present study, the rate of acquisition was slow, the conditioned responding tended to develop as a result of prolonged training and to occur in blocks of consecutive trials, and the maximum scores that were attained were low. Pseudoconditioning procedures carried out on these animals failed to result in any increase in EMG responses to the tone and it was concluded that the conditioned eye blink only appeared as a result of the CS-US pairings (*12*). Moreover, there was some evidence for retention of the conditioned response between training sessions, although, as in the present study, this effect was much smaller than in the intact animals (*12*).

Thus, the brainstem appears to have some independent capacity to integrate information and to organize new patterns of behavior. This ability may normally be more intact in the young brainstem which is not yet heavily committed to forebrain influences.

The decerebrate cat represents a relatively simple mammalian system more amenable to electrophysiological analysis than the intact brain, but still capable of a relatively complex form of learning and of relatively long-term retention. This preparation strongly implicates the brainstem reticular formation as a region in which conditioned association, discrimination, discrimination reversal, and retention can develop independently of the more complex association networks and feedback loops situated in rostral brain areas. This is not to suggest that in the intact brain these areas do not normally function together as an integrated whole; we believe that they do. However, the present experiments emphasize the independent capacity of the brainstem to support a simple form of learning.

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3. The term "neutral stimulus" has traditionally required some qualification. The auditory stimulus used in this study occasionally evoked an acoustic-blink reflex, which is, however, quite different in form and latency than the type of response that is recorded after conditioning. Thus, the CS is neutral in that it does not evoke a conditioned-like response before formal conditioning.
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8. In contrast to our earlier reports of essentially normal eye-blink conditioning after bilateral hemispherectomy (*5*), conditioning in the decerebrate cat required a longer postoperative recovery period, was more labile, and produced lower average levels of responding. While it is sufficient, for our purposes, that the isolated brainstem is shown to be capable of acquiring and maintaining a conditioned blink response which is formally similar to the conditioned blink response of the intact animal, our lesions appear to be impinging upon some component which is highly significant to the conditioning process. The deficit in decerebrate conditioning may be due to the partial deafferentation of the brainstem or it may be due to a reduction of an essential "neural net" projecting from brainstem reticular formation to midline thalamus [E. Ramon-Moliner and W. J. H. Nauta, *J. Comp. Neurol.* **126**, 311 (1966)].
9. Frequency discrimination, in a conditioned avoidance testing procedure with the animal in a double-grill box, is lost after bilateral section of the brachium of the inferior colliculus [J. M. Goldberg and W. D. Neff, *J. Comp. Neurol.* **116**, 265 (1961)], but is not lost after bilateral ablation of the auditory cortices with accompanying degeneration of the medial geniculate body [J. M. Goldberg and W. D. Neff, *J. Neurophysiol.* **24**, 119 (1961)]. From these data, Goldberg and Neff suggest that above the level of the inferior colliculus, frequency discrimination does not require the primary auditory pathway, although some upward projection, possibly through the medial thalamic nuclei, does seem to be necessary. The ability of our decerebrate cats to make a frequency discrimination may reflect a task-dependent difference between the conditioned avoidance procedure of Goldberg and Neff and the present classical conditioned response in which the brainstem reticular formation may be relatively more important.
10. While it would have been useful to test animals with a pseudoconditioning control procedure before demonstrating conditioning, the apparent conditionability of the decerebrate animal progresses uncertainly in the postoperative period. Thus, a cat that did not respond during the initial pseudoconditioning procedures would be unconvincing as a control if conditionability were not present at this time. Owing to the difficulties of maintaining these preparations and the accidental deaths of a number of animals before all the testing paradigms were completed, as well as the interpretive problems on nonresponding in the control experiments, we have focused on the more positive procedure and simultaneous control provided by frequency discrimination.
11. The rate of extinction of the conditioned eye blink in both normal and decerebrate cats is highly idiosyncratic and the lack of extinction in 200 trials is within normal range. In general, one might expect faster extinction in the decerebrate animal because loss of forebrain mechanisms seems to be associated with reduced transfer of the learned task across days (*12*).
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