

## Retention of classically conditioned eyelid responses following acute decerebration

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We show that classically conditioned eyelid responses are retained in albino rabbits following decerebration. The presence of these responses represents retention rather than reacquisition in that they are present in the initial trials following decerebration. This excludes the possibility that the post-decerebration conditioned responses are mediated by pathways different from those involved in the intact animal. These data indicate that the conditioned response pathway, and sites of plasticity, for eyelid conditioning are spared by decerebration and are contained within the brainstem and cerebellum.

### INTRODUCTION

Lesion studies are a useful means for localizing neural pathways necessary for learning. A brain region is considered necessary if a lesion in that region always abolishes learning; conversely the preservation of learning following a lesion may identify regions that are not necessary. The former approach has pitfalls, because lesions in a non-essential region may nevertheless abolish learning by an indirect means. The attempt to identify non-essential regions seems safer, and in the extreme may yield preparations that are so reduced that the feasibility of identifying neural circuits responsible for learning is greatly improved.

Yet, there are pitfalls with this approach as well. The problem involves distinguishing what the nervous system is capable of doing under the special circumstances of the lesion and how it operates normally. For example, conditioned changes in flexor nerve responses have been observed in spinal animals<sup>1,7,26,27</sup>. Yet, it is clear that the spinal cord alone does not contain the circuits that are necessary and sufficient for leg flexion conditioning. This position is supported by two observations. First, lesions in the cere-

bellar nuclei produce complete and specific abolition of leg flexion responses<sup>6</sup>. Second, animals that undergo leg flexion training prior to spinal transection show no retention or savings of conditioned responses following transection (J. Steinmetz, personal communication). Thus, the isolated spinal cord is capable of mediating a form of conditioning, but supraspinal circuits are involved in the production of responses in the intact animal.

The demonstration that decorticate and decerebrate animals are able to learn classically conditioned eyelid responses is one line of evidence that has led to intense investigation of neural circuits in the brainstem and cerebellum<sup>3,8,10–13,21–24,31</sup>. These data have been interpreted as evidence that the telencephalon and diencephalon are not necessary — and thus the brainstem and cerebellum contain the circuitry necessary and sufficient — for the development and expression of conditioned responses<sup>15–19,34</sup>. Is it possible that the presence of conditioned responses in these animals reflects a capability of brainstem and cerebellar circuits apparent only under the conditions of decerebration, or have the necessary and sufficient pathways been left undisturbed? These questions are especially important in light of the cur-

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rent emphasis on brainstem and cerebellar circuits in eyelid conditioning.

This issue is made even more salient by several features of the previous decerebration experiments which encourage the belief that the conditioned responses observed were mediated by circuits other than those normally involved in the intact animal. In many experiments acquisition was unusually slow; in the order of weeks rather than days<sup>21</sup>. However, it is possible that this simply reflects compromised health of the spared tissue. Far more importantly, several of the decerebrations reported appear to have involved removal of the red nucleus<sup>21</sup> — a structure known to be a necessary portion of the eyelid-conditioned response pathway in otherwise intact animals<sup>9,30</sup>. These data suggest that the conditioned responses observed in the decerebrate animals may have been mediated by circuits that are at least partially different from those involved in the intact animal.

In the present experiments we test retention of conditioned eyelid responses following acute decerebration. The advantage of testing retention, rather than acquisition, is that the conditioned responses are established when the animal is intact. If the decerebration spares all essential pathways then conditioned responses should be present in the initial post-surgery trials. If necessary structures are damaged, then no conditioned responses should be present.

We report that rabbits conditioned while intact retain conditioned eyelid responses following acute decerebration. Retention is detected almost immediately; there does not appear to be reacquisition. Naive animals and those given unpaired training do not respond to the conditioned stimulus following decerebration, excluding the possibility that decerebration simply disinhibits auditory-eyeblink reflexes. We suggest that these data provide strong evidence that the brainstem and the cerebellum contain the circuits that are necessary and sufficient for the expression of conditioned eyelid responses.

#### MATERIALS AND METHODS

Male, albino rabbits (*Oryctolagus cuniculus*) weighing 2–3 kg were used in all experiments. Each rabbit was initially anesthetized with halothane (1.5–5%) and a headstage assembly designed to accommodate field effect transistors (FET) and an air

puff–nozzle assembly was cemented to the skull. Wire supports circumscribing the region of skull to be removed during decerebration were also cemented in place. These provided support for the remaining portion of the skull which was considerably weakened during decerebration.

Following 3–5 days of recovery, training procedures were initiated. Training consisted of delay classical conditioning using a tone-conditioned stimulus (CS; 1 KHz, 85 dB SPL, 350 ms duration), followed 250 ms after tone onset by a corneal air puff unconditioned stimulus (US; 2.1 kg/cm<sup>2</sup>, 100 ms duration, co-terminating with the CS). Daily training sessions consisted of 13 blocks of trials. Each block was comprised of one CS-alone trial and 8 paired CS–US trials. Trials were delivered every 20–40 s (mean = 30 s). Some animals were given training in which the CS and US were presented but never paired. For these sessions each block consisted of 8 CS-alone and 8 US-alone trials presented in a pseudorandom sequence. Rabbits were trained for at least one half-session beyond asymptotic performance. This typically required 3–4 sessions. Control animals were given either equivalent amounts of unpaired sessions or no pretraining.

During training, animals were immobilized in Plexiglas restrainers and the left upper eyelid was restrained by a metal clip attached to an elastic band. Eyelid closure was measured by recording EMG activity from the orbicularis oculi muscle. Recordings were obtained by attaching the metal eyelid restrainer to standard FET-input preamplifiers. The signals were amplified and band-pass filtered (0.5–5 kHz) and then recorded on audio tape.

Responses were subsequently quantified by passing the EMG recordings through an integrating amplifier (time constant 45 ms) then digitized using a DEC 11/03 microcomputer. The amplitude of the integrated responses was determined relative to a 0.1 mV, 1 kHz calibration signal which was amplified, recorded and integrated in the same manner as the EMG responses. Conditioned responses were assessed by determining the amplitude and area of the responses in the interval between CS and US onset.

The day following the final training session, the decerebration and testing were performed. Animals were re-anesthetized with halothane (5.0%) and the sutures reopened to expose the skull. A region of

skull approximately  $2 \times 1.2$  cm was removed. The dura was excised with a scalpel and the entire cerebral cortex and hippocampi were removed by aspiration. A razor blade was then positioned stereotaxically 6–7.5 mm posterior to bregma and a transection through the brainstem was produced by rapidly advancing the manipulator. The tissue anterior to the blade was then aspirated, the blade was removed and bleeding was arrested by application of oxidized cellulose (Oxycel). Once bleeding was under control, the cranium was filled with warm mineral oil and the wound was loosely closed. The surgery typically required 7–10 min and the animals were under halothane for no more than 25 min.

Rabbits were then placed in a Plexiglas restrainer and monitored for a recovery period of 3–4 h. Only rabbits that showed minimal bleeding, appeared relatively healthy, and that exhibited strong reflex eyelid responses to manual probing were tested for retention of conditioned responses. Retention was assessed with a standard 13-block training session. The decerebrate animals were generally calm and adapted well to the training procedures.

Following this test session, animals were sacrificed with an overdose of sodium pentobarbital and perfused through the heart with 10% formalin (2–3 liters). The fixed brains were removed and placed in 10% formalin for 1–3 weeks. The brains were then embedded in an albumin-gelatin mixture and allowed an additional 1–2 weeks to fix in paraformaldehyde. The extent of the decerebration was then assessed using standard histological procedures.

## RESULTS

The results of these experiments may be summarized as follows. (1) Robust conditioned responses were observed in the initial post-decerebration trials in 4 animals. The conditioned responses following the decerebration were normal with respect to latency and form. (2) The red nucleus contralateral to the eye trained was undamaged in each of these animals. Conditioned responses were never observed when the decerebration involved damage to the contralateral red nucleus. (3) Naive animals and those presented unpaired CS/US training (and whose decerebration did not damage the contralateral red nucleus) showed no post-decerebration responses to the CS.

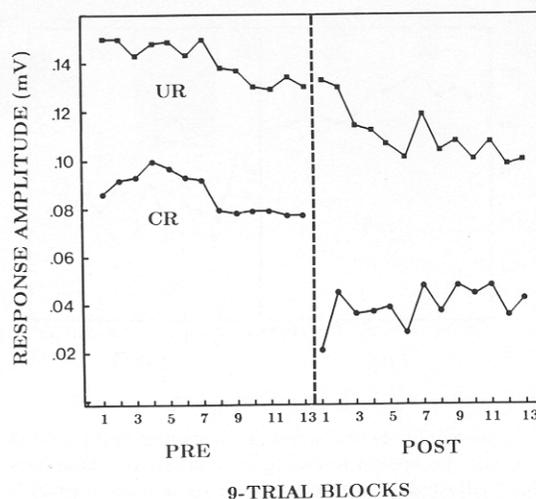


Fig. 1. Mean amplitude of the conditioned (CR) and the unconditioned (UR) eyelid responses in the session prior to, and the session following decerebration. The data in this figure, as well as in Figs. 2 and 3, are presented as blocks of 9 trials. Each training session consisted of 13 of these blocks or 117 trials.

Figs. 1 and 2 show the group means of the conditioned and unconditioned responses in the session prior to and the session following decerebration. Several features of these data merit comment. First, there was an initial reduction in both the mean amplitude and the percentage of conditioned responses. The percentage of conditioned responses quickly recovered to pre-decerebration level, whereas the re-

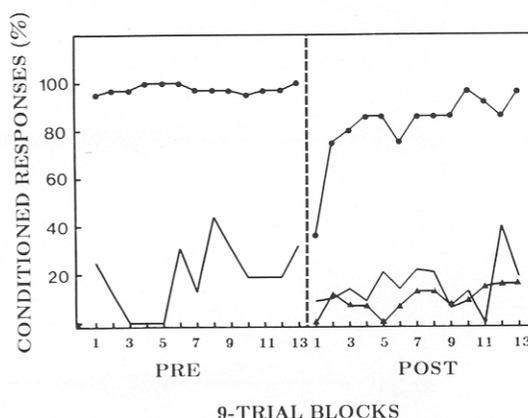


Fig. 2. A comparison of the percentage of conditioned responses present for 3 groups of animals: those given paired training (circles), those given unpaired training (no symbols) and those given no pretraining (triangles). Responses in the left box are prior to and those in the right box are following decerebration.

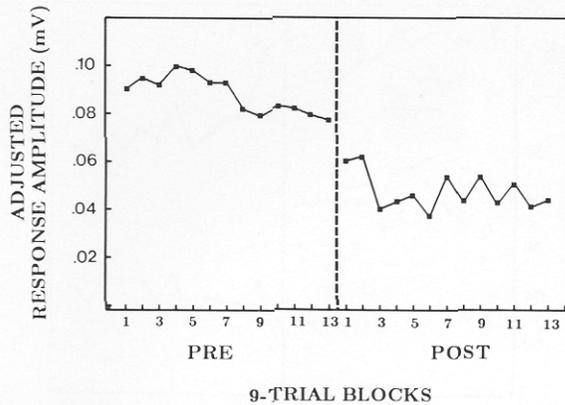


Fig. 3. Mean amplitude of conditioned responses in the session prior to, and the session following decerebration. These data have been adjusted so as to include only those trials in which a conditioned response occurred.

duction in mean amplitude was maintained throughout. The initial reduction in responding was due primarily to the transient decrease in the occurrence of conditioned responses. This is illustrated by Fig. 3 which presents mean response amplitude adjusted to include only trials in which a conditioned response occurred. As can be seen, the amplitude of conditioned responses suffered a small decline, but remained stable following decerebration. Thus, the effect of decerebration for the 4 animals was to produce a transient reduction in the occurrence of conditioned responses that quickly recovered to near baseline. Superimposed on this was a small and stable decrease in the mean amplitude of each conditioned response.

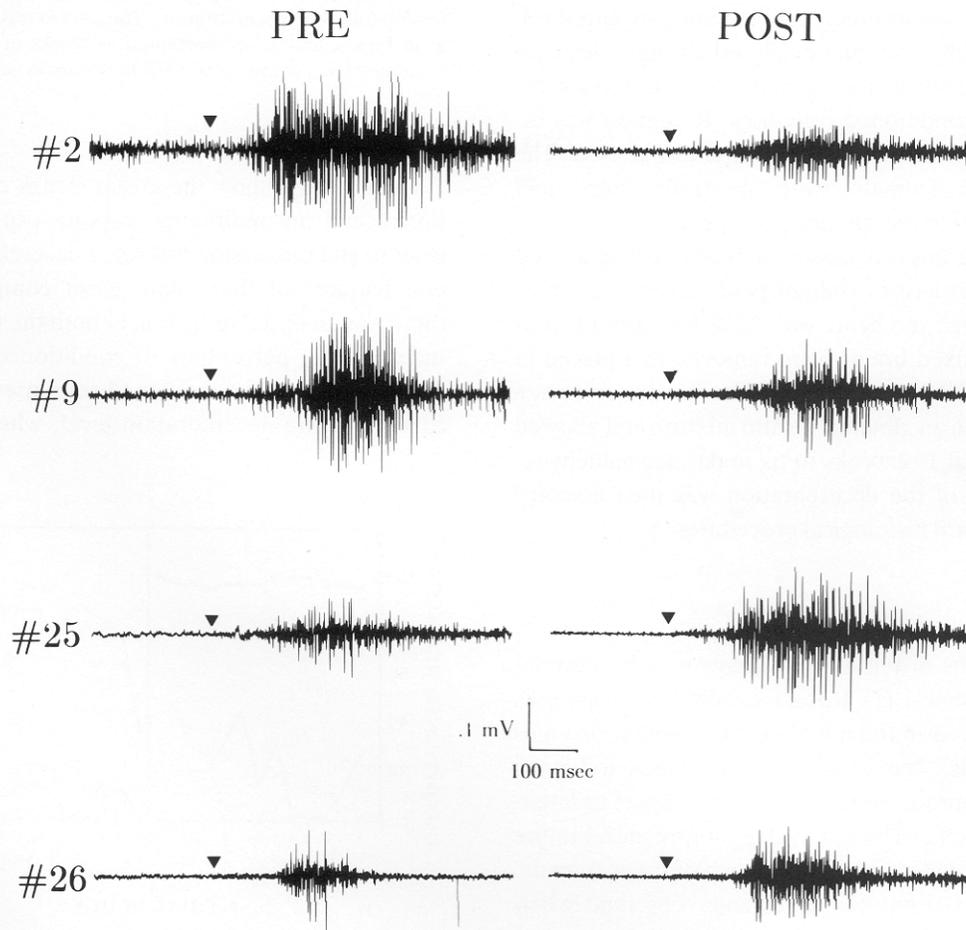


Fig. 4. Representative examples of conditioned responses prior to (left column) and following (right column) decerebration. Eyelid closure is indicated by an increase in EMG activity recorded from the orbicularis oculi muscle. Examples are presented for each of the 4 animals showing retention of conditioned responses. The animal numbers indicated at left correspond to those in Fig. 5. The onset of the tone-conditioned stimulus is indicated by the arrowheads. The trials shown involve only presentation of the CS. Thus, the EMG activity represents a conditioned response elicited by the CS and is not contaminated by an unconditioned response.

Despite this reduction following decerebration, responding to the CS was reliably greater than in the two control conditions (Fig. 2). One control group ( $n = 2$ ) received unpaired presentation of tone and air puff in both pre- and post-decerebration sessions. The number of sessions presented were matched with animals in the retention group. Other animals ( $n = 7$ ) were naive prior to decerebration and received normal paired CS/US training following decerebration. These two control groups were indistinguishable and their data have been combined for the sake of statistical analysis. A two-way, mixed ANOVA revealed reliable differences in post-decerebration responding ( $F_{1,11} = 51.4, P < 0.01$ ). The difference between groups was reliable for each post-decerebration block as assessed by separate  $t$ -tests ( $P < 0.01, df = 11$ ).

The latency and form of the conditioned responses were unaffected by decerebration. Examples of individual trials prior to and following decerebration are shown in Fig. 4. The responses are representative; in each case both larger and smaller responses could have been presented. As can be seen, the responses following decerebration appear normal.

Histological analysis of the 4 animals that retained conditioned responses reveals two main points. First, in each animal the red nucleus contralateral to the trained eye was undamaged. Second, the entire cerebral cortex and hippocampi, as well as much of the brainstem rostral to the red nucleus, were removed. Reconstructions of the planes of section for each animal are presented schematically in Fig. 5.

#### DISCUSSION

As suggested earlier, the presence or absence of conditioned responses in the initial trials is of considerable importance conceptually. It is clear from Fig. 1 that conditioned responses are nearly normal within, at most, 9 trials. This is in contrast to the initial training prior to decerebration, where the appearance of conditioned responses required 100 trials even in the fastest animals. Considering the trauma of surgery, the presence of conditioned responses following decerebration in these 4 animals appears to reflect retention, rather than reacquisition.

Retention of conditioning following decerebration indicates that the responses were produced by the

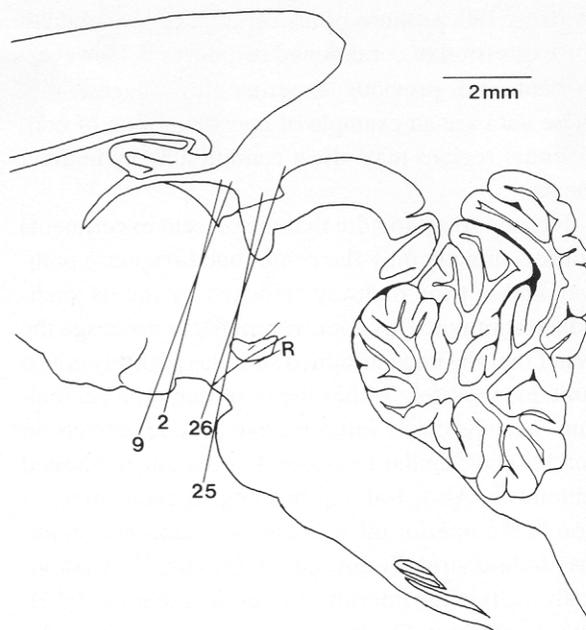


Fig. 5. A schematic reconstruction (parasagittal view) of the plane of transection for each of the animals, showing retention of conditioned responses. The animal numbers correspond to those in Fig. 4. All tissue to the left of (rostral) the plane of transection was removed; this includes the cerebral cortex and hippocampi. The parasagittal view shown was chosen to include the red nucleus contralateral to the eyelid trained.

same neural pathways and plasticity that are responsible in the intact animal. This is supported by the observation that conditioned responses were never present when the decerebration damaged a portion of the known conditioned response pathway — the red nucleus. We suggest, therefore, that the neural pathways necessary and sufficient for the expression of conditioned responses are contained within the tissue spared by the surgery — namely, the brainstem and cerebellum. Portions of this pathway have been identified<sup>15-19,29,30,32-38</sup>. Lesions of the middle cerebellar peduncle, anterior interpositus nucleus of the cerebellum, superior cerebellar peduncle, red nucleus and of the rubro-bulbar fibers projecting to motor nuclei all produce complete abolition of conditioned responses. The present data indicate that the remaining, as yet unidentified, portions of this pathway are within the brainstem and do not involve the thalamus, hippocampus or cerebral cortex. In a previous report, abolition of conditioned responses during cortical spreading depression was presented as

evidence that portions of the cerebral cortex mediate the expression of conditioned responses<sup>20</sup>. However, present and previous experiments<sup>23</sup> suggest that these data are an example of how disruption of non-essential regions may affect conditioning by indirect means.

It is important to note that the present experiments address directly only the conditioned response pathway and not the pathway activated by the unconditioned stimulus. However, recent data encourage the belief that the unconditioned stimulus pathway is also contained entirely within the brainstem and cerebellum. Lesions of the inferior olive produce effects on conditioning similar to removal of the unconditioned stimulus<sup>17</sup>. Also, training procedures using stimulation of the inferior olive as the unconditioned stimulus produce virtually normal conditioning<sup>14</sup>. Anatomically, cells in the inferior olive project to the cerebellum as climbing fibers<sup>5</sup>, and receive input from the spinal trigeminal nucleus which, in turn, contains terminals from primary afferents in the face and cornea<sup>2,4,25,28</sup>. This arrangement suggests that the unconditioned stimulus pathway for eyelid conditioning may also be contained within the brainstem and cerebellum.

This notion is also supported indirectly by the present experiments. Damage to the unconditioned stimulus pathway disrupts not only the ability to acquire conditioned responses, but also the ability to maintain them. In well-trained animals, lesions in the inferior olive produce a gradual decline in conditioned responses which occurs with additional training<sup>17</sup>. In the course of one training session this decline is considerable. In the present experiments no such decline was observed, suggesting that the unconditioned stimulus pathway was spared by the decerebration. Thus, the present data indicate that the conditioned response pathway for eyelid conditioning is contained within the brainstem and cerebellum, and provide indirect evidence that the unconditioned stimulus pathway may be as well.

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