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Effect of Bilateral Lesions of the Dentate and Interpositus Cerebellar Nuclei on Conditioning of Heart-Rate and Nictitating Membrane/Eyelid Responses in the Rabbit

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It has been shown that unilateral lesions of the medial dentate/lateral interpositus nuclear region of the cerebellum abolish the learned nictitating membrane (NM)/eyelid response of the eye ipsilateral to the lesion. The present study examined the effects of bilateral cerebellar lesions on acquisition of heart-rate conditioning (often viewed as a measure of 'conditioned fear') and both its short- and long-term effects on NM/eyelid learning and relearning. The results demonstrate that cerebellar lesions that completely and permanently abolish acquisition or retention of the somatic response (NM/eyelid) bilaterally have no effect on heart-rate conditioning. The neuronal circuits necessary for learning of the heart-rate response and for learning of the adaptive somatic response are thus in significant part different. Results are tentatively interpreted within the context of a two-process theory of aversive learning: an initial phase indexed by conditioned autonomic and 'non-specific' responses such as heart-rate and a subsequent phase of learning the specific adaptive responses.

INTRODUCTION

Two manipulations will eliminate the classically conditioned nictitating membrane (NM)/eyelid response without affecting the unlearned, reflexive response: lesions of the lateral cerebellum^{2,3,13,17,18} and administration of opiates^{14–16}. Unilateral lesions of the cerebellum ipsilateral to the trained eye completely prevent the recall and relearning of the learned response, regardless of level of training or overtraining, and prevent initial learning of that response, but do not impair learning in the contralateral eye. The administration of opiates, on the other hand, abolishes only the newly acquired NM/eyelid response and does not interfere substantially with the conditioned response when it is well-learned¹⁴.

We have proposed tentatively a 'dual-trace' neuronal hypothesis^{28–32} based on behavioral two-process theories of aversive learning^{9,19,20,22,23} to account for these facts. In this view, aversive learning occurs in two phases, an initial phase in which a basic associative process develops rapidly between the condi-

tioned stimulus (CS) and the aversive character of the unconditioned stimulus (UCS) (often termed 'conditioned fear' or conditioned emotional state) and a subsequent phase in which a discrete, adaptive, somatic response is learned to deal effectively with the aversive UCS (here the learned NM/eyelid response). The initial phase, which typically involves conditioned autonomic responses (here the heart-rate) and also diffuse somatic motor activity, has been termed 'non-specific' because the conditioned responses do not deal specifically and effectively with the UCS³⁴. We suggested that the first process (conditioned non-specific responses) may be necessary for subsequent learning of the specific, adaptive response and that the neuronal substrates of the two processes differ, at least in part^{10,11,14,29–32}.

To date, the most effective site of action of centrally administered opiates on the newly learned NM/eyelid response is the vicinity of the fourth ventricle¹⁵. The effect is due entirely to central action and can be obtained with opiate agonists having specific high affinity for the mu receptor^{15,16}. In an initial

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test of the 'dual-trace' neuronal hypothesis, it was shown that administration of these same opiates in the same site also abolished classically conditioned bradycardia in the rabbit^{10,11}. There are of course other brain structures essential for the conditioned heart-rate response, including the amygdala and hypothalamus^{4,8,25} and the amygdala is also opiate sensitive for the heart-rate response⁶ (but not for the newly learned NM/eyelid response¹⁶).

The conditioned heart-rate response is viewed by many workers as an index of 'conditioned fear' and/or conditioned non-specific responses^{8,21,24,25,34}. Others emphasize that it may indeed be adaptive, providing cardiovascular support for accompanying somatic responses⁴. It is even to some degree specific — a bradycardia in animals like the rabbit that tend to freeze when fearful^{8,24} and a tachycardia in animals like the pigeon and baboon that tend to be more active when fearful^{4,25}. However, as Weinberger has emphasized, it does not deal specifically with the UCS, whereas the more slowly learned specific somatic response does³⁴.

The fact that identical fourth ventricular administration of opiates abolished both conditioned bradycardia and the newly learned NM/eyelid response is at least consistent with possible common action on an hypothesized conditioned fear circuit in the brainstem. If the cerebellar system is the locus of the memory trace for discrete, adaptive conditioned responses, if it is in fact to some degree separate from the 'conditioned fear' memory trace, and if the conditioned heart-rate response reflects 'conditioned fear', then cerebellar lesions which prevent learning and abolish memory of the conditioned NM/eyelid response ought not to impair learning of the classically conditioned bradycardia response. Evaluation of this specific hypothesis was the primary purpose of the present study. Because the conditioned bradycardia response presumably does not have a unilateral memory trace, it is necessary to make bilateral lesions of the lateral cerebellum. This also permits test of the possibility that abolition of the conditioned NM/eyelid response by an ipsilateral cerebellar lesion is due to asymmetrical activation of some more remote memory trace system (i.e. the 'Sprague effect'²⁶). Finally, recovery of function, the possibility that some relearning of the NM/eyelid response might occur with time and repeated retraining fol-

lowing cerebellar lesions, was also evaluated.

MATERIALS AND METHODS

Subjects

The subjects for this experiment were adult New Zealand White rabbits ($n = 21$) that were individually housed in stainless steel cages (6048, 6912 or 9216 cubic inches, depending upon animal size) at a constant temperature of 23 °C with a 12 h on, 12 h off light/dark cycle, maintained on ad libitum food and water, and cared for by the staff and veterinarians of the Stanford University Department of Laboratory Animal Medicine.

Procedure

The general paradigm for this study, for the experimental group, was first to destroy bilaterally the lateral cerebellar regions that had previously been found to be critical for learning and retention of the conditioned NM/eyelid response. Second, train the animals in the heart-rate conditioning paradigm (conditioned bradycardia). Third, train rabbits in the conditioned NM/eyelid response paradigm.

Surgery. All experimental rabbits were first operated upon, using deep halothane anesthesia, for radical bilateral cerebellar ablations ($n = 6$) or for localized electrolytic bilateral lesions of the lateral deep cerebellar nuclei ($n = 10$). The radical ablations by aspiration were intended to remove all cerebellar tissue lateral to the vermis and including the lateral deep cerebellar nuclei as previously¹⁸. The restricted electrolytic lesions (2 mA, 150 s) were intended to destroy the cerebellar medial dentate and lateral interpositus nuclei as previously^{2,3}. The animals were additionally fitted with a headstage that would hold a transducer later in training and a small loop of nylon suture was placed in both left and right nictitating membranes. The rabbits were administered antibiotics in the surgical wound (Panalog), in the eyes (Chloricol), and injected with prophylactic bicillin.

Training. Following at least one week of recovery from the surgery each rabbit was habituated in the experimental setting for at least 1 h on the day prior to training for heart-rate conditioning. Habituation consisted of placing the animal into a restraining box, attaching the clips for surface recordings of heart-rate (approximately a Lead II configuration), and

placement of subcutaneous stainless steel wires for later delivery of periorbital faceshock as used previously^{10,11}.

Training for the conditioned heart-rate response ('conditioned fear') began the next day with 20 tone-alone trials in order to adapt reflexive cardiac deceleration to the tone. Next followed 20 trials of paired training in which the offset of a 5-s tone (1 kHz, 85 dB SPL) coincided with the onset of a 0.5 s faceshock (2 mA, 60 Hz). Rabbits typically learn cardiac deceleration in 5-10 paired trials²⁴. Indeed, if a compromise procedure is employed where both conditioned bradycardia and eyelid responses are learned using a constant paradigm in rabbits, the conditioned heart-rate response develops in a few trials and fades as the conditioned eyelid response is subsequently learned²¹. Heart-rates were determined by converting into frequency the distances of 10 heart-beats before tone onset (PreCS, baseline) and the 10 heart-beats before shock onset (PreUCS).

The day following heart-rate training the rabbits began 8 days of training for NM/eyelid conditioning. The training paradigm used in this experiment was similar to that used previously²⁷ with the exception that 15 blocks of 8 trials per block were given (total of 120 trials per day) in which trials 1 and 4 of each block were tone-alone test trials (i.e. training was 75% paired). In each trial a tone (CS) was paired with an airpuff (UCS) to the cornea and the NM/eyelid was monitored for learned or reflexive movement. The tone (1 kHz, 85 dB SPL) was the same as that used for heart-rate conditioning except that for nictitating membrane conditioning it was 350 ms in duration. The unconditioned stimulus was a corneal airpuff (210 g/cm²) given during the last 100 ms of the tone CS. The eyelids of the rabbit were held open with eyeclips to prevent interference with measuring the nictitating membrane, the 'third eyelid' of the rabbit, a cartilaginous membrane that originates nasally and extends temporally across the eyeball. Nictitating membrane extension was measured by attaching a nylon thread from the lever of a minitorque potentiometer to the loop of nylon suture that had previously been implanted (in surgery) in the rabbit's membrane. Movement of the NM was transduced by the potentiometer into an electrical potential that, along with synchronizing pulses marking stimulus events, was recorded on polygraph paper and a tape

recorder. Intertrial intervals were varied in a pseudo-random sequence with a range of 20-40 s (mean = 30 s). The rabbits were trained 4 days on the left eye and 4 days on the right eye.

An occasional animal will appear to have severely impaired hearing, due to unknown causes. Animals that can hear typically show reflex heart-rate deceleration to initial tone-alone presentations given at the beginning of the heart-rate training procedure. This reflex deceleration to tone was required as evidence that the animals could hear, since the lesions are presumed at least to interfere with learning of the NM/eyelid response to tone.

Controls. In order to evaluate the effect of previous heart-rate conditioning on subsequent conditioning of the NM/eyelid response we used the control rabbits from another study^{10,11}. For that study, these animals (n = 4) were given a control mixture of a methylated morphiceptin and naltrexone (opiate agonist and antagonist, respectively), via a previously implanted cannula in the fourth ventricle, once they had reached criterion for conditioned bradycardia. The mixture had no effect on conditioned bradycardia. One additional animal used as a control for the present study was originally given morphiceptin alone, which temporarily abolished the heart-rate CR. For the purposes of the present experiment these 5 animals were then trained for nictitating membrane conditioning on the left side in order to determine any possible interaction between the paradigms on the rate of learning of the NM/eyelid response. Except for administration of the drugs during the first day of training (conditioned bradycardia day) these control animals were treated essentially identically to experimental animals, i.e., surgery, one week of recovery, heart-rate training and finally NM/eyelid training.

Long-term retention. One additional animal was first trained in the NM/eyelid response on both eyes, left eye and then right eye, and was then given the bilateral aspiration and then retrained extensively over a 3-month period. One animal from the electrolytic lesion group was also given extensive retraining on the NM/eyelid response 5 weeks later. Three additional animals were given the same bilateral electrolytic cerebellar lesions, and were repeatedly tested after 4-6 weeks of recovery (4 days of training on each eye) and after more than 14 weeks of recovery

(2 days of training on each eye). These 5 animals served to evaluate possible long-term recovery of the conditioned NM/eyelid response or of the ability to learn it.

Histology. Following training, each animal was overdosed with sodium pentobarbital and perfused intracardially with 0.9% saline followed by 10% formalin. The brains were removed, with great care taken to preserve any remaining cerebellar tissue. The brains were stored in Formalin for several days, then embedded in a gelatin-albumin mixture. Frozen sections through the site of the lesion were taken and mounted on chrome-alum subbed slides. The sections were stained for cell bodies (with either neutral red or cresyl violet) and counterstained for iron deposit in those brains with electrolytic lesions using potassium ferrocyanide and hydrochloric acid (Prussian blue reaction).

RESULTS

Rabbits were included in the study if they met two criteria: first, lesions of the cerebellum had to include bilaterally the cerebellar medial dentate/interpositus nuclear region shown to be critical in earlier unilateral lesion studies^{2,3,17,18}, and second the animal must

have shown an orienting response (heart-rate deceleration) to the first tone habituation trial during heart-rate conditioning.

Of the 13 rabbits given lesions in this experiment, 5 were shown to fulfill these two criteria: radical ablation ($n = 1$) included substantial bilateral damage to the paramedian and ansiform lobes and to the dentate and interpositus nuclei (Fig. 1E); electrolytic lesions of the deep cerebellar nuclei (excluding the fastigial nucleus) were bilateral and complete throughout the extent of these nuclei ($n = 4$) (Fig. 1A-D). Five rabbits did not meet the histological criterion and 3 did not show an orienting response. Thus the final experimental group consists of 5 lesioned rabbits. The 5 control animals all showed an orienting response.

Fig. 2 (left) includes a plot of the mean performance of the lesion and control rabbits on the heart-rate task. Rabbits with lesions that include the dentate/interpositus are denoted with filled circles, controls with open circles. All of the animals learned the heart-rate conditioned response (repeated measures ANOVA $F(3,30) = 23.2$, $P < 0.01$) within the range for normal rabbits^{11,24}. There were no differences between the two groups ($F < 1$) and no interaction ($F < 1$).

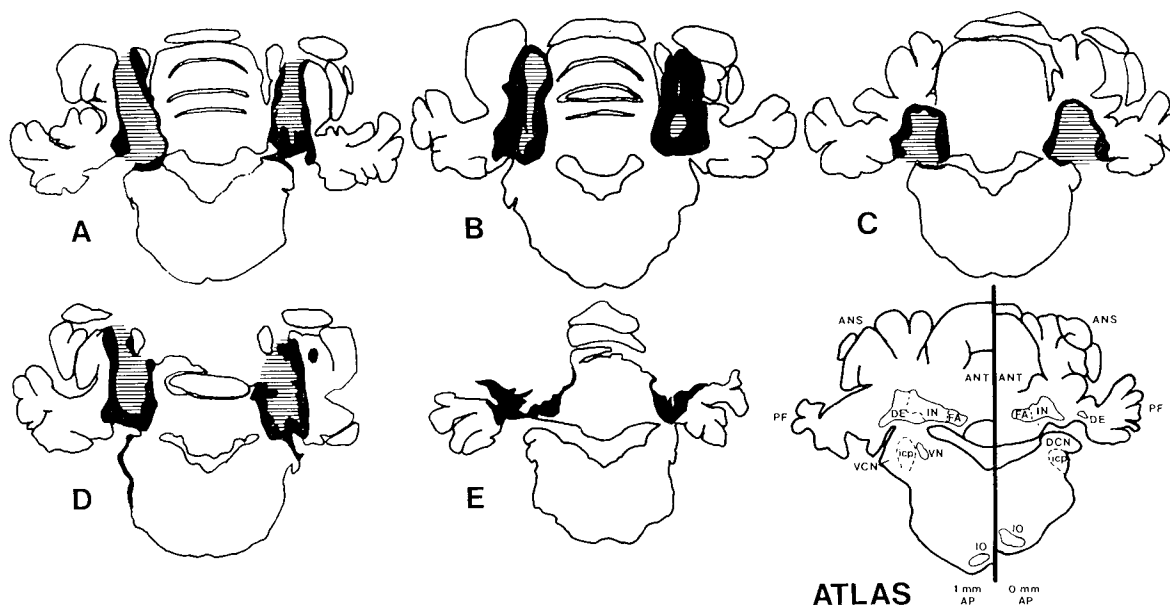


Fig. 1. Histological results showing the extent of lesions produced electrolytically (A, B, C, D) and by aspiration (E). The accompanying atlas shows planes of section at 0 mm and 1 mm anterior to lambda for reference. Blackened areas indicate gliosis, stripes indicate a space produced by the electrolytic lesions.

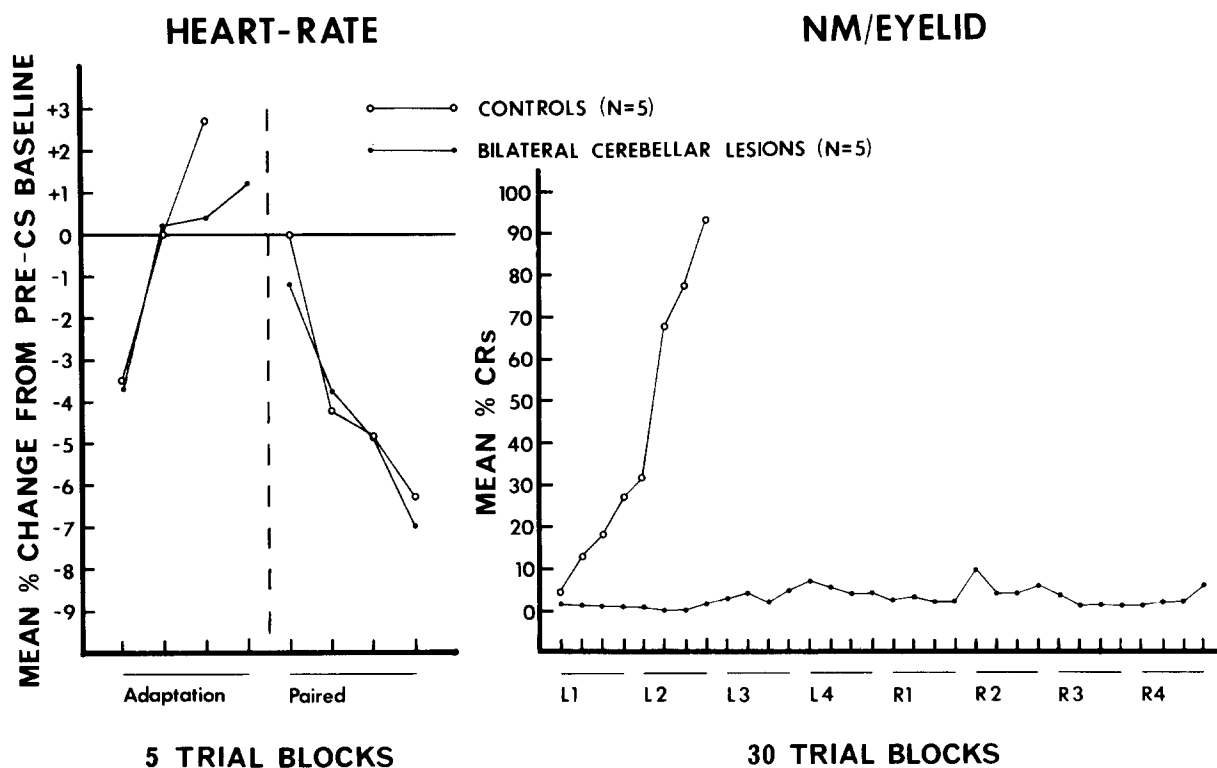


Fig. 2. Results of heart-rate conditioning (left) and nictitating membrane/eyelid conditioning (right) for rabbits with bilateral cerebellar lesions (closed circles) and for controls (open circles). Heart-rate is expressed as the mean percentage difference between baseline (the rate before CS presentation) and the rate before UCS presentation, with positive values reflecting heart-rate acceleration. Both groups adapt to tone-alone presentations, and both groups learn when given pairings of the CS and UCS. When trained on NM/eyelid conditioning, the controls learned in less than two days, but rabbits with bilateral cerebellar lesions failed to learn after 4 days of training each on the left (L1-L4) and right (R1-R4) eyes.

None of the animals with cerebellar lesions that included the deep cerebellar nuclei learned the nictitating membrane/eyelid response on either eye within a total of 960 trials in 8 days (Fig. 2, right). One of the rabbits with electrolytic lesions was allowed to recover for an additional 5 weeks, after first failing to learn the nictitating membrane/eyelid response, and retrained for an additional 8 days (1920 trials total). This rabbit never showed any signs of learning. The 3 additional animals that were trained after 4-6 weeks and after 14 weeks of recovery from bilateral cerebellar lesions showed no learning of the NM/eyelid response with either eye. In order to test further for possible recovery of functioning we first trained and overtrained one rabbit on the NM/eyelid response with both eyes. The rabbit was then subjected to bilateral radical cerebellar aspirations and allowed to recover for one week. This animal failed to relearn the habit with 8 additional days of training, 4 days on

each eye. This rabbit was retrained at intervals of 1 week, 1 month and 3 months and never relearned (Fig. 3). We are confident that bilateral cerebellar lesions that involve the dentate/interpositus nuclei result in a permanent abolition in the ability to learn or relearn the conditioned NM/eyelid response. There is no recovery of function of the conditioned response.

Rabbits in which the histology revealed incomplete lesions were not included in the data above. It is worth noting, however, that these rabbits were indistinguishable from lesioned rabbits on heart-rate conditioning (all rabbits learned the task). The rabbits without sufficient damage to the dentate/interpositus region, however, also were capable of learning the nictitating membrane/eyelid response on the side (sides) ipsilateral to the spared deep cerebellar nuclei. The degree to which the NM/eyelid response was learned was proportional to the degree of spar-

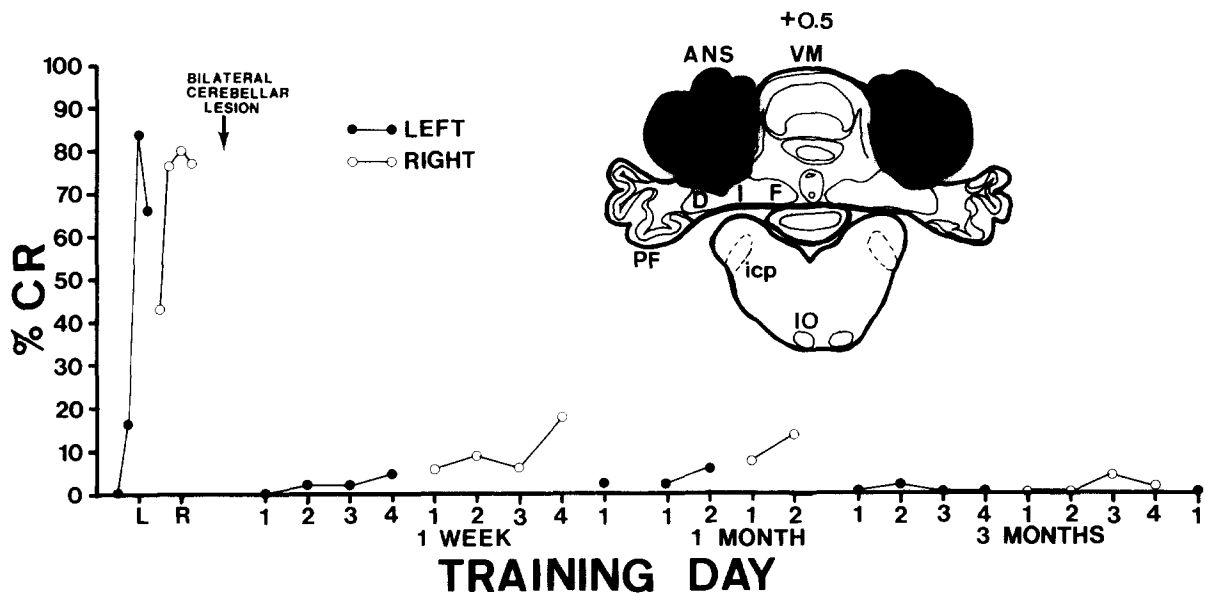


Fig. 3. Results of nictitating membrane/eyelid retraining for a rabbit previously trained on both eyes before bilateral cerebellar aspirations. After repeated retraining over a period of 3 months following the lesion this animal failed to relearn. Four other animals given lesions before training were retrained over an extended period postlesion without learning.

ing of the critical medial dentate-interpositus nuclear region. Specifically, 2 animals had some sparing only of the left medial dentate/interpositus region and showed some degree of learning of the left but not right NM/eyelid response, 1 animal had some degree of sparing of only the right cerebellar nuclear region and showed some degree of learning of the right but not left NM/eyelid response and 1 animal had some degree of bilateral sparing and showed some degree of learning.

Finally, all the control rabbits ($n = 5$) given conditioned bradycardia training followed by NM/eyelid training learned the NM/eyelid response within 2 days, comparable to the normal rate of learning. Prior training in the conditioned bradycardia paradigm has no noticeable effect on rate of learning of the NM/eyelid response under the present experimental conditions.

DISCUSSION

Recent experiments have shown that classical conditioning of the NM/eyelid response in the rabbit can be abolished by injections of opiates early in learning but not with a well learned response^{14,15,16} and abolished permanently by lesions of cerebellar systems^{2,3,12,17,18} all without affecting the reflexive re-

sponse to an unconditioned stimulus. In an experiment that evaluated the effect of opiates on the conditioned heart-rate response, we showed that centrally administered opiates abolished the learned heart-rate response^{10,11}. It was suggested there that opiates may differentially affect an early phase of learning—a conditioned non-specific or emotional response—that may be important for development of the subsequently learned adaptive response, here the NM/eyelid response, and that the cerebellar circuitry may be the locus of the memory trace for the latter (and also the conditioned leg-flexion response⁵).

The present experiment was designed to help evaluate this neuronal 'two-process' theory of learning by distinguishing between the effects of cerebellar lesions on heart-rate conditioning and NM/eyelid response conditioning. If the two paradigms involve systems that are at least in part different we would expect that bilateral cerebellar lesions of the medial dentate/lateral interpositus region that prevent conditioning of the nictitating membrane/eyelid response without preventing conditioning of the heart-rate task. This is exactly what we found.

In sum, these cerebellar lesions have no effect on the rapidly learned non-specific response (here conditioned heart-rate) but prevent (and abolish) subse-

quent learning of the specific, adaptive learned behavioral response (here NM/eyelid). These results are at least consistent with the notion that rapidly learned non-specific or emotional responses (to the extent that conditioned heart-rate is indeed an index of such) may be necessary for subsequent learning of discrete, adaptive behavioral responses. However, an equally plausible alternative at the present is that the two specific types of learned responses have different neuronal substrates. The fact that fourth ventricular administration of opiates abolishes both conditioned heart rate and the newly learned NM/eyelid response may, under this latter interpretation, imply two separate opiate actions. In any event, the present results demonstrate clearly that the neuronal substrates of the conditioned heart rate and NM/eyelid responses are in significant part different. It is possible that lesions elsewhere in the cerebellum of the rabbit, e.g., the fastigial nucleus, might impair the conditioned heart-rate response. However, complete removal of the cerebellum has no effect on heart-rate conditioning in the pigeon⁷.

The effect of bilateral cerebellar lesions in preventing learned NM/eyelid responses appears to be permanent. In one animal trained prior to lesion on the task, retraining periodically for a period of 3 months did not restore any behavioral learning. Another rabbit that first received bilateral electrolytic lesions and then training failed to learn the task with more than 5 weeks of training after the lesion. The 3 additional animals given the same bilateral cerebellar lesions prior to training and allowed one month or more of recovery prior to NM/eyelid training showed no learning at all with repeated training. What is particularly striking about all of these rabbits is that they showed no persisting signs of motor dysfunctions. The conclusion of previous studies^{2,3,17,18}, with short-term behavioral training and retraining, that the lateral aspects of the cerebellar deep nuclei are essential neuronal circuitry for this memory trace holds

also for much longer recovery intervals. The effect cannot be explained easily by a concept like diaschisis³³.

The results of the current study are also important in eliminating another possible interpretation of the basic effect of cerebellar lesions on learned behavior. That possibility suggests that unilateral lesions of the cerebellum create an asymmetry of neural functioning between damaged and surviving tissue that leads to the appearance of unilateral lost functioning. That is, the rabbits with unilateral lesions may not have expressed learning on the side of the lesion because of overpowering asymmetrical activation of sensory or motor systems either contralateral or ipsilateral to the lesion. The 'Sprague effect' is a model for this type of phenomenon²⁶. There it was demonstrated that unilateral lesions of the visual cortex in cats caused an apparent blindness in the contralateral visual field for moving objects, although the ipsilateral superior colliculus was intact. Subsequent lesions of the contralateral superior colliculus, or of the commissure of the superior colliculus, restores perception in the contralateral field. It has been demonstrated electrophysiologically that the contralateral superior colliculus abnormally inhibited the normal functioning of the ipsilateral colliculus following removal of the ipsilateral visual cortex¹. Obviously such effects could not occur in rabbits with symmetrical, bilateral lesions of the lateral cerebellum, nor are they seen.

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