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Initial localization of the memory trace for a basic form of learning

(classical conditioning/cerebellum/dentate/interpositus nuclei)

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ABSTRACT **Electrophysiological recording of neuronal unit activity during paired training trials from various regions of the ipsilateral cerebellum in rabbits well trained in the classically conditioned eyelid/nictitating membrane response have revealed both stimulus-evoked responses and responses that form an amplitude/temporal model of the learned behavioral response. Ablation of the ipsilateral, lateral cerebellum completely and permanently abolished the behavioral conditioned response in well-trained animals but had no effect at all on the unconditioned reflex response. In marked contrast, conditioned responses were easily trained in the eye contralateral to the cerebellar lesion. We suggest that at least part of the essential neuronal plasticity that codes the learned response may be localized to the cerebellum.**

Localization of the memory trace—the circuitry that contains the essential neuronal plasticity coding learning and memory—has proved to be a baffling problem (1, 2). Many regions of the mammalian brain appear to play roles in learning and memory, particularly in relatively complex tasks (3, 4), and certain regions are capable of physiological and anatomical modification as a result of experience (5–8). However, it has not yet been possible to localize the memory trace for even very simple forms of associative learning. Evidence is described here indicating that the cerebellum may be the locus of the memory trace for a basic form of associative learning.

Eyelid [and nictitating membrane (NM)] conditioning is perhaps the most widely used paradigm for the study of basic properties of classical or Pavlovian conditioning of striated muscle responses in both humans and animals (9–12). Even in this seemingly simple paradigm, higher regions of the brain appear to play important roles (13–16). However, animals from which the cerebral neocortex or hippocampus has been removed are able to learn the standard delay conditioned response (14, 17) as indeed are animals from which all brain tissue above the level of the thalamus or midbrain has been removed (18, 19). It would seem that the "primary" memory trace for classical conditioning of the eyelid and NM responses is localized below the level of the thalamus. Recent preliminary observations in our laboratory have implicated the cerebellum (20, 21). We report here the results of experiments indicating that the cerebellum is an essential component of the memory system for this basic form of learning.

METHODS

Standard procedures for classical conditioning of the rabbit NM and eyelid response were used (15, 22): an acoustic conditioned stimulus for 350 msec, coterminating with a 100-msec corneal air-puff unconditioned stimulus, an intertrial interval of ≈60 sec, and 120 trials per day. Extension of the NM was measured with a micropotentiometer and eyelid closure was also monitored; they behave essentially identically (23) and all effects reported here occur equivalently for both responses.

All animals were trained to a criterion of eight conditioned responses in any nine consecutive trials and then given 1 full day of overtraining. The animals in the ablation study ($n = 6$) were then subjected to ablation by aspiration (Fig. 1) of the ipsilateral (left), lateral cerebellum, allowed to recover for 7 full days, and given 4 days of retraining to the left eye. Three of these animals were then shifted to training of the contralateral eye for 72 trials and back to training to the left eye for the remaining 48 trials.

Recordings of neuronal activity from the cerebellum were acquired by two separate means: chronically implanted multiple unit electrodes aimed for the cerebellar deep nuclei (12 animals) and through the use of a chronic microdrive system allowing systematic mapping/recording of multiple unit activity over a wide range of the cerebellar cortex and deep nuclei (10 animals). In all cases, the surgery to implant the electrodes (or the microdrive support base) was performed at least 5 days prior to training, using halothane anesthesia. Recordings were taken from the beginning of training with the chronically implanted electrodes. For the microdrive experiments, the recordings were taken from regions of the cerebellum only on the second and subsequent days after the animals reached criterion performance. Four electrodes were simultaneously advanced in 0.5- to 1-mm steps with recordings of unit activity taken for eight paired trials after each step. In this manner, up to 60 different sites of the cerebellum were recorded in the same animal in a single session. For both recording techniques, the placement, or tracks, of the electrodes were marked by passing 100 μA of direct current for 3 sec.

After training and recording were completed, all animals were sacrificed with an overdose of sodium pentobarbital (Nembutal) and perfused through the heart with 10% formalin. The brains were removed, embedded in albumin, sectioned at 40 μm, and stained for cell bodies with a standard Nissl stain. Computer (PDP 11/03)-generated histograms of each neuronal recording were then matched with the recording site (see Fig. 2).

RESULTS

Recording Study. The neuronal unit recording data from the chronic microdrive procedure (10 animals) indicate that neurons in selected portions of at least the ansiform and anterior lobes and discrete regions of the dentate/interpositus nuclei respond in a manner that parallels the behavioral conditioned response (Fig. 2, day 3). These neuronal responses typically precede the behavioral NM response by 45–55 msec. In addition, stimulus-evoked responses (tone, air puff) have been found throughout parts of the vermal lobes and discrete parts of the deep nuclei (Fig. 2, day 1).

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Abbreviation: NM, nictitating membrane.

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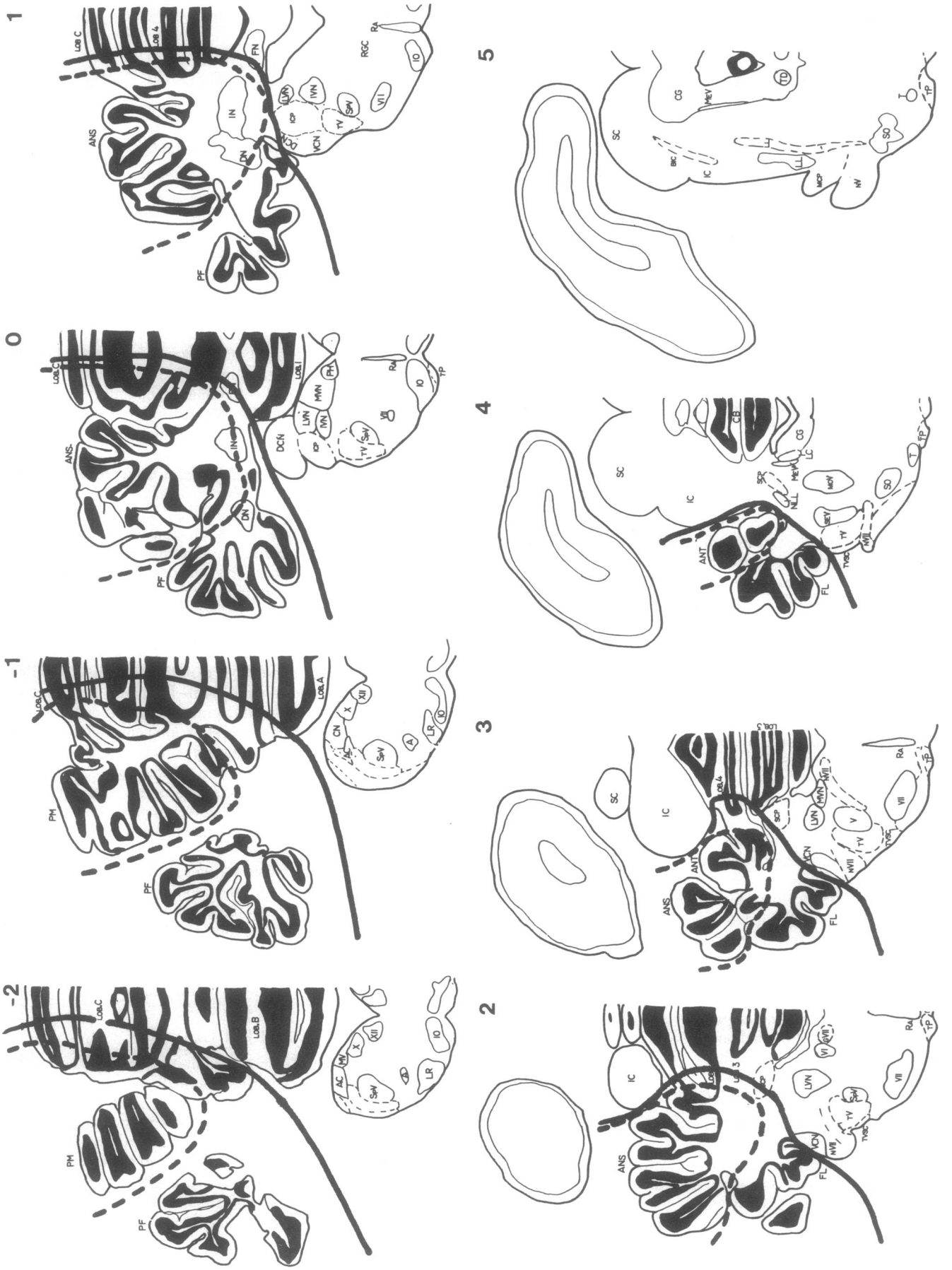

FIG. 1. (*Legend appears at the bottom of the next page.*)

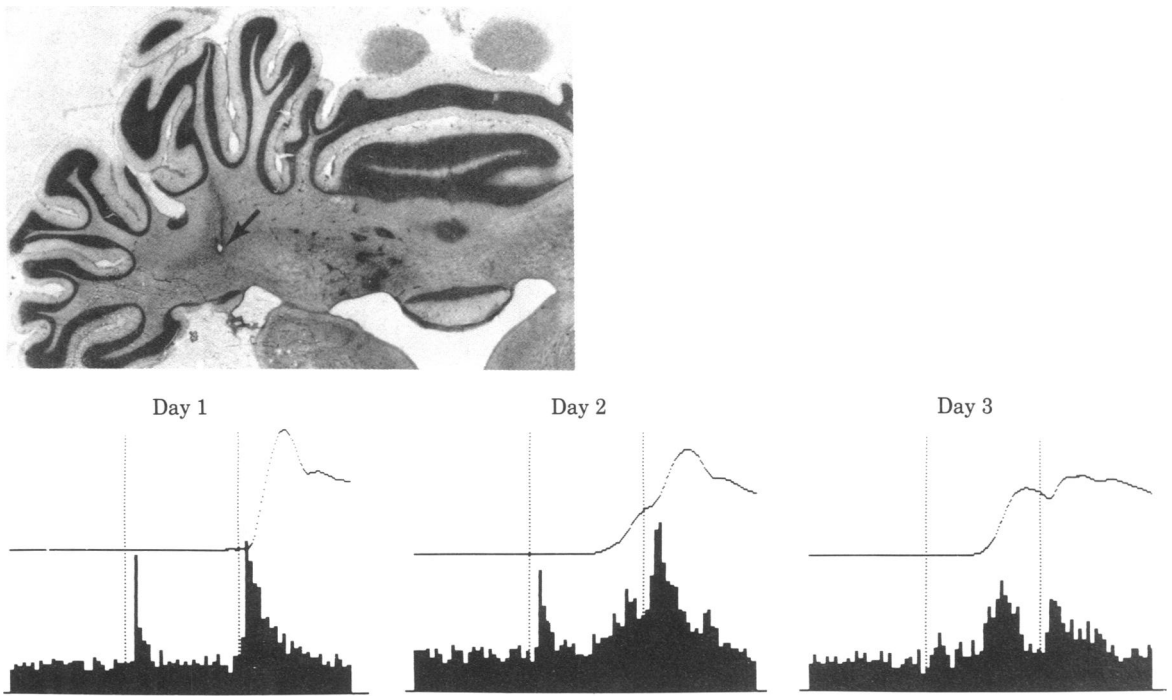

FIG. 2. Unit histograms obtained from the medial dentate nucleus during classical conditioning of eyelid/NM response. The recording site is indicated by the arrow. Each histogram bar is 15 msec wide, and each histogram is summed over an entire day of training. The first vertical line represents the onset of the tone and the second vertical line represents the onset of the air puff. The trace above each histogram represents the averaged movement of the animal's NM for an entire day, with up being extension of the NM across the cornea. The total duration of each histogram and trace is 750 msec.

The recordings obtained by chronic implanted electrodes (12 animals) have revealed various degrees of stimulus-evoked and behaviorally related responses in portions of the cerebellar deep nuclei, the most impressive of which is shown in Fig. 2. Our results with this animal were particularly fortunate in that it showed no learning on day 1, learned over the course of day 2, and exhibited a well-learned response throughout day 3. Before learning had occurred, the cells of this region responded in a stimulus-evoked (tone, air puff) manner and did not model the unconditioned NM response (day 1). However, as learning developed, the stimulus-evoked responses gave way to a clear temporal neuronal model of the learned behavioral response (days 2 and 3).

Ablation Study. All animals in the ipsilateral cerebellar ablation study learned the original response in 2 days of training. The number of trials to reach criterion was 93.7 ± 44.0 (mean ± SD). Ipsilateral (left) ablation of the lateral cerebellum (six animals) completely and permanently abolished the conditioned response (Fig. 3). This effect is of course statistically highly significant [$F(4,20) = 39.6$; $P < 0.001$]. However, ablation had no effect at all on the amplitude of the unconditioned reflex response [$F(4,20) = 2.38$; not significant; see Fig. 3]. Three of these animals were then shifted to training of the other (right) eye and learned the response very rapidly. The number of trials to criterion performance on the right side was 17.0 ± 14.8, which is significantly less than for the original training of the left side ($t = 3.3$; df = 8; $P < 0.01$). The animals were then shifted back to training of the left eye and again showed no learned responses (see Fig. 3). All ablations included the paramedian and ansiform lobes and the most lateral aspects of the pyramis, median lobe, and anterior lobe, together with damage to the dentate and interpositus nuclei (Fig. 1). An additional animal (not included in the six in Fig. 3) with a lesion limited to much of the dorsal/lateral cerebellar cortex (no direct damage to the deep nuclei) showed the same behavioral effect.

DISCUSSION

The lesion study reported here shows that the cerebellar hemisphere ipsilateral to the eye being trained is essential for retention and relearning of the classically conditioned eyelid/NM response. The unconditioned reflex response is completely unaffected by the lesion, indicating that the memory loss is not due simply to motor impairment—i.e., inability to perform the response. The fact that the conditioned response is learned easily by the contralateral eye would seem to rule out nonspecific effects of the lesion. Interestingly, this contralateral conditioned response was learned with significant savings here, when initial training to the ipsilateral eye was given prior to the cerebellar lesion. In another study, unilateral cerebellar lesions were made prior to any training completely prevented original learning of the ipsilateral conditioned response, but the contralateral conditioned response was then learned as though the animals were normal and new to the situation (unpublished data).

In other work, we have found that retention of the conditioned response is abolished by lesions of the ipsilateral superior

FIG. 1. (*on preceding page*). Reconstruction of the smallest and largest aspirations. All tissue encompassed by the dashed line was removed in the animal with the smallest aspiration, and all tissue encompassed by the solid line was removed in the animal with the largest aspiration. Numbers represent millimeters anterior to λ with the top of the skull at λ 1.5 mm lower than that at bregma. ANS, ansiform lobes; ANT, anterior lobe; DCN, dorsal cochlear nucleus; DN, dentate nucleus; FL, flocculus; FN, fastigial nucleus; IC, inferior colliculus; ICP, inferior cerebellar peduncle; IN, interpositus nucleus; MCP, middle cerebellar peduncle; PF, paraflocculus; PM, paramedian lobe; SCP, superior cerebellar peduncle; VCN, ventral cochlear nucleus.

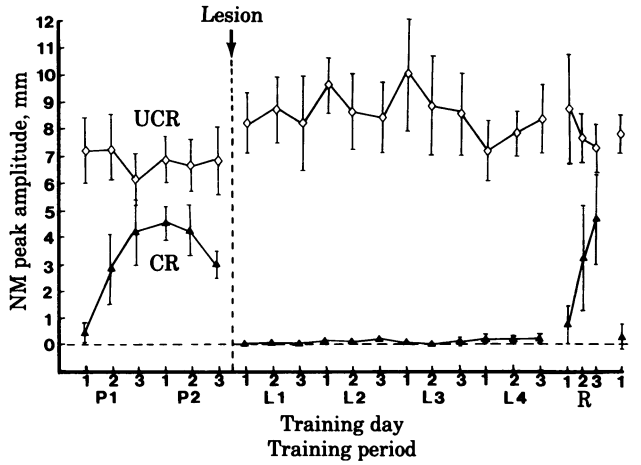

FIG. 3. Effects of ablation of left lateral cerebellum on the learned NM (and eyelid) response (six animals). ▲, Amplitude of conditioned response (CR); ◇, amplitude of unconditioned response (UCR). All training was to the left eye (ipsilateral to lesion), except where labeled "right eye" (R). The cerebellar lesion completely and permanently abolished the CR of the ipsilateral eye but had no effect on the UCR. P1 and P2, initial learning on the 2 days prior to the lesion; L1–L4, 4 days of postoperative training to the left eye; R, right eye training (rapid learning). After training the right eye, the left eye was again trained and it showed no learning. Left eye, 40-trial training periods; right eye, 24-trial training periods.

cerebellar penduncle (the major efferent pathway from the cerebellum) at the level of its decussation (24). In addition, retention and relearning of the conditioned response is abolished by selective destruction of the ipsilateral superior cerebellar peduncle at a very different locus—the point at which it enters the brain stem from the cerebellum (unpublished data). One other group of workers has independently reported a similar behavioral effect of ipsilateral pontine lesions (25), and those workers kindly made their results available to us prior to publication. It appears that all effective lesions damaged the superior cerebellar peduncle. Finally, electrolytic lesions of the cerebellar deep nuclei (dentate/interpositus and vicinity) ipsilateral to the trained eye produce the same effect on the learned response (unpublished observations).

In sum, unilateral lesions of the cerebellum at several loci—cerebellar hemisphere, deep nuclei, and superior cerebellar penduncle—abolish retention and relearning of the classically conditioned ipsilateral eyelid/NM response but have no effect on the reflex response and do not impair learning by the contralateral eye. The ipsilateral cerebellum is essential for the learned response. If the memory trace, the essential neuronal plasticity that codes a learned response, is in fact localized to one region of the brain, then destruction of that region should prevent initial learning of the response and permanently abolish memory and relearning of the response. This is precisely what we have found. Our results are most parsimoniously interpreted by assuming that the memory trace for classical conditioning of the eyelid/NM response is localized to the ipsilateral cerebellar hemisphere. We suggest that this is the case.

Other possible alternatives exist. The cerebellum might be an essential afferent or efferent system for a memory trace localized elsewhere in the nervous system. Even so, the cerebellum is an obligatory part of the memory circuit. In other work, we have presented evidence that argues against the possibility that the conditioned stimulus channel—the primary auditory relay nuclei—is a part of the memory circuit (21) and also evidence against essential involvement of the relevant motor nuclei and unconditioned reflex pathways in the memory circuit (23, 26). Decerebrate animals can learn the conditioned responses (18, 19). If the memory trace is localized elsewhere than in the cerebellum, these findings reduce the number of possibilities considerably. Perhaps the most likely alternatives to cerebellar localization are the inferior olive, the pontine nuclei, and the red nucleus. A final possibility is that the ipsilateral cerebellar lesion somehow results in critical dysfunction of some other region of the nervous system that is itself the locus of the memory trace. In general, there is little evidence for such a process. The fact that lesions limited to the superior cerebellar peduncle disrupt the conditioned response indicates that, if such a process does occur, it must be efferent from the cerebellum.

The neuronal unit recording data reported here indicate that both sensory-evoked responses and motor-like responses that correlate closely with the learned behavioral response occur in selected portions of the cerebellar cortex and deep nuclei. Indeed, in the example shown in Fig. 2, a temporal neuronal response "model" of the learned behavioral response (but not the reflex response) appears to develop in neurons of the medial dentate nucleus over the course of training. This result is consistent with the view that the memory trace is established in the cerebellum.

The cerebellum has been suggested by several authors as a possible locus for the coding of learned motor responses (27–30). Cerebellar lesions have been reported to impair a variety of skilled movements in animals (31, 32) and to prevent plasticity of the vestibular/ocular reflex (33). In addition, neuronal recordings from Purkinje cells of the cerebellar cortex have implicated these cells in the plasticity of various behavioral responses (34, 35). Our results indicate further that cerebellar damage can selectively abolish simple learned responses. Since there is no reason to suppose that the cerebellum has any special role for such movements as NM extension and eyelid closure, we argue that the present finding may hold for all simple learned responses involving discrete striated muscle movements, at least with an aversive unconditioned stimulus. Behavioral analyses of such learning suggest that it may occur as two processes or phases, the first involving "conditioned fear" and the second concerned with learned performance of adaptive motor responses (12, 36–38). In these terms, it seems reasonable to suggest that the cerebellum is essential for the latter aspect of learning.

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