magnetic field map necessarily obscures potentially interesting variations from one spike to the next, the resultant fields represent extremely stable components of the discharge pattern that were replicated precisely on successive days. Similar patterns have been seen in half of the subjects we have studied.

Although the anatomical location of the measured sources is clear, the possibility remains that other sources may also be active either before or after the observed discharges, since the MEG apparatus we used measures only tangential sources (*13*) and is more sensitive to lateral than to mesial generators (*14*). Nevertheless, the multiple sources measured neuromagnetically in this study are similar in complexity to what would be expected from invasive recordings performed with electrodes implanted deep in the brain.

These noninvasive neuromagnetic measures provide a detailed characterization of interictal discharge patterns over extended lengths of time and enable the evaluation of these patterns in patients with various seizure disorders, in addition to the small number of patients under consideration for neurosurgery. Thus, questions may now be asked concerning the long-term stability of spatial and temporal relations within the interictal focus, the influence of antiepileptic drugs and successful control of seizures on the distribution of interictal spikes, and the presence or absence of spatially distributed sources underlying benign spikes in the nonepileptogenic brain.

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31 August 1983; accepted 13 October 1983

## Cerebellum: Essential Involvement in the Classically Conditioned Eyelid Response

Abstract. *Classical conditioning of the eyelid response in the rabbit was used to investigate the neuronal structures mediating basic associative learning of discrete, adaptive responses. Lesions of the ipsilateral dentate-interpositus nuclei, but not of the cerebellar cortex, abolished the learned eyeblink response. Recordings from these nuclei have revealed neuronal responses related to the learning of the response. Stimulating these recording sites produced the eyelid response. The dentate-interpositus nuclei were concluded to be critically involved in the learning and production of classically conditioned responses.*

The nature of the neuronal changes in the mammalian nervous system encoding learned responses, even simple learned motor responses, has proven to be one of the most elusive and baffling problems in science (*1, 2*). Perhaps the greatest impediment to the elucidation of these neuronal changes has been the problem of localization. Within basic associative learning (classical conditioning), however, this problem finally appears to be yielding (*2–5*).

Classical conditioning of the eyelid or nictitating membrane (NM)—a third cartilaginous eyelid—has long been used to study the basic laws of associative learning in lower animals as well as in humans (*6*). Rabbits or cats can learn and retain the eyeblink response normally after removal of the hippocampus, neocortex, or all tissue above the level of the thalamus (*7*), although electrophysiological results indicate that at least some of these structures are normally involved (*8, 9*). Therefore, some neuronal circuitry capable of encoding this learned response must exist at or below the level of the thalamus.

Recent results from our laboratory have shown that the ipsilateral lateral cerebellum, including the dentate and interpositus (D-I) nuclei, is necessary for the acquisition, retention, and reacquisition of the classically conditioned NM-eyelid response in the rabbit (*3*), a finding that has been replicated by others (*4*). Lesions of the ipsilateral lateral cerebellum, D-I nuclei, or the superior cerebellar peduncle—the main output pathway of the D-I nuclei—all abolish a previously learned NM-eyelid response without affecting the unconditioned response to the airpuff or the ability of the animal to learn the NM-eyelid response rapidly on the contralateral side. We now report a region of the D-I nuclei that (i) is critical for the learned response to occur, (ii) exhibits neuronal activity that predicts the performance of the learned response, and (iii) when activated can generate the learned response.

In all studies, behavioral training consisted of pairing an acoustical conditioned stimulus (350-msec, 1000-Hz, 85-dB tone for recording studies, 36-dB spectral-level white noise for lesion studies) with a coterminating corneal airpuff (100-msec, 2.1-N/cm$^2$) unconditioned stimulus. The conditioned response was an extension of the NM, with synchronous contraction of the external eyelids and of some facial musculature (*10*). The amplitude-time course of the NM response was measured and recorded, along with any unit activity, for later analysis on a PDP 11/03 computer. The animals were trained to a criterion of eight conditioned responses on any nine consecutive trials and overtrained by one session, with each daily session consisting of 120 trials. For the recording studies, the manipulator base (short-

term multiple-unit recordings) (*5*) or long-term multiple-unit electrodes (long-term recordings) (*8*) were lowered and cemented to the skull while the animals were under halothane anesthesia. In the lesion studies, selected localized folia of the cerebellar cortex were ipsilaterally aspirated after training. All animals were allowed 7 days to recover before behavioral training started or resumed. All recording and lesion sites were confirmed through a standard Nissl stain (cresyl violet) of 80-μm frozen brain sections.

In the short-term recording study, 323 unit recordings distributed throughout the ansiform, paramedian lobules, anterior lobe, and lobule C (*11*) were collected from 15 animals (Fig. 1). Of the 323 recordings, 66 responded in relation to the amplitude-time course of the learned

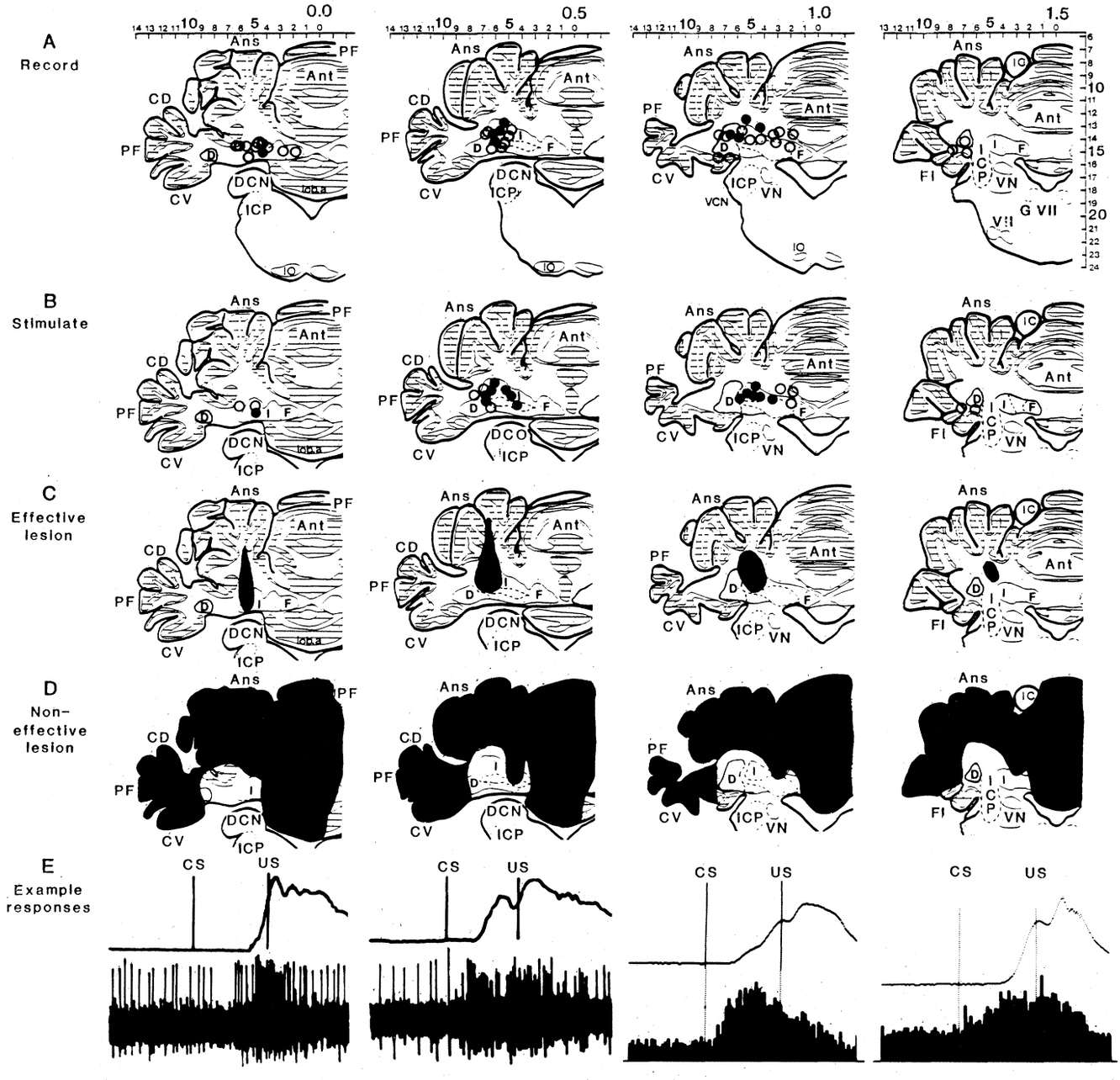


Fig. 1. Spatial distribution of recording, stimulating, effective, and noneffective lesion sites within the ipsilateral cerebellum. (A) Recording sites that did (filled cicles) and did not (open circles) develop neuronal "models" (E) of the learned eyeblink response. Only the recording sites that developed robust responses or no response at all are plotted. The larger numbers above each section represent millimeters anterior to lambda and the numbers to the side represent millimeters below bone at lambda. (B) Sites that, when stimulated, did (filled circles) and did not (open) elicit eyeblink responses. (C) An example of a lesion of the dentate and interpositus (D-I) nuclei which permanently abolished the learned response (*3*). (D) Composite from three animals of cortical lesions that were not effective in abolishing the learned eyeblink response. (E) Neuronal responses of four different recording sites within the cerebellum. The first recording is an example of multiple-unit activity from the ansiform cortex. The second recording and the two histograms on the right were obtained from the D-I nuclei. The histograms are averages of an entire session of training. The first verticle line represents the onset of the tone, and the second represents the onset of the airpuff. Each histogram bar is 9 msec wide, and the length of the entire trace is 750 msec. The top trace in each set is the movement of the NM with "up" being extension across the eyeball. Abbreviations: *Ans*, ansiform lobule (crus I and crus II); *Ant*, anterior lobule; *Fl*, flocculus; *D*, dentate nucleus; *DCN*, dorsal cochlear nucleus; *F*, fastigial nucleus; *I*, interpositus nucleus; *IC*, interior colliculus; *IO*, inferior olive; *lob a*, lobulus A (nodulus); *PF*, paraflocculus; *VN*, vestibular nuclei; *CD*, dorsal crus; *CV*, ventral crus; *G VII*, genu of the tract of the seventh nerve; *ICP*, inferior cerebellar peduncle; *VII*, seventh (facial) nucleus; *VCN*, ventral cochlear nucleus; *CS*, conditioned stimulus; and *US*, unconditioned stimulus.

response. The largest, as well as the majority (72 percent) of these responses were found within the ansiform cortex (crus I). The onset latency of these responses was 29.3 ± 16.5 msec before the onset of the behavioral NM response (*12*). Similar responses were also found within the anterior lobe, lobule C, and discrete regions of the D-I nuclei. These data are consistent with the suggestion that the cerebellar cortex or deep nuclei may be capable of storing "motor programs" or "motor memories" (*13*).

We find, however, that localized aspiration of the cerebellar cortex and certain nuclear regions—including the ansiform-paramedian lobules ($N$ = 7), anterior lobe ($N$ = 5), lobule A (nodulus, $N$ = 1), lobule B (uvula, $N$ = 1), lobule C (medius medianus and pyramis, $N$ = 5), paraflocculus ($N$ = 3), fastigial nucleus bilaterally ($N$ = 2), and the lateral dentate nucleus ($N$ = 1)—does not abolish the previously well-learned eyeblink response. This failure to abolish the learned response by large cortical lesions contrasts markedly with the complete and permanent abolition of the learned response by small lesions of the D-I nuclear region (*3*). We did find in some animals that removal of the ansiform-paramedian lobules or just the ansiform lobule, areas that project to the critical D-I nuclear region, significantly altered the amplitude-time course of the conditioned response such that, although normal eyelid closure occurred after the onset of the conditioned stimulus, the eyelids were often reopened before the onset of the corneal airpuff. This response was not seen in any intact control animal ($N$ = 10) or after removal of any other part of the cerebellar cortex. The flocculus, due to its inaccessible position in the cerebellum, has not yet been removed. However, it is probably not involved in the retention of the learned eyeblink response, since the known effective lesions track the course of the D-I nuclei and their output and do not include the major projections of the flocculus (*3, 14*).

To investigate further the role of the D-I nuclei, 54 long-term recordings were obtained from 32 animals over the course of training. Of these recordings, 20 of 54 (37 percent) exhibited neuronal activity that related to the performance of the learned eyeblink response (Fig. 1E). These neuronal responses always developed as the animal learned the eyeblink response ($r$ = 0.90). Their average onset latency varied widely among recording sites, with some consistently preceding the NM response by 40 to 60 msec and others occurring after the onset of the NM response by as long as 29 msec. Of the 54 recording sites, 22 (41 percent) responded to the tone (onset latency, 12.0 ± 3.7 msec), and 42 (78 percent) responded to the airpuff (onset latency, 4.6 ± 1.9 msec). Many (16 of 22) of the responses to the tone were small, however, and seen only on averages of a number of trials. Misdirection of the airpuff away from the cornea to above the animals' head abolished or substantially reduced the neuronal response to the airpuff in 9 of 18 (50 percent) recording sites tested, suggesting that these neuronal responses contained significant somatosensory components (*15*).

After behavioral training had ended, the recording site was marked in the awake, unanesthetized animal by passing 100 μA of direct current for 3 seconds. In some animals, the onset of this stimulation produced well-isolated eyeblinks of the ipsilateral NM and eyelids. Therefore, future animals were tested with a 60-Hz, 150-msec, 100-μA pulse of a-c stimulation. Stimulation of 14 of 23 sites tested resulted in extension of the NM and closure of the eyelids. The lowest threshold for eliciting an eyeblink response in any animal was 10 μA. Other body movements were also noted, as has been described by others (*16*). Of the 20 recording sites in which neuronal activity increased during learning of the eyeblink response, stimulation in eight of nine tested yielded eyeblink responses. In contrast, recording sites that did not yield eyeblink responses when stimulated after behavioral training also did not develop neuronal activity during learning of the response (nine of nine tested). Stimulation of the D-I nuclei in five additional untrained animals elicited eyeblinks. Lesions of the superior cerebellar peduncle in two of these animals abolished the ability of D-I stimulation to induce eyeblinks.

Figure 1 compares the spatial distribution of ineffective lesions, a representative effective lesion (*3*), the recording sites, and the stimulation sites. The ineffective and effective lesions are complementary, and the long-term recording sites that developed neuronal activity related to the learning of the response and effective stimulation sites are located within this effective lesion zone of the D-I nuclei. Furthermore, the region of the cerebellar cortex from which this region of the D-I nuclei receives its most prominent input is the ansiform cortex (crus I) (*17*), which not only exhibited robust responses related to the performance of the conditioned response but also, when removed, altered the amplitude-time course of the response.

The cerebellar cortex and deep nuclei receive a large input from the trigeminal sensory nuclei; thus it is possible that the responses may be somatosensory (*18*). However, some of the responses within the D-I nuclei predict the amplitude-time course of the conditioned response, but do not clearly predict the amplitude-time course of the unconditioned response. This observation, taken with the short latency of some of the responses, may reflect the development of the associative process.

The results of previous lesion studies have indicated that the D-I nuclei are essential for the learning, retention, and reacquisition of both classically conditioned eyeblink and leg flexion responses to both auditory and visual conditioning stimuli in both the rabbit and the dog (*3, 19*). Our results further indicate that the cerebellar cortex is not essential for the production of a learned response in the well-trained animal, but that it may participate in the control of the amplitude-time course of the response. Therefore, lesions of the cerebellum that abolish the learned eyeblink response seem to do so by interrupting the normal output of the D-I nuclei and not by interrupting a critical signal from the cerebellar cortex. However, this does not mean that the cerebellar cortex does not contain changes in neuronal function that may be important within more demanding training procedures. Recent results indicate that this essential D-I output may reach the critical motoneurons via the magnocellular division of the red nucleus (*20*).

Our results indicate that the D-I nuclei (i) have the neuroanatomical connections that allow them to cause eyeblink responses and (ii) are essential for and active during learning and retention of the eyeblink response. Thus the D-I nuclei seem to be essentially involved in the production of classically conditioned responses in mammals. Since the neurons of this region also receive both auditory and somatosensory information, we suggest that the critical plasticity encoding the learned eyeblink response may be localized to this neuronal region. Alternatively, the critical plasticity may be localized to afferent structures for which the D-I nuclei are an essential efferent (for example, pontine nuclei or inferior olive), with the D-I nuclei critically involved in the production and shaping of the learned response (*21*).

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22. Supported in part by NSF grant BNS-81-17115, Office of Naval Research contract N00014-83-K-0238 to R.F.T., and NIMH fellowship 1-F31-MH08673 to O.A.M.


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2 May 1983; accepted 25 October 1983


# A Monoclonal Antibody to Limbic System Neurons


Abstract. *A monoclonal antibody produced against hippocampal cell membranes labeled the surface of neurons in the rat limbic system. With a few exceptions, all nonlimbic components were unstained. This specific distribution of immunopositive neurons provides strong evidence of molecular specificity among functionally related neurons in the mammalian brain and supports the concept of a limbic system.*


The limbic system, a group of interrelated brain areas, was first described by Broca (*1*) more than 100 years ago. Fifty years later, Papez (*2*) hypothesized that the limbic system is the neuroanatomical substrate of emotion. The organization of the limbic system and other functional neural systems is continually being redefined in anatomic and physiological terms. One reason for this may be the disagreement over how the parts of a system become interrelated and, indeed, what constitutes a functional system in the brain.

If limbic structures do become integrated into a functional system then they might also be expected to display a unique relation at the molecular level, ultimately adding a new dimension to structure-function relations in the central nervous system (CNS). This now can be examined with new molecular approaches. For example, monoclonal antibodies generated thus far have revealed the molecular heterogeneity of brain cell types (*3*), chemical gradients in the developing CNS (*4*), and adhesion factors that may be involved in axon growth (*5*). We are developing monoclonal antibodies that are specific for cell classes or systems in both the differentiating and mature CNS, and have recently produced a monoclonal antibody that demonstrates molecular specificity at the systems level. The antibody, described in this report, exposes an antigenic determinant found almost exclusively in cortical and subcortical regions composing the limbic system.

Monoclonal antibodies were produced by conventional procedures (*6*). BALB/c mice were immunized three times with a crude membrane preparation (0.5 mg per injection) obtained from adult rat hippocampus. After fusion of the mouse spleen cells with the myeloma cell line NS-1, cells were distributed into 96-well plates containing HAT (hypoxanthine, aminopterin, and thymidine) selection medium. Screening for antibodies was performed 10 to 14 days after fusion by indirect immunofluorescence on paraformaldehyde-fixed cryostat sections through the hippocampus. Hybridomas that elicited positive staining of any cellular elements were expanded and cloned by the limiting dilution method (*6*). Eighteen of the original 48 positive clones remained stable and continued to produce antibodies. Only one line, clone 2G9, produced an antibody that stained in a regionally specific fashion. The others stained more ubiquitously, although a few were either neuron- or glia-specific. Clone 2G9 produced an antibody of the immunoglobulin G (IgG) 2a subclass (*7*) that labeled all neurons in the hippocampus.

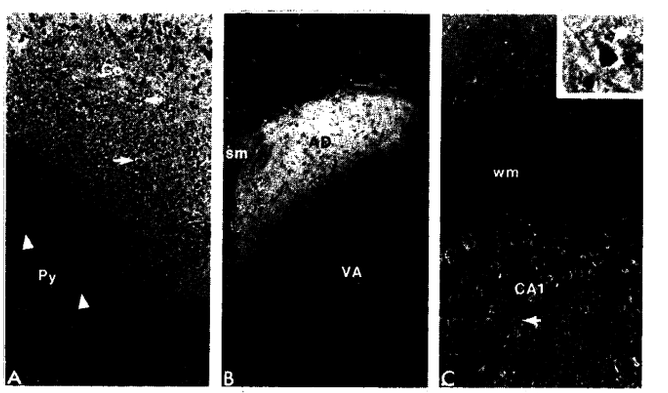


Fig. 1. Photomicrographs showing the specificity of 2G9 monoclonal antibody staining in limbic system regions of the rat brain. (A) Coronal section through the basal forebrain (×125). Note the clear border between the dense immunofluorescence in the corticoamygdaloid nucleus (*Co*) and the unstained pyriform cortex (*Py*). Punctate immunofluorescence surrounds the silhouettes of amygdaloid neurons (arrows). Neurons in the pyriform region are not specifically stained (arrowheads). (B) Lower power micrograph of a coronal section through the rostral thalamus (×80). There is heavy immunofluorescent staining of the anterodorsal thalamic nucleus (*AD*). This contrasts with the dark, unstained region corresponding to the motor thalamus, the ventroanterior nucleus (*VA*). Note that only the neurons in the AD are surrounded by the immunoreactivity (*sm*, stria medullaris). (C) Stained neurons in the CA1 region of the hippocampus (×125). Overlying white matter (*wm*) and motor cortex are unstained. Immunofluorescence is particularly evident on the surface of large neurons and their apical dendrites (arrows). A hippocampal cell viewed at higher magnification (inset) reveals the surface distribution of stain on all 2G9-positive neurons (×320).