operation required for inducing the plastic change *elsewhere* in the central nervous system. In addition, Thompson's argument that the climbing fiber system is involved in establishing the plastic changes underlying motor learning through the mechanisms proposed by Ito is not consistent with some of his own data. His findings clearly imply that the permanent memory trace required for nictitating membrane reflex conditioning cannot occur in the cerebellar cortex, since ablation of this structure does not permanently abolish the conditioned response. This would not be expected if the synapses modified in long-term depression, namely those on the dendrites of Purkinje cells, were responsible for the storage of the memory trace underlying this conditioned behavior.

Thompson discusses long-term depression as an example of a potential synaptic mechanism of plasticity, but several critical and controversial features of the experiments pertaining to this issue are not mentioned. To date, no one has challenged the precise set of observations reported by Ito and his colleagues (*4*). However, because long-term depression has been observed only when a technique referred to as conjunctive stimulation is employed, the functional relevance of these findings can be challenged. This technique employs coincident stimulation of mossy and climbing fiber inputs to the cerebellar cortex. In the published applications of this technique, the climbing fiber input is activated at a higher rate over a more prolonged stimulus period than occurs under behavioral conditions.

Studies from our laboratory support a considerably different view of climbing fiber function. These experiments did not employ electrical stimulation to activate climbing fibers at a specified rate over a specified duration. Rather they examined the effects of spontaneously occurring or naturally evoked climbing fiber inputs, including those evoked by stimuli applied during locomotion in high decerebrate animals. Our findings showed that the effect of the climbing fiber input on Purkinje cell simple spike discharge can be described as a short-term enhancement of the Purkinje cell's response to the peripheral event rather than a prolonged suppression, as implied by the data from the conjunctive stimulation experiments. At the very least these findings indicate that it is premature to conclude that long-term depression characterizes the functional action of the climbing fiber system and that this mechanism is responsible for establishing memory traces in the cerebellum.

Finally, I would like to raise some additional questions concerning the role of the cerebellum in VOR adaptation. Although the studies of Miles *et al.* (*6*) are cited by Thompson, the extent to which they challenge the view that plasticity occurs in the cerebellum is not fully addressed. These investigators recorded from Purkinje cells in the flocculus of animals whose VOR had been adapted by wearing prisms. The changes in the response characteristics of these cells and the latency of their responses were not as predicted from hypotheses proposing that these neurons are localized to the site at which the adaptation occurred. Furthermore, Demer and Robinson's experiments (*7*) strongly arguing against a "teaching" role for the climbing fibers in VOR adaptation are not discussed. In my view these are critical points in an overview of this issue.

In conclusion, I feel that at this time there is no direct proof that the cerebellum is a storage site for motor memory traces. The data only support the likelihood that the cerebellum is a component in a pathway necessary for the conditioning of the nictitating membrane reflex. Given the interest of neurobiologists in the broad issue of motor learning, it is imperative that this issue remain open and that hypotheses regarding the mechanisms underlying this process take all pertinent data into account.

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Response: When we began our search for the memory traces for basic associative learning (using classical conditioning of discrete behavioral responses) 17 years ago, we had no idea it would lead us to the cerebellum. We have been forced there by our findings, beginning in 1980, and into the midst of a long-standing and apparently sometimes heated controversy regarding the cerebellum and motor learning. Several eminent neuroscientists (for example, Eccles and Ito) favor the hypothesis that the memory traces for learned movements are stored in the cerebellum. Others (for example, Llinas) take the opposite position. Our findings to date are consistent with, and supportive of, the former view.

Bloedel's comment focuses on limited aspects of the field and of our work. It does not address much of the evidence presented in my article (*1*) favoring the hypothesis (which I clearly state is not yet proved) that memory traces for associative learning of discrete, behavioral responses are localized to the cerebellum. Our earlier electrophysiological recording data, together with lesion and stimulation data, provided strong evidence that the traces are formed in the cerebellum or in structures afferent to the cerebellum for which the cerebellum is a mandatory efferent, that is, the traces are not formed efferent from the cerebellum. This evidence is sketched briefly in my article and at greater length in earlier publications (*2*).

In brief, our findings are as follows.

1) Lesions (of cerebellum) completely, selectively, and permanently abolish the learned response in trained animals and completely prevent learning in naive animals, but have no effect on the reflex unconditioned response and do not cause sensory or motor impairments relative to the behavioral response.

2) Within trials, over the course of training, neurons in localized regions of the interpositus nucleus and cerebellar cortex develop changes in frequency of discharge (increases in interpositus) that "model" the behavioral conditioned response (CR), but not the reflex response, that is the increase in discharge frequency precedes the onset of the behavioral CR (by as much as 60 milliseconds), predicts the amplitude-time course of the behavioral CR within trials, and predicts its development over the trials of training.

3) Appropriate lesions [of the dorsal accessory olive (DAO)] in trained animals result in experimental *extinction* of the behavioral CR with continued paired conditioned stimulus (CS)–unconditioned stimulus (US) training. To our knowledge this result, which ought to be so if the essential US pathway is destroyed (it ought to be like removing the US in ordinary behavioral extinction training), is new.

4) By appropriate microstimulation of mossy fibers as the CS and climbing fibers as the US, the two types of direct input to the cerebellum, normal behavioral associative learning of virtually any phasic, coordinated skeletal muscle response can be established. Further, these learned responses (and the unlearned responses evoked by climbing fiber stimulation) are abolished by destruction of the interpositus nucleus.

5) Initial evidence suggests that the central CS pathway beyond the primary sensory (auditory) system involves the lateral pontine nuclear region and mossy fibers.

Bloedel, in his comment, selects limited

portions of these findings to criticize. Nowhere does he describe the "shortcomings" of the conditioning paradigm. I made clear in the article that a "demonstration that the cerebellum is required for adaptation or association is not unique" and that it is also "required for adaptation of the vestibulo-ocular reflex [VOR]." But, to our knowledge, our work is the first to show that the cerebellum is necessary for associative learning and memory. VOR adaptation and associative learning differ markedly in their behavioral properties.

"[W]hether these data prove that the learning process actually occurs within the cerebellum itself" is, of course, the $64 question. It has not yet been settled definitively for any form of learning or any brain structure in mammals, but our evidence is clearly supportive of this hypothesis.

Bloedel cites a paper by Gellman *et al.* (*3*) that is a comprehensive study of the responses of olivary neurons to contact and passive body deplacement in the awake cat. The inferior olive stimulation was an incidental part of the study reported in one paragraph without anatomical reconstructions of electrode locations. The strongest current used in that study was 60 uA. The lowest effective current we have found to elicit behavioral movements, with otherwise relatively similar stimulus parameters, is about 60 uA, in the rabbit (*4*) (The parameters *are* specified in the article.) Bloedel does not note one of our other findings, namely, that whatever movements are elicited by DAO stimulation, these exact movements are learned to a neutral tone CS. Stimulation outside the DAO, for example, in the reticular formation, also elicits movements, but these cannot be trained to neutral stimuli.

Our argument for the mossy fiber CS pathway cites the immediate transfer of the CR from pontine stimulation on one side to stimulation of the other side. Bloedel's argument—that [this finding] "may reflect the fact that both stimulus sites are activating pontine projections to both sides of the cerebellum" (because of the bilateral course of the pontine projections)—only has merit if the trace is formed in the cerebellum rather than in the pontine nuclei or in other brain stem structures possibly activated by the initial training electrode. If, as he suggests, the second electrode is stimulating exactly the same fibers stimulated by the first electrode, which seems unlikely, then the plasticity could be at the mossy fiber terminals, rather than beyond. But they are *in* the cerebellum. He ignores the other lines of evidence cited in the article for the mossy fibers being the essential CS pathway.

Bloedel's statement that the permanent memory trace cannot be in the cerebellar cortex "since ablation of this structure does not permanently abolish the conditioned response" is a non sequitur. Neither we nor anyone else has removed all cerebellar cortex. Our findings to date indicate that removal of H VI, Crus I, Crus II, and paramedian lobules does not permanently abolish the CR. But the trace could have multiple cortical representations including areas not yet removed (*5*).

Bloedel discounts the elegant studies by Ito and his associates of long-term depression with conjoint stimulation (of mossy or parallel and climbing fibers) in vivo and in the cerebellar explant and then describes his own in vivo studies on locomotion in high decerebrate animals, where he finds a short-term enhancement of Purkinje cell response after a climbing fiber discharge. This is another non sequitur. Bloedel's observations and paradigms concern locomotion and have nothing to do with adaptation or learning and memory. In both Ito's work and in our own, changes over time are looked at as a result of repeated stimulation, that is, the effects of *training*.

Bloedel argues that I do not fully address the extent to which the studies of Miles *et al.* (his reference 6) challenge the view that plasticity occurs in the cerebellum in VOR adaptation. The focus of my article was not on adaptation of the VOR, but the close parallel with classical conditioning of discrete behavioral responses—the fact that the cerebellum is essential for both—merits emphasis. Two major current views are that the locus of VOR plasticity is either the cerebellar cortex of the flocculus (Ito) or the medial vestibular nuclei. In terms of connections (monosynaptic inhibition from cerebellar cortex), flocculus target neurons are equivalent to deep cerebellar nuclei, and the parallels between the VOR and other motor systems suggest that the deep cerebellar nuclei may be the site of many kinds of motor learning. Thus, both views support my contention that the memory traces for discrete learned responses are in the cerebellum, either in cortex or interpositus nucleus or both.

In sum, the evidence from our laboratory and the related work from other laboratories, which includes electrophysiological recording, lesion behavior, microstimulation, infusion of pharmacological agents, and anatomical pathway tracing, is consistent with, and supportive of, the view that the memory traces for classical conditioning of discrete behavior responses learned associatively to neutral conditioned stimuli, with the use of aversive unconditioned stimuli, are stored in the cerebellum. Has this been proved to everyone's satisfaction? Of course not. Scientific truth is a matter of probability. We have now succeeded in identifying key aspects of the essential memory trace circuit (which includes the cerebellum) for this category of learning and memory beyond a reasonable doubt and are approaching the point when it will be possible to localize the essential memory traces themselves.

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Monoclonal Antibodies as Phylogenetic Labels

The report by Susan Hockfield (*1*) of the development of a monoclonal antibody, Rat-302, specific for a subset of cells in the granule cell layer of the vermis, paraflocculus, and flocculus of rat cerebellum, raises several points of interest. She notes that "in the flocculus and vermis, Purkinje cells project directly" to the vestibular nuclei, bypassing the deep cerebellar nuclei.

Vestibular input to this area is also partially direct. Nauta (*2*) describes the vestibular nuclei as projecting "to part of the vermis . . . and also to the flocculonodular lobe, the caudalmost part of the cerebellum." He notes that (in humans) some fibers of the vestibular nerve bypass the vestibular nuclei, constituting a direct cerebellar input, adding that "no other instance is known in which the cerebellum gets primary sensory input."

The cerebellar structures involved appear to be phylogenetically old. In *Amblystoma*, Herrick (*3*) defines "three of the primordia from which the mammalian cerebellar complex has been assembled."

These are, "1) the vestibulo-lateralis system in the auricles, primordia of the floccular part of the flocculonodular lobes; 2) the median body of the cerebellum which is ancestral to the larger part of the vermis and adjoining regions; and 3) the nucleus cerebelli, internal to the other two, and in intimate relations with both. . . ."