

CARLO, THE CEREBELLUM, AND ME

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To many who knew him, Carlo Terzuolo was the consummate scientist. To me he was also motivator, mentor and friend. Perhaps to the disappointment of some, his passion for his discipline compelled him to be uncompromising on issues that he considered important. To those of us who knew him well, this quality translated into an intense commitment to his work as well as to the people close to him. His intensity and commitment to his discipline were the traits I found most compelling when we first met in 1963, the year I entered his lab on a summer internship as I explored the possibility of changing from biochemistry to neurophysiology.

Even though he was immersed in the spinal cord at that time, it was clear from his discussions that the cerebellum was always more than a passing interest to him, an interest that was stimulated by his work in the laboratories of Bremer and Moruzzi. Carlo's own work on the cerebellum in the late 50s and early 60s offered a clear continuity with that of his mentors. While other outstanding scientists were focused on unraveling the excitatory and inhibitory actions of cerebellar neurons and their electrophysiological characteristics, he and his colleagues continued to ask questions that more directly related to the contribution of this structure to the regulation of motor behavior and spinal integration. The experiments being conducted in Carlo's laboratory when I arrived in the early 60s reflected one of the two major influences he had on me and my work – an emphasis on using a very strong conceptual framework. These studies not only addressed the action of descending projections activated by cerebellar efferent pathways, they also examined critical concepts of spinal integration dealing with the action of differentially distributed inputs to the dendrites and soma of alpha motor neurons (13-15, 21). He was insistent that any project worthy of an intense effort must have a strong conceptual framework and must address a question that was highly relevant functionally. In the 60s, this was a particularly important perspective because of the emphasis at that time on employing electrophysiological tools, particularly intracellular recording, to ask primarily connectionist-related questions regarding the excitatory and inhibitory action of a wide variety of central projections.

His other emphasis that had a significant impact on my thinking and the development of our laboratory was the application of quantitative techniques to the study of neurophysiological questions. This focus also served as the foundation for the first Brainerd Conference in 1969. Carlo had a strong hope that linear systems analysis

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could provide a universal quantitative approach to studying and defining central nervous system integration. This enthusiasm was shared by many and stimulated fascinating discussions among the meeting's participants. As it became clear in subsequent years that the application of this method to central nervous system integration would be limited due to the nonlinearity of many central projections, Carlo continued to stimulate all of his colleagues to generate new quantitative approaches that at the very least represented the data in a meaningful way, even if it failed to define the input-output function for the system being investigated. This passion led Carlo to develop one of the major programs in human motor behavior. This program and its legacy represented by the work of several of Carlo's students and postdoctoral fellows have a highly recognizable signature, a clear conceptual framework together with a strong application of quantitative techniques.

Although I remember being extremely disappointed when I found that the responses of Purkinje cells to natural proprioceptive stimuli were highly nonlinear, my interactions with Carlo and Rodolfo Llinas regarding the cerebellum raised too many questions and engendered too much excitement for me to leave the study of this structure to pursue a more linear system! Furthermore, my interactions in Carlo's laboratory as well as the discussions at the first Brainerd Conference had a very strong influence on the first post-thesis studies I pursued. Together with Bill Roberts, we performed a series of experiments that emphasized the importance of carrier frequency to the interactions occurring in the cerebellar cortex (6). We hypothesized that the tonic discharge rate of cerebellar neurons determined both temporal and spatial features of integration in the cerebellar cortex and nuclei. Although in retrospect this concept appears very simplistic, the findings from the study offered a significant challenge to what was then the current postulates of cerebellar cortical function. In these experiments, a barbiturate anesthetic was used to modify the background level of spontaneous activity, and the effects of activating parallel fibers on the responses and the excitability of Purkinje cells was tested as the rate of spontaneous discharge decreased. As shown in Figure 1, activating parallel fibers in the absence of anesthesia produced predominantly an excitatory response in Purkinje cells (B-F) and an absence of the inhibitory effect mediated by basket cell inhibition, as evidenced by the failure of the surface stimulus to suppress the antidromic response of these neurons (G). When small amounts of the barbiturate were administered, the same stimulus evoked only a single spike from Purkinje cells, and basket cell inhibition (suppression of the antidromic response) became apparent (H and I). This study clearly showed for the first time that it was necessary to consider the very important factor of carrier frequency when considering the spatial and temporal interactions of central neurons.

In this manuscript I've chosen to commemorate Carlo's commitment to the use of strong conceptual frameworks in research by reviewing the series of concepts evolved in our laboratory over the past few decades through studies examining the cerebellum's role in motor learning. At the time we began to address this issue, one of the most important questions related to the action of climbing fibers in establishing plastic changes in the cerebellar cortex. This postulate, often referred to as the

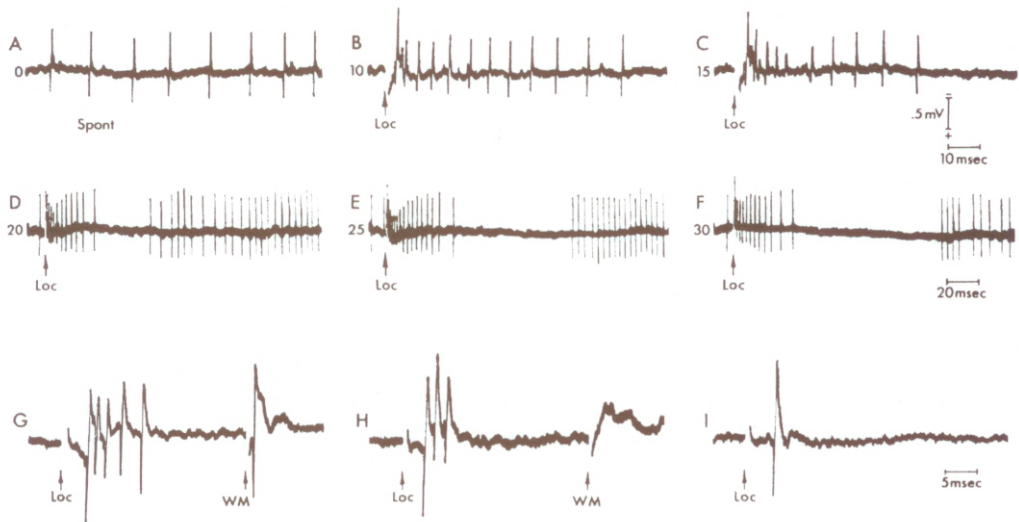


Fig. 1. - Responses of Purkinje cells to the activation of parallel fibers (Loc) and white matter (WM) stimulation.

Strength of the surface stimulus is shown in B through F in relative units to the left of each record. Other parts of this experiment illustrated that the pause in spike discharge could not be ascribed to the action of basket cells. Most critical to the argument in the text, G-I show the effects of incremental amounts of pentobarbital on the responses to the surface stimulus and the inhibition of the of antidromic response to WM stimulation. G: control. H: 14 mg/kg. I: 21 mg/kg. Note that, as the excitatory response to the surface stimulus decreased, the antidromic response was inhibited (H). From 6.

Marr-Albus hypothesis, proposed that during learning climbing fiber inputs induced long lasting changes in the responses of Purkinje cells to specific patterns of parallel fiber inputs converging on the same dendritic tree, and that these changes were the basis for establishing engrams required for the subsequent execution of the practiced behaviors (See 4 for review). Later, the concept of long-term depression emerged as a mechanism by which climbing fibers were felt to exert their proposed effect on the excitability of Purkinje cells (12). The experiments on which this concept was based employed very unusual stimulus rates to activate climbing fibers. Furthermore, these rates were maintained for very prolonged periods of time. We hypothesized that the observed effects could have resulted from activating climbing fibers in an unphysiological manner both spatially and temporally (See 4 for review).

In order to examine this issue under more functionally relevant conditions, Tim Ebner and I designed a series of experiments using paradigms that employed only spontaneously occurring or naturally evoked climbing fiber inputs in unanesthetized preparations to assess this question (5, 9-11). In all of these studies the activation of climbing fiber inputs failed to suppress responses evoked by mossy fiber-parallel fiber inputs; in fact in many cells these responses were enhanced. Subsequently, in studies using intact monkeys adapting to changes in the relationship between the movement of a manipulandum and the movement of a cursor, Tim Ebner's labora-

tory failed to show a change in climbing fiber activation that supported the notions related to the finding of long-term depression (18).

Recently we have examined the relationship of climbing fiber inputs to task acquisition using the classical conditioning of the eyeblink reflex in intact rabbits as a model (3). In these experiments multiple single unit recording techniques were employed to record simultaneously from related areas in the cerebellar cortex and nuclei during the conditioning process. Methods were used to ensure that the recording sites were within the regions of these structures known to be critical for the acquisition and retention of this behavior. Responses evoked by climbing fibers were correlated with several aspects of the acquisition process. Similar to the findings of Ebner's laboratory using a volitional movement, the occurrence of complex spike responses did not change during the acquisition process in a manner consistent with the Marr-Albus hypothesis. Interestingly learning-related changes in the discharge of nuclear neurons and the simple spike activity of Purkinje cells were found, but they were unrelated to the action of the climbing fibers. Although the precise role of climbing fibers in cerebellar cortical integration remains an elusive objective for investigators, the above experiments show the importance of asking questions pertinent to this issue using a strong conceptual framework and paradigms that are as physiological as possible.

Most of the experiments in our laboratory over the past decade attempted to define a concept of the cerebellum's role in motor learning that was applicable across behaviors. Together with Vlastik Bracha, we developed approaches that permitted us to ask related questions using a variety of motor learning paradigms: eyeblink conditioning, several reaching behaviors, and psychophysical studies in human subjects. We have used these diverse paradigms to evaluate a new concept, namely that the role of the cerebellum in motor learning is task-dependent. At the time we initiated these experiments, the prevailing view was that the cerebellum played a singular role in the learning of many different motor behaviors and that this role was one related to the storage of engrams required to execute newly learned tasks. To a significant degree this perspective was based on a series of experiments that examined the effects of lesions in the cerebellar nuclei on the retention of the classically conditioned eye blink reflex in the intact rabbit (See 1 for review). These data were so compelling that they drove the interpretation of experiments investigating the cerebellum's role in learning other types of movements, including volitional movements such as such as mirror tracing (19). These types of experiments frequently compared the performance of patients with substantial cerebellar damage to that of normal controls after comparable periods of practice and concluded that differences in performance were related to a learning deficit in the patients.

To us this appeared counterintuitive, particularly since the inability to learn new behaviors is not a common complaint of patients with cerebellar pathology. Furthermore, comparing the absolute performance of these groups after periods of extensive practice ignored the fact that a significant degradation of the responses in cerebellar patients could be related to their movement deficit. In our view it was highly unlikely that the performance of these patients could ever attain the qual-

ity of movements exhibited by normal subjects independent of the amount of practice or the presence of a learning deficit. Consequently, we decided to employ a complex tracing task using a different type of learning measure (22). Patients and control subjects were asked to practice tracing an irregularly shaped two dimensional object, then mentally rotate it 90 degrees, and redraw the shape from memory. Three different error measurements were used to assess performance based on the deviation of the tracing from the shape of the original object. Rather than comparing absolute measurements taken after each subject completed the learning, the rate of improvement in the task was compared across trials. This approach revealed something that was highly controversial at the time the findings were reported, namely that the performance of the task improved at comparable rates in both groups of subjects. This provided the initial support for our working postulate that the cerebellum could contribute to the learning of motor behaviors in a task-dependent manner, since the same type of patients are unable to acquire the classically conditioned eye blink reflex.

Subsequent experiments performed in cats supported this view (23). In these experiments muscimol was injected in the interposed and dentate nuclei during all training sessions in which these animals were required to improve their performance on a template task. The task required that cats learn to perform a movement sequence in which they were required to move the manipulandum through a groove in the shape of an inverted "L". As the task was practiced, the angle between the two grooves progressively decreased until the animal could no longer improve its performance. The data demonstrated (Fig. 2) that cats were able to learn the task despite the fact that the dentate and interposed nuclei were inactivated throughout the practice period. Conceptually, it is important to emphasize that these findings further support the task-dependency hypothesis by showing that, in contrast to the effects of cerebellar nuclear inactivation on the acquisition of the eyeblink reflex, blocking cerebellar nuclear output did not prevent the animal from learning the learned task despite its dysmetria. Thus, there is a clear difference between the role of this structure in the learning of this volitional movement and its role in the acquisition of the classically conditioned eye blink reflex.

Figure 2 also demonstrates another important finding. When the same cats were given the opportunity to perform the task after they acquired the behavior during nuclear inactivation, they were unable to execute the behavior at the same angle without additional practice. It was necessary to increase the angle and essentially retrain the animal to perform the task. The latter finding suggests, but does not prove, that the incorporation of the cerebellum into the circuits employed in learning and performing the task requires practice and that this incorporation is a preferred condition both for the acquisition as well as for the performance of the movement. Thus, even though the cerebellum may not be *required* for either the retention or the acquisition of the behavior, it may still play a unique role in its acquisition. The importance of the cerebellum in task acquisition will be addressed again below.

An additional study demonstrated that the difference between the contribution of the cerebellum in eye blink conditioning and the drawing and reaching tasks intro-

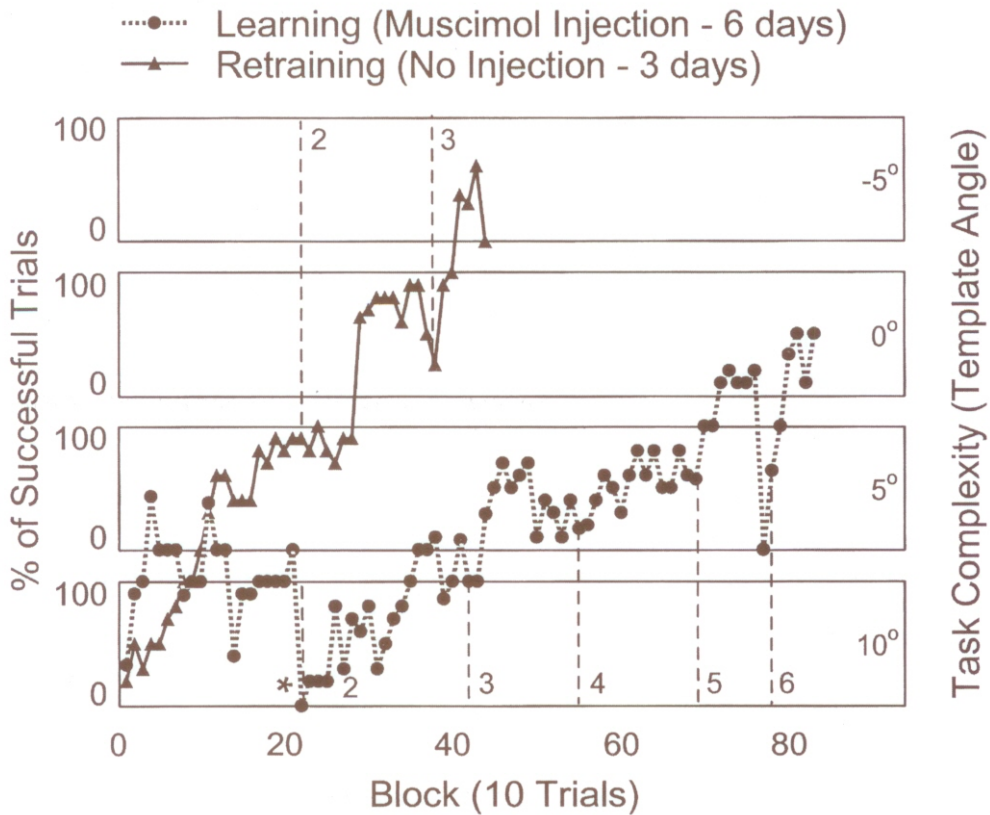


Fig. 2. - Capacity of a cat to learn to move a manipulandum through inverted "L" templates at successively smaller angles while the dentate and interposed nuclei were infused with muscimol. Degrees shown are relative to 90 degrees. The overall learning curve is constructed by stacking the learning curves obtained as the animal practiced at each successively smaller angle. Dashed lines and associated numbers from each curve indicate the days over which the learning occurred. Retraining in the absence of the muscimol injection was performed following the last day of training during muscimol infusion. From 23.

duced above cannot be ascribed solely to the fact that the behavior being learned was a volitional task. In these experiments (20), cerebellar patients and age-matched controls were asked to learn the shape of two-dimensional, irregular objects by moving a stylus through a groove outlining the object's shape while blindfolded (Active kinesthesia, Fig. 3). This protocol required that the subjects use only kinesthetic cues to develop an image of the shape. They were then asked to recall the shape by moving the stylus through various templates and determining whether or not the template's shape was the same as the original. Unlike the tracing task described above, cerebellar patients could not acquire this behavior. Furthermore, when asked to draw the shape of the original template with either eyes opened or closed, the patient group was unable to draw the shape accurately (Fig. 3). Perhaps most interesting, the shape was only *partly* distorted. Although one part of the original template was accu-

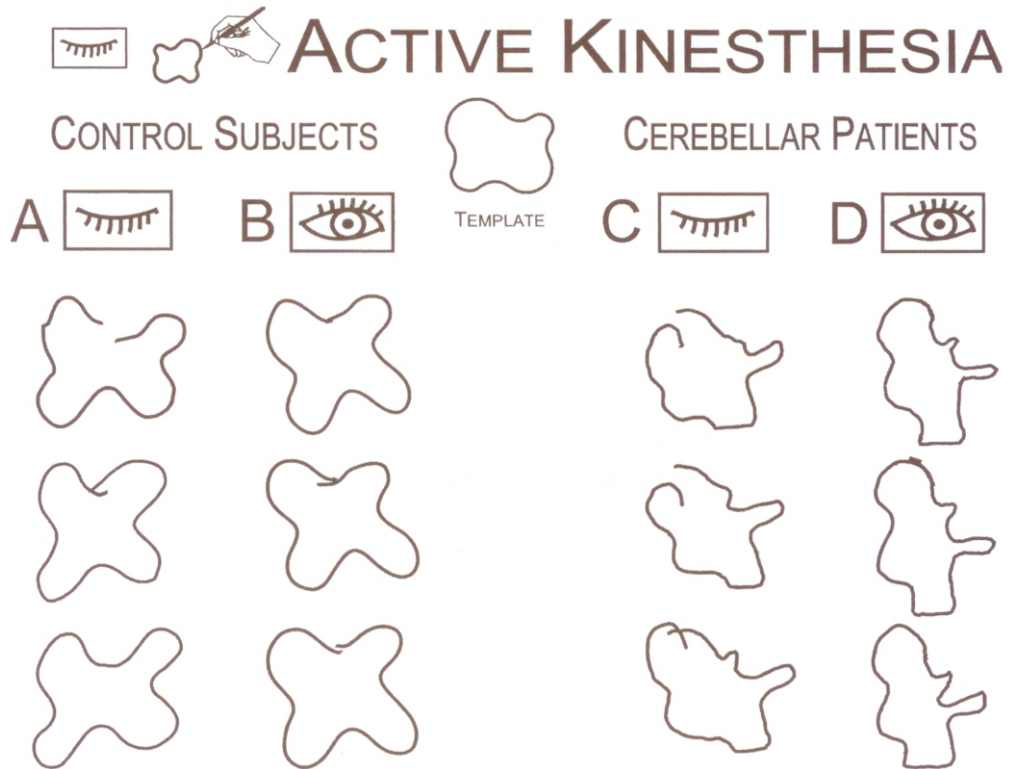


Fig. 3. - Comparison of control subjects and cerebellar patients to learn the shapes of templates under an active "kinesthesia condition", a condition in which the subject attempted to learn the shape by tracing the original template (in the middle between B and C) over five successive sets of trials while blindfolded (These data are not shown in this figure. See 20 for details).

Cerebellar patients could not learn shapes using this paradigm. Following an assessment of the subjects' capacity to learn, they were asked to draw the shape of the original template, first blindfolded (A and C) and then with eyes open (B and D). From 20.

rately represented, another major portion of the drawn figure had no relationship with it. It appeared to us that the patients with selective cerebellar damage were unable to establish a complete and accurate internal representation of the shape when learning required the use of kinesthetic cues. Thus, although the task was volitional in nature, it could not be acquired by patients with significant cerebellar pathology.

These findings substantiate that the characteristics of the task are critical in determining the cerebellum's role in learning a specific movement. Based on these observations and those reviewed above, we consider the task-dependency hypothesis to be a critical concept in defining the cerebellum's contribution to learning motor behaviors. This concept also unifies what to some has been a variety of disparate findings. As stated above, the focus previously was to assign a specific function to the cerebellum that was applicable across the learning of several different behaviors.

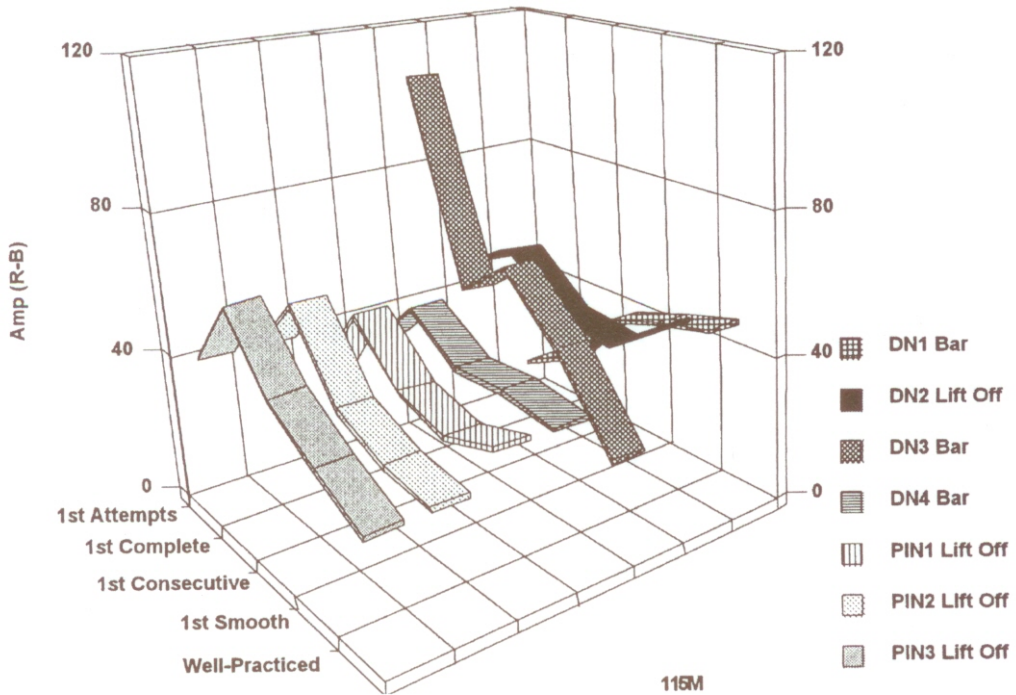


Fig. 4. - Ribbon plots showing the amplitudes of the event-related modulation of four dentate neurons (DN) and three posterior interposed neurons (PIN) recorded throughout the period required for a cat to learn to move a template through an "L" shaped template.

The stages of learning are indicated along the axis. Blocks of trials were combined to generate the histograms related to each stage. Smooth movements were defined as those in which there was only one peak in the velocity profile for each limb of the template. Response amplitude is expressed as mean frequency of the response minus the background discharge rate (R-B). Notice that all but one of the neurons showed an increased modulation at the time the first correct behavioral responses were being performed (1st complete movements through the template, 1st 10 consecutive trials in which criterion performance was achieved). This includes DN3, in which there was a very large modulation initially. See 17 for additional details.

Our data clearly show that the cerebellum can be involved in motor learning in a variety of ways, and that its contribution extends substantially beyond the issue of storage to issues related to phenomena underlying the acquisition process itself.

We currently believe that the cerebellum's contribution to the learning of volitional movements is related to its role in acquisition-related processes. One of our recent studies supports this view (17). In this experiment, multiple single unit recording techniques were used to record the activity of cerebellar nuclear neurons during the acquisition of a complex, volitional task consisting of moving a manipulandum through 1-3 straight grooves in a two dimensional template (17). These experiments demonstrated that the task-related modulation of these cells increased substantially during the initial stages of the acquisition process, peaking at the time the first correct responses were being executed somewhat consistently (Fig. 4). Although much additional work is required to understand the precise contribution of

this change in modulation to the acquisition process, these observations provide a strong indication that the cerebellum's role in learning these types of volitional movements is acquisition-related.

To summarize, our data support the task dependency hypothesis, which is not only consistent with our data but also synthesizes a variety of other findings related to the cerebellum's role in learning motor behavior. Furthermore, it is apparent that the cerebellum's role is not restricted only to roles related to the process of retention and storage. Rather, it can be very important in acquisition-related processes, particularly during the learning of volitional movements.

Carlo delighted in discussing these ideas, and there is no question that he had hoped we would uncover an understanding of the cerebellum's input-output relationships in the tradition of systems analysis, particularly in the initial years of these experiments. However, as our experiments evolved his fears that his pupil was treading down a dangerous connectionist path were allayed as the concepts we developed addressed some of the enigmatic aspects of the cerebellum's role in motor learning. I miss our discussions on these questions, and I truly miss Carlo as a friend and mentor.

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