Relations Among Climbing Fiber Responses of Nearby Purkinje Cells

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ACTIVITY PATTERNS in the cerebellar cortex may be studied by simultaneously recording from two or more cells whose spatial separation and orientation are known. Appropriate statistics such as the cross-correlogram may then be used to describe the temporal relationships. Further computational and experimental methods sometimes permit an interpretation of the temporal relationships in terms of the underlying anatomical connections (20, 23, 24).

Two quite different types of discharges may be recorded from cerebellar Purkinje cells, simple spikes and complex spikes. Simple spikes are similar to nerve impulses recorded elsewhere in the central nervous system and are evoked primarily through parallel fiber activation. Complex spikes have the same sharp initial component, but this is followed by a slow wave lasting 10–15 msec on which ripples or small spikes are superimposed. The complex spike has been shown to be the response of the Purkinje cell to a single spike or a brief burst of spikes in the climbing fiber axon (11), and is also referred to as a climbing fiber response (CFR). In our previous work (6) we found that the simple spikes of nearby Purkinje cells were often correlated in time. It was suggested that the temporal relationship between two such discharges depended on the parallel fiber input shared by the two cells and consequently could be related rather precisely to their spatial separation and orientation on the cerebellar sheet. Freeman (14) has made the same suggestion and provided experimental support for it.

The present experiments also used the method of statistically analyzing simultaneous recordings from two or more cells but concentrated on the relationships among climbing fiber responses. The CFRs were correlated in time and this correlation was more restricted in the direction of the parallel fibers, i.e., in the longitudinal axis of the folia than in the transverse. Our finding is closely related to that of Oscarsson (22) who found that the different climbing fiber pathways from the spinal cord project onto transverse strips of cerebellar cortex. A statistical argument and some stimulation experiments suggested a possible mechanism for the correlation of CFRs, namely, a sharing of recurrent collaterals by the cells which give rise to the climbing fibers.

Describing and interpreting the pattern of climbing fiber activity were the main goals of this study. However, pairs of simple spikes were also recorded and the correlations between these are reported.

METHODS

Fifty-eight guinea pigs were anesthetized and used in acute experiments. Two additional animals were prepared for chronic experiments and were recorded from without anesthesia. Of the acute animals, 36 were used in recording simultaneously the spontaneous activity of two or three Purkinje cells and 22 in studying the CFRs evoked by stimulation of nearby points on the cerebellar surface.

In acute experiments the guinea pigs were anesthetized with pentobarbital (intraperitoneally, 40 mg/kg). After such a dose, the guinea pig still responds to a strong noxious stimulus with flexion. A dose sufficient to suppress such reflexes also suppresses respiration to a degree that may be fatal. Supplemental doses (5–10 mg/kg) were given when necessary. When the animal was anesthetized, 0.04 mg atropine was given and 2% procaine with epinephrine infiltrated beneath the skin of the throat. A

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tracheotomy was done and the animal placed in a stereotaxic apparatus. After infiltrating the skin of the head and neck with procaine, the atlanto-occipital membrane was exposed and the cistern opened widely. A square of bone just anterior to the lambdoidal ridge was removed exposing the vermis of lobules V and VI. Three or four folia were usually visible and from observing the surface one could not be sure which was lobule V and which VI. Hence, the label "next folium" is used in summarizing the results, although this certainly includes cases in which "next lobule" would be correct. Only in some of the stimulation experiments was the primary fissure determined with certainty after perfusing and removing the brain. In all experiments, a small block of wood was attached by dental cement to the anterior skull and held by a clamp mounted on the stereotaxic frame. The mouth piece and ear bars were then removed. Agar in Ringer solution was placed on the brain to protect and stabilize it. Micropipettes filled with 1 M potassium acetate saturated with methyl blue were used. The signals from one or more electrodes were led through emitter follower amplifiers with 2-msec time constants to an oscilloscope and a tape recorder.

Recording spontaneous activity

In some recordings two or three electrodes were made with long shafts, cemented together, and lowered as a unit. While pairs and triplets of cells with separations as small as 20 μm could be recorded in this way, it was quite difficult to obtain the recordings. Therefore, in most experiments the electrodes were mounted on Narishige (Tokyo) two- or three-electrode micromanipulators, making independent control possible. The distance between the electrodes, and their folial orientation, was varied. Each pair of electrodes was either oriented in the longitudinal axis of a folium, or perpendicular to this, in the transverse axis. Distance between electrodes was measured with a reticule in the operating microscope. This was done both before and after a penetration. If the two measurements differed, the later one was used. This method was adequate for the longitudinal and shorter transverse separations in which recording sites were never deeper than 2.0 mm below the surface and it was thus almost certain that both recordings were from the same folium. A more accurate method was necessary, however, for larger transverse separations in which the electrodes were sometimes driven to greater depths. In such cases marks were made at both recording sites by passing current through the dye-filled pipette (32). Marks were located on sagittally cut frozen sections of the fixed brain. The distance between the two marks was measured by projecting the slide onto a flat surface and placing a string along the Purkinje cell layer joining the two marks. All transverse distances between 1.2 and 6 mm were measured in this way. Purkinje cells were identified by the presence of a CFR. In the acute experiments, 96 pairs and 6 triplets were recorded. In summarizing the results, the 3 pairs in each triplet have been included with the others, making a total of 114 pairs.

Stimulation experiments

A pair of sharpened tungsten electrodes, each insulated to approximately 30 μm from the tip and with a separation of about 100 μm was lowered 0.4 mm below the cerebellar surface. The stimulating pair remained fixed while a recording electrode was put at various positions nearby. After finding a CFR with the recording electrode, it was tested for responsiveness to the cortical stimulus. Single pulses of 0.1–0.5 msec duration and 0.1–4.0 ma intensity were used. Stimuli were delivered through an RF stimulus isolation unit from a Tektronix pulse generator.

Chronic experiments

Two animals were prepared a few days before recording. Under pentobarbital anesthesia a circle of bone was removed over the cerebellum and a small plastic cylinder fitted over it and fixed to the bone with dental cement. The cylinder was filled with saline and capped with a plastic top. A block of wood for stabilization was fixed to the bone anteriorly using dental cement and a stainless steel screw with wire attached was then fastened to the bone to serve as an indifferent electrode. Records were made from each guinea pig with the head stabilized on four separate occasions. Two micropipettes were put in an electrode holder and placed in contact with the dura. After filling the plastic cylinder with agar solution, the electrodes were lowered independently into the brain, through the dura. The animal would often remain quiet for an hour or more; sometimes, however, a sedative dose of pentobarbital was necessary (2.5–5.0 mg/kg). The animals were always released after recording a CFR pair and would then walk about and eat. The findings from the chronic experiments are reported separately at the end of the RESULTS section.

Analysis

The cross-correlograms and other statistics describing the pattern of climbing fiber activity

were plotted by hand from filmed data. In most cases, there was more than 200 events in each member of the CFR pair and in no case were there fewer than 100. The relationship between simple spikes was studied by digitizing the occurrence times of all spikes (see RESULTS) and calculating cross- and autocorrelograms on an IBM computer.

RESULTS

Correlations among climbing fiber responses and their spatial distribution

The typical extracellular CFR with its associated simple spike described in the introduction is recorded deep in the molecular layer from the region of the Purkinje cell body. However, a microelectrode at a more superficial level frequently records a broad (2–10 msec) wave with two or more superimposed ripples which is not associated with simple spikes (Fig. 9*B, D*). Several features of these waves indicate that they are CFRs recorded from a dendritic rather than somatic region of the Purkinje cell. The first component is all or none and later components are added or removed in the same fashion. Sometimes one of these waves may be tranformed into a typical CFR with a large, sharp intial spike as the electrode is lowered. Such all-or-none waves have been evoked with monosynaptic latency by white matter stimulation (15). They give double responses to stimulation of the cerebellar surface and the latencies and following capacities of these responses are entirely similar to those of the more typical CFR (see below). Their slow and irregular discharge pattern, and the broad symmetric cross-correlograms between pairs of them, are entirely similar to the discharge patterns and correlograms of typical CFRs. For these reasons, such waves are considered to be CFRs and are not put in a special category. They are probably the response of the Purkinje cell's dentritic tree (15) but whatever their origin, they reflect the discharge pattern of the climbing fiber to that particular cell, as does the typical CFR. Occurrence of a CFR indicates the immediately prior occurrence of a single impulse or brief burst of impulses in the climbing fiber and also in the cell of origin of the climbing fiber.

The climbing fiber responses from a pair or triplet of Purkinje cells often occurred at about the same time. This can be seen in the original record of Fig. 1, and in the cross-correlograms of Fig. 2. There was a remarkable uniformity in the shapes of cross-correlograms from different pairs of cells. Broad symmetric peaks (about 50 msec wide) were in almost all cases centered at the point of zero delay. Symmetric valleys were always present and secondary peaks about 130 msec were common in both the forward and backward directions. These valleys and secondary peaks may be seen in Fig. 2 and more clearly in Fig. 6.

We were interested in how the correlation between two CFRs varied with the folial orientation and the distance separating the two Purkinje cells. To do this, a quantitative estimate of the temporal relationship was necessary. The uniformity of shape among different cross-correlograms suggested the method illustrated in Fig. 3. The number of CFRs of one cell which

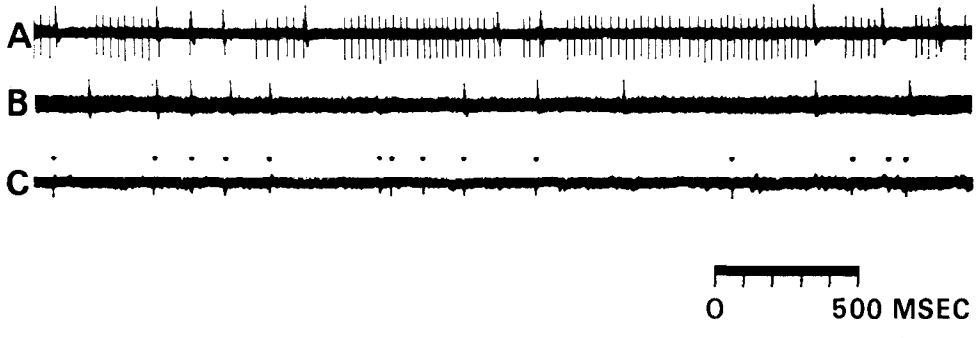

FIG. 1. Spontaneously occurring CFRs recorded simultaneously on three separate electrodes. *A:* both CFRs and simple spikes; *B:* CFRs only; and *C:* a small, negative event treated here as a CFR because of its discharge properties and the shape of its cross-correlograms with CFRs in *A* and *B*. Its occurrence is marked with dots. Figures 6 and 7 are based on much longer samples of trains A and B.

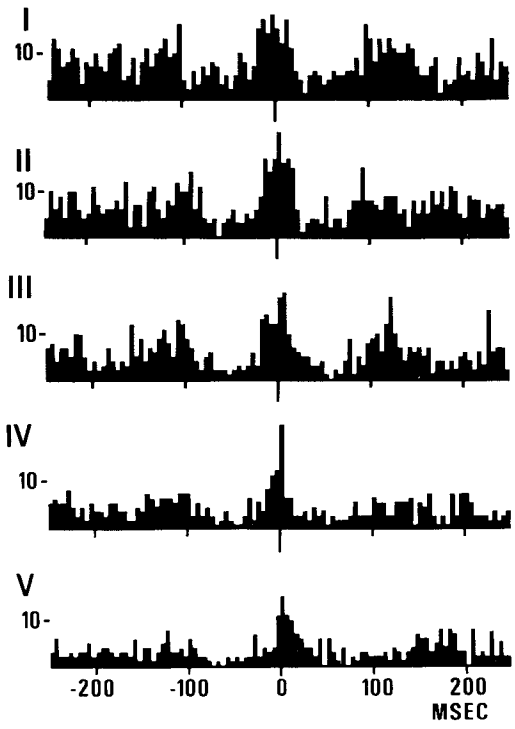

FIG. 2. Cross-correlograms between climbing fiber responses of two neighboring Purkinje cells. The pair in *IV* were separated in the transverse direction by 500 μ; others were less than 50 μ apart in the longitudinal direction. The total number of events during the analysis period in the reference train, i.e., the train correlated from, was 713, 690, 538, 424, and 387 in *I, II, III, IV,* and *V,* respectively.

occurred between 20 msec before and 20 msec after the CFR of the second or reference cell was taken as the number in the peak of the cross-correlogram (Np). The number in the valleys (Nv) was estimated by counting those CFRs of one cell which occurred either between − 70 and − 50 or between + 50 and +70 msec with respect to a CFR in the reference cell. Np and Nv are thus both based on the same interval of time, 40 msec. In the absence of a temporal correlation between a pair of CFRs the expected value of Np and Nv would be the same since there would be no reason for more events to occur in a particular 40-msec period with respect to the reference cell than in any other such period. In the absence of a temporal relation the ratio Np/Nv would therefore be close to 1. This method is similar to one used by Noda and Adey (21) in evaluating cross correlations among cerebral cortical cells.

The ratio Np/Nv was calculated for each of 114 pairs of CFRs. In the upper graph of Fig. 4, the ratio for each longitudinally separated pair is plotted against the distance in millimeters between the two cells. In the lower graph the ratio for each transversely separated pair is also plotted against distance. The two dots with small lines attached refer to cross-correlograms in which there was a clear central peak with surrounding valleys but it was displaced 10–20 msec from the zero delay point. In these cases the ratio was computed with the central peak as the reference rather than the zero delay point.

It can be seen that the amplitude of the correlation falls off rapidly and smoothly in the longitudinal direction, reaching an asymptote of 1 at about 1.5 mm. In the

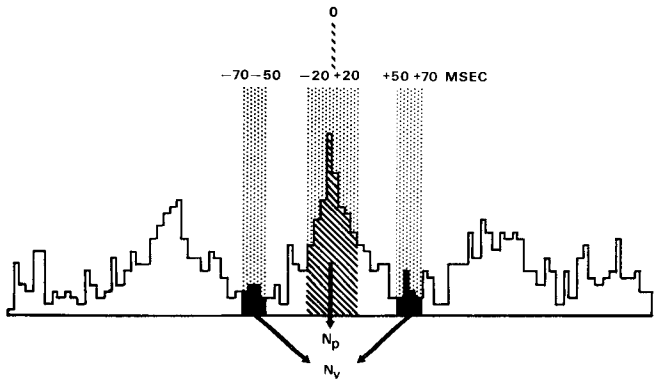

FIG. 3. Diagram showing how the number of events in the peak, Np, and the number of events in the valleys, Nv, were determined for each cross-correlogram. Np was the total number of events between −20 and +20 msec, Nv was the sum of the events between −70 and −50 with those between +50 and +70.

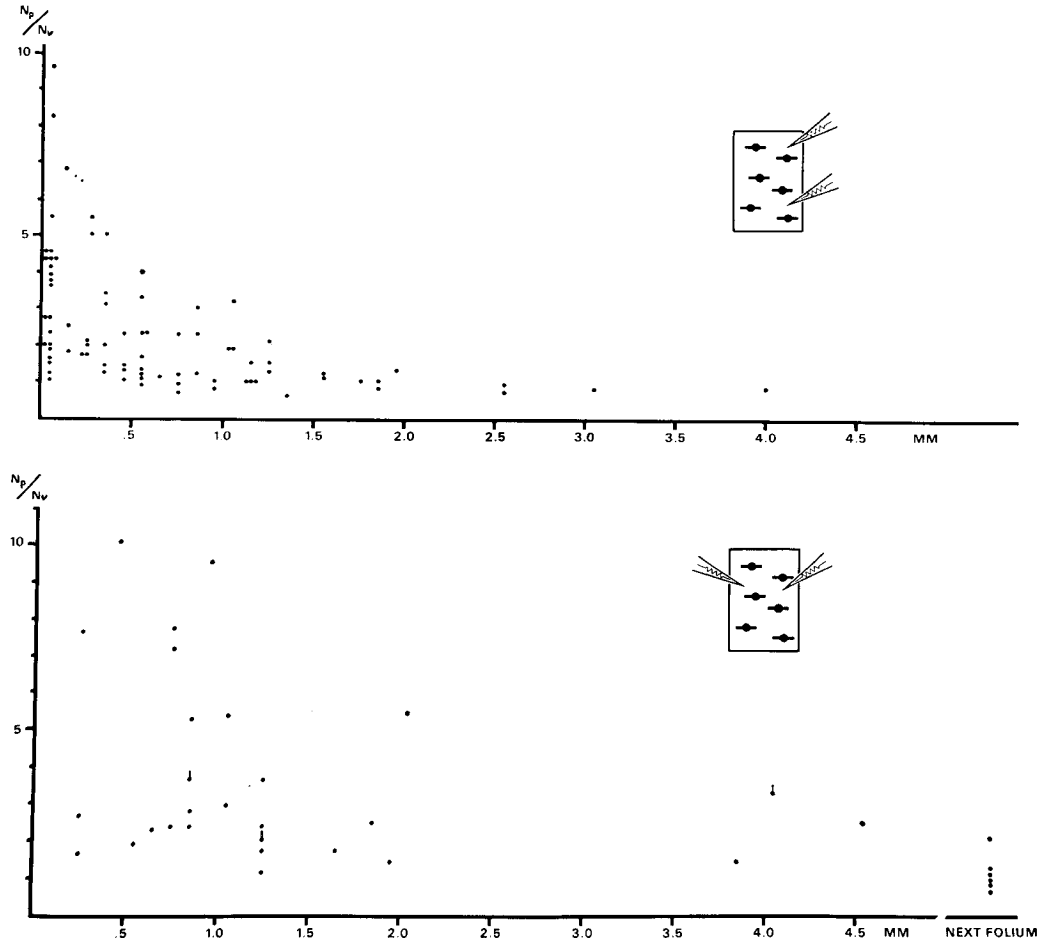

FIG. 4. Degree of temporal correlation between the CFRs of two Purkinje cells as a function of spatial separation in the longitudinal (upper graph) and transverse (lower graph) directions. The small lines attached to two points in the lower graph show two CFR pairs in which the central peak was displaced 10–20 msec from the point of zero delay. Insets show folial orientation of recording electrodes.

transverse direction, however, there is not such a rapid decline. For example, in the longitudinal direction all pairs with ratios greater than 5, indicating a strong correlation, were found at less than 0.4 mm distances. In the transverse direction, however, such pairs were found as far apart as 2.0 mm. Similarly, pairs of CFRs with a ratio of 2.0 were not found beyond 1.2 mm in the longitudinal direction but were seen at distances as large as 4.0 and 4.5 mm in the transverse. In examining a cross-correlogram one usually has the subjective impression of a temporal correlation when the ratio attains a value of 2.0 or more.

The temporal correlation between CFRs, while clear, was also rather rough, as shown by the broad peaks of the cross-correlograms. In one case, however, the CFRs of two different Purkinje cells occurred in nearly perfect synchrony. Figure 5 shows a few examples of recordings from this pair. Both the onset of the response and the peaks in the lower traces occurred 0.3 msec before those of the upper. Neither CFR ever occurred without the other, and in every instance the number and timing of peaks within the CFRs were the same in both. Where the third peak was delayed in one, it was also delayed in the other. This can be seen in the second and third parts of Fig. 5.

The two electrodes had a transverse orientation and the histology showed the marks

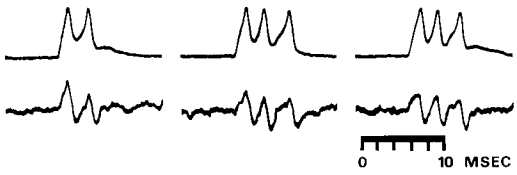

FIG. 5. Simultaneously recorded CFRs from two Purkinje cells. One is shown in the upper trace, another in the lower. The three sections of the figure are successive. Only the long intervening parts of traces without CFRs have been deleted. Positivity is up. The peak in the upper trace is about 2 mv, while the positive excursion in the lower trace is about 0.4 mv.

to be on opposite sides of the same folium, each deep in the molecular layer. Measuring along the Purkinje cell layer, the marks were 5.6 mm apart. Measured directly, through the white matter, they were 0.7 mm. All measurements were made in the fixed brain and there was no correction for shrinkage. The separate marks showed that two different Purkinje cells were recorded from and the synchronous CFRs indicated that each cell received a branch of the same climbing fiber. Such branching has been shown by others using anatomical (13) and less direct physiological methods (4, 12). It is likely that the recordings of Fig. 5 represent a dendritic response to climbing fiber input. If so, the perfect correspondence in number and timing of the peaks indicates that each peak is caused by a presynaptic impulse and that the whole complex is not a repetitive discharge following a single presynaptic impulse.

Rhythmicity of climbing fiber activity and a possible mechanism

There was a rough rhythmicity in the climbing fiber activity in addition to a rough synchronization. The common occurrence of an interval of about 130 msec can be seen in the discharge sequences of all three CFRs in Fig. 1. The likelihood of such intervals between spontaneous CFRs has also been noted in the frog (26). Examination of Fig. 1 also shows that the CFRs of one cell tended to occur not only at about the same time but also 130 msec before or after the CFR of another cell. A similar rhythmicity was present in most CFR sequences and pairs of such sequences. It can be seen in a more quantitative manner in the statistical analyses of Fig. 6. In Fig. 6, *II* and *III* each shows a first-order interval histogram (filled columns) and autocorrelogram (filled columns plus dotted region). Both interval histograms show the frequent occurrence of intervals between CFRs of about 130 msec. The interval histogram of 6*III* suggests that inter-

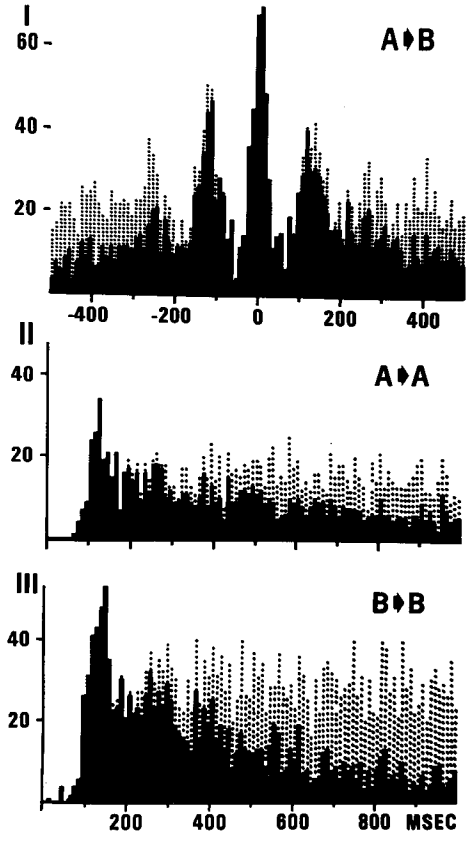

FIG. 6. Cross- and autocorrelograms together with appropriate interval histograms for a pair of CFR trains. *I:* the cross-correlogram is shown by the filled columns plus dots while the cross-interval histograms in both directions are shown by the filled columns alone. Filled columns to the right of zero delay plot forward cross intervals (time between each CFR in A and the first succeeding CFR in B) filled columns to the left plot backward cross intervals (time between each CFR in A and the immediately preceding CFR in B). There are 1,500 events in the reference train. *II:* the autocorrelogram of CFRs in train A is also shown by the filled columns plus dots, while the interval histogram is shown by the filled columns. Autocorrelogram and interval histogram based on 1,570 events. *III:* the same but of CFRs in train B. Autocorrelogram and interval histogram based on 1,300 events.

vals with durations 2 and 3 times the basic interval are also likely to occur. The secondary peaks in the cross-correlograms of Figs. 2 and 6*I* reflect the tendency of a CFR of one cell to occur either 130 msec before or 130 msec after a CFR in the other. In 6*I* the filled columns alone show the first-order cross-interval histograms in both directions, while the filled columns plus the dotted region show the cross-correlogram. The cross-correlogram represents the probability of any spike in B (not just the first) relative to A, and is thus the sum of cross intervals of all orders (24). The small peak in the cross-interval histogram to the left at about 260 msec indicates the common occurrence of a cross interval between the CFR of one cell and the CFR of another of twice the basic 130 msec duration. The rhythmicity in CFR activity described here is predominantly seen in the common occurrence of a single interval of about 130 msec and only rarely as a sequence of two or more such intervals such as appears at the left of Fig. 1. It must also be pointed out that the rhythmicity, like the synchronization, is rather rough, as shown by the broadness of both the interval histogram peaks and of the secondary peaks in the cross-correlogram.

Nevertheless, the rhythmicity is clear and it is possible to inquire into its origin. A priori, there appears to be two equally likely explanations: *1*) an intrinsic pacemakerlike process in each cell such that after the cell fires it tends to fire again at an interval of about 130 msec; *2*) a periodic, extrinsic input common to both cells. An editing procedure carried out on the cross intervals between the pair of CFRs shown in Fig. 1*A* and *B* and in Fig. 6 shows the second explanation to be correct. The method is similar to one which has been used to remove the influence of the intrinsic, recovery processes in a cell from its poststimulus time histogram (16). By removing these cross intervals in which the first cause, the intrinsic rhythmicity of the two cells, could be a determining factor, one is able to evaluate the strength of the second possible cause, namely rhythmicity in the input shared by the two cells. Considering only the histogram of forward cross intervals between events in train A and events in train B, one can remove the effect of an intrinsic rhythmicity in A by plotting only those cross intervals (first order) in which there is no intervening event in A before the occurrence of the next event in B. Recall that the definition of a forward cross interval between A and B is the time between any event in A and the next event in B, regardless of other events in A occurring before the event in B. By ruling out all cross intervals in which such intervening A events occur one rules out the possibility of a second event in A occurring at 130 msec (due to A's intrinsic recovery process) and by exciting B, causing the secondary peak. The influence of a possible intrinsic rhythmicity in A can thus be edited out. One can remove the effect of cell B's intrinsic rhythmicity by plotting only those cross intervals in which no event in B occurs between time 0 and 200 msec before the time of A. This rules out the possibility that spikes in B occurring between time 0 and 200 msec before a spike in A could, because of B's intrinsic periodicity, cause the secondary peak. The upper histogram of Fig. 7 is a plot of the cross

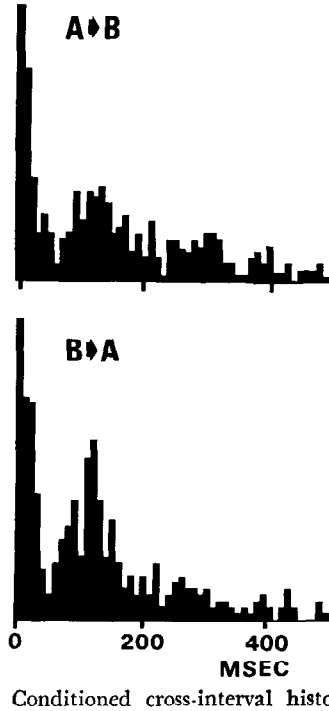

FIG. 7. Conditioned cross-interval histograms of climbing fiber responses whose cross- and autocorrelograms are plotted in Fig. 6 (see text for explanation of figure). A to B, 314 cross intervals; B to A, 363 cross intervals.

intervals from A to B which remain after the above deletions. It might be referred to as a conditioned cross-interval histogram (first order). The lower histogram is of the same type but made in the opposite direction (train B to train A). The conditions are perhaps more strict than necessary and many intervals were rejected, nevertheless, the secondary peaks are still large. This suggests that if an intrinsic pacemaker is present, it cannot be a complete explanation and that shared periodic input to the cells giving rise to the two climbing fibers must play an important role in causing the secondary peaks. No direct inference about the central peak can be made from the histograms of Fig. 7; an effect of direct excitatory interconnections between the two cells cannot be excluded. However, the suggestion is strong that the periodic shared input from other sources also plays a role in forming the central peak. Such a periodic shared input could not only determine the cross-correlograms but also the rhythmicity seen in the individual trains.

As shown by the second and marginal third peaks of Fig. 7 the rhythmic input shared by the two cells giving rise to the climbing fibers has approximately the same period of 130 msec noted earlier in the individual CFR trains. This, together with the evidence for recurrent collaterals among the cells of origin of the climbing fibers (3, 9, 10, and below), suggests a possible source of the shared input, namely, the roughly synchronized discharges among other cells of origin whose collaterals contact both neurons. Such discharges would have the right periodicity. Some evidence for the role of such collaterals in causing the synchronized, spatially oriented, and rhythmic activity of climbing fibers is given below in the section on stimulation of the cortical surface.

Correlations among simple spikes

The simple spikes of 15 different pairs of Purkinje cells were digitized and processed by computer. This is the total number of recordings in which it was judged that the spikes were of constant amplitude, of approximately constant mean rates, and of sufficient number (more than 1,500 spikes in each separate channel). All pairs were oriented in the longitudinal direction of the folium and the distance between electrodes was never more than 65 μm. Unfortunately, it was not feasible to delete the CFRs from these data. However, since the CFR only occurs from $\frac{1}{10}$ to $\frac{1}{20}$ as frequently as the simple spike and the correlation between CFRs, while present, is not dramatic, including the CFRs would probably not have a marked effect on the simple spike cross-correlogram. In addition, the durations of the two correlations are completely different. The peaks in the simple spike correlograms of Fig. 8 are only 4 msec wide, while those in the CFR correlograms are about 50 msec (Fig. 2).

Among the 15 pairs analyzed there were 3 with large, nearly symmetrical bimodal peaks (as in Fig. 8*I*) and two with small monomodal peaks (as in Fig. 8*II*). In 10 pairs the correlograms were marginal or flat, showing little or no sign of interaction. In one of the marginal pairs, an inhibitory relationship is suggested (Fig. 2*III*). These simple spike results are entirely similar to earlier findings in the cat (6). Several pairs of cells which showed a strong correlation in simple spikes also showed a correlation in their CFRs. For example, the CFR correlogram of Fig. 2*II* is derived from cells whose simple spike correlogram is shown in Fig. 8*II*, and the correlogram of Fig. 2*I* from cells showing a simple spike correlogram similar to that of Fig. 8*I*.

CFRs following surface stimulation

If the correlation between CFRs is due to shared recurrent collaterals among the cells giving rise to climbing fibers and if its transverse orientation is correct, a set of such cells, connected together by recurrent collaterals, should project onto a transversely oriented region of the cortical surface. The aim of the stimulation experiments was to test this hypothesis.

Cerebellar surface stimulation often evokes two CFRs from nearby Purkinje cells (Fig. 9), an early one with a latency of 2–8 msec and a later one at 8–16 msec. The first response follows the stimulus to frequencies as high as 50/sec, while the second fails to occur at rates above 4/sec. The first response is due to activation of other branches of the same climbing fiber axon

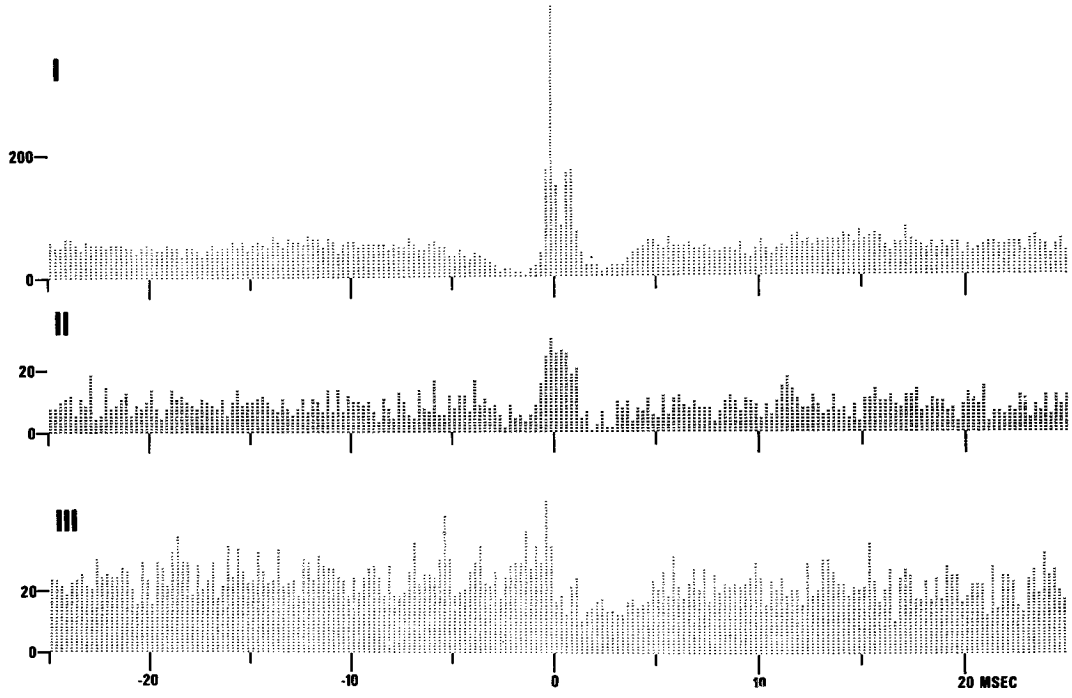

FIG. 8. Cross-correlograms between simple spikes of two neighboring Purkinje cells. Electrode separations were all in the longitudinal direction and were 25, 35, and 30 μm in *I, II,* and *III,* respectively. Bin width is 0.25 msec. There were 2,390, 1,881, 2,400 reference events in *I, II,* and *III,* respectively.

(4, 12). The late response may be attributed at least in part to synaptic activation via the recurrent collaterals among cells of origin of the climbing fiber. Some of the late responses are probably due to another mechanism, namely rebound spikes generated within individual cells by prolonged depolarization following antidromic invasion. Such rebound spikes have been seen in cells of the inferior olive nucleus, a major source of climbing fibers (3, 9). However, a synaptic origin for a large portion of the late CFRs is indicated by the following: *a*) The two responses often had different thresholds and sometimes only one or the other type could be evoked, regardless of

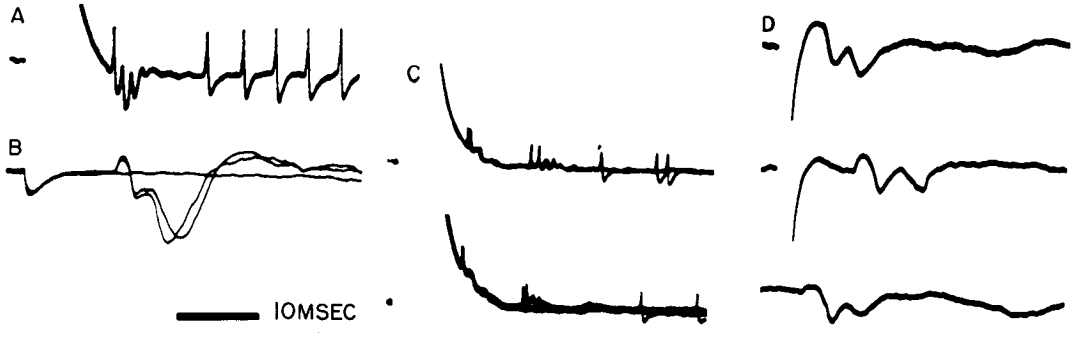

FIG. 9. Examples of late and early CFRs evoked by stimulation of nearby surface points. *A:* CFR with some simple spikes; *B:* three superimposed traces at same stimulus intensity—a molecular layer CFR is evoked on two occasions; *C:* superimposed sweeps showing both early and late responses (a simple spike just before the late CFR occurs in both traces and should be ignored). Stimuli in the upper trace were given every 2.5 sec and the late response occurs each time. It fails to follow stimulation at 2/sec as shown in lower trace. *D:* the first trace shows just the early response of a molecular layer CFR, the second shows just the late response (with a lower threshold), and the bottom trace, a spontaneous CFR.

intensity, indicating separate fiber pathways for the two responses. *b*) In addition to antidromic invasion, Crill (9) consistently found EPSPs in inferior olive cells following cerebellar stimulation. At higher stimulus intensities the EPSPs were large enough to evoke spikes. The latencies of these EPSPs were consistent with conduction of an antidromic impulse down the climbing fiber axon and with monosynaptic activation of the olive cell. Because of our interests here, only the explanation of the late CFR as a synaptically evoked response via recurrent collaterals is considered further.

Both early and late responses showed similar spatial distributions. In the transverse direction both could be evoked from as far away as the next folium but in the longitudinal direction, only a few points beyond 0.7 mm were effective and these required higher currents than points close to the stimulus electrodes. Fig. 10 is a plot of the number of late responses which occurred against distance from the stimulating electrode for both longitudinal (upper graph) and transverse (lower graph) directions. A completely similar pair of graphs could have been included for the early or direct response. Where only one CFR occurred, instead of two, it was classified as a late response if the latency was greater than 8 msec. If the response had a threshold higher than 2 ma, it was categorized as "no response." Folia are rarely more than 1.2 mm across, making it difficult to measure distances greater than this in the transverse direction. It was thought that finding responses on neighboring folia made it unnecessary to study intermediate distances.

In 11 experiments (5 on VI, 6 on V) in which the lobule was checked after perfusion, there was no obvious difference between lobule V and VI in the effective longitudinal distance.

Chronic experiments

In five pairs of CFRs recorded from unanesthetized animals, there was sufficient data to plot cross-correlograms. In all cases the separations were less than 0.3 mm and in the longitudinal direction. One plot showed only a marginal temporal relationship; the remaining four plots are in

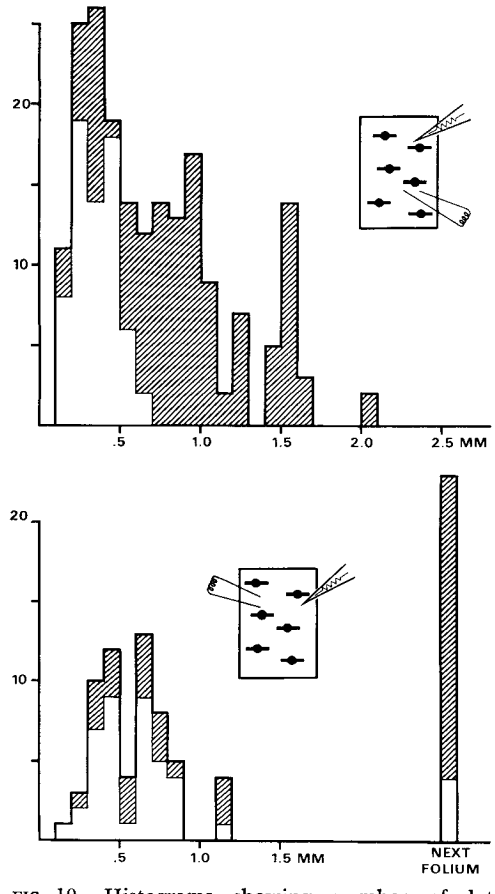

FIG. 10. Histograms showing number of late responses recorded at different distances from the stimulating electrodes in the longitudinal direction of the folium (upper graph) and in the transverse direction (lower graph). Open areas show the number of such responses at each distance, hatched areas indicate those CFRs which either could not be evoked by the stimulus, which showed only a short latency response, or whose late response had a threshold between 2 and 4 ma (five cases).

Fig. 11*I*. The central peaks and valleys of the upper three are similar in width and amplitude to those found in the anesthetized animals, while the lowest plot is rather different. The central peaks of these correlograms are not as consistently centered on zero. Furthermore, the rhythmicities or secondary peaks are not so large as in the anesthetized cases, although a most common interval can be seen in the interval histograms of Fig. 11*II*. In summary, the major feature of a rough synchronization in climbing fiber activity appears to be present in the unanesthetized as well as the anesthetized preparation.

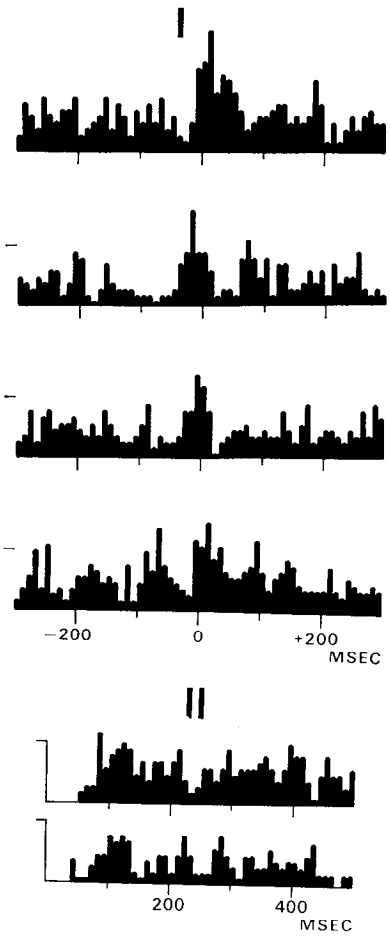

FIG. 11. *I:* cross-correlograms between pairs of spontaneously occurring CFRs in awake unanesthetized animals. On one occasion the animal was completely free of medication (third from top). In the others the animal was lightly sedated with 2–5 mg/kg of pentobarbital 1–5 hr before recording. From the top down, the numbers of reference events was 266, 397, 449, and 271, respectively. *II:* interval histograms of the two CFRs whose cross-correlogram is at the top of *I*. There are 296 intervals in the upper graph, 221 in the lower. No intervals longer than 500 msec are plotted.

DISCUSSION

Synchronization and rhythmicity of climbing fiber activity

Different authors (2, 3, 9, 28) have noted that the response to a given input of the inferior olive, a major source of climbing fibers (10), is deeply depressed for about 100 msec following a response to the same or a second input. Some have also noted a facilitatory period following the 100-msec depression and a second period of decreased responsiveness beginning after the facilitation. These effects may be explained by intracellular studies of inferior olive cells which show a highly consistent depolarization-hyperpolarization sequence following any effective input including antidromic activation from the cerebellar cortex (9). The hyperpolarization lasts about 100 msec and is followed by a second depolarization on which action potentials may be superimposed. The hyperpolarization has been shown to be a true IPSP and cannot be evoked by intracellular stimulation. These results could be explained if the firing of each cell evoked an EPSP-IPSP sequence of similar time course in other olive cells. As mentioned above, such recurrent effects provide a mechanism for the periodicity and synchronization of climbing fiber activity. The mechanism is similar to that proposed for the synchronized, rhythmic activity of thalamic cells (1) with the addition, however, of recurrent excitatory effects. After the discharge of one olive cell, neighboring cells might also fire because of the recurrent excitation. The increase in excitability within such a group would be followed by a prolonged reduction because of recurrent inhibition. After inhibition the whole group of cells will recover their excitability at about the same time and some will fire causing a large burst of activity. The result is an activity pattern with a period determined by the duration of the IPSP, and a synchronization which is the result of recurrent excitation, and the fact that the whole group has simultaneous periods of reduced excitability.

In considering these suggestions, it should be mentioned that histologists (25, 27) describe very few recurrent collaterals of olive cell axons. Furthermore, as pointed out by Armstrong and Harvey (3), there is no likely candidate for the inhibitory interneurons since all olivary neurons degenerate after cerebellar ablation (7). The presence of sites of close apposition of dendrites in the inferior olive suggests the possibility of dendro-dendritic contacts playing a role (34).

It is clear that the synchronization and rhythmicity of the CFRs are far from perfect. The cross-correlograms of CFRs in the

anesthetized animals have broad peaks and the rhythmicity is not detectable beyond the first two periods. In unanesthetized animals, the rhythmicity is even less obvious and correlations are just as broad. Input from outside the olive, not examined here, is superimposed on and probably modulates the regularity generated within it. For example, sharper correlograms might be associated with particular stimuli or motor activity. There seems to be a great deal of intrinsic variability in the climbing fiber system, however. Thach (31) found in chronic studies with trained monkeys that while CFRs often occurred in relation to a simple wrist movement, the time between a CFR and the movement could vary widely from trial to trial, as much as 100 msec.

Organization of climbing fiber activity into transverse strips

The stimulation experiments were undertaken to see if the presumed coupling via recurrent collaterals showed a spatial distribution similar to that of the spontaneous cross-correlation and this prediction was confirmed. In these experiments, as in those of others (4, 5, 12), an early response was evoked. This is probably caused by branching of olive cell axons providing climbing fibers to more than one Purkinje cell. The only point added here is the physiologic demonstration that such branching can occur within a single folium. This has already been shown anatomically (13) and Armstrong et al. (4) have presented indirect evidence for it. The latter authors have shown that separate branches of olive cell axons can go to both the anterior paravermal region and to the paramedian lobules. Faber and Murphy (12) have shown such branching to separate anterior lobe folia.

Both Armstrong et al. and Faber and Murphy found that the branches of a single axon were confined within relatively narrow transverse strips (defined with respect to the folia). Faber and Murphy suggested that such branching could explain the finding of Oscarsson's (22) that the projection of forelimb and hindlimb over particular spino-olivo-cerebellar pathways is onto transversely oriented strips of cerebellar cortex. Synaptic coupling via recurrent collaterals provides another mechanism for this. Input to a restricted part of the olive would activate a strip of Purkinje cells, both because of the branching of single axons and because of organization of synaptic connections in the inferior olive. The rarity of climbing fiber branching compared to correlation of activity among our recorded pairs suggests that synaptic connections play the major role. An anatomical basis for the above physiological findings is provided by the work of Voogd (33) who concluded from degeneration studies that a small region of the inferior olive projects onto a transverse strip of cerebellar cortex extending over several lobules.

Possible significance of climbing fiber activity patterns

There is physiological (17) and anatomical (18, 33) evidence that the efferent projection from the cerebellar cortex onto cerebellar and vestibular nuclei is organized in a sagittal manner. Ito et al. (17) found that the Purkinje cells whose axons impinge on a single neuron in Deiters' nucleus are located within a strip of cerebellar vermis oriented perpendicularly to the folial axis. This is also the orientation of the climbing fiber input. If Ito et al.'s finding is also true for other cerebellar nuclei, as the anatomical results suggest (18, 33), a particular region of the inferior olive and the imput to it would be able to influence single nuclear cells in a strong and preferential manner. This was in fact the suggestion of Ten Bruggencate et al. (30). On stimulating peripheral nerves while recording intracellularly from lumbar projecting Deiters' neurons, they found two types of IPSPs, both of which depended on an intact cerebellum. They interpreted the early, nonspecific, slowly developing IPSP as due to activation over the mossy fiber system and the later, specific, and sharp IPSP as due to climbing fiber activation. The combination of a synchronized, sagitally projecting, afferent system and a similarly oriented efferent system could thus result in a strong and specific influence of the inferior olive on cells of Deiters' nucleus and of the deep cerebellar nuclei. Deiters' nucleus gives rise to the lateral vestibulospinal tract and the

deep cerebellar nuclei have strong excitatory effects on those cells which give rise to the reticulospinal, rubrospinal, and corticospinal tracts (10). Because of these pathways between the cerebellar cortex and the motoneuron, a small region of the inferior olive could have an important influence on motor activity.

One would not expect such an influence to be exerted in a continuous, tonic manner because of the slow and synchronized discharge pattern among climbing fibers. In addition, it is well known that climbing fiber pathways to the cerebellum from the periphery or from other parts of the brain have a very poor following capacity, always failing at rates above 10/sec (2, 10, 22). Llinas (19) has, in fact, suggested that the climbing fiber system is important in phasic motor activity while the parallel fibers and the associated simple spikes of Purkinje cells are concerned with a continuous type of control.

There is some evidence from behavioral studies for phasic or intermittent control in the human and monkey manual control systems (8, 29). The phenomenon is seen, for example, in manual tracking where a person is asked to follow a moving stimulus with a pointer under his own control. When the stimulus is an unpredictable position ramp the response of the subject is seen to be a series of small, discrete, corrective steps (29). The frequency of such steps is limited, usually occurring at about 2/sec but sometimes going as high as 7/sec (29). The possibility of a strong influence of the climbing fiber system on motor output described above and the clear frequency limitation which is imposed on it suggests the possibility that climbing fiber activation is involved in this phasic activity.

The phasic aspect of motor activity is clearly seen when the response must be closely controlled but not when it is executed in an open loop or ballistic manner (8, 29). It is not clearly present, for example, in manual tracking when the stimulus ramps are repetitive and completely predictable to the subject (29). It is interesting in this respect that Thach (31) found CFRs to precede a wrist movement when it was made in response to an unpredictable external stimulus. However, when the same movement was made independently of external control and as part of a self-generated rapid alternation of the wrist, there was no correlation between CFRs and movement. It is possible then that climbing fiber activation occurs when some change in motor activity is required as a consequence, for example, of an unpredictable environmental change. It may play no role, however, in activity which is carried out in a preprogrammed manner, independently of the environment.

SUMMARY

Pairs of climbing fiber responses (CFRs) from nearby Purkinje cells were found to be correlated in their times of occurrence. The cross-correlograms were rather broad, symmetric, and showed evidence of rhythmicity with a period of about 130 msec. In one case only, a perfectly synchronized pair of CFRs was recorded from two separate Purkinje cells. This was probably due to branching of the climbing fiber axon. The rhythmicity could be seen in interval histograms and autocorrelograms of individual CFR trains, as well as in the cross-correlograms between pairs. A statistical argument showed that the rhythmicity did not arise from an intrinsic, pacemakerlike mechanism, but rather from rhythmic driving of both cells by a third source. The recurrent collaterals of other olive cells were suggested as such a third source. The correlation of CFRs was more limited in the longitudinal direction of the folium than in the transverse, that is, there seemed to be strips of correlated activity. Similarly, the synaptically evoked response of a CFR to surface stimulation could be evoked at greater distances in the transverse than in the longitudinal direction of the folia. Such a response is presumed to occur via impulses in recurrent collaterals. The similar spatial distributions of the spontaneous correlations and of the responses evoked over recurrent collaterals provides additional evidence for the proposed mechanism of the correlation, namely a sharing of input from recurrent collaterals. Similar correlations were found among pairs of CFRs

recorded from awake, unanesthetized animals.

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REFERENCES

1. ANDERSEN, P. AND ANDERSSON, S. A. *Physiological Basis of the Alpha Rhythm.* New York: Appleton-Century-Croft, 1968.
2. ARMSTRONG, D. M. AND HARVEY, R. J. Responses of a spino-olivo-cerebellar pathway in the cat. *J. Physiol., London* 194: 167P–168P, 1968.
3. ARMSTRONG, D. M. AND HARVEY, R. J. Responses in the inferior olive to stimulation of the cerebellar and cerebral cortices in the cat. *J. Physiol., London* 187: 553–576, 1966.
4. ARMSTRONG, D. M., HARVEY, R. J., AND SCHILD, R. F. Distribution in the anterior lobe of the cerebellum of branches from climbing fibers to the paramedian lobes. *Brain Res.* 25: 203–206, 1971.
5. ARMSTRONG, D. M., HARVEY, R. J., AND SCHILD, R. F. Branching of individual olivo-cerebellar axons to terminate in more than one subdivision of the feline cerebellar cortex. *J. Physiol., London* 202: 106P–108P, 1969.
6. BELL, C. C. AND GRIMM, R. J. Discharge properties of Purkinje cells recorded on single and double microelectrodes. *J. Neurophysiol.* 32: 1044–1055, 1969.
7. BRODAL, A. Experimentelle Untersuchungen uber die olivo-cerebellare Lokalisation. *Z. Ges. Neurol. Psychiat.* 169: 1–153, 1940.
8. BROOKS, V. B. AND STONEY, S. D. Motor mechanisms: the role of the pyramidal system in motor control. *Ann. Rev. Physiol.* 33: 337–392, 1971
9. CRILL, W. E. Unitary multiple-spiked responses in cat inferior olive nucleus. *J. Neurophysiol.* 33: 199–209, 1970.
10. ECCLES, J. C., ITO, M., AND SZENTAGOTHAI, J. *The Cerebellum as a Neuronal Machine.* New York: Springer, 1967.
11. ECCLES, J. C., LLINAS, R., AND SASAKI, K. The excitatory synaptic action of climbing fibers on the Purkinje cells of the cerebellum. *J. Physiol., London* 182: 168–296, 1966.
12. FABER, D. S. AND MURPHY, J. T. Axonal branching in the climbing fiber pathway to the cerebellum. *Brain Res.* 15: 262–267, 1969.
13. FOX, C. A., ANDRADE, A., AND SCHWYN, R. C. Climbing fiber branching in the granular layer. In: *Neurobiology of Cerebellar Evolution and Development,* edited by R. S. Llinas. Chicago: Am. Med. Assoc., 1969, p. 603–611.
14. FREEMAN, J. A. The cerebellum as a timing device: an experimental study in the frog. In: *Neurobiology of Cerebellar Evolution and Development,* edited by R. S. Llinas. Chicago: Am. Med. Assoc., 1969, p. 397–420.
15. FUJITA, Y. Activity of dendrites of single Purkinje cells and its relationship to so-called inactivation response in rabbit cerebellum. *J. Neurophysiol.* 31: 131–141, 1968.
16. GRAY, P. R. Conditional probability analyses of the spike activity of single neurons. *Biophys. J.* 7: 759–777, 1967.
17. ITO, M., KAWAI, N., AND UDO, M. The origin of cerebellar induced inhibition of Deiter's neurones. III. Localization of the inhibitory zone. *Exptl. Brain Res.* 4: 310–320, 1968.
18. KORNELIUSSEN, H. K. Cerebellar organization in the light of cerebellar nuclear morphology and cerebellar corticogenesis. In: *Neurobiology of Cerebellar Evolution and Development,* edited by R. S. Llinas. Chicago: Am. Med. Assoc., 1969, p. 515–523.
19. LLINAS, R. Neuronal operations in cerebellar transactions. In: *The Neuroscience, Second Study Program,* edited by F. O. Schmitt, New York: Rockefeller Univ. Press, 1970, p. 409–426.
20. MOORE, G. P., SEGUNDO, J. P., PERKEL, D. H., AND LEVITAN, H. Statistical signs of synaptic interaction in neurons. *Biophys. J.* 10: 876–900. 1970.
21. NODA, H. G. AND ADEY, W. R. Firing of neuron pairs in cat association cortex during sleep and wakefulness. *J. Neurophysiol.* 33: 672–684, 1970.
22. OSCARSSON, O. The sagittal organization of the cerebellar anterior lobe as revealed by the projection patterns of the climbing fiber system. In: *Neurobiology of Cerebellar Evolution and Development,* edited by R. S. Llinas. Chicago: Am. Med. Assoc., 1969, p. 525–538.
23. PERKEL, D. H., GERSTEIN, G. L., AND MOORE, G. P. Neuronal spike trains and stochastic point processes. I. The single spike train. *Biophys. J.* 7: 391–418, 1967.
24. PERKEL, D. H., GERSTEIN, G. L., AND MOORE, G. P. Neuronal spike trains and stochastic point processes. II. Simultaneous spike trains. *Biophys. J.* 7: 419–440, 1967.
25. RAYMON Y CAJAL, S. *Histologie du systeme Nerveux de l'Homme et des Vertebres.* Paris: Maloine, 1911.
26. RUSHMER, D. S. AND WOODWARD, D. J. Responses of Purkinje cells in the frog cerebellum

to electrical and natural stimulation. *Brain Res.* 133: 315–335, 1971.

27. SCHEIBEL, M. E. AND SCHEIBEL, A. B. The inferior olive, a Golgi study. *J. Comp. Neurol.* 102: 77–132, 1955.
28. SEDGWICK, E. M. AND WILLIAMS, T. D. Responses of single units in the inferior olive to stimulation of the hind-limb nerves, peripheral skin receptors, cerebellum, caudate nucleus and motor cortex. *J. Physiol., London* 189: 261–280, 1967.
29. STARK, L. *Neurological Control Systems, Studies in Bioengineering.* New York: Plenum, 1968.
30. TEN BRUGGENCATE, G., SONNHOF, U., TEICHMEN, R., AND WELLER, E. A study of the synaptic input to Deiter's neurones evoked by stimulation of peripheral nerves and spinal cord. *Brain Res.* 25: 207–211, 1971.
31. THACH, W. T. Discharge of cerebellar neurons related to two maintained postures and two prompt movements. II. Purkinje cell output and input. *J. Neurophysiol.* 33: 537–547, 1970.
32. THOMAS, R. C. AND WILSON, V. J. Precise localization of Renshaw cells with a new marking technique. *Nature* 206: 211–213, 1965.
33. VOOGD, J. The importance of fiber connections in the comparative anatomy of the cerebellum. In: *Neurobiology of Cerebellar Evolution and Development,* edited by R. S. Llinas. Chicago: Am. Med. Assoc., 1969, p. 397–420.
34. WALBERG, F. Further electron-microscopical investigation of the inferior olive of the cat. In: *Progress in Brain Research: Topics in Basic Neurology,* edited by W. Bargmann and J. P. Schadé. Amsterdam: Elsevier, 1964, vol. 6.