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PURKINJE CELL ACTIVITY DURING MOTOR LEARNING

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SUMMARY

Monkeys were trained to grasp a handle and move it in a horizontal arc to a central position by flexing or extending the wrist. A torque motor applied forces to the handle that switched at random intervals to alternately load flexor and extensor muscles. At each load switch, the handle was displaced transiently from the central position, and then moved back by the monkeys and held there steadily again. Recordings were made from cerebellar Purkinje cells (P-cells) whose simple spike (SS) activity was related to the task.

The magnitude of one of the oppositely directed loads was then altered and the monkeys took about 12–100 trials with the novel load before performing as regularly as previously. During this period with one known and one novel load, some P-cells underwent increases in complex spike (CS) frequency at specific times after the load switch. This increased CS frequency would last for a similar number of trials as that taken by the monkey to adapt to the novel load before decreasing to near its previous level. Associated with the increased CS frequency there were decreases in SS frequency that persisted after the CS frequency had decreased to near its previous level.

These results are consistent with theoretical proposals that motor learning takes place in the cerebellum through *changes* in the strength of transmission of parallel fiber synapses on P-cells caused by the climbing fiber input. These results further suggest that climbing fiber firing causes a *decrease* in the strength of parallel fiber synapses.

INTRODUCTION

A number of theories have shown how the neuronal circuits of the cerebellum could be used to store and retrieve information about movements, enabling the cerebellum to play a part in motor learning[2,3,8,9,11,15]. For these theories a key hypo-

thesis has been that the information is stored through changes in the strength of transmission of the granule cell synapses on cerebellar Purkinje cells (P-cells) caused by firing of the climbing fiber. Each P-cell has two types of excitatory input which generate different action potentials: simple spikes (SSs) are produced by the multiple parallel fiber inputs from granule cells and complex spikes (CSs) by the single climbing fiber input[5]. During learning, the SS output frequency of the P-cell in response to a specific parallel fiber input (pattern of firing in the parallel fibers) would be altered if there were CS activity at the time of occurrence of that input, but the SS output in response to other parallel fiber inputs would be left unchanged. This alteration in response of the P-cell to a parallel fiber input would persist with subsequent presentations of that input without any associated CS activity. The role of the climbing fiber would be to transmit the signals for the required changes in synaptic strength and not to play any other part in producing muscular activity. This hypothesis can be tested experimentally because SSs and CSs are distinguishable in extracellular recordings from P-cells. In two previous studies the activity of P-cells has been recorded from monkeys as they learned motor tasks[10,14]. However, no tests were made in these experiments to see if there was a correlation between CS activity and long-term changes in SS activity. In the present experiment, measurements were made of CS frequencies during motor learning and of long-term SS frequency changes, to enable testing of the hypothesis.

METHODS

Three monkeys *(Macaca mulatta)* were used in this experiment. The techniques were similar to those of previous experiments[18,19]. Each monkey was seated in a primate chair and was able to put his right hand through a small hole and grasp a vertical handle, which could be freely moved by flexion or extension of the wrist through a horizontal arc with a radius of 2.5 cm. He was trained to hold the handle within a fixed angular zone of 10°, whose location was indicated by a light signal when the handle was within this zone. This position had to be maintained against forces which were applied via a torque motor in either direction to the handle, so as to oppose either flexion or extension (these forces will be called flexor and extensor loads, respectively). After the handle had been kept in the correct zone for a random (uniformly distributed) time-interval ranging between 1.5–4.0 sec, the constant force which had been applied to the handle was abruptly switched to one in the opposite direction. This load switch displaced the handle from the correct zone, whereupon the monkey would move it back and hold it there steadily against the oppositely directed load until, after another similar random time-interval, the load switched back to the previous one and the procedure was repeated. Therefore each trial with a particular load consisted of a *transient period* during which the handle was moving and a *maintained period* lasting 1.5–4.0 sec during which the handle was being held steadily in the correct zone. The monkey was rewarded with several drops of fruit juice every 3 trials, and would usually perform over 3000 trials continuously each day, alternately holding against flexor and extensor loads.

Several months of training were given on this task with the flexor and extensor loads kept constant. Then an 18 mm diameter cylinder was attached to a hole cut in the skull vertically above the right anterior lobe of the cerebellum (centered 7 mm posterior to the interaural line and 10 mm lateral to the midline). Extracellular recordings of P-cells were obtained with glass-coated platinum–iridium microelectrodes which had been advanced down the cylinder and through the dura into the cerebellar cortex; the monkey's head was kept still through bolts attached to the skull.

A single penetration with the microelectrode was made daily over the course of several months in each monkey. If the SS discharge of the P-cell were not well-related to the movement, then long-term recordings were usually not made, and the electrode would be moved to record from other P-cells.

After a stable, well-related P-cell had been isolated the magnitude of either the flexor or extensor load was changed to a novel value while the other known load was kept constant to serve as a control. Changes in CS and SS activity were then observed as the monkey adapted to performing with the novel load. A number of trials were required with the novel load before the performance became as uniform and stereotyped as with the previous known load, and this adaptation to a novel load was used as a paradigm of motor learning for the present experiment. Changes were made in either the flexor or extensor loads, with the smallest change in load being 100 g. Loads would be increased or decreased and overall loads ranged between 100–600 g.

CSs were discriminated electronically from SSs on the basis of the greater positivity of the initial phase of their action potential, and rasters of CS and SS activity (as shown in Figs. 3 and 5) were generated on a storage oscilloscope and photographed throughout the course of the recordings. Activity of the P-cells was recorded on magnetic tape for subsequent computer determination of CS and SS frequencies. Signals indicating the times of application to the handle of the different loads, and a signal representing the angular position of the handle, were also recorded on separate channels. Electromyographic (EMG) activity of muscles performing in the task was recorded for a number of different loads.

RESULTS

Motor learning after introduction of a novel load

A first step in the experiment was to find out the approximate number of trials over which motor learning occurred so that recordings could be made from P-cells for as long as learning was taking place. EMG recordings showed that many arm and trunk muscles were active during performance of the task, especially during the transient period. During adaptation with a novel load alterations in the activity of various muscles occurred at different rates. No attempt was made to follow changes in activity of individual muscles as there was no way to decide which P-cells were involved in the control of particular muscles. Instead, the time course of motor learning was followed by observing changes in movement of the handle by the monkey.

Prior to a load change the monkey would perform the task in a uniform and stereotyped manner, as illustrated in Fig. 1 by the reproducible nature of the position

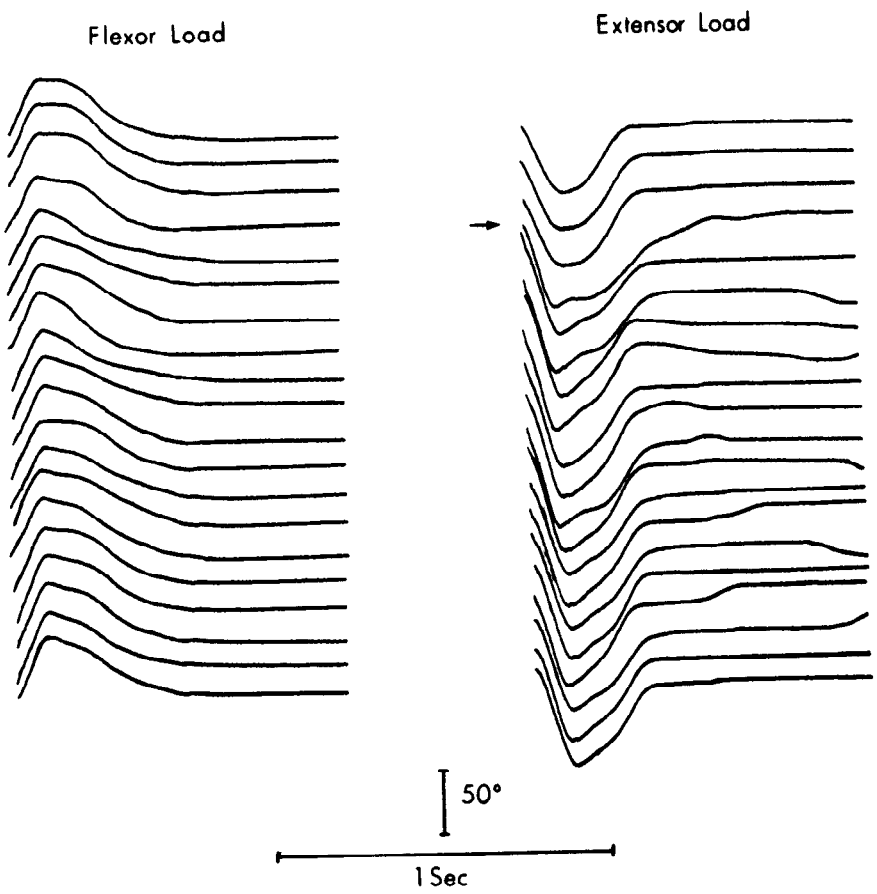

Fig. 1. Changes in task performance with the introduction of a novel load. The task was to move a handle in a horizontal arc by flexing or extending the wrist to a central position, and to hold it there despite flexor and extensor loads applied to the handle. Each trace starts as the load switched to one in the opposite direction, displacing the handle from the central position for a transient period of about 300 msec. Position traces of the handle are shown for successive trials (top to bottom) alternately against the flexor and extensor loads. Each flexor trace on the left is followed by an extensor trace on the right. With known loads the position traces were smooth and reproducible from trial to trial (above arrow). When the extensor load was increased from a known 300 g to a novel 450 g (at arrow), there were immediate irregularities in the position traces during the transient and maintained periods, which gradually diminished with further trials (below arrow). For flexor trials, there were a few irregularities in the transient period only, as the load switched from novel to known.

traces of the handle (above arrow). The monkey would carry out the task in an automatic fashion, frequently not looking at the light which indicated the correct position of the handle.

When the magnitude of the flexor or extensor load was altered, this stereotyped performance became irregular (see position traces below arrow in Fig. 1). There would be irregularities in both the transient and maintained portions of the position traces for initial trials with the novel load. Also the monkey would pay closer attention to the light which signalled the correct holding position. However, after a number of

trials these irregularities became smaller and eventually disappeared. The monkey was considered to have adapted to the novel load when the performance became stereotyped from trial to trial. The numbers of trials required for this adaptation varied between about 12–100. More trials were usually required for the adaptation the greater the difference in magnitude between the known and novel loads. In Fig. 1, after introduction of a novel extensor load (at the arrow), it can be seen that there were changes in performance under both the novel extensor load and the known flexor load. However, there were fewer irregularities in the position traces under the known flexor load, and the adaptation was more rapid.

There appeared to be several components in the adaptation to the novel load. Many of the irregularities in the position traces occurred during the maintained period, when the handle should have been held steadily in the correct zone. However, there were also irregularities in the transient period which occurred at very short time intervals after the start of each trial and many P-cells exhibited changes in CS and SS activity at similar time intervals with the introduction of a novel load. Some of these irregularities are visible in the position traces of Fig. 1 100–200 msec after the start of each extensor trial with the novel load (below arrow).

The maximum amplitude of the transient portion of the position traces (which occurs about 100 msec after the load switch at the start of each trial) also changed during trials immediately succeeding the introduction of a novel load. Thus, if the load applied to the handle was increased, there was an initial increase in maximum amplitude which gradually reduced with successive trials until it reached a stable value after about 10–20 trials; with a decrease in load there was an initial decrease and then a gradual increase in amplitude over a similar period.

There were general aspects of the task which were not altered when the load was changed, and any learned changes in motor performance were presumably superimposed upon this prior learning. Also there was the possibility that adaptation to a novel load would take place more quickly after the monkey had experienced a number of load changes and become familiar with this type of learning. However, provided there were sufficient trials between each introduction of a novel load (100 or more) the monkey would require similar numbers of trials to adapt to the novel loads throughout the course of the experiment.

Purkinje cell activity with known loads

Recordings were made for a number of trials from each P-cell, with known flexor and extensor loads before one of the loads was changed to a novel value and the resultant alterations in SS and CS frequencies observed. This was to ensure that there was a stable recording with good discrimination of CSs from SSs and that there were no spontaneous alterations in activity.

Out of recordings from 133 P-cells, 90 had SS discharges which were related to the task. The SS activity of 78 P-cells varied only in the transient period of each trial when the handle was displaced by the load switch and then returned to the correct zone; the SS frequency of these transient-related P-cells was the same when the handle was held steadily against the flexor and extensor loads. The SS activity of 12 P-cells

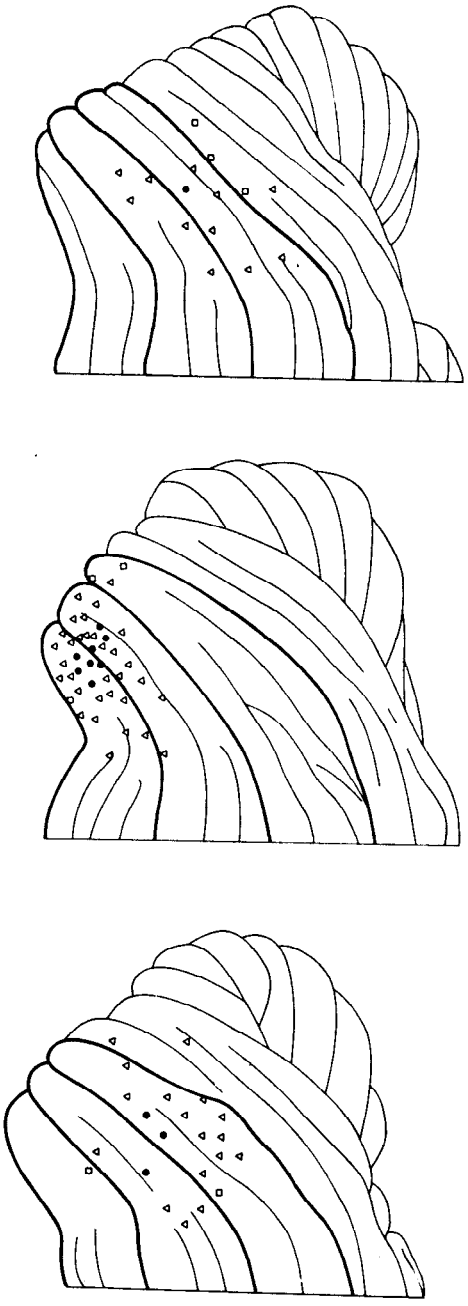

Fig. 2. Dorsal views of the cerebella of 3 monkeys, showing the location of penetrations in which P-cells were encountered. Filled circles represent penetrations where a P-cell had two different maintained SS frequencies while holding against two oppositely directed loads (load-related P-cell); open triangles, where a P-cell changed SS frequency only at the switch of loads (transient-related P-cell); and open squares, where no P-cell was consistently related to the task.

was related to the load applied to the handle when it was being held steadily in the correct zone, with two different SS frequencies for holding against the flexor and extensor loads. These latter P-cells will be referred to as load-related P-cells although the SS activity of these P-cells may have been related to other task variables besides load. All the load-related P-cells also had related SS transient activity.

The locations of the transient-related and load-related P-cells are indicated in Fig. 2 for the 3 monkeys. There was a widespread distribution of transient-related P-cells in lobules III, IV and V of the anterior lobe, while the load-related P-cells were restricted to a smaller area in the intermediate portion of the anterior lobe. EMG recordings showed that many more muscles were active during the transient than the steady holding period, which probably accounted for the difference in number of the two types of P-cell. Presumably the load-related P-cells were involved in the control of arm or wrist muscles, as these were the only ones which showed appreciable differential activity with flexor and extensor loads. The load-related P-cells were in a region of the cerebellum known to receive information about the arms[1,4,13].

Sixty-five of the 78 transient-related P-cells also had related CS activity. No P-cells with unrelated SS activity had related CS activity. When one of the known loads was changed to a novel value some of the CS-unrelated P-cells developed related CS activity, as will be described below. Transient-related P-cells had related CS activity only during the transient portion of the trials. Usually (48 P-cells) this related CS activity took the form of increased CS frequency at one point in either the transient with the flexor (19 P-cells) or extensor (29 P-cells) load, though for 17 P-cells there were related CSs for both flexor and extensor trials. Related CSs would occur from about 50 to 250 msec after the beginning of a trial at the load switch. The accuracy with which they were related to a particular period of the trial varied; for some P-cells related CSs were clustered within 20 msec of each other for successive trials, whereas for other P-cells they would occur at times which could differ by more than 100 msec. In general the earlier the times of occurrence of transient-related CSs within trials, the closer together were these times of occurrence. The frequency of the related CS activity also varied widely for different P-cells, from about one CS every sixth trial, up to an average of one or more CSs every trial. Three P-cells showed a reduction in CS frequency, compared with the spontaneous background frequency, during portions of the transient period. However, because of the very low spontaneous CS frequency (< 1 Hz for most cells), it was necessary to average over a large number of trials in order to determine significant reductions in CS frequency. For this reason it is possible that longer recordings would have revealed suppressions in CS activity for more of the P-cells.

Of the 12 load-related P-cells 7 had CSs related to the transient. None of these P-cells had significantly different maintained CS frequencies for many trials when holding steadily against flexor or extensor loads. There were different maintained CS frequencies over small numbers of trials for 5 of these P-cells when a novel load was introduced, as will be described below.

An interesting feature of transient-related CSs was that there was usually a very low or zero SS frequency at about the same time in the transient period of each trial

enetrations in which
1 two different main-
-related P-cell); open
sient-related P-cell);

as the increased CS frequency. The duration of this reduced SS frequency would correspond approximately with the period of increased CS frequency, and lasted from about 40 to 300 msec. The reduced SS frequency was not directly caused by the CSs because there would be a similar low SS frequency whether or not a CS occurred within a particular trial. For all the P-cells there were 89 transient-related CSs and for 70 of these the SS frequency was lower at the same time in the transient period than the average SS frequency for the P-cell.

Purkinje cell activity during adaptation to novel loads

Of recordings from 90 related P-cells, 28 (20 transient-related, 8 load-related) were of sufficient stability, with good discrimination of CSs from SSs, to enable observation of alterations in CS and SS activity with changes to novel loads. Usually, there were immediate alterations in CS and SS activity when the magnitude of one of the loads applied to the handle was changed, with alterations continuing for a number of trials after the novel load was introduced. Separate analyses of these changes were carried out for the transient and maintained periods of the task.

SS and CS changes in transient period. Fourteen transient-related P-cells and 5 load-related P-cells showed alterations in transient-related CS and SS frequencies with the introduction of a novel load; the remainder exhibited alterations in SS frequency only. Both types of P-cell showed similar alterations in transient-related activity with load changes, and the results will be combined.

Two of the P-cells had reduced transient-related CS frequencies after a load change. These reductions persisted for more than 100 trials after the load changes for both P-cells. There were no significant SS frequency changes associated with these reductions in CS frequency.

Seventeen P-cells had increases in transient-related CS frequencies which lasted for varying numbers of trials after novel loads were introduced. A raster of CS and SS activity for a P-cell with increased transient-related CS frequency is shown in Fig. 3. The CS and SS frequencies during successive trials for this P-cell are plotted in Fig. 4A. For trials where the monkey was performing with known loads (above arrow) there was a low frequency of related CSs about 100 msec after the start of extensor trials. When the extensor load was increased from 300 g to a novel 450 g there was an immediate increase in CS frequency at this time (see 50–150 msec bin for the extensor trials in Fig. 4A). This increased CS frequency lasted about 70 trials and then returned to about the same level as that prior to the load change. In the same 50–150 msec bin which had this increased CS frequency, there was a decrease in SS frequency (Fig. 4A). The SS frequency decreased throughout the period of increased CS frequency and remained at a reduced level after the CS frequency had declined to its original value. The SS frequency for the 50–150 msec bin (and bins with related CSs in other P-cells) was not determined by averaging from successive trials as for the other bins with unrelated CS activity. Instead, care was taken to exclude the effects on the SS frequency determination of the depression in SS frequency immediately following each CS[5] (which can be seen after many CSs in Figs. 3, 5). Therefore the SS frequencies for this bin were found by averaging only from trials in which a CS did *not* occur in that bin. The reason for

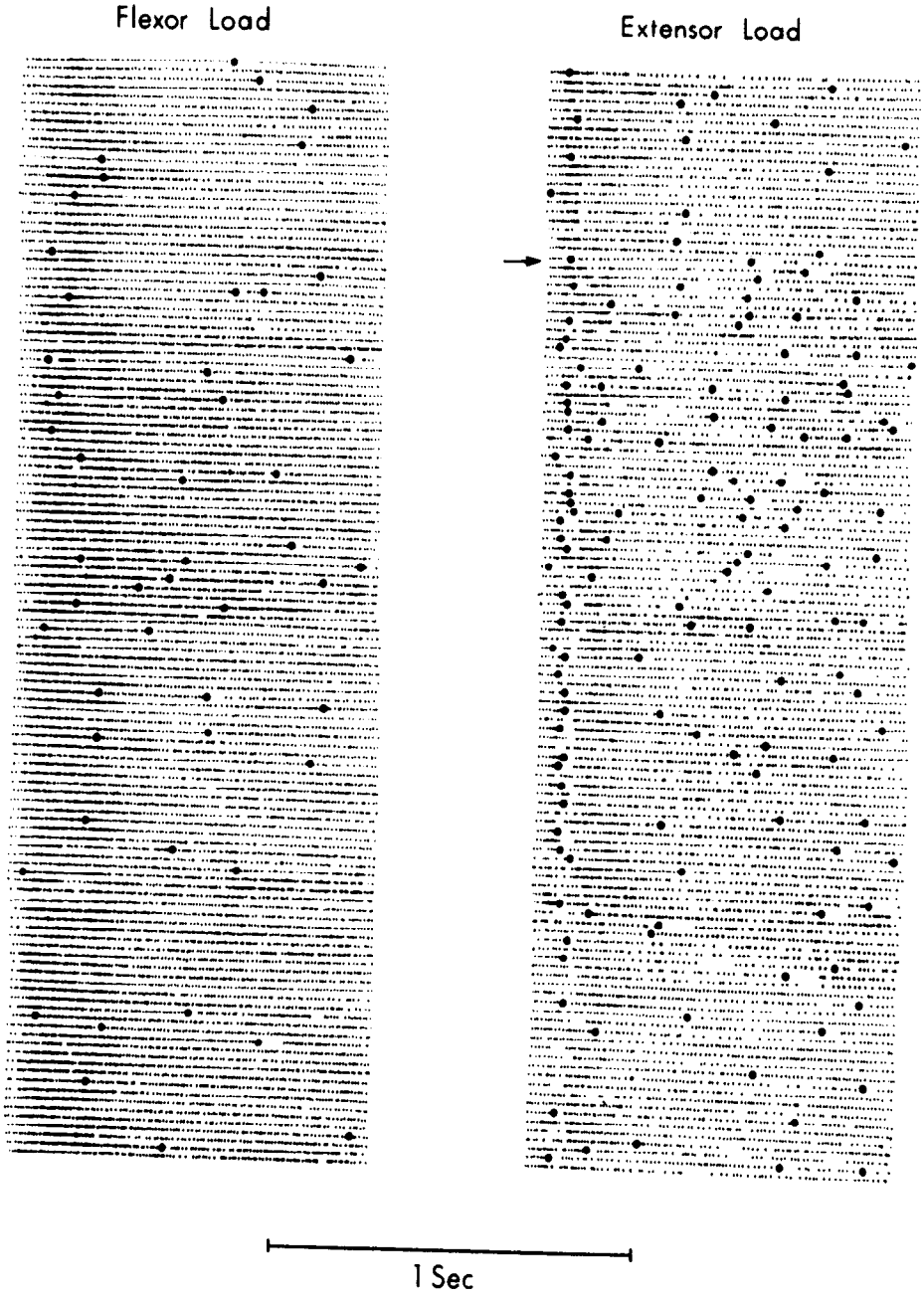

Fig. 3. CS and SS frequency changes for a P-cell after a change in load. Each dot represents a spike potential (SSs: small dots; CSs: large dots); each row of dots represents the discharge during a trial, beginning at the change in direction of load. Successive trials are represented top to bottom, each flexor trace on the left is followed by an extensor trace on the right. This P-cell was load-related with higher SS frequency in the maintained period for flexor than for extensor trials. At the arrow, the known extensor load of 300 g was changed to a novel 450 g, while the known flexor load of 310 g was kept constant. Before the load change (above arrow), there was a low frequency of related CS activity at about 100 msec after the start of extensor trials. After the load change (below arrow), the CS frequency at that time increased greatly and persisted for about 70 trials. There was also an increased CS frequency in the extensor maintained period for about 40 trials. Associated with these CS frequency changes there were changes in SS frequency.

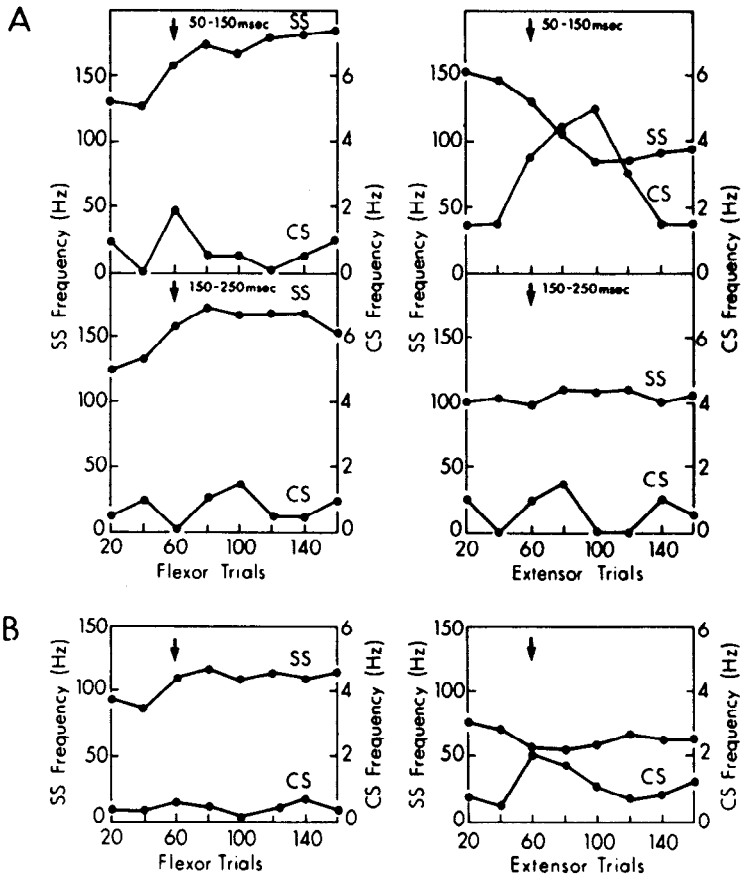

Fig. 4. Graphs of CS and SS frequencies for the same P-cell in Fig. 3. A: transient period. This period was divided into two bins of 50–150 msec and 150–250 msec after the switch in direction of load. For these two bins, the average CS and SS frequencies (for blocks of 20 trials) were plotted against successive flexor and extensor trials as shown. In these graphs the horizontal time axis corresponds to the vertical time axis of the raster in Fig. 3. When the known extensor load was increased to a novel one (arrows indicate the first block of trials with the novel load), there was an increase in CS frequency and decrease in SS frequency for the 50–150 msec bin of the extensor transient; the CS frequency returned to its previous level after 70 trials whereas the SS frequency remained lower. B: maintained period. For this and other cells that had different maintained SS frequencies under oppositely directed loads, the average CS and SS frequencies were determined for successive blocks of 20 trials for both flexor and extensor trials (because the trials were of random duration it was necessary to compute these frequencies from the last 1 sec of activity in each trial). When the known extensor load was increased to a novel one (at the arrow) there was an increase in CS frequency for extensor holding for 40 trials and a decrease in SS frequency which lasted after the increased CS frequency had returned to its prior level. The CS frequency for flexor trials was unaffected by the load change, but the SS frequency showed an increase.

excluding the effects of CSs in the SS frequency determination was that the experiment was seeking to test whether there were lasting changes in the strength of the parallel fiber synapses on the P-cells caused by CS firing, with the SS frequency of the P-cell in response to a constant parallel fiber input being taken as a measure of the strength

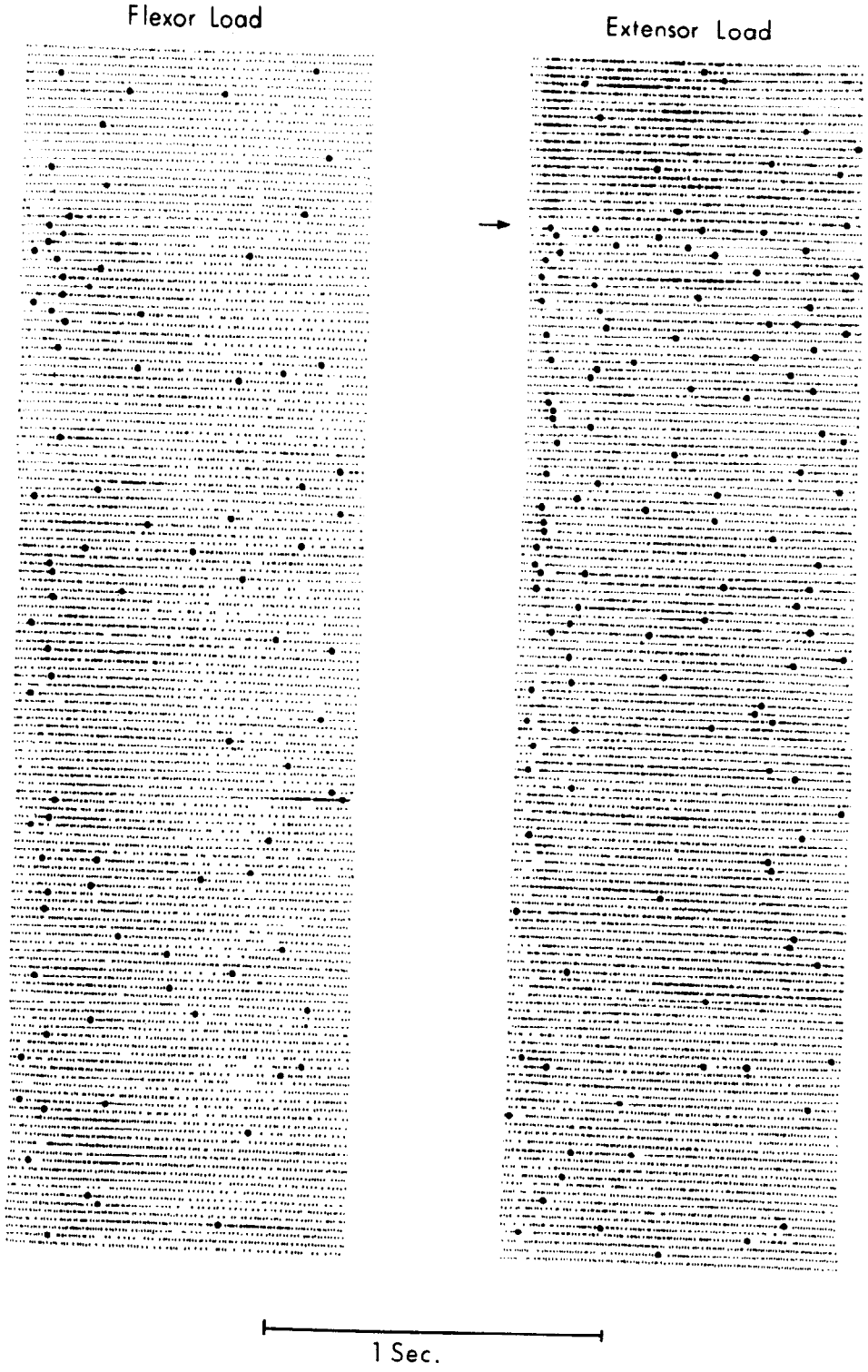

Fig. 5. Similar rasters to those in Fig. 3 are shown for another load-related P-cell. The known extensor load was reduced from 400 g to a novel 290 g (at arrow), while the known flexor load remained constant at 150 g. After the change to a novel extensor load there were changes in CS and SS frequencies at several times in both the flexor and extensor transient periods and in the maintained periods as well.

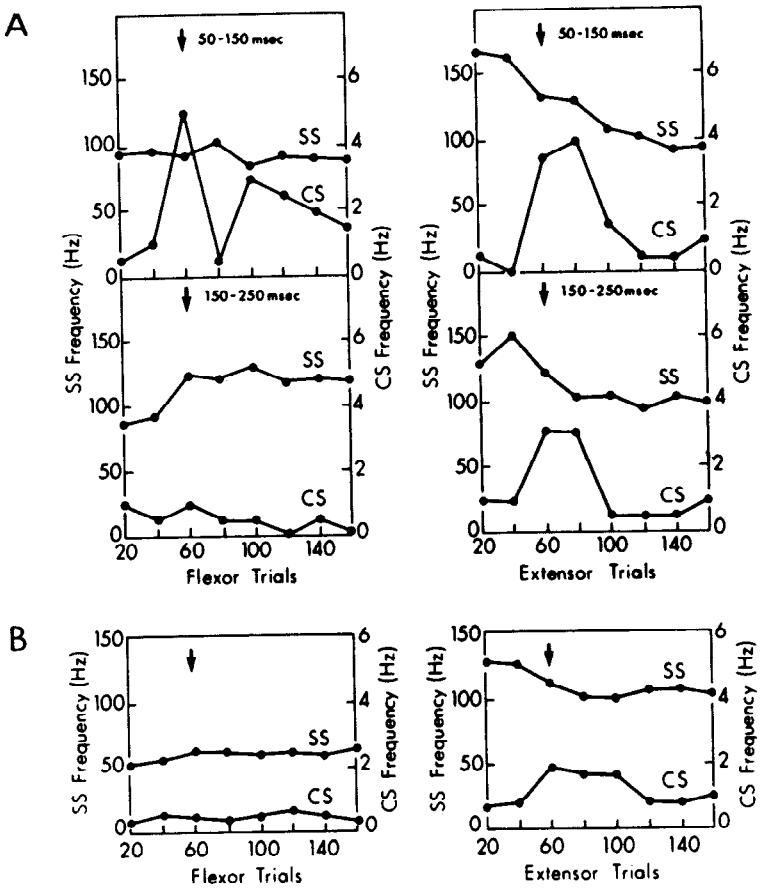

Fig. 6. Graphs of CS and SS frequency for the same P-cell in Fig. 5. A: transient period. When the known extensor load was decreased (at arrow) there were increases in CS frequency in both bins of extensor trials and the early bin of flexor trials that persisted for about 20–100 trials before returning to their previous levels. For the SS frequency, there were decreases for both bins of extensor trials that persisted for the period of observation. B: maintained period. After the change to a novel load there was an increase in CS frequency for 60 of the extensor trials and a decrease in SS frequency which persisted for the period of observation. For flexor trials, the CS and SS maintained frequencies were virtually unaffected by the change in extensor load.

of the parallel fiber synapses. The depressions in SS frequency immediately following each CS would have given an erroneous indication of the strength of the parallel fiber synapses if they had been included in the SS frequency measurements. Changes in CS and SS frequency are shown in Fig. 4A for 3 other 100 msec bins from the flexor and extensor transient periods. No other portions of the transient periods for this P-cell showed similar increases in CS frequency or decreases in SS frequency, though the SS frequency for the two bins of the flexor transient period showed an increase after the load change. Similar measurements were made of the CS and SS transient activity for the rest of the P-cells which showed increases in transient-related CS frequencies with novel loads. Twelve other P-cells were similar to that shown in Fig.

3 and had an increase in CS activity at one point of either the flexor or extensor transient periods.

Four P-cells showed increases in CS frequency at several different times in the transient periods of trials after a change to a novel load. An example is shown in Fig. 5, where there were CS frequency increases centered at roughly 100 msec in the flexor transient period and 70 msec, 200 msec in the extensor transient period. These CS increases lasted for different numbers of trials; the initial CS increase in the flexor transient period for the 50–150 msec bin decreased after 10 trials and then increased again after about 30 trials; the CS increases in the extensor transient period lasted about 30 trials and 20 trials for CSs in the 50–150 msec and 150–250 msec bins, respectively. In a similar fashion to the P-cell described previously there were large decreases in SS frequency in two of the 3 bins in which there was increased CS activity (Fig. 6A).

In general the same patterns were observed when the CS frequency increased with changes to novel loads for all 17 P-cells. Increases in CS frequency would occur at specific times after the start of a trial in the transient periods when there was an increase or decrease in either the flexor load (7 P-cells, 5 increases, 2 decreases in load) or extensor load (10 P-cells, 7 increases, 3 decreases in load). A total of 23 100 msec bins in the transient periods of all 17 P-cells showed increases in CS frequency (by at least an average frequency of one CS every 5 trials), after a novel load was introduced. For 19 of these bins the increased CS activity lasted for periods ranging between 15–90 trials, while in 4 bins, increased CS activity lasted until the end of the recordings for those cells (more than 100 trials).

For each of these twenty-three 100 msec bins, the SS frequency was determined before (S_b), and immediately after (S_a), the change to a novel load and also after the period of increased CS frequency had ended (S_e). Usually these frequencies S_b,S_a,S_e

TABLE I

SS frequency changes compared for bins in the transient period with and without increased CS frequencies after changes to a novel load.

Results for 17 P-cells are combined. For each P-cell at least one out of the 4 bins had an increase in CS frequency (of at least 2 Hz) after a change to a novel load for a number of trials; the bins were divided into two categories according to whether or not they showed such an increase in CS frequency. Average CS and SS frequencies were measured for bins 50–150 msec and 150–250 msec after the start of both the flexor and extensor trials for each P-cell (as shown in Figs. 4A and 6A for two P-cells). The directions of change in SS frequencies occurring in the bins *before* and *after* the change to a novel load (S_b and S_a, respectively), and at the *end* of the period of increased CS frequency (S_e) are shown for the two bin categories.

Bin category	*Numbers of bins showing the specified SS frequency changes*			
	$S_a > S_b$	$S_a < S_b$	$S_e > S_a$	$S_e < S_a$
Increased CS frequency after change to a novel load	3	20	7	16
Unchanged CS frequency after change to a novel load	24	21	29	16

were determined by averaging 20 trials, but in a few cases smaller numbers of trials were used where there was only a short period of increased CS activity. The changes in SS frequency which occurred in these bins were compared with the SS frequency changes which took place over the same trials in bins which had no increased CS activity after the load change (see Table I), and two results emerged.

(1) For those bins which had an increased CS frequency after a change to a novel load the proportion which had a lower SS frequency immediately after the load change than before (i.e. $S_a < S_b$) was 87%, while for bins with unchanged CS frequencies the proportion was significantly different at 47% ($P < 0.005$, χ^2-test). This result is similar to that already described for the performance of the task with known loads, where a high proportion of P-cells had a low SS frequency at the same times in the task that there was a high CS frequency.

(2) For those bins which had an increased CS frequency after a change to a novel

TABLE II

CS and SS frequencies are given for 8 load-related P-cells before and after change to a novel load

Each column gives data for one P-cell. N_1 is the approximate number of trials for which CS frequency increased after the change to the novel load (5 P-cells only), and N_2 is the number of trials before irregularities in the position traces disappeared. L represents the load (g) applied to the handle. The first subscripts *b* and *a* refer, respectively, to loads *before* and *after* the switch to a novel load. The second subscripts *f* and *e* refer, respectively, to the oppositely directed *flexor* and *extensor* loads (the magnitude of one load was changed; the direction was never changed). S and C are the SS and CS frequencies (in Hz, average of 20 trials) respectively. The first subscripts *b* and *a* refer, respectively, to frequencies *before* and immediately *after* the change to a novel load and *e* to frequencies after a temporary increase in CS frequency had *ended*. The second subscripts *f*, *e* indicate that the load was opposing *flexion* or *extension*, respectively, when the measurements were made. The data show that when an increase in CS frequency occurred after a change to a novel load there was an associated reduction in SS frequency.

	Cell No.							
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
N_1	40	75	16	20	50	—	—	—
N_2	15	36	21	32	22	35	46	19
L_{bf}	310	150	200	140	150	220	450	250
L_{af}	310	450	200	250	150	220	450	320
L_{be}	300	325	250	220	400	340	300	300
L_{ae}	450	325	350	220	290	200	400	300
S_{bf}	85.6	88.7	69.7	101.3	53.2	171.9	78.2	65.1
S_{af}	107.8	78.2	67.4	68.3	62.1	165.4	65.8	69.9
S_{ef}	107.0	79.8	69.0	65.1	59.5	—	—	—
S_{be}	69.5	95.5	58.2	131.2	123.0	110.6	100.4	34.5
S_{ae}	57.3	98.5	50.4	137.4	108.4	124.1	106.8	39.2
S_{ee}	58.2	99.0	51.7	139.6	103.7	—	—	—
C_{bf}	0.4	0.4	0.5	0.2	0.5	0.4	0.3	0.6
C_{af}	0.6	1.9	0.5	1.8	0.4	0.4	0.7	0.5
C_{ef}	0.1	0.8	0.4	0.6	0.6	—	—	—
C_{be}	0.5	0.3	0.1	0.2	0.7	0.5	0.4	0.7
C_{ae}	2.1	0.4	2.2	0.4	1.9	0.3	0.6	0.8
C_{ee}	1.1	0.6	0.9	0.7	0.8	—	—	—

load the proportion which had a lower SS frequency at the end of the period of increased CS frequency than at the beginning (i.e. $S_e < S_b$) was 70%, while for bins with unchanged CS frequencies the proportion was significantly different at 36% ($P < 0.01$, χ^2-test). Thus there were continuing decreases in SS frequency throughout the periods of increased CS frequency, which persisted after the period of increased CS frequency had ended.

SS and CS changes in maintained period. After the introduction of novel loads, none of the transient-related P-cells exhibited alterations in maintained CS or SS frequencies. But after changes to a novel load, the 8 load-related P-cells studied showed changes in maintained SS frequency, and 5 also had changes in CS frequency (Table II, cell Nos. 1–5).

Four of the P-cells developed an increased CS frequency in the maintained period for a number of trials after the load was increased (Table II, cell Nos. 1–4). A raster for P-cell No. 1 is shown in Fig. 3. For trials prior to the change to a novel load (above arrow), there was a low CS frequency in both the flexor and extensor maintained periods. When the extensor load was increased the CS frequency increased about 4-fold for about 40 trials while the SS frequency decreased and stayed at a lower level for the rest of the recording (Fig. 4B). The CS frequency in the flexor maintained period remained at a low level after the force change, while the SS frequency showed an increase. Of the other P-cells, one showed an increase in CS frequency in the maintained period for only 16 trials after the extensor load was increased and two had increases in CS frequency for about 20 and 75 trials after the flexor load was increased. For all these P-cells, the SS frequency was lower in the maintained period which had the increased CS frequency after the change to a novel load and remained lower even after the period of increased CS frequency had ended (Table II).

One of the P-cells (Table II, cell No. 5) developed an increased CS frequency in the extensor maintained period after a reduction in the extensor load (a raster of its firing is shown in Fig. 5). The increased CS frequency lasted about 60 trials after the load change and for this P-cell also there was a reduction in SS frequency which persisted after the increase in CS frequency was over (Fig. 6B).

The SS frequencies plotted in Figs. 4B, 6B were not determined in the same manner as those for the transient periods in Figs. 4A, 6A, because no correction for the immediate effect of CS firing on SS frequency was made. This was not possible in the same manner for the maintained periods as for the transient periods because CSs occurred in the maintained period in almost every trial. Therefore, part of the reductions in SS frequency shown in Figs. 4B, 6B were due to immediate depressions in SS frequency caused by CS firing. Nevertheless, there were reductions in SS frequency at the ends of the periods of increased CS frequency, indicating that the immediate effect of CS firing on SS frequency was not the sole cause of the reductions in SS frequency.

Table II gives the CS and SS frequency changes which occurred with novel loads for all 8 load-related P-cells. Whenever a novel load was introduced which increased the difference between the flexor and extensor loads, then the difference between the flexor and extensor SS frequencies in the maintained periods also increased

(and vice versa if the difference between the loads was reduced). Thus for the 5 P-cells which showed an increase in CS frequency after introduction of a novel load the associated reductions in SS frequency followed this rule. It is interesting that for the 3 P-cells which did not show an increased CS frequency during the maintained period after the load was changed, reductions in SS frequency in the maintained period holding against the novel load would not have followed this rule (see Table II).

For the 5 load-related P-cells the reductions in SS frequency in the maintained periods, which occurred immediately after introduction of a novel load that increased the CS frequency, is the same finding as that described previously for the transient periods. However, there was no evidence in the maintained periods for a continuing decrease in SS frequency throughout the period of increased CS frequency, in contrast to the result for the transient periods. Thus only two of the P-cells had lower SS frequencies in the maintained period at the end of the period of increased CS frequency than at the beginning (see Table II, cell Nos. 4, 5). One reason for this difference between the results for the transient and maintained periods could have been the absence of a correction for the latter to take account of the depressions in SS frequency immediately following CSs. Thus the CS frequency usually showed its largest increase immediately after a change to a novel load, and hence these depressions usually had their largest effect on SS firing at this time. In the analysis of the transient periods these short-term effects of the CSs were excluded from the SS frequency determination, as described previously.

For both the transient and maintained periods, there was no direct correspondence between the period of increased CS frequency and the number of trials required to adapt to the novel load (as estimated from the changes in position traces of the handle). Often the increased CS frequency would persist after the monkey had apparently adapted to the novel load (see Table II). However, this observation did not necessarily invalidate the idea that CSs were involved in learning, as will be discussed below.

DISCUSSION

The only direct evidence that the cerebellum may be involved in motor learning is that the gain of the vestibulo-ocular reflex is no longer modifiable when the cerebellar flocculus is destroyed[12,17]. These results support the learning theories[2,3,8,9,11,15] but have not tested the crucial role proposed for the P-cells in learning. Recordings from flocculus P-cells have been made under different conditions of visual and vestibular stimulation[7], but no recordings from single P-cells have been made throughout the period of modification of the vestibulo-occular reflex. In this experiment there were several features of the changes in CS and SS frequency observed in P-cells which are consistent with theoretical proposals which have been made about learning in the cerebellum.

Thus, for a number of P-cells, CSs in both the transient and maintained periods were found to increase and then decline in frequency, over roughly the same time course as the period required to re-learn the task with a novel load. Although CSs

have been found to occur in relation to certain periods of a motor task (e.g. at the beginning of a signal-initiated movement[19]), changes in frequency of the related CSs with successive trials have not been reported in previous experiments. These CS frequency changes support the cerebellar learning theories which predict that CS frequency changes of this type should occur during learning. However, the observation would point to a possible role of CSs in learning even in the absence of these theories.

For most P-cells the number of trials over which the increased CS activity lasted was in the same range as the number of trials required for adapting to the novel load (as judged by the disappearance of irregularities in the position traces of the handle). However, sometimes the number of trials of increased CS activity would differ widely from the number of trials required for the adaptation. This was not inconsistent with the proposal that the increases in CS frequency were involved in the relearning of the task with a novel load because there were many P-cells active in the task which may have been contributing to the learning at different rates. Even the same P-cell sometimes had several different increases in CS frequency which lasted different numbers of trials (e.g. the P-cell of Fig. 5). Also the position traces of the handle probably revealed only gross changes in motor performance during learning, and P-cells which showed increased CS activity under a novel load for longer periods than the irregularities lasted could have been involved in modifying aspects of motor activity which required more sensitive means of measurement.

In a tracking task pushing against forces which suddenly varied Marsden et al.[16] showed that for humans rapid "stretch reflex" responses could occur with a latency of 50–60 msec, which were much faster responses than those occurring after the "ordinary voluntary reaction time" of about 140 msec or more. If the changes in CS activity observed in this experiment do reflect motor learning then the P-cells could be storing information relating to both reflex and voluntary movements. Thus many of the changes in CS and SS activity in the transient period occurred very early in trials (sometimes 50 msec after the load switch at the start of trials) and could have been involved in modifying the stretch reflex response to the novel load. However, there were also much later changes in the maintained periods (more than 300 msec after the start of trials) which could have been responsible for storing information about voluntary movements. It is interesting that both the early and late changes in CS and SS activity were sometimes seen in the same P-cells (for example, see Figs. 3 and 5) indicating that this P-cell could possibly be storing information about both types of movement.

The main hypothesis of the learning theories is that information is stored in P-cells through changes in the strength of parallel fiber synapses when the climbing fiber fires. Several pieces of evidence indicate that there could be a reduction in strength of the parallel fiber synapses on a P-cell when the climbing fiber fires during learning. Thus in the analysis of the transient periods long-term (often lasting more than 100 trials) reductions in SS frequency occurred preferentially in the 100 msec bins which had increased CS frequencies, and were not dependent on the continuation of that increased CS frequency. This is the type of specific change in P-cell output which has been predicted by the learning theories. The SS output frequency of a P-cell in response

to a particular parallel fiber input would be reduced after there had been an increased CS frequency at the same times as that input, while the response of the P-cell to other parallel fiber inputs not subjected to increased CS frequencies would remain unchanged. It was not strictly accurate to consider each 100 msec bin as having a constant parallel fiber input as there was probably a continuously varying parallel fiber input throughout this period; a bin width of 100 msec was chosen because the SS frequency did not change appreciably over periods less than 100 msec for most P-cells, indicating that it was probably a reasonable approximation to regard the parallel fiber input as constant for that period. A P-cell could respond specifically even to a somewhat variable parallel fiber input if the appropriate changes were made in the strength of the parallel fiber synapses[3,8].

The load-related P-cells also showed long-term reductions in SS frequency in maintained periods of steady holding which had previously had increased CS frequencies; while for the maintained periods with the oppositely directed loads, which had not been associated with increased CS frequencies, there were no reductions in SS frequency. However, unlike the situation for the CS and SS changes in the transient periods, the SS frequency tended to increase after the period of increased CS frequency was over. Nevertheless, there were still overall reductions in SS frequencies, for the maintained periods which had had increased CS frequencies, compared to those prior to the change to a novel load. As mentioned previously, the reason for the difference in results could have been because no correction was made for the immediate effect of CSs on the SS frequencies in the maintained periods.

There was further indirect evidence that CS firing may reduce the strength of parallel fiber synapses. Thus even after a novel load had been learned, a large proportion of P-cells which had transient-related CSs also independently had very low or zero SS frequencies at the same points in the transient period. These CSs appeared to be permanently related to the task and the frequencies of most of them did not change when a novel load was introduced. This type of inverse relationship between CS and SS frequency has been observed in previous studies[6,7], and could be explained on the basis of these CSs causing a reduction in the responses of the P-cells to the parallel fiber inputs occurring simultaneously with the CSs. The reason that further changes with time in SS frequency were not observed in these bins with related CSs may have been because the SS frequency in response to the parallel fiber input at that point had been reduced by the maximum amount possible.

If CSs do indeed cause reductions in strengths of certain parallel fiber synapses on P-cells it is probable that there would also be a mechanism for increasing the strength of parallel fiber synapses, or otherwise spontaneously occurring CSs would eventually cause all the synapses to become inactive. Increases in SS frequency were observed in both the transient and maintained periods for many P-cells during learning. However in this experiment it could not be determined whether these increases were due to a specific mechanism for increasing synaptic strengths, or some other cause such as injury to the P-cells. Another possible way in which the effects of spontaneously occurring CSs would be overcome has been suggested[9]: changes in synaptic strengths of parallel fibers on a P-cell caused by CSs would not be consolidated or

made permanent unless there were an input to the P-cell signalling that the information stored was of value to the animal.

The results obtained in this experiment are not compatible with all of the theoretical proposals about learning in the cerebellum. Marr's original theory[15] cannot readily be modified to allow for learning to be caused by reductions in strength of parallel fiber synapses, but with other theories[2,8] learning could occur with reductions in strength. More detailed information about the characteristics of these possible changes in synaptic strength during learning is required before further speculation is justified about the details of various theories. However, it should be emphasized that the data of this experiment indicate a possible involvement of CSs in learning despite the theoretical proposals.

This experiment has not proved a causal relationship between the increased CS frequencies and the temporally associated reduced SS frequencies found in the transient and maintained periods during learning. During the period of adaptation to a novel load both the CS and SS frequency changes might have been produced by feedback to the P-cell from the periphery due to changes in muscular activity not caused by that P-cell, but under the control of some other part of the motor system. The mossy fiber and climbing fiber connections to the P-cell could have been arranged in such a manner as to give this inverse pattern of CS and SS frequencies, but there does not appear to be a plausible reason for such a scheme of connections. Also it is difficult to account for short-term increases in CS frequency being associated with long-term decreases in SS frequency, unless a causal relationship exists between the occurrence of CSs and decreases in SS frequency. A causal relationship may perhaps be established in an experiment where recordings are made from P-cells whose climbing fiber and parallel fiber inputs are excited by direct electrical stimulation.

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