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THE ACTIVITY OF CEREBELLAR NEURONES OF AN ELASMOBRANCH FISH (*SCYLIORHINUS CANICULA*) DURING A REFLEX MOVEMENT OF A FIN

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SUMMARY

1. Response of neurones in the corpus cerebelli of *Scyliorhinus canicula* (Elasmobranchii) have been recorded in decerebrate unanaesthetized fish during the performance of pectoral fin reflexes (p.f.r.) evoked by electrical stimulation of the fins.

2. Of 421 single units recorded in the posterior dorsal quadrant of the corpus, 111 (26%) had their discharges modulated when the reflex was evoked; fifty nine were Purkinje (P) cells discharging single spikes, thirteen were P cells discharging complex responses, thirty-six were stellate (S) cells and three were not positively identified.

3. The responses of fifty-one units (thirty-seven P cells and fourteen S cells) were analysed in detail. In thirty-one of the P cells and all the S cells the initial response was excitatory, six P cells gave an inhibitory response only and a further three were inhibited after the initial excitation. Only one S cell had an inhibitory component in its response.

4. The discharges of the S cells completely overlapped the inhibitory responses of the P cells.

5. Only one cell (a P cell) was evoked at a latency shorter than that of the p.f.r. In one other P cell discharging single spikes spontaneously, the evoked response was a long latency (146 msec) complex response. Four other P cells discharged only complex responses but the pattern of their response to the p.f.r. was similar to that of P cells discharging single spikes only.

6. No cerebellar responses were evoked if the fin stimulus was below the threshold necessary to evoke a p.f.r. In curarized fish, unit responses were recorded that were qualitatively similar to those recorded in unparalysed fish.

7. These results suggest that (i) because of the long latency of the responses, the cerebellum is unlikely to have a role in the initiation of the p.f.r.; (ii) the responses during the p.f.r. were evoked via a mossy fibre-parallel fibre pathway; (iii) the responses were correlated with motor activity rather than sensory input.

INTRODUCTION

Studies on the activity of mammalian cerebellar neurones during movement have been limited by the difficulty of obtaining 'normal' movements from acute experimental preparations. However in lower vertebrates this is not such a restrictive limitation. For example, there is no evidence that decerebration disrupts reflex movement and in *Scyliorhinus canicula*, the elasmobranch fish chosen for this study, reflex movements similar to those seen in normal animal can be obtained from restrained decerebrate preparations (Paul & Roberts, 1979*b*). Moreover, since elasmobranch fish are comparatively primitive vertebrates and have none of the gravitational problems associated with a terrestrial existence, their range of motor behaviours is limited.

In almost all vertebrates the basic structure of the cerebellum is remarkably similar and data obtained from one vertebrate class have often been found to correlate broadly with data from other classes. We report here experiments in which cerebellar unit discharges have been recorded in decerebrate, unanaesthetized fish whilst they performed a reflex movement, the pectoral fin reflex (p.f.r.), which has been studied in detail previously (Paul & Roberts, 1979*b*). Marked modulations of Purkinje and stellate cell discharges were observed, but the latencies of the responses were almost always longer than the latency of the motor output, as assessed by the e.m.g. recorded from the active muscles. In addition, one response only was seen that could have been attributed to climbing fibre activity. A preliminary account of some of this work has been published previously (Paul & Roberts, 1974).

METHODS

Animals. All experiments were done on specimens of the lesser spotted dogfish (*Scyliorhinus canicula*, L.) that had been kept for several days after capture in a large tank of circulating sea water.

Preparation. The fish was initially anaesthetized in sea water containing tricaine methane sulphonate (Tricaine, Sigma; 166 mg/l.), decerebrated as described previously (Paul & Roberts, 1977) and the dorsal surface of the cartilaginous skull removed to expose the cerebellum. The head was mounted rigidly in a holder and the fish immersed, except for the dorsal part of the head, in a tank of circulating sea water (Paul & Roberts, 1979*a*). Water temperatures ranged from 13 to 18 °C. Sea water was flowed over the gills continuously. To abolish spontaneous body movements, the spinal cord was destroyed caudad to an incision made half-way between the pectoral and pelvic fins.

Experiments were begun after the fish had recovered from anaesthesia as indicated by the resumption of spontaneous respiratory movements and the presence of a brisk p.f.r.

Stimulating and recording. To evoke and record the p.f.r., wires were inserted into the pectoral fin and into its levator muscle as described in detail elsewhere (Paul & Roberts, 1979*b*). The p.f.r. was evoked with a train of 3–5 pulses of 200 μsec duration at a frequency of 200 Hz, presented every 6 sec to minimize 'habituation' of the reflex, and the stimulus voltage was adjusted to produce a reflex with both phasic and tonic components in the e.m.g. recorded from the levator muscle (Paul & Roberts, 1979*b*).

Unit recording were made with glass micropipettes filled with either 4 M-NaCl or 3 M-KCl and having d.c. resistances of 5–12 MΩ. Conventional recording equipment was used, the recordings being photographed from an oscilloscope or stored on magnetic tape for subsequent analysis using a digital computer (NOVA, Data General Corporation).

The depth of the micro-electrode was read from a micrometer attached to the manipulator. In some experiments, the extent of the electrode track was checked by leaving the electrode tip *in*

situ and by making measurements of the depth of penetration from frozen sections cut from the excised cerebellum.

In some fish, which were curarized for greater stability during recording (D-tubocurarine, 7 mg/kg I.V.), a concentric bipolar electrode was used to stimulate the surface of the cerebellum in order to identify unit responses (LOC stimulation).

Analysis. The computer was programmed to construct post-event histograms and cumulative frequency distributions from the trains of spikes which had been recorded on magnetic tape. Spikes were converted to standard pulses by a window discriminator and the pulses used for input to the computer. Interspike intervals were measured to an accuracy of 1 msec. The event to which the analysis was time-locked was either a pulse coincident with the presentation of a stimulus, giving a post-stimulus time histogram (PoSTH) or a pulse generated at a set point in time prior to the application of the stimulus, giving a peri-stimulus time histogram (PeSTH). Control PoSTHs were compiled using the same synchronizing pulses but without the stimulus being applied. Statistical procedures employed are described in the results. Means are quoted as ±S.E. of the mean.

RESULTS

In these experiments, micro-electrode penetrations were made into the molecular and ganglionic (P cell) layers, which are, in elasmobranchs, physically separate from the granular layer, except for a narrow region immediately adjacent to the mid-line sulcus. This central region is devoid of P cells and was not explored. Thus the electrode would be expected to have encountered activity generated in (1) the molecular layer (600–900 μm thick) by parallel fibres, S cells, P cell dendrites and perhaps climbing fibres (but see Disscussion); (2) the ganglionic layer (650–1000 μm deep) by P cell bodies and primary dendrites and deep S cells; (3) the infra-ganglionic, periventricular fibre plexus by afferent and efferent fibres and ventral dendrites of the P cells, which are present in these fish.

Unit discharge

Three distinct classes of units were encountered in this study, differing in their extracellularly recorded amplitudes and/or wave forms and, in curarized preparations, in their responses to LOC stimulation.

Small amplitude units (< 2mV peak-to-peak) were located mainly in the molecular layer. They were distinctly biphasic and responded to LOC stimulation at a latency approximately the same as that of the parallel fibre volley, which was usually at least 5 msec. The number of spikes discharged depended upon stimulus strength. These units were classed as S cells (c.f. Nicholson, Llinás & Precht, 1969) and an example is illustrated in Fig. 1*A*.

Large amplitude units (i.e. > 2 mV, typically 5–10 mV and occasionally up to 20 mV) were encountered at the level of the P-cell bodies. They could sometimes be driven by LOC stimulation at very short latency (< 1·0 msec) as well as at a latency similar to that of the S cells. The unit illustrated in Fig. 1*B* responded to LOC stimulation without jitter at a latency of 0·32 msec. Short latency responses were presumed to result from antidromic activation of the axon and the units were classed as P cells (cf. Nicholson Llinás & Precht, 1969).

The unit discharges of the third class were all complex, varying from a double spike to a long lasting steady potential on which were superimposed one or more spikes or spikelets. None of these complex responses could be driven antidromically, but some were driven at longer latency by LOC stimulation. Usually, a complex response

was the only, or the most prevalent activity of the unit but on rare occasions both complex responses and typical single spikes were recorded from the same unit. In some respects these complex discharges resembled the climbing fibre responses seen in higher vertebrates and their large amplitudes plus the fact that sometimes they could be recorded intracellularly suggest an origin within the P cells. However their true identity remains to be established. An example of a complex response can be seen in Fig. 5.

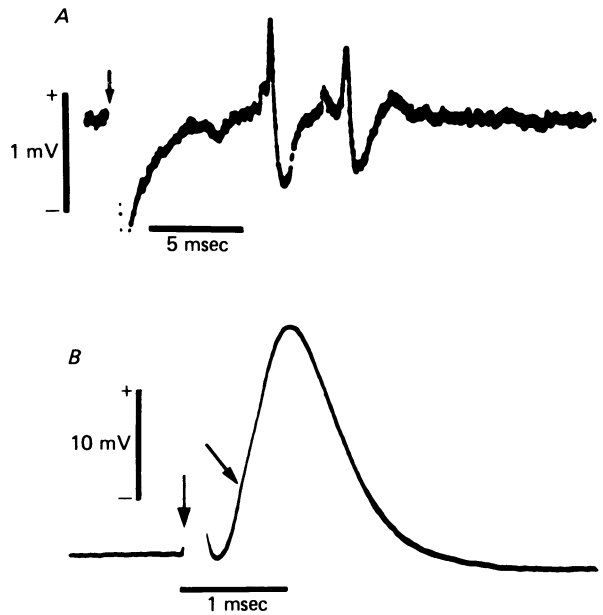

Fig. 1. Recordings of cerebellar neurones evoked by a single shock to the cerebellar surface (LOC). *A*, stellate cell activated synaptically via the parallel fibres. Note the characteristic biphasic nature of the spikes. Stimulus at arrow. *B*, Purkinje cell driven antidromically by surface stimulation. Note the very small inflexion on the rising phase. (oblique arrow) which has been retouched for clarity. Stimulus at downwards arrow. See text for further details.

Although LOC stimulation was employed only in experiments where curare had been given, the distinctions between the three kinds of units were sufficiently clear to enable virtually all units in all experiments to be classified.

Distribution of units evoked by pectoral fin stimulation

In preliminary experiments in which the whole of the dorsal surface of the corpus cerebelli was explored it quickly became apparent that the electrode penetrations into the rostral half consistently failed to locate any evoked responses to pectoral fin stimulation. Consequently in the main study, exploration was restricted to the caudal half of the dorsal corpus, where a total of 421 single unit discharges were recorded from 194 electrode penetrations in twenty-three preparations. Of these, 111 (26·4 %) were observed to respond in association with the p.f.r. and they were identified as fifty-nine P cells with single spike discharges, thirteen complex responses and thirty-six S cells; in only three cases was the identity uncertain. In most experiments,

penetrations were made into the right half of the corpus but some were made on the left side.

The distribution of the 194 electrode tracks in the caudal corpus is illustrated in Fig. 2 plotted on a 'standardized diagram' of the corpus. In each experiment the

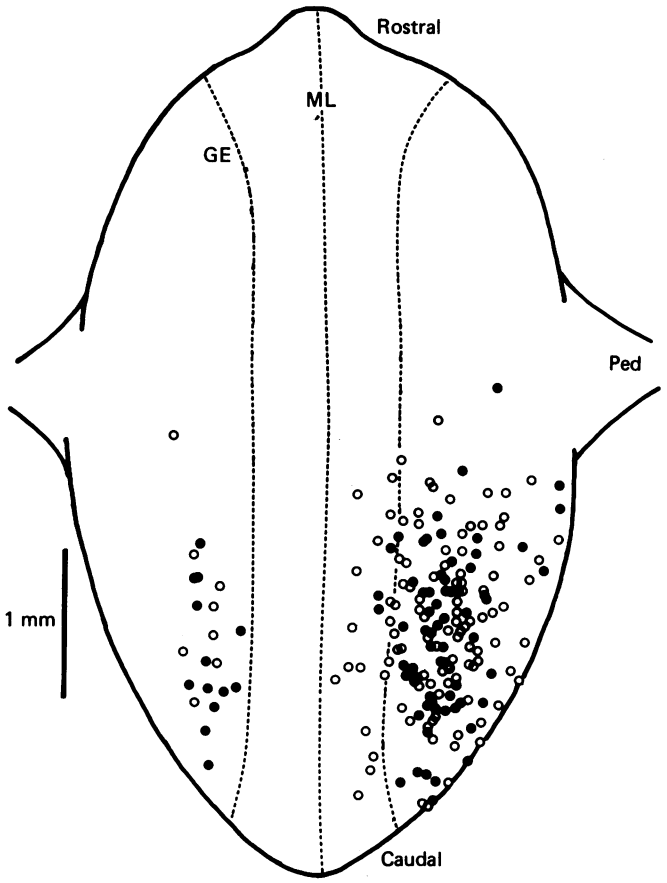

Fig. 2. Locations of all electrode tracks plotted on a standardized drawing of the dorsal surface of the cerebellar corpus. ●, tracks with evoked responses; ○, tracks with no evoked responses. ML, mid-line; GE, edge of granular eminence; Ped, peduncle. See text for further details.

surface of the corpus was either carefully drawn or photographed using Polaroid film, and the surface locations of the vertically made electrode penetrations were marked using the numerous blood vessels as reference points. To obtain the composite plot of Fig. 2 the longitudinal and transverse dimensions of each corpus were measured and used as vertical and horizontal co-ordinates to specify each electrode track. These measurements were then scaled to the standardized diagram, where it can be seen that tracks yielding evoked responses (positive tracks) appeared to group together. The significance of this apparent grouping was tested in the following way. For each positive track, the nature of the nearest neighbouring track in each of the four adjacent quadrants (rostral, caudal, medial and lateral) was noted. Assuming that the

nature of any given track was independent of its neighbours and knowing the total numbers of positive and negative tracks, a theoretical distribution was calculated and compared to the observed distribution by the χ^2 test. The null hypothesis of no difference between the theoretical and observed distributions was rejected at the $P < 0{\cdot}01$ level, indicating a statistically significant tendency of the positive tracks to group together.

Analysis of single unit responses

Out of the 111 units which responded in relation to the p.f.r., sufficient data were obtained from fifty-one (thirty-seven P cells and fourteen S cells) for a quantitative analysis. Of the P cells, thirty-two discharged single spikes only, four discharged complex respones only and one discharged single spikes spontaneously but had an evoked complex response. Spike trains were analysed by compiling PeSTHs, PoSTHs and cumulative frequency distributions as described in the Methods.

The spontaneous activity was irregular, but over many samples, a control PoSTH or the pre-stimulus section of a PeSTH should approximate to a histogram in which each bin has the same value, assuming a random distribution of the spikes relative to the pulses triggering the acquisition of samples. This theoretical bin value was estimated by dividing the total number of intervals analysed by the number of bins into which they were deposited and the resulting theoretical distribution was compared to observed distributions by the χ^2 test. In no case did the distribution of spontaneous intervals deviate significantly from the theoretical case (at $P < 0{\cdot}05$ level), but in all fifty units the post-stimulus distribution of intervals deviated either significantly ($P < 0{\cdot}05$, one case), highly significantly ($P < 0{\cdot}01$, four cases) or very highly significantly ($P < 0{\cdot}001$, forty-five cases) indicating a significant change in the spike discharge rate following the presentation of the stimulus. In the Figures the theoretical distribution is indicated on the cumulative frequency plots by a dashed line.

Spontaneous discharge rates

Both P cells and S cells discharged irregularly and rather slowly. From the control data, the calculated mean discharge rates were $6{\cdot}6 \pm 0{\cdot}7$ spikes/sec, (range 1·7–15·8 spikes/sec) for P cells and $5{\cdot}7 \pm 0{\cdot}7$ spikes/sec (range 0·5–10·1 spikes/sec) for S cells. There was no significant difference between the variances of these two estimates (F test) or between the means (unpaired two-tailed t test).

Response patterns

In the Figures of histograms and cumulative frequency plots, the vertical axes have been normalized by expressing each bin value as spikes per sweep. In order to determine which of the bin values following the fin stimulus differed significantly from control, the mean ($\bar{x}$) and standard deviation (σ) of the control bin values were calculated. Post-stimulus bin values which exceeded $\bar{x}+2\sigma$ were taken to represent excitation and those which were were less than $\bar{x}-2\sigma$ were taken to represent 'inhibition'. The mean and one or both of these limiting values are indicated on the histograms by dashed lines. For a normally distributed population, the probability of a value lying outside the range $\bar{x} \pm 2\sigma$ is $< 0{\cdot}041$. This method of assessing

significant deviations from control is similar to the '*Z* score' used by Olds (1973).

Because the discharge rates of the cells were low, and the responses often prolonged, the histogram bin width was routinely set at 50 or 100 msec and occasionally at 20 or 200 msec. Thus for most cells the parameters of response latency and duration could be estimated only by determining the first and last histogram bins having values significantly different from control.

P cells. The latency of the p.f.r. varies from about 30 to 40 msec depending on the water temperature (Paul & Roberts, 1979*b*), but only five of the thirty-seven P cells analysed had a detectable response during the first 50 msec following fin stimulation. Most of the cells responded later, during the tonic phase of the p.f.r.

The responses could be divided into several groups which are summarized in Fig. 7. These were:

(i) twenty-three cells (62 %) discharging single spikes only, in which the discharge rate increased; in most there was a single period of excitation (seventeen cells, 46 %), but in five (14 %) there was second period and one cell showed two further periods of excitation;

(ii) four cells (11 %) discharging complex responses only, in which the discharge rate increased;

(iii) three cells (8 %) discharging single spikes in which there was an initial excitation followed by inhibition;

(iv) six cells (16 %) discharging single spikes which were inhibited;

(v) one cell which discharged single spikes spontaneously and had an evoked complex response.

Fig. 3*A* illustrates a group (i) unit which responded at a latency of 500–550 msec with a single increase in the discharge rate lasting for 600-800 msec. Amongst the units of this type (Fig. 7) the latency of the response varied from less than 50 to 800 msec. The durations of the responses were mostly less than 500 msec and in five cells the response was confined to one to two bins only. The majority of the cells yielded a PoSTH that was either symmetrical or skewed to the right with a well defined peak. In three cells however, there was only a general increase in the discharge rate which could persist for a second or more; in one cell the response was detectable after 3 sec had elapsed (Fig. 7).

Fig. 3*B* illustrates a group (iii) P cell in which a burst of 2–4 spikes occurred 50–150 msec after the stimulus, followed by a period of inhibition for 250–500 msec. A second, less well marked period of excitation occurred from 600 to 900 msec. An example of a group (iv) unit that was inhibited only is shown in Fig. 3*C*. Separate plots of the control and experimental data are shown and the period of inhibition lasted for 100–1000 msec.

In one cell, illustrated in Fig. 4 the response latency was short and consistent enough to be measured. The p.f.r. had an e.m.g. latency of 30 msec (Fig. 4*A*) and the response latency of the unit was $18{\cdot}8 \pm 1{\cdot}3$ msec ($n = 12$). This was the only unit response with a latency that was both well defined and shorter than the latency of the p.f.r. The duration of the complete response however, was well over 1 sec (Fig. 4*C*).

The one evoked complex response of a unit that otherwise discharged single spikes

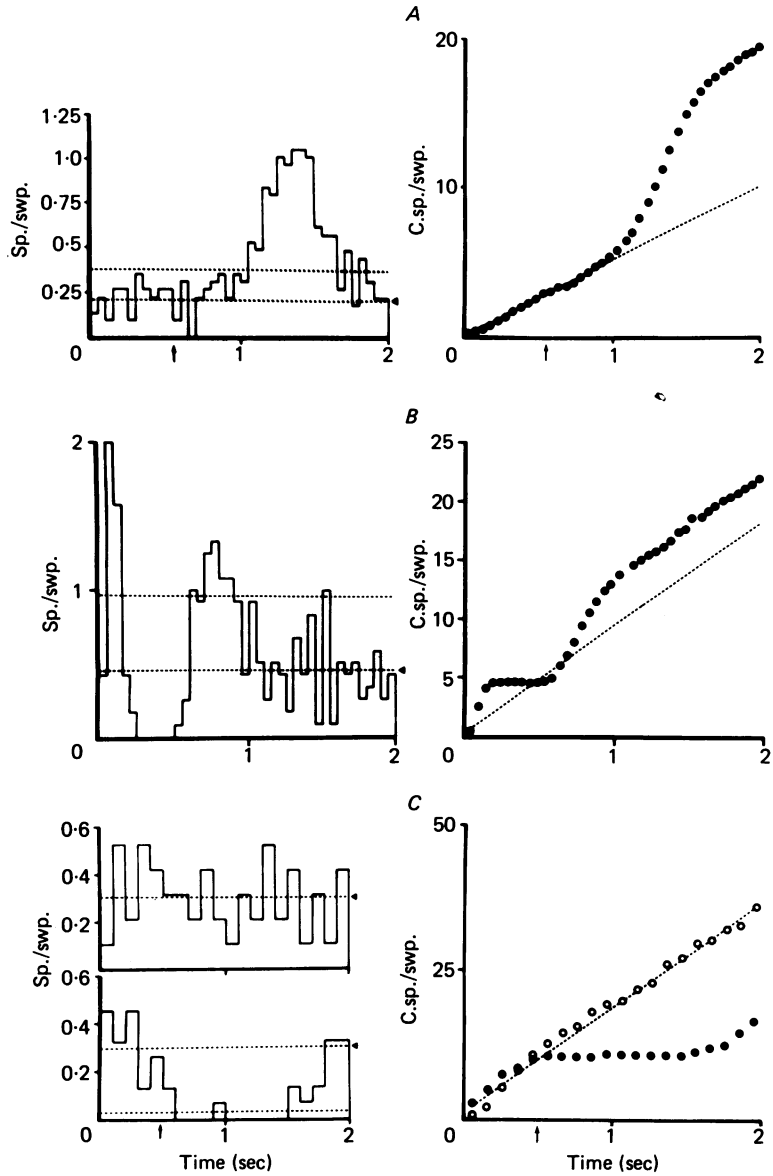

Fig. 3. Histograms and cumulative frequency distributions of Purkinje cell responses to pectoral fin stimulation. *A*, group (i) unit; PeSTH (50 msec bin width), stimulus at arrow. *B*, group (ii) unit, PoSTH (50 msec bin width). *C*, group (iii) unit, upper histogram is control lower is PeSTH (100 msec bin width). Stimulus at arrow. Cumulative distribution, (○) control, (●) experimental values. In the histograms, the mean control bin value is shown by horizontal dashed line marked by arrowhead. Cumulative frequency plot, 'theoretical' control shown by dashed line. Ordinates show spikes per sweep (Sp./swp.) in histograms and cumulative spikes per sweep (C.sp./swp.) in cumulative distribution. Further explanation in text.

is illustrated in Fig. 5. The latency of the complex response was long and variable, having a range of 99·5–194·5 msec with a mean of 146·1 ± 8·4 msec ($n = 14$). The detailed wave form of the response was itself variable, but in general consisted of a spike followed by a large-amplitude positive wave on which were superimposed further spikes or spikelets.

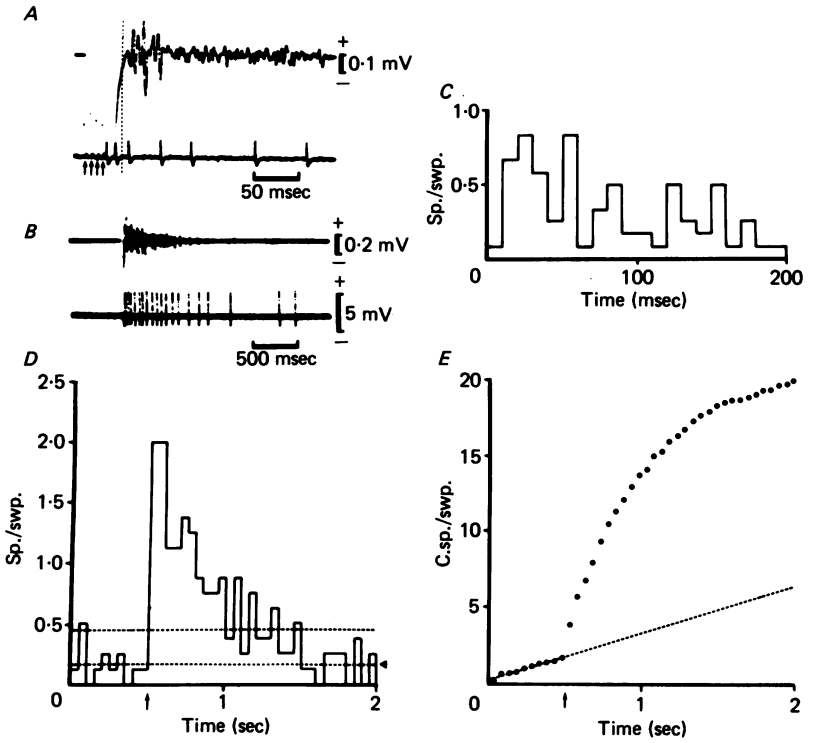

Fig. 4. Short latency response of a Purkinje cell to pectoral fin stimulation. *A* and *B*, e.m.g. of fin muscle (upper traces) and Purkinje cell discharges (lower traces) showing start of (*A*) and complete (*B*) response. Vertical dashed line in *A* is coincident with earliest component of fin e.m.g.; upwards arrows mark stimulus train in *A*. Unit calibration same for *A* and *B*. *C*, PoSTH (10 msec bins) of early part of response. *D* and *E*, PeSTH (*D*) and cumulative distribution (*E*) of complete response (50 msec bins). Stimulus at arrow; other explanations, as for Fig. 3.

S cells. All fourteen observed were excited by the stimulus, one cell having a subsequent period of inhibition. None responded at either a consistent latency or one less than that of the p.f.r. In seven of the cells there was more than one period of excitation. An S cell response is illustrated in Fig. 6 in which there was a marked initial excitation at a latency of 100–150 msec, followed by a period of inhibition and then a further period of excitation.

The response patterns of all the S cells are shown diagrammatically in Fig. 7 where it can be seen that the durations of the S cell excitatory responses were sufficient *in toto* to overlap all of the periods of inhibition of the P cells. Thus if the S cells are inhibitory to the P cells, as they have been shown to be in higher vertebrates, their

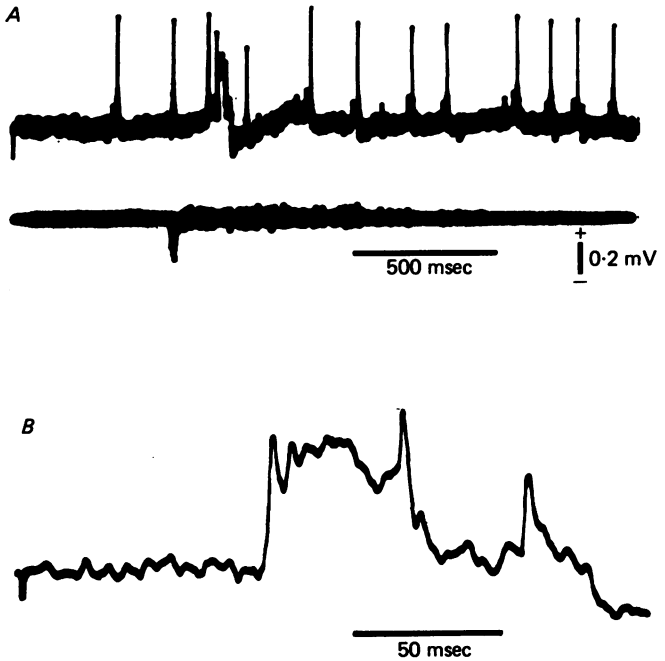

Fig. 5. Complex response evoked by pectoral fin stimulation. *A*, upper trace, unit discharge; lower trace, fin muscle e.m.g. *B*, expanded section of another response showing details of the complex wave. Calibration pulse at start of unit traces in *A* and *B* is 1 mV, +ve upwards.

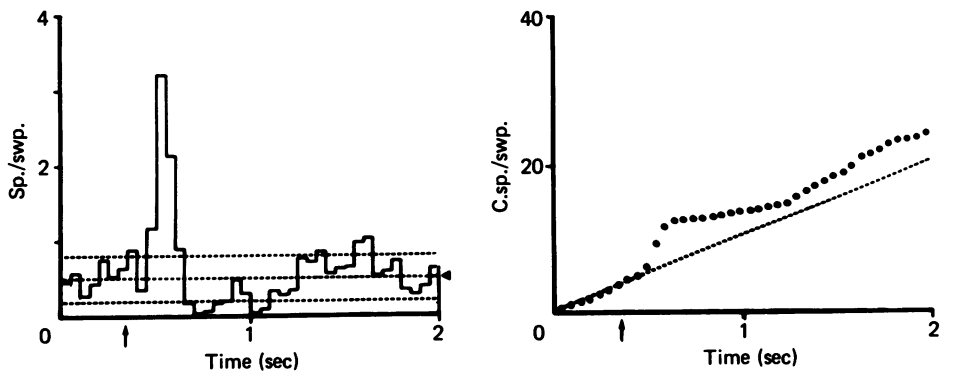

Fig. 6. Stellate cell response to pectoral fin stimulation, showing PoSTH on left, cumulative distribution on right. Stimulus at arrow. Histogram bin width is 50 msec. Other explanations as for Fig. 3.

responses during the p.f.r. can account for all the inhibitory responses recorded in P cells.

Laterality of the responses

Eighteen P cells were recorded in experiments in which both pectoral fins could be stimulated. Of these, fifteen responded to stimulation of one fin only, nine with

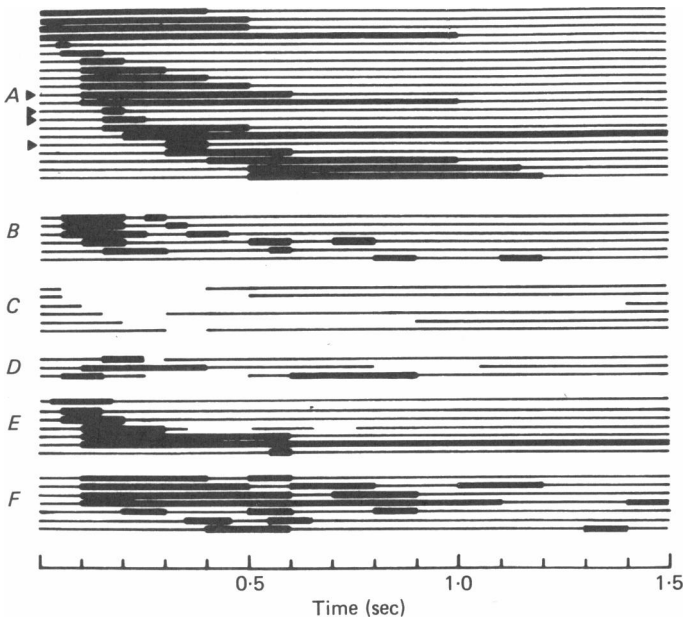

Fig. 7. Diagrammatic summary of the discharge patterns of Purkinje and stellate cells. Thin line represents discharge at spontaneous rate; thick line, discharge above spontaneous rate (excitation); break in line, discharge below spontaneous rate ('inhibition'). *A*, discharge patterns of twenty-one Purkinje cells having a single period of excitation. Discharges comprising complex responses only, marked by arrowheads (on left). *B*, discharge patterns of six Purkinje cells having more than one period of excitation. *C*, discharge patterns of six Purkinje cells having periods of inhibition only. *D*, discharge patterns of three Purkinje cells having periods of excitation followed by periods of inhibition. *E*, discharge patterns of 7 stellate cells having a single period of excitation. One (illustrated in Fig. 6) had periods of inhibition also. *F*, discharge patterns of seven stellate cells having more than one period of excitation. Periods of excitation and inhibition plotted from start of bin in which response first occurred.

an excitatory response and one with an inhibitory response to ipsilateral fin stimulation and five with an excitatory response to contralateral fin stimulation. The remaining three cells were bilaterally affected, two giving an excitatory response to both ipsilateral and contralateral fin stimulation whilst one gave excitation to ipsilateral stimulation and inhibition to contralateral stimulation.

Seven S cells were also tested for bilateral effects. Four were excited by ipsilateral fin stimulation only, one by contralateral fin stimulation only and two bilaterally. There was no significant difference between the proportions of P cells and S cells giving

bilateral responses in the two samples (χ^2 tests, $P > 0{\cdot}9$) or between the proportions of unilaterally excited cells responding to a specified fin (χ^2 test, $P > 0{\cdot}9$).

Relationships between the p.f.r. and the cerebellar unit responses

It was consistently observed that the cerebellar unit responses were abolished if the fin stimulus was decreased below the threshold level required to evoke a p.f.r. Increasing the fin stimulus strength to evoke a reflex resulted in the reappearance of the cerebellar response. The amplitude of the p.f.r. varied with the strength of the stimulus (Paul & Roberts, 1979*b*) but there was no equivalent variation in the cerebellar response. Although in some units small changes were observed in the response with changes in stimulus strength, there was no evidence of a marked relationship between the two.

It is also known that with constant peripheral stimulation, the strength of the reflex contraction varies over a period of time (Paul & Roberts, 1979*b*). In the present experiments this variability of the reflex under conditions of constant stimulation was frequently observed, but there were no indications that the cerebellar responses varied in parallel.

In curarized preparations, nine P cells and six S cells were recorded which responded in relation to a fin stimulus which had evoked a p.f.r. before the curare had been administered; there were no qualitative differences between the responses of these units and the responses of units recorded in uncurarized preparations. Thus although the fin stimulus had to exceed the threshold for evoking a p.f.r. in order for a cerebellar response to be obtained, it seems unlikely that feed-back, either from the contracting muscle or from the moving fin, played a significant role in generating the cerebellar responses.

DISCUSSION

Identification of single units

It is probable that all of the unitary discharges recorded in this study can be attributed to activity within the P cells, except for the small biphasic spikes which were considered to arise from S cells. The large amplitude single spikes probably originated from the initial segment/cell soma region of the P cell for they were recorded at the appropriate depths and could be activated antridomically by surface stimulation (cf. Nicholson *et al.* 1969).

The nature of the complex responses is much less certain. In higher vertebrates, following the studies of Eccles, Llinás & Sasaki (1966), complex responses in P cells are generally acknowledged to signal climbing fibre activity. Other workers have reported complex responses in fish cerebella (Nicholson *et al.* 1969; Eccles, Táboříková & Tsukahara, 1970; Bastian, 1974; Kotchabhakdi, 1976*a*; Russell & Bell, 1978; McGlone & Paul, 1980) but not all agree that they invariably represent climbing-fibre activity. In the present study, complex responses have been seen frequently in the molecular layer but only rarely interspersed among single spike discharges of P cells; one complex response only was evoked by fin stimulation. Thus almost all the activity described in this study appears to reflect the action of mossy fibre input on the granule cells, which is then relayed by the parallel fibres to the molecular layer.

Evoked responses

The p.f.r. is a reflex withdrawal response of the pectoral fin to a peripheral stimulus, the essential features of which are formulated within the spinal cord (Paul & Roberts, 1979*b*). Nevertheless, even during this simple movement, the response patterns of those cells that were evoked in the cerebellar corpus (26% of total recorded), were surprisingly complicated and varied from cell to cell, but certain principles of organization were evident. First, except for one cell the responses occurred after the movement had commenced, making it unlikely that the cerebellum played any part in initiating the movement. Secondly, in the majority of the units the responses were excitatory and often long lasting. Thirldy, responses appeared to be correlated with motor outputs rather than with sensory inputs.

The long latency of the cerebellar responses is presumably due in part to their correlation with the motor output. Primary afferent input reaches the cord within 5–6 msec after the start of the stimulus train (D. H. Paul & B. L. Roberts, unpublished observations) but motor activity is evident in the fin muscle e.m.g. only after some 30 msec. During the intervening period, spinal interneurones become active (D. H. Paul & B. L. Roberts, unpublished observations) and if, as seems likely, impulses destined for the cerebellum originate in spinal interneurones, then as much as 20 msec of the cerebellar latency could be due to their activation times.

The nature of the cerebellar responses indicates that P cells and S cells are being driven via the parallel fibres. The slow conduction velocity of these fibres (0·2 m/sec, Paul, 1969) and the long conduction distance due to the granule cells being confined to the granular eminences, could account for a further 20–25 msec of the cerebellar latency, giving a total of 40–45 msec. Most responses, however, occurred at a considerably longer latency and there must be additional factors about which we have at present no direct information.

The consequence of these delays in the pathway between spinal cord and cerebellum is that a cerebellar response is not generated until the phasic part of the p.f.r. is well under way. Thus any influence that the cerebellum might have upon a reflex already in progress will be limited to the later tonic response. This is exactly what was found when the cerebellum was ablated. The phasic response was little affected but the tonic response was greatly attenuated or even abolished (Paul & Roberts, 1979*b*).

The almost total lack of any evoked complex responses which could be equated with climbing-fibre activity contrasts strongly with the mammalian situation where low threshold shocks to peripheral nerves too weak to evoke reflexes even in the awake animal generate climbing-fibre responses much more readily than responses via the parallel fibres (e.g. Armstrong & Rawson, 1979). As discussed above, the status of the climbing-fibre system in fish is still conjectural. Bastian (1974) described 'climbing-fibre-like' activity in cerebellar units of *Eigenmannia* in response to distortions of the field due to its own weak electric discharges and these had a long mean latency of 132 msec, similar to that of the one evoked complex response seen in the present study. However, Kotchabhakdi (1976*b*) working on *Carassius* found no evidence of climbing-fibre responses to tactile and proprioceptive stimuli.

Some cells which gave complex discharges only responded in a similar way to cells

discharging trains of single spikes and presumably these responses were driven via the parallel-fibre system. Complex responses have been shown to be evoked by the parallel fibres in *Platyrhinoidis* (Nicholson *et al.* 1969) and in *Carassius* (Kotchabhakdi, 1976*a*).

How the cerebellum can exert an influence over the p.f.r. is still a matter for speculation. However, it is known that the cerebellar nucleus projects to the reticular formation (Paul & Roberts, 1975) and that activities of certain reticular neurones which respond concurrently with the reflex can be modulated by stimulating the cerebellar nucleus (Paul & Roberts, 1976). Since the p.f.r. is completely expressed in the spinal animal, but grossly attenuated in the decerebellate animal the cerebellar influence is likely to be an indirect one, probably controlling other central systems which themselves influence the gain of the reflex.

In a recent survey of cerebellar function, MacKay & Murphy (1979) utilizing a wide range of data from clinical and experimental studies have developed a view of the cerebellum as an accessory or meta-system that operates by adjusting the 'gain' of motor control loops established by extracerebellar centres (accessory adjustor hypothesis). As they point out, the studies of the dogfish cerebellum previously published (Paul & Roberts, 1974, 1975, 1976) and further elaborated by Paul & Roberts (1979*b*) and the present paper are consistent with the view that the cerebellum functions to supplement and modify the motor activity elaborated by other regions of the central nervous system.

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