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THE ACTIVITY OF CEREBELLAR NUCLEAR NEURONES IN RELATION TO STIMULI WHICH EVOKE A PECTORAL FIN REFLEX IN DOGFISH

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SUMMARY

1. Extracellular single-unit recordings from the cerebellar nucleus were classified into type I and type II units on the basis of their spontaneous discharges. Type I units discharged at a very regular frequency, giving interspike interval histograms with narrow distributions. Type II units had irregular discharges.

2. Type I units were identified as cerebellar nuclear units by their antidromic responses to stimulation of the contralateral brachium conjunctivum (b.c.) in the mid-brain and by their inhibitory responses to stimulation of the cerebellar cortex. Type II units were not driven antidromically by b.c. stimulation but were inhibited by stimulating the cerebellar cortex.

3. Activity of the nuclear neurones was monitored following subcutaneous electrical stimulation of a fin that elicits a reflex elevation. 67% of units responded, the majority with an increased discharge frequency (excitation, 59%) but some with a decreased discharge frequency ('inhibition', 8%).

4. Latencies of both excitatory and inhibitory responses were > 50–400 msec. Most excitatory responses lasted for at least 500 msec; several lasted for more than 10 sec. Inhibitory responses lasted for about 500 msec.

5. With units tested by bilateral fin stimulation, the same qualitative response was obtained whichever fin was stimulated.

6. These results are discussed in relation to the known responses of cerebellar Purkinje cells recorded under similar experimental conditions.

INTRODUCTION

It is now generally accepted that the participation of the cerebellum in the regulation of movement depends upon the action of the cortex upon the cerebellar nuclei (and, to a limited extent, the vestibular nuclei) and thence upon the interplay between these nuclei and the rest of the central nervous system. Each cerebellar nucleus therefore occupies a key position and plays a role which, since the discovery of the inhibitory impact of Purkinje cells (Ito, Yoshida & Obata, 1964; Ito, Yoshida, Obata & Udo, 1970), is now recognized as being more than that of a simple relay.

Most previous investigations of cerebellar nuclear neurones have been made in mammals, and in a number of studies recordings have been obtained during

behavioural movements in chronic preparations. Activity has been recorded from interpositus neurones in the cat (Burton & Onoda, 1977, 1978; Amassian, Eberie & Rudell, 1980; Armstrong & Rawson, 1979) and the monkey (Thach, 1968, 1970, 1978; Harvey, Porter & Rawson, 1979) and from dentate neurones in the monkey (Thach, 1968, 1970, 1975, 1978; Grimm & Rushmer, 1974; Robertson & Grimm, 1975; Harvey *et al.* 1979) and rat (Hernandez-Mesa & Bures, 1978). Recordings have also been made from fastigial and interpositus neurones in walking mesencephalic cats (Orlovosky, 1972) and from fastigial, interpositus and dentate neurones of the cat during 'fictive' movements (Antziferova, Arshavsky, Orlovsky & Pavlova, 1980; Arshavsky, Orlovsky, Pavlova & Perret, 1980). Phasic changes in the spontaneous activity of nuclear neurones were observed in all of these studies, but the temporal relations between the discharges and movements were complex. Even apparently simple movements performed by the experimental animals involved some degree of pre-programming, while others incorporated sensory feed-back with central (often cortical) and peripheral effects.

In contrast to mammals, very few experimental studies have been made of cerebellar nuclear cell responses in lower vertebrates, and almost all previous speculations on their existence and functional importance have been on the basis of anatomical studies of normal material. The classical view derived from such studies was put succinctly by Herrick (1924) who proposed that the proportion of Purkinje cell axons terminating in the cerebellar nucleus increased throughout the vertebrate series in direct proportion to the 'progressive development' of these nuclei. Since the cerebellar nuclei of cartilaginous fishes, such as the dogfish used in the present study, were considered to be relatively undeveloped it was supposed that a substantial proportion of the Purkinje cell axons terminated directly on extracerebellar neurones. However, given the inhibitory nature of the Purkinje neurones, such a progressive development would imply a parallel functional reorganization of the brain centres to which the cerebellum projects as an inhibitory action became replaced by an excitatory one. In view of this, the report by Ebbesson & Campbell (1973) that in the nurse shark *Ginglymostoma*, all of the Purkinje axons from the cerebellar corpus (i.e. the 'non-vestibular' cerebellum of fishes) terminate in the nucleus was of great significance.

In the present paper, recordings from nuclear neurones are described which were related to activity underlying a reflex movement of the pectoral fins (the pectoral fin reflex of Paul & Roberts (1979*a*)). This preparation offers some advantages over the mammal for, whereas the cerebellum has a comparable histological construction, there is only a single nucleus. Moreover the fin movements of fishes employ far fewer muscle groups than do mammalian limb movements: the movement studied in this paper for example involves the action of just two sets of muscles, one for elevation and one for depression. Part of this work has been published previously in abstract form (Paul & Roberts, 1976).

METHODS

Animals. The experiments were done on twenty 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. Each fish was initially anaesthetized in sea water containing tricaine methane sulphonate (Tricaine, Sigma; 166 mg/l.), then decerebrated and mounted in a head holder in a tank of sea water, with the cerebellum exposed. Full details of these experimental procedures have been given previously (Paul & Roberts, 1977*a*, *b*; 1979*b*). Sea water was allowed to flow continuously over the gills to aid the full recovery from the anaesthetic, after which an intravenous injection of curare was made into the suborbital sinus (*d*-tubocurarine, Wellcome; 7 mg/kg) to abolish body movement.

The cerebellar nucleus was exposed by making an incision through the dorsal mid line of the cerebellum and reflecting the cut edges laterally. In some experiments the incision was restricted to the central portion of the cerebellum, so as to leave most of the cerebellar cortex intact.

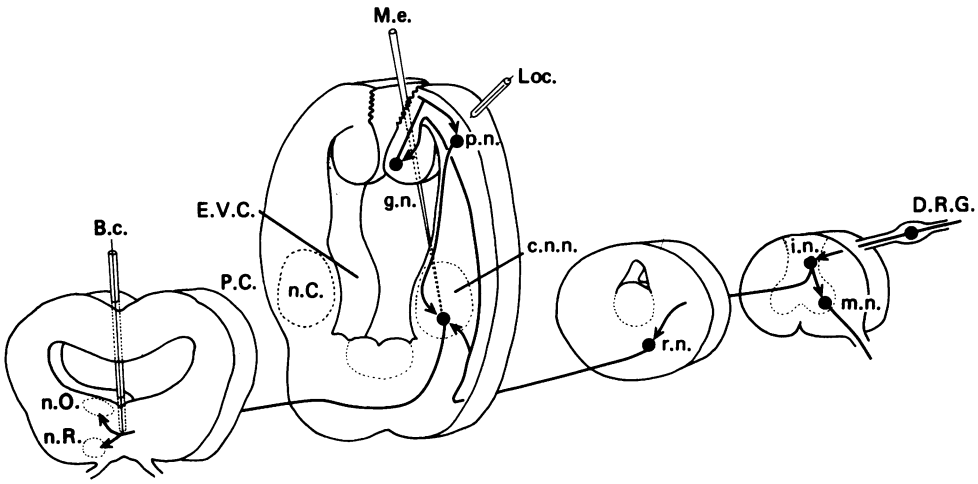

Fig. 1. Schematic diagrams of cross-sections of the central nervous system at levels of spinal cord, hind-brain, cerebellar peduncle and mid-brain, showing the main anatomical features at each level, the experimental arrangements and the postulated connexions between neurones at each level involved in responses of cerebellar nuclear neurones during the pectoral fin reflex. C.n.n., cerebellar nuclear neurone; D.R.G., dorsal root ganglion; E.V.C., eminentia ventralis cerebellaris; g.n., granule neurone; i.n., spinal interneurone; m.n., motoneurone; n.C., cerebellar nucleus; n.O., oculomotor nucleus; n.R., red nucleus; P.C., cerebellar peduncle; p.n., Purkinje neurone; r.n., reticular neurone. B.c., stimulating electrode in brachium conjunctivum; Loc., stimulating electrode on cerebellar surface. M.e., micro-electrode in cerebellar nucleus.

Stimulating and recording. Unit recordings were made with glass micropipettes filled with 4 M-NaCl and having d.c. resistances of 5–12 MΩ; they were lowered into the nucleus with a hydraulic microdrive under visual control. Electrode depth measurements were read from the microdrive display. As an aid to identification, nuclear neurones were activated antidromically by stimulating the brachium conjunctivum with a concentric bipolar electrode lowered into the contralateral mesencephalic tegmentum (b.c. stimulation). In some fish another concentric bipolar electrode was placed on the cerebellar surface to activate cortical neurones (loc. stimulation).

Stimulating and recording wires were placed in both pectoral fins to evoke and record an elevating reflex movement as described previously (Paul & Roberts, 1979*a*). Stimulus parameters necessary to evoke the reflex were established after the fish had recovered fully from anaesthesia and before curarization, and these were then kept constant throughout the experiment after the onset of curare paralysis. Signals from the micro-electrode were amplified, photographed from an oscilloscope and stored on magnetic tape for subsequent analysis using a digital computer (PDP 11/34) programmed to give interspike interval (i.s.i.) and post-stimulus time (p.s.t.) histograms. In most experiments records of instantaneous discharge rate were also available. Additional measurements were obtained from filmed records of oscilloscope traces. The experimental arrangements are illustated in Fig. 1.

RESULTS

Anatomy of the cerebellar nucleus

The cerebellum of cartilaginous fishes consists of an unpaired corpus cerebelli, enclosing a large ventricular cavity, connected to the brain stem by a single peduncle, and paired auricles which join the hind-brain at the level of the dorsal nucleus. On each side, the medial wall of the cerebellar peduncle protrudes into the ventricle as the eminentia ventralis cerebelli (Fig. 1) which contains an aggregation of cells now generally called the nucleus cerebelli and first described by Edinger (1901). He considered the nucleus to be a subcerebellar structure but developmental studies by Rudeberg (1961) have shown that it originates from the cerebellar anlage and lies within the cerebellum.

In *Scyliorhinus* the dorsal margin of the nucleus lies about 100 μm below the ventricular border and the nucleus extends to a depth of approximately 1400 μm, and has a width of about 500 μm. In silver-stained material the cerebellar nuclear neurones appear spindle-shaped. Measurements of the widest point of the soma of these neurones in one fish gave a mean value for the diameter of $17 \pm 3{\cdot}5$ μm ($\pm$S.D.; $n = 100$). Golgi preparations show these cells to be, in fact, multipolar neurones with dendrites extending for 500 μm or more from the soma. Most of the neurones are arranged with their major dendrites lying across the bundles of cerebellar cortical afferent and efferent axons that course through the peduncle.

Spontaneous activity and connexions of cerebellar nuclear neurones

Electrodes inserted into the eminentia ventralis cerebelli recorded spontaneous single unit activity of two distinct patterns. In one (type I neurones) the spontaneous activity was very regular, at rates varying from 1 to 40 spikes/sec, producing i.s.i. histograms with narrow distributions (Fig. 2*A*, *B* and *C*; Fig. 5*A* and *B*). In the other group (type II neurones) the spontaneous discharges were irregular (Fig. 2*D*). Type I units were far more numerous than type II but both were found throughout the nucleus at depths ranging from 73 to 1201 μm from the ventricular surface (Fig. 3*B*). The distribution of discharge rates of sixty-six type I units is shown in Fig. 3*A*. Most cells discharged at rates less than 25 impulses/sec. Some type I neurones tended to discharge for brief periods at rates up to three times their basic spontaneous rate, seen in their i.s.i. histograms (Figs. 2*A* and 5*A*) as a slight skewness to the left (i.e. shorter intervals) and shown very clearly by the rate record of Fig. 7*A*. Further evidence for the identification of these units as cerebellar nuclear neurones came from studies of their connexions with the tegmentum and cerebellar cortex. It is known in the nurse shark *Ginglymostoma* that axons from cerebellar nuclear cells pass as a bundle, the brachium conjunctivum, to the brain stem (Ebbesson & Campbell, 1973). After decussating, this tract splits into a large ascending and a small descending pathway. The ascending pathway provides a massive crossed projection to the presumed red nucleus and to the tegmental reticular formation and also distributes to trochlear and oculomotor nuclei. In another study on *Scyliorhinus* it has already been established that most neurones in this region of the brain stem are activated orthodromically by stimulation of the contralateral cerebellar nucleus (Paul & Roberts, 1978). A bipolar concentric electrode placed in the mid-brain in the

region of the contralateral brachium would be expected, therefore, to activate antidromically at least some of the cerebellar nuclear neurones.

In Fig. 4*A–C*, b.c. stimulation is seen to evoke spikes in a nuclear neurone with the short latency (< 1·5 msec) expected for antidromic excitation. When two stimuli were applied at an interval of 3 msec and stimulus strength was progressively raised from sub- to supra-threshold levels, responses appeared to both stimuli at the same

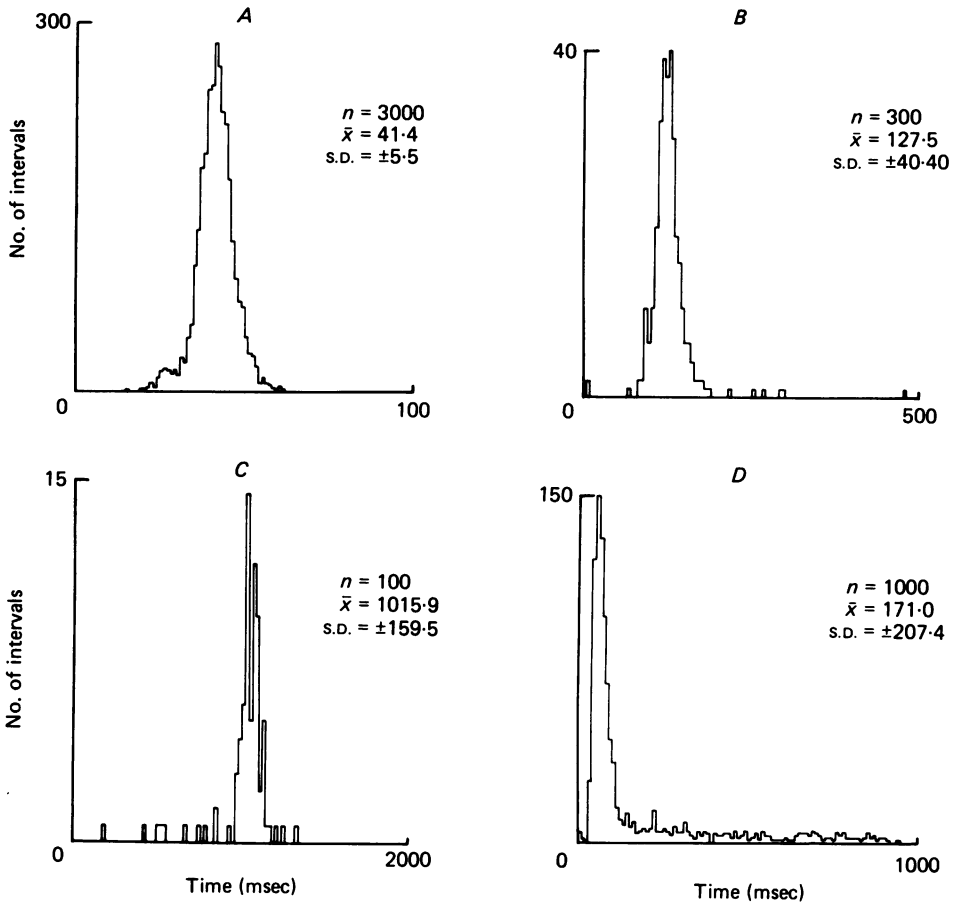

Fig. 2. I.s.i. histograms of spontaneous discharges of cerebellar nuclear neurones. *A*, *B* and *C*, are from type I units; *D* is a type II unit. The sample size (n), mean interval ($\bar{x}$) and standard deviation (S.D.) are given for each unit. Bin widths were: *A*, 1 msec, *B*, 5 msec, *C* and *D*, 10 msec.

time, although the response to the second was attenuated, presumably due to relative refractoriness (Fig. 4*A*). When the inter-stimulus interval was reduced to 2·5 msec (Fig. 4*B*) no response to the second stimulus occurred, except when the response to the first was blocked by a spontaneous spike. In the latter event, the second stimulus evoked a full sized spike, due to the response to the first stimulus colliding in the axon with the spontaneous spike coupled with a decline of relative refractoriness to a low level by the time the spike evoked by the second stimulus reached the cell body. In

Fig. 4*C*, collision between a spontaneous and an antidromic spike is shown. The spontaneous spike occurred more than 5 msec before the expected antidromic response, an interval greater than the refractory period, which can be estimated from Fig. 4*A* and *B* as 2·5–3 msec.

It has been found generally throughout the vertebrates that cerebellar Purkinje cells exert an inhibitory action on cerebellar nuclear neurones. In some experiments

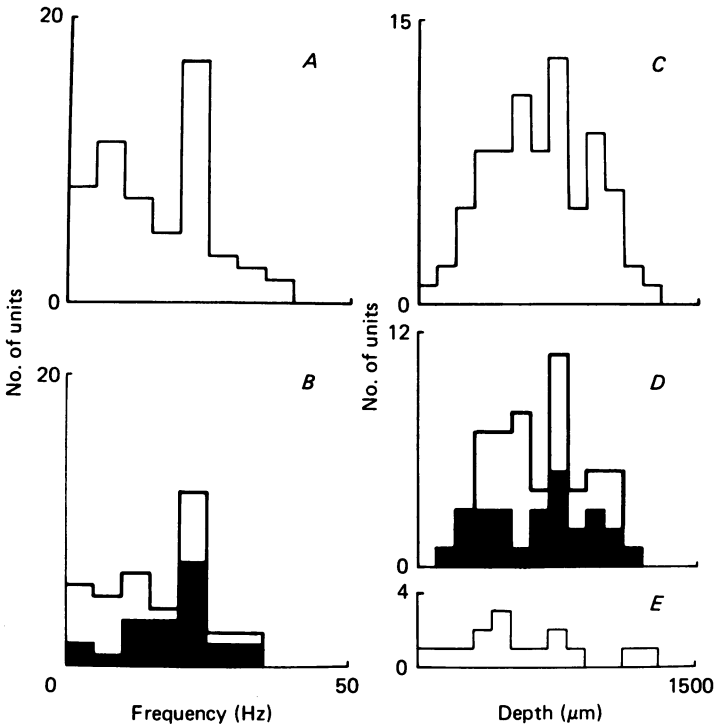

Fig. 3. Distributions of units relative to their discharge rates and their depths from the dorsal (ventricular) surface. *A* and *B*, distributions of type I units relative to their discharge rates; *A*, all units; *B*, units tested with b.c. stimulation (open columns) and those responding to b.c. stimulation with an antidromic spike (filled columns). *C*, *D* and *E*, distributions of units relative to depth; *C*, all type I units; *D*, type I units tested with b.c. stimulation (open columns) and those responding with an antidromic spike (filled columns); *E*, all type II units.

where a substantial part of the cerebellar corpus was left intact, a concentric bipolar electrode was used to stimulate the cortex and so excite Purkinje cells (loc. stimulation). Of fourteen type I cerebellar nuclear neurones tested in this way, twelve were inhibited. The inhibition of spontaneous activity lasted for 50–100 msec and began immediately after the loc. stimulus was applied. An example of this response is shown in Fig. 4*D*. Three of the five type II neurones tested were similarly inhibited.

In three instances, loc. stimulation excited nuclear cells, evoking responses with a latency of about 7 msec. This action probably results from the stimulation of afferent fibres running in the stratum fibrosum which have axon collaterals projecting to the nucleus. An example is illustrated in Fig. 4*E*.

Since a substantial proportion of the type I neurones tested were driven antidromically by b.c. stimulation and were inhibited by loc. stimulation, these cells were considered to be cerebellar nuclear neurones. Although none of the type II neurones were driven antidromically by b.c. stimulation they were also regarded as being nuclear neurones because they were inhibited by loc. stimulation.

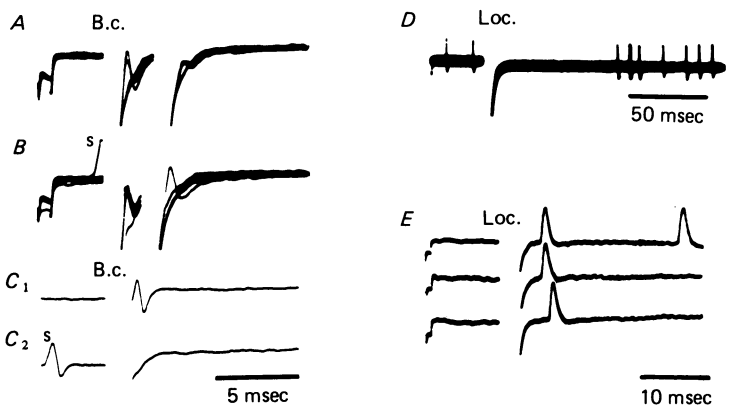

Fig. 4. Responses of nuclear units to b.c. and loc. stimulation. *A*, four superimposed responses of a unit to b.c. stimulation with a pair of identical stimuli separated by 3·0 msec. Stimulus strength increased from below (two traces) to above (two traces) threshold. Note response to second stimulus is attenuated but appears at same time as response to first stimulus. *B*, as *A* but stimulus interval now 2·5 msec and the strength always suprathreshold. Response to second stimulus only when a spontaneous spike (s) precedes first stimulus. Note response to second stimulus now full size. Interval between spontaneous spike and spike evoked by second stimulus is 4·5 msec. C_1 spike evoked by single b.c. stimulus; C_2, spontaneous spike (s) occurring 5·2 msec before expected response to b.c. stimulation blocks response. *D*, five superimposed responses of a nuclear unit to loc. stimulation. Note spontaneous activity is inhibited for about 90 msec after loc. stimulation. *E*, excitatory responses of a nuclear unit to loc. stimulation. Note response latency (6–8 msec). Calibration pulses at beginning of *A*, *B*, *D* and *E* are 1 mV negative downwards. Voltage calibration in *B* also applies to *C*. Time calibration in *C* also applies to *A* and *B*. See text for further explanation. Some spikes retouched for clarity.

Responses to fin stimulation

Sixty of the ninety cerebellar nuclear cells tested responded to stimulation of the pectoral fin and the results are summarized in Table 1. The most significant findings were that fifty-three of these units gave an excitatory response to fin stimulation and that all of the units responding to stimulation of both pectoral fins gave the same qualitative response (twenty-six excitation, two inhibition) whichever fin was stimulated. On only one occasion did any nuclear cell respond to stimuli of strengths below that needed to evoke the pectoral fin reflex. The type II neurones were less responsive to fin stimulation, only six out of seventeen showing any response. However, those responses appeared similar in all respects to responses of type I units.

Excitatory responses. The excitatory responses observed in forty-nine type I units consisted of an increase in discharge frequency to levels double or at most treble the spontaneous firing rate.

Responses of two type I neurones to stimulation of the pectoral fins are illustrated

in Fig. 5. Fig. 5*A*, *C*, *E*, *G* and *I* illustrate a unit discharging spontaneously at 31 impulses/sec which responded to stimulation of either pectoral fin with an increase in discharge frequency that lasted for about 150–200 msec. As was found for most units responding bilaterally, the response to stimulation of the ipsilateral fin was the more pronounced. Fig. 5*B*, *D*, *F*, *H* and *J* illustrate another unit, discharging spontaneously at 14 impulses/sec, which also responded to stimulation of either pectoral fin with an increase in discharge frequency. But in this case the response to the ipsilateral fin stimulation, whilst peaking earlier, was less pronounced than the response to stimulation of the contralateral fin, which lasted for around 3000 msec.

TABLE 1. Summary of responses of nuclear neurones to stimulation of the ipsilateral and/or contralateral fin. Ipsilateral and contralateral relates the fin stimulated to the position of the recording electrode. Bilateral stimulation summarizes responses of all units tested by stimulation of both fins. Unilateral stimulation summarizes responses of units where one fin only had been stimulated

Bilateral stimulation

Response of unit	Excitation		Inhibition	
Type of unit	I	II	I	II
No. responding to:				
Ipsilateral only	7	1	—	2
Contralateral only	6	2	1	—
Bilateral	25	1	2	—

Unilateral stimulation

Response of unit	Excitation		Inhibition	
Type of unit	I	II	I	II
No. responding to:				
Ipsilateral	9	—	2	—
Contralateral	2	—	—	—

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 so the parameters of response latency and duration could be estimated for most cells only by determining the first and last histogram bins having values different from controls. Fig. 6 shows the distribution of latencies in 100 msec bins. Only five responses occurred with latencies shorter than 100 msec and three of these, when analysed using 50 msec bins, had latencies greater than 50 msec. Thus, as with responses of Purkinje cells reported previously (Paul & Roberts, 1981), virtually no nuclear cell responses occurred until after the fin reflex would have begun.

The durations of the responses ranged from 150 msec to 16 sec but in only five of the forty-nine units was the duration less than 500 msec and in seventeen it exceeded 1000 msec. Responses of nuclear cells, following fin stimulation, have lasted longer, on average, than those observed previously in Purkinje cells (Paul & Roberts, 1981). The intermittent fluctuations in spontaneous discharge rate exhibited by some of the cells were eliminated throughout the responses to pectoral fin stimulation but reappeared immediately those responses terminated. This is illustrated by the records of instantaneous firing frequency in Fig. 7 for a unit in which the basic spontaneous rate was about 25 impulses/sec. In the control record occasional episodes are seen, rarely lasting more than 100 msec, when the firing rate increased by 2- to 3-fold

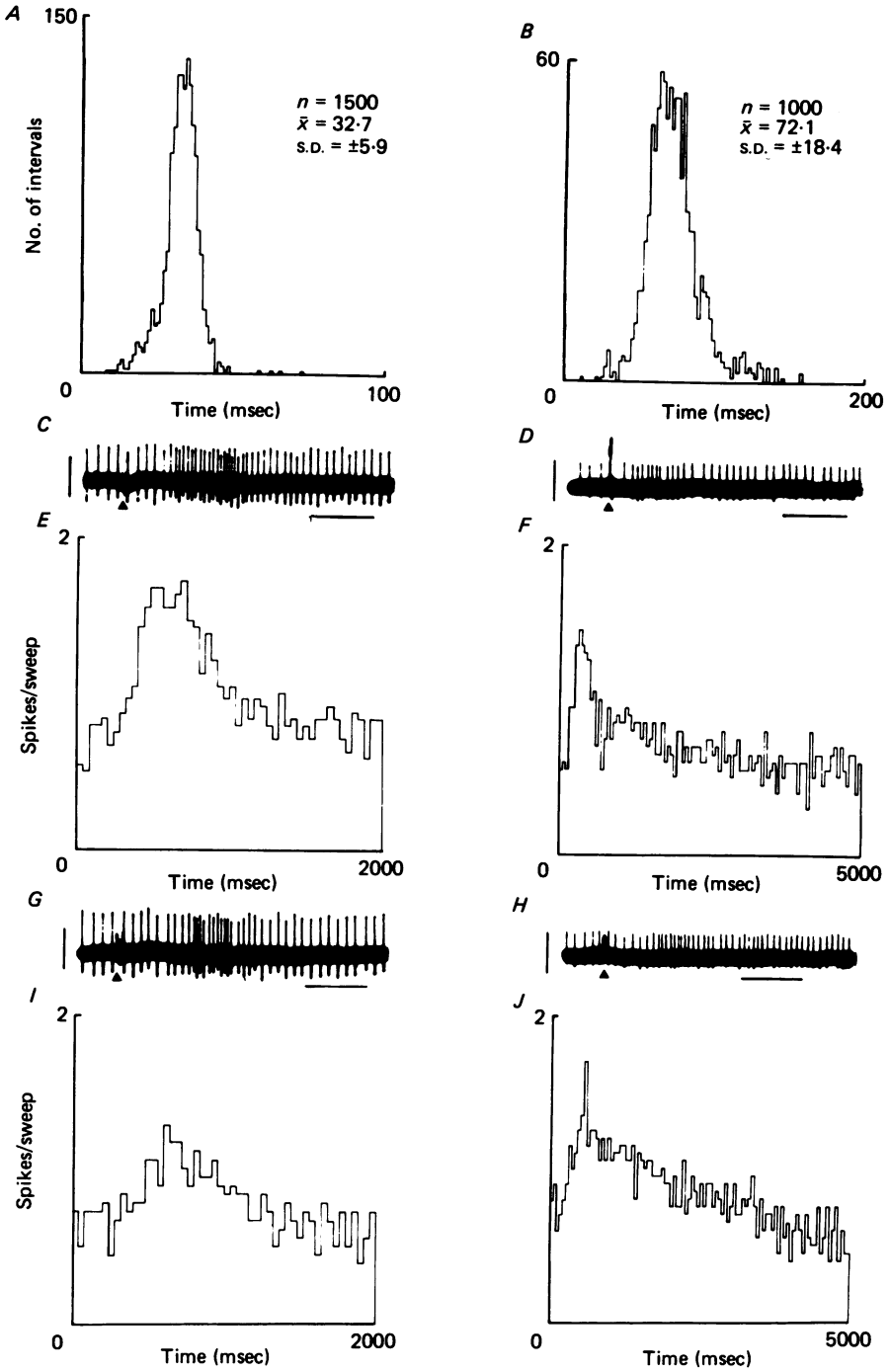

Fig. 5. Responses of type I neurones to pectoral fin stimulation. *A* and *B*, i.s.i. histograms of spontaneous activity; data as for Fig. 2. Responses of unit in *A* shown in *C*, *E*, *G* and *I*, and of unit in *B* in *D*, *F*, *H* and *J*. *C* and *D*, responses to stimulating (at arrowhead) the ipsilateral fin. *E* and *F*, p.s.t. histograms of responses to ipsilateral fin stimulation. *G* and *H*, responses to stimulating (at arrowhead) the contralateral fin. *I* and *J*, p.s.t. histograms of responses to contralateral fin stimulation. Calibrations: vertical, *C*, *D*, *G* and *H*, 1 mV positive upwards; horizontal, *C* and *G*, 200 msec, *D* and *H*, 500 msec. Histogram bin widths: *A*, 1 msec; *B*, 2 msec; *E* and *I*, 20 msec; *F* and *J*, 50 msec. Note time scales of responses are slightly different to those of corresponding histograms.

(Fig. 7*A*). After stimulation of either pectoral fin, the unit discharged a long lasting response of 12–15 sec, during which time the spontaneous fluctuations were absent, to return once more when the response terminated (Fig. 7*B* and *C*).

Five type II units gave excitatory responses to pectoral fin stimulation, with latencies ranging from 100 to 300 msec and with durations lasting from 200 to 500 msec.

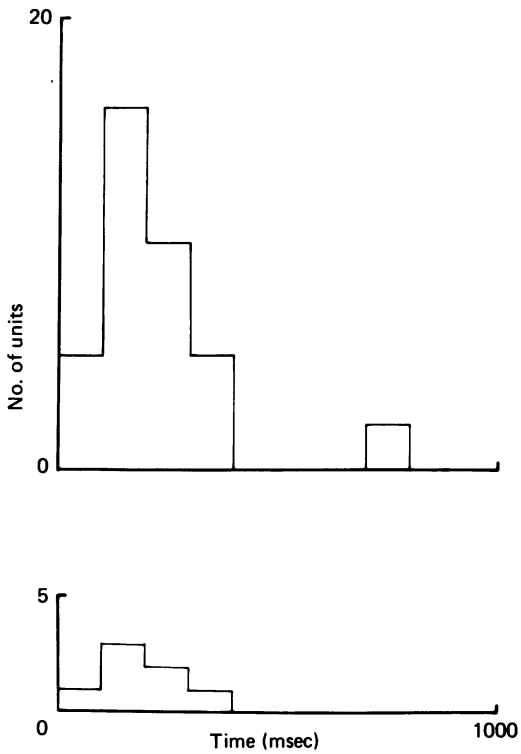

Fig. 6. Latencies of excitatory and inhibitory responses of type I units. Upper histograms, excitatory responses; lower histogram, inhibitory responses.

'*Inhibitory*' *responses.* Only seven units (five type I and two type II) gave responses to stimulation of the pectoral fins in which the discharge rate decreased. In Fig. 8*A–C* a unit having an extremely regular discharge of 23 impulses/sec was inhibited by stimulation of the ipsilateral fin. Although the inhibition was well marked in most of the individual responses, in some it was represented only by a slowing of the discharge rate (Fig. 8*A*) and neither the latency nor duration of the response was consistent from sweep to sweep. From the p.s.t. histogram (Fig. 8*B*) and the cumulative frequency plot (Fig. 8*C*) it can be seen that the response latency was between 250 and 300 msec, whilst the duration of the response was 400–450 msec.

In the unit illustrated in Fig. 8*D–F* the influence of fin stimulation was usually simply to slow the spontaneous discharge. The effect was inconsistent and the latency and duration showed considerable variation, as can be seen in the raster display of Fig. 8*F*. From the p.s.t. histogram (Fig. 8*E*) and the responses illustrated (Fig. 8*D*), the latency of the response can be seen to fall in the period 100–150 msec after the

stimulus and its duration to be 500 msec. The latencies of all the inhibitory responses are plotted in Fig. 6*B*. These all fall within the range for excitatory responses of Purkinje cells recorded in a similar series of experiments and reported previously (Paul & Roberts, 1981).

DISCUSSION

Connexions of the cerebellar nuclei

Ebbesson & Campbell (1973) concluded that in the nurse shark *Ginglymostoma* all the Purkinje cell axons terminate in the cerebellar nucleus. This is an important conclusion since it establishes the organization of the cerebellar output paths in these

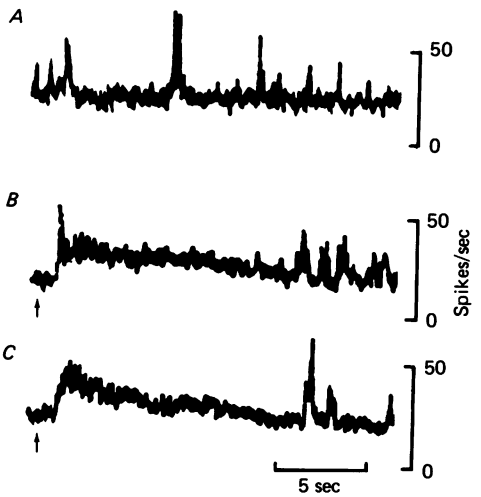

Fig. 7. Rate-meter records of spontaneous activity and responses to fin stimulation of a type I neurone. *A*, spontaneous activity. Note the underlying steady discharge rate and the epochs of increased discharge rate. *B* and *C*, responses to ipsilateral (*B*) and contralateral (*C*) fin stimulation. Note the increases in steady discharge rate and the absence of epochs of increased discharge rate. Stimuli given at arrows.

fishes as being directly comparable to that of mammals. We have obtained similar evidence in *Scyliorhinus*, using degeneration methods (D. H. Paul & B. L. Roberts, unpublished observations). It has not been possible to trace the axons of nuclear cells to their terminations in any of our silver-stained or Golgi material from *Scyliorhinus* and little information is available in the literature about connexions of the cerebellar nuclei with the brain stem in this species. In *Ginglymostoma*, on the basis of an experimental neuroanatomical study, Ebbesson & Campbell (1973) have described projections from the cerebellar nuclei to the reticular formation of the mid-brain (including a putative red nucleus) and hind brain and to the trochlear and oculomotor nuclei. The nuclear cell axons form a well developed brachium conjunctivum which, after decussating, sends a larger bundle rostrally to the mesencephalic tegmentum and a smaller bundle caudally to the medulla. A major part of this projection has been confirmed electrophysiologically in *Scyliorhinus*. Stimulation of the cerebellar

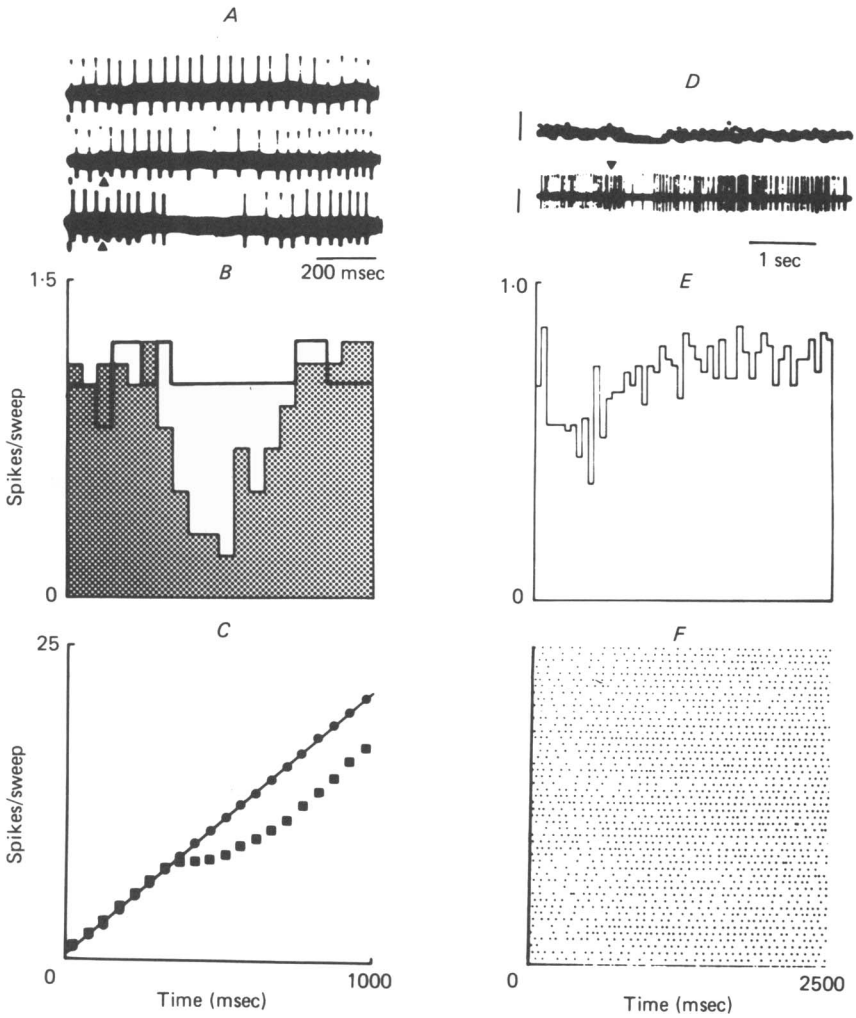

Fig. 8. Inhibitory responses of type I units. *A*, examples of spontaneous activity (top trace) and responses to ipsilateral fin stimulation (given at arrow heads). Calibration pulse at beginning of each trace is 1 mV, negative downwards. *B*, control (heavy line) and p.s.t. (stippled) histograms of unit shown in *A*. Bin width, 50 msec. *C*, data from *B* presented as cumulative frequency plots. ●, control; ■, ipsilateral fin stimulation. Note the highly regular spontaneous activity of this unit. *D*, five superimposed records of responses to ipsilateral fin stimulation of another unit. Stimulus given at arrow head. Top trace, rate-meter record; vertical calibration, 0–50 spikes/sec. Bottom trace, unit discharges; vertical calibration 1 mV, positive upwards. *E*, p.s.t. histogram of unit in *D*. Bin width 50 msec. *F*, raster display of fifty consecutive records of unit discharge of unit shown in *D*. Note the variability of the response of this unit. Stimulus given at time zero.

nucleus produces orthodromic driving of reticular neurones in the hind brain (Paul & Roberts, 1975) and of reticular and rubral neurones in the mid-brain (Paul & Roberts, 1978). In the present study we have used antidromic driving of units by stimulation of the contralateral brachium conjunctivum as an identifying criterion for nuclear neurones.

The sources of input to the cerebellar nuclei which are responsible for the responses to fin stimulation are not known with certainty. In the mammal, a substantial input to the cerebellar nuclei via collaterals of afferents relaying to the cortex has been proposed as the major source. In elasmobranch fish, spinal connexions with the nucleus have been described by Hayle (1973) and by Ebbesson & Hodde (1981), presumably as collaterals of the spino-cerebellar projections which reach the cortex.

Stimulation of the hind-brain reticular formation generates orthodromic potential fields in the cerebellar nucleus (Paul & Roberts, 1975) and in the present study an occasional excitatory response of a cerebellar neurone was seen to follow stimulation of the cerebellar cortex, a result that also suggests the existence of collaterals to the nucleus from cortically projecting fibres.

Electrophysiological properties of cerebellar nuclear neurones

The spontaneous activity of most of the nuclear neurones recorded in this study was remarkably regular. Interspike interval histograms had quite narrow distributions, even in cells discharging at frequencies as low as 1 impulse/sec. Because of the inhibitory nature of Purkinje cells, it is of course necessary for nuclear cells to maintain a degree of spontaneous activity against which this inhibitory cortical output can be expressed.

The highly regular spontaneous discharges of the nuclear neurones are difficult to explain purely on the basis of an intermittent afferent input, and it has to be considered that there may be an element of pace-maker activity present. The finding that in many of these cells the steady basic spontaneous discharge was occasionally interrupted by short periods of high-freqency firing could then be interpreted as the addition of intermittent afferent inputs to the endogenous spontaneous activity.

It is known that Purkinje cells discharge spontaneously in these fish but the mean discharge rate is low, averaging only 6·6 impulses/sec (Paul & Roberts, 1981). Whether or not this level of activity is sufficient to maintain an ongoing inhibitory regulation of nuclear cell discharges could not be determined in these experiments, but the possibility that the surgical procedures had removed a significant proportion of the Purkinje cell inhibition must be considered. Comparisons between the discharge rates of nuclear cells in experiments in which cortical damage was minimal and in others in which it was more extensive did not reveal any obvious differences and even in those experiments where a maximum exposure of the nucleus was made some 80 % of the cortex remained intact. Furthermore, the reflected parts of the cortex were not necessarily damaged and the one region indisputably damaged, the mid-line region, contains no Purkinje cells. Thus there is no reason to suppose that a large fraction of the Purkinje cell inhibitory drive had been removed.

Responses to fin stimulation

Although the cells from which the recordings were obtained were divided into two groups on the basis of their spontaneous discharges, no differences were noted between the evoked responses of the two groups and they are therefore considered together in this section.

Because the fish were curarized in these experiments, it was not possible to make the comparisons between the evoked responses and the actual fin reflexes as was done

for Purkinje cells (Paul & Roberts, 1981). However, at the beginning of each experiment the stimulus parameters were set to produce a well formed fin reflex with both phasic and tonic components and it is known from earlier experiments (Paul & Roberts, 1979*a*) that under these conditions the latency of the response is highly consistent and does not exceed 41 msec at the lowest water temperature used.

The major findings to emerge from this series of experiments fall into three main categories: the proportion of cerebellar nucleus neurones responding to the fin stimulus, the kinds of responses recorded from these neurones and the parameters of the responses.

Numbers of responding cells. Two-thirds of the ninety cells tested in these experiments responded in some way to the fin stimulus. This is in contrast to the 26 % of cortical units which responded in similar experiments reported previously (Paul & Roberts, 1981), even though in the latter study recordings were made exclusively from a region of the cerebellar corpus in which responsive cells were particularly concentrated. The inference is that whereas quite a small proportion of the cortical Purkinje cells respond to this stimulus, a large proportion of the nuclear cells do so.

The kinds of response recorded. Nearly 90 % (fifty-three out of sixty) of the nuclear cells that responded to the fin stimulus were excited. A preponderance of excitatory responses amongst nuclear cells has been remarked on previously by several authors who have studied mammals performing movements. Thach (1970), Mortimer (1975) and Grimm & Rushmer (1974) have all reported that 70 % or more of the nuclear cells they recorded exhibited excitatory responses. This preponderance of excitation amongst the nuclear cells is noteworthy because of the reciprocal relationship between nuclear and Purkinje cells. If there was close coupling between the Purkinje and nuclear cells that was influenced by a particular set of afferent inputs, then inhibition might be expected to play a prominent role in the nuclear responses. On the other hand, there would clearly be a latency difference between the onset of nuclear excitation in response to afferent impulses and the arrival of inhibitory impulses from the cortex, and Mortimer (1975) claimed to have detected this. In the present experiments, there was no evidence for any *widespread* inhibitory influence from the cortex following the initial excitation of the nuclear cells.

In neurones which were tested by stimulation of either fin, 60 % were found to respond and in all cases the response was qualitatively the same no matter which fin was stimulated. Again this contrasts strongly with the findings for Purkinje cells where only 17 % responded to stimulation of both fins and in which the responses were not necessarily the same.

Parameters of the responses. The latencies of the excitatory responses were mostly in excess of 100 msec and therefore the vast majority of the nuclear neurones did not respond until a time at which the tonic part of the reflex would have been about to commence or had indeed begun. This is exactly comparable to the latencies of the Purkinje cell responses reported earlier (Paul & Roberts, 1981) and as argued then, it seems highly unlikely that these responses could be related to direct afferent inputs.

Comparing the durations of the responses, the nuclear cells were excited for much longer periods than Purkinje cells. Responses lasting well over 10 sec have been recorded in nuclear cells, whilst response durations over 1 sec were rare in Purkinje cells. This seems to suggest that either there is a difference between the pathways

which carry the inputs to the cortex and nucleus or that the effects of the inputs have profoundly different outcomes in the two types of neurones.

The inhibitory responses recorded all had latencies in the 100–400 msec range and since Purkinje cells also begin discharging during this period the evidence is consistent with the hypothesis that the inhibitory responses were the result of Purkinje cell activity. The durations of the responses, which were all about 500 msec or less, were also consistent with the reported durations of Purkinje cell discharges (Paul & Roberts, 1981).

The nature of the type II neurones

Type II neurones consistently failed to respond to b.c. stimulation, raising the possibility that they may represent a population of interneurones. In mammals, where populations of neurones of different sizes have been reported, some small neurones have been considered to be putative interneurones (e.g. by Matsushita & Iwahori, 1971). In the dogfish cerebellar nucleus, however, all the neurones appear morphologically similar.

Recent experimental anatomical evidence has shown that all classes of cerebellar nucleus neurones project to extracerebellar sites in the mammals; what is clear is that different neurones may well project preferentially to different regions. Many small neurones have been retrogradely labelled from the medulla, but in double-labelling experiments only a fraction of the labelled neurones projected to both mid- and hind brain (Bharos, Kuypers, Lemon & Muir, 1981; Bentovoglio & Kuypers, 1982). Thus some neurones must project exclusively to the medulla. Therefore an alternative suggestion is that the type II neurones of the dogfish cerebellar nucleus project only to the hind brain. Projections to the reticular formation of the medulla in elasmobranch fishes have been demonstrated anatomically by Ebbesson & Campbell (1973) and electrophysiologically by Paul & Roberts (1975). However, it must be stressed that in the present experiments no evidence was obtained to support or deny either of these two possibilities.

Cerebellar operations during the fin reflex

On the basis of the evidence from this paper and from the previous descriptions of the reflex and its relations to the cerebellum (Paul & Roberts, 1979*a*) and of the responses of Purkinje cells during the reflex (Paul & Roberts, 1981) it may be suggested that some time after a reflex has begun, widespread excitation of cerebellar nuclear cells begins and that this excitation may outlast the reflex by a substantial margin. Through the efferent projections of the nucleus, this excitatory activity will be relayed to the reticular formation of both the mid- and hind brain. It has been reported for example that well over 90% of mid-brain reticular and rubral neurones receive an excitatory projection from the cerebellar nucleus (Paul & Roberts, 1978). Amongst all this excitatory activity there will be small islands of decreased activity representing the final outputs generated by cerebellar cortical operations. Since a major role of the reticular system appears to be to constantly oppose the tendency of spinal neurones to become excessively excitable (Paul & Roberts, 1979*a*), a role which is considerably influenced by the cerebellum, the function of the cerebellum in these relatively primitive vertebrates may be to so regulate the activity of the

reticular formation that 'desired' behavioural movements are promoted within the context of the current status of the motor system. Accordingly the long-lasting, widespread excitatory activity produced by the cerebellar nuclei would represent a non-specific background against which the cerebellar cortically induced inhibitions of a select few nuclear neurones would appear in high contrast. We propose that it is these latter responses which are important in the freely swimming fish for the regulation of its ongoing motor behaviour.

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