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EFFECTS OF AFFERENT VOLLEYS FROM THE LIMBS ON THE DISCHARGE PATTERNS OF INTERPOSITUS NEURONES IN CATS ANAESTHETIZED WITH α-CHLORALOSE

BY D. M. ARMSTRONG, BARBARA COGDELL* AND R. J. HARVEY

From the Department of Physiology, the Medical School, University of Bristol, University Walk, Bristol BS8 1TD

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SUMMARY

1. In cats anaesthetized with α-chloralose, micro-electrodes have been used to record the discharge patterns of single neurones in the region of the nucleus interpositus.

2. Almost all cells tested could be antidromically invaded following electrical stimulation of the contralateral red nucleus, showing that they were cerebellar efferent neurones.

3. A little over half of the interpositus neurones were spontaneously active, usually at rates of less than 20 impulses/sec.

4. About 40 % of the cells had no spontaneous activity, although they gave brisk responses to electrical stimulation of cutaneous nerves. Such silent units were encountered most frequently in the earlier stages of an experiment, but a number were found more than 15 hr after the beginning of an experiment.

5. Stimulation of cutaneous and mixed nerves of the fore and hind limbs provoked impulse discharges of the cells and also produced phases of deceleration of the resting discharge of spontaneously firing cells.

6. The typical response of an interpositus neurone consisted of a short latency (6–35 msec) discharge, usually separated from a long latency (50–500 msec) discharge by a period of inhibition or return to the resting discharge rate. The two phases of excitation appeared to be independently generated, since in a number of cells one phase appeared without the other. In addition, the later phase of excitation was abolished in all cells tested by a small dose of pentobarbitone which produced very little effect on the earlier phase. The long latency response was quantitatively much greater, sometimes consisting of 50 or more impulses in a response which lasted several hundred msec, but was very variable from one trial to another.

* MRC Scholar; present address, School of Medicine, Dept. of Physiology and Biophysics, SH – 40, University of Washington, Seattle, Washington 98 195, U.S.A.

7. The long latency discharge and sometimes the preceding inhibition could readily be mimicked by single shock stimulation of the region of the contralateral inferior olive. Short latency discharges were, however, rarely evoked by olivary stimulation.

8. It is suggested that the short latency responses of the interpositus neurones were a result of synaptic excitation via cerebellar afferents, while the ensuing inhibition was a result of post-synaptic inhibition resulting from the Purkinje cell excitation due to the afferent volleys. It is suggested that the long latency excitation is due at least in part to disinhibition resulting from long pauses in Purkinje cell firing following their activation by climbing fibre afferents.

9. The possibility that these long latency responses have a physiological significance in relation to locomotion is discussed.

INTRODUCTION

The cerebellum is heavily implicated in the control of limb movements and receives a great deal of afferent information from the limbs. Numerous investigations (see Oscarsson (1973) for a review) have shown that this information reaches predominantly the 'intermediate' region of the cerebellar cortex (paravermal anterior lobe and the paramedian lobules). The output from this part of the cerebellar cortex is carried via Purkinje cell axons to the neurones of the nucleus interpositus, which are monosynaptically inhibited (Ito, Yoshida, Obata, Kawai & Udo, 1970). Thus, afferent inflow from the limbs would be expected to have powerful effects on interpositus neurones.

In view of this, we have investigated responses evoked in nucleus interpositus by afferent volleys from the limbs. Individual nuclear cells have been studied by extracellular recording and we have attempted to characterize both the spontaneous activity and the effects wrought by stimulation of limb nerves. The majority of units recorded in the interpositus nucleus were positively identified as cerebellar efferent neurones since they were antidromically invaded following an electrical stimulus to the region of the contralateral red nucleus, to which interpositus neurones are known to project.

Responses evoked by stimulation of forelimb cutaneous afferents in interpositus neurones of decerebrate cats have been studied by Eccles, Rosén, Scheid & Táboříková (1972), who found in most cells a series of increases and decreases in the discharge rate terminating with a period of depression with latency *ca.* 35 msec and duration *ca.* 100 msec. These responses were attributed to activity in the forelimb homologues of the direct spino-cerebellar paths, the spino-reticulo-cerebellar paths and the

spino-olivocerebellar paths. The two former groups of pathways terminate in the cerebellar cortex as mossy fibres, while the last group terminates as climbing fibres; all the pathways are believed to provide direct collaterals to the nuclear neurones.

In the present experiments we have used α-chloralose anaesthesia, because barbiturates are known to exert a depressant effect on a number of aspects of cerebellar cortical activity (Bloedel & Roberts, 1969; Murphy & Sabah, 1970; Gordon, Rubia & Strata, 1973), whilst decerebration indiscriminately severs many descending pathways to the cerebellum.We have made an attempt to assess the contribution of the climbing fibre pathways to the responses we observed, by comparing the responses to peripheral nerve stimulation with those evoked by electrical stimulation of the inferior olive.

Some of the results have previously been reported in brief (Armstrong, Cogdell & Harvey, 1973).

METHODS

Experiments were performed on twenty cats weighing between 2·0 and 3·0 kg. Anaesthesia was induced using ether or halothane in air and was maintained by intravenous injection of α-chloralose, 50 mg/kg, as a 10% solution in propane-1,2-diol. The chloralose was recrystallized from commercial chloralose (British Drug House) to remove the β-isomer, so as to reduce the convulsant activity (Kruger & Albe-Fessard, 1960). Maintenance doses of chloralose were administered intravenously as necessary, typically a further 20 mg/kg. When pentobarbitone sodium was administered (see Results) it was delivered intravenously.

Gallamine triethiodide (Flaxedil; May & Baker) was administered intravenously to most animals in repeated doses of 15 mg/kg to maintain complete paralysis. Paralysed animals were artificially respired using oxygen.

In each animal a craniotomy was performed to expose the cerebellar cortex overlying the left nucleus interpositus; wide access was obtained in four animals by removing the occipital pole of the left cerebral hemisphere by suction and subsequently removing the left side of the bony tentorium. In the majority of animals a small craniotomy was made in the base of the skull to provide free drainage of cerebrospinal fluid from the cranium. Small craniotomies were made to allow stereotaxic insertion of needle electrodes (see below) into the right red nucleus and into the right inferior olive.

In each experiment some or all of the following nerves were dissected for electrical stimulation: *nerves of the forelimbs*, ipsilateral and contralateral superficial radial (iSR and cSR), ipsilateral deep radial (DR); *nerves of the ipsilateral hind limb*, gastrocnemius-soleus (GS), sural (SUR), femoral (FEM), semimembranosus and semitendinosus together (HAM), and the sciatic nerve (SCI). The electrical thresholds of the most excitable fibres in the nerves were determined by recording the compound action potential from a point on the nerve proximal to the stimulating electrodes. For forelimb nerves, the threshold was sometimes determined by recording from the surface of the dorsal columns at the level of the second cervical segment. Stimulating voltages are expressed in terms of the electrical threshold (T) for the nerve.

Concentric needle electrodes for intracranial stimulation were constructed from stainless-steel wire 100 μm in diameter and insulated except at the tip with Bakelite

Laquer (B & K Resins Ltd; grade L 3128), which was inserted into stainless-steel 27 G hypodermic needle tubing which was similarly lacquered. The wire was used as cathode relative to the more diffuse anode produced by removing a small patch of insulation from the outer electrode between 0·5 and 1·0 mm behind the tip. Constant current stimulators were employed for brain stimulation and currents were routinely monitored. Stimulus duration was 0·2 msec and stimuli normally ranged in intensity between 10 and 500 μA. The tip positions of concentric electrodes were often verified at the end of the experiment by passing a direct current to produce iron deposits which were made visible by the Prussian blue technique. The brain stem was embedded in celloidin and sectioned transversely at 100 μm. Sections containing iron spots were counterstained with gallocyanin.

Interpositus neurones were recorded using glass micro-electrodes filled with 4 M-NaCl (tip diameter *ca.* 2 μm) or metal micro-electrodes consisting of tungsten or stainless-steel needles varnished except at the tip (Frederick Haer Ltd). Micro-electrode penetrations were made in the stereotaxic vertical in the four experiments in which the bony tentorium was removed but in the remaining sixteen experiments the electrodes were angled forwards by 30°. In a small number of experiments, when stainless-steel electrodes were used, the position of the tips was marked by an iron deposit.

Impulse activity was amplified using a Grass P 16 pre-amplifier and fed to an oscilloscope screen for photography, to a tape recorder and after the first experiments, via an analogue-digital converter to a digital computer (Modular One; Computer Technology Ltd). The latter was controlled from a teletype peripheral and programmed to produce interval histograms and autocorrelograms of resting discharges and post-stimulus histograms of evoked activities. Results were displayed temporarily on an oscilloscope (Tektronix Type 611). A computer controlled graph plotter was used to prepare permanent histograms.

RESULTS

Location and identification of units

Micro-electrodes were introduced into the cerebellum through the region of cerebellar surface just rostral to the paramedian lobule. The tracks were between 4·0 and 5·5 mm from the cerebellar mid line but the majority of the units were collected around 4·5 mm from the mid line. The units were between 6 and 9 mm below the cerebellar surface. In six experiments the last track was made using a stainless-steel micro-electrode for marking purposes (see Methods). The distribution of the iron deposits showed that the units were in the region occupied by nucleus interpositus. The majority of units were collected within nucleus interpositus anterior (see Flood & Jansen, 1961).

A total of 212 single units were studied for periods ranging from a few minutes to over 2 hr. Action potentials were negative-going or biphasic (positive–negative) and ranged in amplitude from 0·5 to 2·0 mV. No attempt was made to obtain large spikes because mechanical stimulation by the micro-electrode may produce changes in firing pattern (Alanis & Matthews, 1952). The units were isolated on the basis either of their resting

discharge or of the impulses fired in response to mid-brain or peripheral nerve stimulation. When the electrode was moved units could be recorded over a distance of at least 100 μm, suggesting that the recordings were from cell bodies.

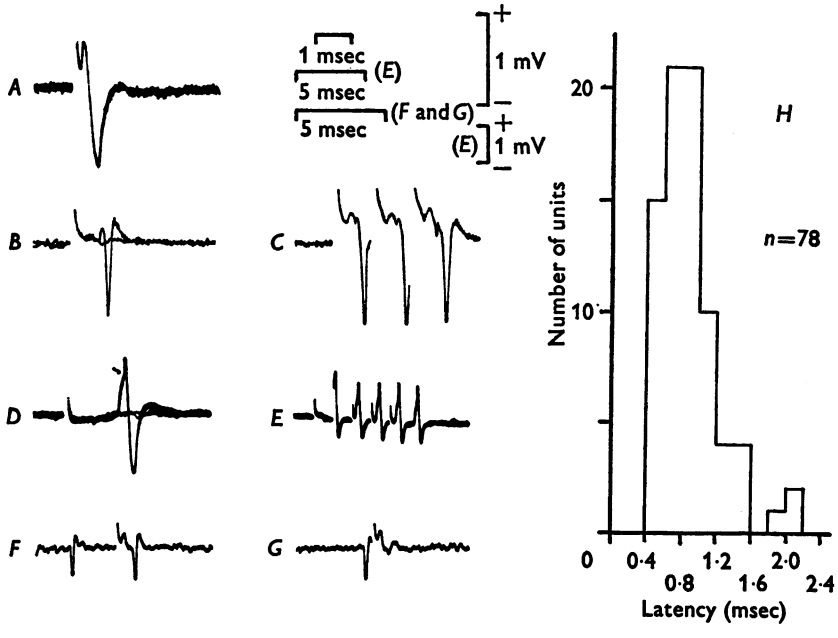

Fig. 1. Responses evoked in the nucleus interpositus by stimulation of the red nucleus. *A*, five superimposed field potentials. *B*, three superimposed responses of a single interpositus unit, with the stimulus just at threshold for the unit. *C*, three superimposed responses of the unit in *B* to three stimuli at 1000/sec. *D*, five superimposed responses of a unit in a cat cooled to 32° C with the stimulus just at threshold for the unit. Arrow indicates inflexion on the rising phase of the spike (see text). *E*, five superimposed responses of the unit in *D* to five stimuli at 700/sec. *F*, *G*, single responses evoked by stimuli occurring a short time after a spontaneous firing of the unit (see text). *H*, histogram of the distribution of latency of antidromic response for seventy-eight interpositus units.

Top time calibration applies to all records except *E*, *F* and *G*. Top voltage calibration applies to all records except *E*.

In the first eight experiments an attempt was made to identify all units as cerebellar efferent neurones by determining whether or not an antidromic action potential could be evoked by mid-brain stimulation. In five of these experiments the position of the stimulating electrode was checked histologically (see Methods) and in each case the tip lay in the caudal part of the red nucleus. Ninety-nine units were studied and in ninety-two of these a single impulse could be evoked by a shock of intensity

1 mA or less. Responses of three different units are shown in Fig. 1*B*, *D* and *F*. Fig. 1*A* shows the mass response in nucleus interpositus; single units were invariably found on a field potential of this kind. The unit responses were characterized by an abrupt threshold, which ranged from 25 to 700 μA in the sixty-one cases in which it was accurately measured, and was usually less than 200 μA. The latency of the responses was short (range 0·5–2·1 msec) and did not vary between successive trials, except with just supra-threshold stimulation when variations of around 0·1 msec were often detected. Fig. 1*H* is a histogram showing the distribution of response latencies in the seventy-eight units (from all twenty experiments) for which latency was accurately measured.

In many cases, occlusive interaction between impulses evoked by mid-brain stimulation and impulses fired spontaneously or in response to stimulation of peripheral nerves was demonstrated. This showed that the responses were antidromically generated and is illustrated for one unit by the traces of Fig. 1*F* and *G*. In Fig. 1*F* a mid-brain stimulus occurs 2·5 msec after a spontaneous impulse and succeeds in evoking an antidromic response. In Fig. 1*G* the interval is 0·5 msec and the stimulus fails to produce an antidromic action potential. In other units impulses were shown to follow mid-brain stimulation at frequencies of 700–1000/sec (as shown in Fig. 1*C* and *E*).

Of the seven cells which could not be antidromically identified in the first eight experiments, three were recorded in an experiment in which the thresholds for antidromic invasion were unusually high. In the twelve remaining experiments, antidromic identification was attempted for only 60 of the 113 units studied, but was always successful.

In one experiment the body temperature of the animal fell to 32° C due to a malfunction of the homoeothermic blanket control. Under these conditions the antidromic impulses displayed a pronounced *A–B* inflexion on the rising phase (see for example the arrow in Fig. 1*D*) and could usually be fractionated by presenting a train of three or four mid-brain stimuli at a frequency of 1000/sec, when the *B* component of the third or fourth spike was frequently stunted or failed to develop. This confirms that the recordings were from cell bodies rather than axons. Impulse fractionation was not observed at normal body temperature.

Spontaneous activity of neurones in nucleus interpositus

Records of resting activity were made for 154 units. For ease of description, the cells have been divided into four classes.

(*a*) Fifty-seven cells (37 % of the total) were essentially silent, generating less than a dozen sporadic impulses when monitored continuously for periods between 5 and 30 min.

(*b*) Seventy-one cells (46 %) displayed continuous discharge or fired in bursts separated by no more than 2 or 3 sec. The mean frequency of firing

was measured over 1000 discharges for fifty-six of these cells and was found to range from 2 to 35/sec. Only eight cells showed mean rates greater than 20/sec whilst thirty-one had rates of 10/sec or less. The discharge was in general rather irregular; the standard deviation of the interval lengths was frequently greater than the mean. Typical interval histograms are shown in Fig. 2*A* and *B*.

(*c*) Twenty cells (13%) generated occasional single impulses or short bursts of impulses between silences lasting for several seconds.

(*d*) Six cells (4%) showed variable behaviour, with periods of activity lasting for several minutes separated by periods of silence of a similar duration.

These subdivisions almost certainly have little functional basis because the level of spontaneous activity was not constant throughout each experiment. Recordings began between 5 and 9·5 hr after the initial dose of α-chloralose and usually continued for around 10 hr. At the beginning of the recording period it was usually found that the majority of nuclear cells displayed little or no spontaneous activity although the overlying Purkinje cells were tonically active. However, the level of activity increased slowly so that 3–5 hr later many cells displayed an irregular spontaneous discharge with bursts of impulses between short pauses. In view of this variation with time, it is clear that the numbers of cells in the different categories is partly a reflexion of the time at which the individual units were recorded. Nevertheless, some silent cells were encountered even towards the end of the experiments. The increase in spontaneous activity with time appeared roughly to parallel the frequency and intensity of 'chloralose jerks' (e.g. Adrian & Moruzzi, 1939) in unparalysed animals. However, the activity did not depend on sensory input produced by these movements since it was unchanged by the administration of paralysing doses of gallamine.

The spontaneous activity of the nuclear cells could be greatly increased by small doses of pentobarbitone. This effect was studied in twelve cells from as many different animals and the discharge pattern was invariably converted from irregular discharge or silence to a much more regular discharge at a higher mean rate. The effect is illustrated for three different cells in Fig. 2*C*, *D*, *E*, *F* and *G*. Fig. 2*C* shows a cell which was silent initially but began a fairly regular discharge at a rate of around 80/sec after an injection of pentobarbitone, 10 mg/kg. Fig. 2*D* shows the effect on another unit of three successive injections of 5 mg/kg. The first and second doses silenced the intermittent firing of the unit whilst the third provoked a dramatic increase in the firing, initially to around 70/sec. Fig. 2*E*, *F* and *G* each show pairs of successive sweeps in which the right-hand trace of each pair shows the activity of an interpositus unit whilst the

left-hand trace shows the activity recorded from the overlying surface of the cerebellum with a ball electrode. Parts *E*, *F* and *G* respectively show the activity before pentobarbitone, after one dose of 7 mg/kg and after a second similar dose. The first dose abolished the irregular 'bursting' discharge shown initially by the unit and simultaneously reduced the

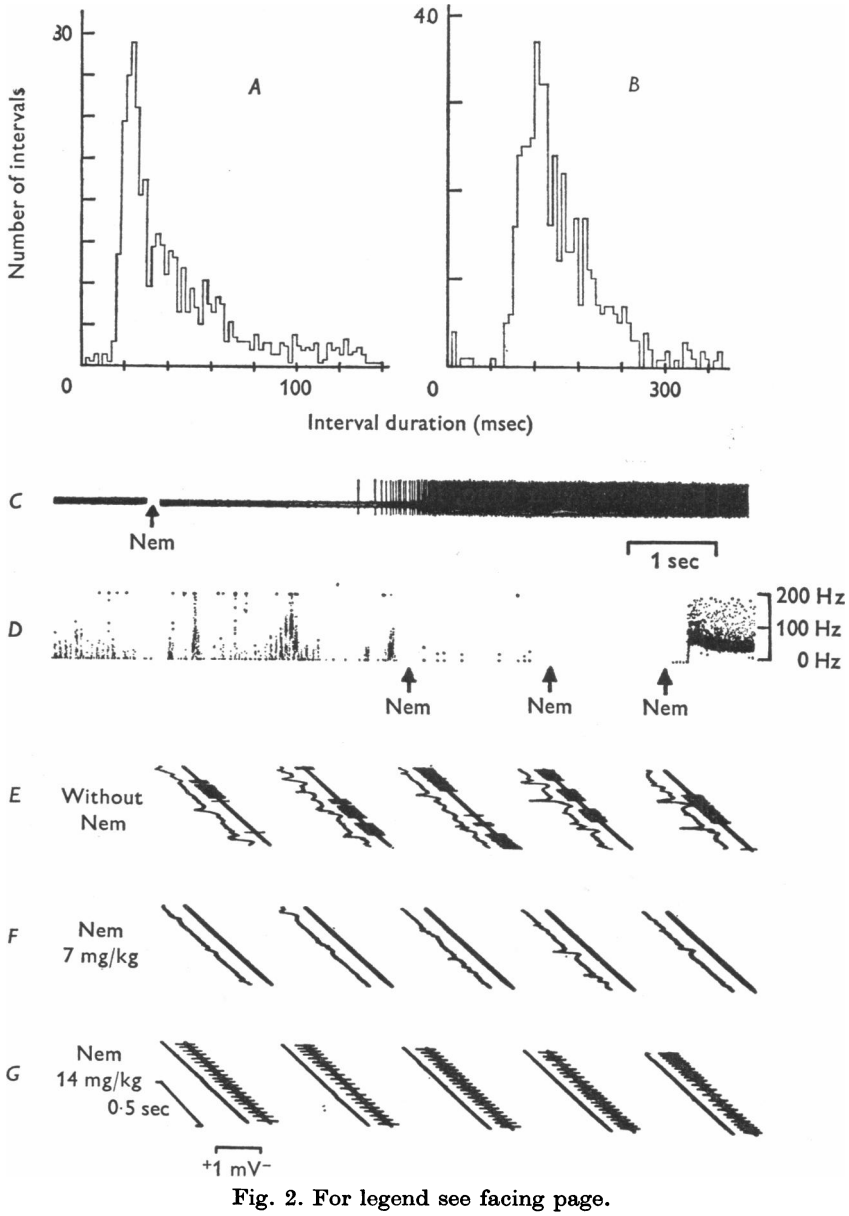

Fig. 2. For legend see facing page.

activity recorded from the cerebellar surface. The second dose abolished the surface activity and initiated a regular discharge of the unit at about 20/sec.

Responses of interpositus neurones to stimulation of peripheral nerves

In the majority of units, records were made of the impulse patterns evoked by stimulation of one or more limb nerves. In general it was found that broadly similar responses were evoked by any nerve which was effective in modulating the discharge pattern. For this reason we first describe the responses to stimulation of the ipsilateral superficial radial nerve (iSR). Such responses were studied in 150 cells, but the following account is based mainly on sixty-one cells studied in detail using the computer to sample groups of fifty successive responses and construct post-stimulus histograms (see Figs. 4, 8, 12 and 13).

At stimulus intensities up to 6*T* (the maximum employed), it was usually possible to distinguish two phases of excitation. A discharge with latency less than 35 msec was followed by a much more striking burst of impulses with latency usually 100–200 msec. Both excitations are visible in the multi-unit records of Fig. 3 which show a group of three interpositus neurones responding in qualitatively similar fashion. The early response is poorly displayed in these slowly swept traces but fast sweeps showed that the small unit responded in most, and the intermediate unit in all trials; both gave only a single impulse. The large unit generated an early impulse only very occasionally (and not in any of the trials shown).

Responses similar to those of Fig. 3 were encountered in forty-one out of sixty-one units (67 %). Post-stimulus histograms for two such units are

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Fig. 2. Spontaneous discharge of interpositus neurones. *A*, *B*, interspike interval histograms prepared from 500 successive spontaneous spikes of two different interpositus neurones. In *A* intervals longer than 140 msec (8 % of the total) and in *B* intervals longer than 375 msec (1 % of the total) have been omitted. *C*, the onset of maintained spontaneous firing in an initially silent interpositus neurone following the administration of pentobarbitone, 8 mg/kg, to the preparation at the point marked by the arrow. Nem = Nembutal. *D*, the alteration of the firing pattern of an interpositus neurone produced by infusion of three doses of pentobarbitone (each 5 mg/kg). The display is generated by an instantaneous frequency meter. Each spot represents a spike fired by the cell and the height of the spot above the base line is proportional to the reciprocal of the interval since the preceding spike. Time calibration below *C* applies to *C* and *D*. *E*, *F* and *G* each consist of five successive pairs of traces at 2 sec intervals showing the spontaneous activity of a single interpositus neurone (right hand trace of each pair) and potentials recorded simultaneously from the surface of the paramedian lobule (left hand traces). *E* was recorded before injection of Nembutal, *F* after one injection and *G* after two injections of Nembutal.

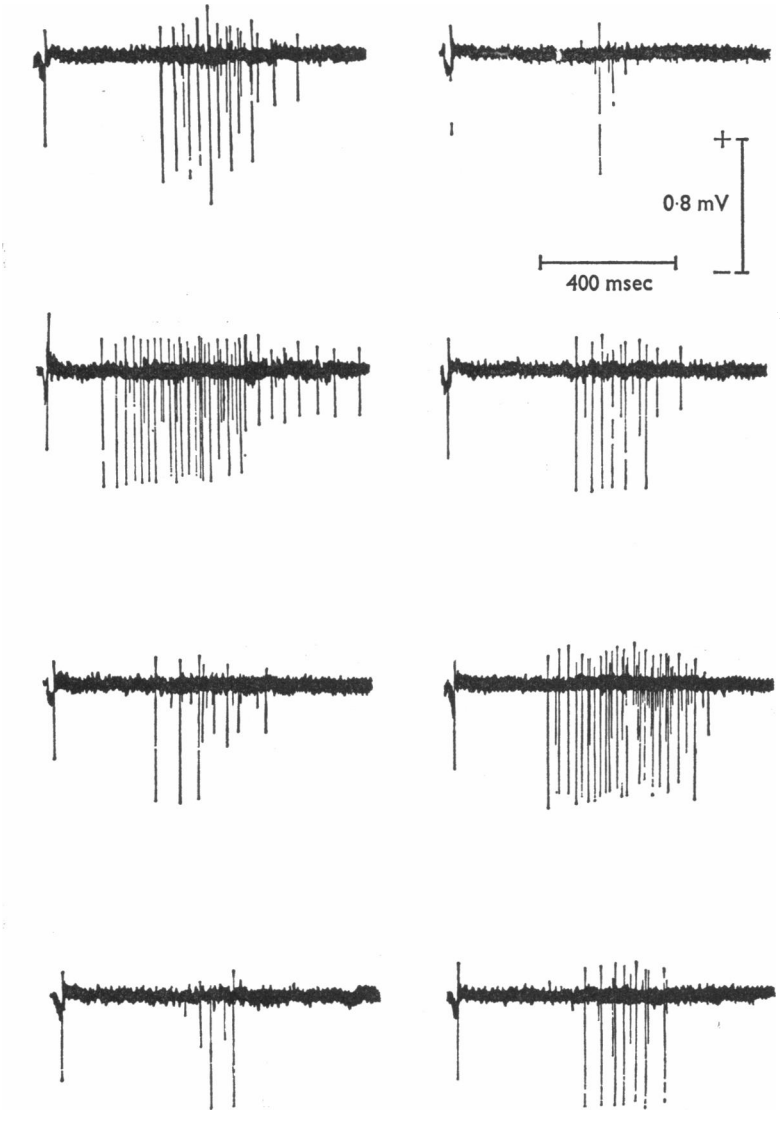

Fig. 3. Responses evoked in three simultaneously recorded interpositus neurones by stimulation of the ipsilateral SR nerve. Eight successive sweeps at intervals of 1·5 sec are presented to show the variability of the responses (time runs from top to bottom, with the left hand column first). The stimulus was presented at the beginning of each sweep and the initial response always contains a spike from the unit with the intermediate sized spike.

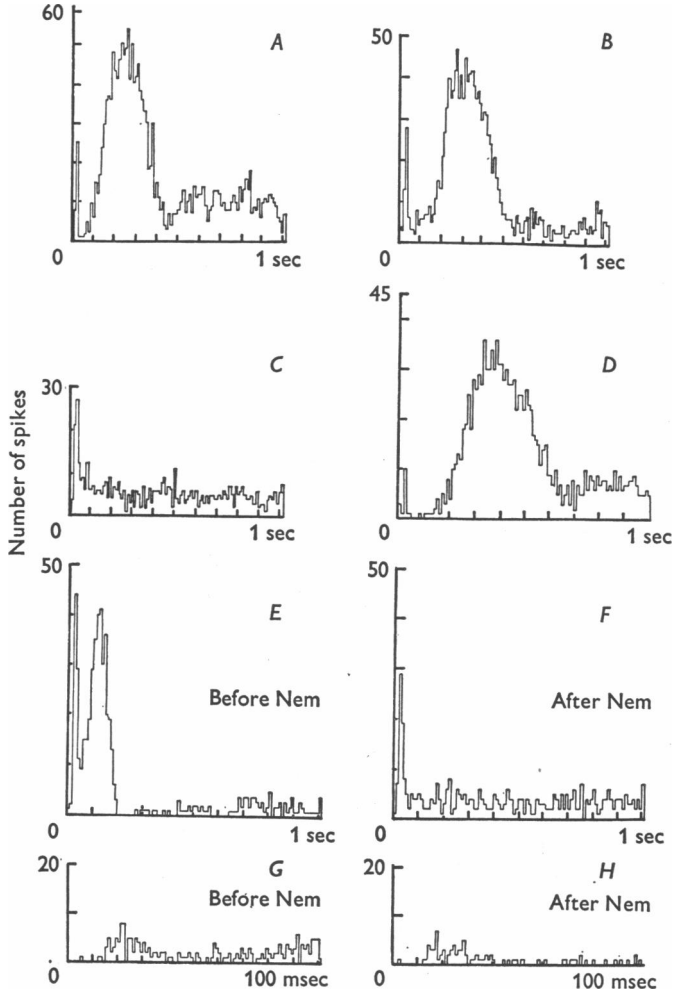

Fig. 4. Post-stimulus histograms prepared from fifty successive responses evoked in interpositus neurones by stimulation of the ipsilateral superficial radial nerve. *A*, *B*, *C* and *D* are for four different cells. *E*, *F*, *G* and *H* are from one cell before (*E* and *G*) and after (*F* and *H*) the administration of Nembutal, 15 mg/kg. *G* is derived from the same data as *E* and shows the first part of *E* on an expanded time scale. *H* is similarly derived from the same data as *F*.

shown in Fig. 4*A* and *B*. These differed from the units in Fig. 3 in showing background activity. Nevertheless, in each histogram a clear trough is visible between the two discharge phases.

In one third of the cells the pattern of response was different. Thus in seven units (12 %) an early excitation occurred in the absence of any late

discharge (Fig. 4*C*). In the remaining thirteen cells (21 %) there was no early discharge although a well developed late discharge was present (Fig. 4*D*).

The separate occurrence of early and late discharges in some units suggests that these responses can be discussed independently and a similar conclusion follows from the experiments in which small doses of pentobarbitone were administered. In each of the twelve cells tested, the late burst was completely abolished by doses as small as 2 mg/kg (Fig. 4*E* and *F*), despite only modest reductions in the early responses. This can be more easily seen when the data are displayed on a faster time base (Fig. 4*G* and *H*). The dose of pentobarbitone required to abolish the late burst was always distinctly less than that needed to accelerate the background discharge of the neurones.

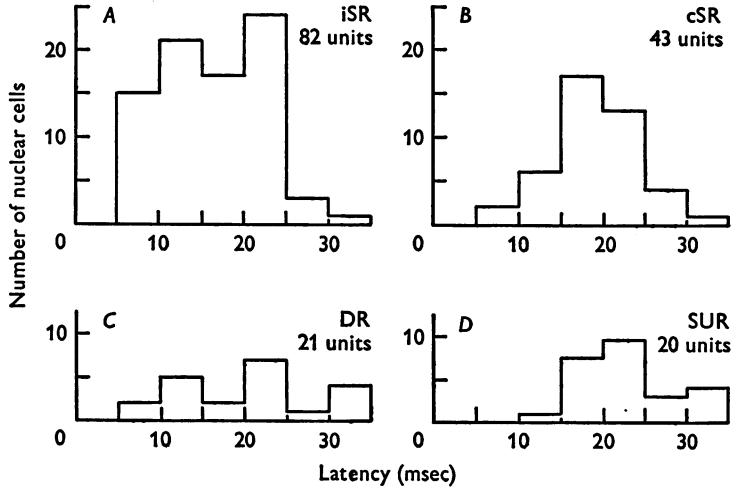

Fig. 5. Histograms showing the distribution of the latency of onset of the phase of early excitation of interpositus neurones in response to stimulation of different peripheral nerves.

The phase of early excitation

This response varied in different units from a single impulse appearing in a small proportion of trials, to a sequence of 4–5 impulses appearing after most presentations of the stimulus and generating a substantial peak in the post-stimulus histogram (see for example Fig. 8*C*). Fig. 5*A* is a histogram showing the shortest latency for the early response as recorded for each of eighty-two units (forty-eight studied using the computer plus thirty-four studied photographically). Threshold for generating an early peak in the post-stimulus histogram was less than 1·5*T*. Response amplitude reached a plateau at around 2·5*T*.

The intermediate phase

Following iSR stimulation, early and late discharges were found together in forty-one of the cells studied with computer assistance. Thirty-six of these showed background activity against which inhibition might be manifest. In all but two cases the two excitations were separated by a well marked trough in the post-stimulus histogram. During this trough, the discharge rate might be below (twenty cells, e.g. Fig. 4*A*), the same (Fig. 4*B*) or above (Fig. 4*E*) the background level.

Amongst the seven units showing an early but no late discharge, six were spontaneously active but only one showed an inhibition of the background discharge following the early response. Ten of the thirteen cells showing only a late discharge were spontaneously active. In five of these the late burst was preceded by a suppression or reduction of the background.

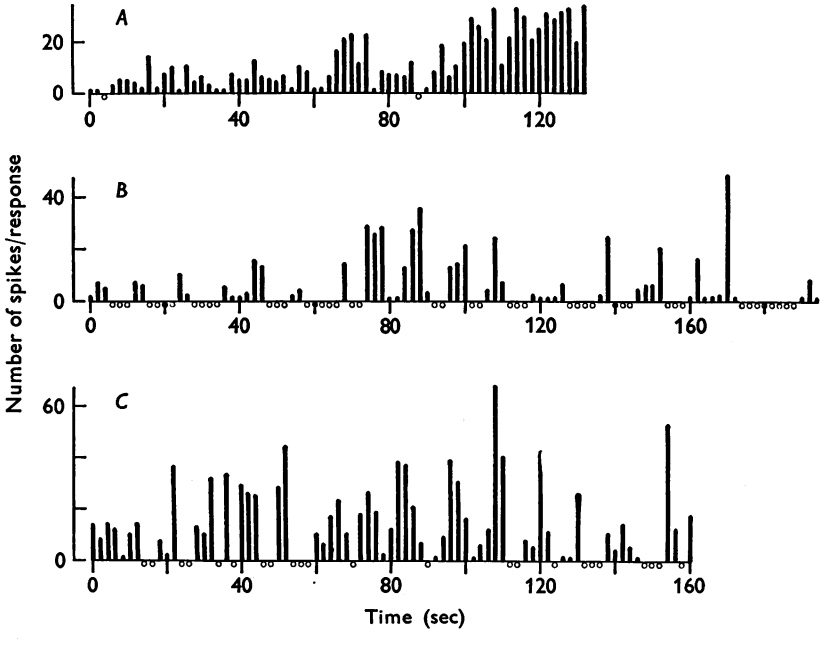

Fig. 6. Variations in the number of impulses in the late burst response of interpositus neurones in response to a series of repetitions of a constant stimulus to a peripheral nerve. The height of each bar represents the number of impulses evoked in response to each trial. Trials in which no late burst response occurred are indicated by a small open circle below the base line. Interval between trials was 2 sec. *B* and *C* are from responses of the same unit, *B* to stimulation of iSR, *C* to stimulation of cSR. In each case the stimulus intensity was 2 × threshold for the most excitable fibres in the nerve (2*T*).

The late burst

This was both the commonest and most striking response to peripheral nerve stimulation. In any one unit the response was usually rather variable both in latency and in spike content, as can be seen by comparison of the successive single sweeps in Fig. 3. In some cases the variation extended from complete failure of the response in some trials to a burst of up to 70 impulses lasting around 600 msec. During well developed bursts the rate of discharge often reached 200/sec. Variations in the spike content of the burst are plotted for two units in Fig. 6. Fig. 6*A* and *B* show responses of two different units to iSR stimulation whilst Fig. 6*C* shows the responses of the same unit as in *B* to cSR stimulation. In Fig. 6*B* large responses appear to recur in a roughly cyclical manner with a period of about 10 sec. There is some suggestion of a similar alternation in Fig. 6*C*.

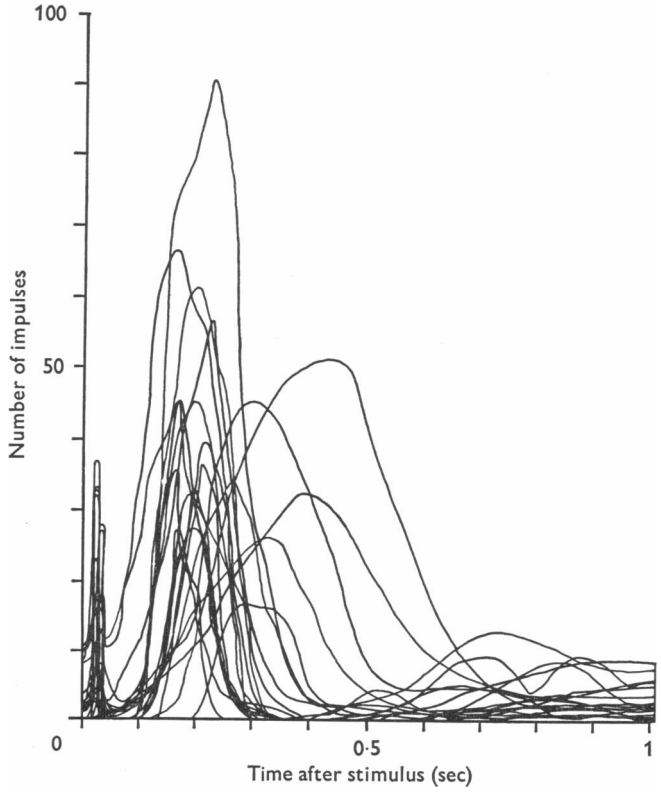

Fig. 7. Smoothed post-stimulus histograms prepared from responses evoked in 20 interpositus neurones by stimulation of the ipsilateral superficial radial nerve at intensity 2·5*T*. These histograms were fitted to the original histograms by eye.

In addition to the response variations in each unit, there were marked differences in latency and amplitude between different units. These are readily appreciated when the post-stimulus histograms from several different units responding to the same iSR stimulus are smoothed and superimposed as in Fig. 7. The frequency distribution for the minimum latencies in sixty-four units is shown in Fig. 9, where it can be seen that latencies ranged from 50 to 500 msec although values greater than 250 msec were uncommon; the mean latency was 151 msec (S.D. 99 msec) and the mode was around 100 msec. As shown in Fig. 9, the duration of the response was variable, ranging from less than 50 msec to almost 1 sec (mean

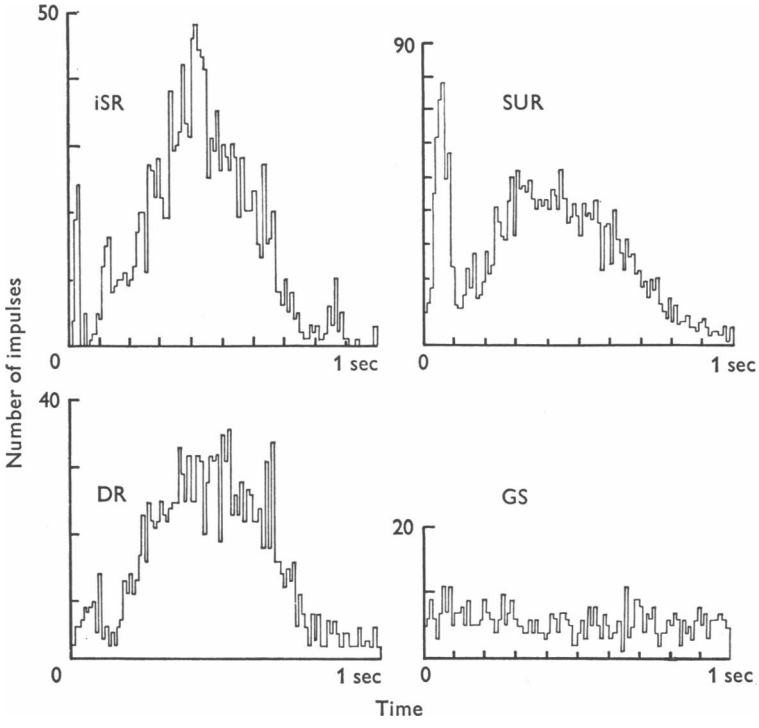

Fig. 8. Post-stimulus histograms prepared from responses evoked in a single interpositus neurone by stimulation of four different peripheral nerves. Stimulus intensity for iSR, 1·6T; SUR, 2T; DR, 3·5T; GS, 8T. Note differing vertical scales.

325 msec; S.D. 200 msec). Such values of course reflect the *maximum* duration of the response in each unit. The threshold stimulus required to evoke a late burst was comparable with that for the early discharge (i.e. less than 1·5T) and the response usually saturated at intensities below 3T.

Responses to stimulation of nerves other than the ipsilateral superficial radial

In any one unit, all effective nerves almost invariably produced closely similar responses. Thus, units responding to iSR stimulation with only an early or only a late discharge responded in similar fashion to the other inputs tested. This is illustrated by the histograms of Fig. 8, which show the responses of a single cell to input from four different nerves.

In relation to the early responses there were some latency differences between different nerves. Fig. 5 shows latency histograms for the early discharge evoked from four different nerves. Responses evoked from the three forelimb nerves were distributed over the same latency range, but when responses from these nerves were compared in individual units the actual latencies were always greater for DR and cSR than for iSR. Latencies for hind limb nerves were a few msec longer as can be seen for SUR by comparing Fig. 5*D* with Fig. 5*A*, *B* and *C*.

In individual units the timing of the late burst was very similar whichever nerve was stimulated. As a result there was little variation in the mean latency of the response as calculated for the whole cell population, e.g. 161 msec for DR, 166 for FEM, 158 for SCI as compared with 151 msec for iSR and 159 msec for cSR. Nevertheless, as judged by the spike content of the burst, different nerves differed considerably in effectiveness (see Fig. 8). In general, cutaneous nerves (SUR, SRs) produced larger responses than muscle nerves (DR) or mixed nerves (FEM, SCI) when stimulated at comparable intensities. Two hind-limb muscle nerves (GS, HAM) evoked neither early nor late discharges. They were tested on fifteen single units in three different preparations and at many recording sites where multi-unit activity was under observation. Nevertheless, it was noted for both early and late responses that individual nerves were not equally effective in all units. Thus iSR was usually the most effective, but cSR was sometimes more potent. Again, in many cells the SRs were more effective than SUR but this order was sometimes reversed.

Investigations into the origin of the late burst

Two experimental procedures were used in an attempt to determine the origins of the late bursts.

(i) *Recordings from the cerebellar cortex*

It is possible that the late bursts were due to long latency excitation of the interpositus neurones. It is believed that the excitatory input to the cerebellar nuclei is derived from collaterals of mossy and climbing fibres which terminate in the overlying cerebellar cortex. We have therefore used ball electrodes to record from the surface of the intermediate cortex which projects mainly to nucleus interpositus (Courville, Diakiw & Brodal, 1973) and have searched for evoked potentials coincident with the

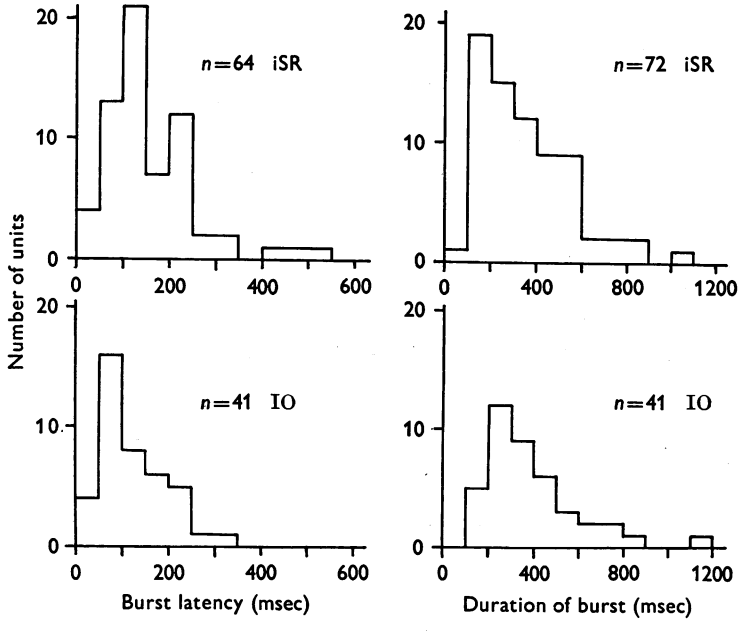

Fig. 9. Histograms showing distribution of latency and duration of late burst responses evoked in interpositus neurones by stimulation of the ipsilateral superficial radial nerve (iSR) at intensities between 2 and 3T and of the inferior olive (IO) at intensities between 300 and 350 μA.

late bursts. The majority of recordings were made from the left paramedian lobule and complex series of relatively short latency ($<$ 30 msec) potentials evoked by nerve stimulation were readily detected. However, no responses were detected at the time of the late bursts in interpositus neurones.

(ii) *Electrical stimulation of the inferior olivary nucleus*

Stimulation of the right inferior olive, which projects to the left half of the cerebellum, was attempted in ten experiments. Post-stimulus

histograms were prepared from the responses to olivary stimulation of fifty-three interpositus neurones. For thirty-seven cells, the responses to nerve stimulation were also studied so that the effects of the two types of stimulation could be compared directly. As judged from the post-stimulus histograms, only three units gave unequivocal evidence of an early excitation evoked by olivary stimulation. The latency was 5 msec in two of these cells and 8 msec in the third. However, late bursts were readily evoked from the olive; their latencies ranged from 40 to 300 msec (mean 113;

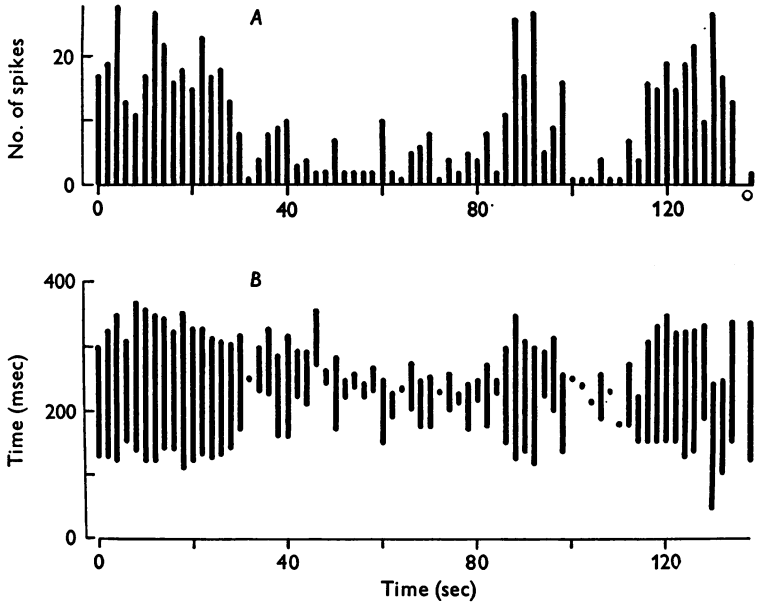

Fig. 10. Variations in the late burst response evoked in an interpositus neurone by stimulation of the inferior olive. *A*, number of spikes in each response. *B*, latency and duration of each response. Latency is indicated by the height of each bar above the base line and duration by the length of the bar. Interval between trials 2 sec. One trial which produced no response is indicated by a small open circle below the base line in *A*. Intensity of olive stimulus 350 μA.

S.D. 69 msec) and their durations from 100 to 1100 msec (mean 369; S.D. 210 msec). These values are very similar to those for the late bursts evoked by limb nerve stimulation (see above) as may be seen from Fig. 9.

The bursts evoked from the olive like those evoked from nerves, showed marked variability in regard to latency, duration and spike content. Fig. 10 (compare Fig. 6) plots for one unit the variations between successive late bursts evoked at 2 sec intervals by two 350 μA stimuli 2 msec apart.

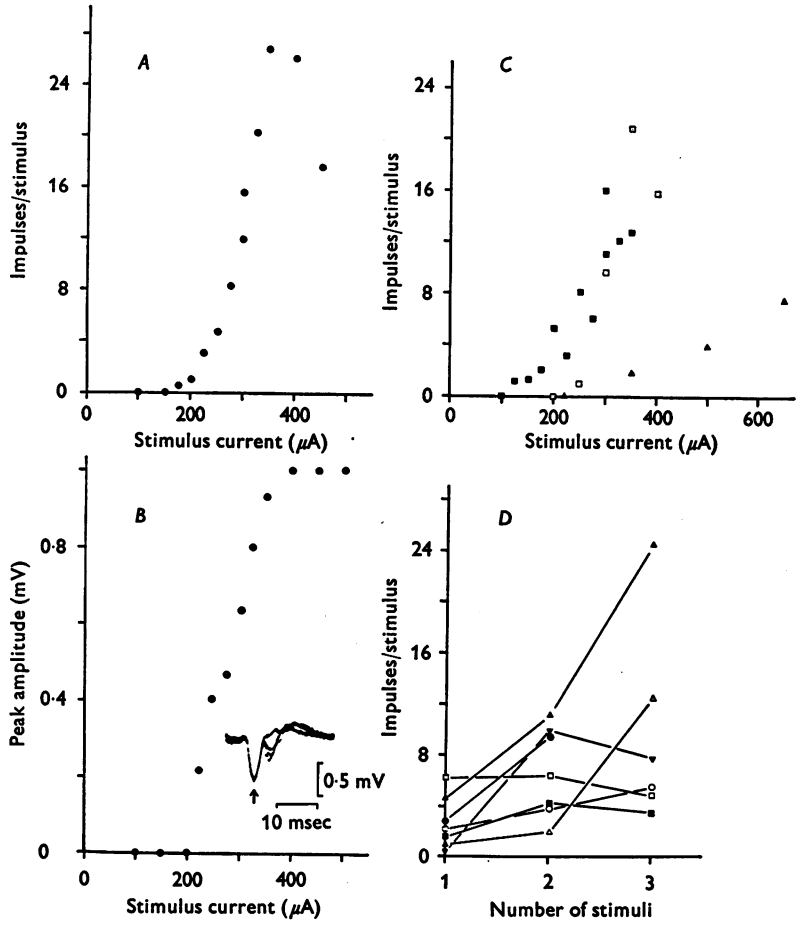

Fig. 11. Characteristics of late bursts evoked by olivary stimulation. *A*, number of impulses in late burst response of an interpositus neurone plotted against intensity of olivary stimulus (each point is the mean of fifty responses). *B*, amplitude of climbing fibre evoked potential recorded from the surface of the paramedian lobule simultaneously with the responses on which *A* is based. Inset record shows a sample CF response. Arrow indicates point at which amplitude measurements were made. Note that the initial CF response is followed by a second response more variable in amplitude (see text). *C*, amplitude of late burst response plotted against intensity of olivary stimulus for three further interpositus neurones (each point is the mean of fifty responses). *D*, amplitude of late burst responses evoked in six interpositus neurones by different numbers of olivary stimuli at 500/sec (each point is the mean of fifty responses. Upright filled triangles and filled squares show data obtained from the same unit at different stimulus intensities).

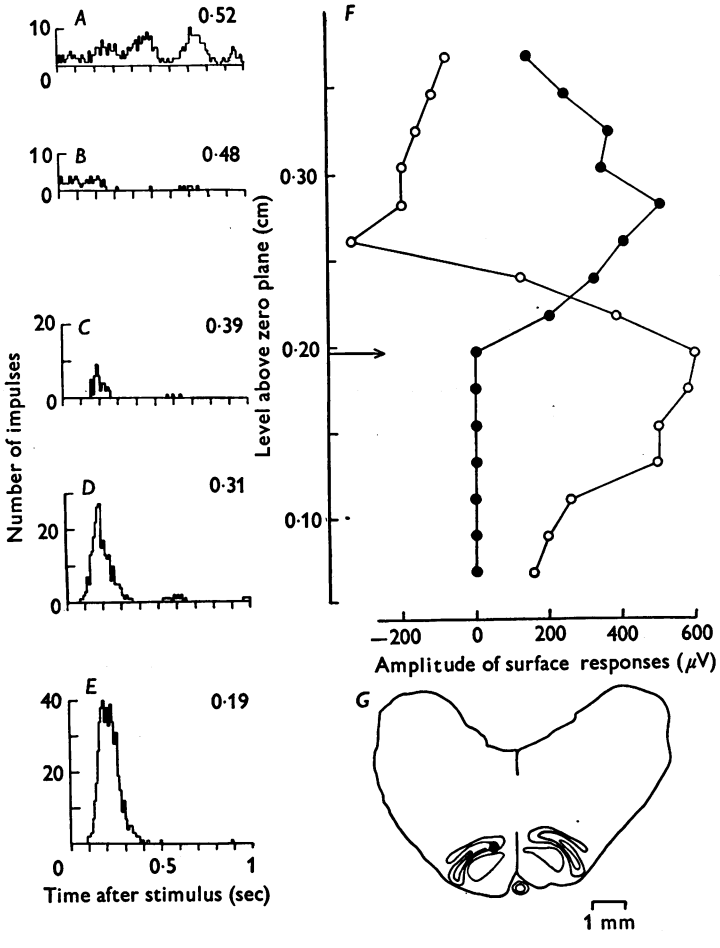

Fig. 12. Effect of locus of brain stem stimulation on responses of interpositus neurones. *A–E*, post-stimulus histograms prepared from the responses of an interpositus neurone to stimulation at different points in the brain stem. The level of the tip of the stimulating electrode above the stereotaxic zero plane is given alongside each histogram. Note also that the cell which was initially spontaneously active at a mean rate of about 6/sec (*A*) ceased its spontaneous firing during the course of the recordings. *F*, amplitude of short-latency (*ca.* 4 msec) CF evoked potentials recorded from the surface of the paramedian lobule in the termination zone of the DF-SOCP (filled circles) and the LF-SOCP (open circles), plotted against the level of the tip of the stimulating electrode. *G*, diagrammatic transverse section through the brain-stem showing the position of the inferior olives and the location of a Prussian blue spot which was made at the level indicated by the arrow in *F*.

In four units the relationship between the amplitude of the late burst and the intensity of the olivary stimulus was investigated. Fig. 11*A* plots this relationship for one unit, whilst Fig. 11*B* shows, for comparison, the relation between stimulus intensity and the amplitude of CF potentials recorded simultaneously from the paramedian lobule. Fig. 11*C* collects the results for the three other units studied. Responses comparable with those produced by nerve stimulation were elicited by currents between 150 and 300 μA.

In ten units an investigation was made of the relationship between the number of stimuli applied to the inferior olive and the size of the late burst. Results from six units are plotted in Fig. 11*D*. In most units two stimuli 2 msec apart produced a larger response than a single stimulus. A third stimulus dramatically increased the response in two cases but produced a small reduction in three other cases.

Although the inferior olive is a well defined nucleus, care was exercised to avoid excitation of nearby reticular nuclei, which provide mossy fibres to the cerebellum (Brodal, 1943; Sasaki & Strata, 1967; Azzena & Ohno, 1973). The stimulating electrode was inserted stereotaxically. In eight experiments its location was confirmed histologically and the tip was found to have been located in the middle third of the right olive in all but one case where it lay in the rostral part of the olivocerebellar decussation. During each experiment the risk of stimulus spread was minimized by routinely monitoring the stimulus currents which were never above 650 μA and were usually less than 350 μA.

Ball electrodes were used to record from the cerebellar surface to monitor the location of the olivary electrode. Records obtained in this way can show whether mossy fibre (MF) or climbing fibre (CF) afferents are being stimulated (see Eccles, Provini, Strata & Táboříková, 1968). MF evoked potentials were never recorded unless currents in excess of 500 μA were employed, but minimum thresholds for evoking CF responses were less than 100 μA in six experiments and the overall range was 10–160 μA. The latency of the earliest responses was *ca.* 4 msec (see Fig. 11*B*) which corresponds well with the olivo-cerebellar conduction time (see Armstrong, 1974).

At the beginning of each experiment, the CF responses evoked on the paramedian lobule by SR stimulation were charted to delineate the termination zones of the dorsal funiculus spino-olivocerebellar pathway and the lateral funiculus spino-olivocerebellar pathway (DF-SOCP and LF-SOCP respectively; see Armstrong, Harvey & Schild, 1973). The olivary stimulating electrode was moved to maximize the CF evoked responses recorded in these two zones.

For several interpositus neurones, both the unit responses and the cerebellar surface responses were recorded for a number of different dorso-ventral positions of the stimulating electrode. This was done in an attempt to show whether the late burst responses were due to stimulation of structures within the confines of the olive. Typical results are shown in Fig. 12 from which it can be seen that large late bursts (latency *ca.* 100 msec) were obtained only when stimulating at depths which yielded large CF evoked potentials on the paramedian lobule. The location of the

stimulating points in relation to the olive can be seen from Fig. 12*G*, which shows the position of an iron deposit made at the end of the experiment.

In five units the response to olivary stimulation was recorded both before and after the intravenous infusion of pentobarbitone (15 mg/kg or less) and, as with the responses to peripheral nerve stimulation, the late burst was immediately abolished. This is shown for one unit in Fig. 13*A* and *B*, where the resting discharge was greatly increased and the burst

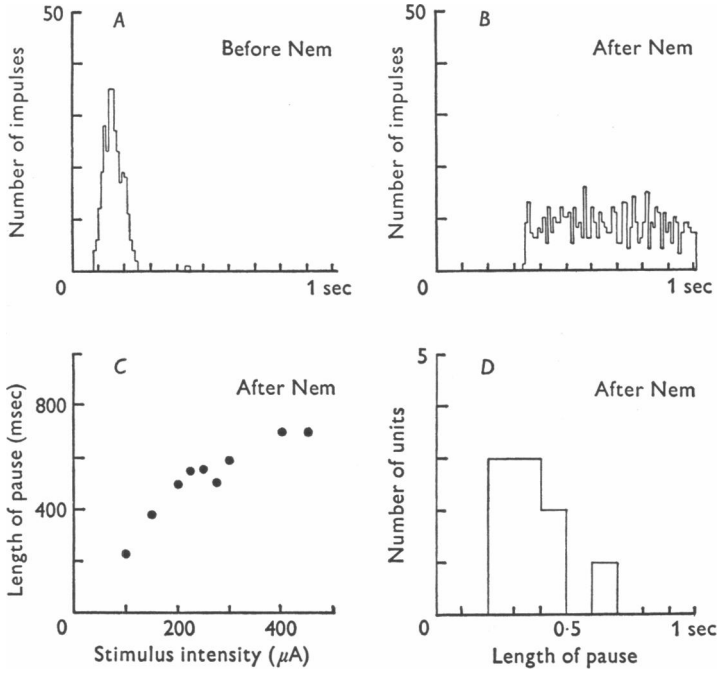

Fig. 13. Responses to olivary stimulation following infusions of pentobarbitone. *A*, *B*, post-stimulus histograms prepared from the responses of the same interpositus neurone to stimulation of the inferior olive, *A* before and *B* after the administration of pentobarbitone 15 mg/kg. *C*, length of the pause in spontaneous firing of an interpositus neurone in a preparation to which pentobarbitone had been administered, plotted against the intensity of the stimulus applied to the inferior olive. *D*, distribution of lengths of pauses generated in nine interpositus neurones by olivary stimulation in preparations which had received pentobarbitone (stimulus intensity, 300–350 μA).

was replaced by a prolonged inhibition. In eight more units responses were recorded after pentobarbitone administration but not before. In each case the response qualitatively resembled that shown in Fig. 13*B*. As the stimulus current was increased above threshold for the inhibition, the duration of the latter increased up to a maximal value (see for example

Fig. 13*C*) which ranged from 130 to 700 msec in the nine cells studied (see Fig. 13*D*). In several units it was shown that the inhibition was due to stimulation in the olivary region rather than to current spread outside it by moving the stimulating electrode vertically (see Fig. 12). When no CF potentials were evoked on the paramedian lobule no inhibition was produced in nucleus interpositus.

DISCUSSION

Spontaneous activity of interpositus neurones

Levels of spontaneous activity in interpositus neurones under chloralose anaesthesia similar to those found here were described by Yu, Tarnecki, Chambers, Liu & Konorski (1973). They found many cells with relatively slow spontaneous activity, and a number of antidromically identified units which were silent. In contrast, Rosén & Scheid (1972) found spontaneous discharge rates around 100/sec.

In barbiturate anaesthetized preparations, Tsukuhara, Toyama & Kosaka (1967) found spontaneous discharge rates of about 100/sec and Allen, Azzena & Ohno (1972) found briskly active neurones. It is possible that rates are high under barbiturate anaesthesia because of the depressant effect which it exerts on Purkinje cell discharge (Murphy & Sabah, 1970; Gordon *et al.* 1973). This would be expected to disinhibit the nuclear cells and therefore to increase their spontaneous activity. Our observation that administration of pentobarbitone leads to an increase in interpositus discharge is consistent with this. On the other hand, we have encountered many silent nuclear cells in preparations anaesthetized with pentobarbitone alone (unpublished observations; see also McCance, 1972). It seems possible that the rate of spontaneous firing is very critically dependent both on the depth of anaesthesia and on the anaesthetic agent employed. A similar conclusion was reached in respect of fastigial neurones by Eccles, Sabah & Táboříková (1974*a*).

The finding that not all interpositus neurones are spontaneously active raises the possibility that some cells may be silent in unanaesthetized animals. In the published reports of nuclear cell activity in conscious animals (Thach, 1970; Mortimer, 1973) the cells were located on the basis of their spontaneous activity so silent cells would not have been detected. However, in a recent study in this laboratory (J. A. Rawson, in preparation) some antidromically identified interpositus neurones of conscious cats have been silent for long periods.

Evoked responses of interpositus neurones

Responses evoked in interpositus neurones by natural and electrical stimulation of cutaneous afferents have previously been studied by Eccles, Rosén *et al.* (1972) in cats decerebrated under pentobarbitone and by Allen *et al.* (1972) in thiopentone anaesthetized cats. Both these groups describe two phases of excitation, sometimes separated by a brief period of inhibition, and succeeded by a longer and more profound inhibition which terminated the response. Comparison with our results strongly suggests that our early excitation includes both phases of excitation described by these authors while our intermediate inhibition clearly corresponds to their terminal inhibitory phase. As can be seen from Fig. 5, a majority of our units had an early excitation corresponding in latency to the second excitation of these authors (i.e. > 15 msec). Although some units responded at a time corresponding to their first excitation (< 15 msec), there was no inhibition detectable within our early excitation. These authors ascribed their first excitation to the action on the nuclear cells of activity in collaterals from fibres of the direct spino-cerebellar paths or their forelimb homologues (Matsushita & Ikeda, 1970*b*). This activity, on reaching the cerebellar cortex, evoked discharges of Purkinje cells, which in turn gave rise to the brief inhibitory phase in the nuclear cells. Their second excitation and the succeeding terminal inhibitory phase, they ascribed to a similar sequence of events, but arising from activity relayed in fibres of the spino-reticulo-cerebellar and spino-olivocerebellar paths and their nuclear collaterals (Matsushita & Ikeda, 1970*a*). Our results are consistent with this explanation, even if details of the response differ.

Recordings from the cerebellar surface showed that, in our preparations, relatively small potentials were evoked via the direct spino-cerebellar pathways and this perhaps accounts for the small number of cells excited with a latency of less than 15 msec and the lack of any inhibition within our early excitation. Our results also suggest that the olivocerebellar pathways are relatively ineffective at exciting short latency discharges of interpositus neurones, at least on their own, since olivary stimulation gave little short latency excitation despite its evoking large cerebellar surface potentials. In this, nucleus interpositus may differ from the fastigial nucleus, since Eccles *et al.* (1974*b*) found that stimulation in the olive gave 'two major excitatory responses' of fastigial neurones at short latency.

The most striking feature of the response of the interpositus neurones which we found was the late burst, which has no parallel in the results of Eccles, Rosén *et al.* (1972) and Allen *et al.* (1972). Our early excitation usually consisted of one or two spikes and never more than five spikes, while up to 70 spikes were discharged during a late burst. Our experiments

produced no evidence that the bursts were due to a large excitation of interpositus neurones via collaterals of afferents to the cerebellar cortex but cannot eliminate the possibility that the bursts were due to activity in afferents *terminating* in the nucleus interpositus. However, there is no anatomical evidence for such afferents.

Following peripheral nerve stimulation, potentials evoked on the cerebellar surface via both mossy and climbing fibre afferents could readily be distinguished. However, after olivary stimulation only climbing fibre potentials were observed. While very small contributions from mossy fibres cannot be excluded, this observation does not support recent reports that a significant number of olivocerebellar fibres terminate as mossy fibres (e.g. Murphy, O'Leary & Cornblath, 1973). Bursts evoked by peripheral nerve and by olivary stimulation were remarkably similar in terms of post-stimulus histograms (representing a statistical similarity), in their sensitivity to pentobarbitone and in being very variable from one trial to another (see Figs. 6 and 10). These points of similarity lead us to conclude that the late bursts evoked in interpositus neurones by peripheral nerve stimulation were probably mediated via the inferior olive. While our experiments cannot exclude the possibility that mossy fibre pathways may have contributed to the bursts evoked by nerve stimulation, it seems very likely that these bursts and those evoked by olivary stimulation were a result of climbing fibre activation.

Indeed, the variability in the bursts recalls the variations in transmission through the olivary relay in many spino-olivocerebellar pathways (e.g. Oscarsson, 1973) and in other pathways relaying in the inferior olive. It is perhaps surprising that olivary stimulation should evoke variable responses. However, recordings from the cerebellar surface showed that olivary stimulation evoked not only typical CF responses of constant latency and amplitude, but also later CF responses which were very variable in latency and/or amplitude (see, for example, the inset record in Fig. 11*B*), and which in some cases were of greater amplitude than the earlier responses. It seems likely that the initial CF response resulted from direct stimulation of olivary cells or their axons, while the later responses were due to indirect excitation of olivary neurones following stimulation of afferent fibres within the olive (see also Dow, 1939).

If olivocerebellar volleys evoke late bursts in interpositus neurones as a result of their action on the cerebellar cortex, then the burst must be a disinhibitory phenomenon. Indeed, Ito *et al.* (1970) have shown by intracellular recording that, following a volley in the olivocerebellar afferents, there is an inhibitory post-synaptic potential lasting about 100 msec succeeded by a sizeable disinhibitory depolarization. The inhibition was attributed to the Purkinje cell firings evoked by the afferent volley. It is well known that when a Purkinje cell is excited via its climbing fibre afferent, the climbing fibre response is usually succeeded by a cessation

of tonic discharge (e.g. Granit & Phillips, 1956; Bell & Grimm, 1969), the so-called CF pause. This pause, if occurring in a number of Purkinje cells simultaneously, would lead to disinhibition of the underlying nuclear cells. To give rise to the prolonged bursts we have observed in interpositus neurones, the pauses would have to be of considerable length (i.e. hundreds of msec) before the resumption of Purkinje cell firing which would terminate the burst. CF pauses have often been reported to be only 20–100 msec in duration (e.g. Bloedel & Roberts, 1971) but most of these studies were of pauses following spontaneous CF responses.

The first 10 msec or so of a CF pause appears to be a result of the spike generator being blocked by the intense depolarization associated with the CF response (Bloedel & Roberts, 1971). However, synaptic mechanisms have been implicated in the production of the later stages of a pause. Climbing fibres provide collaterals both to basket cells and to Golgi cells (Palay & Chan-Palay, 1974); the former post-synaptically inhibit Purkinje cells, while the latter inhibit granule cells, thereby disfacilitating Purkinje cells (Eccles, Ito & Szentágothai, 1967). Currently, there are differing views as to which of these mechanisms is the more important (see Armstrong (1974) for a review), but both might be expected to generate a longer pause in Purkinje cell firing for a larger climbing fibre afferent volley. Indeed, Murphy & Sabah (1971) found that in decerebrate or thiopental anaesthetized cats the length of the CF pause increased with stimulus strength to the inferior olive. Pauses following CF responses evoked by stimulation elsewhere might be relatively long also and this has been found, especially under chloralose anaesthesia. Thus, Talbott, Towe & Kennedy (1967) found pauses lasting 'several hundred msec' following peripheral nerve stimulation and Freeman (1970) often found pauses lasting more than 200 msec following a variety of stimuli. Finally, we ourselves (D. M. Armstrong, B. Cogdell & R. J. Harvey, in preparation) have found in chloralose anaesthetized animals that CF pauses lasting hundreds of msec can be evoked both by peripheral nerve and by olivary stimulation.

Interpositus neurones provide a potent excitatory input to the red nucleus (e.g. Tsukuhara *et al.* 1967) so that, whatever the mechanism by which the late bursts arise, they would profoundly influence the firing patterns of rubro-spinal neurones. It is satisfying to note that Massion & Albe-Fessard (1963) and Stenhouse & Eccles (1971) found when recording from rubro-spinal neurones in chloralose-anaesthetized cats that nerve stimulation evoked bursts similar in latency and duration to those we have found in nucleus interpositus. However, such bursts were not reported when rubro-spinal neurones were studied using pentobarbitone anaesthesia (Eccles, Scheid & Táboříková, 1973) and this provides an additional point of resemblance between rubro-spinal bursts and the interpositus

bursts which we have observed. Furthermore, Massion & Albe-Fessard (1963) showed that rubro-spinal bursts were abolished by destruction of nucleus interpositus anterior.

Finally, it remains to consider whether the responses we have observed are of any physiological significance or whether they are an artifact of chloralose anaesthesia. Some evidence is available from the work of Massion & Albe-Fessard (1963), who found broadly similar rubro-spinal responses in paralysed unanaesthetized cats. Some interpositus neurones of unanaesthetized unrestrained cats have recently been shown to yield similar responses (J. A. Rawson, in preparation).

One possible role for the responses is suggested by considering the inputs known to be adequate for production of volleys in the spino-olivocerebellar paths. Recent investigations have shown that tap stimuli which phasically excite cutaneous mechanoreceptors in the feet provide a very potent input to these paths (Eccles, Sabah, Schmidt & Táboříková, 1972). In our preparations it was repeatedly observed that late bursts were readily evoked by manually tapping the feet. In life, a phasic excitation of foot mechanoreceptors will occur whenever a foot comes sharply into contact with a surface as during locomotion or exploratory manipulation. If a foot tap leads to the production in interpositus neurones of a brief excitation followed by a pause followed by a late excitation, it will lead via the rubro-spinal system to a similar sequence of excitability changes in the flexion reflex apparatus of the spinal cord (see Massion, 1967). It is worth noting that in thalamic cats, Orlovsky (1972) has recorded rhythmic bursts of rubro-spinal activity occurring in phase with walking movements. These bursts were completely abolished by removal of the cerebellum suggesting that they depended on bursts in interpositus neurones. The bursts were also abolished or greatly diminished by reducing the afferent input associated with walking movements.

Cat locomotion has been studied electrophysiologically by Lundberg & Engberg (1969). During walking, the flexion phase of the step made by a (hind) limb begins around 200 msec after the foot contacts the floor: clearly late bursts such as we have studied would be suitably timed to aid in producing this flexion phase whilst the preceding inhibition would coincide with the stance phase when flexor activity is low. However, during galloping, the flexion phase begins around 60 msec after foot placement so that the late bursts in rubro-spinal neurones could contribute to control of the late part of this phase, but not to its initiation. This is consistent with Orlovsky's (1972) finding that the rubro-spinal path facilitates rhythmic locomotory movements but is not essential for their initiation.

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