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SYNAPTIC ACTIONS OF PERIPHERAL NERVE IMPULSES UPON DEITERS NEURONES VIA THE CLIMBING FIBRE AFFERENTS

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SUMMARY

1. The cerebellar integration of sensory inputs to Deiters neurones was studied in cats under Nembutal anaesthesia.

2. Stimulation of peripheral nerves produced in the Deiters neurones a sequence of an initial excitatory post-synaptic potential (e.p.s.p.) and a later inhibitory post-synaptic potential (i.p.s.p.), or a relatively small e.p.s.p.

3. The Deiters neurones were classified as forelimb (FL)- or hind limb (HL)-type cells according to the location of the most effective peripheral nerve. In the FL cells stimulation of the forelimb nerves produced the e.p.s.p.–i.p.s.p. sequence (dominant response), while stimulation of the hind limb nerves was ineffective or produced the small e.p.s.p. (non-dominant response). In contrast, in the HL cells the non-dominant response was evoked from the forelimb nerves, and the dominant response from the hind limb nerves.

4. The stimulus intensity–response relation indicates that Group I and II muscle afferents and low and high threshold cutaneous afferents contribute to the dominant and non-dominant responses.

5. Antidromic identification of these Deiters neurones revealed that 90 % of the HL cells and 85 % of the FL cells project to the lumbo-sacral and cervico-thoracic segments of the spinal cord, respectively, while 10 % of the HL cells and 15 % of the FL cells innervate the cervico-thoracic and lumbo-sacral segments, respectively.

6. The mean latency of the e.p.s.p. evoked from the forelimb nerves was 14 msec in the FL cells and 13 msec in the HL cells, and the latency of the e.p.s.p. evoked from the hind limb nerves was 17 msec in the FL cells and 18 msec in the HL cells. The later i.p.s.p. regularly followed the onset of the e.p.s.p. with a delay of 3–5 msec.

7. The dominant and non-dominant responses in both types of cells exhibited the following three characteristic features: (i) a strong depression after conditioning stimulation of the inferior olive, (ii) an increase of the inferior olivary excitability during the responses, and (iii) a striking frequency depression with stimulation at relatively low frequency (5–10/sec).

8. Consequently it was concluded that all of the responses were produced through the climbing fibres originating from the inferior olive, the i.p.s.p.s due to inhibition from Purkyně cells activated by the climbing fibres and the e.p.s.p.s due to excitation from the collaterals of the climbing fibres.

INTRODUCTION

The cerebellar integration of sensory input from peripheral nerves has been extensively investigated on Purkyně cells (Eccles, Provini, Strata & Táboříková, 1968*a*, *b*; Eccles, Faber, Murphy, Sabah & Táboříková, 1969, 1970, 1971*a*, *b*). Stimulation of the peripheral nerves produced excitation and/or inhibition in Purkyně cells, and the sets of effective nerves were different from cell to cell. Therefore it was suggested that Purkyně cells might integrate specific subsets of sensory inputs (Eccles *et al.* 1969, 1971*b*). A question that arises is how the processing of information proceeds at the subcerebellar nuclear cells, namely, fastigial, interpositus, lateral and Deiters neurones, each of which receives the convergence of some tens of Purkyně cells (Eccles, Ito & Szentágothai, 1967). It was found that the responses to incoming signals are quite similar in the neurones of these four nuclei (Ito, Obata & Ochi, 1966; Ito & Yoshida, 1966; Eccles *et al.* 1967; Ito; Yoshida, Obata, Kawai & Udo, 1970). They receive background excitation from the mossy and climbing fibres and strong inhibition from Purkyně cells. Thus the excitation from the two types of cerebellar afferents provides a base for the Purkyně cells to exert an inhibitory modulation of the excitability in the subcerebellar nuclei cells. Deiters nucleus occupies a special position among the subcerebellar nuclei in that it projects directly to the spinal motoneurone (Grillner, Hongo & Lund, 1970; Wilson, Yoshida & Schor, 1970) and thus constitutes the shortest pathway for cerebellar control of the spinal motoneurones. Therefore, the study of Deiters neurones may provide the simplest model of cerebellar control over the motoneurones.

In the work to be reported here synaptic actions evoked in Deiters neurones during stimulation of the fore- and hind limb nerves were investigated intracellularly, and they were compared with the level of spinal projection of the Deiters neurones determined by their antidromic activation. The main concern of this work was confined to the synaptic inputs through the climbing fibre system. Therefore relatively deep barbiturate

anaesthesia was employed to suppress transmission through the mossy fibre system. Preliminary reports of this work have been presented (Allen, Sabah & Toyama, 1971*a*, *b*).

METHODS

The fourteen cats used in this investigation were anaesthetized by I.P. injection of sodium pentobarbitone (20 mg/kg) and sodium thiopentone (20 mg/kg) with supplemental doses (10 mg/kg) of the latter every 2 or 3 hr. The cats were mounted on a stereotaxic frame in the supine position, immobilized with Flaxedil (May & Baker) and artificially respirated. The operative procedures for approaching the left Deiters nucleus from the ventral surface of the brain stem were the same as those described previously (Ito, Hongo, Yoshida, Okada & Obata, 1964). A pair of insulated metal wires exposed at their tips was placed on the ventral surface of the C 3 spinal segment, and in some cats another pair of metal electrodes was placed on the ventral surface of the L 1 spinal segment. These electrodes were used for antidromically exciting Deiters neurones projecting to the spinal cord, and for discriminating between those cells projecting to the cervico-thoracic level (CT cells) and those to the lumbo-sacral level (LS cells). Stimulating electrodes composed of a pair of steel needles insulated except for their very tips were inserted into the contralateral inferior olive (IO). The following nerves were used for stimulation:

Forelimb nerves

Superficial radial (SR): mainly cutaneous with some muscle nerve.
Deep radial (DR): mainly muscle with some cutaneous nerve.
Ulnar (UL): mixed muscle and cutaneous nerve.
Median (MED): mixed muscle and cutaneous nerve.

Hind limb nerves

Muscle nerves.
- Quadriceps (QUAD): knee extensors.
- Posterior biceps – semitendinosus (PBST): knee flexors.
- Semimembranosus – anterior biceps (SMAB): hip extensors.
- Gastrocnemius – soleus (GS): ankle extensors.
- Plantaris (PL): ankle extensor and plantar flexor toes.
- Flexor digitorum – hallucis longus (FDHL): plantar flexor toes.
- All of deep peroneal except the long branch to the foot (PDP): dorsi-flexors ankle and toes.

Cutaneous nerves.
- Suralis (SUR).
- Superficial peroneal (SP).
- Plantar cutaneous (PC).

They were dissected on the ipsilateral side and mounted on platinum stimulating electrodes in a paraffin pool. Four forelimb nerves (DR, SR, MED, UL) and one hind limb nerve (QUAD) were stimulated in special electrode assemblies buried subcutaneously. The threshold was determined for each nerve by monitoring its action potential at a suitable location. A single pulse (duration, 0·1 msec) or a train of 2–4 pulses (interval, 2 msec) and at intensities of 1–35 times the determined threshold value (i.e. about 1 V) was employed for stimulation of peripheral nerves. Single pulses of 1–30 V (0·1 msec) were used for stimulation of C 3 and IO. The single pulse or train used to stimulate the peripheral nerves, C 3 or IO was usually repeated at a frequency of 0·2–30/sec.

Glass micro-electrodes were filled with 2 M-NaCl and those with an electrical resistance of 12–20 MΩ were used for intracellular recording. The micro-electrode was connected through a thin silver–silver chloride wire to a cathode follower which was modified for both recording and injecting current through a single micro-electrode (Ito, 1960). The output of the cathode follower was connected directly to a main amplifier and it was displayed on the screen of a cathode ray oscilloscope. The output was also monitored by a pen recorder. The d.c. level of the output from the cathode follower was adjusted by applying a bucking off potential between the cat and the ground. This was done automatically through a negative feed-back circuit with a phase delay (time constant, 2, 0·2 or 0·02 sec), which makes the whole recording system an a.c. amplifier with the same time constant (Allen & Toyama, 1972). Thus the a.c. time constant of the whole recording system was selected by changing the time constant of the phase delay in the automatic bucking off circuit. This automatic bucking off circuit could practically eliminate any d.c. drift in the recording system, even during passage of current and greatly expanded the dynamic range of the cathode follower from ± 4 to ± 19 V. Manual control of bucking off was employed when d.c. recording was desired. The penetrated cells were usually held for 1–2 hr in a reasonably good condition for experimental observation. At the end of the penetration of each cell, the micro-electrode was withdrawn to a position just outside of the penetrated cell and a complete sequence of extracellular recordings was made for every trial of stimulation of the peripheral nerves, C3 or IO. In each experiment the electrode used for the final penetration to Deiters nucleus was left buried in the brain tissue, and the track of the electrode was examined histologically.

RESULTS

Among 250 Deiters neurones identified by antidromic activation from C3, 122 were maintained with a resting potential larger than -50 mV for the period of observation and were selected for analysis. Twenty-one of the 122 cells were sampled from the ventral portion of Deiters nucleus. In these cells no detectable change in membrane potential was produced by stimulation of any of the fore- and hind limb nerves. On the basis of their location, these cells presumably are the Deiters neurones which receive no Purkyně cell projection from the anterior lobe of the cerebellum (Brodal, Pompeiano & Walberg, 1962; Ito, Hongo & Okada, 1969).

Response to peripheral nerve stimulation

In the remaining 101 cells more dorsally located, stimulation of the fore- and hind limb nerves invariably produced changes of membrane potential. These neurones were classified into two groups: thirty-five as forelimb type (FL) and sixty-six as hind limb type (HL) according to whether the most effective nerve, i.e. the nerve eliciting the largest membrane potential changes, was in the forelimb or hind limb.

Fig. 1 illustrates the effect of peripheral nerve stimulation on a typical HL cell. Stimulation of the forelimb nerves (SR and DR) produced a small depolarization of about 1 mV which declined gradually, lasting for more

than 50 msec (Fig. 1*A–D*). In contrast, stimulation of QUAD (*E*, *F*) evoked a greater depolarization (3 mV) which rose sharply and was curtailed by a large hyperpolarization (7 mV) lasting for 70 msec or more. The hyperpolarization was followed by a small rebound depolarization (2 mV) (Fig. 1*F*). A similar sequence of membrane potential changes of smaller

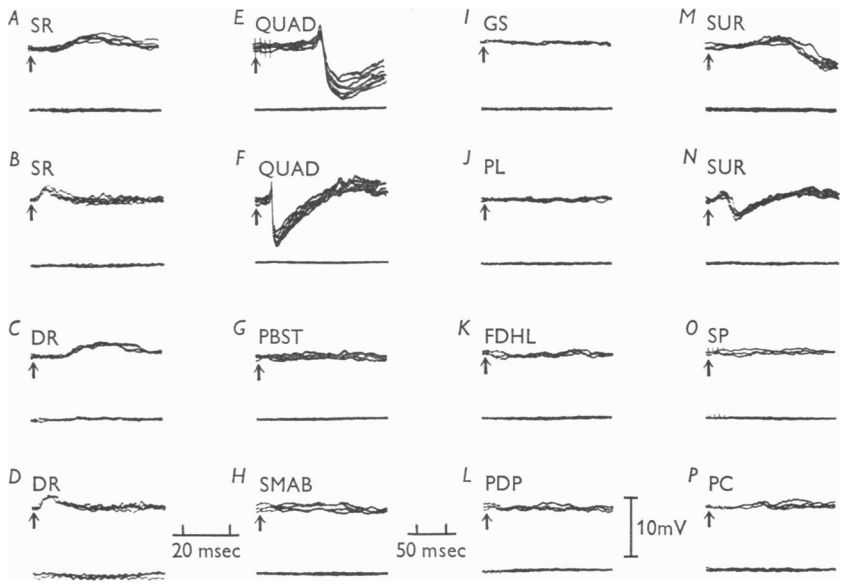

Fig. 1. Intracellular responses from an HL Deiters neurone evoked by stimulation of the peripheral nerves. The upper traces are the intracellular potentials and the lower traces the extracellular. The nerves were stimulated by a train of four pulses at a frequency of 500/sec and at an intensity about 7 times threshold (7*T*). The nerve stimulated is indicated on each record. In this and subsequent Figures, the upward arrow in each record indicates the moment of the first pulse. The voltage scale of 10 mV applies to all traces. The time scale of 20 msec applies to *A*, *C*, *E*, *G–M*, *O* and *P*, while that of 50 msec applies to *B*, *D*, *F* and *N*. The recording time constant in all Figures is 0·2 sec and upward deflexion represents depolarization. Records were made by superposing four to ten traces at intervals of 1·3 sec, unless otherwise specified.

amplitude was also evoked from SUR (*M* and *N*), while the remaining eight nerves (PBST, SMAB, GS, PL, FDHL, PDP, SP, PC) produced no detectable response in this cell (*G–L* and *O–P*).

Fig. 2 is an example of the responses of a typical FL cell. All of the four forelimb nerves (SR, DR, UL, MED) evoked responses in the FL cells which are quite similar to those produced in the HL cells from the hind limb nerves (Fig. 2*A–F*), while some hind limb nerves (SUR, SP and PC) evoked a small response resembling that produced in the HL cells by the

forelimb nerves (*I–L*). The remaining two nerves (QUAD and PDP) produced no response at all in this cell (*G* and *H*). The above observation indicates that the forelimb and hind limb nerves produce a clear differential effect upon HL and FL cells. The nerves in the dominant limb (hind limb nerves for HL cells and forelimb nerves for FL cells) invariably produced a sequence of a depolarization, hyperpolarization, and sometimes

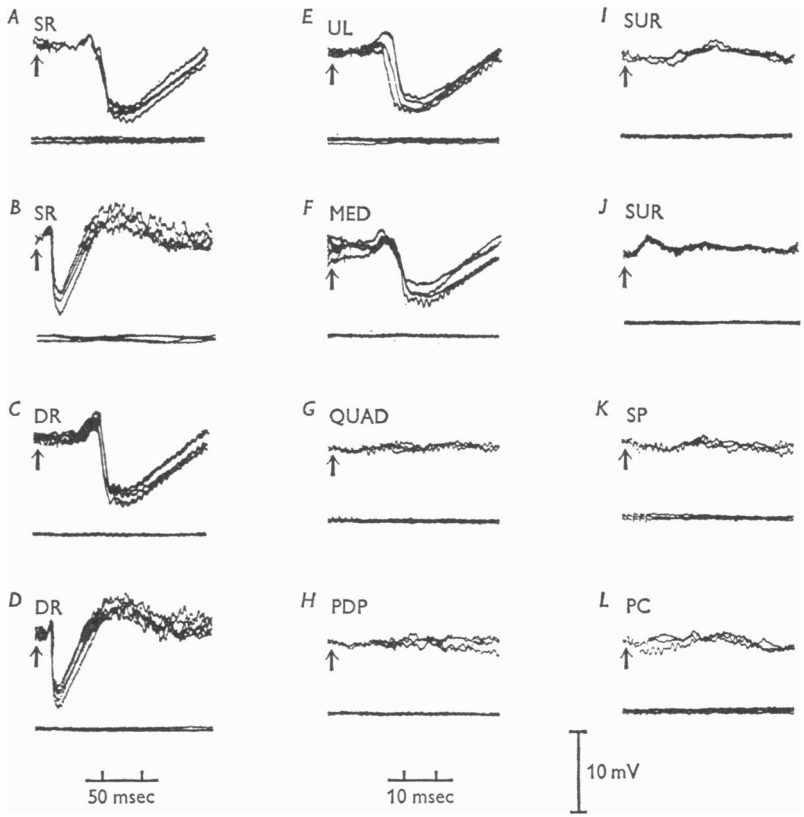

Fig. 2. Responses of an FL Deiters neurone. Upper traces, the intracellular potentials. Lower traces, the extracellular controls. The nerves were stimulated by a train of four pulses (6*T*). The voltage scale of 10 mV applies to all traces. The time scale of 10 msec applies to *A*, *C*, *E–I*, *K* and *L*, while that of 50 msec applies to *B*, *D* and *J*.

rebound depolarization, regardless of the amplitude of the evoked responses. This sequence is hereby designated as the dominant response. The nerves in the non-dominant limb (forelimb nerves in HL cells and hind limb nerves in FL cells) evoked a relatively small, long-lasting depolarization, which was designated as the non-dominant response.

Fig. 3 exemplifies diagrammatically some variations of the responses of

four different HL and FL cells by representing the amplitude of the initial or long-lasting depolarization by the size of open circles and that of the later hyperpolarization by filled circles. The spectrum of responses to nerve stimulation was quite variable from cell to cell. In some of the HL cells (twenty-two of the sixty-six cells) dominant effects were confined to only a few nerves (Cell 1), while in the others (forty-four) they were produced from several nerves (Cell 2). In some HL cells (forty of the sixty-six cells) some forelimb nerves produced the non-dominant response (Cell 1), while in the others (twenty-six) they had no effect (Cell 2). The most effective

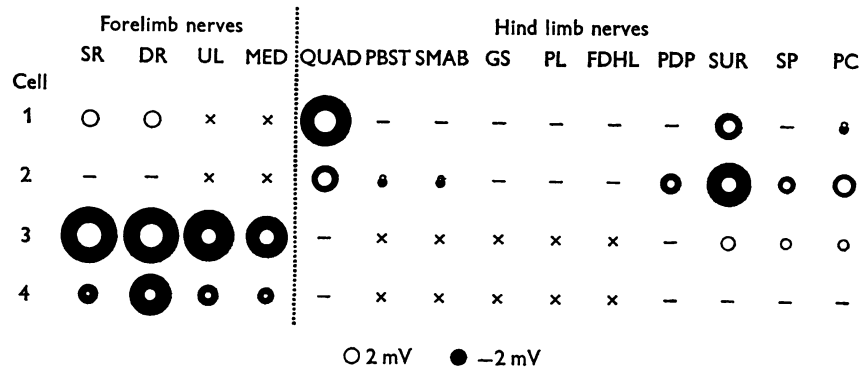

Fig. 3. Diagram representing response spectra of Deiters neurones to peripheral nerve stimulation. The amplitudes of depolarization and hyperpolarization are indicated by the diameters of the open and filled circles, respectively. The nerves were stimulated by trains of two or four pulses (6–7 *T*). The open and filled calibration circles represent depolarization of 2 mV and hyperpolarization of −2 mV, respectively. The minus sign (−) indicates no response and × indicates that the nerve was not stimulated.

nerves in producing the dominant response were QUAD in 34 % of the sixty-six HL cells, SUR in 25 %, PDP in 20 %, PC in 15 %, SP in 5 % and PL in 1 %. In none of the HL cells investigated was PBST, SMAB, GS or FDHL the most effective nerve, although they were frequently the second or third most effective nerves. The order of effectiveness of the hind limb nerves in producing the dominant responses was quite variable from cell to cell. No special relationship was found in the order of effectiveness between the muscle and cutaneous nerves, between the nerves innervating synergic and antagonistic muscle groups, or among the nerves innervating the neighbouring muscles.

In FL cells the dominant effects were usually evoked from all of the four forelimb nerves (Cells 3 and 4 of Fig. 3). A non-dominant response was produced in ten of the thirty-five cells from a few hind limb nerves (Cell 3), and in the remaining twenty-five no effect was detected from any of the

hind limb nerves (Cell 4). Generally the hind limb nerves producing strong dominant effects in HL cells (QUAD, SUR and PDP) also had potent non-dominant effects upon FL cells.

For some of the HL (twenty) and FL (twelve) cells the relation between their peripheral receptive area and the destination of their axons was investigated. It was found that eighteen of the twenty HL cells and two of the twelve FL cells projected to the lumbo-sacral spinal cord, while two HL cells and ten FL cells innervated the cervico-thoracic spinal cord. Thus there is a strong correlation between the receptive area of the Deiters neurone and the destination of its projection.

Latency of excitatory and inhibitory post-synaptic potentials

The nature of these dominant and non-dominant responses was studied by injection of hyperpolarizing current or Cl^- ions through the recording micro-electrode (Eccles, 1964). The control intracellular trace of Fig. 4*A* shows an initial depolarization and later hyperpolarization evoked in an HL cell from a muscle nerve in the hind limb, QUAD. In Fig. 4*B* the later hyperpolarization was reversed into depolarization during injection of hyperpolarizing current. Fig. 4*D* superposes the traces of the records in *A* (continuous line), *B* (interrupted line), and that of the extracellular potential in *C* (dotted line). It is seen that the initial depolarization remains virtually unaffected, while the later hyperpolarization is reversed completely. Therefore the initial depolarization should be an excitatory post-synaptic potential (e.p.s.p.), and the later hyperpolarization should be an inhibitory post-synaptic potential (i.p.s.p.) (Eccles, 1964). Fig. 4*E–H* shows similar analysis of the rebound depolarization following the i.p.s.p.; during application of hyperpolarizing current the rebound depolarization changed into hyperpolarization, indicating that the rebound is caused by disinhibition (Wilson & Burgess, 1962). Similar disinhibition of Deiters neurones was reported by Ito, Kawai, Udo & Sato (1968) during cerebellar and spinal stimulation. Fig. 4*I* and *J* provide another example of reversal of the later hyperpolarization evoked in an HL cell by stimulation of a cutaneous nerve in the hind limb, SUR. Fig. 4*K* and *L* show the effect of the hyperpolarizing current upon a long-lasting depolarization evoked in the HL cell from a forelimb nerve, SR. Comparison between the control response (real trace) and that during application of the hyperpolarizing current (interrupted line) indicates that the hyperpolarizing current did not affect this depolarizing response, and therefore it should be an e.p.s.p. For each of the HL cells studied it was found that the initial depolarization and later hyperpolarization evoked from the hind limb were always a sequence of an e.p.s.p. and i.p.s.p., respectively, while the depolarization evoked from the forelimb was an e.p.s.p. Comparable observations made

upon the responses of the FL cells revealed that the depolarization and subsequent hyperpolarization induced from the forelimb nerves always consisted of a sequence of an e.p.s.p. and i.p.s.p. (Fig. 4*M* and *N*), and the depolarization from the hind limb nerves was an e.p.s.p. (not shown in Figure). Thus a general conclusion can be made for both the FL and HL cells that the nerves in the dominant limb produced an e.p.s.p. and i.p.s.p. sequence while those in the non-dominant limb evoke simply a long-lasting e.p.s.p.

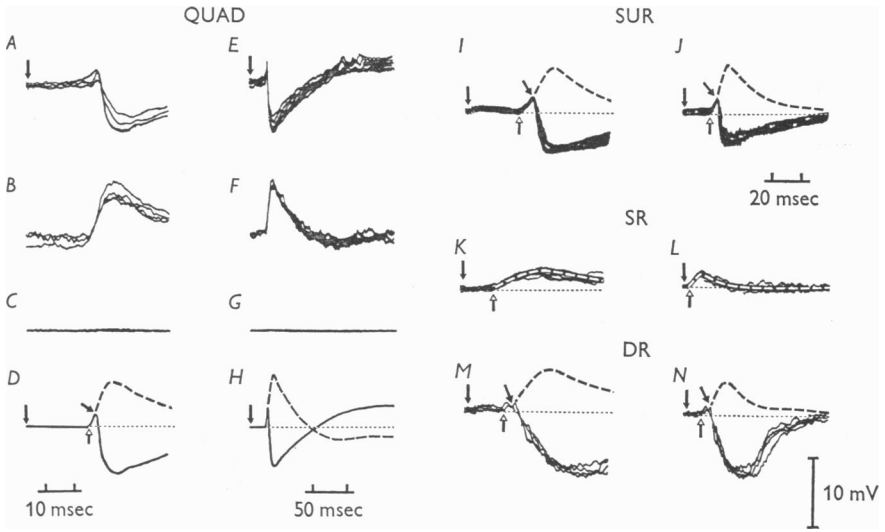

Fig. 4. Effect of hyperpolarizing current upon dominant and non-dominant responses. *A*, dominant response evoked in an HL cell by stimulation of QUAD, without injection of current. *B*, same as in *A* but during injection of hyperpolarizing current ($1{\cdot}7 \times 10^{-8}$ A). *C*, the extracellular potential. *D*, superposition of *A* (continuous line), *B* (interrupted line) and *C* (dotted line). The open arrow indicates the point of deviation of trace *A* from trace *C* and the closed arrow indicates the point of deviation of trace *B* from trace *A*. *E–H*, similar to *A–D*, respectively, but with a slower sweep. *I* and *J*, similar to *D* and *H*, but evoked from SUR (current injected, $0{\cdot}7 \times 10^{-8}$ A). *K* and *L*, non-dominant responses from SR in an HL cell ($1{\cdot}7 \times 10^{-8}$ A). *M* and *N*, dominant responses from DR in an FL cell ($0{\cdot}7 \times 10^{-8}$ A). The nerves were stimulated by a train of four pulses (8*T*). The voltage scale of 10 mV applies to all traces. The time scale of 10 msec applies to *A–D*, *I*, *K* and *M*, that of 20 msec to *J* and *N*, and 50 msec to *E–H* and *L*.

As shown in Fig. 4*D*, *H–N*, the onsets of the e.p.s.p. after stimulation of the peripheral nerves were determined at the point of divergence between the control intracellular and extracellular traces (open arrow). No systematic difference was found between the latencies of the above two types of e.p.s.p.s evoked in the HL and FL cells from the same nerve. Except for a small number of cells (12 of 101) which will be mentioned below (see p. 328),

the latency of the e.p.s.p. evoked by stimulation of the hind limb nerves with a train of two to four pulses was 18 msec in HL cells and 17 msec in FL cells (Table 1). Similarly the latency of the e.p.s.p.s evoked from the forelimb nerves was 13 msec in HL cells and 14 msec in FL cells (Table 1). The onset of the i.p.s.p.s was determined at the point of divergence between the control and reversed intracellular traces (filled arrows in Fig. 4*D*, *I*, *J*, *M*, *N*). The onset of the i.p.s.p. was regularly delayed by 3–5 msec from that of the e.p.s.p. (Table 1).

TABLE 1. Latency of p.s.p.s evoked from peripheral nerves. The latencies were measured for the p.s.p.s evoked from the most effective nerve in the fore- and hind limbs, and the mean and S.D. are indicated for the number of cells enclosed in brackets

	Hind limb nerve		Forelimb nerve	
	E.p.s.p. (msec)	I.p.s.p. (msec)	E.p.s.p. (msec)	I.p.s.p. (msec)
HL cell	18·4 ± 2·4 (66)	22·6 ± 2·4 (66)	12·8 ± 2·3 (40)	—
FL cell	16·9 ± 2·2 (10)	—	13·8 ± 1·9 (35)	19·2 ± 2·4 (35)

Analysis of pathway

The latencies of i.p.s.p.s evoked from the forelimb (19 msec) and hind limb nerves (23 msec) agree approximately with the latencies of climbing fibre responses evoked in Purkyně cells by nerve stimulation (11–19 and 20–27 msec, respectively, Eccles *et al.* 1969, 1971*a*, *b*), after allowing about 1 msec for impulse conduction and synaptic delay from Purkyně cell to the Deiters neurone (Ito & Yoshida, 1966). This agreement suggests that the i.p.s.p.s are produced in Deiters neurones as a consequence of the climbing fibre activation of Purkyně cells. The e.p.s.p.s preceding the i.p.s.p.s may be evoked a few milliseconds earlier than the i.p.s.p.s through the collaterals of the climbing fibres to Deiters neurones as assumed by Ito, Obata & Ochi (1966). Close agreement of the latency of the long-lasting e.p.s.p.s of the non-dominant responses (13 msec from the forelimb nerve and 19 msec from the hind limb nerve) with that of the early e.p.s.p.s of the dominant responses (14 msec from the forelimb and 18 msec from the hind limb) indicates that the former e.p.s.p.s are also produced through collaterals of the climbing fibres originating from IO. The above view was supported by the following two experiments.

Depression following inferior olivary stimulation. First, direct stimulation of IO modified the effectiveness of peripheral nerve stimulation in evoking the e.p.s.p.–i.p.s.p. sequence. Fig. 5*A* shows the response in an HL cell to conditioning IO stimulation (S_1 in Fig. 5*E*) which by itself produces a sequence of an e.p.s.p. and an i.p.s.p. The e.p.s.p. has been shown to be

produced monosynaptically through collaterals of the climbing fibres and the i.p.s.p. disynaptically through the Purkyně cells activated by the climbing fibres (Ito *et al.* 1966). Fig. 5*B* represents the control testing e.p.s.p.–i.p.s.p. sequence evoked from QUAD (S_2 in Fig. 5*E*). In Fig. 5*C* the test response evoked from QUAD was suppressed completely when

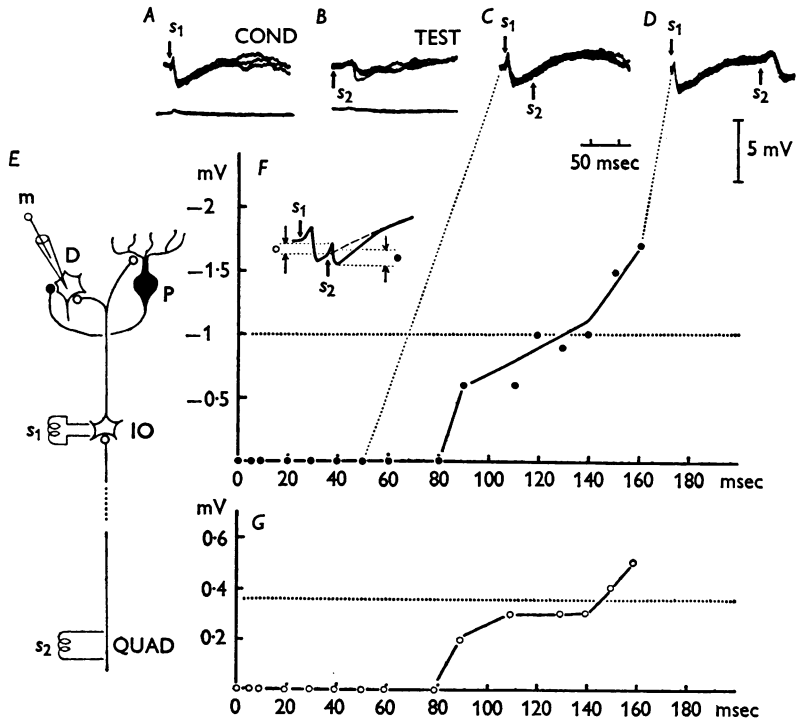

Fig. 5. Depression of response to peripheral nerve stimulation following inferior olivary stimulation. The experimental arrangement is shown diagrammatically in *E*. Conditioning stimuli (S_1) were applied to IO and test stimuli (S_2) to QUAD. m, recording micro-electrode. D, Deiters neurone. P, Purkyně cell. IO, inferior olive. *A*, response to IO stimulation (single pulse, 14 V). Upper trace, the intracellular potential. Lower trace, the extracellular potential. The downward arrow (S_1) indicates the moment of conditioning stimulation. *B*, dominant response to test stimulation of QUAD with a single pulse (5*T*). The upward arrow (S_2) indicates the moment of test stimulation. *C* and *D*, pairing of conditioning and test stimuli. The intervals of the two stimuli are indicated by dotted lines in *F*. *F*, plot of the amplitudes of the i.p.s.p.s evoked by QUAD test stimuli as a function of the interval between conditioning and test stimuli. The horizontal dotted line indicates the control size of the i.p.s.p. The inset in *F* indicates the convention used for measuring the amplitudes of the e.p.s.p. (open circle) and i.p.s.p. (filled circle). *G*, similar plot to *F* but for the e.p.s.p.s. The voltage scale of 5 mV and the time scale of 50 msec apply to *A–D*.

superposed at a short interval upon the conditioning response elicited by IO stimulation. At a longer interval it recovered from the depression and even was facilitated (Fig. 5*D*). Fig. 5*F* and *G* shows the time course of this early depression and later facilitation, plotting the peak amplitude of the testing i.p.s.p. and e.p.s.p., respectively, as a function of the conditioning-testing time interval. The depression of the test i.p.s.p. and e.p.s.p. commenced immediately after the conditioning IO stimulation, continued for 130 msec, and then passed over to a facilitation. The early phase of the depression may be attributed to occlusion of the transmission of the peripheral nerve impulses from QUAD across IO following conditioning activation of IO. The later phase of depression may be due to a relatively long refractoriness in the IO neurones caused by the plateau potential which follows their spike potentials (Crill, 1970) or to an i.p.s.p. similar to that produced after their activation from the contralateral hind limb nerves (Armstrong, Eccles, Harvey & Matthews, 1968). Similar depression of the dominant response was observed upon three other cells, 2 HL and 1 FL.

Inferior olive excitability. Secondly, after stimulation of limb nerves excitability of IO neurones was modified in parallel with the dominant or non-dominant response in the Deiters neurone. As illustrated in Fig. 6*E*, conditioning stimuli were applied to SUR (S_1) to produce the dominant response of Fig. 6*A* in an HL cell, while excitability in IO neurones was assessed by the size of the e.p.s.p. and i.p.s.p. (Fig. 6*B*) evoked in the Deiters neurones by testing stimulation of IO (S_2). In Fig. 6*C*, the testing e.p.s.p. and i.p.s.p. were greatly enhanced (compare with the control in Fig. 6*B*), when superposed upon the conditioning p.s.p.s. evoked at short intervals. At longer intervals there was a depression of the testing responses (Fig. 6*D*). In Fig. 6*F*, a plot of the amplitude of the IO-evoked i.p.s.p.s as a function of the SUR-IO stimulation interval, it will be noticed that there was a short facilitation of the i.p.s.p., starting at 15 msec after SUR stimulation and reaching the maximum (70% increase over control) at 17 msec. This initial facilitation was followed by a long depression (35% decrease) lasting about 60 msec. There was a comparable facilitation and depression in the IO-evoked e.p.s.p.s (Fig. 6*G*). The time course of the excitability of IO neurones after conditioning stimulation of SUR was compared with that of the p.s.p.s evoked by the conditioning stimulation in Fig. 6*H*. Allowance was made thereby for the time delay of 3 msec (Ito *et al.* 1966) for the peripheral nerve impulses to be transmitted from IO to the Deiters neurone, by shifting the moment of SUR stimulation (vertical dotted line in Fig. 6*H*) 3 msec ahead of the zero time of Fig. 6*F* and *G*. After this compensation the increase in excitability of IO neurones coincides exactly with the onset of the e.p.s.p. evoked from SUR. The total time course of IO excitability (early facilitation and late depression) also

agrees with the sequence of depolarization and hyperpolarization observed in IO neurones after stimulation of the hind limb nerves (Armstrong *et al.* 1968).

A comparable increase of excitability of IO neurones was found during the non-dominant response evoked in the HL cells by SR stimulation. As

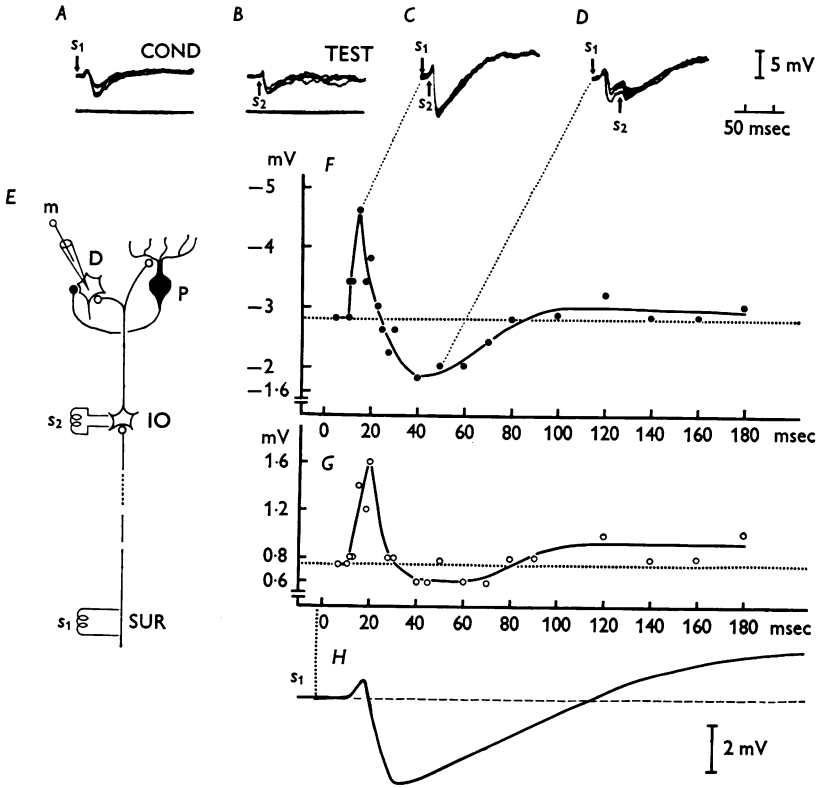

Fig. 6. Inferior olivary excitability following peripheral nerve stimulation. The experimental arrangement is shown diagrammatically in *E* using the same conventions as Fig. 5*E*. *A*, dominant responses to conditioning stimulation of SUR with S_1. Upper trace, the intracellular potential. Lower trace, the extracellular potential. SUR was stimulated by a train of two pulses (2*T*). *B*, control response to test stimulation of IO with S_2 (single pulse, 1·2 V). *C* and *D*, pairing of conditioning and test stimuli. *F*, plot of test i.p.s.p. amplitudes, similar to 5*F*. *G*, plot of e.p.s.p. amplitudes, similar to Fig. 5*G*. *H*, trace of response to SUR conditioning stimulation drawn to the time scale of plots *F* and *G*. The moment of S_1 stimulation (vertical dotted line in *G*–*H*) is displaced to the left by 3 msec from zero time in *F* and *G* to allow for time of conduction and transmission from IO to the Deiters neurone. In Fig. 6*H* and 7*G* a correction was made for distortion of the response caused by a relatively short time constant (0·2 sec). The voltage scale of 5 mV and the time scale of 50 msec apply to *A*–*D*. The voltage scale of 2 mV applies to *H*.

shown in the records of Fig. 7*A–D* and the plots of the amplitudes of the test i.p.s.p. in Fig. 7*E* and e.p.s.p. in Fig. 7*F*, respectively, conditioning SR stimulation produced a facilitation which started at about 12 msec after stimulation, reached the maximum at 14 msec, and declined gradually over the next 60 msec. The onset of the excitability increase agrees exactly

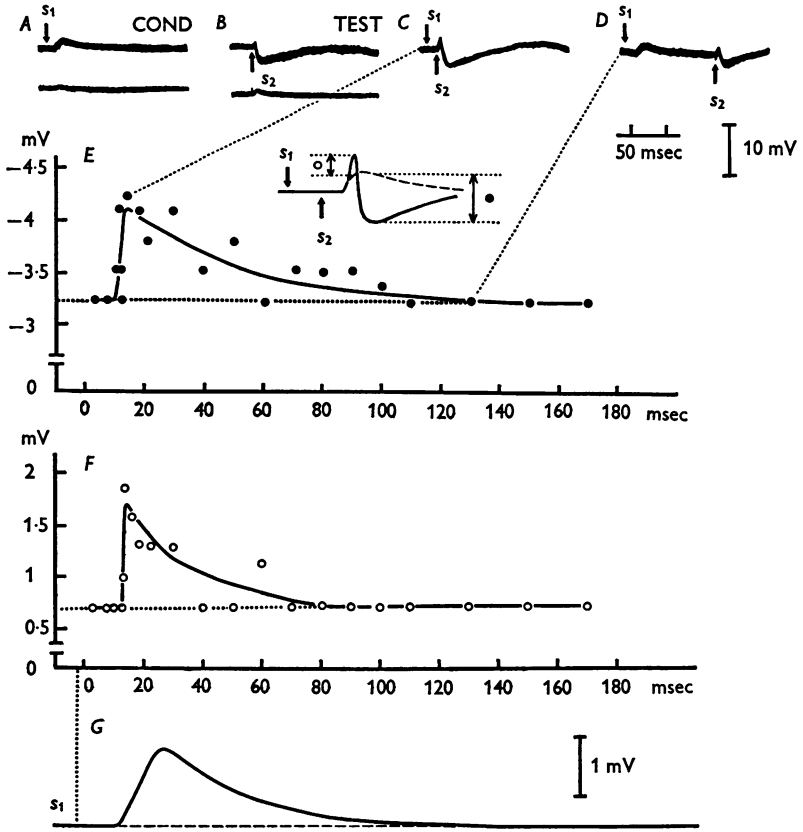

Fig. 7. Inferior olivary excitability changes during non-dominant response. The experimental arrangement is the same as that shown diagrammatically in Fig. 6*E* but the conditioning stimulus is applied to SR. *A*, non-dominant response to conditioning stimulation of SR (S_1) with a train of four pulses (14*T*). Upper trace, the intracellular potential. Lower trace, the extracellular potential. *B*, control response to test stimulation of IO (single pulse, 5·0 V). *C* and *D*, pairing of conditioning and test stimuli. *E*, plot of test i.p.s.p. amplitudes as a function of the interval between conditioning and test stimuli, as in Fig. 6*F*. The inset in *E* indicates the convention used for measuring the amplitudes of the e.p.s.p. (open circle) and i.p.s.p. (filled circle). *F*, plot of e.p.s.p. amplitudes, similar to Fig. 6*G*. *G*, trace of response to SR conditioning stimulation drawn to the time scale of plots *E* and *F*. The voltage scale of 10 mV and time scale of 50 msec apply to *A–D*. The voltage scale of 1 mV applies to *G*.

with that of the e.p.s.p. produced in the HL cell by the SR stimulation (Fig. 7*G*), if the allowance is made for the conduction time and synaptic delay from IO to the Deiters neurone. Similar excitability changes in IO neurones were demonstrated for two other HL cells during the dominant and non-dominant response.

Thus it is quite likely that the dominant and non-dominant responses produced in the HL and FL cells are all produced through the inferior olive. Probably transmission through the mossy fibre system was depressed by the relatively deep barbiturate anaesthesia employed in this experiment (Körlin & Larson, 1970).

Frequency depression. As is expected from the strong depression of IO excitability after conditioning stimulation of the peripheral nerves (Fig. 6), there was a striking frequency depression of the dominant and non-dominant responses. As shown in Fig. 8*A–D*, the initial e.p.s.p. and later i.p.s.p. evoked in the HL cells from SUR were markedly depressed at a relatively low frequency of stimulation. As the frequency was increased from 1/sec (*A*) to 3/sec (*B*) and further to 10/sec (*C*) both the e.p.s.p. and i.p.s.p. were gradually reduced. A further increase in frequency to 23/sec (*D*) depressed the amplitude of the p.s.p.s to about a quarter of the original amplitude at 1/sec. Such a frequency effect is characteristic of the climbing fibre responses evoked in Purkyně cells by peripheral nerve stimulation (Eccles *et al.* 1968*a*). A similar though more remarkable frequency effect upon the long-lasting e.p.s.p.s evoked in the HL cell from SR is illustrated in Fig. 8*E–H*. A reduction of the e.p.s.p. was found with an increase of the frequency from 0·2/sec (*E*) to 1/sec (*F*). A further increase of the frequency to 2/sec (*G*) and 5/sec (*H*) almost abolished the e.p.s.p.s. Similar observations were made upon three other HL cells and five FL cells. In all of them comparable frequency depression was found for the e.p.s.p.–i.p.s.p. sequence evoked from the nerves in the dominant limb as well as for the long-lasting e.p.s.p. produced from the non-dominant limb.

Stimulation intensity–response relationship

As shown in Fig. 9*A*, weak stimulation (1·5*T*) of QUAD produced in an HL Deiters neurone only a very small e.p.s.p. and i.p.s.p. of about 0·2 and −0·4 mV, respectively. In Fig. 9*B* and *C*, there was a large increase of the e.p.s.p. and i.p.s.p. with increase of the stimulus intensity. In Fig. 9*D* the amplitudes of the e.p.s.p. (open circles and triangles for stimulation with a train of two and four pulses, respectively) and i.p.s.p. (filled circles and triangles) were plotted as functions of the stimulus intensity. With a train of two pulses the threshold for evoking the e.p.s.p. and i.p.s.p. was 2·5*T*. Both e.p.s.p. and i.p.s.p. increased gradually with increase of the stimulus intensity and reached the maximum at about 7*T*. With a train of four

pulses the threshold was lower (1·5T) than with two pulses, and the p.s.p.s increased more sharply. The low threshold for evoking the dominant response indicates that the low threshold muscle afferents, Group I and II, are involved. Fig. 9E–H demonstrates with SUR nerve that even with a train of two pulses the threshold was very low (1T) and both e.p.s.p. and i.p.s.p. increased gradually up to the intensity of 5T. This indicates contribution of the low and some of the high threshold cutaneous afferent

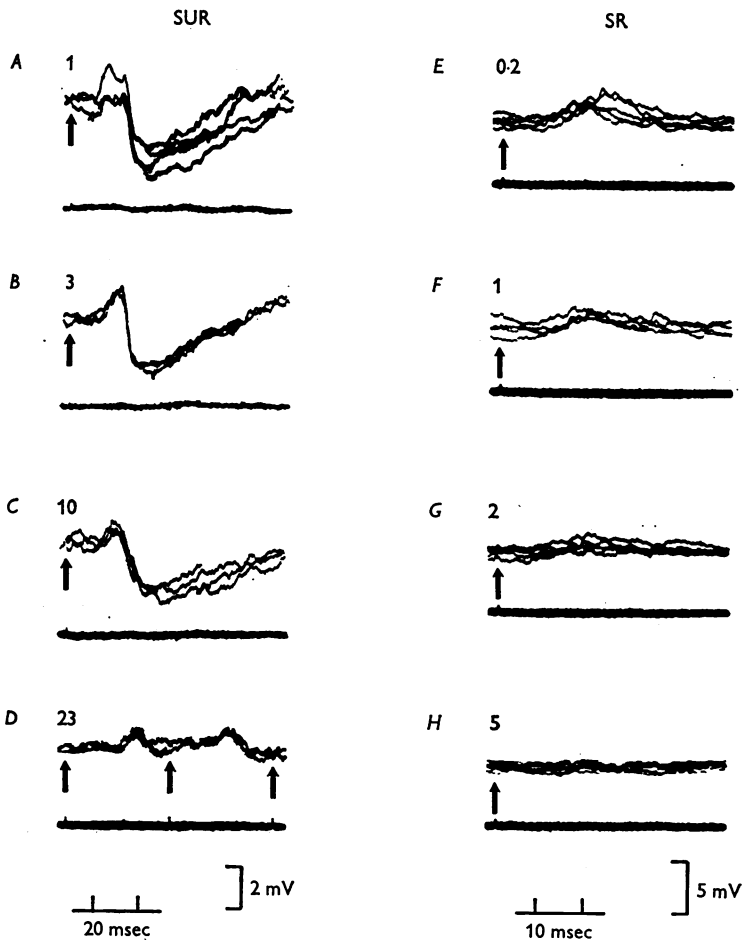

Fig. 8. Effects of stimulus frequency. Upper traces, the intracellular potentials. Lower traces, the extracellular controls. A–D, the dominant responses evoked in an HL cell by SUR stimulation at various frequencies. The frequency is indicated on each record in cycles per second. The nerves were stimulated by a train of four pulses (7T). E–H, non-dominant responses evoked in an HL cell from SR. The nerves were stimulated by a single pulse (4T). The voltage scale of 2 mV and the time scale of 20 msec apply to A–D, while those of 5 mV and 10 msec apply to E–H.

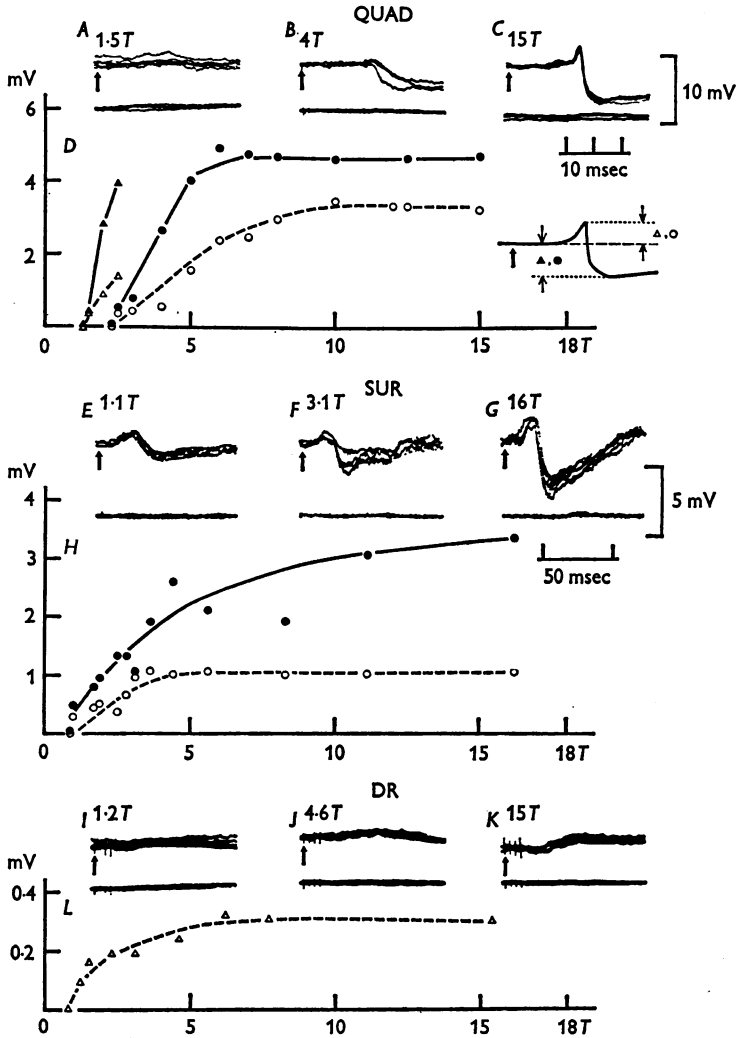

Fig. 9. Effect of stimulus intensity. *A–C*, specimen dominant responses in an HL cell, evoked from QUAD at various intensities. Upper traces, the intracellular potentials. Lower traces, the extracellular controls. The intensities are indicated on each trace. In *A*, the nerve was stimulated by a train of four pulses, whereas in *B* and *C*, two pulses were used. The amplitudes of the e.p.s.p.s (open symbols) and i.p.s.p.s (filled symbols) were measured as indicated by the inset of *D* and plotted in *D* as circles for two pulses and triangles for four pulses. *E–G*, similar to *A–C* but from SUR (two pulses). *H*, plot similar to *D*, but for the responses evoked from SUR using two pulses. *I–K*, non-dominant responses evoked from DR (four pulses) in an HL cell. *L*, plot of e.p.s.p. amplitude in response to DR stimulation with four pulses. The voltage scale of 10 mV applies to *A–C*, while that of 5 mV applies to *E–G* and *I–K*. The time scale of 10 msec applies to *A–C* and *I–K*, while that of 50 msec applies to *E–G*.

impulses. It might be noted in Fig. 9*D* and *H* that there was always an association of the e.p.s.p. and i.p.s.p. even with the weakest nerve stimulation and some tendency for correlation between the amplitudes of both p.s.p.s especially at intensities below 5*T*. In Fig. 9*I–L* a similar experiment upon the non-dominant response evoked in the same HL cell from DR also demonstrates the contribution of the low and high threshold muscle afferent impulses in the production of the non-dominant response in HL cells. A similar study on four other HL cells and three FL cells also revealed contribution of the Group I and II muscle afferent impulses and both low and high threshold cutaneous afferent impulses in producing the dominant and non-dominant responses in Deiters neurones.

Early excitation

Finally, mention should be made of some exceptional cells (ten of the sixty-six HL cells and two of the thirty-five FL cells studied), in which peripheral nerve stimulation produced e.p.s.p.s at a latency shorter than that expected for excitation through the climbing fibres. Fig. 10 exemplifies such a response for an HL cell. The essential features of the response were quite similar to those of the HL cells shown in Fig. 1, except that SR and PBST stimulation produced a late sequence of e.p.s.p. and i.p.s.p. (see open and filled arrows in Fig. 10*B* and *G*). The latencies of the e.p.s.p.s of the dominant and non-dominant responses in this group of Deiters neurones were invariably shorter than those in the other group of Deiters neurones described above.

The latencies of the e.p.s.p.s in this group of Deiters neurones ($6{\cdot}6 \pm 1{\cdot}4$ ($n = 5$) msec from the forelimb nerves (Fig. 10*A*, *C*) and $9{\cdot}5 \pm 1{\cdot}0$ ($n = 10$) msec from the hind limb nerves (*D*, *F*, *M*)) are considerably shorter than the values given in Table 1 for the forelimb nerves (13 msec) and the hind limb nerves (18 msec). On the other hand, the latencies of the i.p.s.p. in this group of Deiters neurones ($21{\cdot}6 \pm 3{\cdot}6$ ($n = 10$) msec from the forelimb nerves and $21{\cdot}5 \pm 2{\cdot}2$ ($n = 2$) msec from the hind limb nerves, *D*, *E*, *F*, *G*, *M*, *N* of Fig. 10) approximately agree with 19 and 23 msec, respectively, from the forelimb and hind limb nerves in the other group of Deiters cells (Table 1). Therefore it appears that in this group of Deiters neurones early e.p.s.p.s are superposed on the dominant and non-dominant responses produced through the climbing fibres originating from IO. Judging from their early onset, the e.p.s.p.s are probably produced through the collaterals of the spinocerebellar tracts to the Deiters neurones (Eccles *et al.* 1967; Ito, Kawai, Udo & Mano, 1969).

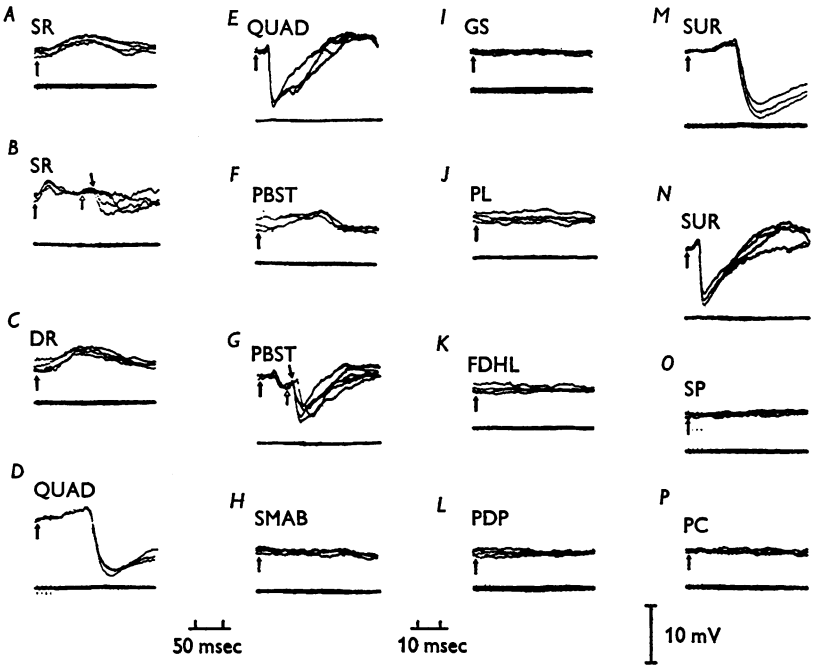

Fig. 10. Mixed mossy and climbing fibre responses of an HL neurone. Upper traces, the intracellular potentials. Lower traces, the extracellular controls. The nerves were stimulated by a train of four pulses (7 *T*). The nerve stimulated is indicated on each trace. In *B* and *G*, the upward open and downward filled arrows indicate the onsets of late e.p.s.p.s and i.p.s.p.s, respectively. The voltage scale of 10 mV applies to all traces. The time scale of 10 msec applies to *A*, *C*, *D*, *F*, *H*–*M*, *O* and *P*, while that of 50 msec applies to *B*, *E*, *G* and *N*.

DISCUSSION

The present study has revealed that stimulation of peripheral nerves can evoke two different responses in Deiters neurones, the dominant response consisting of a sequence of e.p.s.p. and i.p.s.p. and the non-dominant response consisting only of an e.p.s.p. A clear discrimination was found among Deiters neurones according to whether they received the dominant and non-dominant responses from the forelimb and hind limb nerves, respectively (FL cells), or from the hind limb and forelimb nerves, respectively (HL cells). There seems to be further specialization of the HL cells in the extent of the convergence of input from the hind limb nerves on to individual cells. The spectrum of response to stimulation of the hind limb nerves was narrow in some cells, being confined to only a few nerves (Fig. 3, Cell 1), and it was wide in others, spreading over many nerves (Cell 2). The nerves most preferred by the sixty-six HL cells comprised

QUAD, PL, PDP, SUR, SP and PC. Although SMAB, PBST, GS and FDHL were never the most preferred nerves, they frequently constituted the second- or third-most preferred nerves. HL cells of a narrow response spectrum collect information selectively from QUAD, PL, PDP, SUR, SP and PC, while those of a broad response spectrum receive information widely, including SMAB, PBST, GS and FDHL, as well as the former group of nerves. No preference was found in the HL cells between the muscle and cutaneous nerves, between the nerves originating from the synergic and antagonistic muscle groups, or among the nerves originating from neighbouring muscles. Therefore, it appears that each individual HL cell represents a different combination of inputs from the hind limb nerves, and thereby the whole population of HL cells represents all the possible combinations of inputs from the hind limb nerves. The same features were found in the non-dominant response of the FL cells: the response spectrum was narrow in some cells while it was broad in others, there being no general rule for the order of preference. Thus the mode of integration of the non-dominant inputs to the FL cells is probably not very much different from that of the dominant inputs to the HL cells.

From the close agreement between the onsets of the i.p.s.p.s in the dominant responses in a great majority of FL and HL cells and the latency of the climbing fibre activation of Purkyně cells, it is postulated that the i.p.s.p.s are evoked from Purkyně cells as a consequence of climbing fibre activation and that the e.p.s.p.s preceding the i.p.s.p.s are produced through the collaterals of climbing fibres to Deiters neurones. Furthermore, the close agreement in latency between the e.p.s.p.s of the dominant and non-dominant responses in the FL and HL cells suggested that both e.p.s.p.s are produced through the same pathway. This view was supported by observations of (i) strong depression of the dominant response after conditioning IO stimulation (Fig. 5) and (ii) increase of IO excitability which precedes the onsets of the e.p.s.p.s in the dominant (Fig. 6) and non-dominant responses (Fig. 7) by the time required for impulse conduction and synaptic transmission from IO to the Deiters neurone.

The clear discrimination of Deiters neurones into FL and HL cells and the differential effects between the nerves in the dominant and non-dominant limbs on both FL and HL cells would probably require some specific convergence in the afferent pathway from the peripheral nerves to those cells. Our results suggest that one particular population of IO neurones would receive its input predominantly from a certain group of hind limb nerves and in turn excite the HL cells by collateral synapses, while inhibiting the same HL cells by exciting the Purkyně cells projecting to the HL cells. This is also supported by the association of the e.p.s.p. and i.p.s.p. in every dominant response in the HL cells and correlation of

the amplitudes of the e.p.s.p.s and i.p.s.p.s during stimulation at different intensities. In addition, our results suggest that there is a second population of IO neurones collecting inputs from the forelimb nerves and acting upon the FL cells in the same way as the former group of IO neurones acted upon the HL cells. The non-dominant responses evoked from the forelimb in the HL cells can be explained by assuming collaterals of the climbing fibres originating from the second group of IO neurones and similarly the non-dominant responses in FL cells may be attributed to collaterals coming from the first group of IO neurones.

Antidromic identification performed on some of the FL cells revealed that about 85 % of them project to the cervico-thoracic level of the spinal cord and the remaining 15 % to the lumbo-sacral level. Similarly 90 % and 10 % of the HL cells were found to innervate the lumbo-sacral and cervico-thoracic levels, respectively. Apparently the majority of Deiters neurones receive Purkyně inhibition selectively from the nerves in the target area of their axonal projection through the spino-olivo-cerebello-vestibular pathway.

The present experiment employed relatively deep pentobarbitone anaesthesia to minimize the contribution of mossy fibres which provide abundant projection to Purkyně cells and also some collaterals to Deiters neurones. The observation of barbiturate depression of mossy fibre-granule cell transmission (Körlin & Larson, 1970) is thus extended to the mossy fibre-Deiters neurone transmission. Excitation was produced through the collaterals of the mossy fibres in a minority of Deiters neurones investigated, in association with the responses produced through the climbing fibres, but inhibition with sufficiently short latency to be mediated through the mossy fibre system never appeared. The p.s.p.s induced in Deiters neurones through the MF system will be the subject of the following paper (Allen *et al.* 1972).

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REFERENCES

ALLEN, G. I., SABAH, N. H. & TOYAMA, K. (1971*a*). Effect of fore- and hindlimb nerve stimulation on Deiters' neurones. *Brain Res.* **25**, 645–650.

ALLEN, G. I., SABAH, N. H. & TOYAMA, K. (1971*b*). Afferent nerve volleys acting on Deiters neurones via the cerebellum. *Proc. XXV Int. Congr. Physiol. Sci.* vol. **9**, pp. 27. Munich.

ALLEN, G. I., SABAH, N. H. & TOYAMA, K. (1972). Synaptic actions of peripheral nerve impulses upon Deiters neurones via the mossy fibre afferents. *J. Physiol.* **226**, 335–351.

ALLEN, G. I. & TOYAMA, K. (1972). An automatic d.c. level compensation circuit for electrophysiology. *I.E.E.E. Trans. Bio-Med. Engng* (in the Press).

ARMSTRONG, D. M., ECCLES, J. C., HARVEY, R. J. & MATTHEWS, P. B. C. (1968). Responses in the dorsal accessory olive of the cat to stimulation of hind limb afferents. *J. Physiol.* **194**, 125–145.

BRODAL, A., POMPEIANO, O. & WALBERG, F. (1962). In *The Vestibular Nuclei and their Connections: Anatomy and Functional Correlations*, p. 83. Edinburgh: Oliver and Boyd.

CRILL, W. E. (1970). Unitary multiple-spiked responses in cat inferior olive nucleus. *J. Neurophysiol.* **33**, 199–209.

ECCLES, J. C. (1964). *The Physiology of Synapses*, pp. 49–53. Berlin, Göttingen, Heidelberg: Springer-Verlag.

ECCLES, J. C., FABER, D. S., MURPHY, J. T., SABAH, N. H. & TÁBOŘÍKOVÁ, H. (1969). Firing patterns of Purkyně cells in response to volleys from limb nerves. *Brain Res.* **14**, 222–226.

ECCLES, J. C., FABER, D. S., MURPHY, J. T., SABAH, N. H. & TÁBOŘÍKOVÁ, H. (1970). The integrative performance of the cerebellar Purkyně cells. In *Excitatory Synaptic Mechanisms*, ed. ANDERSEN, P. & JANSEN, J. K. S., pp. 223–236. Oslo: Oslo University Press.

ECCLES, J. C., FABER, D. S., MURPHY, J. T., SABAH, N. H. & TÁBOŘÍKOVÁ, H. (1971*a*). Afferent volleys in limb nerves influencing impulse discharges in cerebellar cortex. II. In Purkyně cells. *Expl Brain Res.* **13**, 36–53.

ECCLES, J. C., FABER, D. S., MURPHY, J. T., SABAH, N. H. & TÁBOŘÍKOVÁ, H. (1971*b*). Investigations on integration of mossy fiber and climbing fiber inputs to Purkyně cells in the anterior lobe. *Expl Brain Res.* **13**, 54–77.

ECCLES, J. C., ITO, M. & SZENTÁGOTHAI, J. (1967). *The Cerebellum as a Neuronal Machine*, pp. 227–294. New York: Springer-Verlag.

ECCLES, J. C., PROVINI, L., STRATA, P. & TÁBOŘÍKOVÁ, H. (1968*a*). Analysis of electrical potentials evoked in the cerebellar anterior lobe by stimulation of hindlimb and forelimb nerves. *Expl Brain Res.* **6**, 171–194.

ECCLES, J. C., PROVINI, L., STRATA, P. & TÁBOŘÍKOVÁ, H. (1968*b*). Topographical investigations on the climbing fiber inputs from forelimb and hindlimb afferents to the cerebellar anterior lobe. *Expl Brain Res.* **6**, 195–215.

GRILLNER, S., HONGO, T. & LUND, S. (1970). The vestibulospinal tract. Effects on alpha-motoneurones in the lumbosacral cord in the cat. *Expl Brain Res.* **10**, 94–120.

ITO, M. (1960). New electronic device for stimulating and recording potentials for a single ganglion cell. In *Medical Electronics*, ed. SMYTH, C. N., pp. 84–85. London: Iliff.

ITO, M., HONGO, T. & OKADA, Y. (1969). Vestibular-evoked postsynaptic potentials in Deiters neurones. *Expl Brain Res.* **7**, 214–230.

ITO, M., HONGO, T., YOSHIDA, M., OKADA, Y. & OBATA, K. (1964). Antidromic and transsynaptic activation of Deiters' neurones induced from the spinal cord. *Jap. J. Physiol.* **14**, 638–658.

ITO, M., KAWAI, N., UDO, M. & MANO, N. (1969). Axon reflex activation of Deiters neurones from the cerebellar cortex through collaterals of the cerebellar afferents. *Expl Brain Res.* **8**, 249–268.

ITO, M., KAWAI, N., UDO, M. & SATO, N. (1968). Cerebellar-evoked disinhibition in dorsal Deiters neurones. *Expl Brain Res.* **6**, 247–264.

ITO, M., OBATA, K. & OCHI, R. (1966). The origin of the cerebellar-induced inhibition of Deiters neurones. II. Temporal correlation between the trans-synaptic activation of Purkinje cells and inhibition of Deiters neurones. *Expl Brain Res.* **2**, 350–364.

ITO, M. & YOSHIDA, M. (1966). The origin of the cerebellar induced inhibition of Deiters neurones. I. Monosynaptic initiation of the inhibitory postsynaptic potentials. *Expl Brain Res.* **2**, 330–349.

ITO, M., YOSHIDA, M., OBATA, K., KAWAI, N. & UDO, M. (1970). Inhibitory control of intracerebellar nuclei by the Purkinje cell axons. *Expl Brain Res.* **10**, 64–80.

KÖRLIN, D. & LARSON, B. (1970). Differences in cerebellar potentials evoked by the group I and cutaneous components of the cuneocerebellar tract. In *Excitatory Synaptic Mechanisms*, ed. ANDERSEN, P. & JANSEN, J. K. S., pp. 237–241. Oslo: Oslo University Press.

WILSON, V. J. & BURGESS, P. R. (1962). Disinhibition in the cat spinal cord. *J. Neurophysiol.* **25**, 392–404.

WILSON, V. J., YOSHIDA, M. & SCHOR, D. H. (1970). Supraspinal monosynaptic excitation and inhibition of thoracic back motoneurons. *Expl Brain Res.* **11**, 282–295.