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

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SUMMARY

1. The cerebellar integration of sensory inputs to Deiters neurones was investigated in decerebrate cats. In some preparations decerebration was combined with transection of the olivocerebellar fibres.

2. In the latter preparations peripheral nerve impulses generally produced a response consisting of a sequence of the following post-synaptic potentials: (i) an initial e.p.s.p. ($d_1$), (ii) early i.p.s.p. ($h_1$), (iii) later i.p.s.p. ($h_2$).

3. The mean latencies of $d_1$, $h_1$ and $h_2$ were 5·7, 7·3 and 9·8 msec from the forelimb nerves, and 7·5, 9·0 and 13·4 msec from the hind limb nerves, respectively.

4. The stimulus intensity–response relation indicates that the Group I muscle afferents as well as the low threshold cutaneous afferents contribute to the response.

5. In the preparations with the intact inferior olive there were additional components of the post-synaptic potentials: a later e.p.s.p. ($d_2$) and another later i.p.s.p. ($h_3$), their mean latencies being 15·3 and 19·7 msec from the forelimb nerves, and 18·0 and 21·3 msec from the hind limb nerves, respectively.

6. The $d_1$ and $h_2$ components were attributed to the mossy fibre afferents and $d_2$ and $h_3$ to the climbing fibres; $d_1$ and $d_2$ were due to excitation through the collaterals of the mossy and climbing fibres, and $h_2$ and $h_3$ to inhibition from Purkyně cells activated by the mossy and climbing fibres, respectively. $h_1$ was too early to be produced through the cerebellum, and was probably mediated by inhibitory neurones in the reticular formation.

## INTRODUCTION

By using relatively deep pentobarbitone anaesthesia the previous study revealed synaptic actions of peripheral nerve impulses upon Deiters neurones through the climbing fibre system (the spino-olivo-cerebello-vestibular pathway) (Allen, Sabah & Toyama, 1972). Under this experimental condition transmission through the spinocerebellar pathway was greatly reduced (Körlin & Larson, 1970) and consequently no post-synaptic potentials attributable to the mossy fibre system (spino-cerebello-vestibular pathway) were detected except in a small number of Deiters cells in which early excitation was produced, probably through the collaterals of the spinocerebellar afferents (Allen *et al.* 1972). In these cells there was always association of the responses produced through the mossy and climbing fibre systems during peripheral nerve stimulation, suggesting some cooperation between the above two pathways in their actions upon the Deiters neurones.

The aim of the present study was to investigate the synaptic actions of the mossy fibre system upon Deiters neurones and the mode of its cooperation with the climbing fibre system as suggested above. In order to avoid a depression of synaptic transmission through the mossy fibre system by anaesthesia, the animals were decerebrated for the present investigation. In addition, in some experiments the decussation of the inferior olive was severed contralateral to the Deiters nucleus studied in order to avoid the contribution of the climbing fibre system (Batini & Pumain, 1968).

## METHODS

The ten cats used in this experiment were first anaesthetized with halothane, and decerebrated at the level of the caudal end of the thalamus (Frontal plane A 8·0, according to Berman, 1968) by electrocoagulation (Koller & Jenny, 1969). The electrodes used for the electrocoagulation were composed of six metal needles (diameter, 0·5 mm) spaced at 2 mm intervals along the frontal plane of the stereotaxic co-ordinate and insulated except for their distal 13 mm. These electrodes were stereotaxically inserted into the brain on both sides, dorsoventrally with a latero-medial angle of 7° to the vertical axis, in order to position their tips 0·5 mm above the ventral surface of the brain stem. An a.c. current of 120 mA (frequency, 500 kHz) was passed monopolarly through each electrode for 30 sec. This caused electrocoagulation of about 2 mm thickness on both sides of the electrodes producing a complete severance of the brain stem. In four cats the fibres projecting from the right inferior olive (IO) were severed with a microknife. The knife was inserted from the ventral surface of the brain stem after electrical coagulation of the surface vessels, and a cut 3 mm deep was made cranio-caudally for 7 mm very close (0·5 mm) to the mid line. As shown in Pl. 1*A–D*, it was confirmed by histological examination after each experiment that all of the fibres projecting from the contralateral IO to the cerebellum across the median raphe of the brain stem were severed in this way. Other experimental procedures were the same as in the previous experiments (Allen *et al.* 1971, 1972), and the same abbreviations were used for the peripheral nerves.

## RESULTS

Fifty-four cells sampled from the dorsal part of Deiters nucleus were maintained in reasonable condition for the period of study and were identified as Deiters neurones by antidromic activation from the C3-spinal segment (Ito, Hongo, Yoshida, Okada & Obata, 1964).

Forty-four of the fifty-four cells were sampled from cats with an intact IO and the other ten cells after severance of the inferior olivary decussation.

### *Responses after transection of the inferior olivary decussation*

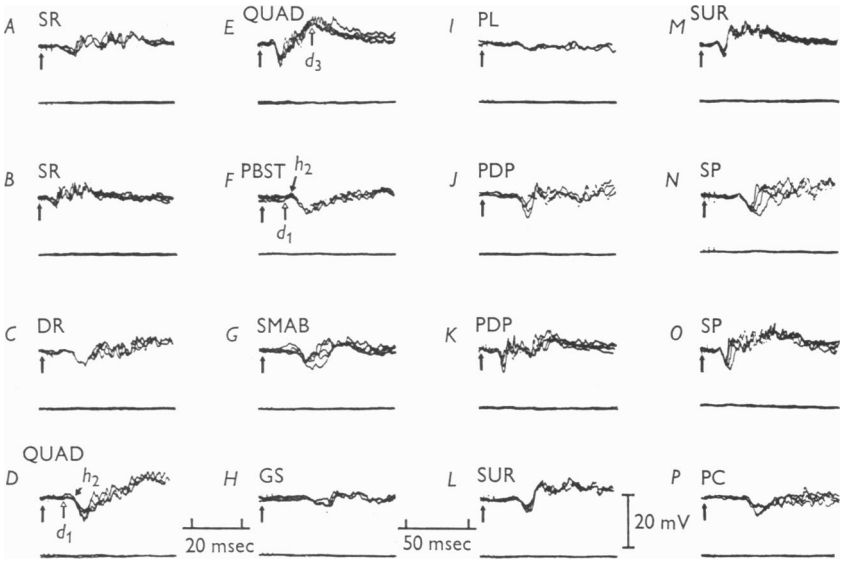
In view of the complex nature of the combined responses evoked in Deiters neurones through both the mossy and climbing fibre systems, a description will be made first of the ten Deiters neurones studied after severance of the inferior olivary decussation. This operation was intended to abolish the contribution of climbing fibres from the contralateral inferior olive and thereby isolate the effects of the mossy fibre system upon Deiters neurones.

As shown in Text-fig. 1 stimulation of the nerves in fore- or hind limb usually produced in a Deiters neurone an initial short depolarization (open arrow $d_1$ in Text-fig. 1*D* and *F*) and a later hyperpolarization lasting for 10–50 msec (filled arrow $h_2$). The later hyperpolarization sometimes passed over to a strong rebound depolarization continuing for more than 50 msec (open arrow $d_3$ in *E*). The response was largest from QUAD in this Deiters neurone. However, smaller responses were evoked widely from the forelimb nerves (SR in *A*, *B*; DR in *C*) as well as from the hind limb nerves (PBST in *F*; SMAB in *G*; GS in *H*; PL in *I*; PDP in *J*, *K*; SUR in *L*, *M*; SP in *N*, *O*; PC in *P*).

Among the ten cells examined in this manner the most effective nerve was QUAD in 4, PDP in 2, SUR in 2 and SP in 2. The spectrum of response spread widely in all cells over several nerves in the hind limb as well as the forelimb.

The nature of the above responses was investigated in Text-fig. 2 by applying hyperpolarizing current through the recording micro-electrode. As shown in Text-fig. 2*A* and its tracing in Text-fig. 2*D*, QUAD stimulation produced a sequence of initial depolarization (denoted as $d_1$ in Text-fig. 2*D*) and later hyperpolarization ($h_2$) which was similar to that described in Text-fig. 1, except for an additional hyperpolarization ($h_1$) superposed upon the top of $d_1$. Hyperpolarizing current was injected in Text-fig. 2*B*. When the traces in *A*, *B* and *C* (extracellular potential) are superposed in *D* by continuous, interrupted and dotted lines, respectively, it is seen that the injection of the current reversed $h_1$ and $h_2$ into depolarization, while there was little effect upon $d_1$. Therefore, $d_1$ should be an e.p.s.p. and $h_1$ and $h_2$

should be i.p.s.p.s (Eccles, 1964). The same reversal of $h_1$ and $h_2$ is shown in Text-fig. 2*E* with a slower time scale. Text-fig. 2*F* and *G* provide examples of similar analysis made upon the responses evoked from PBST and SUR, which consisted of $d_1$ and $h_2$ but lacked $h_1$. Fig. 3*H* and *I* show another example of the analysis on the DR-evoked responses composed of

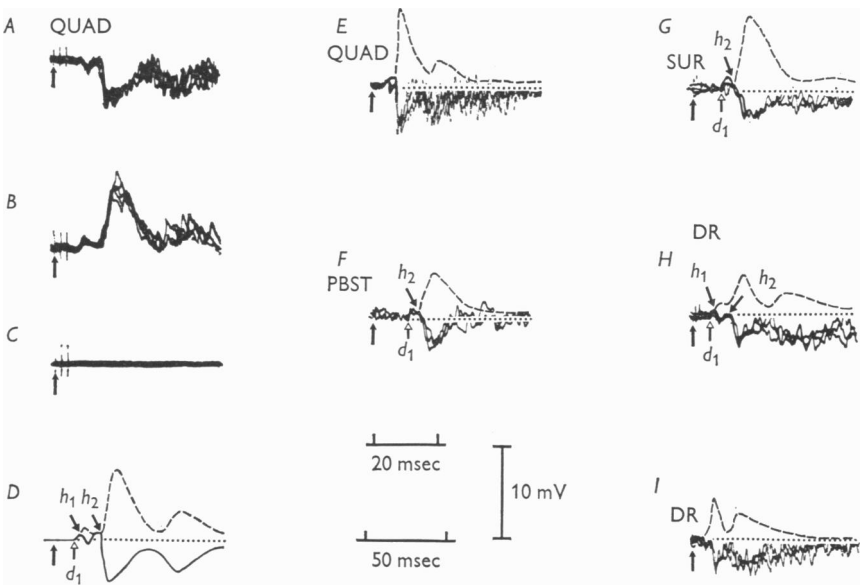



Text-fig. 1. Intracellular responses from a Deiters neurone evoked by stimulation of the peripheral nerves. The upper traces are the intracellular potentials and the lower traces the extracellular. The nerves were stimulated by a train of three pulses at a frequency of 500/sec and at an intensity about 8 times threshold (8*T*). The nerve stimulated is indicated on each record. In this and subsequent Text-figures, the upward arrow in each record indicates the moment of the first pulse. In *D–F*, depolarization ($d_1$ and $d_3$) is indicated by an open arrow and hyperpolarization ($h_2$) by a filled arrow. For further explanation of these potentials, see text. The voltage scale of 20 mV applies to all records. The time scale of 20 msec applies to records *A*, *C*, *D*, *F–J*, *L*, *N* and *P*, while that of 50 msec applies to records *B*, *E*, *K*, *M* and *O*. In Text-figs. 1–4, recording was made after transection of the inferior olivary decussation. The recording time constant in all Text-figures is 0·2 sec and upward deflexion represents depolarization. Records were made by superposing 4–10 traces at intervals of 1·3 sec, unless otherwise specified.

$d_1$, $h_1$ and $h_2$. The sequence of $d_1$, $h_1$ and $h_2$ was found in four of the ten cells, while the rest of the cells lacked $h_1$. It was always found that $d_1$ was an e.p.s.p. and $h_1$ and $h_2$ were i.p.s.p.s.

The moment of onset of $d_1$ was determined at the point of divergence (open arrows $d_1$ in Text-fig. 2*D–I*) of the intracellular control traces (continuous line in Text-fig. 2*D*, or real traces in *E–I*) from the extracellular traces (dotted lines in Text-fig. 2*D–I*). Similarly, the onsets of $h_1$ and $h_2$

were determined at the first (filled arrows $h_1$) and second (filled arrows $h_2$) points of divergence between the intracellular control traces and the traces obtained during injection of hyperpolarizing current. The latency of each component thus measured is represented in Table 1. The onset of $d_1$ ranged from 5 to 7 msec (mean 6·3 msec) from the nerves in the forelimb and 7 to 10 msec (mean 7·5 msec) from those in the hind limb. The latency of $h_1$, on the other hand, was 6–8 msec (mean, 7·6 msec) from the forelimb




Text-fig. 2. Effect of hyperpolarizing current on the post-synaptic potentials. *A*, a response to stimulation of QUAD, without injection of current. *B*, same as *A* but during injection of hyperpolarizing current ($1{\cdot}4 \times 10^{-8}$ *A*) through the recording micro-electrode. *C*, the extracellular potential. *D*, superposition of *A* (continuous line), *B* (interrupted line) and *C* (dotted line). *E*, same as *D* but with a slower sweep. *F*, similar to *D* but from PBST. *G*, from SUR. *H* and *I*, from DR with fast and slow sweeps, respectively. Each nerve was stimulated using a train of three pulses at an intensity of 8*T*. Conventions for denoting depolarization ($d_1$) and hyperpolarization ($h_1$ and $h_2$) are the same as in Text-fig. 1. The voltage scale of 10 mV applies to all traces. The time scale of 20 msec applies to *A*–*D* and *F*–*H*, while that of 50 msec applies to *E* and *I*.

nerves and 7–9 msec (mean, 8·5 msec) from the hind limb nerves, while that of $h_2$ was 9–12 msec (mean, 9·6 msec) from the forelimb nerves and 10–14 msec (mean, 11·7 msec) from the hind limb nerves.

The latency of $d_1$ (6·3 and 7·5 msec, respectively from the fore- and hind limb nerves) is much shorter than that for the climbing fibre field evoked in the cerebellum by the fore- and hind limb nerves (11–19 and 18–25 msec), but is very close to the latency of the mossy fibre field in the

cerebellum (5–7 and 7–10 msec) evoked from the fore- and hind limb nerves (Eccles, Provini, Strata & Táboříková, 1968*a*, *b*). Similarly the latency of $h_2$ (9·6 ± 1·2 msec from the forelimb nerves and 11·7 ± 1·0 msec from the hind limb nerves, Table 1) agrees approximately with 6–11 and 10–15 msec given for the latency of activation of Purkyně cells through the mossy fibres (Eccles, Faber, Murphy, Sabah & Táboříková, 1969, 1970, 1971*a*, *b*). Thus $d_1$ and $h_2$ appear to be produced through the mossy fibre system; $d_1$ directly and $h_2$ as a consequence of Purkyně cell activation. The latency of $h_1$ (7·6 msec from the forelimb nerve and 8·5 msec from the hind limb nerves, Table 1), on the other hand, seems to be too early to be mediated through Purkyně cells (see Discussion).

TABLE 1. Latency of p.s.p.s in Deiters neurones evoked by peripheral nerve stimulation in decerebrate preparations with transection of inferior olivary decussation. 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

| Nerve | $d_1$ (msec) | $h_1$ (msec) | $h_2$ (msec) |
|---|---|---|---|
| Forelimb | 6·3 ± 0·7 (3) | 7·6 ± 0·7 (2) | 9·6 ± 1·6 (3) |
| Hind limb | 7·5 ± 1·3 (7) | 8·5 ± 1·0 (4) | 11·7 ± 1·0 (7) |

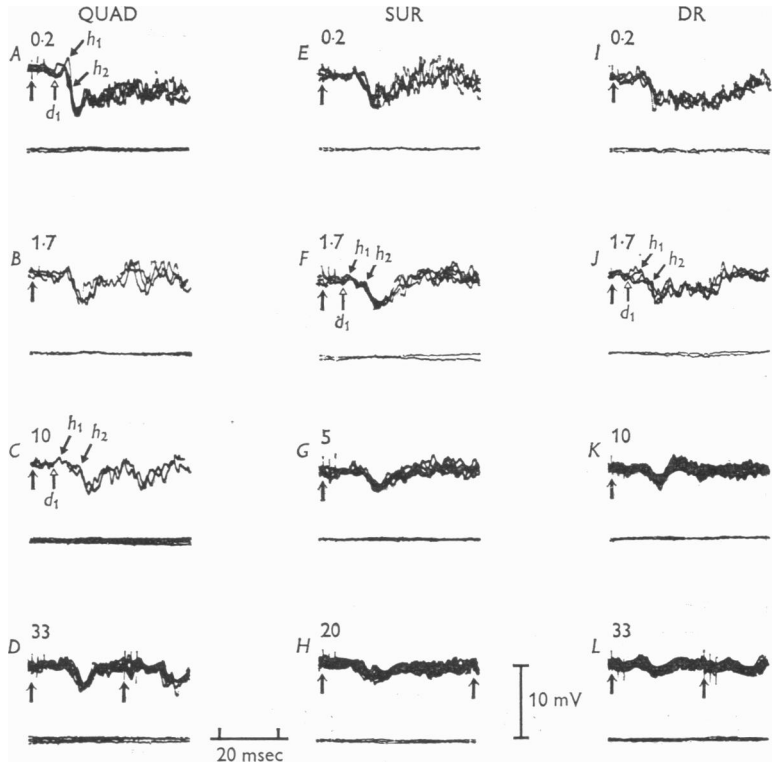
*Frequency effect of stimulation*

The efficiency of the pathways mediating these e.p.s.p.s during tetanic stimulation of the peripheral nerves was investigated in Text-fig. 3. In Text-fig. 3*A–D* where QUAD was stimulated at increasing rates from 0·2 to 33/sec, there was a progressive decrease in all of the $d_1$, $h_1$ and $h_2$ components. However, even at 33/sec (Text-fig. 3*D*) these components still maintained amplitudes about 65% of those at 0·2/sec. This depression is much less than that observed for the p.s.p.s evoked through the climbing fibre system (see Fig. 8 in Allen *et al.* 1972). Similar relatively efficient transmission was found for SUR (Text-fig. 3*E–H*) and for DR (*I–L*). In these cases the depression was greater than that with QUAD stimulation, but still the amplitudes of the above three components at 20 or 33/sec was never reduced below 50% of those at 0·2/sec.

*Stimulus intensity–response relationship*

In Text-fig. 4*A–C* QUAD was stimulated with a train of four pulses or two pulses at different intensities. The response produced by QUAD stimulation consisted of the $d_1$ and $h_2$ components which the above analysis suggests are evoked through the mossy fibre system. In Text-fig. 4*D* amplitudes of $d_1$ and $h_2$ were plotted as functions of the intensity of stimulation.

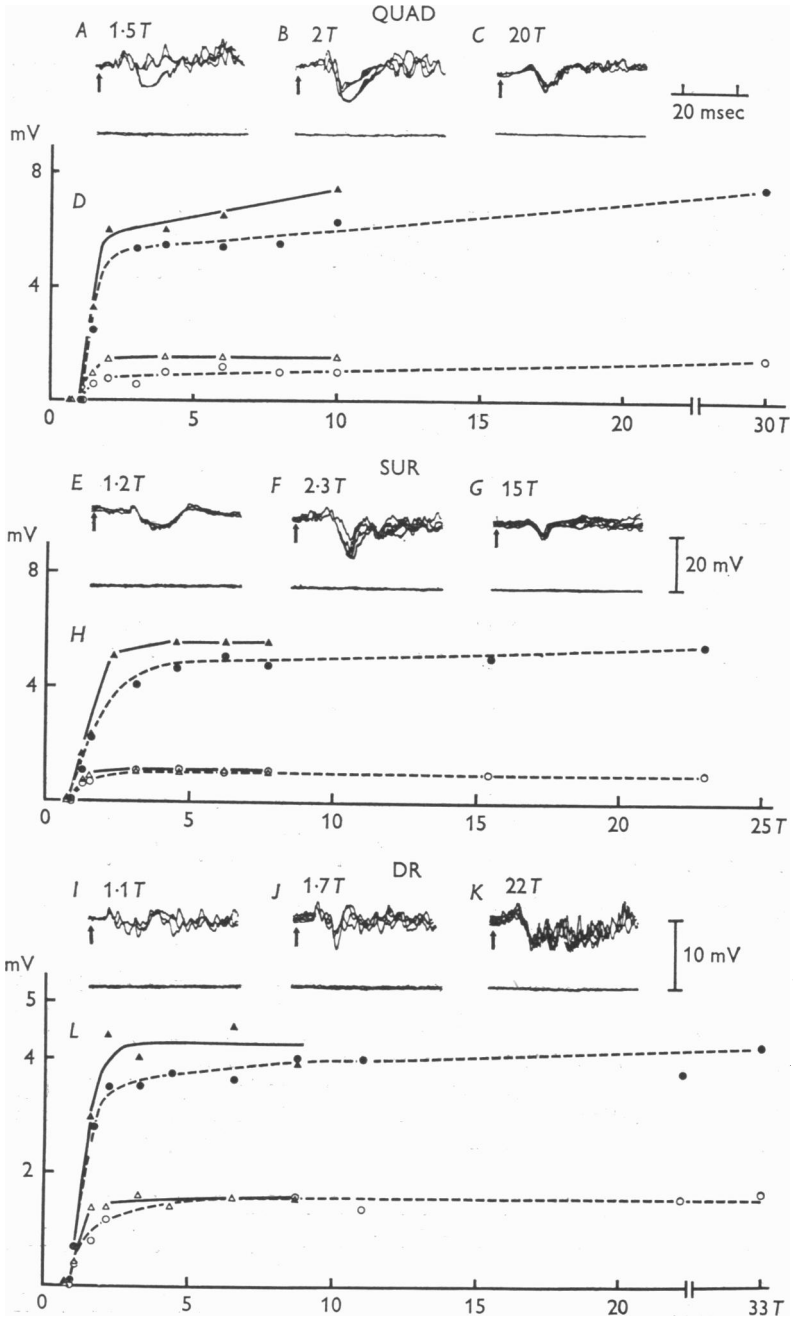
With stimulation of four pulses, the threshold for evoking both $d_1$ and $h_2$ was $1{\cdot}5T$. There was a sharp increment in both $d_1$ and $h_2$ up to $2T$, and a slow increase from $2T$ to $10T$. A similar build-up of both $d_1$ and $h_2$ was also found with stimulation of two pulses, but the increase of $d_1$ and $h_2$ was slower than with four pulses and it continued up to the intensity of $30T$.




Text-fig. 3. Effects of stimulus frequency. Upper traces, the intracellular potentials. Lower traces, the extracellular controls. *A–D*, the p.s.p.s evoked by QUAD stimulation at various frequencies. The frequency is indicated on each record in cycles per second. *E–H* and *I–L*, similar to *A–D* but from SUR and DR, respectively. The nerves were stimulated by a train of three pulses ($8T$). Conventions for denoting depolarization ($d_1$) and hyperpolarization ($h_1$ and $h_2$) are the same as in Text-fig. 1. The voltage scale of 10 mV and the time scale of 20 msec apply to all traces.

This indicates that Deiters neurones receive abundant impingement of Group I impulses, as well as some Group II and probably Group III, all through the mossy fibre system.

Similar investigation with cutaneous nerves (SUR in Text-fig. 4*E–H*) revealed strong impingement of the low threshold afferent impulses upon Deiters neurones, and some weak influence of the high threshold afferents.




Text-fig. 4. For legend see opposite page.

The same experiment with the muscle nerve in the forelimb, DR, also indicates impingement of impulses from the low and high threshold muscle afferents (Text-fig. 4*I–L*).

*Responses with intact inferior olive*

The responses evoked in most of the forty-four Deiters neurones with the intact inferior olive during stimulation of the peripheral nerves were complicated by the contributions of the climbing fibre system in addition to the mossy fibre system. Text-fig. 5 presents a typical example of such responses. Stimulation of QUAD (Text-fig. 5*D*, *E*), PDP (*J*, *K*), SP (*M*, *N*) and PC (*O*, *P*) produced strong responses in the Deiters neurone. In the response evoked from PDP (Text-fig. 5 *J*, *K*) two new components, $d_2$ and $h_3$, may be identified in addition to the components $d_1$, $h_1$, $h_2$ and $d_3$, which were investigated in the preparation with the severed inferior olivary decussation. Traces of the $d_2$ and $h_3$ components are also seen in the responses evoked from QUAD (Text-fig. 5*D*, *E*), SP (*M*, *N*), PC (*O*, *P*) and SUR (*L*). These two components invariably displayed a striking frequency depression (see Text-fig. 7) similar to the responses mediated by the climbing fibre system (Allen *et al.* 1972). Therefore $d_2$ and $h_3$ are presumably of climbing fibre origin (see below). The responses to SR (*A*) and DR (*B*, *C*) include $d_1$ and $h_2$ but fail to show $d_2$, $h_3$ and $d_3$ components. Even weaker responses were induced from PBST (*F*), SMAB (*G*), GS (*H*) and PL (*I*). Thus the response spectrum of this Deiters neurone virtually spread over all of the nerves in the forelimb as well as hind limb.

Analysis of the nature of these components similar to that in Text-fig. 2 was performed in Text-fig. 6. As exemplified for the responses evoked from QUAD (Text-fig. 6*A*, *B*), PC (*C*, *D*), and SR (*E*, *F*), injections of hyperpolarizing current reversed the $h_1$, $h_2$ and $h_3$ components into depolarization,

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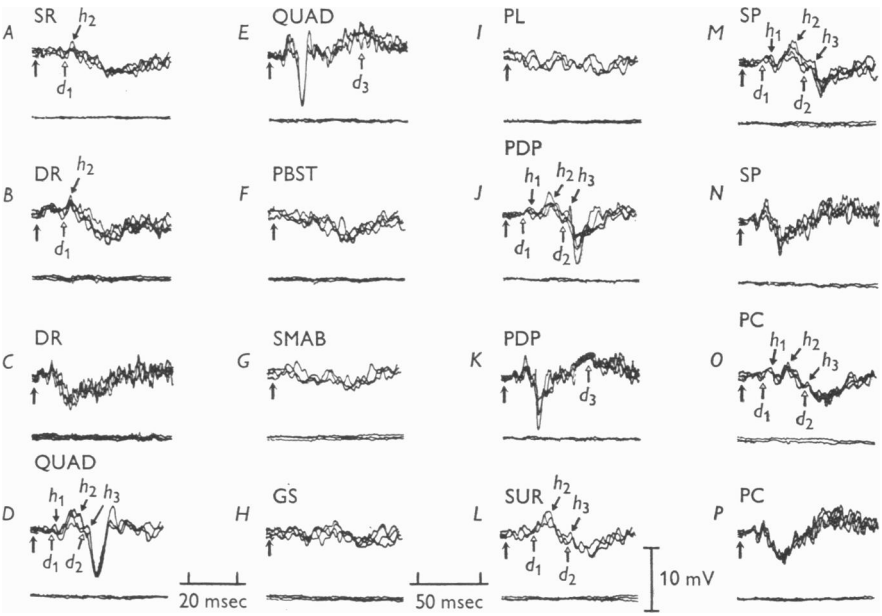
Legend to Fig. 5.

Text-fig. 5. Effect of stimulus intensity. *A–C*, specimen intracellular responses evoked from QUAD at various intensities. The intensity is indicated on each trace. In *A–C*, the nerve was stimulated by a train of two pulses. Upper traces, the intracellular potentials. Lower traces, the extracellular controls. The amplitudes of the e.p.s.p.s and i.p.s.p.s evoked with two pulses were plotted in *D*, as open and filled circles, respectively. A similar plot was made for the e.p.s.p.s and i.p.s.p.s by four pulses, using open and filled triangles, respectively. *E–G*, similar to *A–C*, but from SUR. *E*, with four pulses. *F* and *G*, with two pulses. *H*, plot similar to *D*, but for the responses evoked from SUR in the same cell. *I–K*, similar to *A–C*, but from DR. *I*, with four pulses. *J* and *K*, with two pulses. *L*, plot similar to *D*, but for the responses evoked from DR. The voltage scale of 10 mV applies to traces *A*, *B*, *E*, *F* and *I–K*, while that of 20 mV applies to *C* and *G*. The time scale of 20 msec applies to all traces.

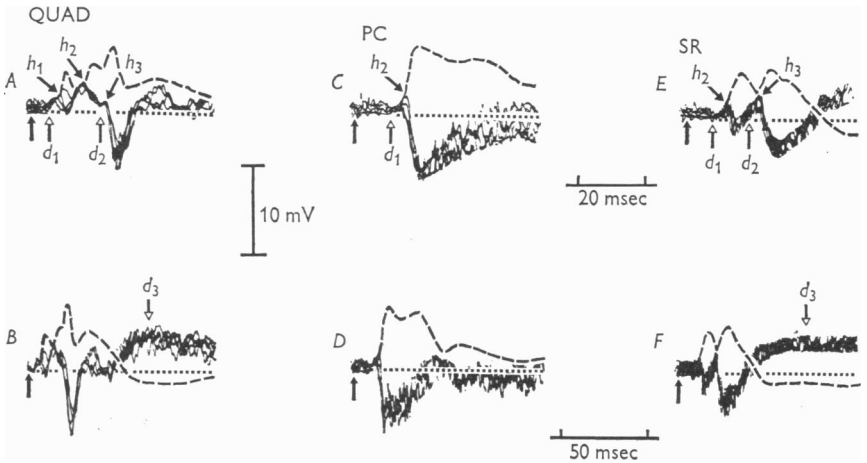
and the $d_3$ component into hyperpolarization. Therefore they are all related to i.p.s.p.s in their nature: $h_1$, $h_2$, and $h_3$ components produced by onsets of i.p.s.p.s and $d_3$ by removal of the background i.p.s.p.s, i.e. disinhibition (Wilson & Burgess, 1962). On the other hand, the $d_1$ component was very little affected by the hyperpolarizing current, and therefore it is an e.p.s.p. The effect of hyperpolarizing current upon the $d_2$ component could not be analysed accurately, because it was superposed upon the $h_2$ component.




Text-fig. 5. Mixed mossy and climbing fibre-evoked responses from peripheral nerve stimulation. Upper traces, the intracellular potentials. Lower traces, the extracellular controls. The nerves were stimulated by a train of three pulses ($8T$). Conventions for denoting depolarization ($d_1$, $d_2$ and $d_3$) and hyperpolarization ($h_1$, $h_2$ and $h_3$) are the same as in Text-fig. 1. The voltage scale of 10 mV applies to all traces. The time scale of 20 msec applies to *A*, *B*, *D*, *F*–*J*, *L*, *M* and *O*, while that of 50 msec applies to *C*, *E*, *K*, *N* and *P*. Figs. 6–8 were obtained from decerebrate cats with intact inferior olive.

The onset of $d_1$ was determined as in Text-fig. 2 at the point of divergence between the intra- and extracellular traces (open arrow in Text-fig. 6) and that of $h_1$, $h_2$ and $h_3$ at the points of divergence between the intracellular traces with and without hyperpolarizing current (filled arrows). The onset of $d_2$ was determined at the point where the upward deflexion from the $h_2$ component begins (open arrow). The mean latency of these components are shown in Table 2. The onsets of $d_1$ (5·7 msec), $h_1$ (7·3 msec), $h_2$ (9·8 msec) from the nerves in the forelimb and those from the hind limb (7·5, 9·0,

13·4 msec, respectively) in the preparation with intact inferior olive agree with the latencies of the respective components in the preparation with severed inferior olivary decussation (compare Table 2 with Table 1). On the other hand the onsets of the new components $d_2$ and $h_3$ (15·3 and 19·7 msec from forelimb nerves and 18·0 and 21·3 msec from hind limb nerves) are approximately equal to the respective latencies of the climbing




Text-fig. 6. Effect of hyperpolarization. *A*, response to stimulation of QUAD. Superimposed are the same response obtained during injection of hyperpolarizing current of $1{\cdot}3 \times 10^{-8}$ A (interrupted line) and the extracellular control (dotted line). *B*, similar to *A* but with a slower sweep. *C* and *D*, similar to *A* and *B*, respectively, but evoked from PC. *E* and *F*, from SR with fast and slow sweeps, respectively. Conventions for denoting depolarizations ($d_1$, $d_2$ and $d_3$) and hyperpolarizations ($h_1$, $h_2$ and $h_3$) are the same as for Text-fig. 1. The voltage scale of 10 mV applies to all traces. The time scale of 20 msec applies to *A*, *C* and *E*, while that of 50 msec applies to *B*, *D* and *F*.

fibre-evoked e.p.s.p.s and i.p.s.p.s studied in the previous paper (Allen *et al.* 1972) which gave the latencies 13·8 and 19·2 from the forelimb nerves and 18·4 and 22·6 msec from the hind limb nerves for the e.p.s.p.s and i.p.s.p.s, respectively. Therefore it is likely that the $d_2$ and $h_3$ components are an e.p.s.p. and i.p.s.p. evoked through the climbing fibre system.

In the majority (thirty-one) of the forty-four cells, stimulation of the peripheral nerves produced these mixed mossy fibre (MF)- and climbing fibre (CF)-evoked responses as shown in Text-fig. 5. The response pattern of these cells agrees with that of Deiters neurones studied under chloralose anaesthesia (Bruggencate, Sonnhof, Teichmann & Weller, 1971). In these cells there was always a coherence between the MF- and CF-evoked responses. In Text-fig. 5 QUAD (*D*, *E*), PDP (*J*, *K*), SUR (*L*), SP (*M*, *N*)

and PC (*O*, *P*) stimulation produced strong CF-evoked responses, while MF-evoked responses were effectively produced from these nerves as well as from SR (*A*), DR (*B*, *C*) and PBST (*F*). Thus the effective nerves for the CF-evoked response were among those eliciting MF-evoked responses. However, in a small fraction of cells (seven) MF-evoked responses appeared without association of the CF-evoked responses ($d_2$ and $h_3$ components).

No systematic difference was found between the MF-evoked responses in Deiters neurones with and without associated CF-evoked responses, regarding the most effective nerves or the shapes of the response spectrum. The most effective nerves were QUAD in 44 % of the thirty-eight cells, SUR in 16 %, SR in 11 %, PDP in 8 %, PC in 8 %, DR in 8 % and SP in 5 %. The

TABLE 2. Latency of p.s.p.s in Deiters neurones evoked by stimulation of peripheral nerves in decerebrate preparations. 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

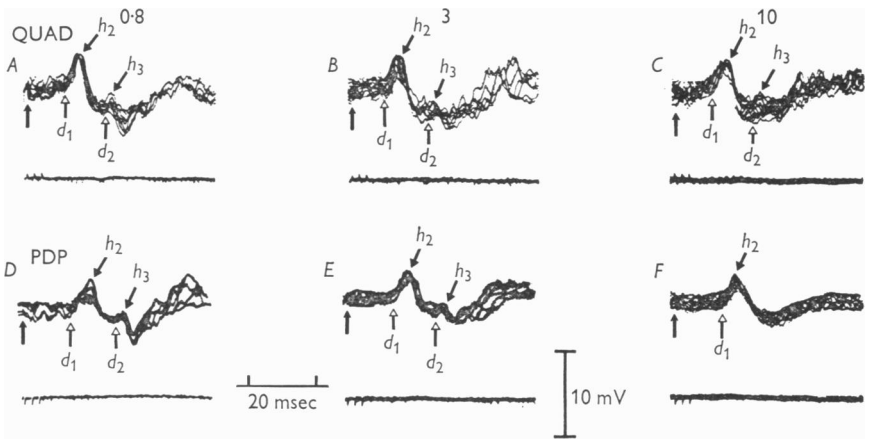
| Nerve | $d_1$ (msec) | $d_2$ (msec) | $h_1$ (msec) | $h_2$ (msec) | $h_3$ (msec) |
|---|---|---|---|---|---|
| Forelimb | 5·7 ± 0·9 (7) | 15·3 ± 1·7 (8) | 7·3 ± 0·7 (3) | 9·8 ± 1·2 (8) | 19·7 ± 2·0 (8) |
| Hind limb | 7·5 ± 1·4 (30) | 18·0 ± 2·0 (25) | 9·0 ± 0·8 (20) | 13·4 ± 1·8 (30) | 21·3 ± 1·8 (25) |

response spectrum usually spread broadly over several nerves and usually involved the nerves in both the fore- and hind limbs. This is in marked contrast to the differential effects of the fore- and hind limb nerves upon the CF-evoked responses of FL and HL Deiters neurones (Allen *et al.* 1972) where, for example, in an HL neurone the e.p.s.p.–i.p.s.p. sequence was only evoked from hind limb nerves while forelimb nerves elicited only an e.p.s.p. As was found with CF-evoked responses, there was no rule in the order of the effectiveness in the peripheral nerves, and there was much variability from one cell to another.

In another small fraction of cells (six of forty-four) stimulation of the peripheral nerves produced only the CF-evoked responses as under pentobarbitone anaesthesia. In three of the six Deiters neurones stimulation of the forelimb nerves produced a non-dominant response consisting of an e.p.s.p. while stimulation of some of the hind limb nerves evoked a dominant response composed of an e.p.s.p. and subsequent i.p.s.p.; i.e. the response pattern of the HL cells. The remaining three cells showed the pattern of the FL cells.

*Frequency effect*

The differential effects of stimulus frequency which were revealed between the CF-evoked responses under pentobarbitone anaesthesia (Fig. 8, Allen *et al.* 1972) and the MF-evoked responses in the decerebrate preparation with severance of the inferior olivary decussation (Text-fig. 3) were more demonstrably shown in Text-fig. 7 on a Deiters neurone which had mixed MF- and CF-evoked responses. QUAD stimulation at 0·8/sec produced both the MF- ($d_1$ and $h_2$) and CF- ($d_2$ and $h_3$) evoked responses




Text-fig. 7. Effects of stimulus frequency. Upper traces, the intracellular potentials. Lower traces, the extracellular controls. *A–C*, the p.s.p.s evoked by QUAD stimulation at various frequencies. The frequency is indicated above each column in cycles per second. *D–F*, similar to *A–C* but from PDP. The nerves were stimulated by a train of three pulses (6–8*T*). Conventions for denoting e.p.s.p.s ($d_1$ and $d_2$) and i.p.s.p.s ($h_2$ and $h_3$) are the same as in Text-fig. 1. The voltage scale of 10 mV and the time scale of 20 msec apply to all traces.

(Text-fig. 7*A*). At 3/sec the CF-evoked response was reduced (*B*), and at 10/sec it was reduced further (*C*), while the MF-evoked response remained unchanged. Similar differential effects upon the MF- and CF-evoked responses were exemplified in Text-fig. 7*D–F* for PDP evoked response. Again an increase of the stimulus frequency from 0·8/sec (*D*) to 3/sec (*E*) reduced the amplitude of the CF-evoked response, and a further increase to 10/sec (*F*) completely suppressed the CF-evoked response. These differential effects were found in all of the five Deiters neurones studied, with the cut off frequency for the CF-evoked responses ranging from 5 to 20/sec.

## DISCUSSION

### *Transection of inferior olivary decussation*

Destruction of the inferior olive or transection of the inferior olivary decussation has been performed by several workers (cf. Szentágothai & Rajkovits, 1959; Batini & Pumain, 1968; O'Leary, Dunsker, Smith, Inukai & O'Leary, 1970). Subsequently there have been conflicting conclusions about the origin of the climbing fibres to the cerebellum. Some authors insist that at least half of the climbing fibres originate from structures other than the inferior olive (Batini & Pumain, 1968; O'Leary *et al.* 1970), while others maintain that the main source of the climbing fibres is the inferior olive (Szentágothai & Rajkovits, 1959). In the present experiment care was taken to transect the inferior olivary decussation over the whole extent of the inferior olive (about 7 mm). Consequently the CF responses evoked in Deiters neurones from the peripheral nerves were completely abolished. This indicates that the climbing fibre afferents, at least those impinging on Deiters neurones, arise almost exclusively from the contralateral inferior olive.

### *Origin of $h_1$ component*

The experiments on decerebrate cats with intact inferior olive as well as with transected inferior olivary decussation revealed an i.p.s.p. ($h_1$) which was evoked too early to be attributed to the MF system. The onset of the i.p.s.p. was about 1·5 msec later than that of the e.p.s.p. which was attributed to excitation through the MF system ($d_1$). Assuming minimum time lags of 0·5 msec (Eccles, 1964) for synaptic transmission between mossy fibres and granule cells and between parallel fibres and Purkyně cells, and impulse conduction times of 0·3 msec from the region of Deiters nucleus to granule cells, 0·2 msec from granule cells to Purkyně cells and 0·7 msec from Purkyně cells to Deiters nucleus (Eccles, Ito & Szentágothai, 1967), the total delay for the i.p.s.p. produced through the above pathway should be more than 2·2 msec later than the e.p.s.p. which is produced through excitatory collaterals of the mossy fibres. The observed delay of 1·5 msec indicates that the i.p.s.p. is produced through some other pathway with a shorter neuronal chain. In this regard, monosynaptic inhibition of Deiters neurones by giant cells of the reticular formation in the medulla has been reported by Ito, Udo & Mano (1970). These cells are excited monosynaptically by the spinoreticular afferents which have a slightly slower conduction velocity than the spinocerebellar afferents (Udo & Mano, 1970). Therefore inhibition mediated through the giant cells of the reticular formation could be responsible for the $h_1$ inhibition.

*Spectrum of mossy evoked responses*

One striking feature of MF-evoked responses is their relatively broad spectrum spreading over many nerves. Only a few of the fifty-four cells studied had a narrow response spectrum confined to a few nerves, while the majority had a broad response spectrum sometimes involving the nerves in the hind limb as well as the forelimb. This finding is contrary to the view of a highly organized way of information transfer in the mossy fibre system as suggested from its anatomical structure (Eccles *et al.* 1967). Because of a large amount of divergence and convergence in the transmission line of the MF system, it has been considered that the MF system carries detailed topographical information to Purkyně cells (Marr, 1969). In contrast the present investigation revealed that Purkyně cells relay no topographically circumscribed information from the mossy fibre to Deiters neurones. The MF system was found to transmit even broader topographical information to Deiters neurones than the CF system does. However, this might be true only for experiments using electrical stimulation of peripheral nerves which initiates impulses in the peripheral sensory nerves in a highly synchronous manner. This may induce a massive synchronous impingement of MF impulses upon granule cells and impair the discriminative capability of the mossy fibre-granule cell–Purkyně cell transmission (Marr, 1969). Further investigation using natural stimulation is required for solution of this problem.

Another feature of the MF-evoked responses to be considered is the finding of some cooperation of the mossy fibre system with the climbing fibre system. In thirty-one of forty-four Deiters cells studied in the decerebrate animal, stimulation of peripheral nerves produced mixed MF- and CF-evoked responses. As shown in Text-fig. 5 above, there was invariably a high tendency for coherence of both responses. Furthermore, there appeared to be some correlation between the amplitudes of both responses, although it was difficult to confirm the correlation in a quantitative manner because of the complex nature of the potentials.

It should be pointed out, however, that there was a minority of Deiters neurones which received only MF- or CF-evoked responses. Therefore the cooperation between the MF and CF systems is not necessarily an essential feature of the cerebellar action upon the Deiters neurones.

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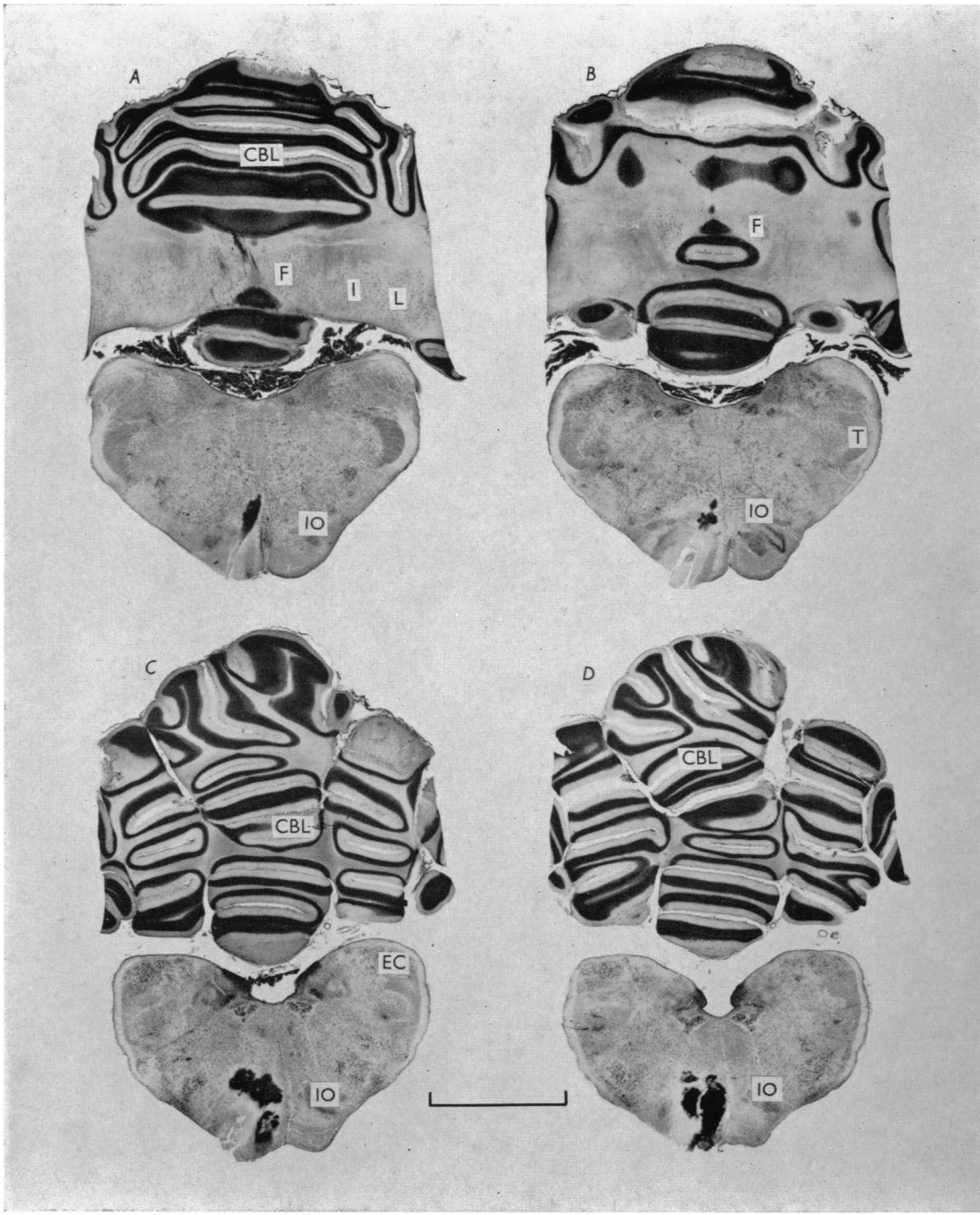
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## EXPLANATION OF PLATE

Histological sections showing transection of the inferior olivary decussation. Cresyl violet stain. *A–D*, frontal sections through the inferior olive taken cranio-caudally at an interval of about 1·3 mm. The horizontal bar between *C* and *D* represents a calibration of 5 mm. CBL, cerebellum. F, fastigial nucleus. I, interpositus nucleus. L, lateral nucleus. EC, external cuneate nucleus. T, spinal trigeminal tract. IO, inferior olive.