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SENSORY INTEGRATION IN THE SPINO-OLIVOCEREBELLAR PATHWAYS OF THE ANAESTHETIZED CAT

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SUMMARY

1. The responses evoked by peripheral nerve stimulation in the c_1 and c_3 zones of the cerebellar cortex have been examined in barbiturate-anaesthetized cats. The responses evoked via the spino-olivocerebellar pathways (SOCPs), which terminate in the cortex as climbing fibres, were recorded as positive multiunit field potentials from the cerebellar surface of lobule V.

2. Low-strength conditioning stimulation of the superficial radial, ulnar or median nerve frequently modified the climbing fibre-mediated responses evoked by a subsequent test stimulus to one of the other nerves. In most cases this modification involved a depression of the evoked response. The depression was not dependent on the conditioning stimulus evoking climbing fibre-mediated responses in the cortex.

3. The depression of the evoked responses increased as the conditioning stimulus intensity was raised within the range of 1·1–1·5 × threshold (1·1–1·5*T*).

4. Topical application of bicuculline to the surface of the dorsal column nuclei reduced the depression evoked by conditioning stimulation and it is therefore concluded that GABAergic inhibition in the cuneate nucleus contributes to the depression.

5. The inhibition is discussed in relation to its possible contribution to movement-related regulation of the excitability of SOCPs which occurs during locomotion in awake cats.

INTRODUCTION

In awake but passive cats, the cells of the inferior olive are readily caused to discharge by peripheral tactile stimulation (Gellman, Gibson & Houk, 1985) and therefore generate complex spikes in the Purkinje cells of the cerebellar cortex (Armstrong, Edgley & Lidierth, 1988) which they excite through their climbing fibre terminals. However, self-generated tactile inputs that arise during an animal's own movements fail to evoke olivary discharges (Gellman *et al.* 1985) or complex spikes in Purkinje cells (Andersson & Armstrong, 1987; Armstrong *et al.* 1988). Instead, a gating mechanism may operate during movement and suppress the effect of such self-

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generated inputs on the spino-olivocerebellar pathways (SOCPs) which transmit the peripheral inputs to the cerebellum, although inputs arising from unexpected movement perturbations may still be transmitted (Gellman *et al.* 1985; Andersson & Armstrong, 1987). It has recently been shown that the excitability of the SOCPs varies during stepping in awake cats in a manner that is consistent with the operation of such a gating mechanism (Apps, Lidierth & Armstrong, 1990; Lidierth & Apps, 1990).

Several sources of inhibitory synaptic input to the SOCPs may contribute to such gating. Thus, motor cortical (Leicht, Rowe & Schmidt, 1973), rubral (Weiss, McCurdy, Houk & Gibson, 1985), cerebellar (Andersson, Garwicz & Hesslow, 1988), peripheral (Armstrong & Harvey 1966; Leicht *et al.* 1973; Andersson, 1984) and even visceral (Newman & Paul, 1966) stimuli may modify the response evoked through the SOCPs by a subsequent peripheral stimulus. The present study re-examines the effects of peripheral stimuli on the responses evoked in the c_1 and c_3 zones of the cerebellar paravermal cortex in lobule V of the anterior lobe of anaesthetized cats. The olivary neurones which supply climbing fibres to these zones are activated by flexor reflex afferents through the dorsal funiculus SOCP (Oscarrson, 1969) and by distal cutaneous afferents through the dorsolateral funiculus SOCP (Larson, Miller & Oscarrson, 1969*a*). The SOCPs innervating lobule V are particularly sensitive to inputs from the ipsilateral forelimb and climbing fibre-mediated responses are evoked in the cerebellar cortex at latencies of 9–16 ms via the dorsal funiculus pathway and 15–22 ms via the dorsolateral funiculus pathway (Larson *et al.* 1969*a*; Oscarsson, 1969) in response to high-strength stimulation of forelimb nerves.

The purpose of the present study was to identify an inhibitory mechanism which could contribute to the changes in excitability of the SOCPs seen during locomotion (Lidierth & Apps, 1990). Leicht *et al.* (1973) have recorded the complex spikes of single Purkinje cells and demonstrated that tactile stimulation can depress the probability of evoking a complex spike in response to a subsequent tactile stimulus. This depression occurred in 40% of Purkinje cells in the vermal and paravermal cortex of lobule V when both stimuli were delivered in close spatial proximity in the periphery. As peripheral inputs arising during locomotion are movement related, this peripheral modification of the excitability of the SOCPs could contribute to the excitability changes seen during stepping. However, in the study of Leicht *et al.* (1973) the initial tactile stimuli were likely to have evoked an olivary discharge and it is possible that the depression reported by these authors was due to recurrent inhibition in the inferior olive which is known to be powerful (Armstrong, Eccles, Harvey & Matthews, 1968; Armstrong & Harvey, 1968). Such recurrent inhibition is unlikely to explain the step-related variation in excitability because the olivary discharge during locomotion is not step-related (Armstrong *et al.* 1988) and cannot therefore generate a step-related recurrent feedback.

In the study described here the effects of peripheral stimuli on the climbing fibre-mediated responses evoked by a subsequent peripheral stimulus were re-examined using a protocol that should have minimized the contribution of recurrent inhibition in the inferior olive. Low-strength electrical stimulation of forepaw nerves was used to condition the responses evoked by a subsequent forepaw nerve stimulus. A depression of the excitability of the SOCPs was demonstrated in response to such

low-strength stimuli and was shown not to be dependent on recurrent inhibition in the inferior olive. For the dorsal funiculus SOCP, which relays in the dorsal column nuclei (Oscarsson, 1969), a bicuculline-sensitive inhibition in the cuneate nucleus was shown to contribute to the depression. A preliminary account of this work has been published (Lidierth, 1990).

METHODS

Preparation

Experiments were performed in cats of either sex which were anaesthetized with sodium pentobarbitone (40 mg/kg I.P., supplemented as required I.V.). The femoral vein and artery were cannulated for drug administration and monitoring of blood pressure respectively and the animals were tracheotomized. Body temperature was maintained by a thermostatically controlled heating blanket. The superficial radial, ulnar and median nerves in each forearm were dissected free, cut and mounted for electrical stimulation in bipolar cuff-type electrodes which were sutured in place. A cervical laminectomy exposed the spinal cord. Most of the posterior lobe of the cerebellum was exposed through a craniotomy. The occipital pole of the cerebral cortex was also exposed and, in most preparations, unilaterally ablated by suction to expose the bony tentorium which was removed to facilitate recording from the cerebellar anterior lobe. The spinal cord and cerebellum were bathed in warmed paraffin oil to prevent drying.

Some animals were paralysed with gallamine triethiodide (2–3 mg/kg I.V., Flaxedil) and artificially respired. Adequacy of anaesthesia was ensured by monitoring blood pressure and pupillary diameter. Paralysis was periodically allowed to wear off to allow the corneal reflex to be tested.

Recording

Recordings of potentials evoked by nerve stimuli were made from the cerebellar surface using Ag–AgCl ball electrodes. In some experiments micropipettes with their tips broken to *ca* 50 μm diameter and filled with 1 % agar in 3 M-NaCl were used but these offered no advantages over the ball electrodes. Recordings were amplified, filtered at 10 Hz–3 kHz (sometimes DC–3 kHz with the agar electrodes), digitized and stored on an IBM AT computer. In some experiments tungsten-in-glass microelectrodes were used to record the discharges of single Purkinje cells. In these experiments recording stability was improved by covering the cerebellum in a 4–7 % agar solution in isotonic saline which was allowed to set.

Electrical stimuli of 0·05–0·1 ms duration were delivered to the peripheral nerves at strengths expressed in multiples of the threshold required to evoke a just-detectable cord dorsum potential (T). The cord dorsum potentials were digitized and stored together with the cerebellar potentials.

Experimental protocol

Stimuli of adequate strength to evoke cerebellar surface potentials were delivered to peripheral nerves at a rate of approximately 1 Hz. Stimuli were alternately conditioned by a preceding low strength stimulus to another nerve or left unconditioned. Responses evoked by the unconditioned stimuli were computer averaged to provide a 'control' average. Conditioned responses were separately pooled to provide a 'test' average. The interval between conditioning and test stimuli was varied to allow construction of a time course for the effect of the conditioning stimulus on the test response. At each interstimulus interval, thirty control and thirty test stimuli were delivered over a period of 1 min in order to construct the averaged records and the amplitude of the test response was expressed as a percentage of the amplitude of the control response.

A similar protocol was used when studying the evoked responses of single Purkinje cells but in these cases fifty control and fifty test stimuli were delivered at each interstimulus interval. The single-cell responses were filtered (5 kHz high-cut) and digitized. A time–voltage threshold function was implemented in software to allow the detection of complex spikes in the Purkinje cell discharge. Post-stimulus time histograms and rasters for the complex spikes were separately constructed for the control and test stimuli.

RESULTS

Electrical stimulation of nerves innervating the forepaws was used to evoke climbing fibre-mediated responses in lobule V of the cerebellar anterior lobe. The form, latency and distribution of these responses when recorded from the pial surface were consistent with those in earlier studies (Larson *et al.* 1969*a*, *b*; Oscarsson, 1969) and will therefore be described here only briefly.

Three major parasagittal zones may be distinguished by electrophysiological methods in the paravermal cortex of lobule V in the cerebellar anterior lobe. Figure 1 shows typical potentials recorded with a Ag–AgCl ball electrode placed on the pial surface over each of these zones which are labelled c_1, c_2 and c_3 following Oscarsson (1980). The recording positions are indicated on the surface drawing to the left of the figure. Stimuli were applied to the superficial radial, ulnar and median nerves in both forearms and to the sciatic nerve in the ipsilateral hindlimb at a strength of $20T$ for the lowest threshold afferents in each nerve (see Methods). In the c_1 and c_3 zones stimulation only of the nerves in the ipsilateral forelimb was effective in generating climbing fibre-evoked potentials. In the c_2 zone, however, nerves in both forelimbs and in the ipsilateral hindlimb were effective (nerves in the contralateral hindlimb were not stimulated). The latency of the potentials evoked by ipsilateral forelimb nerve stimulation were typically ~ 5 ms shorter in the c_1 and c_3 zones than those evoked in the c_2 zone by stimuli of similar strength (Fig. 1). Responses in the c_2 zone were often labile, possibly because of the use of barbiturate anaesthesia (cf. Larson *et al.* 1969*b*); thus supplementary doses of anaesthetic frequently reduced or abolished the potentials evoked in the c_2 zone and for this reason they were studied less extensively than those in the c_1 and c_3 zones and will not be described further.

Although stimuli of $20T$ were used in Fig. 1, stimuli of much lower strength were generally as effective. Maximal response amplitude in the c_1 zone was seen with stimuli of $\sim 2T$.

Effects of nerve stimulation on the excitability of the SOCPs

The upper trace of Fig. 2 (marked Con) shows the averaged potential recorded from the surface of the c_1 zone of lobule V in response to a $1{\cdot}5T$ stimulus to the ipsilateral superficial radial nerve. The stimulus evoked an initial mossy fibre-mediated response (marked MF) followed by a sharp surface-positive potential (marked CF) due to climbing fibre-mediated activation of the Purkinje cells underlying the electrode. In the remaining traces of Fig. 2, similar averages are shown for trials in which the superficial radial nerve stimulus (▼) was preceded by a conditioning stimulus (▽) of $1{\cdot}1T$ delivered to the ipsilateral ulnar nerve over a range of interstimulus intervals as indicated to the right of each trace. With intervals of 10–30 ms, the climbing fibre-mediated response was clearly depressed compared to the control response (Con). With increasing intervals in the range of 45–65 ms the test response amplitude showed a progressive recovery towards its control level. Note that the conditioning stimulus did not evoke climbing fibre-mediated responses.

Similar results from a single recording site in another experiment are shown quantitatively in Fig. 3. At this c_3 zone recording site the ipsilateral ulnar, superficial radial and median nerves were each stimulated at $1{\cdot}1T$ in order to condition the response evoked by a subsequent test stimulus at $2{\cdot}0T$ to each of the other two

nerves. The amplitude of the resulting test responses are plotted relative to their control amplitudes for a range of intervals between conditioning and test stimuli. Example averages are shown above each plot with the control response shown to the left and the test response, conditioned by a stimulus (△) delivered 50 ms before the test stimulus (▼), shown to the right.

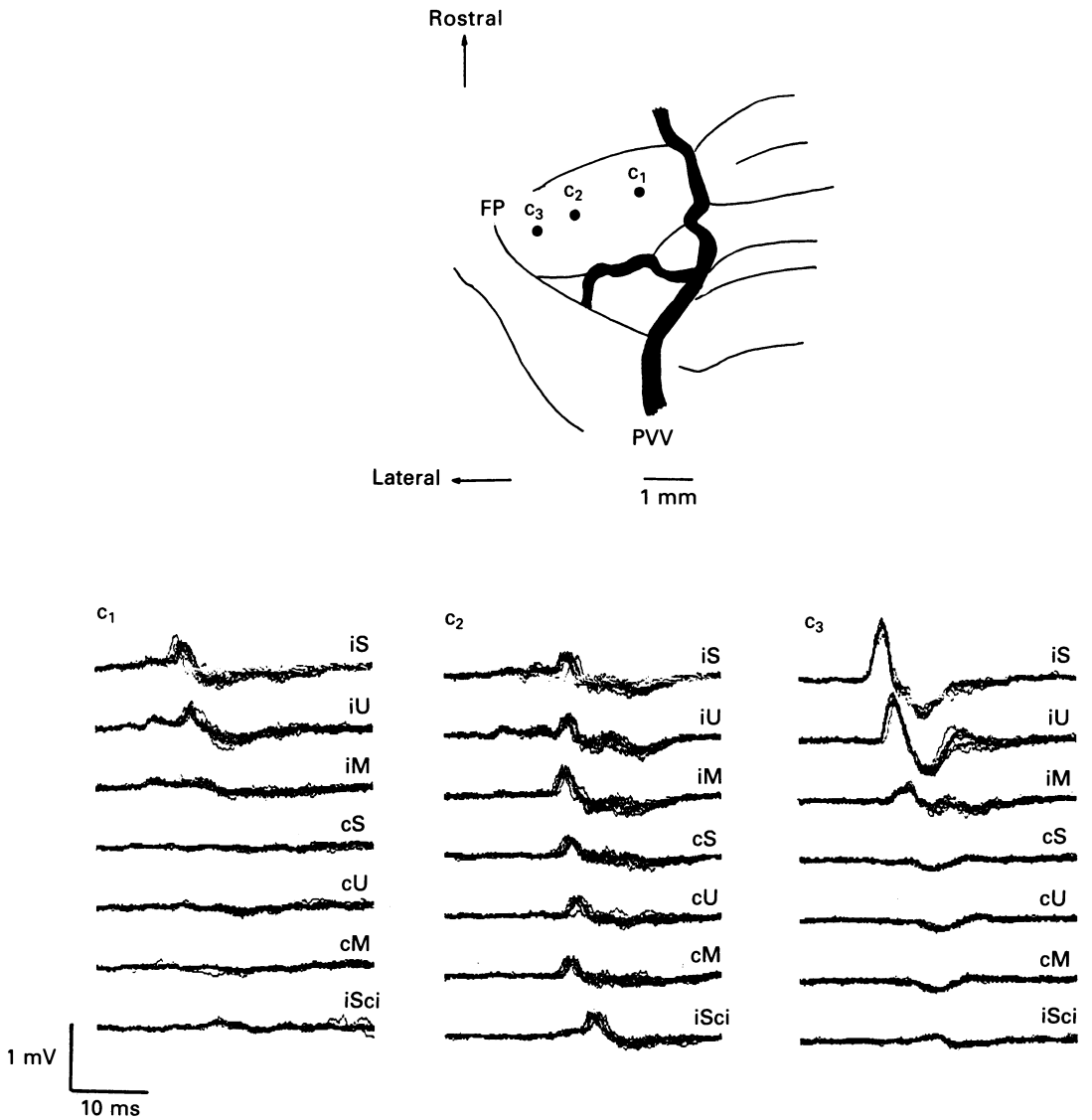

Fig. 1. A surface view of the caudal folia of the cerebellar anterior lobe (lobule V) is shown at the top. The fissura prima (FP) and paravermal vein (PVV) are marked. The records below show the climbing fibre-mediated surface potentials evoked by peripheral nerve stimulation in each of the c_1, c_2 and c_3 zones as indicated. The ipsilateral (i) and contralateral (c) superficial radial (S), ulnar (U) and median (M) nerves were each stimulated at 20*T*. The sciatic nerve in the ipsilateral hindlimb was also stimulated (iSci).

Figures 3*A* and *B* show the effect of a conditioning stimulus applied to the ulnar nerve on the response evoked by a subsequent superficial radial and median nerve stimulus respectively. Note that in these cases the conditioning stimulus itself evokes a small excitatory response. With increasing intervals between conditioning and test

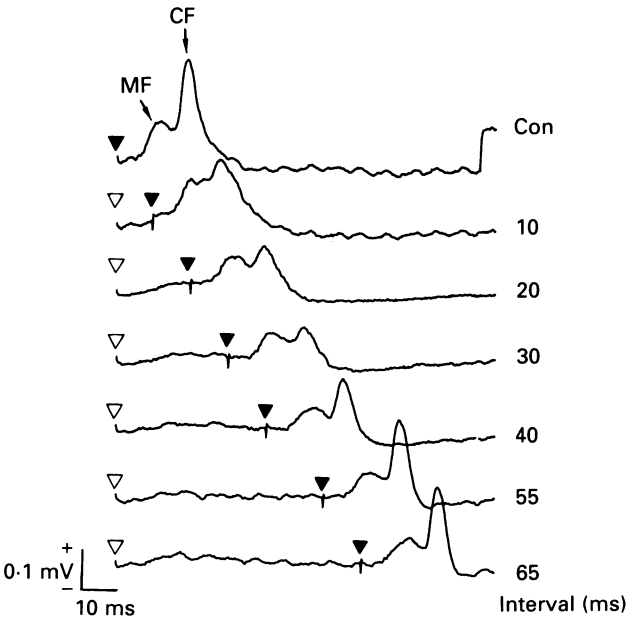

Fig. 2. The effect of conditioning stimuli applied to the ipsilateral ulnar nerve at a strength of 1·1*T* on the response in the c_1 zone of lobule V evoked by stimulation of the ipsilateral superficial radial nerve at 1·5*T*. Each record shows the response recorded at the cerebellar surface and is an average of thirty trials. The top record shows the control response (Con) for which the superficial radial nerve stimulus was delivered alone. In the remaining traces the conditioning stimulus to the ulnar nerve (▽) was applied at the trace onset and the test stimulus to the superficial radial nerve (▼) after a delay which varied between 10 and 65 ms as shown to the right of each trace. The superficial radial nerve stimulus evoked an early response (MF) due to the action of fast mossy fibre pathways. Superimposed on this was a sharp positive potential (CF) due to activation of the Purkinje cells underlying the electrode by their climbing fibres.

stimuli the size of the test response progressively declined to reach a minimum at intervals of 20–40 ms. With test stimuli delivered to the superficial radial nerve (Fig. 3*A*) the evoked responses were reduced to approximately half of their control amplitude. The median nerve-evoked response (Fig. 3*B*) declined more rapidly with increasing interstimulus intervals and at intervals of 20–40 ms the test responses were abolished. When a conditioning stimulus was applied instead to the superficial radial nerve, the response evoked by a subsequent stimulus to the ulnar (Fig. 3*E*) or median nerve (Fig. 3*F*) was also depressed. In these cases, the size of the depression was similar irrespective of which nerve was presented with the test stimulus. The time course of the depression resembled that seen with conditioning stimuli to the ulnar nerve (Fig. 3*A* and *B*).

Figure 3*C* and *D* shows the effect of a conditioning stimulus applied to the median

nerve on the test response evoked by a subsequent superficial radial or ulnar nerve test stimulus. In both cases conditioning stimulation produced a decrease in the amplitude of the test response but the effect was small. Raising the intensity of the conditioning stimulus to the median nerve increases its effectiveness in reducing the

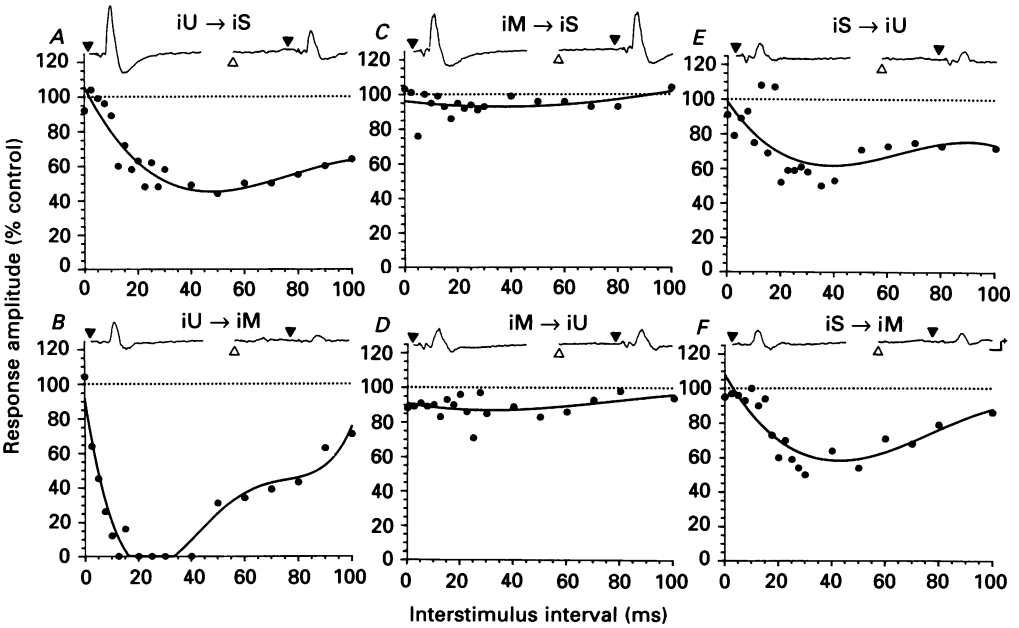

Fig. 3. The effects of conditioning stimuli of 1·1T applied to each of the ulnar (A and B), median (C and D) and superficial radial (E and F) nerves in the ipsilateral forelimb on the test responses evoked by stimulation of each of the other two nerves at 2·0T at a single recording site on the c_3 zone of lobule V. Panels A–E show the effects of varying the interval between conditioning and test stimuli. The amplitudes of the resulting test responses are plotted relative to their control amplitudes. Curves were fitted by a least-squares polynomial regression. The example records above each plot show the control (left) and test (right) responses with an interstimulus interval of 50 ms. The positions of conditioning (△) and test (▼) stimuli are marked. Voltage and time calibrations in F are 0·1 mV and 10 ms respectively and apply throughout A–F.

response evoked by a test stimulus to the superficial radial nerve (see below) but even when increased to 10·0T the conditioning stimulus produced a depression of the test response to only 35% of its control level when delivered 20 ms before the test stimulus. The action of higher-strength conditioning stimuli on the response evoked by an ulnar nerve stimulus was not examined. In other experiments the effects of low-strength conditioning stimuli to the median nerve were greater (see below).

The effects of conditioning shown in Fig. 3 are typical of those seen frequently throughout this study but other patterns of interaction between nerve stimuli were also observed. Figure 4 shows a selection of curves which illustrate the range of effects seen. In Fig. 4A, a conditioning stimulus applied to the ulnar neve at 1·1T potentiated the test response when applied up to 40 ms before a test stimulus of 2·0T to the superficial radial nerve. When the interstimulus interval was increased beyond

50 ms there was a small decrease in the amplitude of the test response. In Fig. 4*B*, which shows data from another experiment but using the same stimulus protocol, a similar early potentiation is observed but is interrupted by a depression at intervals of 10–30 ms and, as in Fig. 4*A*, is followed by a depression with intervals greater than

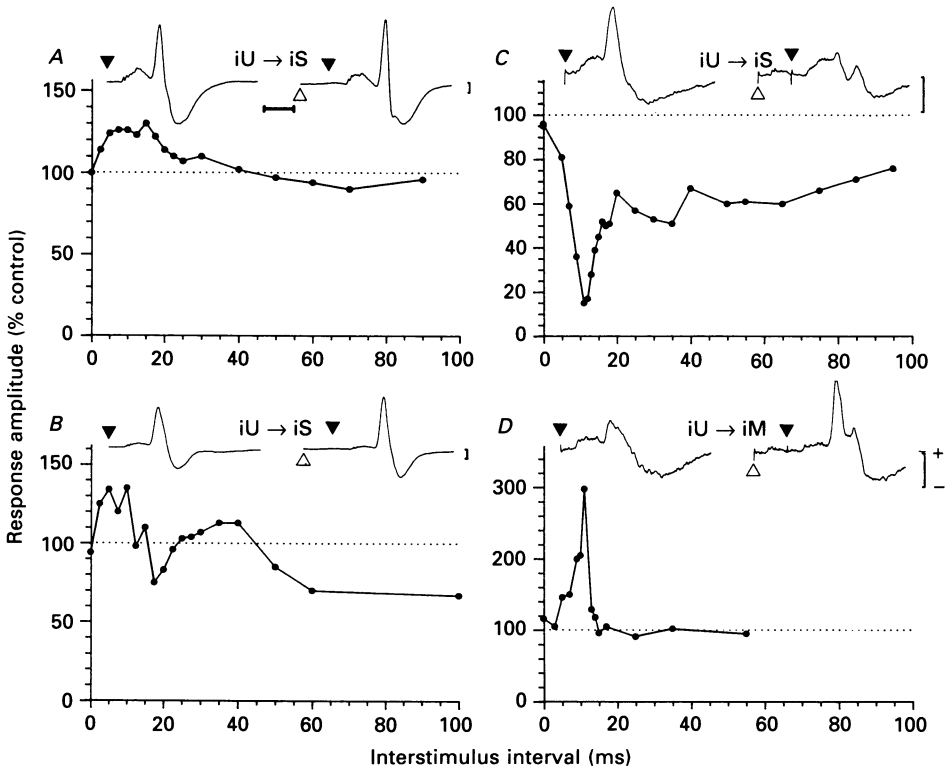

Fig. 4. Illustrating the range of effects that could be obtained by weak conditioning stimuli. *A–C* each show the effect of conditioning stimuli to the ulnar nerve (at 1·1*T* in *A* and *B* and 1·05*T* in *C*) on the response evoked by a subsequent superficial radial nerve stimulus (at 2·0*T* in *A* and *B* and 2·3*T* in *C*). *D* shows data from the same site as in *C* and with the same conditioning stimulus but with a test stimulus applied to the median nerve (at 2·0*T*). *A–C* are from different experiments. *A* and *B* are from the c_1 zone and *C* and *D* from the c_3 zone. The example traces above each record show the control (left) and test (right) responses with 10 ms (*A* and *B*) and 11 ms (*C* and *D*) separation between conditioning (△) and test (▼) stimuli. Time scale bar in *A* is 10 ms and applies throughout *A–D*. Voltage calibration bar is 0·2 mV in *A* and 0·1 mV in *B–D*.

50 ms. This complex pattern of potentiation and depression may result from the addition of a facilitatory effect similar to that in Fig. 4*A* with a biphasic depression similar to that in Fig. 4*C*, which again is from another experiment using the same stimulus protocol. In this example there was a potent depression of the test response with intervals of 5–15 ms between conditioning and test stimuli. This was followed by a partial recovery and a weaker but long-lasting depression as the interval was increased to 20–100 ms and beyond. Figure 4*D* illustrates an unusual result in which an ulnar nerve conditioning stimulus powerfully potentiated the response to a

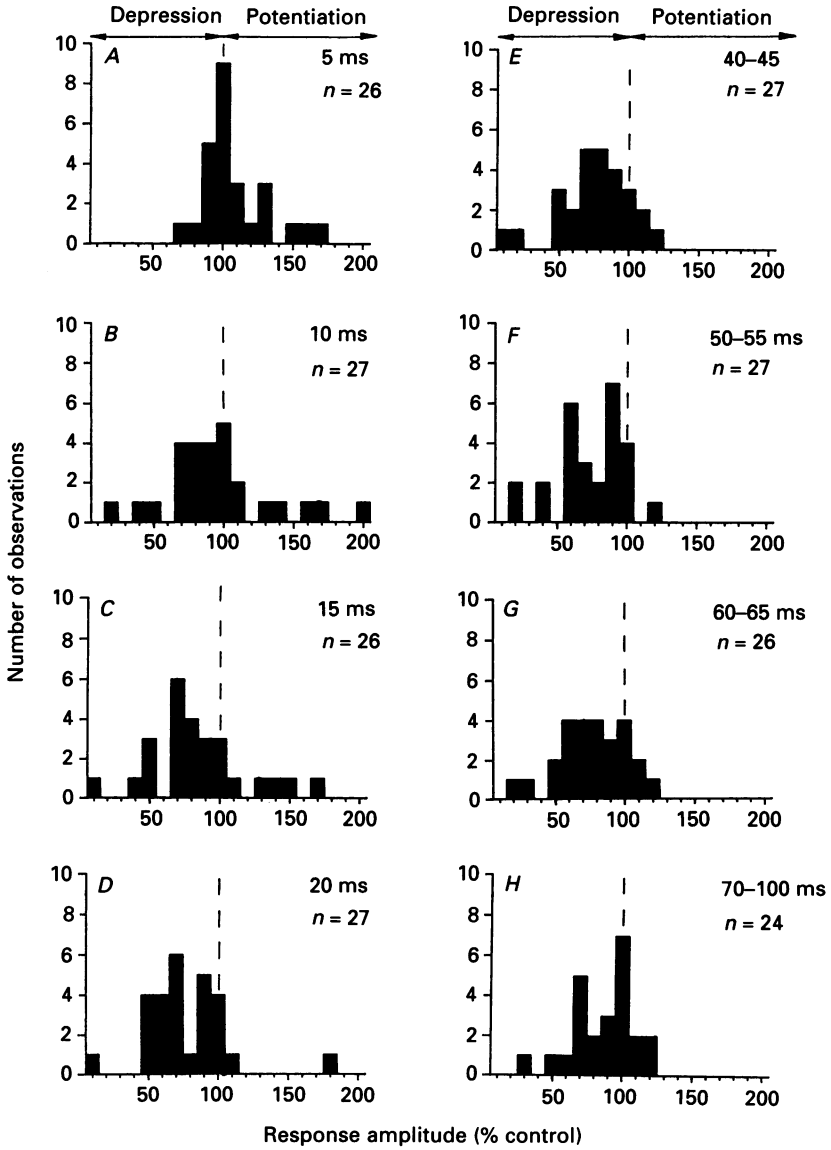

Fig. 5. The frequency distribution of the effects of low-strength conditioning stimuli on a subsequent test stimulus. *A–H* each show a grouped frequency distribution histogram illustrating the effect of a 1·05–1·1*T* conditioning stimulus to the ulnar nerve on the test response evoked by a 1·5–2·5*T* stimulus to the superficial radial nerve at twenty-seven c_1 and c_3 zone recording sites in twenty-two preparations. The interval between conditioning and test stimuli was as indicated at the top right of each graph except that there was one sample at 6, 11 and 16 ms in *A*, *B* and *C* respectively. The number of samples contributing to each histogram varied as indicated (*n*). The vertical dashed line marks the control response amplitude (100 %). Entries to the left of this represent depressed and those to the right potentiated test responses.

subsequent median nerve stimulus. The result is particularly unusual because the data were from the same site as in Fig. 4*C* where the ulnar nerve stimulus generated a depression.

Frequency of the effects of conditioning

The frequency and potency with which low-strength conditioning stimuli either potentiated or depressed the response to a subsequent test stimulus was assessed by grouping data from trials in which nerves were stimulated in particular combinations. Each of the histograms in Fig. 5 is a frequency distribution histogram showing the effect of conditioning a test response evoked by a superficial radial nerve stimulus with a preceding stimulus to the ulnar nerve at a fixed interstimulus interval or within a narrow range of intervals as shown at the top right of each graph. The data are from twenty-seven c_1 or c_3 zone recording sites in twenty-two preparations, in five of which recordings were made from both the c_1 and c_3 zones. At two sites recordings were made with a microelectrode placed in the molecular layer of the cerebellar cortex; for the remainder recordings were made from the cortical surface. Note that not all recording sites were tested at each interval and the sample size (n) therefore varies from 24–27 as shown. Conditioning stimuli were in the range of 1·05–1·1*T* and test stimuli in the range of 1·5–2·5*T*.

With short interstimulus intervals, as in Fig. 5*A*, the effects of a conditioning stimulus were often negligible. At 35% (9/26) of the recording sites included in Fig. 5*A*, the test response amplitude was within 5% of its control level while at 38% (10/26) of sites the test response was potentiated to more than 5% above its control amplitude. The test response was reduced by more than 5% at only 27% (7/26) of sites. However, as the interstimulus interval was increased towards 20 ms, the number of sites at which the conditioning stimulus had no effect decreased and there was an increase in the proportion of sites at which the test response was depressed. Thus with an interstimulus interval of 20 ms (Fig. 5*D*), potentiated test responses were observed at only 7% (2/27) of recording sites while the responses were depressed at 78% (21/27). As the interstimulus interval was increased further, the effect of conditioning stimulation declined (Fig. 5*E–H*). The time course of the effect was generally not followed in detail to its end, but with intervals in the range of 70–100 ms (Fig. 5*H*) the proportion of sites at which the test responses were depressed had fallen to 54% (13/24).

Similar data were obtained when other combinations of nerves were stimulated at intensities in the same ranges as above. Thus when an ulnar nerve stimulus was used to condition the responses evoked by a median nerve stimulus with an interstimulus interval of 20 ms, the responses were depressed by more than 5% of their control amplitude at each of ten tested recording sites. When the conditioning stimulus was delivered to the median nerve, responses evoked by stimulation of the superficial radial nerve were depressed at 50% (4/8) of sites, potentiated at 25% (2/8) and unchanged (by more than 5%) at 25% (2/8). Those evoked by stimulation of the ulnar nerve were depressed at four of six sites and unchanged at two. Conditioning stimuli applied to the superficial radial nerve resulted in depression of an ulnar or median nerve-evoked response at five of six tested sites in each case while the remainder showed no change.

It should be noted, however, that the interpretation of these data is complicated

by the dependence of the effects of conditioning not only on the interval between stimuli but also on the intensity of both conditioning and test stimuli as described below.

Effects of varying the conditioning stimulus intensity

In general, the strength of the depression evoked by a conditioning stimulus rose rapidly as its intensity was increased. Figure 6*A* and *B* shows data from a c_3 zone

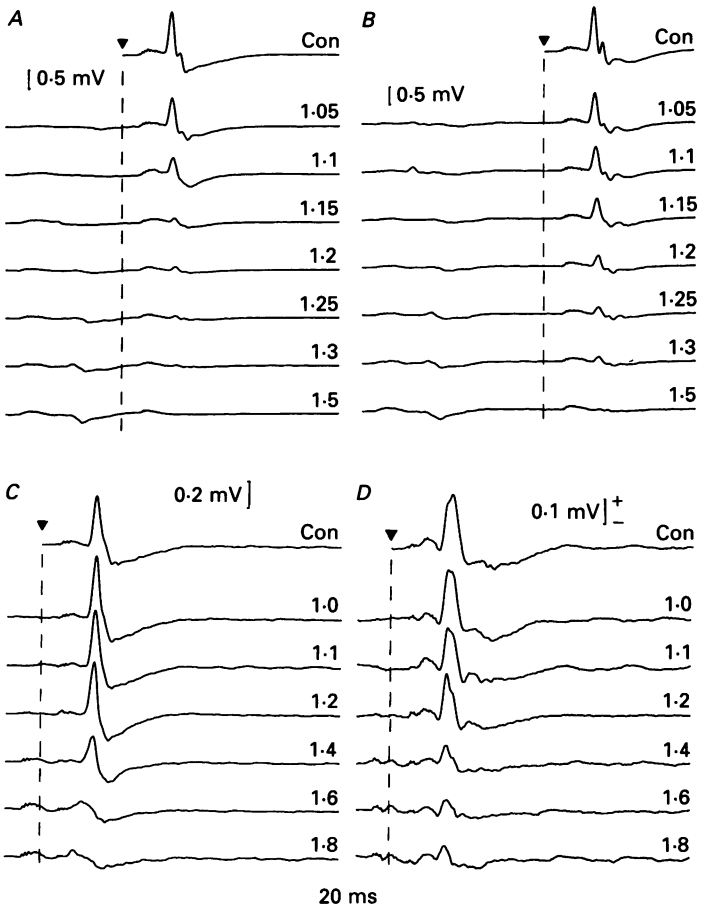

Fig. 6. Effects of varying the intensity of the conditioning stimulus. *A* and *B* show averaged traces (of thirty trials each) from a single c_3 zone recording site at which the interval between conditioning and test stimuli was 35 ms in *A* and 55 ms in *B*. Each record shows the response to a 2·0*T* stimulus to the ipsilateral superficial radial nerve (▼). In the upper traces the test stimuli were not conditioned (Con). In the remaining traces the test stimulus was conditioned by a stimulus to the ipsilateral ulnar nerve delivered at the trace onset and at the intensity indicated to the right of each trace. *C* and *D* show similar data from another experiment in which simultaneous recordings were made from a c_3 zone and a c_1 zone recording site respectively, again with a superficial radial nerve test stimulus (▼) of 2·0*T* and conditioning stimulation of the ulnar nerve with an interstimulus interval of 10 ms.

recording site at which a stimulus of 2·0T to the ipsilateral superficial radial nerve (▼) evoked a clear climbing fibre-mediated potential on the cerebellar surface (Con). The remaining traces of Fig. 6A and B show the effect of a conditioning stimulus delivered to the ipsilateral ulnar nerve at the trace onset. This preceded the superficial radial nerve stimulus by 35 ms in A and 55 ms in B. The intensity of the conditioning stimulus to the ulnar nerve was varied between 1·05 and 1·5T as indicated to the right of each trace. In Fig. 6A, the test response is reduced by a preceding conditioning stimulus of 1·05T and is reduced further as the intensity of the conditioning stimulus is raised to 1·15T. The test response was absent with conditioning stimuli of 1·5T. In Fig. 6B, the interval between conditioning and test stimuli has been increased to 55 ms. When compared with Fig. 6A, the depression of the test response rises less rapidly as the conditioning stimulus intensity is raised but the test response is still abolished with conditioning stimuli of 1·5T.

Figure 6C and D shows further examples from another experiment in which surface recordings were made from both the c_3 zone (C) and the c_1 zone (D). The recordings were made simultaneously. In Fig. 6C, conditioning stimuli of up to 1·2T delivered to the ulnar nerve facilitated the response evoked by a superficial radial nerve stimulus (▼) delivered 10 ms later. The full time course of this effect for a conditioning stimulus of 1·1T is shown in Fig. 4C. When the intensity of the conditioning stimulus was increased above 1·2T (Fig. 6C) the facilitation of the test response was replaced by a potent depression and the test response was absent with conditioning stimuli of 1·6T or more. Thus the effect of the conditioning stimulus was dependent both on the interval between conditioning and test stimuli (Fig. 4C) and on the intensity of the conditioning stimulus (Fig. 6C). For the c_1 zone site in this experiment (Fig. 6D), the conditioning stimulus evoked only a depression which increased rapidly in strength as the conditioning stimulus intensity was increased but saturated at intensities greater than 1·6T. Even with conditioning stimuli of 3·0T (not illustrated) the test response amplitude was reduced to only about 30% of its control level.

Effects of varying the test stimulus intensity

The effect of low-strength conditioning stimuli was reduced when the test stimulus intensity was increased. In general, a substantial reduction of the effect of a conditioning stimulus occurred as the intensity of the test stimulus was increased within the range of 1·5–2·0T. The use of test stimuli within this range may have reduced the extent to which depression was observed in the present study.

Role of GABAergic inhibition in the cuneate nucleus

For reasons that will be given in the Discussion, it is unlikely that the low-strength conditioning stimuli used here could have evoked inhibition in the inferior olive of sufficient power to explain the depression described above. Experiments were therefore done to see if inhibition acting at the level of the cuneate nucleus could contribute to the depression. Topical applications of filter paper soaked with bicuculline methiodide (40 mM in 100 mM-NaCl) were made to the dorsal column nuclei immediately caudal to the cerebellum in order to antagonize GABAergic inhibitory transmission (Curtis, Duggan, Felix & Johnston, 1971) in the cuneate

nucleus. The efficacy of the bicuculline application was monitored by recording the P wave evoked on the surface of the cuneate nucleus (not shown) by a forelimb nerve stimulus (Andersson, Eccles, Schmidt & Yokota, 1964).

Figure 7*A* shows a control response (left) evoked in the cerebellar c_1 zone by a stimulus of 2·0*T* to the ipsilateral median nerve (▲). Both mossy (MF) and climbing

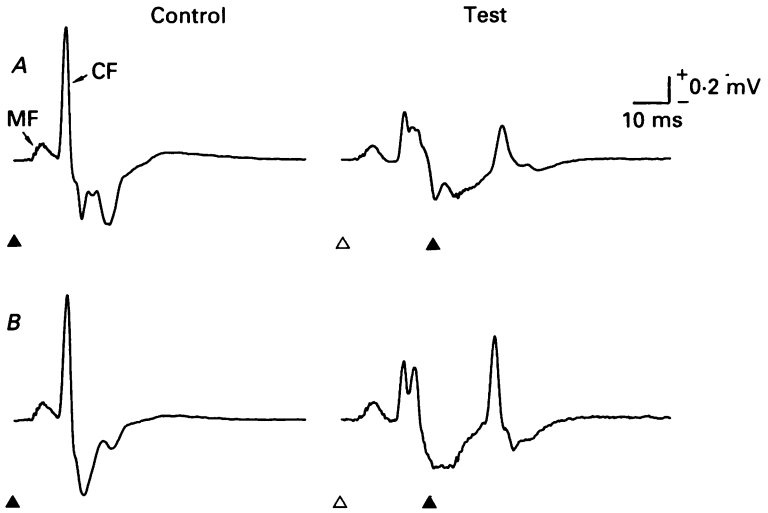

Fig. 7. The effects of a topical application of bicuculline methiodode to the surface of the cuneate nucleus on the responses evoked in the cerebellar cortical c_1 zone. Conditioning stimuli were applied to the ulnar nerve at 1·5*T* and test stimuli to median nerve at 2·0*T*. The interval between conditioning and test stimuli was 25 ms. Each record is an average of 300 individual trials taken over a period of 10 min. *A* shows the responses evoked before the bicuculline application. The control stimulus (▲) evoked an initial mossy fibre response (MF) which was followed by a sharp climbing fibre-evoked response (CF). When preceded by a conditioning stimulus the evoked test response was of only 35% of the control response amplitude. Note that the conditioning stimulus (△) also evoked a climbing fibre-mediated response. *B* shows similar averages collected after a 20 min application of bicuculline to the dorsal column nuclei. Note that the responses evoked by the control and conditioning stimuli were largely unchanged but the test response amplitude was approximately doubled.

(CF) fibre-mediated potentials were evoked. When the response was conditioned by a 1·5*T* stimulus delivered 25 ms earlier to the ulnar nerve (△, Fig. 7*A*, right), the response to the median nerve stimulus was reduced to approximately 35% of its control amplitude. Control and test stimuli were delivered alternately as above. Note that in this example the conditioning stimulus was sufficiently powerful to evoke a climbing fibre-mediated potential. Figure 7*B* shows the corresponding potentials recorded after a 20 min application of bicuculline to the dorsal column nuclei. The control response was unchanged by the application; the response evoked by the conditioning stimulus was slightly increased but this was not significant (see below). In contrast, the test response was substantially increased to approximately twice its pre-bicuculline amplitude (Fig. 7*A*).

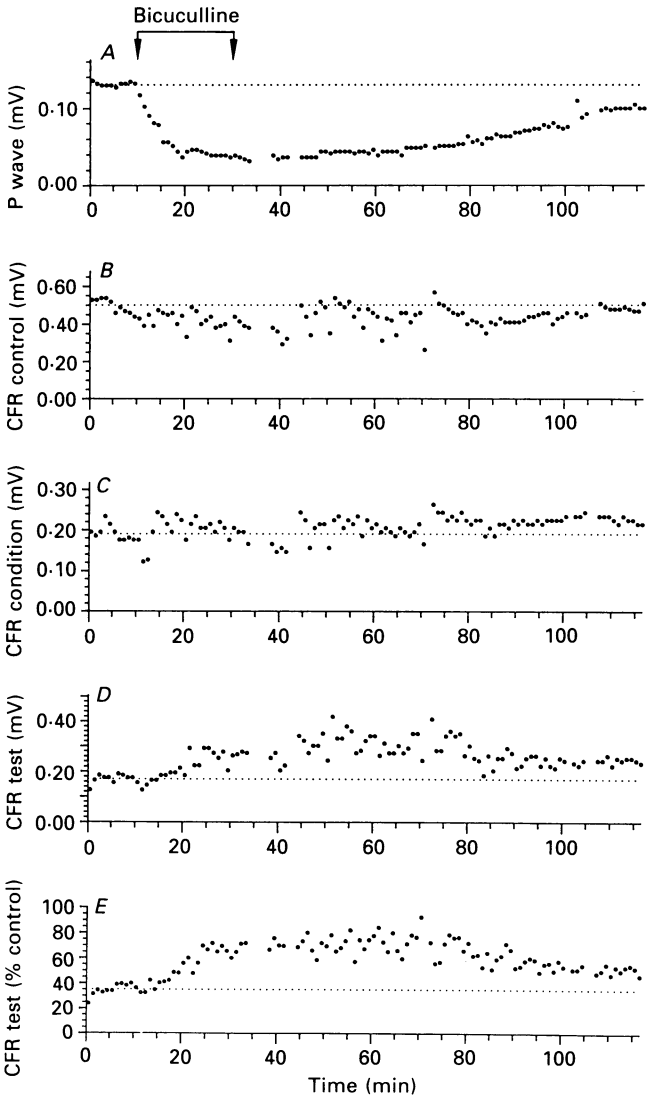

Fig. 8. Illustrates the time course of the effect of bicuculline as shown in Fig. 7. Each point is based on a measurement from averages of thirty trials collected over 1 min. Arrows in *A* mark the period of application of bicuculline to surface of the cuneate nucleus. *A* shows the amplitude of the P wave recorded from the surface of the cuneate nucleus and evoked by the control stimulus. *B–D* show the amplitudes of the cerebellar evoked potentials (CFR). *B* shows the amplitude of the control response, *C* the response evoked by the conditioning stimulus and *D* the test response. *E* shows the amplitude of the test response relative to the amplitude of the corresponding control response.

Figure 8 shows the time course of the effect of the bicuculline application for the trial illustrated in Fig. 7. Each point shows a measurement from an averaged record of thirty individual trials. Figure 8*A* shows the amplitude of the P wave evoked by the control stimulus and recorded with a Ag–AgCl ball electrode placed caudal to the filter paper on the surface of the cuneate nucleus. The bicuculline was applied 10 min

after the onset of data collection (arrows in Fig. 8*A*). There was a clear reduction in the size of the P wave after the application of bicuculline and a slow recovery, which was not followed to completion, after its removal. Figure 8*B* and *C* shows that application of bicuculline did not affect the size of the climbing fibre-mediated

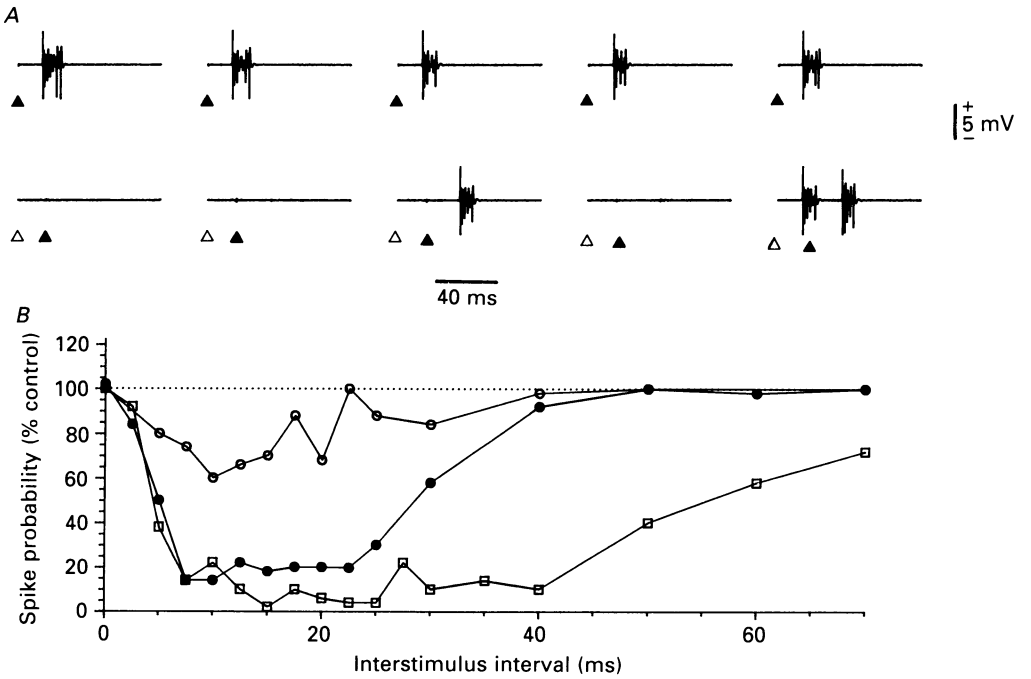

Fig. 9. Suppression of an evoked complex spike in a single Purkinje cell in the c_1 zone of lobule V. The top row of *A* shows five traces in which a complex spike was evoked by a 1·5*T* stimulus (▲) to the ipsilateral ulnar nerve. In the lower row, the same stimulus was delivered 20 ms into the trace, and a 1·35*T* conditioning stimulus to the superficial radial nerve (△) was applied at the trace onset. *B* shows the probability of the test stimulus evoking a complex spike for a range of intervals between conditioning and test stimuli and for three conditioning stimulus intensities: 1·3*T* (●), 1·35*T* (○) and 1·4*T* (□). For each data point fifty control and fifty test stimuli were delivered in alternation and the probability of evoking a complex spike within 30 ms of the test stimulus is shown relative to the probability over the same period in the control trials.

response evoked by the control or conditioning stimuli respectively, although a small reduction in the amplitude of the control response occurred over the 5 min preceding the bicuculline application. However, the amplitude of the test response was increased in the presence of bicuculline as shown in Fig. 8*D* and *E*. Figure 8*D* shows the absolute amplitude of the test response while in *E* the amplitude is shown relative to the size of the corresponding control average. The effect of the conditioning stimulus was progressively reduced as the bicuculline took effect in the cuneate nucleus, as judged from the P wave amplitude (Fig. 8*A*), and was restored as the effect of the bicuculline declined. The effect on the cerebellar test response lagged that on the cuneate P wave by approximately 5 min.

The effects of bicuculline were assessed in a further two trials in the same preparation with at least 3 h separating applications. In these additional trials the conditioning stimulus to the ulnar nerve was reduced to 1·1*T* and it was not effective in evoking climbing fibre-mediated potentials. In the first additional trial a c_1 zone test response grew from 65% of control amplitude before bicuculline to 78% afterwards and in the second from 73 to 90%; in this trial a c_3 zone response was also recorded and grew from 40% before to 58% after bicuculline application.

In another experiment a 1·1*T* stimulus to the ulnar nerve was used to condition the response evoked by a 2·0*T* stimulus to the superficial radial nerve delivered 40 ms later. In the c_1 zone the test response was reduced to approximately 60% of control before and 80% after bicuculline application. In the c_3 zone the corresponding values were 40 and 70%. However, when the experiment was repeated using a test stimulus of 2·3*T* to the median nerve, the bicuculline was without effect up to 17 min after application.

Thus, in all but one trial, application of bicuculline to the surface of the dorsal column nuclei approximately halved the observed depression.

Effects on the evoked complex spikes of single Purkinje cells

The effects of low-strength conditioning stimuli on the responses evoked in single Purkinje cells were examined in a small sample from five experiments. As expected given the results described above, it was found that such conditioning stimuli could potently depress the complex spikes evoked by a subsequent test stimulus to another nerve. Figure 9*A* illustrates example records from the cell which was studied in most detail and in which a 1·35*T* conditioning stimulus to the superficial radial nerve depressed the response to a 1·5*T* ulnar nerve test stimulus. The upper row of traces shows the complex spikes evoked by five successive control stimuli (▲); in the lower row, a conditioning stimulus (△) was applied at the trace onset. The complex spike evoked by the test stimulus is suppressed in three of the first four traces. The conditioning stimulus was effective in evoking complex spikes in approximately 4% of the trials one of which is shown in the far right-hand trace of the figure. Note that despite the response to the conditioning stimulus the cell still responded to the test stimulus.

The time course of the depression is shown in Fig. 9*B* (●) together with those for conditioning stimuli of 1·3*T* (○) and 1·4*T* (□). There is a clear similarity between these graphs and those showing the time course of the effects of conditioning stimuli on cerebellar surface potentials (Figs 3 and 4).

DISCUSSION

Origin of the inhibition

The present study has shown that electrical activation of afferents supplying the forepaw may powerfully depress the transmission of inputs from the forepaw through the SOCPs to the c_1 and c_3 zones of the cerebellar cortex. Similar results were reported by Leicht *et al.* (1973) who examined the responses of both vermal and paravermal Purkinje cells to tactile stimulation. These authors reported that tactile stimulation outside the excitatory receptive field of individual Purkinje cells

inhibited the climbing fibre-mediated response (i.e. the complex spike) evoked by a subsequent tactile stimulus within the excitatory receptive field. Although it was necessary that tactile conditioning stimuli were applied close to the receptive field for such depression to be evoked, they also reported that electrical stimulation of the superficial radial nerve could depress the responses evoked by a subsequent tactile stimulus to the distal hindlimb. Andersson (1984) examined this effect more fully in the lateral vermis (b zone; Oscarsson, 1980) of the anterior lobe. High-strength (20*T*) electrical stimulation of peripheral nerves was shown to depress the climbing fibre-mediated response evoked by a subsequent stimulus to another peripheral nerve. The responses evoked by direct stimulation of the spino-olivary fibres in the spinal ventral funiculus were also depressed which showed that inhibition at olivary level contributed to the depression. A similar, but weak, inhibition between forelimb and hindlimb inputs also occurred in the c_1 and c_3 zones. Andersson (1984) explained these results as due to mutual inhibition between cerebellar microzones evoked through recurrent inhibitory interneurones to the inferior olive. A similar, but unidirectional, inhibition by contralateral eye inputs of the responses evoked from the ipsilateral eye in the olivary dorsal cap had been reported by Takeda & Maekawa (1980).

However, it is unlikely that such mutual inhibition significantly contributes to the depression of the responses in the c_1 and c_3 zones reported here because the present results were obtained with low-strength conditioning stimulation. Thus in all the trials for which data are presented in Fig. 5 the conditioning stimuli to the ulnar nerve were of 1·1*T* or less. Even when recording within the optimal region for observing a response such weak stimuli evoke responses which, if present at all, are small, often insecure and frequently abolished by supplementary doses of anaesthetic. It is therefore unlikely that the often powerful inhibition reported here was due to recurrent (or mutual) inhibition generated by such weak olivary activation. In addition, it has previously been shown that peripherally evoked inhibition of spontaneous neuronal discharge is uncommon in the rostral pole of the dorsal accessory olive, which supplies the c_1 and c_3 zones (Groenewegen, Voogd & Freedman, 1979); thus Gellman, Houk & Gibson (1983) found that although 150 of 156 tested cells responded to natural peripheral stimuli only 4 of the 150 cells (2·7 %) were inhibited. Oscarrson (1969) found a correspondingly low proportion (2/58, 3·6 %) of Purkinje cells in the c_1 zone which exhibited inhibition of their spontaneous complex spike discharge following natural stimulation. Thus peripheral tactile stimuli appear to be effective in depressing the responses evoked by subsequent peripheral stimuli (Leicht *et al.* 1973) but not in depressing the spontaneous discharges of olivary cells projecting to the c_1 and c_3 zones. This suggests that the depression does not occur at the olivary level in the SOCPs supplying these zones.

It is therefore likely that the inhibition of evoked responses observed in the present study occurred substantially at a pre-olivary level and this is supported by the observation that topical application of bicuculline to the cuneate nucleus greatly reduced the inhibition (Figs 7 and 8). None the less, it was not abolished which clearly leaves scope for some inhibition to occur also at olivary level. However, it should be noted that the P wave recorded from the cuneate surface was never totally suppressed either and, consequently, it might be reasonably supposed that the

concentration of bicuculline was not sufficient to abolish GABAergic inhibitory transmission throughout the cuneate nucleus. In addition, for the example illustrated in Figs 7 and 8 both test and conditioning stimuli were strong enough to evoke a climbing fibre-mediated response; an occlusive interaction between conditioning and test stimuli therefore may account for part or all of the residual depression. In this respect it may be noteworthy that during the peak of the effect of bicuculline the sum of the amplitudes of responses evoked by conditioning (Fig. 8*C*) and test (Fig. 8*D*) stimuli was approximately equal to the amplitude of the control response (Fig. 8*B*).

Functional significance of the inhibition

Tactile stimulation may powerfully excite the SOCPs to the c_1 and c_3 zones in awake but passive cats but when tactile inputs arise as a consequence of the animal's own movements they are ineffective (Gellman *et al.* 1985; Armstrong *et al.* 1988), possibly because of a gating mechanism which suppresses the transmission of self-generated tactile inputs (Gellman *et al.* 1985; Andersson & Armstrong, 1987). Recently, the excitability to peripheral stimuli of the SOCPs supplying the c_1 and c_2 zones has been studied in awake, walking cats (Apps *et al.* 1990; Lidierth & Apps, 1990). In the forepaw-receiving area of the c_1 zone, the cortical potentials evoked through the SOCPs by stimulation of the ipsilateral superficial radial nerve were shown to vary during the step cycle and to be depressed at the times of ipsilateral forepaw contact and lift. This depression could clearly contribute to the failure of sensory inputs arising naturally at paw contact and lift to evoke complex spikes in c_1 zone Purkinje cells (Armstrong *et al.* 1988).

The present experiments have revealed an inhibitory mechanism which may account for this decreased excitability of the SOCPs. It has been shown that electrical activation of afferents supplying the forepaw may powerfully depress the transmission of forepaw inputs to the cerebellum through the SOCPs. Activation of the inhibitory pathway may also occur in response to the natural sensory inputs arising at paw contact and paw lift during locomotion. If the consequent inhibition of the SOCPs was stronger than the excitation also arising from the paw, transmission of sensory inputs through the SOCPs might be prevented. Clearly, such inhibition may account for the absence of complex spikes in Purkinje cells at paw contact and paw lift during normal locomotion (Armstrong *et al.* 1988) and for the depressed SOCP excitability in response to peripheral nerve stimuli observed at those times in the step cycle (Lidierth & Apps, 1990).

The afferent fibres responsible for evoking this inhibition have low thresholds for electrical activation and may therefore be assumed to have high conduction velocities. The latency for evoking inhibition will be increased by the need for an inhibitory interneurone in the pathway but this need not imply that the latency will be too long to suppress the transmission of inputs from the forepaw arising at paw contact and paw lift during locomotion. Post-synaptic inhibitory potentials are evoked in cuneothalamic neurones with latencies only 0·85–1·6 ms longer than those of EPSPs following electrical stimulation of peripheral nerves (Andersson, Eccles, Oshima & Schmidt, 1964). Paw contact and paw lift during locomotion are likely to give rise to a much more temporally dispersed afferent input than an electrical stimulus and if the latency differential in cuneo-olivary neurones is similar it may not

be significant. Furthermore, the course through the cuneate nucleus of the dorsal funiculus SOCP may be disynaptic (Ekerot & Larson, 1979).

Although self-generated tactile inputs are not transmitted to the cerebellum through the SOCPs, unexpected tactile inputs arising from movement perturbations are transmitted (Gellman *et al.* 1985; Andersson & Armstrong, 1987). It has been suggested that the SOCPs act as error detectors which compare, and respond when there is a mismatch between, the actual and the expected peripheral feedback during a movement. Such a mismatch might arise in response to a movement perturbation as above, or in response to an error in the execution of a movement. Previously, it has been supposed that the comparison is made between the descending supraspinal locomotor drive and the afferent feedback but a comparison could also be made between two sets of afferent feedback. Thus, during locomotion on a moving belt (cf. Armstrong *et al.* 1988) a substantial part of the plantar surface of the forepaw makes contact with the belt during stance and this may include both an excitatory and an inhibitory receptive field for any cuneo-olivary neurone. The simultaneous activation of both receptive fields may lead to cancellation of the excitatory input as described above. However, during natural locomotion on an uneven surface a situation could clearly arise where incomplete paw contact with the walking surface might activate only the excitatory field and so generate a response in the cuneo-olivary neurone, i.e. generate an error signal. Supraspinal control of the SOCPs may then shape the convergence and relative strengths of excitation and inhibition in such a way that they are appropriate to a particular movement. This is clearly speculative but as the comparison would be based only on peripheral feedback and occur at the earliest stage in the dorsal funiculus SOCP (i.e. the cuneate nucleus) it could constitute both a fast and an economical mechanism for detecting movement errors.

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