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LOCOMOTION-RELATED VARIATIONS IN EXCITABILITY OF SPINO-OLIVOCEREBELLAR PATHS TO CAT CEREBELLAR CORTICAL c_2 ZONE

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SUMMARY

1. Cutaneous nerve stimulation was used to study the excitability of the spino-olivocerebellar pathways (SOCPs) to the c_2 zone of the paravermal cerebellar cortex in the cat. Non-noxious single-shock stimulation of the right and left superficial radial (SR) nerves via implanted cuff electrodes was used to evoke field potentials in the cerebellar cortex via the SOCPs.

2. The evoked potentials were recorded extracellularly either in lobule V of the anterior lobe (three cats) or within the paramedian lobule of the posterior lobe (one cat) with glass-coated tungsten microelectrodes. Measurement of the amplitudes of the responses was used to monitor transmission in the SOCPs in cats at rest and during walking.

3. A total of eleven c_2 recording sites were investigated in detail. At seven of these sites, responses were recorded both during locomotion and at rest. For all seven sites responses during locomotion were smaller, more variable in amplitude and less securely evoked (average reduction 59%).

4. At five out of the eleven recording sites (45%) the mean amplitude of responses elicited during different tenths of the step cycle fluctuated sufficiently that the largest response was more than twice the smallest. In the majority of these cases (4/5) the responses were largest in either mid-stance or late swing. These fluctuations in response size occurred without parallel fluctuation in the amplitude of the peripheral nerve volley. At the remaining sites fluctuation of the cerebellar field size was less and in some cases practically absent.

5. At six recording sites it was possible to record the climbing fibre potentials evoked by stimulation of both the ipsilateral and contralateral superficial radial nerves. In all six cases the fluctuations in size of the response during locomotion occurred in phase, despite the fact that the two limbs move out of phase.

6. The probability that an individual stimulus would evoke any cerebellar response also varied between the different tenths of the step cycle and such variations occurred in parallel with the fluctuations in response size. This shows that the SOCP regulatory mechanism(s) must, at least in part, operate at a precerebellar level.

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INTRODUCTION

Previous reports from this laboratory have established that in awake, walking cats the complex spikes of cerebellar Purkinje cells are not discharged in relation to the course of the step cycle (Andersson & Armstrong, 1987; Armstrong, Edgley & Lidierth, 1988). Instead, the excitatory peripheral drive to the pathways which generate these complex spikes (the spino-olivocerebellar pathways) appears to be gated such that only peripheral events which are unexpected are successful in evoking complex spikes (Andersson & Armstrong, 1987; see also Gellman, Gibson & Houk, 1985). The present report is the first in a series which will examine the properties and mechanisms of such gating.

Complex spikes are generated by the action on Purkinje cells of climbing fibre afferents which arise in mammals from the inferior olivary nucleus. The olivocerebellar projection is topographically highly organized so that the cortex is divisible into several longitudinally (i.e. sagittally) oriented strips or zones which receive their climbing fibres from populations of neurones in different regions of the inferior olive (see Armstrong, 1974; Groenewegen & Voogd, 1977; Groenewegen, Voogd & Freedman, 1979; Brodal & Kawamura, 1980; Voogd & Bigaré, 1980; Trott & Armstrong, 1987*a*, *b* for evidence and reviews). Electrophysiological studies in the cat have shown that olivary neurones receive ascending inputs so that in the paravermal and vermal portions of the cortex the longitudinal zones are the termini for a number of spino-olivocerebellar pathways (SOCPs) transmitting information which includes inputs from low- and high-threshold mechanoreceptors in the skin and deeper tissues of the limbs. These SOCPs have been studied extensively in decerebrate and in anaesthetized preparations by recording the cerebellar responses they generate when peripheral nerves are electrically stimulated (e.g. Oscarsson, 1968; 1969; Larson, Miller & Oscarsson, 1969*a*, *b*; Armstrong, Harvey & Schild, 1973; Ekerot, Gustavsson, Oscarsson & Schouenborg, 1987). Such studies have shown that different SOCPs can be defined according to their location in the white matter of the spinal cord; they include, for example, lateral funiculus (LF)-, dorsal funiculus (DF)- and ventral funiculus (VF)-SOCPs. These paths show some convergence at the olivary level so that each cerebellar cortical zone receives input from a characteristic combination of SOCPs. Except for DF-SOCPs mediated in the cord via ascending collaterals of primary afferent fibres, all the paths involve synaptic relays at the segmental level. In addition, all the paths, except for some VF-SOCPs mediated via spinal neurones that project directly to the inferior olive, involve pre-olivary synaptic relays at the level of the brain stem or midbrain. As a result of these complexities, there is ample opportunity for descending control of all the SOCPs to be exercised at a pre-olivary level and because many olive cells which act as SOCP relays also receive descending inputs (e.g. Miller, Nezlina & Oscarsson, 1969*a*, *b*), substrates also exist for central regulation at the olivary level.

There is indeed some evidence that such regulation may occur. Thus, Andersson & Sjölund (1978) found that transmission in some VF-SOCPs is profoundly altered in chloralose-anaesthetized preparations by administration of clonidine and L-DOPA to change the excitability of interneuronal circuits in the spinal cord. Subsequently, it was shown that similar actions could be evoked by electrical

stimulation of descending tracts (Sjölund, 1978). In awake animals, other less direct evidence suggests that SOCP excitability may vary with behavioural context. Thus, for example, Gellman *et al.* (1985) found that some individual olive cells were readily discharged by tactile stimuli delivered to the passive animal but that similar stimuli generated by the animal's own active movements failed to evoke discharges. Similarly, Andersson & Armstrong (1987) recorded the complex spikes which were evoked in individual Purkinje cells by impulses in the climbing fibres and found during walking that such responses were randomly timed with respect to the step cycle unless the on-going movement was unexpectedly perturbed. Such observations are consistent with (though they do not prove) the notion that transmission in SOCPs is modulated or gated in such a manner that unexpected peripheral inputs are transmitted to the cerebellum whilst predictable inputs resulting from the animal's own motor activities are not.

In light of the above, it is clear that further studies of SOCP transmission in awake behaving animals are needed. Accordingly, this paper presents the results of a study in which the excitability of the SOCPs to one particular longitudinal cerebellar zone, the c_2 zone in the paravermal cortex, was studied in awake cats by recording extracellular field potentials generated in the zone by climbing fibre volleys evoked by electrically stimulating cutaneous nerves at non-noxious intensities. Field potentials were recorded in preference to complex spikes in single Purkinje cells because neighbouring olive cells are electronically coupled (Llinás, Baker & Sotelo, 1974; Llinás & Yarom, 1981*a*), raising the possibility that information may be conveyed to the cerebellum by groups of olive cells acting in concert (cf. Boylls, 1980; Lou & Bloedel, 1986; Bloedel & Lou, 1987). Responses were recorded whilst the animals rested quietly and also during steady walking on a moving belt and in the latter condition the possibility that transmission might vary during the step cycle was studied.

METHODS

Extracellular recordings were made from the cerebellar cortex in four awake, purpose-bred, male cats using glass-insulated tungsten microelectrodes introduced using a miniature micromanipulator affixed to a titanium cylinder chronically implanted over a small craniotomy. The dura mater remained intact and was covered by a protective overlay of medical grade silicone elastomer (Dow Corning 382 Silastic). In three animals the craniotomy allowed access to the paravermal part of lobule V (Larsell, 1953) of the right side of the cerebellar anterior lobe while in the fourth access was to the the rostral folia of the right paramedian lobule. Electromyographic (EMG) signals were simultaneously recorded from the lateral head of triceps brachii muscle in both forelimbs. The animals were trained to walk steadily on an exercise belt moving at a comfortable walking speed which varied slightly between animals but was in the range 0·4–0·5 m/s. Full details of the training and operative methods and the techniques used for microelectrode and EMG recording were given in previous papers (Armstrong & Rawson, 1979; Armstrong & Drew, 1984; Armstrong & Edgley, 1984; Edgley & Lidierth, 1988). No aversive methods were used in training and the animals were unrestrained and gave no signs of any stress or discomfort.

Nerve stimulation

Two pairs of fine multistranded, Teflon-insulated stainless-steel leads (diameter 300 μm; Cooner Wire Corp; A S5633) were implanted at a separation between pairs of at least 5 cm into the connective tissue sheath surrounding the superficial radial nerve in each forelimb. For each nerve the distal pair of leads was used during recording sessions to stimulate the nerve (in continuity)

with single rectangular pulses 0·05 ms in duration, delivered at a repetition rate of 1 per 1·5 s. Compound action potentials evoked in the nerve were recorded via the proximal pair of leads which were enclosed in a cuff of silicone elastomer to reduce pick-up of EMG signals originating in nearby muscles. Intensities of nerve stimulation were expressed as multiples of the threshold value (T) required to evoke a compound action potential detectable by inspection of superimposed traces displayed on a storage oscilloscope. Stimulus intensities used to activate the SOCPs to the c_2 zone ranged from 1·1 to 4 T for the ipsilateral nerve and 1·5 to 5 T for the contralateral nerve. Above 2 T, a weak flexion reflex was often evoked in the limb but stimulus intensity was always well below that needed to excite nociceptive afferents and no sign of stimulus aversion was ever evident; on the contrary, stimulation occasionally appeared to exert a mildly somnolent effect.

Recording techniques

Stimuli were delivered and the resultant cerebellar responses were recorded while the animals walked steadily and also, when possible, while they rested between bouts of walking. Recordings in the absence of movement could, however, not always be obtained because the animals were selected for confidence in the laboratory and often explored their environment.

All bioelectric signals were amplified, filtered and tape-recorded (Racal Store 7D instrumentation recorder; tape speed 60 in/s) for later off-line analysis. Electroneurograms, cerebellar evoked potentials and EMG signals were filtered at 10 Hz–10 kHz. The electroneurograms and evoked potentials were recorded on FM tape channels (tape band width DC to 20 kHz) and the EMGs on either FM or DR channels (DR bandwidth 300 Hz–300 kHz). A stimulus marker pulse was also recorded on a separate channel. Because the interstimulus interval (1·5 s) differed from the duration of the step cycle (typically *ca* 850 ms), stimuli delivered during walking exhibited considerable drift relative to the step cycle (see below).

Data analysis

Tape-recorded data were analysed using a PDP 11/34 minicomputer. The cerebellar signals were fed to the computer via an analog-to-digital converter and the electroneurograms were simultaneously sampled. Tapes were played back at 15 in/s so that computer sampling rate was effectively raised from 10 to 40 kHz. The cerebellar potentials were bandpass filtered at this stage (equivalent to 40 Hz–1 kHz at normal replay speed). Note that, as both signals were recorded on FM tape channels, the slower replay speed did not lead to distortion of the replayed signal. Digitized responses were displayed on an oscilloscope screen (Tektronix 711) and manually controlled cursors were used to measure the peak-to-peak amplitude of compound action potentials recorded from the nerves and also to estimate the amplitude of the corresponding cerebellar evoked potentials. For the latter, two measures were used, namely the area (mV ms) under the whole of the initial peak of the response attributable to climbing fibre input and the area under the first 2 ms of that peak (see Results and Fig. 6). The latency to onset of both types of response was also measured relative to onset of the nerve stimulus.

In the resting animal the amplitude measurements relating to batches of forty to fifty responses were averaged whilst during locomotion at least seventy consecutive responses recorded during steady uninterrupted walking were measured and again averaged. In addition, each step cycle was divided into ten equal time periods and responses to stimuli occurring during each of these epochs were averaged separately to test the possibility that response size might vary systematically during the step cycle. The period during which any particular stimulus was delivered was determined by measurement from a display of the EMG signals and the stimulus marker pulses generated on an ink-jet recorder (Mingograf EEG junior). To enable comparison between cases, onset of the first epoch was always taken as coinciding with the onset of the locomotor EMG burst in the lateral or long heads of the triceps brachii muscle of the right (i.e. ipsilateral) forelimb. The choice of period duration as one-tenth of the step cycle was arrived at empirically and dictated by the fact that the animals walked steadily only for limited periods so that it was necessary to compromise between a wish to divide the step into as many periods as possible and the need to ensure that enough stimuli occurred in each period to allow meaningful averaging of the responses. Given the drift which occurred between the stimuli and the step cycle (see above), division of the step into tenths generally ensured that at least seven stimuli (usually more) occurred within each period.

Among the recordings made during walking it was sometimes necessary to reject occasional responses because the record was contaminated by transient interference due, for example, to stray

contact of the recording leads with some part of the frame surrounding the exercise belt. However, such rejections occurred randomly with respect to the step cycle so they are most unlikely to have introduced any bias into the results.

Histology

At the end of the experiment each animal was deeply anaesthetized with barbiturate and perfused intracardially with isotonic saline followed by neutral buffered formalin. The positions of the nerve and EMG electrodes were checked post-mortem by dissection and the cerebellum was studied histologically to provide anatomical confirmation of the region from which microelectrode recordings had been made. Frozen sections 100 μm thick were cut in the sagittal plane, stained with Cresyl Violet and the locations of the electrode tracks were determined microscopically. Tracks in 'anterior lobe' cases were confined to lobule V except for a few which had crossed the fissura prima into the adjacent part of the lobule VI; in the paramedian lobule tracks were confined to the rostral folia of the lobule.

RESULTS

General characteristics of cerebellar field potentials resulting from nerve stimulation

Tracks were made with glass-coated tungsten microelectrodes into the cerebellar cortex in lobule V of the anterior lobe (three animals) or in the rostral part of the paramedian lobule (one animal) while single, non-noxious, electrical stimuli were delivered to one of the superficial radial nerves. At a total of eleven recording sites extracellular field potentials were detected that were attributable (see below) to activation of the c_2 cortical zone via its spino-olivocerebellar paths (SOCPs) and were sufficiently large relative to background noise to be suitable for measurement. Peak amplitude of the initial component (see below) of such responses was in the range 0·5–1·0 mV.

In Fig. 1*A* and *B* the left-hand records are examples of extracellular fields evoked at one cortical site by stimulation of the contralateral and the ipsilateral (SR) nerve respectively. The right-hand records represent the corresponding compound action potentials monitored from the nerves as described in Methods and recorded both to allow determination of stimulus intensity and to monitor possible variations that might occur in stimulus effectiveness during locomotion occurring, for example, as a result of relative movements between the nerve and its stimulating electrodes. (Note the different time scales for cerebellum and nerve recordings.) In Fig. 1*A* the cerebellar response to contralateral superficial radial (SR) nerve stimulation consists of a negative–positive diphasic wave (starred) with onset latency 23 ms that can be seen to fluctuate somewhat in amplitude between stimuli. Comparison with earlier studies (e.g. Armstrong & Harvey, 1968; Oscarsson, 1968; Eccles, Provini, Strata & Taborikova, 1968) indicates that such responses are typical of field potentials set up in the cortex by a volley in the climbing fibres that constitute the final stage of the SOCPs and further evidence supporting this identification is presented later. Figure 1*B* shows for comparison the pattern of response evoked at the same recording site from the ipsilateral SR nerve and in this case a sequence of two negative-positive diphasic potentials is evident. The second of these (starred; latency 22 ms) is similar in amplitude to the response in Fig. 1*A* and again fluctuated between trials. Other characteristics of this response (see below) again supported its identification as SOCP-mediated. It may be noted that an ability to evoke such responses by bilateral

stimuli is a characteristic of the c_2 zone that is not shared by the c_1 and c_3 zones which flank it medially and laterally respectively. These latter zones are termini for SOCPs which convey input from the ipsilateral forelimb only (see for example Armstrong *et al.* 1973; Oscarsson, 1980).

At different recording sites within the c_2 zone, the latency of SOCP-mediated responses ranged overall from 16 to 24 ms for ipsilateral responses ($n = 11$) and from 16 to 27 ms for contralateral responses ($n = 6$). The first (unstarred) response in Fig. 1*B* has a latency of 7 ms and is therefore too early to be mediated via any known

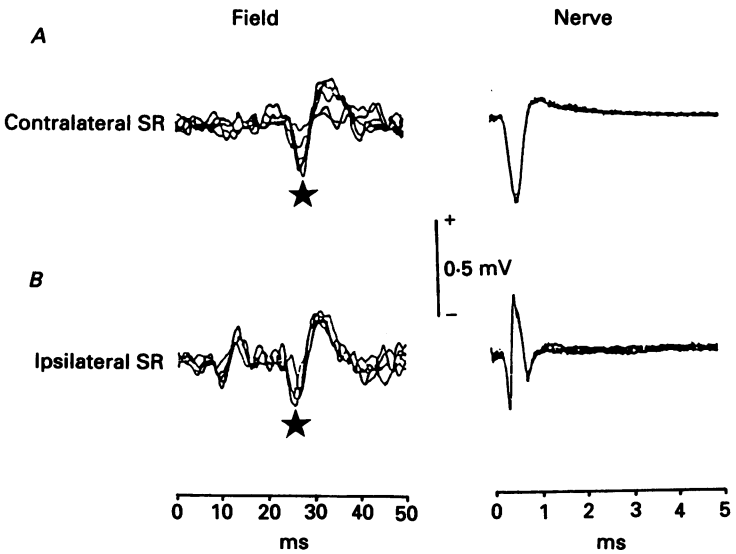


Fig. 1. Oscilloscope traces (five consecutive sweeps, superimposed) of typical cerebellar cortical extracellular field potentials and nerve volleys encountered in the present study. The left-hand column illustrates the various cortical wave forms while the corresponding monitored nerve volleys are illustrated in the right-hand column (note different time bases). Cortical responses marked with a star are attributable to activation via climbing fibres while the responses at a shorter latency in *B* are the result of activation via mossy fibres (latencies and pattern of peripheral nerve convergence characteristic of activation of climbing fibres in the c_2 zone; see text for details). *A*, responses evoked following stimulation (at an intensity of $\times 3$ *T*) of the contralateral superficial radial (SR) nerve; *B*, responses evoked following stimulation (at an intensity of $\times 3$ *T*) of the ipsilateral superficial radial nerve.

SOCP; the latency is, however, compatible with its having resulted from activation of the cortex by a volley in mossy fibres belonging to the exteroceptive division of the cuneocerebellar tract (cf. Ekerot & Larson, 1973, 1979). Such responses were usually present and were always evoked exclusively by ipsilateral stimuli; they were not studied further.

Here it should be noted that microelectrodes were routinely positioned in depth so as to record the largest possible potentials and that the SOCP responses were sometimes initially negative as in Figs 1 and 2 and sometimes initially positive as in Fig. 3. This is not surprising because in previous studies of cerebellar field potentials evoked by volleys in SOCPs (or by climbing fibre volleys elicited by other means such

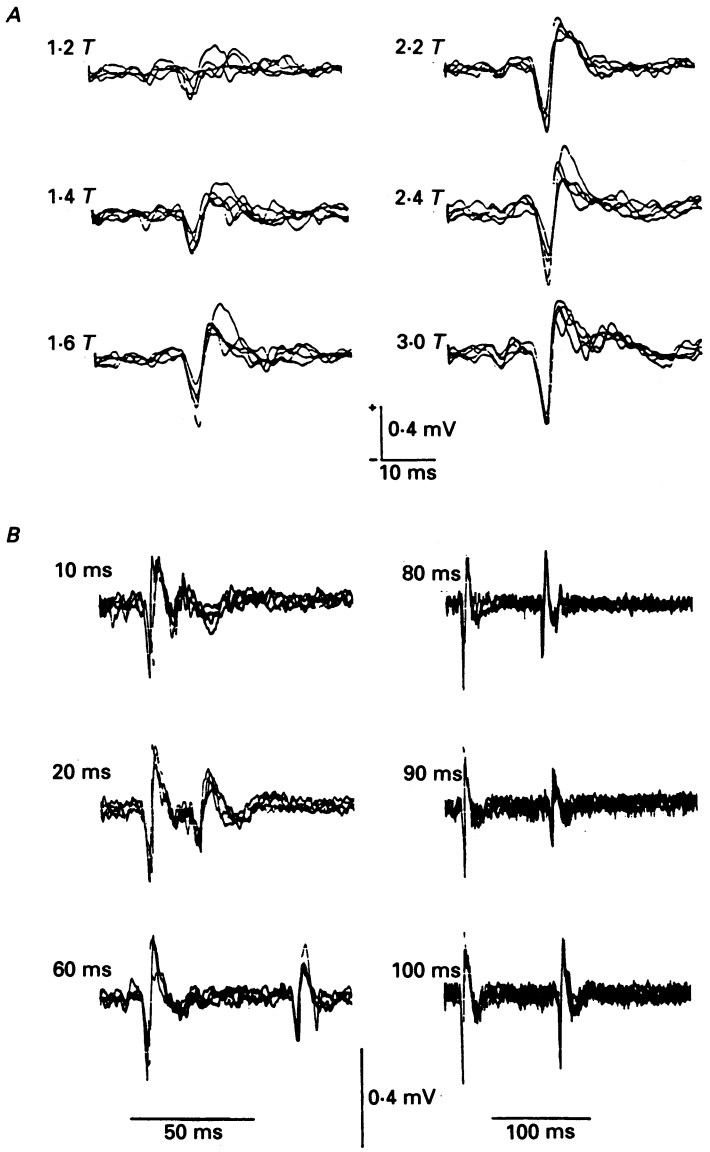


Fig. 2. *A*, typical example showing the effect of increasing stimulus strength on the size of a climbing fibre field potential. Each trace is a superimposition of five consecutive sweeps. Values indicate the stimulus intensity relative to the threshold (*T*) to evoke a just-detectable compound action potential in the nerve. *B*, typical example at a different recording site of the effect of paired stimuli (nerve intensity $\times 2$ *T*) on a climbing fibre field potential. Values indicate the time interval (ms) between the paired stimuli. Each trace is the superimposition of five consecutive sweeps. Note faster time base for right-hand column. Ipsilateral superficial radial nerve stimulated in both panels.

as cerebral cortical stimulation or direct electrical stimulation of the inferior olive) such reversals have been commonplace (cf. Armstrong & Harvey, 1968; Oscarsson, 1968; Eccles *et al.* 1968). In light of these findings responses evoked via SOCPs are treated below in a uniform manner irrespective of polarity.

The explanation of this reversal provided by Eccles *et al.* (1968) is that the initial phase of the response represents an extracellular record of the very large excitatory postsynaptic potentials (EPSPs) evoked in the Purkinje cells by impulses in their climbing fibre afferents. Such EPSPs are seen as a large negativity when the microelectrode tip is in the deeper parts of the molecular layer, level with the climbing fibre–Purkinje cell synapses. However, the action of the synapses, by creating a current sink at this level in the cortex, also creates corresponding sources more superficially at the pial surface and also at deeper cortical levels, so that in these locations initially positive-going responses are recorded. Response polarity is thus simply a function of microelectrode tip position relative to the cortical layers and in accordance with this interpretation the present SOCP responses were often observed to be initially positive when the electrode first contacted the cerebellar surface and then to reverse to initially negative responses which grew in size with advance into the molecular layer. Just after the region of maximum negativity was passed trains of single-unit potentials were often seen, presumably because the tip was then at the level of the layer of Purkinje cell bodies. These discharges were sometimes seen to include complex spikes evoked by nerve stimulation and superimposed on the SOCP field potentials. At deeper levels (presumably in the granular layer) the responses were again initially positive. In tracks which passed through several folia such sequences were encountered repeatedly.

It was important for present purposes to establish that the responses were not unitary but represented the summed activity of a population of Purkinje cells. In this connection, reference has already been made to intertrial variations in response size but in addition it was routinely demonstrated that amplitude was graded with stimulus intensity. Records illustrating the effect at one typical recording site of progressively increasing the stimulus from 1·2 to 3 times the threshold (T) for the most excitable fibres in the nerve are shown in Fig. 2*A* where it is evident not only that the response is graded but also that amplitude increased rapidly over the range 1·2–2·2 T. Similar rapid increases have previously been noted as characteristic of SOCPs to the c_2 zone (cf. Armstrong *et al.* 1973).

Further evidence for the gradeable nature of the responses is given by Fig. 2*B* which shows pairs of responses elicited by two shocks delivered at intervals ranging from 10 to 100 ms: in each case the second response is smaller than the first but recovers progressively as the interstimulus interval is lengthened. Figure 2*B* provides additional confirmation that the responses were mediated via SOCPs (rather than via spinocerebellar paths terminating as mossy fibres). Thus, the prolonged depression of the second response (50% recovery was typically reached only at *ca* 50 ms) is characteristic of SOCPs (cf. Armstrong & Harvey, 1968; Miller & Oscarsson, 1970) and results in part at least from the complex electrophysiological properties of olivary neurones (cf. Armstrong, Eccles, Harvey & Matthews, 1968; Crill, 1970; Llinás & Yarom, 1981*a*, *b*). It contrasts markedly with the much more rapid recovery (not illustrated) of responses mediated via mossy fibres (cf. Eccles *et al.* 1968).

Comparison of SOCP responses elicited during rest and walking

For a total of seven recording sites in three cats, substantial periods of recording were made both while the cat rested and while it walked. At four of these sites

response size estimated by averaging over many stimulus presentations (see Methods) was significantly reduced during locomotion (normal distribution $P < 0{\cdot}01$) while at the remaining three sites response depression was also observed but the interpretation of the effect remained equivocal because of an accompanying reduction in the evoked nerve volley size (see below).

Typical findings from two of the four sites with significant depression are illustrated in Fig. 3 in which *A*, *B* and *C* each show the responses to twelve successive stimuli delivered to the ipsilateral SR nerve at a repetition rate of 1 per 1·5 s. In each case the sequence of responses begins with the bottom trace in the left-hand column and ends at the top of the right hand column. In *B* and *C* the records are accompanied by a continuous vertical trace of the EMG activity in the lateral head of triceps brachii in the ipsilateral forelimb. During walking a rhythmic locomotor burst of EMG occurs in the muscle once per step cycle (during stance) and the position at which each cerebellar trace begins relative to these bursts indicates the time of stimulus delivery relative to the step cycle.

Figure 3*A* and *B* is from one cerebellar site but in *A* the animal was sitting quietly while in *B* it was walking. Inspection shows that in *B* the responses are more variable in size and on average smaller than in *A*. This difference occurred despite the fact that the nerve volley remained constant in size between the two behavioural conditions (not illustrated).

Figure 3*C* involves a different site and demonstrates the temporal link which existed between the onset of movement and the onset of response reduction. In the left-hand column the EMG trace shows only a low level of tonic (i.e. postural) activity (because the animal was sitting quietly on the stationary treadmill belt) and each stimulus succeeded in evoking a response. However, during the period shown in the middle column the animal stood and began to walk as movement of the belt was initiated. It is clear that in this period the cerebellar response was first reduced (lowest trace) and then completely suppressed, this suppression being maintained throughout the remaining traces. At the end of the bout of walking an opposite effect was seen; the responses promptly recovered when walking ceased (not illustrated). It is perhaps worth noting that a few of the stimuli delivered during walking did in fact succeed in evoking a response and that such responses were occasionally as large as those seen during rest. Effects similar to those in Fig. 3 were also observed at other recording sites which could not be studied fully because of restless mobility of the animals.

In addition, for the site shown in Fig. 3*C*, it was noted that when stimulus intensity was increased from $2T$ to $3T$ during walking responses reappeared (at reduced size) suggesting that the suppression could be partly offset by an increase in the size of the peripheral nerve volley. In fact, because the cerebellar response was reduced substantially during locomotion as compared to rest it was sometimes necessary to increase the stimulus strength during walking so that the cortical response was of sufficient size to be reliably measured.

At four sites with depression of the response the overall mean size of the responses was reduced during locomotion to 40, 75, 82 and 84% of the mean size during rest and the differences between the means were in each case statistically significant (normal distribution $P < 0{\cdot}01$). At none of these sites was the nerve volley different in amplitude between locomotion and quiet resting.

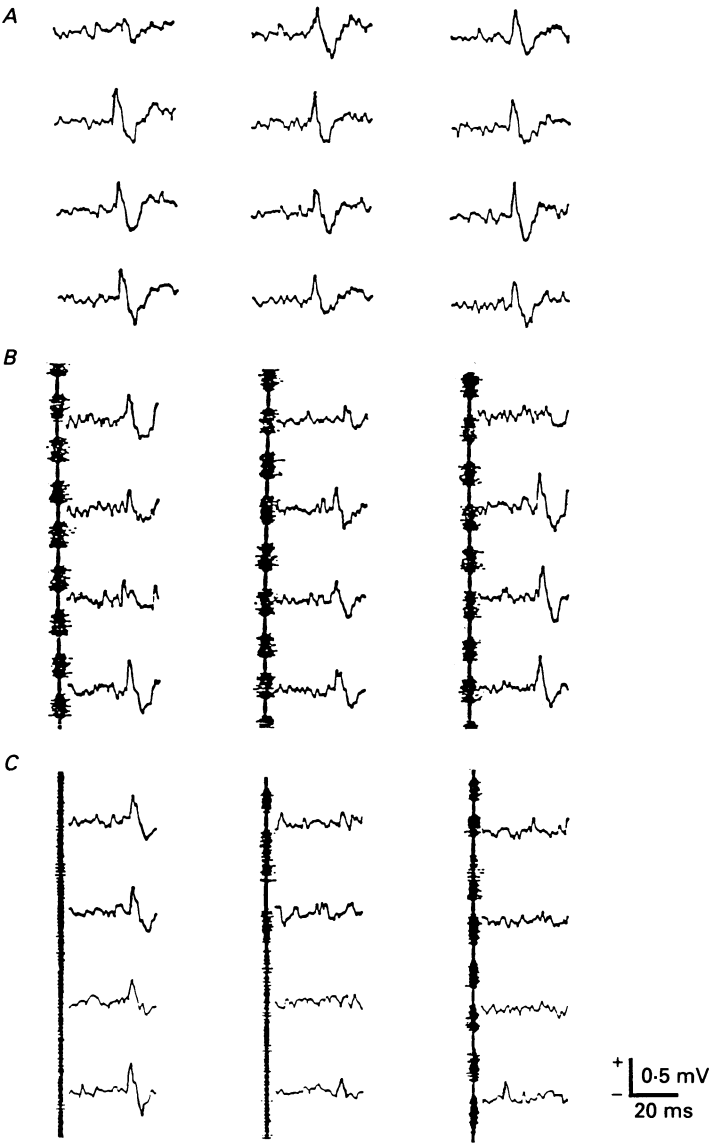


Fig. 3. Consecutive single-sweep recordings of climbing fibre field responses evoked by ipsilateral superficial radial stimulation at intensity $\times 2$ T. For all traces the first sweep is shown in the bottom left-hand corner, the next sweep is above and the last sweep is shown in the top right-hand corner. The responses in A and B were recorded at the same site. In A the responses were recorded while the cat was sitting quietly, while in B the cat was walking steadily on the treadmill. In B and C the activity in the ipsilateral triceps brachii muscle is shown as a continuous vertical trace to the left of the cerebellar recordings. The beginning of each sweep of cortical recording marks the approximate time during the step cycle when the stimuli were delivered. C illustrates the responses recorded at a different recording site following stimulation of the ipsilateral superficial radial nerve at intensity $\times 2$ T. The continuous EMG trace shows that the cerebellar responses were recorded during the transition from rest (left-hand column) to steady locomotion (right-hand column).

At the three remaining sites among the seven studied quantitatively the responses were also substantially reduced during locomotion (to 28, 34 and 68%) but interpretation of these changes was complicated by the fact that nerve volley amplitude was also reduced (to 83, 50 and 62% respectively), perhaps because of relative movement between the stimulating electrodes and the nerve. In these cases it cannot be excluded that the cerebellar change might have resulted from the change in the effective stimulus strength rather than from the operation of a central influence on SOCP transmission.

Step-phase dependence of response amplitude during locomotion

As explained in Methods, the interstimulus interval routinely used (1·5 s) was such that successive stimuli were usually delivered at different times during the step cycle as monitored from the EMG recordings. That the interval was sufficiently long to exclude any cumulative effects of repeated stimulation was established for a random sample of recording sites by measuring the amplitude of successive responses in each sequence (for data obtained during both rest and locomotion) and using the method of least-squares regression to determine the slope of the relationship between the size of each response and its position in the sequence of responses. For all samples the slopes were not significantly different from zero (t test, $P > 0{\cdot}05$), showing that repeated stimulation did not lead to any progressive increase or decrease in response amplitude. Such findings also gave reassurance that there was unlikely to have been any progressive shift in microelectrode tip position during the recordings.

The drift in stimulus timing relative to the step cycle was used to investigate the possibility that response amplitude (and therefore SOCP transmission) might vary systematically during the cycle and indeed when the mean sizes of responses evoked during different tenths of the cycle were compared (see Methods) there was always some variation in amplitude. However, the extent of such variation differed between sites as also did the times during the step when the responses were largest and smallest in mean area.

In Fig. 4 the step-phase dependence of the responses is shown for one site at which response amplitude displayed a relatively modest variation. In this case the contralateral SR nerve was stimulated but in this and all subsequent diagrams the phases of the step cycle are shown relative to the movements of the *ipsilateral* forelimb (see Methods). The filled squares represent for each tenth of the cycle the mean area of the responses evoked by stimuli delivered to the contralateral SR nerve during that tenth. The smallest responses occurred in period 1 which coincides with the F/E_1 portion of the step in the ipsilateral forelimb (when footfall occurs) while the largest response occurred in period 6 which coincides with the E_2/E_3 portion of the step.

In this example the mean amplitude of the response during period 6 showed an 80% increase over the value for period 1. For comparison the filled diamonds plot the mean peak-to-peak amplitude of the compound action potential recorded from the nerve. It is evident that there is some step-related variation in the nerve volley so that the largest value (in period 9) is 10% larger that the smallest (in period 5). However, the temporal pattern of this variation shows no obvious correlation with the pattern for the cerebellar responses. Note that this recording site was one of those at which cerebellar responses were reduced during locomotion: the dotted line across the upper part of Fig. 4 represents response mean amplitude during quiet rest. This

difference in size of cortical response occurred despite constancy of the nerve volley between the two behavioural conditions.

At some other sites there were much more substantial variations in response size and two such examples are shown in Fig. 5*A* and *B*. As in Fig. 4 mean area of the cerebellar response in each step tenth is shown (■——■), as is the corresponding nerve volley amplitude (◆--◆). As in Fig. 4 the volley shows some slight variation during the step but again as in Fig. 4 these changes do not correlate with the much more marked changes in the cerebellar response. In Fig. 5*A* the largest cerebellar

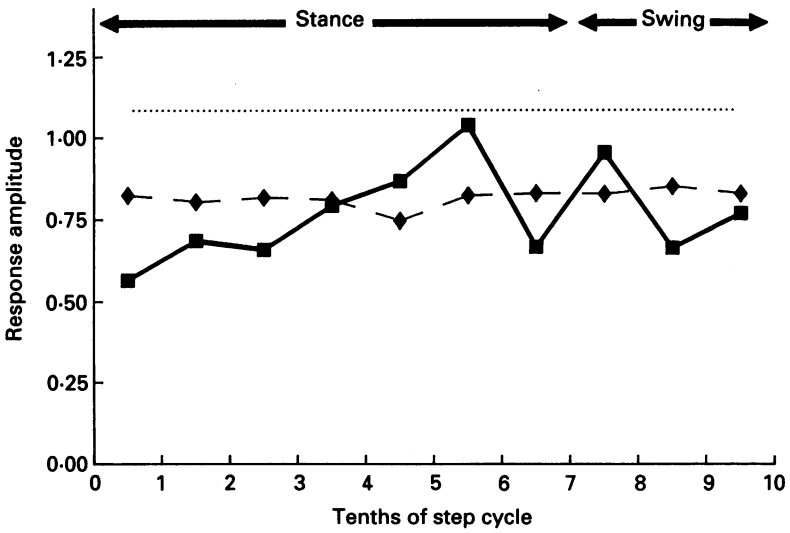


Fig. 4. Graph to illustrate a recording site that showed only a modest variation in the size of a climbing fibre field response during locomotion. The step cycle is divided into tenths relative to onset of activity in the ipsilateral extensor muscle triceps brachii. Dotted line (towards the top of the figure) displays the mean total area of the climbing fibre response at rest as compared to the mean total area of the response for each tenth of the step cycle (■——■). ◆--◆, the corresponding mean peak-to-peak amplitude of the nerve compound action potential (contralateral superficial radial nerve stimulated at intensity $\times 5$ *T*) for each tenth of the step cycle. Periods of stance and swing are approximate timings for trajectory of the ipsilateral forelimb. For all plots the mean size of the cerebellar field potential is measured in units of mV ms and the mean amplitude of the nerve volley is measured in mV.

response (in period 10) is more than five times larger than the smallest response (in period 2), In Fig. 5*B*, which relates to a recording site in a different animal, the response variation is even more marked because none of the stimuli presented during periods 8 and 10 succeeded in evoking any detectable response.

It was mentioned earlier that not only were responses evoked during locomotion often smaller than in the resting animal but there were often reductions in the probability that a stimulus would evoke any response (cf. Fig. 3*C* and accompanying text). It is obvious from Fig. 5*B* that the extent of such probability changes could be step-phase dependent because responses were encountered during most tenths of the cycle but completely failed to appear during periods 8 and 10. Such response failures were observed at other sites and attempts were therefore made to determine

the extent to which changes in response probability were a contributory factor in producing differences in mean response area between the different tenths of the step cycle.

For each tenth the number of stimuli which evoked a response was expressed as a proportion of the total number of stimuli which fell within that tenth to give a

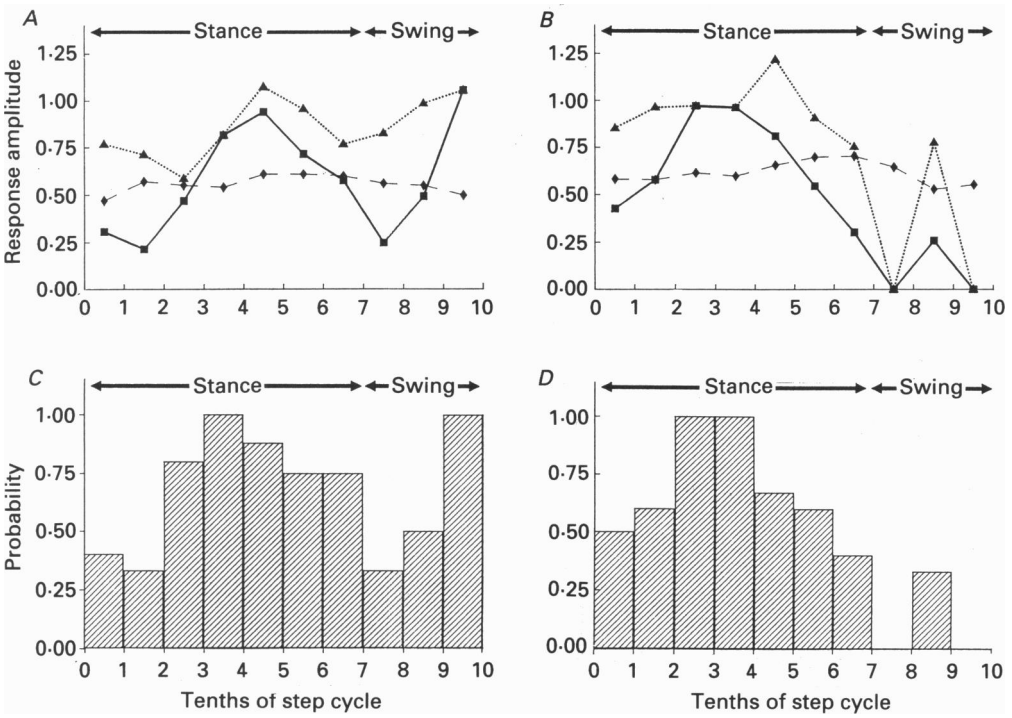


Fig. 5. For all graphs the x axis is divided into tenths relative to the timing of onset of activity in the ipsilateral triceps brachii muscle and the periods of stance and swing are approximate timings for trajectory of the ipsilateral forelimb. *A* and *B*, same format, i.e. for each tenth of the step cycle the size of the climbing fibre field is shown in terms of its mean total area (■——■) whereas the size of the climbing fibre field with all zero responses removed is shown by ▲···▲. For comparison the mean peak-to-peak amplitude of the nerve volley for each tenth is illustrated by ◆--◆. The ipsilateral superficial radial nerve was stimulated in *A* at intensity $\times 1{\cdot}75\ T$ and in *B* at intensity $\times 3\ T$. For all plots the mean size of the climbing fibre field potential is measured in units of mV ms and the mean amplitude of the nerve volley is measured in mV. *C* and *D*, hatched bars indicate for the same sites shown in *A* and *B* the corresponding probabilities of the occurrence of a climbing fibre response for each tenth of the step cycle (a value of 1·0 means that all trials that occurred in a particular tenth evoked a response).

probability value ranging from zero (when no responses occurred) to 1 (when each stimulus was successful). In Fig. 5*C* and *D* these values for the sites in Fig. 5*A* and *B* respectively are represented by the hatched columns and it is evident that they vary in approximate parallel with the fluctuations in mean response size. This finding has considerable significance for the interpretation of the results (see Discussion) but it should be noted that the probability changes were usually not alone sufficient to account for the changes in mean response size. Thus, when trials involving response

failure were excluded from the calculations of response area, variation in mean area was typically still present though reduced in extent. This is illustrated in Fig. 5*A* and *B* (▲···▲). In Fig. 5*B* the procedure has markedly reduced the response variations (except in periods 8 and 10 where it could not because, as noted above, no responses could be evoked in these periods) but in Fig. 5*A* substantial variation remains and its time course relative to the step cycle is essentially unchanged. Findings similar to those of Fig. 5*A* were made for most sites and imply that the variations in mean response area are due both to step-cycle-related variations in the ability of the stimulus to evoke a response and to essentially parallel variations in the amplitude to those responses that were evoked. In other words, the typical finding was that in

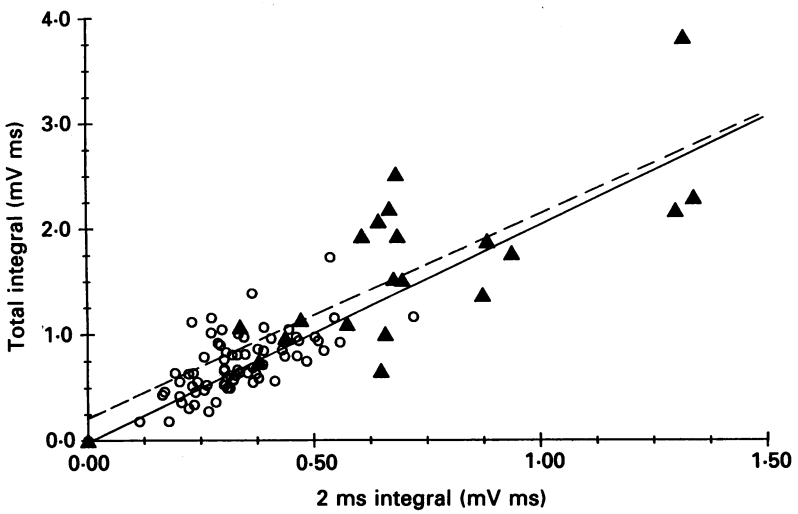


Fig. 6. Scatter plot of the sizes of individual responses recorded at one typical recording site in the anterior lobe following stimulation of the ipsilateral superficial radial nerve to demonstrate the relationship between the total area beneath the initial component and that beneath the first 2 ms of the fields. ▲, the relation between the two measures of response size while the cat was sitting quietly at rest (dashed regression line, correlation coefficient $r = 0{\cdot}63$, significant at the 0·1% level). ○, for the same recording site, the relation between the two measures of response size while the cat was walking on the treadmill (continuous regression line correlation coefficient $r = 0{\cdot}71$, significant at the 0·1% level). Note that for both behavioural conditions all trials that failed to evoke a response have been excluded from the calculation of the regression lines and correlation coefficients.

those periods when probability was high or low the 'successful' stimuli tended to evoke large and small responses respectively.

Equivalence of measures of response size

In Figs 4 and 5 response sizes were estimated throughout in terms of the area under the whole of the initial (positive or negative) component of the diphasic evoked potential which lasted *ca* 4–6 ms. However, as mentioned in Methods and for reasons given in Discussion, an additional measure was also employed, namely the area under the first 2 ms of the response. However, in the course of the study the two measures were in fact found to vary in parallel. This is illustrated for one recording

site by the scattergram of Fig. 6 in which, for individual responses recorded during rest (▲) and during locomotion (○), the two measures are compared.

For each behavioural condition there was a significant direct linear relationship between the two measures, as demonstrated by the clustering of the points around the calculated line of regression (continuous line for responses during locomotion; dashed line for responses during rest). Moreover, the two regression lines are very similar indicating that the relationship between the two measures was essentially the same during rest and during walking. Similar findings were made for all other data samples so compared.

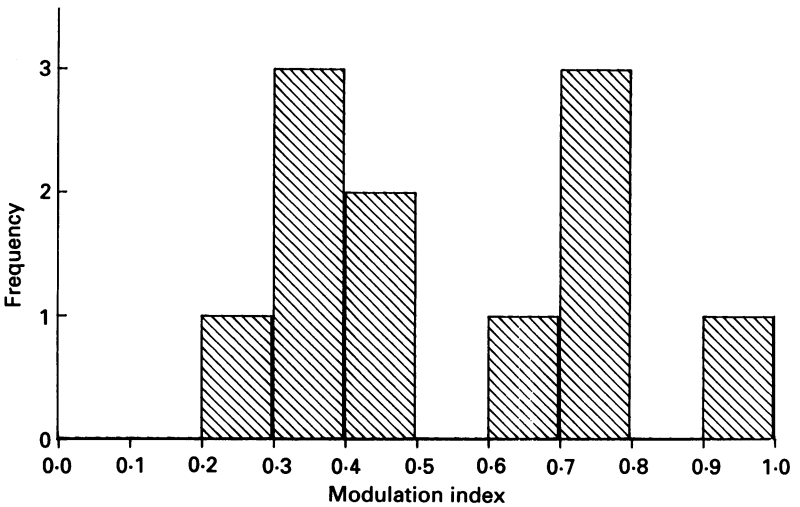


Fig. 7. Frequency histogram for all recording sites ($n = 11$) of the distribution of modulation index, defined as one minus the ratio of the response in the step-cycle tenth with the smallest response to the response size in the tenth with the largest response. (A value of one indicates the maximum variation in response size while a value of zero indicates no variation.) Response sizes were estimated using the area beneath the first 2 ms of the evoked field. Modulation index calculated for each site following stimulation of the ipsilateral superficial radial nerve.

Extent of response size modulation at different recording sites

That different recording sites manifested different degrees of step-cycle-related variation in response size has already been mentioned and this difference was explored quantitatively by calculating a 'modulation index' for each site as one minus the ratio of the amplitude in the 'worst' tenth to that in the 'best'. On this basis a value of unity would correspond to cases in which in the 'worst' tenth no stimuli succeeded in evoking a response (cf. Fig. 5*B*) whilst a value of zero would signify that mean response size was constant throughout the step cycle. Figure 7 is a bar chart showing the frequency distribution of different values of modulation index for the total of eleven sites from which responses to stimulation of the ipsilateral SR nerve were recorded during walking. The values ranged widely, from one case in which the index was 0·25, indicating only modest variation in mean response size with respect to the step cycle, to a value of 1·0 indicating the maximum variation (since all stimuli delivered in at least one tenth failed to evoke a detectable response).

This wide range would seem to imply that the SOCPs projecting to different parts of the c_2 zone are subject to a modulation of transmission that differs widely in the extent to which it varies in intensity during the course of the step cycle.

However, caution in interpretation is required because at sites at which it was possible to employ more than one intensity of stimulation the extent of the modulation was found to vary with stimulus intensity. In general, stimulus intensities at which response probability was high and the responses were large yielded lower values of modulation index than weaker stimuli that evoked smaller responses at lower levels of probability. At the site featured in Fig. 5*A* and *C*, for example, stimulation at 1·75 *T* yielded a modulation index of 0·80 but when during another episode of walking an intensity of 2 *T* was employed the index fell to 0·70. Such reductions could be explained by supposing that larger nerve volleys are better able to override whatever mechanism gives rise to the step-related variations in SOCP transmission. Because Fig. 7 is based on values of modulation index obtained using stimuli delivered to the ipsilateral SR nerve ranging at the different sites from 1·1 to 4 *T*, it is likely that intensity dependence of the index is partly responsible for the wide spread of values present. However, the use of different intensities at different sites was a necessity because the stimulus needed to evoke measurable responses was different for different loci.

Timings of step-related modulations of response amplitude

As already noted, the times of minimum and maximum response amplitude relative to the step cycle in the ipsilateral forelimb varied between recording sites (compare Fig. 5*A* and *B* and Fig. 5*C* and *D*; see also Fig. 9*A*) when responses to stimulation of the ipsilateral SR nerve were studied. Figure 8*A* and *B* therefore shows for all eleven sites the times of minimum and maximum response respectively. These displays show that over all sites there was a wide range of tenths of the step at which SOCP transmission was 'worst' and also a wide range at which it was 'best'. Nevertheless, a tendency is evident for times of 'best' response to cluster in two phases of the step, namely mid-stance and late swing. The possible significance of the clustering is considered in the Discussion.

Comparison of ipsilateral and contralateral responses at the same recording sites

At six sites, recording was sufficiently prolonged that responses to stimulation of both ipsilateral and contralateral SR nerves could be studied and the findings for one such site are summarized in Fig. 9. For ipsilateral stimulation (Fig. 9*A*) the nerve volley was essentially constant throughout the step cycle (for clarity not illustrated) but the cerebellar response nevertheless varied markedly. Mean response size as measured by the area under the whole of the initial component of the field is shown (■——■) as is the size as given by the area under the first 2 ms of the response (■—■). A general similarity exists between the two measures confirming their essential equivalence (see above); for both measures response size was least in the first step tenth and greatest in the fourth tenth. Mean amplitudes (whole response) when response failures were excluded from the calculation are also shown and response size remains modulated (▲···▲).

Figure 9*B* is similar to 9*A* but relates to contralateral stimulation (nerve volley also omitted) and again the different measures of cerebellar response yield essentially

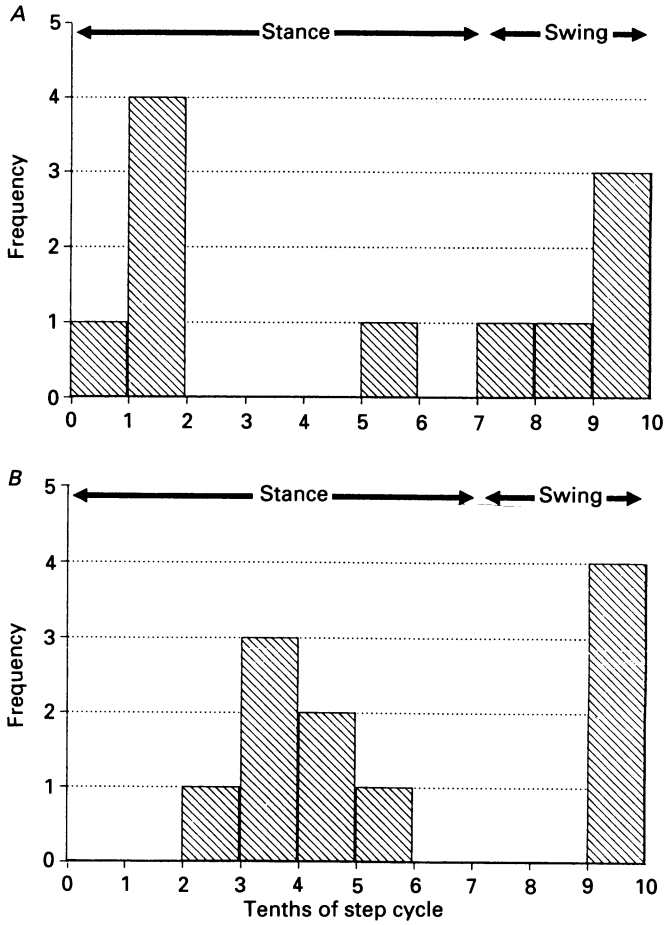
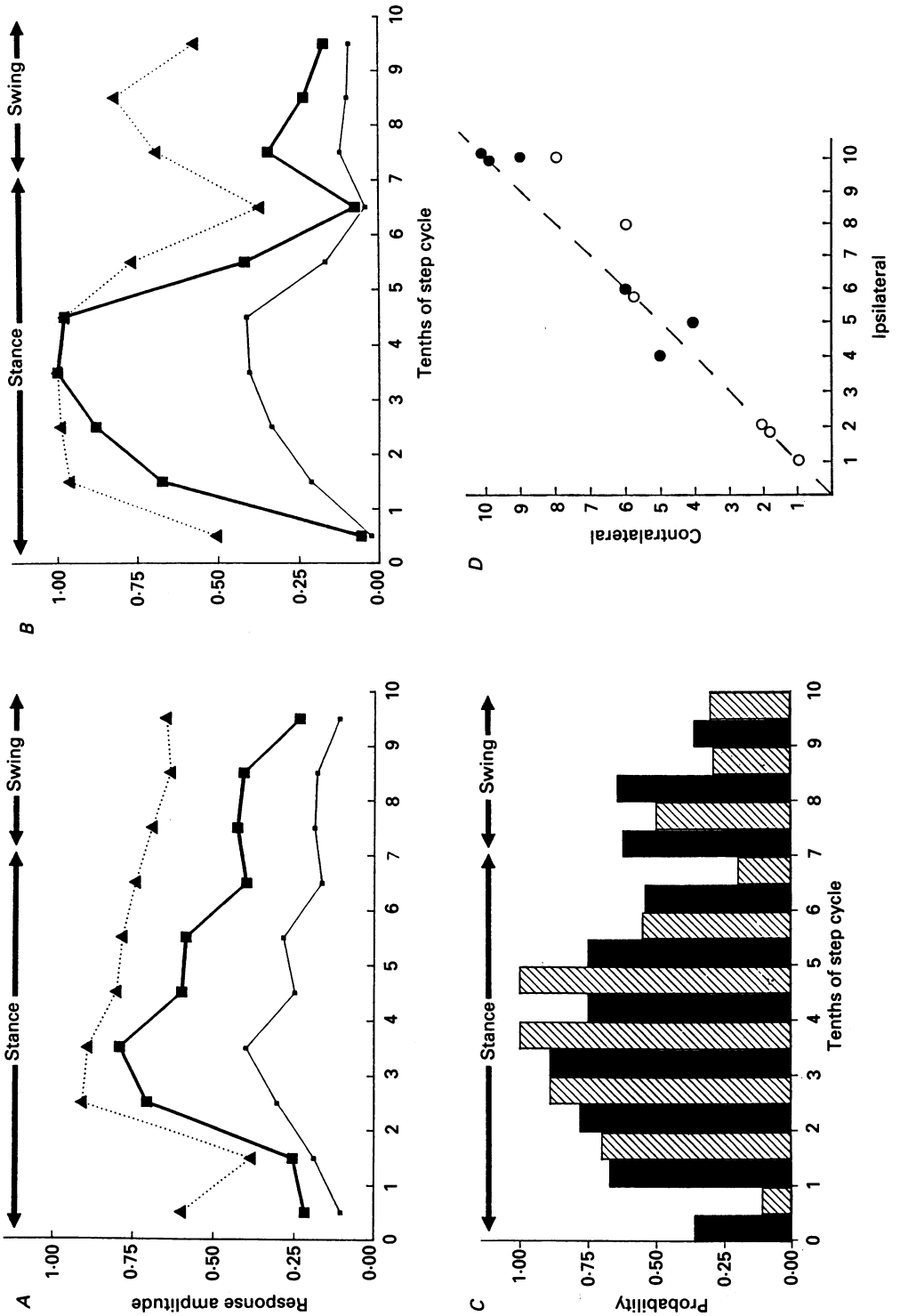


Fig. 8. Frequency distribution for all eleven c_2-identified recording sites at which responses to stimulation of the ipsilateral superficial radial nerve were studied to show the time during the step cycle (measured relative to onset of activity in ipsilateral triceps brachii) when the smallest mean size of climbing fibre field was evoked (*A*) and when the largest mean size of climbing fibre field was evoked (*B*).

similar patterns of response modulation. Moreover, although the patterns are not identical with those for the corresponding measures in Fig. 9*A*, the times of maximum and minimum response are nevertheless the same. Considerable similarity also exists in Fig. 9*C* which is a bar chart for the ipsilateral (filled columns) and contralateral responses (hatched columns) showing the variation in the probability that each stimulus would evoke a response. It is important in connection with Fig. 9*A*, *B* and *C* to note that for both sets of responses timing has been referred to the step cycle in the same (i.e. ipsilateral) forelimb.

The 'timing' findings of Fig. 9*A*, *B* and *C* were typical of those made for all six recording sites as may be seen from the scattergram of Fig. 9 *D* in which the times of maximum (●) and minimum (○) response are compared for the ipsilateral and contralateral responses at each site. All points lie on or close to the diagonal line of



equality because at each site the temporal patterns of response modulation were similar. Note also that again, as in Fig. 8, the times of maximum response are clustered in mid-stance and late swing.

DISCUSSION

Use of cerebellar evoked potentials to monitor SOCP excitability

Given their form, latency and frequency-following characteristics there can be no doubt that the potentials studied resulted from the action on the cerebellar cortex of impulses in the climbing fibre afferents and therefore that they were the outcome of activity in one or both of two SOCPs known to terminate in the c_2 zone: previous studies in anaesthetized and in decerebrate animals have shown that the zone receives input from two SOCPs, one mediated via the lateral and the other via the dorsal funiculus of the spinal white matter. Latencies reported for responses evoked by stimulation of the ipsilateral SR nerve have ranged from 16 to 20 ms for the LF-SOCP and 17 to 27 ms for the DF-SOCP. For the contralateral nerve the corresponding values are 16–25 ms and 21–32 ms (see Larson *et al.* 1969*b*; Armstrong, *et al.* 1973; Ekerot & Larson, 1979). The present findings are clearly entirely compatible with these values but the overlap in reported latencies between the LF and DF paths implies that most of the present responses could be mediated via either (or both) of the paths, with the exception of the earliest contralateral responses which were presumably conveyed via the LF-SOCP. The pathways converge in the rostral part of the medial accessory olive which supplies the climbing fibres of the c_2 zone. Both paths have complex synaptic relays in the brain stem and the LF-SOCP also involves interneuronal relays at the segmental level.

In the present study the excitability of these paths was studied by using electrical stimulation of a cutaneous nerve to set up test volleys which represents a revival of an approach adopted by Carli, Diete-Spiff and Pompeiano (1967) in an early study

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Fig. 9. *A* and *B*, same format as for Figs 4 and 5, i.e. for each tenth of the step cycle the size of the response is shown in terms of the mean area of the initial component (■——■) and also in terms of the mean area beneath the first 2 ms of the field (■—■) in addition to the size of the response with all zero responses removed (▲···▲). The *x* axis is divided into tenths relative to the timing of onset of activity in the ipsilateral triceps brachii muscle and the periods of stance and swing are approximate timings for trajectory of the ipsilateral forelimb. *A* and *B*, fluctuation in the size of the climbing fibre field relative to the step cycle for responses evoked by stimulation of the ipsilateral or contralateral superficial radial nerve at the same recording site. *A*, ipsilateral response. *B*, contralateral response. *C*, the probabilities per tenth of the step cycle of a 'successful' trial to stimulation of the ipsilateral (■) and contralateral (▨) superficial radial nerve recorded at the same site as depicted in *A* and *B*. (A probability of 1·0 indicates that all stimuli delivered during that tenth evoked a detectable cortical response). Step cycle divided into tenths relative to the onset of EMG activity in the *ipsilateral* forelimb extensor triceps brachii. *D*, scatter plot of the step-cycle position (relative to onset of activity in ipsilateral triceps brachii) at which the largest response (●) and smallest response (○) occurred for six recording sites at which both an ipsilateral and a contralateral recording were made. Equal step positions are indicated by the dashed line.

of changes in the excitability of cerebellar afferent pathways during the sleep–waking cycle. That study was, however, carried out before the different actions of mossy and climbing fibre volleys on the cortex had been clarified.

In view of the synaptic complexity of the SOCPs it is clear that changes in the test responses might result from transmission changes at a number of synaptic relays. There are, however, two factors which could in theory generate 'spurious' variations in response size and therefore falsely suggest that pathway excitability was varying. Variations might occur in the effectiveness of the stimuli applied to the SR nerves, resulting in variable-sized volleys. Such variations might arise because of relative movement between the nerves and the stimulating electrodes or (less probably) because of interactions between the natural and the electrically evoked impulse traffic in the nerves (which were stimulated in continuity). Possible variations were therefore controlled for by routinely recording compound action potentials from the nerves and this did reveal that in some cases similar-sized stimuli evoked smaller volleys during locomotion than during quiet rest. Fortunately, however, this was not always the case. In addition, volley size sometimes varied during the step cycle but, when present, such variations were usually modest and, moreover, their temporal pattern during the step cycle did not match with that for variations in the cerebellar responses.

One observation of particular interest in this connection was that at each of six recording sites the temporal pattern of step-related variation in the responses evoked from the ipsilateral and the contralateral nerves was essentially similar. Because the activity of the ipsilateral limb was always used to time the step cycle it is unlikely in these cases that parallel changes in the two nerve volleys were responsible for the parallel between the corresponding cerebellar responses: a stimulus delivered to the contralateral nerve at a particular time during the ipsilateral step cycle will come at a very different time in the movement cycle of the contralateral limb (because the activities of the two limbs are oppositely phased during walking).

Finally, it is noteworthy that when different cerebellar sites were studied in the same animal (sometimes in the same recording session) the responses to stimulation of the same nerve often behaved differently in respect of their degree and temporal pattern of locomotor-related size and modulation.

A second factor which requires discussion because it might spuriously suggest changeable SOCP excitability is the possibility that the Purkinje cells may vary in their responsiveness to input from their climbing fibres. Such variations might result from changing levels of activity in the mossy fibre-granule cell-parallel fibre pathway which directly excites the Purkinje cells and also, by exciting nearby stellate and basket cells, postsynaptically inhibits them. It is relevant here that walking is accompanied by rhythmic (i.e. step-related) discharges of simple spikes in Purkinje cells (Orlovsky, 1972; Armstrong & Edgley, 1984, 1988; Edgley & Lidierth, 1988) in the c_1, c_2 and c_3 zones implying that mossy fibre input does indeed vary rhythmically and therefore that rhythmic activity probably also occurs in basket and stellate cells, which, like the Purkinje cells, receive excitatory synapses from the parallel fibres.

Several studies have examined the influence of volleys in the parallel fibres (evoked by electrical stimulation of the cortical surface; the LOC stimulation of Eccles,

Llinás & Sasaki, 1966) on the responsiveness of Purkinje cells to subsequent input via the climbing fibres. Thus Jansen & Fangel (1961) found that extracellular field potentials tentatively (and correctly) attributed to climbing fibre activation of the cortex could be significantly depressed by surface stimulation, but only briefly and only when the interval between the conditioning and the test stimuli was very short (less than 20 ms). Subsequently, Eccles, Llinás, Sasaki & Voorhoeve (1966) used LOC stimulation to evoke very powerful stellate/basket cell inhibition of Purkinje cells and found that climbing fibre EPSPs recorded intracellularly from Purkinje cells were first depressed (for *ca* 10 ms) and then enhanced for some tens of milliseconds. These changes were attributed respectively to shunting of the EPSPs by the membrane conductance change during the inhibitory synaptic current and to EPSP enhancement resulting from the hyperpolarization of the Purkinje cell during the stellate/basket cell-induced IPSPs. Extracellular fields were not studied so comprehensively but when stellate/basket cell-generated inhibition was at its height they were enhanced by up to 60%.

More recently, Campbell, Ekerot & Hesslow (1983) studied the effect of LOC conditioning on the responses initiated in Purkinje cell dendrites by climbing fibre input and found that although in branches lying superficially in the molecular layer (i.e. in distal branches) the whole response was markedly reduced in amplitude and duration, in deeper (i.e. proximal) dendrites only the later phase was depressed while the first few milliseconds of the response underwent little or no change.

Collectively, these observation suggest that when large numbers of parallel fibres are synchronously activated the resultant mixed excitatory and inhibitory effects on the Purkinje cells can alter the amplitude of the initial component of a field potential evoked via the climbing fibres but that such effects are most unlikely to account fully for the large change often observed in the present study. Moreover, the potency of the climbing fibre synapses is such that the responses they evoke in the Purkinje cells are never completely abolished by even the most intense inhibitory actions of the cerebellar cortical interneurones. It is therefore safe to assume that changes in response *probability* must be due entirely to some precerebellar change in SOCP excitability. Since the time course of step-related variations in response size usually paralleled that of variations in probability the most economical hypothesis would be that the amplitude changes were also largely precerebellar in origin.

When olive cells are synaptically discharged their response varies from one impulse to a burst of up to five impulses at a spacing of *ca* 2 ms and correspondingly the Purkinje cells exhibit from one to five climbing fibre EPSPs (Eccles, Llinás & Sasaki, 1966; Armstrong & Harvey, 1968). Because of this variability a change in the area under the initial peak of the cerebellar field potential might reflect a change in the number of responding olivary neurones and/or a change in the number of impulses discharged by each neurone. A change of the latter kind could not, however, influence the area under the first 2 ms of the response and this was therefore used as an alternative index of response size. As demonstrated by Fig. 6 the two indices were found to vary in parallel and it is therefore likely that variations in the number of olive cells discharging was an important factor in producing the observed variations in response size.

Locomotor-related changes in SOCP excitability

At some recording sites studied quantitatively and at several others studied less thoroughly the behavioural transition from quiet rest to walking was accompanied by a decline in SOCP excitability and there were no sites at which responses were enhanced. In some cases the onset of depression could be seen to precede the onset of regular stepping (cf. Fig. 3*C*) and it is therefore possible that such changes are better regarded as related to the onset of active movement rather than of locomotion *per se*. Depressions were, however, maintained throughout walking. Similar excitability changes have previously been shown to occur in the dorsal column pathway where medial lemniscus volleys set up by cutaneous nerve stimulation are attenuated during active movements, including locomotor movements (Coulter, 1974; see also Ghez & Lenzi, 1971). Evidence has also been presented for modulation at a subcortical level of transmission in the pathway mediating forelimb cutaneous inputs to the motor area of cerebral cortex (Palmer, Marks & Bak, 1985).

At most recording sites the extent to which SOCP excitability was reduced varied considerably during the course of the step cycle. The temporal pattern and the intensity of such variation was different at different recording sites suggesting that within the relevant SOCPs there might be functionally distinct subpaths projecting to different portions of the c_2 zone. This possibility deserves further study, perhaps via determinations for individual Purkinje cells of the level of probability at which complex spikes are evoked by nerve volleys initiated at different times during the step cycle.

Overall, despite the difference between recording sites, the excitability of the SOCPs appeared to be most markedly reduced at around the transition times from stance to swing and from swing to stance (i.e. at around footfall and footlift) in the ipsilateral forelimb. Interestingly, these are the times when stepping is likely to generate the largest levels of input from those receptors from which the SOCPs are most strongly activated, i.e. the phasic, low-threshold cutaneous mechanoreceptors. This might be taken to suggest that the excitability reductions reflect the operation of a pathway-gating mechanism acting to reduce transmission to the cerebellum of self-generated cutaneous inputs. The existence of such a mechanism has previously been suggested by Gellman *et al.* (1985) to account for their observation that in awake cats individual olive cells are often highly sensitive to tactile stimuli imposed on the passive animal but not to similar-sized stimuli resulting from the animal's own movements. Likewise, Andersson & Armstrong (1987) found that complex spikes were evoked in Purkinje cells when stepping was perturbed by an unexpected peripheral event but were not time-locked to undisturbed step cycles (cf. also Lou & Bloedel, 1986; Bloedel & Lou, 1987). However, in respect of the present results it should be noted that the times of least excitability for responses evoked from the *contralateral* forelimb did not coincide with footfall and footlift in that limb but occurred during its stance phase.

Most recently Baker, Seers & Sears (1989) have investigated in anaesthetized or decerebrate cats changes in transmission during the respiratory cycle in SOCPs that arise from the thorax and terminate in the b zone. The phasic activity of the respiratory central pattern generator was found to exert a modulatory influence so

that in the ipsilateral b zone response size was smallest during the inspiratory phase. Thus in agreement with the present findings a central mechanism is postulated that acts to reduce transmission at those times of the respiratory cycle when self-generated sensory traffic is likely to be maximal, and suggests that cyclical changes in excitability may be a general feature of SOCPs.

Returning to the question of the precise precerebellar level at which SOCP excitability was varied it may be noted that, because the timing relative to the step cycle was similar for ipsilateral and contralateral responses at the same recording site, this was probably at or after the level at which bilateral convergence occurred. For the DF-SOCP this would probably imply a modulatory influence exerted at a brain stem level rostral to the cuneate nucleus (the influence of contralateral inputs to the cuneate nucleus is unlikely to be sufficiently potent to account for the present results: see e.g. Andersson, Etholm & Gordon, 1970; Jabbur & Banna, 1970) but for the LF-SOCP the segmental and/or the brain stem might be involved. In both cases the modulation might occur at the level of the inferior olive itself because most olive cells which act as SOCP relays also receive other inputs (which include cerebello-olivary and descending inputs; see for example Miller, Nezlina & Oscarsson, 1969*a*, *b*). However, for 'spontaneous' or 'background' complex spikes in individual Purkinje cells the probability of occurrence when averaged over many steps shows only minor and inconstant fluctuations during step cycles undertaken by awake cats (Andersson & Armstrong, 1987; Armstrong *et al.* 1988), suggesting that olive cell excitability remains approximately constant. It is therefore likely either that the step-related variation in SOCP excitability arises at a pre-olivary level or that it depends on fluctuations in olive cell excitability that are small but occur near-synchronously in a number of cells. In view of the evidence for a significant degree of electronic coupling between olive cells (Llinás & Yarom, 1981*a*) this latter possibility cannot be ignored.

However, perhaps the most attractive (though speculative) explanation that might be offered to reconcile single-unit studies with the present results would suppose that spinal input to the olive (or to some pre-olivary stage of the SOCP) varies cyclically during stepping but that the cells concerned also receive a 'mirror-image' input from another source so that overall excitability is near-constant. This could account for the fact that in walking decerebrate preparations the 'background' probability for complex spikes does vary markedly over the course of the step cycle (Udo, Matsukawa, Kamei, Minoda & Oda, 1981; Kim, Wang & Ebner, 1987): in these preparations activity in several descending paths is presumably disrupted, which might remove or disorganize the mirror-image signal postulated above.

A theoretical advantage of an argument along these lines is that it has obvious affinities with the proposal that some SOCPs convey to the cerebellum error signals derived via a comparison between an efference copy of a central 'instruction to move' and feedback signals from the cord regarding the effect of the instruction on the cord and the actual movement achieved (Miller & Oscarsson, 1970; Oscarsson, 1980). Unexpected perturbation of an on-going movement might be expected to produce a mismatch between intention and achievement and such perturbations indeed result in olivary discharges (Gellman *et al.* 1985; Andersson & Armstrong, 1987). The present finding that test SOCP volleys are less well transmitted at some

times than at others may imply that there are times during the step cycle when it would be 'undesirable' for an error signal to reach the c_2 zone because it might evoke a behaviourally inappropriate change in cerebellar output. A similar argument has been offered by Forssberg (1979) in respect of the observation that although a cutaneous stimulus to the foot dorsum evokes a reflex flexion of the limb during the swing phase of the step cycle, the same stimulus during stance evokes an extensor thrust: a flexion when the limb is load-bearing might imperil the equilibrium of the animal and it is therefore 'appropriate' that the reflex circuitary is modulated (indeed sign-reversed) in the course of the step cycle.

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