J. Physiol. (1979), **287**, *pp.* 1–14
With 5 text-figures
Printed in Great Britain

MOSSY AND CLIMBING FIBRE MEDIATED RESPONSES EVOKED IN THE CEREBELLAR CORTEX OF THE CAT BY TRIGEMINAL AFFERENT STIMULATION

BY FREDERICK W. J. CODY AND HELEN C. RICHARDSON

From the Department of Physiology, the Medical School, Stopford Building, University of Manchester, Manchester M13 9PT

(*Received 7 June 1978*)

SUMMARY

1. The trigeminal input to the cerebellar cortex was studied by recording mass and unitary responses evoked by electrical stimulation of individual trigeminal cutaneous and muscle nerve branches, in cats lightly anaesthetized with sodium thiopentone.

2. The trigeminal projection area of the cerebellar cortex comprised essentially lobule HVI, but included adjacent folia of lobules HV and HVIIA.

3. Each trigeminal branch had a 'patchy' representation throughout the projection area and there was extensive convergence of individual afferents at each site. Exact combinations of convergent inputs varied between loci, the projection from the muscle nerve being weaker. No other differential representation of individual trigeminal branches was evident.

4. Responses were evoked by stimulation of both ipsi- and contralateral trigeminal afferents but contralateral projections were present at fewer sites.

5. Mass responses to stimulation of individual trigeminal branches comprised mossy fibre and climbing fibre-mediated potentials, although both components were not always present. Latencies for mossy and climbing fibre responses, evoked by ipsilateral nerve stimulation, were in the ranges 5–8 msec and 11–29 msec respectively.

6. Unitary responses of Purkinje cells activated by trigeminal inputs also revealed convergence from individual ipsilateral afferent sources (28% influenced by one ipsilateral trigeminal branch, 48% by two branches, 17% by three branches and 7% by four branches).

7. Response patterns comprised one or more of the following: short latency (3–8 msec) simple, mossy fibre-mediated spikes, 'delayed' (10–25 msec) simple spikes and climbing fibre-mediated multiple spike bursts (9–35 msec).

INTRODUCTION

The existence of cerebellar inputs from the face and the general location of this representation within the cerebellar cortex are well known (Adrian, 1943; Snider & Stowell, 1944), but there has been little detailed analysis of the trigemino-cerebellar projection.

Recently, Miles & Wiesendanger (1975*a*, *b*) have studied climbing fibre-mediated cerebellar field potentials and unitary responses elicited by stimulation of individual

trigeminal cutaneous nerve branches in barbiturate anaesthetized cats. The extent of the trigeminal projection area (lobules HV, HVI and HVIIA) was broadly similar to that previously described. A 'patchy' somatotopy similar to that observed in the anterior lobe for limb nerve afferents (Eccles, Provini, Strata & Taborikova, 1968*a*, *b*) was found, in which convergence from individual trigeminal branches was a marked feature. These findings were supplemented by the identification, in rats, of a population of inferior olivary neurones, generally believed to be the major source of climbing fibres, activated by trigeminal afferent stimulation (Cook & Wiesendanger, 1976). No evidence of evoked mossy fibre-mediated responses was obtained, their absence being attributed to the relatively deep level of barbiturate anaesthesia.

In view of several unique anatomical and physiological features of the trigeminal sensory system (Darian-Smith, 1973; Sessle, 1977) it is of relevance to determine whether the functional organization of the trigeminal cerebellar input corresponds to the pattern of projection, by mossy fibre and climbing fibre pathways, established for a variety of spinal somatic afferents (Eccles *et al.* 1968*a*, *b*; Latham & Paul, 1968; Oscarsson, 1968, 1969; Coffey, Godwin-Austen, MacGillivray & Sears, 1971). Therefore the present experiments were undertaken to identify and to characterize both mossy and climbing fibre-evoked components of cerebellar responses arising from stimulation of trigeminal muscle and cutaneous branches. Preliminary results have been briefly reported (Cody & Richardson, 1977).

METHODS

Experiments were performed on eighteen adult cats weighing 2·3–4·0 kg. Animals were anaesthetized with nitrous oxide (80% in oxygen) and halothane (0.5%) during surgical preparation and thereafter a light level of anaesthesia was maintained by I.V. administration of sodium thiopentone (approximately 6 $mg.kg^{-1}$ hr^{-1}). During the recording period animals were immobilized with gallamine triethiodide, thereby eliminating contamination of neural records by muscle action potentials due to excitation of motor fibres following masseter nerve (*i*MSN) stimulation or reflex contractions following trigeminal cutaneous nerve stimulation. Regulated artificial ventilation was applied, blood pressure monitored and maintained above 100 mmHg, and deep body temperature held at 38 ± 1 °C. The animal's head was secured in a stereotaxic frame (La Precision Cinématographique), which allowed access to the face, a unilateral craniotomy made and the occipital cortex reflected to expose cerebellar lobules V, VI and VII. After removing the dura, brain surfaces were protected by a thin layer of immuno-agar in 0·9% saline.

Stimulation procedures. Electrical stimulation of the three major cutaneous trigeminal nerve branches supraorbital (*i*SON), infraorbital (*i*ION) and mental (*i*MLN), ipsilateral to the recording site, was by bipolar stainless-steel electrodes placed percutaneously at the sites of emergence of the nerves from the skull (cf. Miles & Wiesendanger, 1975*a*). A trigeminal muscle branch (*i*MSN) was isolated and a bipolar sleeve electrode placed upon the intact nerve. For each cutaneous branch, stimulus strength was adjusted to produce small reflex twitches of the facial musculature, but to be just subthreshold for the jaw-opening reflex. Stimulation at this intensity (typically $< 5{\cdot}0$ V, 0·05 msec) excites only large diameter myelinated afferents since it is well documented that Aδ activation invariably elicits a jaw-opening reflex (Thexton, 1968; Keller, Vycklicky & Sykova, 1972). *i*MSN stimulation was of a strength (2–3 V, 0·05 msec) just sufficient to cause an orthodromically mediated twitch in masseter muscle, resulting from Aα excitation. It is therefore probable that the afferent volley from *i*MSN was confined to Gr I (and possibly some Gr II) proprioceptive fibres.

Recording procedures. Mass evoked potentials and unitary responses were recorded from lobules V, VI and VII of the cerebellar cortex (Larsell, 1953), using glass-coated tungsten

micro-electrodes (Merrill & Ainsworth, 1972) of impedance 2–5 MΩ at 1 kHz, or occasionally glass micropipettes filled with 2 M-NaCl (DC resistance 2–6 MΩ). Signals were amplified by conventional means and together with stimulus pulses, were recorded on FM magnetic tape (band width 0–5 kHz). Electrode tracks were made essentially perpendicular to the cortical surface to a depth of 4–5 mm. Small electrolytic lesions were made periodically to allow subsequent verification of recording sites in histological sections.

Data analysis. Cerebellar evoked mass responses were routinely averaged (32 or 64 responses; 256 addresses; 0·4 msec in width) and the technique of laminar field potential analysis (Eccles *et al.* 1968*a*) used in the identification of mossy fibre and climbing fibre responses during cerebellar mapping. The technique of recording climbing fibre responses as negative extracellular field potentials in the molecular layer provides an accurate index of the precise location and strength of climbing fibre activation of Purkinje cells. In assessing mossy fibre inputs particular attention was paid to the negative potentials (N_1, N_2) generated at short latency in the granular layer due to excitation of granule and Golgi cells (Eccles *et al.* 1968*a*), in addition to the more indirectly evoked N_3 wave observed in the molecular layer.

Individual unitary responses were recorded from Purkinje cells, identified by the presence of multiple spike bursts characteristic of climbing fibre responses (Eccles, Faber, Murphy, Sabah & Taborikova, 1971). The depression of simple spike activity following a climbing fibre response served to indicate that both types of discharge were generated by the same cell (Eccles *et al.* 1971; Latham & Paul, 1971). Post-stimulus time histograms of the responses were constructed from either thirty-two or sixty-four trials (256 addresses, 2 msec in width).

RESULTS

Trigeminal evoked mass responses

The area of the cerebellar cortex in which trigeminal evoked potentials were recorded, ipsilateral to the stimulating electrodes, during the course of exploration of lobules, V, VI and VII is shown in Fig. 1*A*. Both mossy and climbing fibre responses were evoked by stimulation of each of the trigeminal nerve branches tested throughout a region which comprised essentially the paravermal hemispheral zone of lobule HVI, but in addition, included the immediately adjacent folia of lobules HV and HVII. The general extent of the trigemino-cerebellar projection determined in the present experiments agrees closely with that described by Miles & Wiesendanger (1975*a*) for trigeminal cutaneous climbing fibre inputs.

Form of trigeminal evoked responses and identification of mossy and climbing fibre-evoked potentials

Examples of the complex field potentials generated at two locations in the molecular layer of lobule HVI by stimulation of an ipsilateral trigeminal cutaneous branch (*i*SON) are illustrated in Fig. 2. In both the individual responses (Fig. 2*A*, *C*) and their corresponding averaged records (Fig. 2*B*, *D*) two components may be identified, short latency (5 and 6·5 msec) small amplitude negative waves and later (12 and 13 msec) large amplitude negative potentials.

The later component of most individual responses has a prominent secondary wave, which is particularly clear in the averaged records, a common feature of the large evoked potentials of the inferior olive (Armstrong, Eccles, Harvey & Matthews, 1968). In addition to differences in latency and amplitude, the early and late components showed a number of distinguishing features which allowed them to be characterized as mossy fibre-evoked and climbing fibre-evoked potentials respectively, according to the criteria of Eccles *et al.* (1968*a*): (i) effect of repetitive stimulation

(Fig. 2*E*); (ii) depth profiles (Fig. 1*B*, *C*); and (iii) facilitation of the late component by double nerve volleys.

Distribution of trigeminal evoked cerebellar cortical responses

Fig. 3*A* summarizes the pattern of distribution of trigeminal evoked responses in lobule HVI determined in three representative experiments. Whilst precise details of topographical relationships cannot be extrapolated between experiments, the composite map serves to illustrate several features of the trigeminal projection common to each experiment: (i) responses to both trigeminal cutaneous (*i*SON, *i*ION and

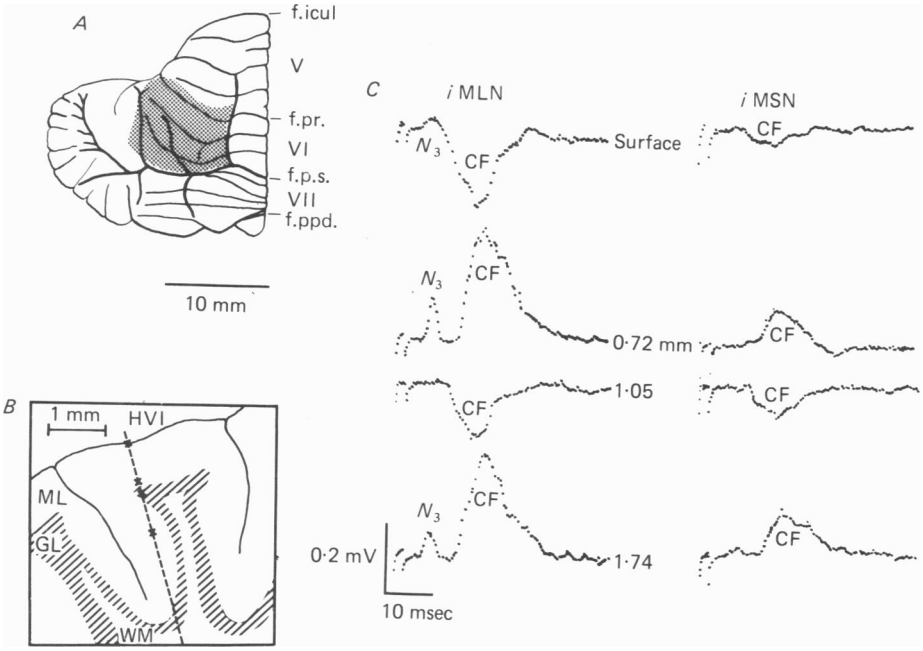

Fig. 1. *A*, line drawing of part of the surface of the left cerebellar cortex (lobules V, VI and VII) in which the stippled region represents the trigeminal projection area. Abbreviations: f.icul. 1, interculminate 1 fissure; f.pr., primary fissure; f.p.s., posterior superior fissure; f.ppd., prepyramidal fissure. *B*, line drawing of a histological section showing the course of an electrode penetration (interrupted line) in lobule HVI. Crosses indicate the depths at which the responses shown in *C* were obtained. Abbreviations: ML, molecular layer; GL, granular layer; WM, white matter. *C*, averaged (thirty-two sweeps) mass responses to pairs of stimuli to *i*MLN and *i*MSN at the depths indicated. The polarity changes of the late component are typical of climbing fibre (CF) responses.

*i*MLN), and muscle (*i*MSN) nerve stimulation were recorded widely throughout the ipsilateral lobule HVI; (ii) the degree of convergence of afferent projections from the four nerve branches varied considerably between individual cerebellar recording sites; and (iii) the exact combination of mossy and climbing fibre inputs from the four nerve branches and their relative efficacies differed uniquely between individual cerebellar cortical foci.

Considering the twenty-two electrode tracks shown in Fig. 3*A*, six sites received

inputs from each of the four trigeminal branches, six from three branches, four from two branches, and four from one branch. No responses were elicited at the remaining two sites. There was a fairly even projection from each of the four individual nerve branches in terms of the number of cerebellar foci at which they evoked responses (*i*SON, fourteen sites; *i*ION, twelve; *i*MLN, sixteen; *i*MSN, twelve). No consistent differential distribution of representation was apparent within the trigeminal projection area, rather each nerve branch had 'mosaic-like' representation, similar to that described in the anterior lobe for spinal nerve inputs (Eccles *et al.* 1968*a*). No single

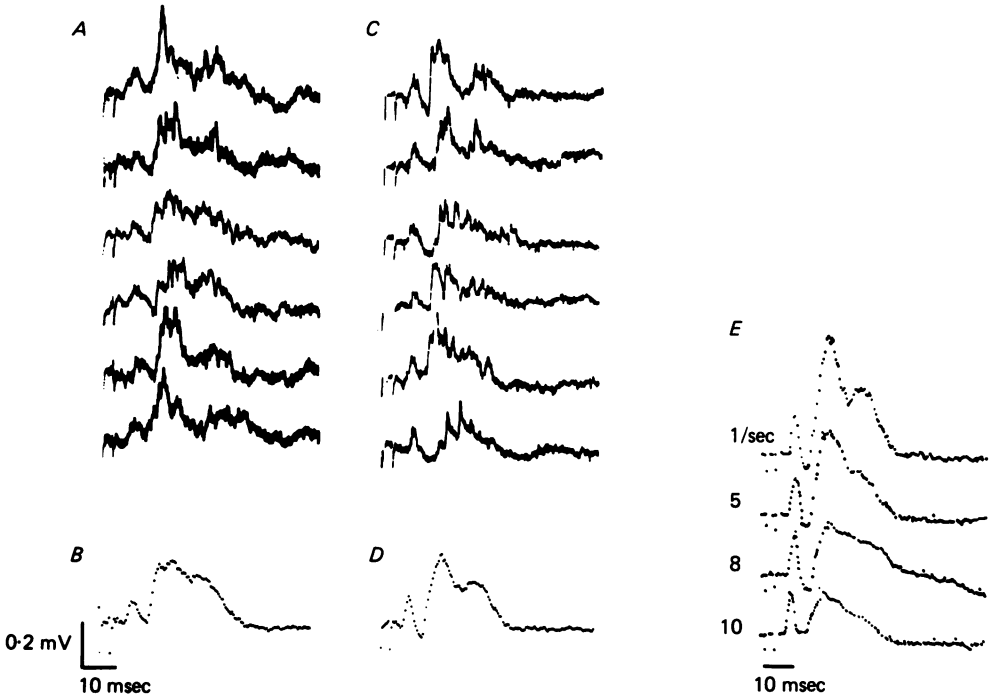

Fig. 2. *A* and *C* show series of consecutive mass responses, evoked by paired stimulation of *i*SON, at two sites within the molecular layer of lobule HVI. Corresponding averages (thirty-two sweeps) are shown in *B* and *D*, respectively. *E*, the effect of varying frequency (Hz indicated at left-hand side) of *i*SON stimulation upon the averaged field potential recorded from the same site as the records of *C* and *D*. Note the progressive reduction in amplitude of the late component with frequency which characterizes climbing fibre-evoked potentials.

focus has been encountered in any experiment at which *i*MSN alone was represented, a muscular input being accompanied always by a projection from at least one of the cutaneous sources. Conversely, it was commonplace for cutaneous inputs to exist in the absence of an effective projection from the muscle nerve.

The specimen averaged records (Fig. 3*B*, *C*, *D*), obtained in a single experiment from sites at which each trigeminal nerve branch evoked a response, indicate that mossy- and climbing fibre-evoked responses were not always co-existent in Fig. 3*C*, for example, small but distinct mossy fibre responses were elicited with *i*SON and *i*ION stimulation, in addition to climbing fibre responses, whereas for *i*MLN and

*i*MSN only climbing fibre-evoked potentials were present. No instances of mossy fibre responses in the absence of climbing fibre responses have been apparent in the mass potentials recorded.

For the recording sites in Fig. 3*A*, the relative incidence of mossy and climbing fibre responses, expressed as a ratio (*MF/CF*) was 10/14 (71 %), 8/12 (67 %), 5/16 (31 %) and 10/12 (83 %) for *i*SON, *i*ION, *i*MLN and *i*MSN respectively. Typically, clear mossy fibre responses were associated with large climbing fibre potentials (e.g.

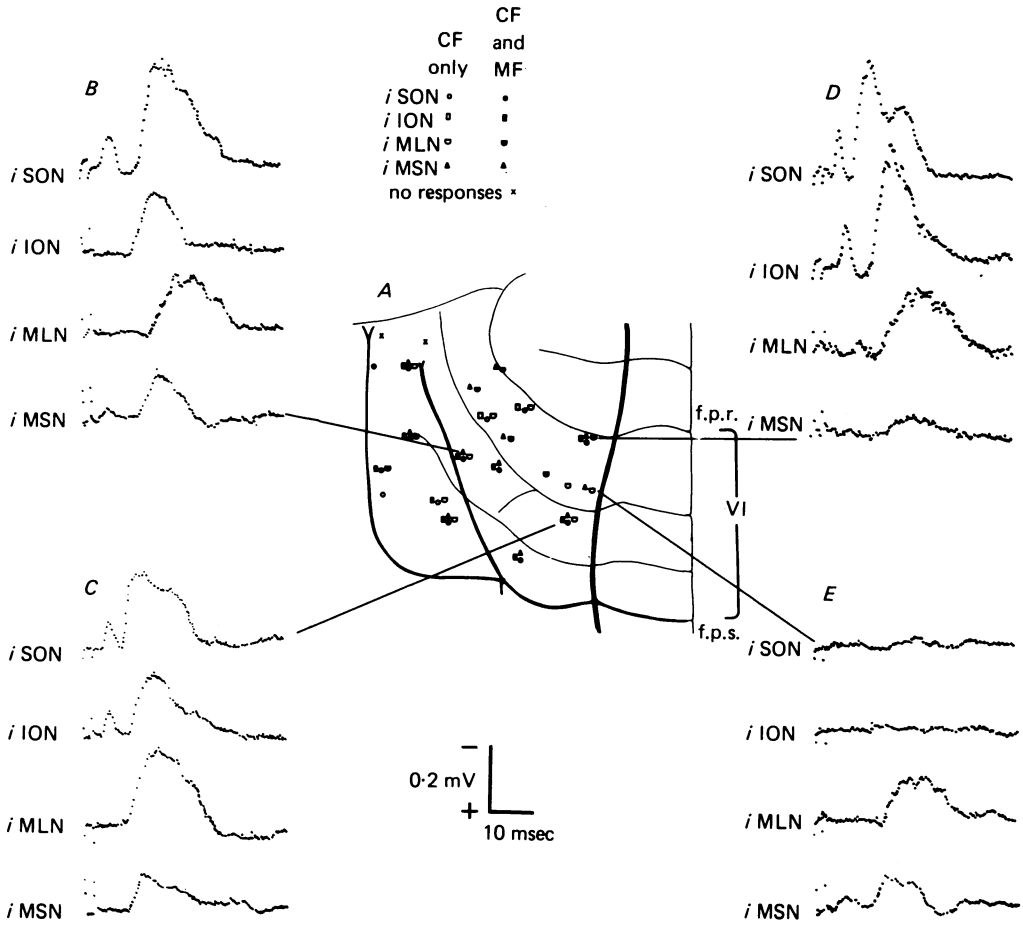

Fig. 3. *A*, composite surface map, obtained in three experiments, showing the combinations of trigeminal-evoked mass responses recorded at different sites. Averaged (thirty-two sweeps) responses, to each trigeminal input, recorded at four sites, in the molecular layer, are shown in *B*, *C*, *D* and *E*. MF, mossy fibre. CF, climbing fibre.

Fig. 3*C*, *D*: *i*SON, *i*ION; Fig. 3*B*: *i*SON), although in several instances large amplitude climbing fibre responses were found in the absence of corresponding mossy fibre activation (e.g. Fig. 3*B*, *C*, *E*). One generalization, illustrated in Fig. 3 and common to all experiments, was that both mossy and climbing fibre responses evoked by

*i*MSN stimulation tended to be smaller than those elicited by cutaneous (*i*SON, *i*ION, *i*MLN) afferent volleys.

Latencies of trigeminal mossy and climbing fibre-evoked responses

The latencies measured from the first stimulus to the onset of the mossy fibre-evoked potentials (N_3 waves) recorded in the molecular layer varied little, either between different trigeminal nerve branches or between different recording sites, the range being 5–8 msec. By contrast the latencies of the simultaneously recorded climbing fibre responses differed widely, both between nerve branches and electrode tracks. Ranges, measured from the first stimulus artifact were: *i*SON, 11–20 msec; *i*ION, 11–28 msec; *i*MLN, 11–25 msec; *i*MSN, 11–29 msec. However, these values slightly overstate latencies, since at the low stimulus strengths employed single volleys were often ineffective in eliciting climbing fibre responses and consequently paired stimuli (separation 2–4 msec) were routinely applied. Analysis of the spatial distribution of latencies of trigeminal evoked climbing fibre potentials indicated that latencies tended to be longer in the band of cortex adjacent to and extending approximately 1 mm laterally from the paravermal vein than those recorded in the remainder of the projection area. The former area corresponds to part of the termination region of forelimb dorsal funicular- and dorsolateral funicular-spino-olivocerebellar pathways (see Armstrong, 1974). No further zonal subdivision of the trigeminal projection area, on the basis of climbing fibre response latencies, analogous to the parasagittal strip-like organization of the various spino-olivocerebellar projections (see Oscarsson, 1973; Armstrong, 1974) was apparent. However, the fairly limited numbers of widely spaced electrode penetrations made in individual preparations, in the present study, may have been inadequate to reveal the presence of such an arrangement. When compared within a given electrode penetration, the latencies of climbing fibre responses to *i*SON were shorter than those to each of the other nerve branches ($P < 0{\cdot}01$) but there was no significant difference between the latencies to *i*ION, *i*MLN and *i*MSN.

Responses evoked by contralateral trigeminal inputs

Bilateral trigeminal inputs to lobule HVI were not routinely studied, but in three experiments two contralateral nerves (a cutaneous (*c*SON) and a muscular (*c*MSN) branch) were tested in addition to the four ipsilateral branches. Comparison of responses evoked by *c*SON and *c*MSN with those to *i*SON and *i*MSN, respectively, indicate: (i) contralateral cutaneous and muscular inputs evoked responses in lobule HVI by mossy fibre and climbing fibre pathways; (ii) when present, the amplitudes of contralaterally evoked mass potentials were generally similar to those elicited by equivalent ipsilateral inputs; and (iii) the latencies of contralaterally evoked mossy fibre responses were no more than 1 msec greater than those elicited by equivalent ipsilateral sources and differences were often indistinguishable in averaged records, whereas those of climbing fibre responses were consistently 2–5 msec greater; (iv) contralaterally evoked mossy and climbing fibre responses were apparent at fewer (approximately one third of) sites, although these were not restricted to the caudo-medial part of the trigeminal projection area as has been reported for cutaneous climbing fibre projections by Miles & Wiesendanger (1975*a*).

Trigeminal evoked responses of Purkinje cells

An analysis has been made of the responses of forty-six Purkinje cells, in lobule HVI (identified by the presence of climbing fibre responses in their discharges, Eccles, Llinas & Sasaki, 1966) to electrical stimulation of individual trigeminal branches.

Thirteen (28 %) of these units were influenced by a single ipsilateral trigeminal branch. The remainder received varying combinations of convergent inputs: twenty-two (48 %) units responded to stimulation of two branches, eight (17 %) units to three branches and three (7 %) units to each of the four nerves tested. The numbers

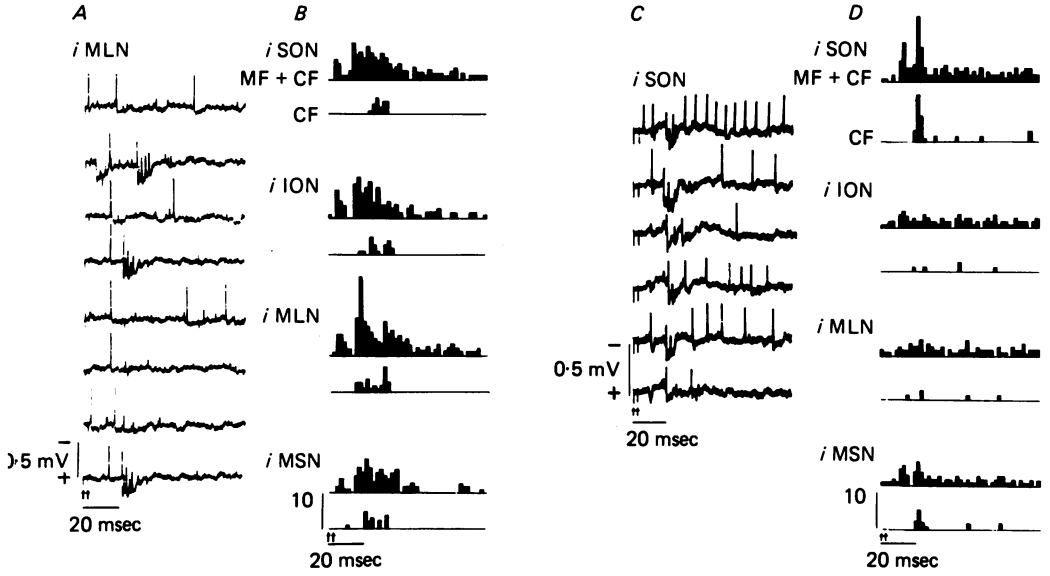

Fig. 4. Responses of two Purkinje cells in lobule HVI, showing mossy fibre (MF) and climbing fibre (CF)-mediated discharges, to stimulation of individual trigeminal branches. *A*, specimen records of the discharge of a Purkinje cell following paired volleys in *i*MLN. *B*, post-stimulus time histogram (bin width, 2 msec; sixty-four consecutive trials) for each trigeminal nerve. Two histograms are presented for each branch, the upper representing a combination of simple spike and CF response discharge and the lower CF responses only. *C*, specimen responses of a different unit to paired *i*SON stimulation. *D*, histograms, as described in *B*, for the cell shown in *C*.

of Purkinje cells activated by the different trigeminal branches were: *i*SON thirty-two (70 %); *i*ION twenty-seven (58 %); *i*MLN twenty-one (46 %); *i*MSN thirteen (29 %).

Effective trigeminal sources evoked unitary discharge patterns comprising three main response elements: (i) short latency (3–8 msec) simple mossy fibre-mediated spikes; (ii) 'delayed' (10–25 msec) simple spikes and (iii) climbing fibre multiple bursts (9–35 msec). Interspersed were periods of depressed discharge. Considering the responses of all Purkinje cells to all inputs tested, the response patterns of forty units could be allocated to one of four basic categories according to the precise combination of the aforementioned components: (i) short latency simple spikes and later climbing fibre responses (seven units); (ii) both short latency and 'delayed' mossy

fibre-mediated spikes and climbing fibre potentials (two units); (iii) 'delayed' simple spikes and climbing fibre responses (twelve units); and (iv) climbing fibre responses only (nineteen units).

For the majority of individual Purkinje cells which received convergent inputs from two or more trigeminal nerves (twenty-one out of thirty-three) a common response pattern was generated by each effective source.

Fig. 4 illustrates two units whose responses to trigeminal stimulation comprised both simple spikes and climbing fibre bursts as shown in the specimen records (*A*, *C*). For the four ipsilateral trigeminal branches (Fig. 4*B*) in which (*a*) combined mossy and climbing fibre-mediated discharges and (*b*) climbing fibre responses alone are displayed separately, post-stimulus time histograms reveal generally similar response patterns for each nerve. In each of the 'combined' histograms, a

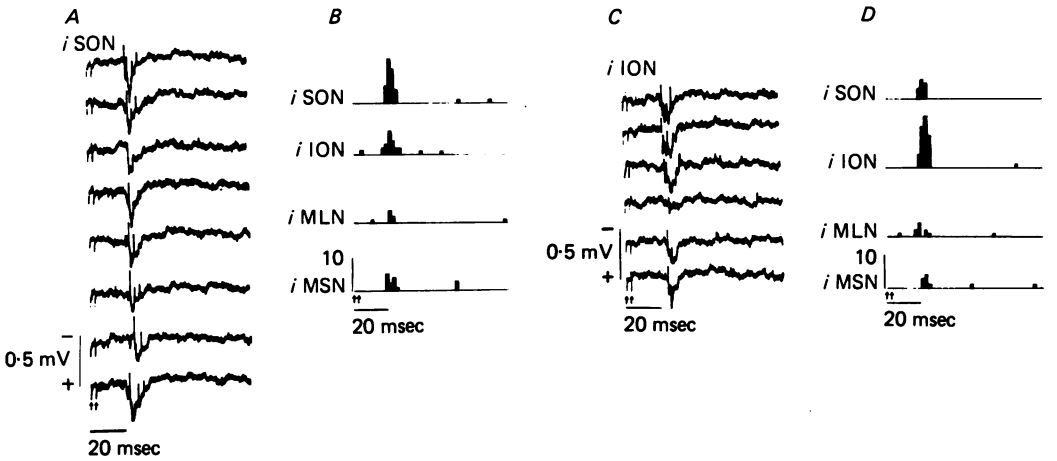

Fig. 5. Responses of two Purkinje cells in lobule HVI, showing only climbing fibre-mediated responses. *A* and *C* show specimen records of the two units following, respectively, pairs of stimuli to *i*SON and *i*ION. Corresponding post-stimulus time histograms (thirty-two consecutive responses) are given in *B* and *D* respectively.

small peak is present at 4–8 msec and a second, more protracted elevation commences at approximately 14 msec. In addition to 'delayed' simple spike activity, the latter peak is associated with climbing fibre responses as indicated by the distribution of their latencies (14–34 msec) shown in the accompanying histograms of climbing fibre responses alone. This response pattern conforms to type II as defined above. By contrast, the responses of the unit featured in Fig. 4*C*, *D* differed in two main respects: (i) only two trigeminal branches (*i*SON and *i*MSN) clearly evoked responses; (ii) whilst the two effective nerves both elicited similar response patterns consisting of mossy and climbing fibre-mediated discharges, histograms (Fig. 4*D*) indicate that there was an absence of evoked discharge at short latency, evoked activity occurring only between 10 and 26 msec. Climbing fibre responses were elicited by both *i*SON and *i*MSN stimulation at 18–26 msec. The response pattern of this Purkinje cell corresponded to type III. In fact for the trigeminal activated Purkinje cells in which mossy fibre-mediated responses were evoked, this pattern (type III) was the most common, being observed in 58 % of these units.

However, in many (42% of total) units, trigeminal stimulation evoked only climbing fibre discharges (response type IV). Two units in which trigeminal stimulation evoked, with high probability climbing fibre responses alone are shown in Fig. 5. For each unit (Fig. 5*A*, *B* and *C*, *D*), each ipsilateral branch elicited climbing fibre responses with latencies in the ranges 16–24 msec and 16–22 msec respectively.

DISCUSSION

Organization of trigeminal projections

Our results demonstrate that the trigeminal input to cerebellar cortical lobule HVI and adjacent folia of lobules HV and HVIIA from muscular and cutaneous afferent sources is mediated by both mossy and climbing fibre systems. The trigeminal projection area determined in the present study corresponds closely to the region (lobulus simplex) in which responses have been evoked in the cat by mechanical stimulation of the face (Adrian, 1943) and by electrical stimulation of cutaneous climbing fibre paths originating in the trigeminal region (Miles & Wiesendanger, 1975*a*), and in the lamb by trigeminal proprioceptive inputs (Azzena, Desole & Palmieri, 1970). Using natural stimulation Adrian (1943) and Azenna *et al.* (1970) described ipsilateral influences only. By contrast, with electrical stimulation the present authors and Miles & Wiesendanger (1975*a*) found that, although ipsilateral actions predominated, bilateral projections were also present. In view of histological evidence for a bilateral distribution of trigemino-cerebellar fibres (Larsell, 1947; Carpenter & Hanna, 1961) these discrepancies may be a consequence of the type of stimulation employed and the depth of anaesthesia.

The organization of mossy and climbing fibre projections from trigeminal afferents resembled, in many respects, that previously described for limb nerve inputs to the anterior lobe (Eccles *et al.* 1968*a*): (i) individual trigeminal branches had a 'patchy' representation and the combinations of convergent inputs varied between loci; (ii) convergence of muscular and cutaneous, mossy and climbing fibres, inputs to individual foci was often apparent; (iii) in many instances trigeminal nerve volleys, particularly in *i*MSN, were relatively ineffective in evoking mossy fibre potentials although they elicited large climbing fibre responses. The latter finding emphasizes the independence of the two afferent systems and reflects not only any differences in the distribution of mossy and climbing fibres within the cerebellar cortex but also differential actions of anaesthetics upon the two systems (Latham & Paul, 1971; Gordon, Rubia & Strata, 1973) and the efficacy of the peripheral nerve stimulation in generating activity within the respective pathways (Oscarsson, 1973).

However, whilst mossy and climbing fibre responses from a single trigeminal branch were not always co-existent, there was no evidence of a systematic spatial segregation of their inputs comparable with that reported for extra-ocular muscle afferents (Baker, Precht & Llinas, 1972). Mossy fibre responses were never encountered in the absence of associated climbing fibre potentials when individual trigeminal branches were tested, although the converse was often found.

Trigeminal cutaneous climbing fibre responses have previously been described in some detail (Miles & Wiesendanger, 1975*a*, *b*). The form, distribution and latency of these potentials evoked by stimulation of the same cutaneous afferents in the current

investigation, are in general accord with these earlier findings; in addition, our observations demonstrate the presence of trigeminal muscular climbing fibre inputs.

Mossy fibre responses have not previously been characterized in trigeminal-evoked cerebellar mass potentials, although Darian-Smith & Phillips (1964) recorded complex surface waves from lobulus simplex of chloralose-anaesthetized cats, following electrical stimulation of the lip, whose earliest component was of comparable latency to the mossy fibre potentials observed in the present experiments.

Patterns of activity evoked in Purkinje cells by trigeminal mossy fibre and climbing fibre inputs

The findings of mass potential analysis were supported by recordings of trigeminal-evoked discharges of Purkinje cells in lobule HVI. The timing of the early simple spikes and complex discharges generally corresponded, respectively, with the N_3 wave (attributed to impulse transmission in granule cells and synaptic excitation of Purkinje cells (Eccles *et al.* 1968*a*)) and negative climbing fibre potentials recorded in the molecular layer. However, post-stimulus time histograms indicated that in two units mossy fibre-mediated discharges preceded the shortest latency averaged fibre (N_3 wave) field potentials. As apparent in Fig. 2*A*, *C*, the latency of the N_3 wave varied slightly between consecutive trials, although latencies measured from averaged records do not reflect such variability. The occurrence of simple spikes before the onset of the averaged N_3 wave is not inconsistent with this field potential resulting from e.p.s.p.s being generated in a large number of Purkinje cells synchronously, since individual Purkinje cells may receive effective synaptic activation before the majority of neighbouring units. No separate component of the mass potential corresponding in latency to the 'delayed' simple spikes could be distinguished, but in view of the similarity of their latency to that of climbing fibre responses, such a contribution to field potentials would almost certainly be masked.

The precise patterns of response elicited in each Purkinje cell by stimulation of individual trigeminal branches and the combination of effective afferent sources varied widely, in a manner analogous to that seen for limb inputs on Purkinje cells in the pars intermedia (Eccles *et al.* 1971).

In some instances unitary discharge sequences comprised each of the above components; but more commonly, responses consisted of climbing fibre multiple bursts alone (cf. Miles & Wiesendanger, 1975*b*) or climbing fibre potentials accompanied by either short latency or 'delayed' simple spikes. When present, the latency of the 'early' mossy fibre discharges was similar to that found for extra-ocular muscle inputs (Baker *et al.* 1972), whilst that of climbing fibre responses corresponded to the range (11–26 msec) determined for trigeminal cutaneous unitary discharges by Miles & Wiesendanger (1975*b*). Analysis of the response patterns generated in Purkinje cells by different trigeminal branches failed to reveal any consistent dissimilarities, except that *i*MSN (muscle nerve) stimulation was relatively ineffective in producing mossy fibre responses, particularly at short latency.

The incidence of convergence from trigeminal afferents onto single Purkinje cells was slightly greater than that reported for cutaneous climbing fibre inputs alone (Miles & Wiesendanger, 1975*b*), even after allowance was made for the muscle nerve actions tested in the present experiments. However, this difference may be accounted

for by the lighter barbiturate anaesthesia employed in our study, in which the extent of convergence most commonly observed was from two ipsilateral branches. This occurred in 48% of trigeminal-activated Purkinje cells.

Pathways mediating trigemino-cerebellar responses

Information concerning the pathways by which trigemino-cerebellar responses are mediated is sparse. Brodal & Saugstad (1966) have reported that cerebellar lesions induce retrograde degeneration of neurones in the mesencephalic nucleus, which has been shown to contain the first-order cell bodies of jaw-elevating muscle spindles and periodontal mechanoreceptors (Cody, Lee & Taylor, 1972). However, no mossy fibre responses were recorded at the extremely short latencies which would be compatible with direct trigemino-cerebellar projections. Other degeneration studies have shown that fibre tracts from various nuclei within the trigeminal brainstem complex enter the cerebellum, e.g. superior trigeminal nucleus (Larsell, 1947), spinal trigeminal nucleus, particularly the interpolaris division (Carpenter & Hanna, 1961). Although these studies have not provided a clear consensus as to the site of termination of the projection fibres, these projections would appear to constitute the trigeminal homologues of spino-cerebellar pathways and may be responsible for the 'early' mossy fibre responses observed in the present study.

An alternative pathway, relaying in the lateral reticular nucleus, which could mediate relatively short latency trigeminal mossy fibre responses is indicated in the electrophysiological data of Darian-Smith & Phillips (1964). Latencies for transmission from the facial periphery to the cerebellar cortex, via individual lateral reticular neurones, were estimated to be in the range 2–22 msec (mode = 4–5 msec).

At present 'early' mossy fibre responses (3–8 msec) cannot be attributed unequivocally to either trigemino-cerebellar or trigemino-lateral reticular nuclear-cerebellar pathways on the basis of latency, since two factors indicate overlap of latencies of cerebellar activity evoked by these routes: (i) the discharge latencies of second order neurones in the trigeminal brainstem sensory complex to electrical stimulation of facial skin are indistinguishable from those of trigeminal activated lateral reticular neurones (Darian-Smith & Mayday, 1960; Darian-Smith, Procter & Ryan, 1963; Darian-Smith & Phillips, 1964); (ii) in view of the short conduction distances from the trigeminal brain stem nuclei and the lateral reticular nucleus to the cerebellum, it is unlikely that an appreciable difference in conduction time exists between these two paths (cf. Oscarsson, 1973). 'Delayed' trigeminal responses (10–25 msec) are presumably mediated by indirect trigemino-reticulo-cerebellar pathways.

It is generally accepted that the inferior olivary nucleus is a major source of climbing fibres (Armstrong, 1974). Histological evidence of trigemino-olivary connexions is limited to a description of a projection from the trigeminal nucleus caudalis to the medial accessory olive (Stewart & King, 1963). However, a recent electrophysiological study in rats has convincingly demonstrated that a group of inferior olivary neurones receive inputs from trigeminal cutaneous afferents (Cook & Wiesendanger, 1976). In approximately half of these units, convergence from individual trigeminal branches was found comparable to that observed in single Purkinje cells in the present study. The wide range of latencies of trigeminal-evoked

climbing fibre potentials observed in the present study may indicate that these responses were transmitted by a number of different pathways.

In conclusion, the present data from evoked mass potentials and Purkinje cell response patterns demonstrate two main features of the trigemino-cerebellar projection not previously described: (i) trigeminal muscle nerve inputs evoke responses in cerebellar cortical lobules HV, HVI and HVIIA by both mossy and climbing fibre systems; (ii) trigeminal cutaneous afferent information is transmitted to the same cerebellar projection area by mossy fibre pathways, in addition to the climbing fibre paths reported by Miles & Wiesendanger (1975*a*, *b*). Collectively these results emphasize the similarity of the pattern of the trigeminal projection to lobules HV, HVI and HVIIA to that of limb afferents to the anterior lobe (Eccles *et al.* 1968*a*; Eccles *et al.* 1971) and thus imply a common mode of cerebellar processing of sensory information. It has been postulated that a major function of the sensory input to the intermediate zone of the cerebellar cortex is in monitoring continuously the progress of 'on-going' movements and thereby to participate in 'on-line' correction (Eccles, 1973). In this context trigeminal cutaneous and proprioceptive signals may be particularly concerned in the control of jaw movements, and in support of this idea, electrical stimulation of the trigeminal cerebellar projection area has recently been shown to modify trigeminal α and fusimotor neuronal discharge (Sessle, 1977).

We are indebted to Professor S. Thomas and Dr D. H. Paul for critical reading of the manuscript and Mr W. Manning for technical assistance. This work was supported by the Science Research Council (grant B/RG/9415.8).

REFERENCES

ADRIAN, E. D. (1943). Afferent areas in the cerebellum connected with the limbs. *Brain* **66**, 289–315.

ARMSTRONG, D. M. (1974). Functional significance of connections of the inferior olive. *Physiol. Rev.* **54**, 358–417.

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

AZZENA, G. B., DESOLE, C. & PALMIERI, G. (1970). Cerebellar projections of the masticatory and extraocular muscle proprioception. *Expl Neurol.* **27**, 151–161.

BAKER, R., PRECHT, W. & LLINAS, R. (1972). Mossy and climbing fiber projections of extraocular muscle afferents to the cerebellum. *Brain Res.* **38**, 440–445.

BRODAL, A. & SAUGSTAD, L. F. (1966). Retrograde cellular changes in the mesencephalic trigeminal nucleus in the cat following cerebellar lesions. *Acta morph. neerl.-scand.* **6**, 147–159.

CARPENTER, M. & HANNA, G. (1961). Fiber projections from the spinal trigeminal nucleus in the cat. *J. comp. Neurol.* **117**, 117–131.

CODY, F. W. J., LEE, R. W. H. & TAYLOR, A. (1972). A functional analysis of the components of the mesencephalic nucleus of the fifth nerve in the cat. *J. Physiol.* **226**, 249–261.

CODY, F. W. J. & RICHARDSON, H. C. (1977). Trigeminal projections to the cerebellar cortex in the cat. *J. Physiol.* **267**, 41–42*P*.

COFFEY, G. L., GODWIN-AUSTEN, R. B., MACGILLIVRAY, B. B. & SEARS, T. A. (1971). The form and distribution of the surface evoked responses in cerebellar cortex from intercostal nerves in the cat. *J. Physiol.* **212**, 129–145.

COOK, J. R. & WIESENDANGER, M. (1976). Input from trigeminal cutaneous afferents to neurones of the inferior olive in rats. *Exp. Brain Res.* **26**, 193–202.

DARIAN-SMITH, I. (1973). The trigeminal system. In *Handbook of Sensory Physiology*, vol. II, ed. IGGO, A. pp. 271–314. Berlin: Springer-Verlag.

DARIAN-SMITH, I. & MAYDAY, G. (1960). Somatotopic organisation within the brainstem trigeminal complex of the cat. *Expl Neurol.* **2**, 290–309.

DARIAN-SMITH, I. & PHILLIPS, G. (1964). Secondary neurones within a trigemino-cerebellar projection to the anterior lobe of the cerebellum in the cat. *J. Physiol.* **170**, 53–68.

DARIAN-SMITH, I., PROCTER, R. & RYAN, R. D. (1963). A single neurone investigation of somatotopic organization within the cat's trigeminal brainstem nuclei. *J. Physiol.* **168**, 147–157.

ECCLES, J. C. (1973). The control of movement by the brain. In *The Understanding of the Brain*, pp. 106–145. New York: McGraw-Hill.

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

ECCLES, J. C., LLINAS, R. & SASAKI, K. (1966). The excitatory synaptic action of climbing fibres on the Purkinje cells of the cerebellum. *J. Physiol.* **182**, 268–296.

ECCLES, J. C., PROVINI, L., STRATA, P. & TABORIKOVA, H. (1968*a*). Analysis of the electrical potentials evoked in the cerebellar anterior lobe by stimulation of hind-limb and fore-limb nerves. *Exp. Brain Res.* **6**, 171–194.

ECCLES, J. C., PROVINI, L., STRATA, P. & TABORIKOVA, H. (1968*b*). Topographical investigations on the climbing fiber inputs from fore-limbs and hind-limb afferents to the cerebellar anterior lobe. *Exp. Brain Res.* **6**, 195–215.

GORDON, M., RUBIA, F. J. & STRATA, P. (1973). The effect of pentothal on the activity evoked in the cerebellar cortex. *Exp. Brain Res.* **17**, 50–62.

KELLER, O., VYCKLICKY, L. & SYKOVA, E. (1972). Reflexes from Aα and Aδ trigeminal afferents. *Brain Res.* **37**, 330–332.

LARSELL, O. (1947). The development of the cerebellum in man in relation to its comparative anatomy. *J. comp. Neurol.* **87**, 85–129.

LARSELL, O. (1953). The cerebellum of the cat and monkey. *J. comp. Neurol.* **99**, 135–199.

LATHAM, A. & PAUL, D. H. (1968). Projection of low threshold cutaneous afferent nerve fibres to the cerebellum of the cat. *J. Physiol.* **196**, 69–70*P*.

LATHAM, A. & PAUL, D. H. (1971). Spontaneous activity of cerebellar Purkinje cells and their responses to impulses in climbing fibres. *J. Physiol.* **213**, 135–156.

MERRILL, E. G. & AINSWORTH, A. (1972). Glass coated platinum-plated tungsten microelectrodes. *Med. biol. Engng.* **10**, 662–672.

MILES, T. S. & WIESENDANGER, M. (1975*a*). Organization of climbing fibre projections to the cerebellar cortex from trigeminal afferents and from the SI face area of the cerebral cortex in the cat. *J. Physiol.* **245**, 409–424.

MILES, T. S. & WIESENDANGER, M. (1975*b*). Climbing fibre inputs to cerebellar Purkinje cells from trigeminal cutaneous afferents and the SI face area of the cerebral cortex in the cat. *J. Physiol.* **245**, 425–445.

OSCARSSON, O. (1968). Termination and functional organisation of the ventral spino-olivocerebellar tract. *J. Physiol.* **196**, 453–478.

OSCARSSON, O. (1969). Termination and functional organisation of the dorsal spino-olivocerebellar path. *J. Physiol.* **200**, 129–149.

OSCARSSON, O. (1973). Functional organisation of spino-cerebellar paths. In *Handbook of Sensory Physiology*, vol. II, ed. IGGO, A., pp. 339–380. Berlin: Springer-Verlag.

SESSLE, B. J. (1977). Modulation of alpha and gamma trigeminal motoneurons by various peripheral stimuli. *Expl Neurol.* **54**, 323–339.

SNIDER, R. S. & STOWELL, A. A. (1944). Receiving areas of tactile, auditory and visual systems in the cerebellum. *J. Neurophysiol.* **7**, 331–357.

STEWART, W. A. & KING, R. B. (1963). Fiber projections from the nucleus caudalis of the spinal trigeminal nucleus. *J. comp. Neurol.* **121**, 271–286.

THEXTON, A. J. (1968). Jaw opening and closing reflexes in the cat. *J. Physiol.* **197**, 34–35*P*.