J. Physiol. (1980), **309**, *pp.* 357–374
With 5 text-figures
Printed in Great Britain

RESPONSES IN THE POSTERIOR LOBE OF THE RAT CEREBELLUM TO ELECTRICAL STIMULATION OF CUTANEOUS AFFERENTS TO THE SNOUT

BY D. M. ARMSTRONG AND T. DREW*

From the Department of Physiology, The Medical School, University of Bristol, University Walk, Bristol BS8 1TD

(*Received 14 March 1980*)

SUMMARY

1. Responses in the cortex of the posterior lobe of the cerebellum to electrical stimulation of afferent fibres from the skin of the snout have been analysed in decerebrate and pentobarbitone-anaesthetized rats by means of surface and microelectrode records. Single shocks were applied either to the exposed follicles of the mystachial vibrissae or to the infraorbital branch of the trigeminal nerve.

2. In *decerebrate* rats responses were mediated only via mossy fibre afferents. Stimulation of one side of the snout yielded responses with mean latency 2·4 ms throughout the uvula (largest ipsilaterally and in lobule IXa). Smaller responses with similar latency were present in both cerebellar hemispheres (largest ipsilaterally). The earliest discharges of Purkinje cells in lobule IXa occurred at latencies between 4·5 and 8·5 ms.

3. All components of the extracellular field potentials generated within the cortex by the mossy fibre input were detectable by surface recording with ball electrodes.

4. The earliest surface potentials had a latency of 0·55 ms (peak latency 0·8 ms); they arose through volume conduction from the brain stem of a potential which signalled arrival of the primary afferent volley. The short delay between this event and the arrival of the mossy fibre volley in the cerebellum suggests that only one synaptic relay occurs in the brain stem.

5. In *pentobarbitone-anaesthetized* rats surface responses mediated via mossy fibres persisted and were accompanied at slightly higher threshold by responses shown to be mediated via climbing fibres. The latter were present in descending order of amplitude in three sagittally directed zones, one in contralateral Crus 2 (minimum latency 13 ms), one in the vermis contralaterally near the mid line in lobule IXa (latency 16 ms) and a third in ipsilateral Crus 2 (latency 20 ms).

6. In the hemisphere the responses mediated via climbing fibres occurred within the somewhat larger zones activated via mossy fibres but in the vermis the two types of trigemino-cerebellar input influenced quite separate areas of cortex.

* M.R.C. Research Student.

0022-3751/80/2660-0856 $07·50 ©1980 The Physiological Society

INTRODUCTION

The snout with its well developed vibrissae is important in the rat as a source of tactile input used in guiding behaviour. Not unexpectedly, therefore, there is an elaborate whisker somatotopy in the thalamus and the somatosensory cerebral cortex (e.g. Waite, 1973; Welker, 1971, 1976). In view of this large central representation it might *a priori* be expected that the snout would provide correspondingly dense projections to the cerebellum and (by analogy with the organization of cutaneous input from the limbs) it is likely that such cerebellar input will be mediated via both the mossy and the climbing fibre afferents. Recent investigations indeed provide some confirmation. Thus Cook and Wiesendanger (1976) have found that electrical stimulation of the infraorbital nerve in the rat evokes discharges of neurones in the rostral half of the contralateral inferior olive with a latency of 19–30 ms; this demonstrates the existence of at least one ipsilateral (twice crossed) trigemino-cerebellar path relaying in the inferior olive and therefore mediated via climbing fibres. The cerebellar termination zone of this path remains to be determined though Axelrad & Crepel (1977) have recorded responses due to climbing fibre input (latency 15–25 ms) from a region near the Crus 1/Crus 2 boundary in the hemisphere.

Other recent studies have demonstrated responses in the cerebellar hemispheres (Shambes, Gibson & Welker, 1978), the uvula (Joseph, Shambes, Gibson & Welker, 1978) and lobulus simplex (Shambes, Beerman & Welker, 1978) to stimulation of the face and perioral structures. Attention was confined to the granular layer of the cerebellar cortex where micro-electrode recordings, mainly of multi-unit activity, indicated the discharge of many small elements believed to be granule cells excited via paths terminating as mossy fibres. Response latency was short (3–5 ms) and, perhaps surprisingly in view of the characteristics of responses mediated via mossy fibres in other species (e.g. Gordon, Rubia & Strata, 1973), they were resistant to pentobarbitone anaesthesia.

The present study was undertaken to determine the distribution of climbing fibre mediated responses evoked in the posterior lobe of the rat cerebellum by electrical stimulation of afferents from the skin of the snout and to provide additional data in regard to trigemino-cerebellar paths terminating as mossy fibres. Recordings were made from the exposed surface of the cerebellum and in addition micro-electrodes were used both to record extracellular field potentials from within the cerebellar cortex and to characterize the responses of individual Purkinje cells. Half the animals were anaesthetized with sodium pentobarbitone whilst the remainder were decerebrate preparations with residual levels of barbiturate present.

METHODS

Animals

Young adult rats weighing 300–550 g were used. All animals were initially anaesthetized with intraperitoneal injection of Na pentobarbitone (60 mg/kg; Sagatal; May & Baker). A tracheostomy was performed and the femoral vein was cannulated for administration of additional drugs. The animals were placed in a head holder (David Kopf Inc.) with the snout tilted downwards to allow optimum access to the posterior lobe of the cerebellum. Body temperature was maintained at 37°C by means of a thermostatically controlled electric blanket.

Beginning at the foramen magnum the cerebellum was exposed as far rostrally as lobule VIa (Larsell, 1952) using rongeurs. The dura and the atlanto-occipital membrane were reflected and the exposure was covered with warm liquid paraffin.

Thirteen animals were anaesthetized for the whole duration of the experiment, the anaesthetic level during the recording period being sufficiently light that a weak flexion was elicitable by pinching the toes. In the remaining eighteen animals a large craniotomy was made in the parietal bone and a mid-collicular decerebration was performed using suction. Recordings were begun between 3 and 5 h after the initial injection of anaesthetic. The decerebrate animals displayed brisk flexion reflexes and often made spontaneous 'walking' movements. These were suppressed by intravenous injection of gallamine triethiodide (Flaxedil; May & Baker) and the animals were artificially respired using pure oxygen.

Stimulation of trigeminal afferents

In the majority of experiments the skin of the snout was shaved, incised and reflected to expose the vibrissal follicles. Electrical stimuli were applied between a silver ball electrode positioned amongst the follicles and a second (diffuse) electrode formed by a wad of saline-soaked cotton wool. Less frequently the infraorbital nerve was dissected and mounted on hook electrodes for stimulation in continuity or after division distal to the electrodes. Rectangular stimulating pulses (duration 0·2 ms) were supplied from isolated stimulators (Devices Ltd). Stimulus intensities were regulated using a surface electrode to record the primary afferent volley from the brain stem (see Results). To confine stimulation to the large myelinated afferent fibres intensities were restricted to a maximum of 3–4 times threshold (T) for a just detectable volley.

Recording

Evoked potentials were recorded differentially using a spring mounted ball electrode on the cerebellar surface and an indifferent electrode placed on exposed bone alongside the cerebellum (amplifier bandpass 8 Hz–10 kHz).

Micro-electrode recordings were made using glass capillary electrodes filled with 4 M-NaCl (impedance 1–6 MΩ measured at 1 kHz). A Grass P 16 amplifier was employed and recordings were made differentially between the micro-electrode and a ball electrode on the bone.

RESULTS

Both in decerebrate rats and in rats anaesthetized with pentobarbitone widespread responses were evoked in the posterior lobe of the cerebellum by electrical stimulation of exposed vibrissal follicles (see Methods) or the infraorbital branch of the trigeminal nerve. The responses were unaffected by paralysis of the animal. Responses to vibrissal stimulation were unaffected by section of either the ipsilateral facial nerve or the contralateral infraorbital nerve but were abolished by section of the ipsilateral infraorbital nerve.

Decerebrate animals

Surface responses

In decerebrate preparations no responses were observed with the characteristics expected for responses mediated via paths terminating as climbing fibres (see below). Nevertheless large evoked potentials were present, the largest being invariably on lobule IXa of Larsell (1952). The threshold for these responses, whether evoked by snout or nerve stimulation, was not detectably different from the threshold for the primary afferent volley recorded from the brain stem (see below). Presumably therefore the lowest threshold afferents in the infraorbital nerve contribute to production

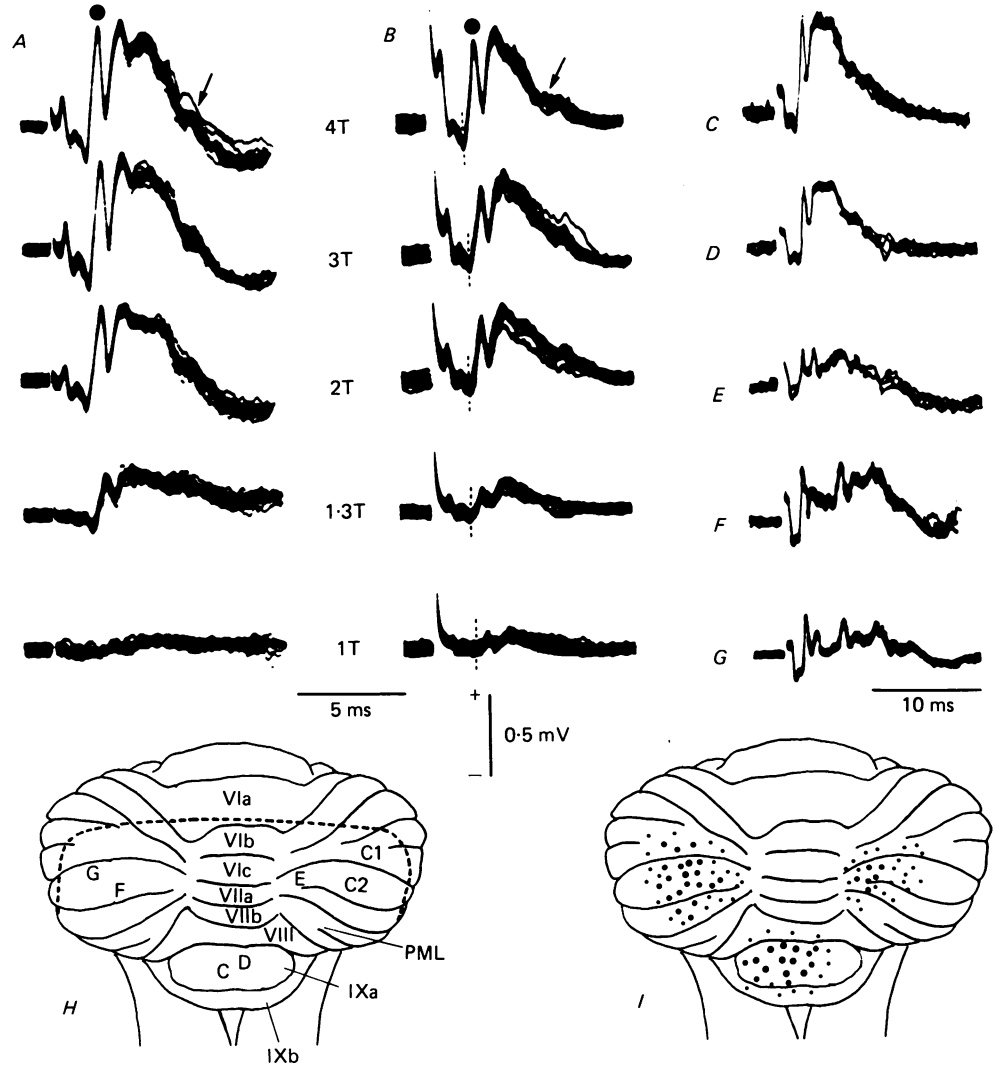

Fig. 1. Cerebellar surface responses to electrical stimulation of trigeminal afferents in decerebrate rats. *A* and *B*, effect of stimulus intensity on responses recorded from ipsilateral side of uvula (lobule IXa) in two animals. Stimulus intensity is in multiples of threshold (*T*) for first detectable response. Stimuli to exposed follicles of the mystachial vibrissae. In this and all other Figures traces are 5–10 superimposed sweeps except where stated. Symbols explained in text. 5 ms time scale applies to both *A* and *B*. *C*, *D*, *E*, *F* and *G* responses to stimulation of left infraorbital nerve recorded from the points designated by corresponding letters in Fig. 1*H* which also identifies the cerebellar lobules (cf. Larsell, 1952). 10 ms time scale applies *C*, *D*, *E*, *F* and *G*. In *H*, C1 is Crus 1; C2, Crus 2; *PML*, paramedian lobule. Maximum width of cerebellum 12 mm. Interrupted line indicates rostral limit of exposure. *I*. Over-all distribution of responses obtained by stimulation of vibrissal follicles on left of snout at intensity 4*T*. Similar results in all experiments. Approximate amplitude of positive deflexion (filled circles in *A* and *B*) is indicated by size of circles. Only responses larger than 100 μV shown. Smallest circles are amplitudes up to 250 μV; intermediate circles 250–500 μV; largest circles, 500 μV–1·3 mV.

of the cerebellar responses. The records of Fig. 1 *A* and *B* show examples of recordings from lobule IXa in two different animals (positive up). They demonstrate that when the stimulus intensity was raised from just threshold to 4*T* the responses remained essentially unchanged in form despite a considerable increase in amplitude. At 4*T* the average latency at the foot of the first really prominent component (interrupted line in Fig. 1 *B*) was 1·8 ms in eighteen preparations (S.D. 0·1 ms) whilst the latency of the peak (filled circles in Fig. 1 *A* and *B*) was 2·4 ms (S.D. 0·2 ms). Despite the complexity of the responses their form was essentially the same at all sites on the lobule and in all animals (compare Fig. 1 *A*, *B* and *C*). The major variation related to the deflexion arrowed in Fig. 1 *A* and *B* which was larger in some preparations than others (cf. Fig. 2 *A* and *B*). This deflexion was abolished or much reduced by small doses of barbiturate (10 mg/kg I.V.) so that its variable size (and threshold) may well reflect differences between the levels of residual anaesthesia present in different animals.

Responses were invariably largest on the ipsilateral side (Fig. 1 *C*) but were almost as large in the medial part of the contralateral half of the lobule (Fig. 1 *D*; see Fig. 1 *H* for recording sites).

Evoked potentials were not confined to the uvula but were present also at somewhat smaller amplitude on the cerebellar hemispheres. They were both larger and more widespread on the side ipsilateral to the site of stimulation. The form of such responses is illustrated by Fig. 1 *E*, *F* and *G* which were recorded respectively from points *E*, *F* and *G* in Fig. 1 *H*. The hemispheral responses were similar in latency and threshold to the responses on the uvula and in their initial phases were also similar in form. However, they were in general both longer lasting and more complex in wave form. The over-all distribution of the responses evoked by a 4*T* stimulus in a typical experiment is shown in Fig. 1 *I*, where the size of the filled circles represents the amplitude of the positive-going peak marked with a filled circle in Fig. 1 *A* and *B*.

Micro-electrode analysis of the evoked potentials

Extracellular field potentials

To clarify the nature of the evoked potentials on lobule IXa micro-electrode penetrations were made normal to its surface and extracellular field potentials were recorded after each 50 μm increment in depth. Typical series of potentials recorded in two different animals are shown in Fig. 2 *A* and *B* (*negative* up; depths indicated in μm); for comparison the top and bottom records in each series show the evoked potential recorded from the cerebellar surface close alongside the point of entry of the micro-electrode. It should be noted in regard to both *A* and *B* that those components occurring after the dashed line (i.e. with latency 1·8 ms or more) resemble very closely both in form and in depth distribution the extracellular field potentials recorded by Eccles, Sasaki & Strata, 1967 and by Sasaki & Strata, 1967 in the cat cerebellum and attributed to activation of the cortex via mossy fibres. Thus in the granular layer (see depths below 350 μm) there is a small positive deflexion succeeded by a larger negative peak. These have generally been attributed to arrival of the mossy fibre volley and termed respectively P_1 and N_1. In the present experiments the peak of the P_1 and foot of the N_1 component had average latency of 2·4 ms (S.D. 0·2 ms; cf. the peak marked by a filled circle in the surface records of Fig. 1 *A* and *B*). The N_1

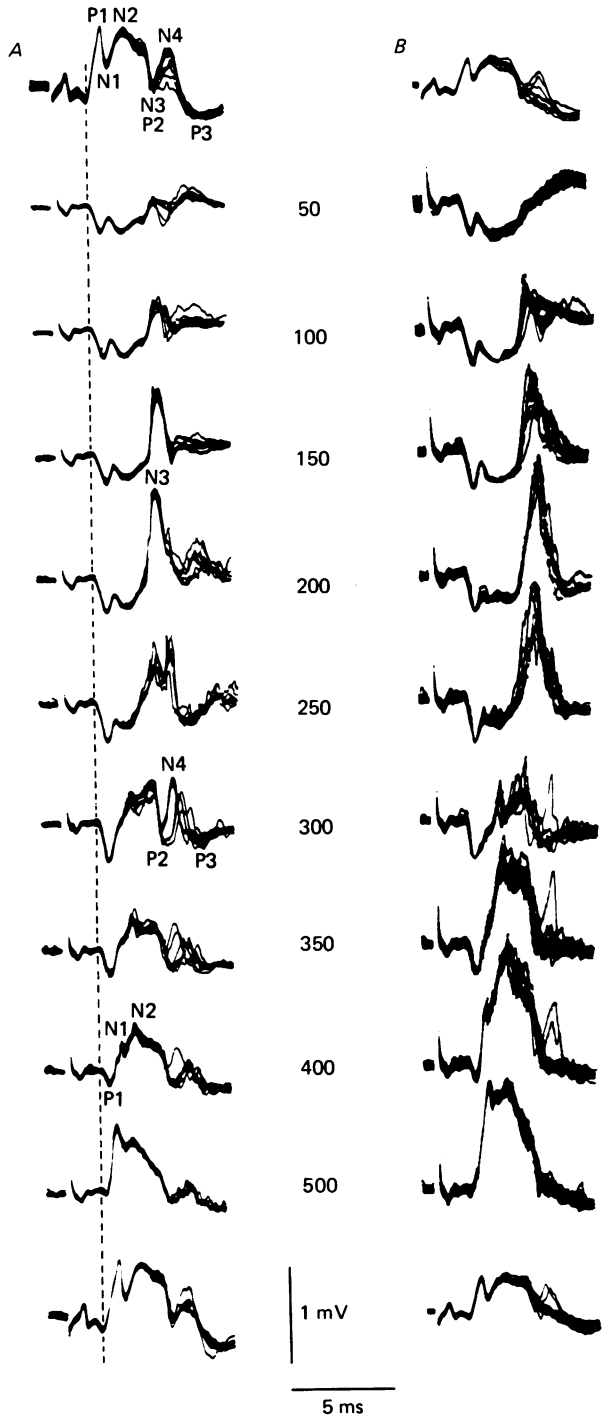

Fig. 2. Comparison of extracellular field potentials recorded within cortex of lobule IXa using micro-electrodes with surface responses recorded using ball electrodes. Stimulation of vibrissal follicles at $4T$; responses from ipsilateral side of lobule. *A* and *B* from different decerebrate animals. Surface responses are shown at top and bottom of each column; Positive up. For each micro-electrode trace estimated depth of electrode tip shown in μm. Note micro-electrode traces are ***negative*** up. For symbols and interrupted line in *A* see text. Voltage and time scales apply to all records.

deflexion is immediately succeeded in the granular layer by a negativity (N_2) ascribed to e.p.s.p.s and consequent discharges occurring in granule and Golgi cells directly excited by the mossy fibres. Discharges of the granule cells lead to propagated impulses in their axons (the parallel fibres) and consequently to activation of the parallel fibre – Purkinje cell synapses and to e.p.s.p.s in the Purkinje cell dendrites. These events are reflected in the molecular layer (i.e. at depths less than 300 μm) by a large extracellular negativity (N_3) largest at depth 200 μm. The peak of this wave occurred with latency 5·0 ms, i.e. delayed by 1·5 ms relative to the N_2 peak. This delay is ascribable to the low conduction velocity of the unmyelinated parallel fibres (0·3 m/s; Eccles, Llinás & Sasaki, 1966*b*). Simultaneously, the granular layer acts as a source to the active current sink in the molecular layer so that the N_3 wave is synchronous with a positive-going deflexion (P_2) at the Purkinje cell layer (i.e. at depth *ca.* 300 μm) and below.

According to the analysis of Eccles *et al.* (1967) if the parallel fibres succeed in discharging the Purkinje cells a negativity (N_4) is generated at and below the Purkinje cell bodies. Such a negativity is present fairly consistently at depths 300–500 μm in Fig. 2*A* and visible in some traces at depths 300–400 μm in Fig. 2*B*. This component is followed by a positivity (labelled at depth 300 μm). Eccles *et al.* did not compare surface responses with those recorded via micro-electrodes but it is clear from Fig. 2 that they are very similar in form to those recorded within the molecular layer and that discrete components are identifiable which coincide in time with the P_1, N_1, N_2, N_3, P_2, N_4 and P_3 responses. Appropriate labels have therefore been applied to the top surface record of Fig. 2*A*.

Initial responses

Inspection of the traces in Figs. 1 and 2 shows that the evoked potentials and extracellular field potentials so far discussed are in each case preceded by smaller deflexions which cannot be equated with any component of the mossy fibre mediated responses analysed by Eccles *et al.* (1967). In surface records these comprised a positive-negative biphasic response (see Fig. 1*A* and especially Fig. 3*A*, *B* and *C*) which was sometimes partly obscured by stimulus artifact (e.g. Fig. 1*B* and *C*). The latency of the first part of this response was 0·55 ms (S.D. 0·05 ms) and the latency of the positive peak was 0·8 ms (S.D. 0·1 ms). In depth profiles this component retained the same polarity throughout the track and showed no change in amplitude. These characteristics suggest it is generated some distance from the recording site and the very short latency suggests it may be a brain stem response recorded in the cerebellum via volume conduction. This explanation receives support from several other characteristics demonstrated for the response as illustrated in Fig. 3.

(*i*) *Following frequency.* The early potential was little diminished by high frequencies of stimulation: Fig. 3*A* shows superimposed responses recorded on the ipsilateral uvula during stimulation at 1/s whilst Fig. 3*B* and *C* show responses recorded at the same locus during stimulation at frequencies of 100 and 200/s respectively. At both latter frequencies the amplitude of P_1 deflexion is appreciably reduced and its time to peak is increased; the subsequent N_2 response is even more severely depressed. By contrast the early response remains unchanged. In fact it was little changed even by frequencies as high as 1000/s.

(*ii*) *Spatial distribution in the cerebellum.* The early response was very widespread on the cerebellum and its amplitude was not correlated with that of the later deflexions: it was often small at loci where they were large (e.g. Fig. 3*D*) or vice versa (e.g. Fig. 3*F* and *G*).

(*iii*) *Presence outside the cerebellum.* The possibility that the early response arose through volume conduction from a brain stem site was emphasized by the finding that a similar but much larger response could be recorded both from brain stem sites

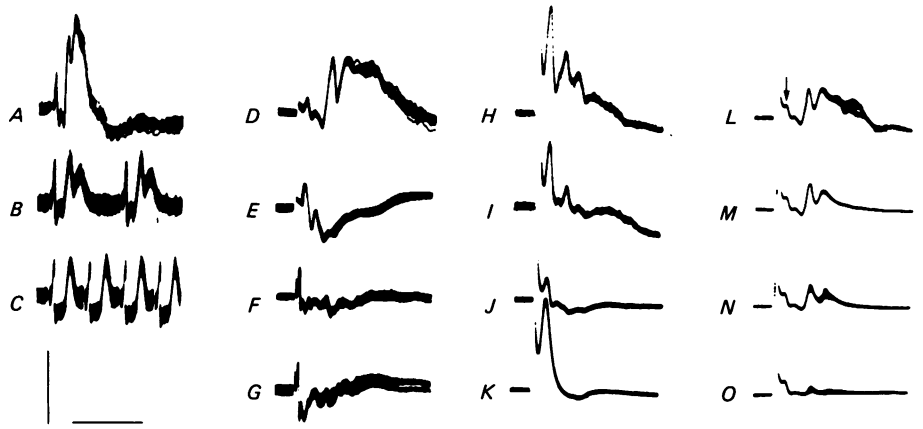

Fig. 3. Responses to stimulation of vibrissal follicles recorded with ball electrodes from cerebellum and brain stem. *A*, *B* and *C*, recorded from a point on ipsilateral side of lobule IXa during stimulation at 1 per sec in *A*, 100 per sec in *B*, 200 per sec in *C*. *D*, *E*, *F* and *G*, responses from different loci in one animal. *D*, from ipsilateral lobule IXa; *E*, dorsal surface of ipsilateral side of brain stem just caudal to cerebellum; *F*, lateral part of ipsilateral Crus 2; *G*, ipsilateral side of lobule IXb. Note different time scales for *D*, *E* and *F*, *G*. *H*, *I*, *J* and *K*, from brain stem after aspiration of cerebellum. *H* from floor of IVth ventricle; *I* and *J* same locus but with increasing thicknesses of Ringer-soaked fibrin foam between brain stem and electrode. *K*, floor of IVth ventricle 3 min after a lethal I.V. overdose of sodium pentobarbitone. *L*, *M*, *N* and *O*, responses from ipsilateral lobule IXa. *L* just before lethal I.V. overdose of anaesthetic; *M*, *N* and *O* at successively later intervals of 30 sec. See text for arrow. Time scale 10 ms for *A*, *B*, *C*, *F* and *G*; 5 ms for all other records. Voltage scale 2 mV for *L*, *M*, *N* and *O*; 1 mV for all other records.

just caudal to the cerebellum (Fig. 3*E*) and from the floor of the fourth ventricle exposed by aspirating the cerebellum (Fig. 3*H*). When this surface was covered by a dressing of gelfoam (Sterispon; May & Baker) soaked in Ringer solution a response was still recordable through the dressing (Fig. 3*I*). When the dressing was increased until its thickness approximated to that of the cerebellum the response (Fig. 3*J*) was very similar in both form and amplitude to those recorded with the cerebellum intact (cf. Fig. 1*A*). The threshold stimulus required to evoke a brain stem response was identical with that for the early cerebellar responses and no difference was detected between this threshold and that for the succeeding P_1 N_1 response.

(*iv*) *Effect of anoxia.* The short latency of the initial response and its resistance to high frequencies of stimulation indicate that it probably signals the primary afferent volley generated by the stimulus. This is also suggested by its relative resistance to

anoxia. Thus Fig. 3*K* shows a response recorded from the brain stem three minutes after the breathing of the animal was stopped by a lethal dose of anaesthetic. The traces of Fig. 3*M*, *N* and *O* were recorded at 30 s intervals from the cerebellum in another animal given an anaesthetic overdose. The initial response (arrow) remains virtually unchanged at 1·5 min (Fig. 3*O*) although the P_1 N_1 complex was much reduced at this time. Note that the N_2 wave was attenuated after as little as 30 s (Fig. 3*M*).

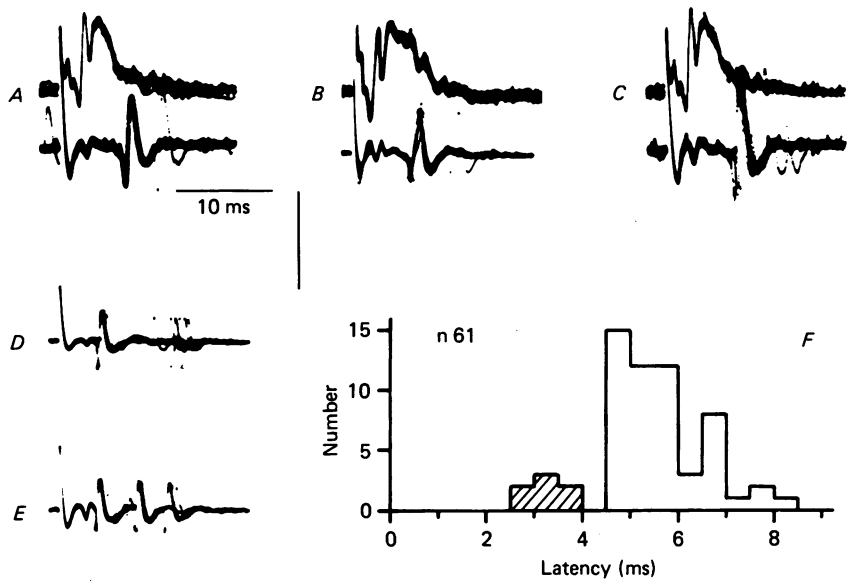

Fig. 4. Single unit responses in lobule IXa. *A*, *B* and *C*, lower traces are responses of three different Purkinje cells to stimulation of vibrissal follicles at 4*T*. Note that these and all other single unit records were high pass filtered. Upper traces, simultaneous records from surface alongside micro-electrode entry point. *D* and *E*, responses of a cell tentatively identified as a Golgi interneurone (see text). Stimulus 2*T* in *D*, 4*T* in *E*. Time scale applies to all records. Voltage calibration 1 mV for all traces in *A*, *B* and *C*; 2 mV for *D* and *E*. Note positive up in surface *and* in micro-electrode recordings. *F*, frequency histogram for minimum latency of first simple spike discharged by each of 61 units in lobule IXa. Hatched columns tentative Golgi neurones; open columns Purkinje cells.

Recordings from individual Purkinje cells

In the course of micro-electrode tracking through the uvula single unit recordings were obtained in both decerebrate and anaesthetized animals from 100 Purkinje cells identified on the basis of depth and pattern of simple spike discharges. In anaesthetized animals the spontaneous discharges always included complex spikes (i.e. responses to input via the climbing fibres) but in decerebrate animals such events were detectable in only one third of the cells (cf. Eccles, Ito & Szentágothai, 1967). Sixty-six cells were discharged by stimulation of the exposed vibrissal follicles. In all cases the response consisted of simple spikes only. Usually there was one impulse at fairly constant latency as can be seen for three different cells in the lower traces of

Fig. 4*A*, *B* and *C*; the upper traces show potentials recorded simultaneously from the surface near the micro-electrode entry point. In most cells a second spike was discharged in some trials, usually around 4 ms after the first. The latencies of the first spikes ranged between 4·5 and 8·5 ms, as shown by the open columns in Fig. 4*F*. Comparison of the latency distribution with the surface records and the extracellular fields within the cortex indicates that the majority of these discharges coincided with the N_4 deflexion attributed to Purkinje cell discharges. For each cell, the threshold for discharge was determined and the values ranged from 1·5*T* upwards which agrees well with the intensity required to evoke an N_4 wave. Thus the results of single unit recordings are consistent with short latency activation of the Purkinje cells via the mossy fibre–granule cell–parallel fibre path and incidentally provide strong support for the interpretation offered above for the extracellular fields.

In addition to the Purkinje cells a further seven units were encountered just deep to the first layer of Purkinje cells. Their discharges were superimposed on fields characteristic of the granular layer. These units discharged simple spikes at fairly regular rates between 3 and 10/s. They are tentatively identified as cortical interneurones (cf. Eccles, Llinás & Sasaki, 1966*a*) and all seven responded to snout stimulation. One example is shown in Fig. 4*D* and *E*. The first spike in these units had latency 2·8–4 ms (diagonally hatched columns in Fig. 4) and it was usually followed by one or two additional impulses with more variable latency. The initial discharges coincided with the N_2 wave of the granular layer as expected if the units were Golgi neurones directly excited by the mossy fibres.

Hemispheral responses were not subjected to micro-electrode analyses in decerebrate preparations but results from anaesthetized animals confirmed that their initial part was mediated via mossy fibres (see below).

Animals anaesthetized with pentobarbitone

Surface responses

There is much evidence from cats to indicate that responses initiated via mossy fibre paths are markedly depressed by barbiturate anaesthesia (e.g. Latham & Paul, 1971; Gordon *et al.* 1973). We were surprised therefore that in anaesthetized rats the responses on the uvula were similar in latency and distribution to those in decerebrate animals and were almost as large. The only significant difference was that surface deflexions corresponding to the N_4 wave were much reduced. Presumably therefore there was sufficient reduction of transmission from the granule to the Purkinje cells to reduce considerably the number of Purkinje cells discharged. It will be recalled that in decerebrate preparations the surface deflexions corresponding to N_3, P_2 and N_4 were much reduced by small doses of barbiturate.

The major difference between the surface potentials in the two types of preparation lay in the additional presence in the anaesthetized animals of prominent deflexions with longer latencies (ranging from 13 to 25 ms depending on recording locus). As shown below these were identifiable as cortical responses to input via climbing fibres and they were present on both vermis and hemispheres. Fig. 5*A*, *C* and *B* show examples from the contralateral hemisphere, the ipsilateral hemisphere and the contralateral vermis respectively. Such responses consisted of a sharply rising positive deflexion followed by a smaller negative deflexion. At near-threshold stimulus intensities response amplitudes fluctuated markedly and latency fluctuated by 2–5 ms, as commonly described for such responses in other species (e.g. Miller &

Oscarsson, 1970). The threshold was higher than for potentials evoked via mossy fibres, averaging 1·8 *T* (S.D. 0·3 *T*).

Response distributions were very similar in all preparations and results from one

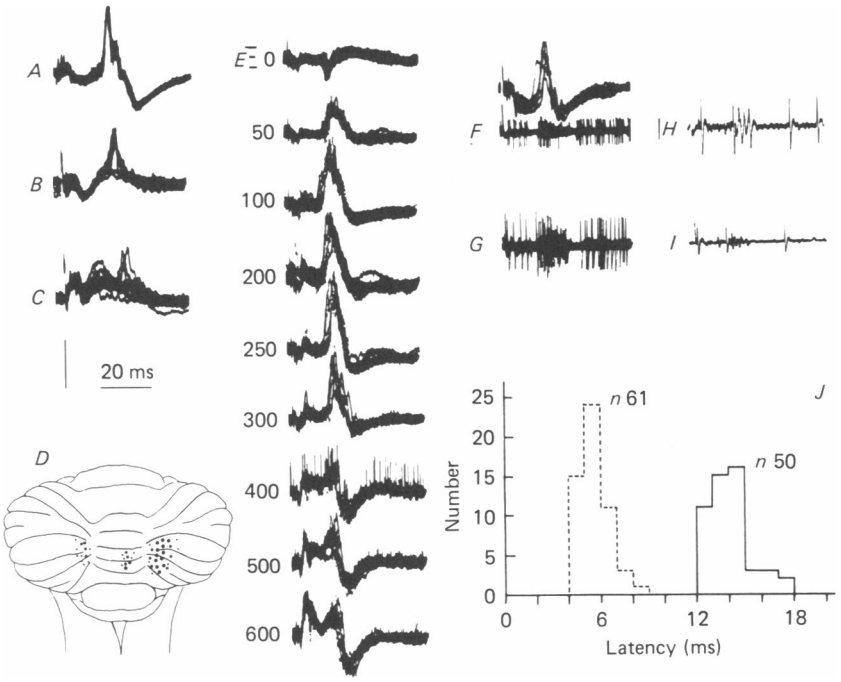

Fig. 5. Cerebellar responses mediated via climbing fibres in pentobarbitone-anaesthetized rats. All parts of Figure involve stimulation of vibrissal follicles on left of snout at intensity 4*T*. *A*, *B* and *C*, surface responses. *A* from right Crus 2; *B* from vermis just to right of mid line in lobule VIIb; *C* from left (i.e. ipsilateral) Crus 2. *D*, overall distribution of surface responses attributable to climbing fibre input (distribution similar in all thirteen experiments). Approximate amplitude of positive deflexion is indicated by size of filled circles. Smallest circles correspond to amplitude between 100 and 300 μV; intermediate circles, 300–700 μV; largest circles, 700 μV–1·5 mV. Note largest responses are on right (i.e. contralateral) hemisphere. *E*, extracellular field potentials within contralateral Crus 2. Estimated depths of electrode tip given in micrometres. *F*, lower trace is response of a Purkinje cell in contralateral Crus 2. Upper trace is surface response from alongside micro-electrode entry point. *G*, *H* and *I*, responses of three further Purkinje cells. Single sweeps. Time calibration below *C* applies to all records. Voltage calibration is 2 mV for micro-electrode recordings in *F* and *I*; 1 mV for all other traces. Note positive-up in all surface records; negative up in all micro-electrode records except *F*. *J*, frequency histogram of minimum latencies for complex spikes evoked in each of fifty Purkinje cells in contralateral hemisphere. Columns in interrupted outline show for comparison latencies of simple spikes evoked in sixty-one Purkinje cells in uvula (cf. Fig. 4*F*); latencies of simple spike responses in hemisphere were similar (fifteen cells).

experiment in which the left side of the snout was stimulated are shown in Fig. 5*D* where the size of the filled circles is proportional to the amplitude of the positive deflexion. Three discrete response zones were present each with a characteristic response latency. In any one preparation the latency was least in the contralateral hemisphere and greatest in the contralateral vermis; considering all thirteen prepara-

tions the latencies ranged from 13 to 19 ms for the contralateral hemisphere, from 16 to 22 ms for the contralateral vermis and from 20 to 25 ms for the ipsilateral hemisphere. The largest responses were always on the contralateral hemisphere and the smallest on the ipsilateral hemisphere. Each responsive area occupied portions of two or more folia and so comprised an essentially strip-like zone with its long axis in the sagittal direction: this type of projection zone is familiar from studies of spino-olivo-cerebellar paths in the cat (see Oscarsson, 1973).

Micro-electrode analysis

The nature of the surface responses was verified by recording extracellular field potentials within the responsive zones and Fig. 5*E* shows a typical depth profile obtained within the contralateral hemisphere. These records demonstrate that the positive-going surface response (latency 13 ms) is synchronous with a large sharply-rising negativity in the underlying molecular layer (largest at depths around 250 μm). Such responses are ascribable to activation of the climbing fibre – Purkinje cell synapses (e.g. Eccles, Llinás & Sasaki, 1966*c*). This potential is preceded in Fig. 5*E* by an earlier response largest at depths corresponding to the granular layer. Examination at faster sweep speed showed this response to be a $P_1 N_1$ complex followed by an N_2 wave, indicating that it is mediated via mossy fibres. Such early responses were invariably present in recordings from the hemisphere but were absent in the vermal zone. Thus responses mediated only via mossy fibres were present in the uvula whilst responses mediated only via climbing fibres were present in vermal lobule VII.

Recordings from Purkinje cells

Within the hemisphere recordings were made from seventy-two Purkinje cells, fifty of which discharged a complex spike in response to contralateral stimulation of the snout at intensities of 4*T* or less; responses of four typical cells are illustrated in Fig. 5*F*, *G*, *H* and *I*. For each cell the threshold was measured and a range of 1·6–4*T* was found. The lowest values agreed well with the threshold for the first detectable surface potential attributable to climbing fibre input.

With near-threshold stimulation the latency of the complex spike in any one cell usually fluctuated up to 5 ms but with increasing intensity such fluctuations decreased (cf. the surface potentials). For each cell the minimum latency for a 4*T* stimulus was determined and the columns in continuous outline in the frequency histogram of Fig. 5*J* indicate the latency distribution for the complex spikes (for comparison the latency distribution for simple spike responses in the uvula is shown in interrupted outline). The distribution agrees well with the latency of the surface potentials and agreement between the surface and the single unit records is also demonstrated by Fig. 5*F* in which the lower trace shows complex spikes in one cell whilst the upper trace shows the potential recorded simultaneously from the overlying surface.

Of the seventy-two cells fifteen responded with simple spikes; only two of these also gave a complex spike so that simple and complex spikes were rarely evoked in the same Purkinje cell. The low number of cells yielding simple spikes presumably reflects the relatively small size in this area of surface potentials attributable to input via mossy fibres. It will be recalled that such potentials could be evoked from both sides of the snout and fourteen of the fifteen Purkinje cells yielding simple spike

responses were driven bilaterally, indicating widespread convergence at the level of the Purkinje cells. By contrast, none of the twenty-three cells tested for bilateral input via the climbing fibres responded to ipsilateral stimulation of the snout. Thus although surface potentials due to climbing fibre input could be produced in the hemisphere from both infraorbital nerves ipsilateral responses seem restricted to a zone which escaped study at the single unit level. Examination of Fig. 5*D* indeed shows that responses were largest in a rostro-medial area which was not explored with micro-electrodes because of its proximity to the paravermal blood vessels.

DISCUSSION

Responses mediated via mossy fibres

Electrical stimulation of cutaneous afferent fibres from the snout of the rat clearly gives rise to a substantial cerebellar input mediated via mossy fibres. In decerebrate preparations considerable numbers of Purkinje cells responded with one or more simple spikes and it is widely accepted that such spikes are generated via the mossy fibre–granule cell–parallel fibre path (cf. Eccles, Ito & Szentágothai, 1967). Furthermore, both in decerebrate and barbiturate-anaesthetized animals snout stimulation generated within the cortex a pattern of extracellular field potentials virtually identical with those evoked in the cat cerebellum by volleys in several mossy fibre pathways (Eccles, Sasaki & Strata, 1967; Sasaki & Strata, 1967). Such extracellular fields, recorded with micro-electrodes, have not until now been compared in detail with the potentials registered by ball electrodes placed on the pial surface and it is interesting therefore to note that all component waves present within the cortex (i.e. P_1, N_1, N_2, N_3, P_2, N_4 and P_3) are detectable on the cerebellar surface. Moreover, the thresholds for surface and intracortical responses were the same suggesting that, for detection of responses via this mossy fibre path, the surface and the micro-electrode recording techniques are similar in sensitivity. However, our findings also show that caution must be exercised in interpreting potentials recorded from the cerebellar surface, since potentials originating in the brain stem can contribute to the evoked response as a result of volume conduction through the cerebellar tissue. Caution has previously been advocated by Rahn & Zuber (1971) on related grounds.

Experiments on cats have demonstrated that mossy fibre inputs are markedly depressed by barbiturate anaesthetics, in part at least because the discharge threshold of the granule cells is increased (Gordon *et al.* 1973). It was unexpected, therefore, to find that the responses mediated via mossy fibres differed so little between the decerebrate preparations (with residual levels of barbiturate present) and those fully anaesthetized with pentobarbitone. However, the P_2 N_4 potentials which signal Purkinje cell discharge were consistently smaller in anaesthetized animals and in addition they were temporarily reduced in decerebrates by small doses of barbiturate. It seems unnecessary, therefore, to suppose that the mossy fibre–granule cell–Purkinje cell path in the rat differs radically in its barbiturate susceptibility from that in the cat.

We were initially surprised that the largest mossy fibre input was directed to vermal folium IXa because our first experiments preceded the reports by Joseph *et al.*

(1978), Shambes *et al.* (1978*a*, *b*). These workers have studied responses evoked in the granular layer of many folia in the rat posterior lobe by natural stimulation of cutaneous mechanoreceptors, including those associated with the mystachial vibrissae: large numbers of small units, believed to be granule cells, are discharged and the uvula contains a detailed somatotopical map of the snout. They did not study the responses of Purkinje cells but our finding that these cells discharge simple spikes after electrical stimulation of vibrissal afferents supports their claim that they recorded from granule cells excited via mossy fibres. The response latencies in the two studies are in good agreement, the major difference being that responses in our animals were distributed bilaterally in the uvula as well as in the hemisphere. Because bilateral responses were recorded in our study even after section of one infraorbital nerve it is unlikely our result is ascribable to stimulus spread across the mid line of the snout. The difference may have arisen simply because natural stimulation of receptors was used in one study and electrical stimulation in the other. However, an alternative explanation may reside in the fact that discharge of a localized population of granule cells is *a priori* likely to excite (via the parallel fibres) a significantly more extensive array of Purkinje cells. Indeed recent anatomical studies (Mugnaini, 1976; Schild, 1980; Armstrong & Schild, in preparation) show that rat parallel fibres are often as long as 4–5 mm so that in a folium as short as IXa a unilateral mossy fibre input might discharge Purkinje cells on both sides of the cerebellar mid line.

In addition to the responses in the uvula responses similar in latency were present (bilaterally) in the medial parts of both crura. This finding is again consistent with those of Shambes *et al.* (1978*b*) though the responsive area they defined was larger and involved the paramedian lobule. Simple spike discharges of Purkinje cells in the same area as in our experiments have also been mentioned by Axelrad & Crepel (1977) as following electrical stimulation of the ipsilateral infraorbital nerve.

The responses in the hemisphere in decerebrate animals were invariably smaller in amplitude than those on the uvula but they were more prolonged. The later parts of the responses were not mediated via climbing fibres and presumably they represent input via slower mossy fibre paths. By analogy with responses to limb nerve stimulation in the cat, they may signal input via reticulocerebellar pathways. In the cat Darian-Smith & Phillips (1964) have recorded responses to trigeminal input in the lateral reticular nucleus, and have suggested that these give rise to long-latency responses which were observed in the culmen of the anterior lobe.

The peripheral part of the pathway which mediates the responses is provided by the infraorbital nerve ipsilateral to the site of stimulation since the responses were immediately abolished by division of that nerve (and were unaffected by section of the contralateral nerve and of the ipsilateral facial nerve). We cannot of course be sure that stimulation was confined to vibrissal afferents but such fibres are likely to have been important since the stimuli were applied to the exposed bases of the vibrissae and around 150 large myelinated afferent fibres are known to enter each follicle in rodents (Vincent, 1913; Lee & Woolsey, 1975). The earliest responses detected by recording from the brain stem had latency 0·55 ms. They were capable of following frequencies as high as 1000/s, and were the last responses to succumb following a lethal dose of barbiturate. It seems certain therefore that they signal

arrival in the brain stem of the primary afferent volley. Assuming a conduction distance of 45 mm in the trigeminal nerve the conduction velocity of the primary afferents concerned in generating the earliest part of the brain stem responses would be 80 m/s and the corresponding value for the peak of the response would be 55 m/s.

As regards the path between brain stem and cerebellar cortex, the zero crossing point between the P_1 and N_1 responses may be taken as representing the time of arrival of a mossy fibre volley in the cortex (cf. Eccles *et al.* 1967; Sasaki & Strata, 1967) and this occurred 2·4 ms after the stimulus. The latency was thus 1·85 ms measured from the *onset* of the brain stem response and 1·6 ms measured from its *peak*. These delays must include any conduction and synaptic delays within the brain stem plus a conduction time from brain stem to cerebellum. It is unlikely therefore that more than one synapse is involved and this is consistent with the high following frequency observed for the P_1 N_1 response (see Fig. 3*C*). Anatomical experiments have recently demonstrated that cells in the principal and spinal interpolar trigeminal nuclei provide both crossed and uncrossed fibres to the uvula and the crura in the rat (Watson & Switzer, 1978).

Responses mediated via climbing fibres

In anaesthetized rats responses mediated via climbing fibres were found at different latency in three separate cerebellar regions following unilateral stimulation of the snout. Because exploration was confined to the posterior lobe it remains possible that additional termination zones are present in lobules I to V. Responses in the vermis have not previously been reported but Axelrad & Crepel (1977) have described responses in an area of the ipsilateral hemisphere which appears to correspond well with the ipsilateral zone described here. In our experiments the responses in the ipsilateral hemisphere occupied a zone which corresponded with the rostro-medial portion of the termination zone in the contralateral hemisphere. However we neither confirmed nor excluded the existence of bilateral convergence onto individual climbing fibres in the hemisphere. In the cat, however, some Purkinje cells in the hemisphere are supplied by climbing fibres which can be driven from both sides of the face (Miles & Wiesendanger, 1975*a*).

In the cat the bulk of the available evidence suggests that all climbing fibres arise as the axons of inferior olivary neurones and therefore that all somatic afferent paths terminating as climbing fibres are relayed via the inferior olive (see Armstrong, 1974). Whether this is true also for the rat has been questioned (Chan-Palay, Palay, Brown & Van Itallie, 1977) but a recent autoradiographic study of the olivo-cerebellar projection in this species has confirmed that most (and probably all) the climbing fibres originate from the olive (Campbell, 1980). By implication it is probable that the present responses were relayed via the olive. No detailed topographical map yet exists for the olivo-cerebellar projection in the rat but assuming similarity with the cat (see e.g. Groenewegen & Voogd, 1977; Groenewegen, Voogd & Freedman, 1979) it is likely the vermal responses were relayed via the caudal part of the medial accessory olive whilst the hemispheral responses were relayed via the rostral half of the olivary complex. It is relevant that Cook & Wiesendanger (1976) have found in the rat that neurones in the rostral half of the olive are discharged by contralateral trigeminal inputs. Latencies ranged from 16 to 30 ms and most cells were in the

medial parts of the dorsal accessory and principal subnuclei. Since the olivo-cerebellar projection is crossed, these authors were presumably studying the relay neurones for responses in the termination zone in the ipsilateral hemisphere.

Both the vermal and the hemispheral zones comprised portions of two or more adjacent folia so that they resemble the strip-like longitudinal termination zones which exist sometimes side by side and sometimes superimposed for each of the large numbers of spino-olivo-cerebellar paths conveying input from the limbs in the cat (e.g. Oscarsson, 1973). In the cat only one termination zone has so far been described for trigeminal inputs (Miles & Wiesendanger, 1975*a*). It is centred in the paravermal portion of lobule VI and extends caudally into lobule VIIa so that it may correspond with our hemispheral zone. Our findings raise the possibility that a more extensive exploration of the posterior lobe in the cat may reveal an additional zone in the vermis.

Regarding the functional significance of the trigemino-olivo-cerebellar paths Miles & Wiesendanger (1975*b*) have commented that climbing fibres receiving trigeminal input 'are capable of transmitting very precise information regarding the location of peripheral sensory events to individual Purkinje cells'. Though we have not attempted such precise investigations the ease with which responses were evoked by electrical stimulation of vibrissal follicles (and by mechanical stimulation of vibrissae; unpublished results) certainly supports their view.

Despite careful exploration responses mediated via climbing fibres were almost never detected in the decerebrate rats, indicating that the excitability of the climbing fibre pathways was low. One possible explanation is suggested by the finding that in the cat there is extensive excitatory convergence between trigeminal inputs and inputs from the somatosensory cerebral cortex, such convergence occurring at or before the level of the inferior olive (Miles & Wiesendanger, 1975*a*).

Comparison of the distribution of responses mediated via mossy and via climbing fibres

In the cerebellar hemisphere the areas receiving mossy and climbing fibre inputs were approximately coextensive and our findings therefore agree with Axelrad & Crepel (1977). In anaesthetized animals we found few Purkinje cells which responded to afferent volleys with both simple and complex spikes but the real extent of such convergence may have been obscured by our use of moderate barbiturate anaesthesia: using cats under light barbiturate anaesthesia Cody & Richardson (1979) found (by computer averaging of successive responses) that both types of discharge were given by twenty-one out of forty Purkinje cells in the hemisphere responding to volleys in branches of the ipsilateral trigeminal nerve. In any event our findings in the hemisphere do not conflict with the general finding (see Strata, 1976) that input from particular areas of the body surface is delivered via the two types of cerebellar afferent to approximately congruent areas of the cerebellar cortex. In the vermis however, there was no overlap between the mossy fibre input which produced large responses in the uvula and the climbing fibre input which activated a portion of lobule VIII. The functional significance of this separation between the two types of afferent path is obscure. However, a recent autoradiographic study of the cerebellar cortico-nuclear projections in the rat (Armstrong & Schild, 1978) has shown that Purkinje cells in vermal lobule VII project to the caudal part of the fastigial nucleus, whilst

the projection from the uvula is directed principally to the vestibular complex. This would imply that the spatial separation between the mossy fibre and climbing fibre trigemino-cerebellar paths to the vermis is maintained at the cerebellar outflow level.

REFERENCES

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

ARMSTRONG, D. M. & SCHILD, R. F. (1978). An investigation of the cerebellar cortico-nuclear projections in the rat using an autoradiographic tracing method. I. Projections from the vermis. *Brain Res.* **141**, 1–19.

AXELRAD, H. & CREPEL, F. (1977). Repiésentation selectif des vibrisses mystaciales au niveau des cellules de Purkinje du cervelet par la voie des fibres grimpantes chez le Rat. *C. r. Acad. Sci., Paris* **284**, 1321–1324.

CAMPBELL, N. C. (1980). The inferior olive as a source of climbing fibres throughout the cerebellar cortex of rats. *J. Physiol.* **303**, 24*P*.

CHAN-PALAY, V., PALAY, S. L., BROWN, J. T. & VAN ITALLIE, C. (1977). Sagittal organization of olivocerebellar and reticulocerebellar projections: autoradiographic studies with ^{35}S-methionine. *Exp. Brain Res.* **30**, 561–576.

CODY, F. W. J. & RICHARDSON, H. C. (1979). Mossy and climbing fibre mediated responses evoked in the cerebellar cortex of the cat by trigeminal afferent stimulation. *J. Physiol.* **287**, 1–14.

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

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.

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

ECCLES, J. C., LLINÁS, R. & SASAKI, K. (1966*a*). The inhibitory interneurones within the cerebellar cortex. *Exp. Brain Res.* **1**, 1–16.

ECCLES, J. C., LLINÁS, R. & SASAKI, K. (1966*b*). Parallel fibre stimulation and the responses induced thereby in the Purkinje cells of the cerebellum. *Exp. Brain Res.* **1**, 17–39.

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

ECCLES, J. C., SASAKI, K. & STRATA, P. (1967). Interpretation of potential fields generated in the cerebellar cortex by a mossy fibre volley. *Exp. Brain Res.* **3**, 58–80.

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.

GROENEWEGEN, H. J. & VOOGD, J. (1977). The parasagittal zonation within the olivocerebellar projection. I. Climbing fiber distribution in the vermis of the cat cerebellum. *J. comp. Neurol.* **174**, 417–488.

GROENEWEGEN, H. J., VOOGD, J. & FREEDMAN, S. L. (1979). The parasagittal zonation within the olivocerebellar projection. II. Climbing fiber distribution in the intermediate and hemispheric parts of the cat cerebellum. *J. comp. Neurol.* **183**, 551–602.

JOSEPH, J. W., SHAMBES, G. M., GIBSON, J. M. & WELKER, W. (1978). Tactile projections to granule cells in caudal vermis of the rat's cerebellum. *Brain. Behav. & Evol.* **15**, 141–149.

LARSELL, O. (1952). The morphogenesis and adult pattern of the lobules and fissures of the cerebellum of the white rat. *J. comp. Neurol.* **97**, 281–356.

LATHAM, A. & PAUL, D. H. (1971). Effects of sodium pentobarbitone on cerebellar neurone activity. *Brain Res.* **25**, 212–215.

LEE, K. J. & WOOLSEY, T. A. (1975). The relationship of peripheral innervation density (vibrissal) to cortical neuron number (barrels) in the mouse. *Anat. Rec.* **181**, 408.

MILES, T. S. & WIESENDANGER, M. (1975*a*). Organization of climbing fibre projections to the cerebellar cortex from trigeminal cutaneous 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.

MILLER, S. & OSCARSSON, O. (1970). Termination and functional organization of spino-olivo-cerebellar paths. In *The Cerebellum in Health and Disease*, ed. FIELDS, W. S. & WILLIS, W. D., pp. 172–200. St Louis: Green.

MUGNAINI, E. (1976). Organization of cerebellar cortex. *Experimental Brain Research, Suppl. I, Afferent and Intrinsic Organization of Laminated Structures in the Brain*, ed. CREUTZFELDT, O., pp. 8–19. Berlin: Springer-Verlag.

OSCARSSON, O. (1973). Functional organization of spinocerebellar paths. In *Handbook of Sensory Physiology*, II. Somato-Sensory System, ed. IGGO, A., pp. 339–380. Berlin: Springer Verlag.

RAHN, A. C. & ZUBER, B. L. (1971). Cerebellar evoked potentials resulting from extraocular muscle stretch: evidence against a cerebellar origin. *Expl Neurol.* **31**, 230–238.

SASAKI, K. & STRATA, P. (1967). Responses evoked in the cerebellar cortex by stimulating mossy fibre pathways to the cerebellum. *Exp. Brain Res.* **3**, 95–110.

SCHILD, R. F. (1980). Length of the parallel fibres in rat cerebellar cortex. *J. Physiol.* **303**, 25*P*.

SHAMBES, G. M., BEERMAN, D. H. & WELKER, W. (1978*a*). Multiple tactile areas in cerebellar cortex: another patchy cutaneous projection to granule cell columns in rats. *Brain Res.* **157**, 123–128.

SHAMBES, G. M., GIBSON, J. M. & WELKER, W. (1978*b*). Fractured somatotopy in granule cell tactile areas of rat cerebellar hemispheres revealed by micromapping. *Brain. Behav. & Evol.* **15**, 94–140.

STRATA, P. (1976). A general review of the physiological function of the neuronal machine in the cerebellar cortex. *Experimental Brain Research, Suppl. I, Afferent and Intrinsic Organization of Laminated Structures in the Brain*, ed. CREUTZFELDT, O., pp. 103–112. Berlin: Springer-Verlag.

VINCENT, S. B. (1913). The tactile hair of the white rat. *J. comp. Neurol.* **23**, 1–34.

WAITE, P. M. E. (1973). Somatotopic organization of vibrissal responses in the ventrobasal complex of the rat thalamus. *J. Physiol.* **228**, 527–540.

WATSON, C. R. R. & SWITZER, R. C. (1978). Trigeminal projections to cerebellar tactile areas in the rat – origin mainly from n. interpolaris and n. principalis. *Neurosci. Lett.* **10**, 77–82.

WELKER, C. (1971). Microelectrode delineation of the fine grain somatotopic organization of SM I cerebral neocortex in albino rats. *Brain Res.* **26**, 259–275.

WELKER, C. (1976). Reception fields of barrels in the somatosensory neocortex of the rat. *J. comp. Neurol.* **166**, 173–190.