Exp Brain Res (1990) 80:415–428

Experimental Brain Research

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Topographical organisation within the cerebellar nucleocortical projection to the paravermal cortex of lobule Vb/c in the cat

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Summary. The projection from the intracerebellar nuclei to the paravermal (intermediate) cerebellar cortex of lobule Vb/c has been investigated in the cat using a combined electrophysiological and neuroanatomical technique. A small (10–30 nl) injection of WGA–HRP was made into one of the three paravermal zones (c_1, c_2 or c_3) after the mediolateral boundaries of the zones had been delimited on the cerebellar surface by recording climbing fibre field potentials evoked in response to percutaneous stimulation of one or more paws. The distribution of retrogradely labelled cell bodies within the intracerebellar nuclei was compared with the distribution of terminal labelling arising from anterograde transport by cerebellar Purkinje cells. The three paravermal zones displayed marked heterogeneity in their receipt of a projection from the intracerebellar nuclei. The c_1 and c_3 zones received virtually no such input, although injections in either zone resulted in significant terminal labelling (which was largely restricted to nucleus interpositus anterior). By contrast, the intervening c_2 zone received a much heavier nucleocortical input which arose almost exclusively from nucleus interpositus posterior (to which the zone also projected). A sparse contralateral nucleocortical input to the c_2 zone was also demonstrated. This arose primarily from nucleus fastigius. It is concluded that the nucleocortical projection to the paravermal cortex of lobule Vb/c displays marked topographical specificity and some functional implications of this are discussed.

Key words: Nucleocortical – Corticonuclear – Cerebellar nuclei – Cerebellar zonation – Cat

Introduction

In recent years, a number of studies in different species have revealed a detailed pattern of topographical organisation within the projection from the cerebellar cortical Purkinje cells to the deep cerebellar nuclei. The cortex appears to consist of a number of narrow sagittal strips each of which projects to a localised region within one of the deep nuclei (see for example, Haines and Rubertone 1979; Dietrichs and Walberg 1979, 1980; Bishop et al. 1979; Voogd and Bigaré 1980; Dietrichs 1981; Haines and Patrick 1981; Trott and Armstrong 1987a,b). By comparison, relatively little attention has been paid to the precise topographical organisation of the projection from the deep nuclei to the cerebellar cortex. The latter pathway has been shown to exist in a number of species: in the cat (Gould and Graybiel 1976; Tolbert et al. 1976; Dietrichs and Walberg 1979, 1980), monkey (Tolbert et al. 1977, 1978a; Chan-Palay 1977), rat (Hess 1982), tree shrew (Haines and Pearson 1979) and bushbaby (Haines 1989).

It has been suggested that the nucleocortical projection may be a substantial one (see Discussion in Tolbert 1982) and light microscopical and EM autoradiographic studies of the pathway (Chan-Palay 1977; Tolbert et al. 1978a; Tolbert et al. 1980; Legendre and Courville 1986) have demonstrated that the cortically directed axons form mossy fibre type terminal arborisations within the granular layer. Some authors however (e.g. Legendre and Courville 1986) have questioned the quantitative importance of the pathway and in an EM study of nucleocortical terminals (Hamori et al. 1981) it was estimated that they may represent less than 5% of the total mossy fibre input.

The precise topographical organisation of the pathway could, nevertheless, carry important functional implications particularly as it has been demonstrated electrophysiologically (Tolbert et al. 1978b; McCrea et al. 1978) and anatomically (Payne 1983) that nucleocortical terminals arise, at least in part, from axon collaterals of cerebellar efferents destined for the contralateral ventrolateral thalamus and/or inferior olive. The nucleocortical projection could thus provide a pathway by which some cerebellar outflow could be fed back to the cerebellar cortex.

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Several axonal transport studies in the cat (e.g. Tolbert et al. 1978a; Gould 1979) have demonstrated that the nucleocortical projection is organised, broadly speaking, into the three longitudinal cerebellar cortical/nuclear axes which were originally described for the corticonuclear projection by Jansen and Brodal (1940). That is, cells in the fastigial nucleus project mainly to the medial (vermal) cerebellar cortex, cells within the interpositus nuclei project mainly to the intermediate (paravermal) cortex whereas those within the dentate nucleus project more laterally to the hemispheral cortex.

The organisation of the nucleocortical projection relative to that from the cortex to the nuclei has been examined at a higher level of resolution in combined anterograde and retrograde transport studies in the cat (Dietrichs and Walberg 1979, 1980; Dietrichs 1981) in which injections of HRP were centred within (but not restricted to) the much narrower (c. 1 mm wide) individual cortical zones which can be defined on the basis of their olivocerebellar input (see Voogd and Bigaré 1980; Oscarsson 1980 for reviews) and their corticonuclear projection. The pattern of reciprocity between corticonuclear and nucleocortical projections appeared to be maintained at this zonal level. Within the paravermal cortex, for example, each of the three constituent sagittal zones – c_1, c_2 and c_3 – were reported to receive input from approximately the same region of the cerebellar nuclei to which the zone, in turn, projected and retrogradely labelled cells were usually found in the cerebellar nuclei intermingled with anterogradely labelled corticonuclear axon terminals. Such a zonal organisation within the nucleocortical projection has also been reported for the bushbaby in a recent study by Haines (1989) which additionally presented interesting evidence that some heterogeneity could be present within the projection, in that the cortical zones differed in the extent to which they were targeted by nucleofugal axons (see Discussion).

The corticonuclear and nucleocortical pathways are not, however, always precisely reciprocal. Several studies have shown that the latter pathway is more diffuse in some important respects than that from the cortex to the nuclei. In the cat, for example, nucleus fastigius has been shown by Dietrichs and Walberg (1979, 1980) to project to the entire mediolateral extent of the cerebellar cortex including areas such as the paramedian lobule and cerebellar hemispheres, which do not themselves project to the fastigial nucleus, whereas in the monkey the nucleocortical projection appears to arise mainly from the dentate nucleus (Chan-Palay 1977; Tolbert et al. 1978a) which receives a corticonuclear projection only from the most lateral parts of the cerebellar cortex. Moreover, in both these species, a small number of cerebellar nuclear cells has been shown to project to the contralateral cerebellar cortex (e.g. Tolbert et al. 1978a; Dietrichs and Walberg 1979) whereas the corticonuclear projection is known to be entirely ipsilateral. A similar weak crossed projection within the nucleocortical pathway has also been described for the tree-shrew (Haines 1978) and more recently for the bushbaby (Haines 1989).

In the present study, the precise topographical organisation of the nucleocortical pathway to the paravermal region of the cat anterior lobe has been investigated at a higher level of resolution than has been possible to date. A combined electrophysiological and neuroanatomical technique was used such that small (10–30 nl) injections of WGA–HRP were made in lobule Vb/c into single cortical zones which were defined electrophysiologically on the basis of their spino-olivocerebellar input (cf. Oscarsson 1980). The extent of the nucleocortical projection associated with each of the three functionally defined zones (c_1, c_2 and c_3) could thus be assessed and comparisons could be made between the locations of retrogradely labelled cells within the cerebellar nuclei and the corresponding terminal labelling resulting from orthograde transport within the Purkinje cells. The study aimed to address the following questions: (1) do the three paravermal zones differ in the extent to which they receive a nucleocortical projection? (2) to what extent is the nucleocortical projection organised reciprocally to that from the cortex to the nuclei? (3) is there evidence for a contralateral nucleocortical pathway?

Material and methods

Experiments were carried out using purpose bred adult cats between 2.5 and 5.5 kg in body weight and, in total, 19 injections were made into the paravermal cortex of lobule Vb/c. All surgical techniques, which were carried out aseptically, were performed under general anaesthesia induced with sodium pentobarbitone (Sagatal; BDH) injected intraperitoneally at an initial dose of 40 mg/kg. Maintenance doses were given as necessary via an intravenous saline drip. In each animal a small craniotomy was performed to expose the dorsal surface of the cerebellum in the paravermal region of lobule Vb/c.

Mapping of surface recordings

The boundaries between the zones of the paravermal (or intermediate) cortex of the anterior lobe were located in lobule Vb/c by mapping, on the cerebellar surface, climbing fibre field potentials evoked in response to cutaneous stimulation of one or more paws as described in Trott and Armstrong (1987a,b). Climbing fibre responses were recorded extracellularly using glass-coated tungsten microelectrodes (tip size 5–20 μm; impedance 50 kΩ–1 MΩ) and their distribution showed that each paravermal region consisted of three electrophysiologically distinct zones (c_1, c_2 and c_3) which were bounded medially by the paravermal vein and laterally by a narrow d_1 zone which extended to the lateral margin of the anterior lobe and is not considered to be part of the intermediate cortex. In agreement with other studies (e.g. Larson et al. 1969; Ekerot and Larson 1979; Trott and Armstrong 1987a) climbing fibre responses in the middle paravermal zone (c_2) could be evoked at latencies between 20 and 25 ms by stimulation of either forelimb whereas the two flanking zones in lobule Vb/c (c_1 and c_3) displayed climbing fibre field potentials at 14–17 ms latency in response only to stimulation of the ipsilateral forelimb. The c_3 zone lay lateral to the c_2 zone and its width (1.5–2.0 mm) was invariably greater than that of either the c_1 zone (0.9–1.5 mm) or the c_2 zone (0.6–1.1 mm).

Injections and histochemical analysis

When the locations and widths of the paravermal zones had been determined, a small (10–30 nl) injection of 1–2% WGA–HRP was

made 500 μm below the pial surface into a chosen zone. The tracer was injected hydraulically using a glass micropipette (tip diameter 25–35 μm) attached to a 1 μl Hamilton syringe (Field instruments). Injections into the c_1 and c_2 zones were made into the centre of the zone but those in the c_3 zone were centred in either its medial or its lateral half. It was usual to make injections in both halves of the cerebellum, sometimes into corresponding zones on each side and sometimes into different zones. However, on occasion a unilateral injection was made in order to assess the extent to which the cells of the deep nuclei projected to the contralateral cerebellar cortex. After removal of the micropipette, the pial surface was covered with gelfoam (Sterispon), the craniotomy was sealed with dental acrylic and the wound was closed in layers.

After recovery from anaesthesia, post-operative analgesia was maintained for a further 24 h with buprenorphine (Temgesic; Reckitt and Colman) injected intramuscularly at a dose of 10 μg/kg. After a survival time of 50–80 h, the animal was deeply anaesthetised and perfused transcardially with 2 l of a saline rinse solution followed by 2 l of fixative (a mixture of 1.0% paraformaldehyde and 1.25% glutaraldehyde) and finally with 2 l of 0.2 M phosphate buffer solution (pH 7.4) containing 10% sucrose. The cerebellum was removed, blocked in 10% gelatin and frozen sagittal sections (two series at 50 μm intervals) were prepared. One series was processed histochemically for HRP using the chromogen tetramethyl benzidine (TMB) according to the protocol described by Mesulam (1982). The other series was stained with cresyl violet.

The sections were scrutinised using a Wild macroscope and a Leitz Diaplan microscope, both equipped with polarising filters. The position and extent of the injection site were noted and compared with the width of the injected zone determined electrophysiologically. The "measured injection site" was taken as being the region of cerebellar cortex which contained visible reaction product and from which labelled axons could be seen to arise. It is probable, however, that not all this region contributed to the "effective injection site" i.e. the area of cortex which gave rise to detectable labelling within the cerebellar nuclei. This conclusion was reached because, on occasion, very small injections of WGA–HRP were made which, although they gave rise to labelled axons leaving the cerebellar cortex, failed to produce any detectable orthograde or retrograde labelling within the cerebellar nuclei. Since the injection sites which gave rise to just-detectable nuclear labelling measured around 0.5 mm mediolaterally, it is likely that the criteria used for measuring the injection site overestimated the size of the "effective injection site" by about this amount. (Cases in which the injection site measured 0.5 mm or less in width have not been included in the present results). The widths of the injection sites listed in Table 1 and those depicted diagrammatically in the Figures are, however, "measured" not "effective" injection sites since this was the only value which could be assessed objectively.

The intracerebellar nuclei (but not the lateral vestibular nuclei) were examined under bright- and dark-field illumination (using partially crossed polarising filters in the latter situation) and the position and extent of terminal labelling (resulting from uptake and orthograde transport of tracer substance by Purkinje cells) and retrograde cell labelling (resulting from uptake and retrograde transport of tracer by nuclear cells providing one or more axon terminals to the cerebellar cortex) were transferred to standard outline maps of the cerebellar nuclei in sagittal section. These maps consisted of representative outlines, at equally spaced intervals, which were compiled by "averaging" camera lucida drawings of sagittal sections through the cerebellum for each of 6 cats. Figure 1 depicts the entire series of 32 outlines. They were found to be broadly similar to those which were compiled by Dietrichs and Walberg (1979) and reproduced in previous reports from this laboratory (e.g. Trott and Armstrong 1987a,b). They differ, however, in some details: for example, the inclusion of a separate, dorsal cell group which is usually present within interpositus posterior between levels 12 and 16; the shape of the nuclear outlines at the junction between nucleus interpositus and fastigius (levels 20–22) and the inclusion of outlines extending to the medial limit of fastigius. The

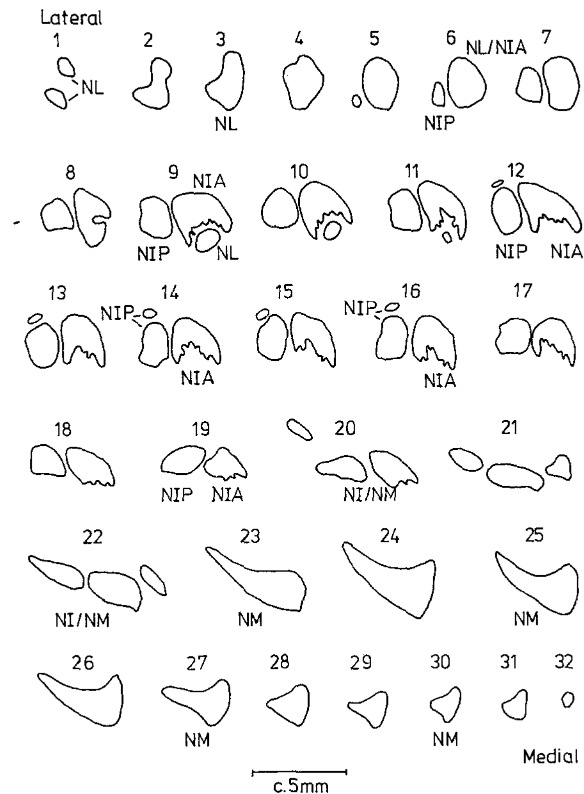

Fig. 1. Equally spaced diagrammatic outlines of sagittal sections through the intracerebellar nuclei arranged sequentially from lateral (1) to medial (32). The results of individual cases are presented in subsequent Figures using selected parts of the series. Approximate distance between adjacent outlines is 250 μm. NL, nucleus lateralis (dentate); NL/NIA, transitional region between dentate and interpositus anterior (taken as being present dorsally within levels 6 to 8; see Trott and Armstrong 1987a); NIA, nucleus interpositus anterior; NIP, nucleus interpositus posterior; NI/NM, junctional area between nucleus interpositus and nucleus medialis (note that at levels 20–22 it is not always possible to assign the various cell groups to either cerebellar nucleus); NM, nucleus medialis (fastigius)

results of individual cases are depicted in subsequent figures using a selected part of the series of standard outlines at the appropriate mediolateral level for each case. The mediolateral distance between adjacent outlines in the series is, on average, 250 μm but this figure varied somewhat between animals.

It should be noted that the numbers of retrogradely labelled cells depicted in the figures and listed in Table 1 were obtained from scrutiny of alternate cerebellar sections only (since only one of the two series of sections was processed histochemically). As the same experimental procedure was applied to all cases, comparisons can be made between cases but the numbers quoted obviously underestimate the absolute number of labelled cells for each case. The total number of labelled cells was estimated in certain cases (see Discussion) by applying an appropriate correction factor (after Abercrombie 1946).

Results

The results revealed a striking heterogeneity between the three paravermal zones (c_1, c_2 and c_3) in lobule Vb/c in the extent to which they were targets for cortically direc-

Table 1. Summary of the characteristics of the cases employed in the present study. First column lists the location and width of 19 different injection sites (arranged with the sites in each zone ranked according to size). Second column shows case numbers used in text together with the original experiment identifiers in which L and R respectively indicate whether the injection was placed to left or right of the cerebellar midline. Third column depicts the location of anterogradely labelled Purkinje axon terminals within nucleus interpositus. Relative spatial extents and densities of terminal labelling in nucleus interpositus anterior (NIA) and posterior (NIP) are indicated by the number of chevrons. Fourth column shows the number and location of retrogradely labelled nucleocortical cell bodies in the nucleus interpositus ipsilateral to the injection site. Note that the cell numbers do not include any labelled cells in the other ipsilateral deep nuclei. Such cells were confined to case 9 (HC 31L); see text. Note also that alternate sections were counted so the total number of labelled cells is underestimated. In 5 of the cases with retrogradely labelled cells ipsilateral to the listed injection site an injection site was also present in the other side of the cerebellum. These cases are asterisked in the second column and further details are provided in the Discussion in the section dealing with crossed nucleocortical projections

Location and mediolateral width of inj. site	Case number	Anterogradely labelled axon terminals	Retrogradely labelled cells	
middle c_1			NIP	NIA
1.2mm	1 (HC29R)*	NIA	3	0
2.0mm	2 (HC11R)	NIA > NIP	0	0
2.9mm	3 (HC17R)	NIA >>> NIP	0	0
3.2mm	4 (HC17L)	NIA >>> NIP	0	0
middle c_2				
1.0mm	5 (HC16L)*	NIP >>> NIA	11	0
1.3mm	6 (HC30R)*	NIP	19	0
1.6mm	7 (HC28L)	NIP >> NIA	28	0
3.8mm	8 (HC29L)*	NIP >> NIA	31	0
3.9mm	9 (HC31L)	NIP >> NIA	86	1
medial c_3				
1.2mm	10 (HC7R)	NIA	0	0
1.3mm	11 (HC19R)	NIA	0	0
1.4mm	12 (HC19L)	NIA	0	0
1.7mm	13 (HC8L)*	NIA >> NIP	4	0
2.3mm	14 (HC13L)	NIA >> NIP	1	0
2.8mm	15 (HC14L)	NIA >> NIP	0	1
2.9mm	16 (HC15L)	NIA >> NIP	0	0
lateral c_3				
0.7mm	17 (HC9R)	NIA	0	0
1.1mm	18 (HC11L)	NIA	0	0
1.4mm	19 (HC27L)	NIA >> NL	0	0

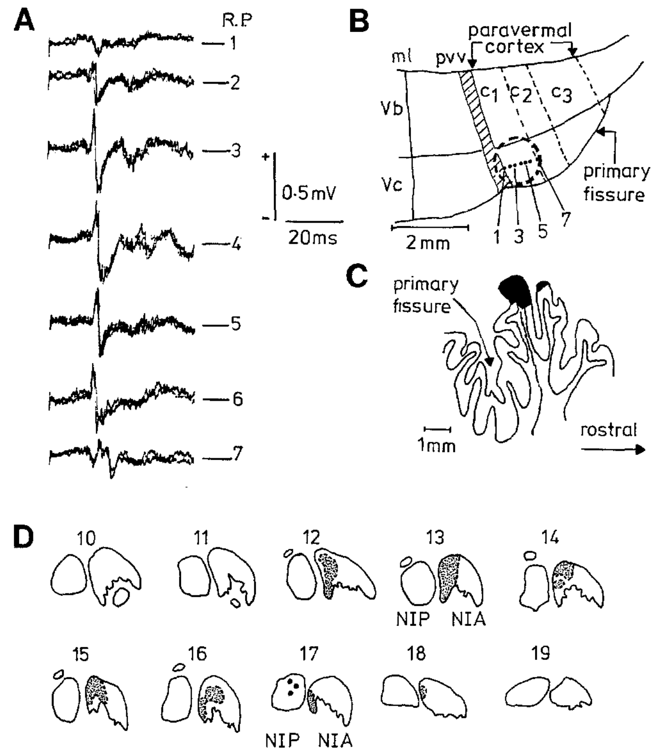

Fig. 2A–D. An injection confined to the c_1 zone (Case 1). **A** Climbing fibre field potentials, evoked at recording positions (R.P.) depicted in **B**, in response to percutaneous stimulation of the ipsilateral forepaw. Each record comprises 3 or 4 superimposed oscilloscope traces and the stimulus occurs at the beginning of each sweep. **B** Diagrammatic dorsal view of the right anterior lobe at the level of lobule Vb/c to show recording positions from which potentials in **A** were obtained. Dashed lines depict mediolateral boundaries between cortical zones, estimated electrophysiologically. Dashed circle depicts location and extent of "measured injection site" within the c_1 zone, estimated histologically, in relation to electrophysiological limits of zone. Pvv and oblique hatching, paravermal vein; ml, midline. **C** Camera lucida drawing of sagittal section through cerebellar cortex in centre of injection site to show region of injection site (filled area) together with tract of labelled axons. **D** Location of terminal labelling (stippling) and retrogradely labelled cells (filled circles-one circle equivalent to one labelled cell) within the intracerebellar nuclei at levels 10–19 (cf. Fig. 1). Abbreviations as in Fig. 1

ted axons of the deep nuclear cells. The results from all 19 cases in the present study are summarised in Table 1. It can be seen that injections of WGA-HRP centred within the c_2 zone invariably gave rise to very many more retrogradely labelled cells within the cerebellar nuclei than injections made within the c_1 or c_3 zones. Furthermore, 99% (153/155) of the labelled cells within nucleus interpositus were found within its posterior division, compared with only 1% in the anterior division of the nucleus.

Injections within the c_1 zone

In 4 cases (Cases 1, 2, 3 and 4 in Table 1), injections were centred within the c_1 zone. In all four cases terminal labelling, arising from orthograde transport within Purkinje cells, was heaviest in (and in one case was restricted to) nucleus interpositus anterior. In three of the experiments no retrogradely labelled cells could be seen in any of the cerebellar nuclei (see Table 1). In the remaining case (Case 1) three HRP-positive cells were identified in interpositus posterior although no terminal labelling was present in that nucleus.

This experiment is illustrated in Fig. 2. Figure 2A depicts the climbing fibre field potentials evoked on the cerebellar surface, at the recording positions shown in Fig. 2B, in response to percutaneous stimulation of the ipsilateral forepaw. The recording positions were either 100 µm or 200 µm apart and the total distance explored (i.e. between positions 1 and 7) was 900 µm. Near the centre of the c_1 zone (positions 3 and 4) a large, initially

positive-going climbing fibre field potential was evoked at a latency of 15 ms in response to ipsilateral, but not contralateral (not illustrated) forepaw stimulation. Such a response is characteristic of the c_1 zone. As the recording electrode was moved medially towards the paravermal vein (positions 1 and 2) the response became progressively smaller. Lateral to position 4 there was a similar reduction in amplitude of the potential at latency 15 ms while at position 7 a later, initially positive-going potential, at latency 20 ms, could be evoked by both ipsilateral and contralateral (not illustrated) forepaw stimulation. This later bilateral response increased in amplitude as the recording electrode was moved further laterally. Such a bilateral response is characteristic of the c_2 zone and the boundary between the c_1 and c_2 zones was considered to lie at or very close to position 7. The medial boundary of the c_1 zone could not be located but was presumed to lie beneath the paravermal vein close to position 1.

An injection of 25 nl of WGA–HRP was made 500 μm below the pial surface near the centre of the c_1 zone at recording position 3. The "measured injection site", which had a mediolateral width of 1.2 mm, is depicted diagrammatically as the dashed circle in Fig. 2B and it can be seen that the injection was almost completely confined to the c_1 zone. The maximal extent of the injection site in the rostrocaudal and dorsoventral planes is depicted in the sagittal line drawing of Fig. 2C. In Fig. 2D (and all subsequent figures) areas of fine stippling represent the location of terminal labelling and filled circles represent HRP-positive cell bodies within the cerebellar nuclei seen in sagittal section. In this case terminal labelling extended mediolaterally for about 1.6 mm (levels 12–18) and occupied the more caudal regions of interpositus anterior. Only three retrogradely labelled cell bodies could be detected and these were all located within medial parts of interpositus posterior (at level 17).

The three other cases of injections within the c_1 zone resulted in injection sites which were larger than that of the case depicted in Fig. 2 (and in each case somewhat larger in mediolateral extent than the c_1 zone as defined electrophysiologically). In each of these experiments terminal labelling was present, not only in the same regions of interpositus anterior as shown in Fig. 2D, but also in dorsomedial parts of interpositus posterior (see Table 1). The latter labelling was however much lighter and less extensive than that in interpositus anterior. None of these injections gave rise to any retrogradely labelled cells within the cerebellar nuclei.

Injections within the c_2 zone

In five experiments (Cases 5, 6, 7, 8 and 9), injections were centred in the c_2 zone of lobule Vb/c. In every case, terminal labelling was either restricted to interpositus posterior or was heaviest and most extensive in that nucleus with some additional, but lighter terminal labelling apparent in interpositus anterior. In each experiment, retrogradely labelled HRP-positive cell bodies were also present within interpositus posterior and, in four of the five cases, such cells were restricted to that nucleus (see Table 1). In the fifth case (Case 9 – in which the largest injection was made) no fewer than 93 labelled cells were present in interpositus posterior, 86 of them located in the main part of the nucleus and 7 in the junctional region between interpositus posterior and nucleus fastigius. The injection in case 9 also gave rise to one HRP-positive cell within interpositus anterior and to 10 cells scattered within the fastigial nucleus, but these represented only 11% (11/104) of the labelled nuclear cells found ipsilaterally in that animal.

Figures 3 and 4 represent respectively the results obtained from a small injection which was restricted to the c_2 zone (Case 6, Fig. 3) and the largest injection which was made into the zone (Case 9, Fig. 4). Figure 3A depicts the climbing fibre field potentials evoked, at the recording positions shown in Fig. 3B, by stimulation of either the ipsilateral or the contralateral forepaw. The

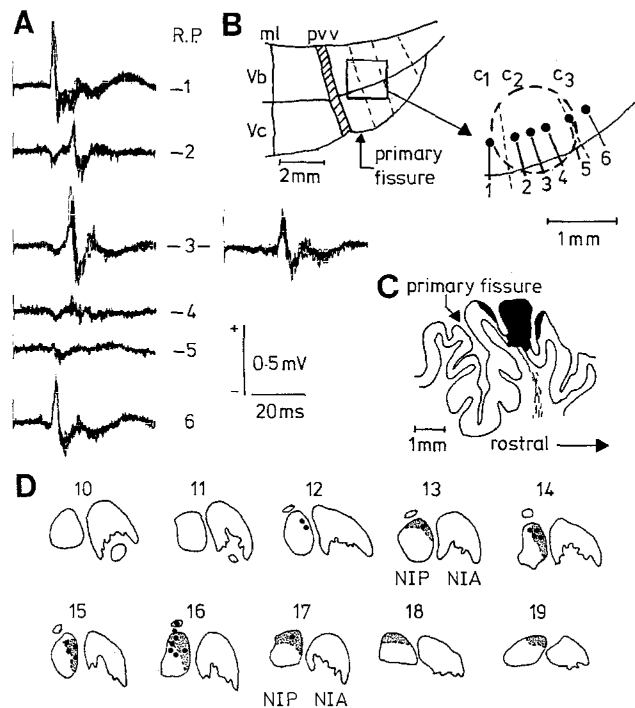

Fig. 3A–D. An injection confined to the c_2 zone (Case 6). **A** Climbing fibre field potentials evoked at the recording positions depicted in **B**, in response to stimulation of either the ipsilateral forepaw (left hand column) or the contralateral forepaw (single record depicted to right for position 3). Each record comprises 3 sweeps with the stimulus at the beginning of each sweep. **B** Diagrammatic dorsal view of lobule Vb/c with inset area within paravermal cortex enlarged to right. Dashed lines indicate locations of mediolateral boundaries between cortical zones (delimited electrophysiologically) and filled circles represent locations of recording positions from which potentials in **A** were obtained. Dashed circle indicates location and extent of "measured injection site" within the c_2 zone, estimated histologically. **C** Camera lucida drawing of sagittal section through cerebellar cortex at centre of injection site to show extent of injection site and tract of labelled axons. **D** Location of terminal labelling (stippling at levels 13–19) and retrogradely labelled cells (filled circles) in nucleus interpositus posterior (NIP). Other conventions and abbreviations as in Figs. 1 and 2

total distance explored between recording positions 1 and 6 was 1.5 mm and the relative spacing between adjacent recording positions is illustrated diagrammatically in the inset to Fig. 3B. Near the centre of the c_2 zone at position 3, ipsilateral or contralateral forepaw stimulation evoked a large climbing fibre field potential with a latency of 20 or 22 ms respectively. As the recording electrode was moved either medially (e.g. position 2) or laterally (e.g. position 4) this response decreased in amplitude. At the most medial recording position (position 1), the bilateral response at latency 20–22 ms was absent, but ipsilateral forepaw stimulation gave rise to an earlier potential, at latency 15 ms, which could not be evoked by contralateral stimulation. Since such a response is characteristic of the c_1 zone, the boundary between the c_1 and c_2 zones was considered to lie between positions 1 and 2. At the most lateral recording position (position 6) a large, purely ipsilateral response was also evoked at latency 15 ms indicating that this recording locus lay within the c_3 zone. Since a very small, purely ipsilateral response could also be evoked at position 5, the boundary between the c_2 and c_3 zones was considered to lie at or near this position. The mediolateral width of the c_2 zone in this case was therefore judged to be approximately 1.0 mm.

A 15 nl injection of WGA–HRP was made at position 3 and the resulting injection site is depicted diagrammatically as the dashed circle in Fig. 3B. The rostrocaudal and dorsoventral extent of the centre of the injection site is represented in the sagittal line drawing of Fig. 3C. The "measured" mediolateral spread of the injected material was estimated to be 1.3 mm. Although this was slightly larger than the width of the zone estimated electrophysiologically, it can be seen from Fig. 3D that the terminal labelling arising from the injection was entirely restricted to nucleus interpositus posterior (levels 13–19) suggesting that the "effective injection site" (see Methods) had, in fact, been confined to the c_2 zone. Figure 3D shows that the injection also gave rise to 19 retrogradely labelled cells within interpositus posterior, many (although not all) of which were distributed in areas of the nucleus which also contained terminal labelling. No HRP-positive cells could be detected in any other cerebellar nuclei.

Figure 4 depicts the results obtained from Case 9 in which a larger injection was made into the c_2 zone. In this experiment, recordings were made from 11 positions in the paravermal cortex of lobule Vc, as depicted in Fig. 4B. The recording positions were either 100 μm apart (positions 3–8) or 200 μm apart (positions 1–3 and 8–11). The peak-to-peak amplitudes of the climbing fibre field potentials evoked by ipsilateral forepaw stimulation are presented graphically in Fig. 4C. Additional (not illustrated) responses at latency 22–23 ms could be evoked at recording positions 2–9 by a stimulus applied to the contralateral forepaw, confirming that these positions lay within the c_2 zone. Representative records of the potentials evoked at positions 1, 5 and 11 by stimulation of the ipsilateral forepaw are displayed in Fig. 4A. The distribution of the climbing fibre field potentials suggested that the c_2 zone extended from position 2 to position 9 i.e. a mediolateral width of 0.9 mm (although small c_1 responses could also be evoked at position 2 and small c_3 responses were also present at position 9). An injection of 30 nl WGA–HRP was made near the centre of the c_2 zone between positions 5 and 6. This gave rise to a "measured" injection site with a width of 3.9 mm, in-

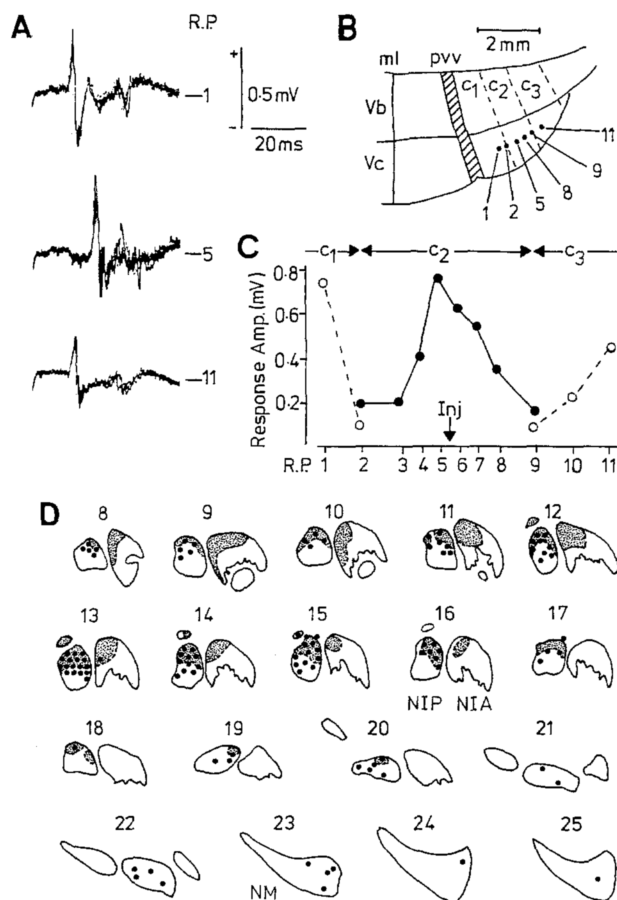

Fig. 4A–D. A large injection centred in the c_2 zone (Case 9). **A** Typical climbing fibre field potentials evoked near the centre of the c_1 zone (R.P.1), the c_2 zone (R.P.5) and the c_3 zone (R.P.11) in response to ipsilateral forepaw stimulation. **B** Diagrammatic dorsal view of lobule Vb/c to show mediolateral extent of recording positions from which climbing fibre field potentials were obtained (some intermediate recording positions not depicted for sake of clarity). Dashed lines depict mediolateral boundaries between cortical zones delimited electrophysiologically. Pvv, paravermal vein; ml, midline. **C** Graphical display of peak-to-peak amplitudes of climbing fibre field potentials evoked at positions 1 to 11 by ipsilateral forepaw stimulation. Horizontal axis depicts recording positions spaced according to their relative positions on cerebellar surface. Broken lines and open circles depict responses evoked at latency 14–15 ms in either the c_1 zone (positions 1 and 2) or the c_3 zone (positions 9–11); unbroken line and filled circles represent potentials evoked in the c_2 zone at latency 20–21 ms. Arrow between positions 5 and 6 indicates centre of injection site. **D** Location of terminal labelling (stippling) and retrogradely labelled cells (filled circles) in intracerebellar nuclei. Terminal labelling was present within interpositus anterior (NIA) at levels 8–16 and within interpositus posterior (NIP) at levels 8–20. Retrograde labelling was present mainly within NIP, but some labelled cells were also present in nucleus fastigius (NM) and interpositus anterior (at level 13)

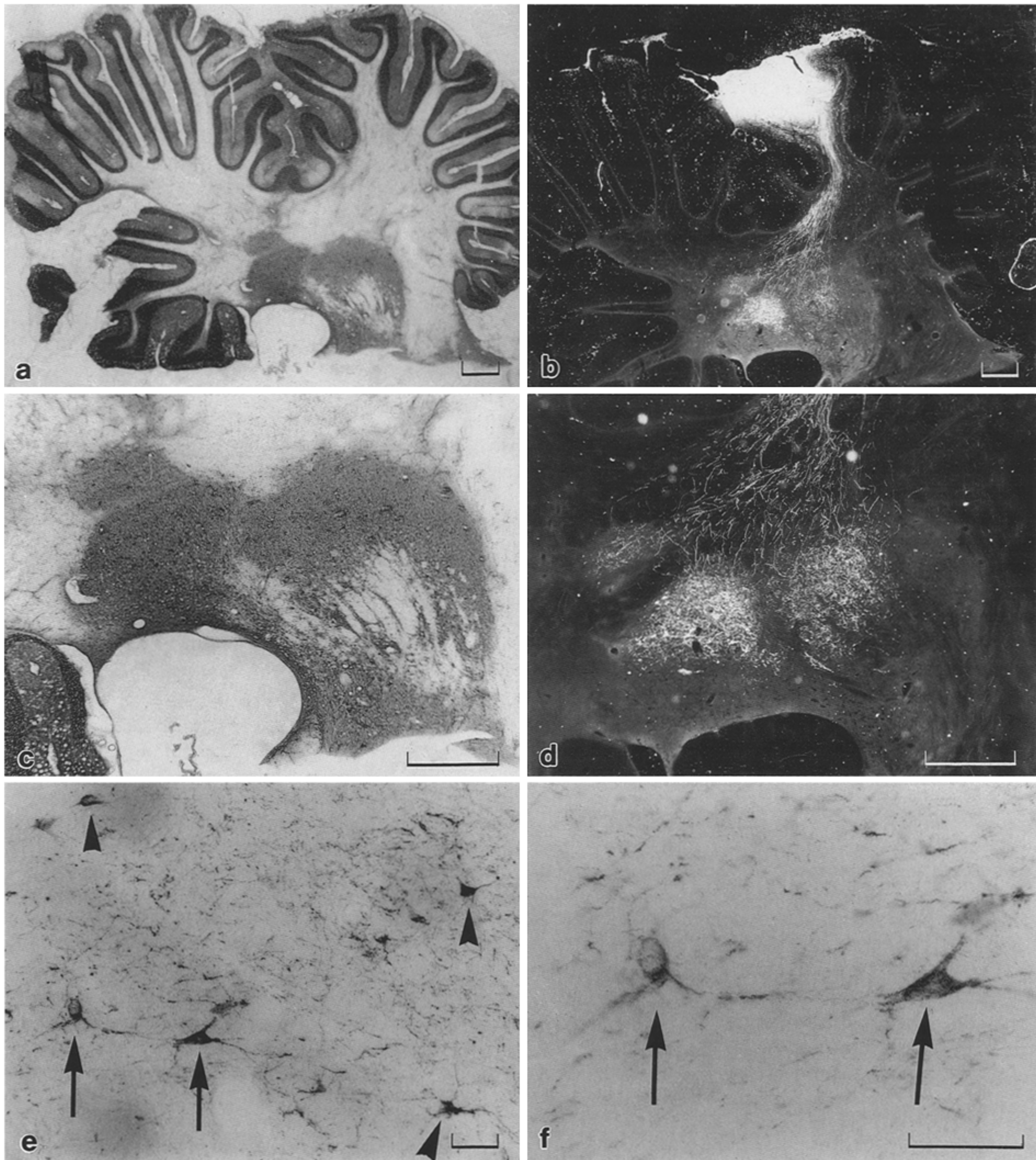

Fig. 5a–f. Photomicrographs from Case 9 (a large injection in the c_2 zone). **a** Low power, bright-field view of cresyl violet stained, sagittal section through cerebellum at the level of the paravermal cortex and nucleus interpositus. Rostral is to the right. **b** Low power, dark-field view of section adjacent to that in **a** viewed under dark-field illumination with partially crossed polarising filters. WGA–HRP injection site can be seen in lobule Vb/c together with tract of labelled axons and terminal labelling within nucleus interpositus. **c** Higher power, bright-field view of same section as in **a** to show interpositus anterior to right and interpositus posterior to left. Note that at this mediolateral level (around level 13 in Fig. 4D) interpositus posterior consists of a large ventral division with a separate, smaller dorsal division. **d** Higher power, dark-field view of section depicted in **b** to show terminal labelling in both divisions of nucleus interpositus (heavier in interpositus posterior) together with retrogradely labelled cells in interpositus posterior. **e**, **f** Higher power bright-field views of section depicted in **b, d** to show examples of retrogradely labelled cells (arrowed) in interpositus posterior. The two cells arrowed in **f** are the same as those marked with full arrows in **e**. Calibration bars: **a–d**, 1 mm; **e–f**, 100 μm

dicating that the injected material had spread significantly beyond the limits of the c_2 zone.

The injection was, however, considered to have been confined within the paravermal cortex (i.e. not to have spread beyond the medial boundary of the c_1 zone or the lateral boundary of the c_3 zone) since the distribution of retrograde labelling within the olive was restricted to the rostral poles of the dorsal and medial accessory olives (see Voogd and Bigaré 1980; Brodal and Kawamura 1980). Since neither the caudal pole of the dorsal accessory olive nor the principal olive contained labelled cells, it is unlikely that the injected tracer spread either sufficiently far medially to involve the b zone or sufficiently far laterally to include the d_1 zone.

The injection in Case 9 gave rise to terminal and to retrograde labelling within the cerebellar nuclei as depicted in Fig. 4D. The presence of terminal labelling within both divisions of nucleus interpositus (in contrast to its restriction within interpositus posterior cf. Fig. 3D) is entirely compatible with the inclusion of much of the c_1 and c_3 zones within the injection site. It is striking, therefore, that many (86) HRP-positive cells were located within interpositus posterior whereas only one retrogradely labelled cell could be found in interpositus anterior (at level 13). The retrogradely labelled cells were usually, although not always, intermingled with labelled terminal branches of Purkinje cell axons and the plexus of terminal labelling was in places so dense that it may have obscured some labelled cell bodies. The filled circles in Fig. 4D represent the number of clearly defined HRP-positive cells and it is therefore possible that the total number of labelled cells is slightly underestimated. Careful scrutiny of the labelled cells enabled many of them to be characterised morphologically as either small, spindle-shaped or larger, multipolar cells. However, it was not possible to quantify the relative numbers of these two cell types since some cells were cut in a plane of section which did not allow them to be assigned to either category. It can be seen from Fig. 4D that a few (7) cells were also found in the transition region between interpositus posterior and nucleus fastigius (levels 20–21) and a further 10 cells were scattered throughout the rostral part of the fastigial nucleus proper (levels 22–25), although this nucleus was entirely devoid of terminal labelling. There were, however, no HRP-positive cells or terminal labelling found within the dentate nucleus.

Case 9 is further illustrated in the photomicrographs of Fig. 5. It can be seen that whereas terminal labelling was present in both divisions of nucleus interpositus, retrogradely labelled cell bodies were restricted to nucleus interpositus posterior (see Fig. 5D). It was possible to categorise some of these cells on the basis of their morphological properties (see for example the multipolar cell arrowed to the right in Fig. 5F).

Injections within the c_3 zone

In a total of 10 experiments an injection of WGA–HRP was made into the c_3 zone which was invariably wider than either the c_1 or c_2 zones. It was therefore possible to centre injections within either the lateral half of the zone (3 cases) or within its medial half (7 cases). (The distinction between the two halves of the zone could be made only on geographical, not electrophysiological, grounds because there is no clear difference between the two halves of the zone in their climbing fibre responses to forepaw stimulation). From all 10 cases, a total of only 6 retrogradely labelled cerebellar nuclear cells was obtained.

Medial c_3 injections. These cases will be considered in two groups: three small injections (of diameter 1.2–1.4 mm) which gave rise to terminal labelling restricted to interpositus anterior (Cases 10, 11 and 12 in Table 1) and four larger injections (diameter 1.7–2.9 mm) which gave rise primarily to terminal labelling within interpositus anterior but in which additional, although lighter and less extensive, terminal labelling was also present in interpositus posterior (Cases 13, 14, 15 and 16 in Table 1).

No retrogradely labelled cells could be found in any of the cerebellar nuclei in Cases 10, 11 and 12. The results from a typical experiment (Case 12) are depicted in Figs. 6A and 6A'. Figure 6A depicts the location and extent of the injection site in relation to the medial and lateral boundaries of the c_3 zone which were determined electrophysiologically as described for previous cases. The zone in this case was found to be approximately 1.8 mm wide and an injection of 20 nl of WGA–HRP was made in lobule Vb, about 400 μm lateral to the medial boundary of the c_3 zone at the position indicated by the arrow in Fig. 6A. The "measured injection site" was approxi-

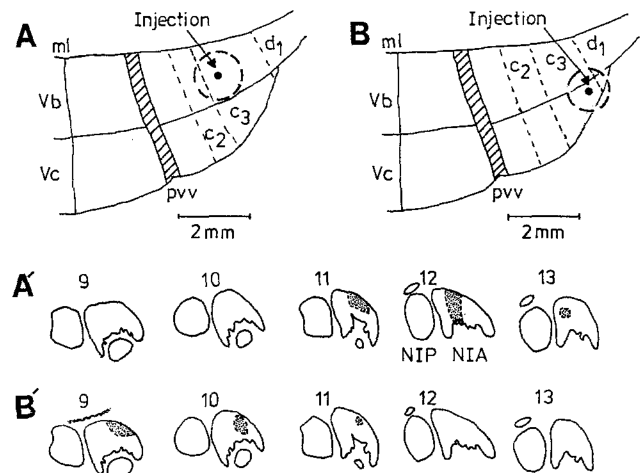

Fig. 6A, B. Two injections in the c_3 zone: one placed medially within the zone (Case 12; **A, A'**) and one placed laterally (Case 18; **B, B'**). **A, B** Diagrammatic dorsal views of lobule Vb/c to show the location and spread of injections made respectively into the medial or lateral part of the c_3 zone, in relation to the mediolateral boundaries of the zone, which were delimited electrophysiologically. **A'** Terminal labelling (stippling) arising from the injection site depicted in **A** was restricted to interpositus anterior at levels 11–13. **B'** Terminal labelling (stippling) arising from the injection site depicted in **B** was restricted to interpositus anterior (NIA) at levels 9–11 (i.e. centred slightly lateral to that depicted in **A'**). Note that no retrogradely labelled cells were identified within the intracerebellar nuclei in either case

mately 1.4 mm in diameter and its location is indicated by the dashed circle. It can be seen that the injected material was located primarily within the medial half of the c_3 zone, although there was also some spread into the c_2 zone. The "measured injection site" is, however, likely to have been an overestimate of the "effective injection site" (see Methods) and, consistent with this, the injection gave rise to terminal labelling restricted to central regions of interpositus anterior, as depicted at levels 11–13 in Fig. 6A'. No retrogradely labelled cells could be detected within the cerebellar nuclei.

In Cases 13, 14, 15 and 16 in which larger injections were made into the medial half of the c_3 zone, heavier and more extensive terminal labelling was present in interpositus anterior at the same mediolateral levels as those depicted in Fig. 6A'. This was accompanied by additional but lighter terminal labelling within more lateral regions of interpositus anterior together with some light terminal labelling in interpositus posterior. The latter labelling was very similar in distribution to that arising from injections made into the c_2 zone (cf. Figs. 3 and 4). Retrogradely labelled cerebellar nuclear cells were present in three of these four cases but in two of them (Cases 14 and 15) only one HRP-positive cell could be located (one in each division of interpositus). In Case 13, 4 retrogradely labelled cells could be identified, all within interpositus posterior (see Table 1) within regions of the nucleus which also contained terminal labelling.

Lateral c_3 injections. Three injections (Cases 17, 18 and 19) were made into the lateral half of the c_3 zone. None of these injections gave rise to any HRP-positive cells within any of the cerebellar nuclei. In every case, terminal labelling was centred further laterally within the cerebellar nuclei than that resulting from injections in the medial half of the c_3 zone. In two cases terminal labelling was restricted to lateral regions of the anterior division of interpositus, whereas in the third case (Case 19), significant terminal labelling was also present in the dentate nucleus proper, suggesting that one or more of the d zones in the most lateral part of the anterior lobe had been included in the effective injection site.

Figures 6B and 6B' depict the results from Case 18. Fig. 6B is a diagrammatic view of lobule Vb/c to show the location and spread of the injected material in relation to the boundaries of the c_3 zone which was found to be about 1.4 mm wide. An injection of 25 nl of WGA–HRP was made approximately 300 μm medial to the boundary between the c_3 and d_1 zones, as indicated by the arrow. This gave rise to a "measured injection site" of 1.1 mm in diameter, depicted by the dashed circle in Fig. 6B. It can be seen that the site was centred in the lateral half of the c_3 zone and, as shown in Fig. 6B', it gave rise to terminal labelling in interpositus anterior centred at slightly more lateral levels (levels 9–11) than the labelling produced by injections made into the medial half of the zone, which was usually located around levels 11–13 (cf. Fig. 6A', Case 12). As can be seen from Fig. 6B' there were no HRP-positive cells within the cerebellar nuclei.

Contralateral retrograde labelling arising from ipsilateral injections into the c_2 zone

In two of the cases where an injection was centred in the c_2 zone, a unilateral cerebellar injection was made so that the extent of any contralateral nucleocortical projection could be assessed. In one of these cases (Case 7), although the injection gave rise to 28 labelled cells within interpositus posterior ipsilaterally, no labelled cells could be detected contralaterally in any of the cerebellar nuclei. In the other case (Case 9), a larger injection was made and 12 HRP-positive cells were present *contralaterally*, of which 8 were located in the fastigial nucleus, 2 in the junctional region between interpositus posterior and fastigius and one in each of the two divisions of interpositus proper. These figures compare with the total of 104 labelled cells distributed throughout the intracerebellar nuclei *ipsilateral* to the injection site in this case, 87 of which were located in nucleus interpositus (see Table 1); the proportion of contralateral cells within the total in this case was therefore 10% (12/116).

Discussion

The present results show clearly that, at least for the paravermal region of lobule Vb/c in the cat anterior lobe, the organisation of the pathway from the deep nuclear cells to the cerebellar cortex is very different from that of the corticonuclear projection provided by the Purkinje cells. The organisation found for the latter pathway was in good agreement with that deduced from autoradiographic studies (Trott and Armstrong 1987a) and from retrograde transport of HRP following injections into the cerebellar nuclei (Voogd and Bigaré 1980): the c_1 and c_3 zones were found to project to partially overlapping regions within nucleus interpositus anterior, whereas the intervening c_2 zone projected to interpositus posterior. Furthermore, good agreement was found between the extent to which terminal labelling was restricted to a single division of interpositus and the extent to which the "effective injection site" was likely to have been confined to a given "electrophysiological" zone. A similar, zonal pattern within the corticonuclear projection of the paravermal region of the anterior lobe has also been described for other species, for example: the bushbaby (Haines and Rubertone 1979) and the squirrel monkey (Haines et al. 1982).

The striking findings in the present study were, however, the marked heterogeneity between the three paravermal zones in regard to their receipt of a projection from the cerebellar nuclei and the dramatic differences between the cerebellar nuclei as sources of such a projection. The principal (and almost sole) source of the nucleocortical projection was found to be nucleus interpositus posterior since 94% (190/202) of all the ipsilateral, retrogradely labelled deep cerebellar nuclear cells were located within that nucleus. The principal cortical target of the projection appears to be the c_2 zone since the only cortical injections of WGA–HRP which

gave rise to significant numbers of HRP-positive cells within the cerebellar nuclei were those ($n=5$) which were centred in the c_2 zone. In total, these injections gave rise to 182 labelled cells within interpositus posterior, 10 labelled cells in nucleus fastigius but only one such cell in interpositus anterior. A further 9 retrogradely labelled cells were found within the ipsilateral cerebellar nuclei following injections made either within the c_1 zone (1 case, 3 cells) or the c_3 zone (3 cases, 6 cells). However, since only a small number of such cells was found and since, in three of the four cases, the c_2 zone is likely to have been included within the effective injection site, such findings do not detract from the overall conclusion that, of the paravermal zones within lobule V, the c_2 zone is the principal cortical target for the nucleocortical projection which, in turn, arises almost exclusively from interpositus posterior.

In so far as the c_2 zone itself projects to interpositus posterior, these results suggest that the pathways linking the cerebellar cortex and nuclei embody a reciprocal arrangement within the "c_2-interpositus posterior axis", although even within this axis some HRP-positive cells were found within interpositus posterior in areas lacking in terminal labelling and conversely, terminal labelling was not invariably associated with the presence of retrogradely labelled cells. A very tightly coupled reciprocal arrangement does not therefore seem to be present. It should, however, be remembered in this respect that there may not be complete congruence between the "effective injection sites" for retrograde and anterograde transport of WGA–HRP so some caution is needed when comparing the precise distributions of the two types of labelling within the cerebellar nuclei.

It is of interest in this context, however, that the distribution of olivary labelling, resulting from uptake and *retrograde* transport by climbing fibres, was almost always compatible with the conclusions reached regarding the extent of each injection site from the pattern of *terminal* labelling within the cerebellar nuclei. For example, injections within the c_2 zone which resulted in terminal labelling restricted to interpositus posterior, also gave rise to olivary labelling confined to the rostral pole of the medial accessory olive (see Voogd and Bigaré 1980; Brodal and Kawamura 1980), whereas injections which gave rise to terminal labelling within more than one of the cerebellar nuclei (a finding compatible with more than one cortical zone having been included within the "effective injection site") also gave rise to retrograde labelling within more than one division of the inferior olivary complex. These findings suggest that a reasonable degree of congruence did exist between the "effective injection sites" for anterograde as compared with retrograde transport arising from a given injection of the tracer used.

It is also of interest that all 4 injections into the c_1 zone resulted in retrograde labelling within both the dorsal accessory olive and the medial accessory olive, in the regions which have been shown (Campbell and Armstrong 1985; Trott and Armstrong 1987c) to project respectively to the medial part of the c_1 zone and to its lateral (cx) part. The two halves of the c_1 zone cannot be distinguished electrophysiologically or on the basis of their corticonuclear projection (they project to common regions within interpositus anterior, see Trott and Armstrong 1987c) but the distribution of olivary labelling in the present cases demonstrates clearly that all the c_1 injection sites included both halves of the zone. It therefore appears that, at least in lobule Vb/c, neither half of the zone receives any significant nucleocortical input.

Imbalance between cerebellar nuclei as sources of nucleocortical projection

It is striking that interpositus anterior provided such a limited nucleocortical projection to any of the paravermal zones studied, with only 2 retrogradely labelled cells being located within this nucleus (one in each of Cases 9 and 15). This must reflect a genuine paucity of the projection since every cortical injection gave rise to significant *terminal* labelling within the appropriate division of nucleus interpositus, implying that the negative findings did not arise from inadequate injection of tracer material or from a failure in the histochemical processing for HRP. Furthermore, in addition to the retrograde labelling observed in the inferior olive (see above), every cortical injection also resulted in significant retrograde labelling within structures, other than the cerebellar nuclei, which provide a mossy fibre input to the cerebellar cortex e.g. the cuneate nucleus, the trigeminal nuclear complex and the lateral reticular nucleus. Such findings imply that the level of WGA–HRP in the granular layer of the cerebellar cortex was sufficiently high to result in the uptake and retrograde transport of tracer substance by mossy fibre terminals from a wide variety of sources, making it most unlikely that a projection from interpositus anterior could have been overlooked.

Since other authors (e.g. Tolbert et al. 1978a; Gould 1979; Dietrichs and Walberg 1979; Dietrichs 1981) have encountered somewhat larger numbers of retrogradely labelled cells within interpositus anterior following injections of tracer substance into the cat paravermal cortex, our findings suggest that the cortically directed axons arising from cells in that nucleus terminate within paravermal regions other than the area of present interest, which was restricted to the tips of the folia within lobule Vb/c of the anterior lobe.

Although such a marked imbalance as we have found between the two divisions of interpositus in providing a projection to the intermediate cortex has not been described before, previous tracer studies in the cat have presented material which suggests that, at least for some cases, *some* imbalance could be present in the projection to the intermediate region of the *posterior lobe* (i.e. the paramedian lobule) as well as to the area of present interest (i.e. the intermediate region of the *anterior lobe*). See, for example, Gould (1979) Figs. 2 and 4; Dietrichs and Walberg (1979) Figure 6 for examples of injections of tracer material spanning the entire mediolateral width of the paramedian lobule (i.e. zones c_1, c_2 and c_3) which gave rise to many more HRP-positive cells within interpositus posterior than within interpositus anterior. A similar, although less striking bias towards interpositus posterior appears to be present in Dietrichs (1981) Figs. 3 and 4 when tracer injections were made into the paravermal cortex of lobule V in the anterior lobe.

It is also of interest that Haines (1989) has provided evidence for zonal heterogeneity in the nucleocortical projection to lobule V in the bushbaby: within the paravermal region, the anatomically defined c_2 zone was reported to receive greater nucleocortical input than ei-

ther the c_1 or c_3 zones, although the discrepancy between the density of the projections does not appear to be as great as that reported here. Furthermore, Haines reported that injections of tracer material which spanned the entire mediolateral width of the intermediate cortex gave rise to appreciably more retrograde labelling within interpositus posterior as compared with the anterior division of the nucleus, suggesting that a disparity between the two divisions of interpositus as sources of axons directed to the cortex of lobule V may be a finding common to more than one species.

The only case in the present study in which significant numbers of HRP-positive cells were located in a cerebellar nucleus other than interpositus posterior was Case 9 in which a large injection was made in the c_2 zone. In addition to 86 labelled cells in interpositus posterior, there were 10 labelled cells within the fastigial nucleus and a further 7 labelled cells in the junctional region between fastigius and interpositus posterior. The fact that such cells were detected only when a large injection was made implies that the fastigial nucleus is not a quantitatively important source of nucleocortical projections to the paravermal cortex, although the presence of *any* such cells demonstrates an element of nonreciprocity between the corticonuclear and nucleocortical pathways since none of the paravermal zones projects to the fastigial nucleus and indeed no terminal labelling was found in that nucleus in any of the experiments. Such a finding is in agreement with the results of Dietrichs and Walberg (1979) who demonstrated that (relatively large) injections of HRP within the paravermal part of the posterior lobe (the paramedian lobule) often resulted in HRP-positive cells within fastigius, in the absence of any terminal labelling in that nucleus.

Contralateral nucleocortical projections and interpretation of labelling in cases of bilateral injections

The results obtained from the two cases in which a unilateral injection was made into the c_2 zone suggest that some cerebellar nuclear cells project to the contralateral cerebellar cortex but that this projection is considerably less dense than the corresponding ipsilateral projection. In one of these animals (Case 7), no labelled cells could be detected contralaterally in any of the cerebellar nuclei. In the other animal (Case 9), in which a larger injection was made, most contralateral cells were located either in the fastigial nucleus or in the junctional region between fastigius and interpositus posterior with only one cell in each of the two divisions of nucleus interpositus proper. Comparison with the ipsilateral side indicates that the crossed nucleocortical pathway is rather weak. Moreover, the topographical relationship between the c_2 zone and interpositus posterior is not preserved in the contralateral pathway. A contralateral nucleocortical projection has been described previously both for the cat (Dietrichs and Walberg 1979; Dietrichs 1981) and for other species e.g. the monkey (Tolbert et al. 1978a), tree shrew (Haines 1978), rat (Buisseret-Delmas and Angaut 1988) and bushbaby (Haines 1989) and it is generally agreed that the projection is weak in comparison with its ipsilateral counterpart.

The existence of any crossed connection does, however, imply that in interpreting the present results it is necessary to take account of the fact that most cases were drawn from animals in which an injection was placed in each side of the cerebellum. The possibility must therefore be considered whether, in such cases, any cells were labelled because they provided a crossed nucleocortical projection. In fact, in many bilateral cases (involving injections into the c_1 and c_3 zones) no retrograde labelling was found in either the left or the right deep nuclei so that no difficulty of interpretation arose. However, other cases involving bilateral injection, in which retrogradely labelled cells *were* identified, require more detailed consideration: these are Cases 1, 5, 6, 8 and 13 which are asterisked in Table 1. In these cases the injection sites contralateral to the labelled cells featuring in Table 1 were located in the c_2 zone (Cases 1 and 6), spanning both the c_2 and the medial c_3 zones (Case 5), in the c_1 zone (Case 8) and in the medial c_3 zone (Case 13). In four of these (Cases 1, 5, 8 and 13) the contralateral site was in lobule Vb/c whilst in the fifth (Case 6) it was in the rostral part of the paramedian lobule.

In light of the two cases, described above, in which a unilateral injection was made in the c_2 zone, it is possible that in the two bilateral cases involving a contralateral injection site in the c_2 zone in lobule Vb/c (Cases 1 and 5) a small proportion of the cells listed in Table 1 were labelled as a result of the contralateral injection. In fact, this seems the most likely explanation for the 3 cells identified in Case 1 in light of the absence of *any* retrograde labelling in the other cases in which an injection was made into the c_1 zone (Cases 2, 3 and 4). However, it is most unlikely that any significant proportion of the 19 labelled cells within interpositus posterior in Case 6 (when the contralateral injection was in the c_2 zone within rostral folia of the paramedian lobule) arose as a result of that contralateral injection, since in 2 cases of *unilateral* injections made into the c_2 zone at a corresponding rostrocaudal level within the paramedian lobule (in preparation) only a single labelled cell was found contralateral to the injection site.

In Case 8, in which the contralateral injection was in the c_1 zone, it is very unlikely that any of the 31 cells in nucleus interpositus posterior (see Table 1) resulted from the contralateral injection, in view of the findings from another animal in which retrograde labelling was entirely absent from both the right and the left deep nuclei when injections were placed in both c_1 zones (animal HC17, Cases 3 and 4).

Finally, in Case 13 there were 4 labelled cells, all in interpositus posterior, after injection into both of the medial c_3 zones. In this instance no evidence is available to decide categorically which of the two injections gave rise to the retrograde labelling but the number of cells involved is clearly small. Furthermore, in view of the fact that the injection in Case 13 gave rise to terminal labelling within both divisions of the ipsilateral nucleus inter-

positus (see Table 1) it is probable that the effective injection site included part of the c_2 zone, raising the possibility that the retrograde labelling arose from the ipsilateral c_2 zone rather than from either the ipsilateral or contralateral c_3 zone. Indeed, in view of the absence of *any* retrograde labelling in animal HC19 (Cases 11 and 12) in which injections were made bilaterally into the medial c_3 zone (and in which terminal labelling was restricted to interpositus anterior) this seems the most likely explanation.

In conclusion, it seems that the procedure of making an injection in each half of the cerebellum and considering the two sides of the cerebellum separately, is most unlikely to have given rise to any significant error in attributing the labelled cells to the ipsilateral injection site in each case.

Density of nucleocortical projection

It could perhaps be argued that the numbers of deep nuclear cells revealed as providing a cortically directed axon terminal are not high in comparison with the total nuclear cell population. The five cases of injections within the c_2 zone (Cases 5–9) yielded respectively counts of 11, 19, 28, 31 and 86 labelled cells within interpositus posterior with larger injection sites giving rise to greater numbers of labelled cells. These figures do not, however, represent the true number of HRP-positive cells in each case since only alternate sagittal sections through the nuclei were processed histochemically and used for cell counts. If the observed number of labelled cells is simply doubled for each case, an overestimate of the true population of labelled cells will be obtained (see Abercrombie 1946) since some cells counted will have been portions of cells actually 'centred' within and therefore 'belonging to' the unstudied sections. The true numbers of labelled cells can, however, be estimated using an approximate correction factor (Abercrombie 1946). Thus the total number of retrogradely labelled cells (T) would be given by the formula:

$$T = 2 \times C \times S/(D+S),$$

where C is the counted number of cells from alternate sections, S is the section thickness (μm) and D is the average diameter (μm) of the cells. The section thickness was 50 μm and if the average diameter of a nuclear cell is taken as 20 μm (although this can only be a rough average since the cerebellar nuclear cells make up a cell population multimodal in their diameter distribution), the true number of retrogradely labelled cells is given by:

$$T = 2 \times C \times 50/70.$$

The total number of labelled cells for each of the five cases described above would therefore be respectively 16, 27, 40, 44 and 123 giving an average value of 50 cells per case. By comparison, it has been estimated (Allen and Tsukuhara 1974) that nucleus interpositus posterior contains, on average, 5000 cells with a total of 23000 cells being distributed throughout all of the intracerebellar nuclei in each half of the cerebellum. However, it should be remembered that the size of the injections in the present study was small in comparison with the total area of the unfolded cerebellar cortical sheet (estimated in the cat as being around 2300 mm^2 by Palkovits et al. 1971). When injections were made in the c_2 zone the injected tracer reached, on average, approximately 8 mm^2 of this sheet, which represents only 0.4% of the total in one half of the cerebellum but nevertheless resulted in retrograde labelling of around 1% of the cells within interpositus posterior (and around 0.2% of the total population of nuclear cells ipsilateral to the injection site).

This suggests that the apparent weakness of the nucleocortical projection described in some studies (e.g. Hamori et al. 1981; Legendre and Courville 1986) may be a reflection of its topographical specificity rather than a generalised low density within the projection throughout the entire cerebellum. It is also possible that the 5% of mossy fibre rosettes which survived pedunculotomy (and which were equated with a population of mossy fibre terminals arising from an "intracerebellar" source) as described by Hamori et al. (1981), may underestimate the proportion of mossy fibres which arise from cells within the cerebellar nuclei. It has been suggested, for example, by Haines (1989) that those cortically directed axons which arise as collaterals of cerebello-fugal axons (e.g. Tolbert et al. 1978; McCrea et al. 1978; Payne 1983) are also likely to have undergone degenerative changes during the two weeks following section of their parent axon by a peduncular lesion. It is therefore possible that the 5% surviving mossy fibre rosettes observed by Hamori et al. (1981) may represent only those nucleocortical terminals arising from a subpopulation of nuclear cells which project *principally or only* to the cerebellar cortex.

Functional implications of nucleocortical projection

The topographical specificity revealed within the nucleocortical pathway in the present study may carry important functional implications. In common with the findings of Tolbert et al. (1978b), HRP-positive cells were found within both the small, spindle-shaped (fusiform) and the larger, multipolar cell populations within the deep nuclei, confirming that each of these cell types can provide cortically directed axonal branches. The demonstration that these cortically directed axons arise, at least in part, as collateral branches of cerebellar efferents destined for the contralateral ventrolateral thalamus and/or inferior olive (Tolbert et al. 1978b; Payne 1983) implies that some form of cerebellar outflow is fed back to at least part of the c_2 zone within the anterior lobe. By contrast, there is little evidence from the present study of such feedback to the flanking c_1 and c_3 zones. Further study at other rostrocaudal levels in the cortical sheet is needed to establish whether this inter-zonal difference is maintained for other parts of the c_1, c_2 and c_3 zones and a study of the nucleocortical projection to the paramedian lobule of the cat is currently in progress in our laboratory.

It is not at present clear whether the nucleocortical pathway exerts an excitatory or inhibitory synaptic effect within the granular layer: classically, all mossy fibre terminals have been considered to be excitatory (see e.g. Eccles et al. 1967; Palay and Chan-Palay 1974) although more recent reports (Chan-Palay et al. 1979; Angaut et al. 1988; Batini et al. 1989) cite evidence for a putative GABAergic (and therefore presumably inhibitory) pathway from the intracerebellar nuclei to the cerebellar cortex in the rat. Regardless of the sign of the nucleocortical projection, however, the present study reveals a clear difference in connectivity, at least for lobule Vb/c, between the paravermal zones in their receipt of such a projection and between the two divisions of nucleus interpositus in the extent to which their cells provide cortically directed axons. The demonstration of such a striking distinction between the cerebellar compartments adds force to the hypothesis put forward by Oscarsson (1980) that the sagittal zones within the cerebellar cortex, together with their associated input and output pathways, may constitute basic functional units of the cerebellum, each with its own particular motor responsibilities.

Acknowledgements. We are grateful to Mrs Lesley Amos for expert technical assistance. The study was funded by the Wellcome Trust.

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Received July 24, 1989 / Accepted October 31, 1989