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RESEARCH ARTICLE

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Sagittal zonal organization of climbing fibre input to the cerebellar anterior lobe of the ferret

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Abstract The organization of climbing fibre input to the cerebellar anterior lobe of the ferret was investigated in barbiturate-anaesthetized animals. Climbing fibre field potentials evoked on electrical stimulation of forelimb and hindlimb nerves were recorded at the cerebellar surface. Based on characteristic latencies of climbing fibre responses and their relative localization along the longitudinal axis of the folia, nine sagittally oriented zones could be distinguished and were tentatively named, from medial to lateral, A, X, B, C1, Cx, C2, C3, D1 and D2. Within the C1, C2 and C3 zones, climbing fibre input from the ipsilateral forelimb was found caudally and from the hindlimb rostrally, while the corresponding topographical representation in the B and D2 zones was medial to lateral. The X, Cx and D1 zones did not receive input from the hindlimb, while input from the forelimb to the A zone was weak. Overall, the sagittal zonal organization of climbing fibre input appears to conform with the compartmentalization of the ferret cerebellum based on the myeloarchitecture of corticonuclear fibres, although the X and Cx zones have not been previously identified. In terms of both general electrophysiological characteristics of input to different zones and intrazonal topographical representation, the organization of climbing fibre input to the ferret cerebellum seems to strongly resemble that in the cat. The findings thus provide evidence of cross-species generality of cerebellar sagittal organization.

Key words Motor control · Spino-olivary pathways · Inferior olive · Cerebellum · Ferret

Introduction

An intricate compartmentalization of the cerebellum has been revealed in studies of corticonuclear and olivocerebellar connections, mainly in the cat (for reviews see Voogd and Bigaré 1980; Ito 1984; Armstrong 1990). Each compartment consists of a sagittally oriented cerebellar cortical zone innervated by climbing fibres from a circumscribed portion of the inferior olive and in turn projecting to a restricted region of the deep cerebellar nuclei. Seven zones were delineated in the early investigations (Voogd 1964, 1969; see also Armstrong et al. 1974), while subsequent work indicates a division of the cortex into at least nine major zones (Voogd 1982; Campbell and Armstrong 1985; Trott and Apps 1991; see also Ekerot and Larson 1979a, 1982).

It is reasonable to assume that the anatomical olivocortico-nuclear compartmentalization reflects a functional division of the cerebellar circuitry. Given the uniformity of the cytoarchitecture throughout the cerebellar cortex, it would indeed be expected that the specific functions carried out by different cortical regions are determined mainly by the local afferent and efferent connections. This notion is strongly supported by the fact that different parts of the inferior olive receive input from specific sets of spino-olivary pathways with different origins and synaptic relays (cf. Andersson et al. 1987) and that different subdivisions of the deep cerebellar nuclei project onto different descending tracts with specific roles in motor control (cf. Ito 1984).

As a consequence of the specificity in their spino-olivocerebellar input, the sagittal zones in the cat cerebellum can readily be identified by the attributes of climbing fibre field potentials evoked on peripheral stimulation (for references, see Oscarsson 1980). The high spatial resolution provided by this methodological approach has played a crucial role both in the identification of new zones (Ekerot and Larson 1979a, 1982; see Discussion) and of microzones, proposed to constitute the functional units of the cerebellar circuitry (Andersson and Oscarsson 1978; Ekerot et al. 1991).

Most investigations of spino-olivocerebellar input have been restricted to the cat, and comparative electrophysiological investigations are scarce. It is noteworthy, however, that studies in the rat indicate that inter-species differences in the organization of climbing fibre input

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may exist (e.g. Ekerot et al. 1996). In the ferret, which was one of the first species to be investigated with anatomical techniques (Voogd 1969), a comprehensive electrophysiological assessment of the organization of climbing fibre input seems to be lacking, in spite of recent use of ferrets in studies of cerebellar function (e.g. Elias et al. 1987; Lou and Bloedel 1992; Ivarsson and Hesslow 1993).

Therefore, the main aim of the present study was to establish whether a zonal organization of the ferret cerebellum compatible with that demonstrated by anatomical techniques is evident on analysis of climbing fibre field potentials evoked on peripheral stimulation. In addition, comparative aspects between ferret and cat cerebellar organization will be discussed. The findings suggest a division of the caudal part of the cerebellar anterior lobe of the ferret into nine sagittally oriented zones. These were tentatively named the A, X, B, C1, Cx, C2, C3, D1 and D2 zones in accordance with nomenclature from the original anatomical studies in the ferret (Voogd 1969) and modifications in subsequent electrophysiological and anatomical studies in the cat ('Cx' often being referred to as 'lateral C1'; Trott and Apps 1991).

Materials and methods

Animals and preparation

Experiments were performed on six adult male ferrets (1.8–2.1 kg) anaesthetized with pentobarbital (35–40 mg/kg i.p.; supplementary doses i.v., as required). The animals were purpose-bred and experimental procedures were approved in advance by the local Swedish Ethical Committee. Throughout the experiment a surgical level of anaesthesia was maintained, characterized by general muscle atonia, completely depressed withdrawal reflexes, constricted pupils and a stable blood pressure, also during electrical stimulation of peripheral nerves or noxious mechanical stimulation of the skin.

To obtain stable recording conditions, the animals were paralyzed with alcuronium and a bilateral pneumothorax was made. After the pneumothorax, the ferrets were artificially ventilated using a mixture of air and oxygen. In order to further check the level of anaesthesia, the muscle relaxant was allowed to wear off at regular intervals. Cannulae were inserted into the trachea and the right femoral artery and vein. The end-expiratory CO_2 concentration, mean arterial blood pressure and rectal temperature were monitored throughout the experiment and kept within physiological limits. A continuous infusion of 5% glucose in Ringer acetate was given.

The head of the ferret was placed in an adjustable stereotaxic frame previously used for cats, and the left cerebellar anterior lobe was subsequently exposed following craniotomy, resection of the occipital lobe and removal of the tentorial dura mater. The cerebellar surface was covered with warm mineral oil to minimize shunting of currents and to prevent drying and heat dissipation. A hole in the dura mater of the caudal brainstem was made in order to provide a drainage of cerebrospinal fluid and thereby increase the mechanical stability of the cerebellum. The left (ipsilateral to the cerebellar exposure) ulnar (Uln), superficial radial (SR) and sciatic (Sci) nerves and the right (contralateral) SR nerve (except in one experiment) were dissected, cut distally and mounted on bipolar hook electrodes for electrical stimulation in a pool of warm mineral oil. Approximate sites of stimulation for the Uln, SR and Sci nerves were, respectively, at the level of the distal upper arm, the proximal forearm and the proximal hindlimb.

Stimulation and recordings

Field potentials evoked on peripheral nerve stimulation were recorded at the cerebellar surface, using a small, ball-tipped electrode. Climbing fibre responses were seen as sharply deflecting potentials with a biphasic positive-negative configuration, often preceded by responses related to mossy fibre input. At the beginning of each experiment, the cerebellar surface was scanned for input from the four nerves, and the stimulus threshold (T) for evoking a detectable climbing fibre response at the locus with the largest potentials was determined for each nerve separately. For data collection, the nerves were stimulated at 10T, and field potentials evoked on five consecutive stimulus presentations at a rate of 0.5 Hz were routinely averaged for off-line analysis. Recording locations were indicated on a photograph of the cerebellar surface. Slow fluctuations of excitability in afferent pathways and in the inferior olive were reduced by administrating the required supplementary anaesthetic in small doses, sometimes using a mini-pump. Furthermore, input from each of the nerves was routinely re-assessed at regular intervals by monitoring a few 'reference' recording locations.

At the end of the experiment, the animals were killed by an overdose of barbiturate and subsequently perfused with formalin (10%) in saline. The cerebellum was removed for verification of recording locations with respect to the cerebellar lobules (cf. Voogd 1969).

Analysis and presentation of data

Response latencies were measured from onset of the stimulus artefact to onset of the evoked climbing fibre field potential. Response size was defined as the amplitude from onset to the positive peak of the potential. Since there was often some overlap between potentials from neighbouring sagittal zones, the plots of response amplitudes within zones A to D2, inclusive, are presented on separate outlines for clarity in the primary analysis (Figs. 2–4). In these figures, the outline of each zone has been drawn so as to encompass mediolaterally all recording sites from which responses belonging to that particular zone were obtained from any of the nerves stimulated. In the rostrocaudal dimension, the outlines are continuous unless discontinuity of the zone was indicated by an absence of responses belonging to that particular zone in any of the folia recorded from.

Figure 5B shows a synthesis of the present findings superimposed on an outline of cerebellar lobules IV-VI, adapted from Voogd (1969). The diagram was constructed from the data presented in Figs. 2–4 in the following way. If the localization of a zonal boundary was not evident from the raw data, i.e. potentials from two neighbouring zones were observed at the same recording site, that site was assigned to the zone displaying the locally generated potential with the largest relative amplitude as compared to the other potentials within that zone in the same folium. The zonal boundaries were then transferred from the original cerebellar outlines (cf. Figs. 2–4, left panel) to the diagram in Fig. 5B by expressing the width of each zone as a fraction of the total length of a given folium, separately for lobules IV, V and VI.

Results

The organization of climbing fibre input evoked on peripheral nerve stimulation was investigated in a total of six experiments, covering lobules IV (n=5), V (n=6) and VI (n=3) of the ferret cerebellum. The number of recording sites in individual animals ranged from 25 to 130, distributed rostrocaudally over three to seven folia. In order to give an impression of the degree of consistency or variability in the data, the results from two experiments are described in detail in the account below. These two experiments, henceforth referred to by roman numerals (experiment I and experiment II, respectively), were selected be-

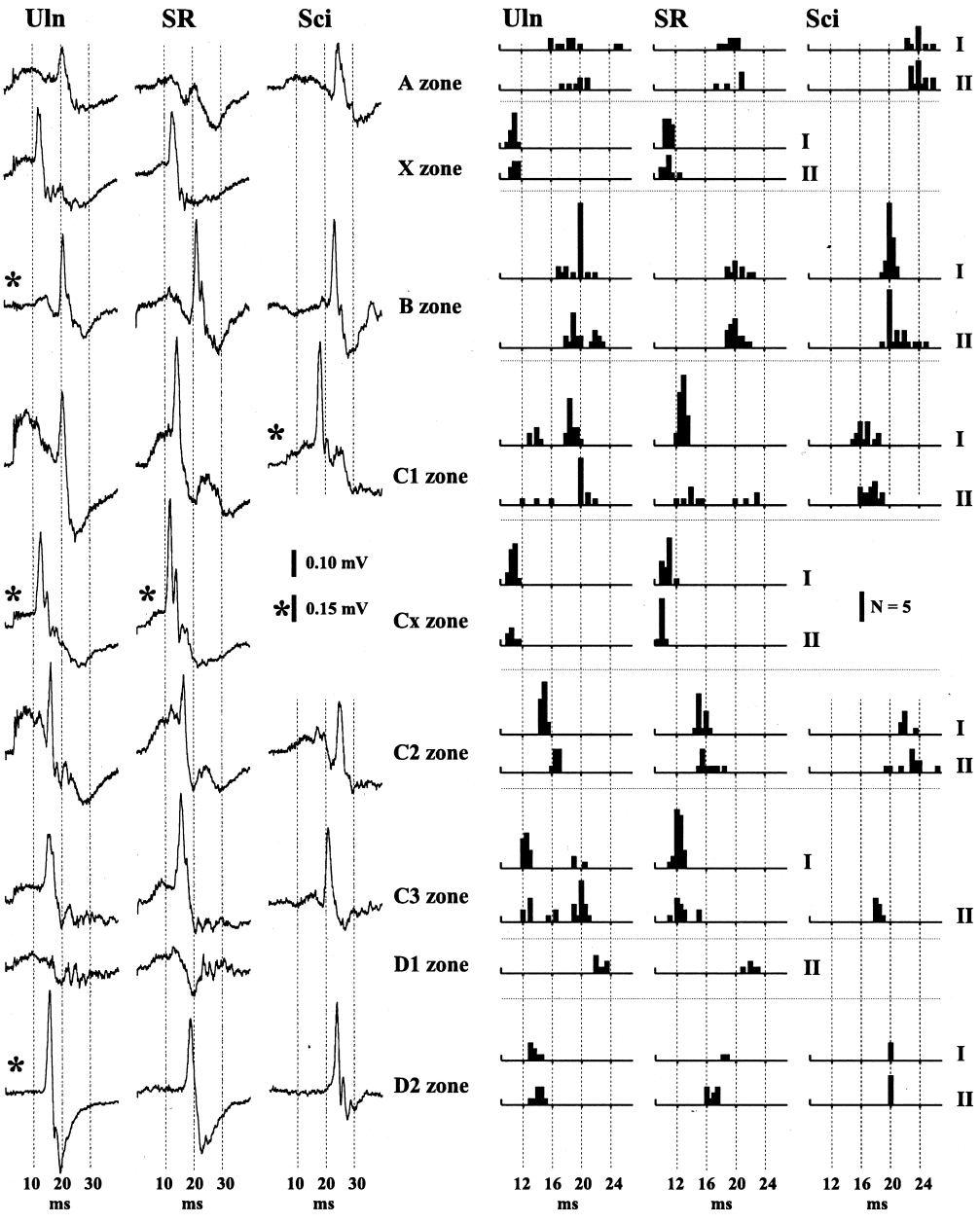

Fig. 1 Raw data and latencies of climbing fibre responses characteristic of different zones. *Left panel:* Climbing fibre field potentials from experiments I and II evoked on stimulation of peripheral nerves. Each record represents a mean of five consecutive sweeps. In most cases, the potentials shown were among the largest ones recorded in the zone. Note different voltage scale for records indicated by *asterisk*. *Right panel:* Distribution of response latencies in experiment I (*I*; *upper row* of histograms for each zone) and experiment II (*II*; *lower row*). Bin width 0.5 ms in all histograms. Note different time scale as compared to *left panel* (*Uln* ulnar nerve, *SR* superficial radial nerve, *Sci* sciatic nerve)

cause they had the two largest numbers of recording sites and the highest resolution of mapping. Unless otherwise indicated, all findings were compatible with data from the four other experiments in the study.

Latencies of climbing fibre field potentials

Examples of averaged field potentials evoked on stimulation of the ipsilateral Uln, SR and Sci nerves and distributions of response latencies in experiment I and experiment II are shown in Fig. 1 (see also Tables 1, 2). Each set of potentials has been assigned to a particular zone mainly on the basis of characteristic onset latencies and relative location along the longitudinal axis of the folia. The classification of climbing fibre responses can be regarded as a working hypothesis of zonal organization, which is tested by evaluating the distribution of response latencies with respect to consistency within zones and differences between neighbouring zones. These consistencies and differences are best appreciated by inspection of the histograms in Fig. 1 (right panel) and the spatial distributions of potentials with different latencies, shown in Figs. 2–4. Henceforth, the zones are tentatively named in accordance with nomenclature established in the original anatomical investigation in the ferret and subsequent anatomical and electrophysiological studies in the cat (for references, see Introduction). The similarities between the present findings and the cerebellar organization derived from previous studies will be evaluated in the Discussion.

Table 1 Comparison between mean latencies (milliseconds) in experiment I (*I*) and experiment II (*II*). *Uln* ulnar nerve, *SR* superficial radial nerve, *Sci* sciatic nerve

Zone:	A	X	B	C1	Cx	C2	C3	D1	D2
Uln I	19.3	10.8	19.5	13.8; 18.8[a]	10.7	14.9	12.4; 19.5[a]	–[b]	13.5
II	19.6	11.1	20.2	14.0; 20.4[a]	10.6	16.7	12.7; 19.9[a]	22.6	14.1
SR I	19.5	11.0	20.4	12.9	10.7	15.4	12.2	–[b]	18.5
II	19.9	10.9	20.1	13.9; 21.9[a]	10.0	16.3	12.7	22.1	16.8
Sci I	23.9	–[b]	20.1	16.6	–[b]	22.1	–[b]	–[b]	20.0
II	24.1	–[b]	21.1	17.4	–[b]	22.9	18.3	–[b]	20.0

[a] Pairs of values given for C1 and C3 zones reflect bimodal distributions of response latencies (Fig. 1). The divisions into two ranges of response latencies were based on visual inspection of histograms
[b] No input found

Table 2 Comparison between latencies (milliseconds) of climbing fibre field potentials in the ferret (experiments I and II) and in the cat

Zone	A	X	B	C1	Cx	C2	C3	D1	D2
Uln Cat	–[d]	10.0–15.0[c]	17.0–25.0[b]	11.0–14.0[c]	cf. X zone	19.0–24.0[c]	11.0–14.0[c]	15.0–20.0[c]	cf. D1[e]
Ferret	16.0–25.5	10.0–11.5	17.0–23.0	12.0–16.0[a]	10.0–11.5	14.5–17.0	12.0–13.0[a]	22.0–23.5	13.0–15.0
SR Cat	–[d]	12.0–19.0[c]	–[d]	10.0–14.0[c]	cf. X zone	17.0–27.0[c]	10.0–13.0[c]	17.0–25.0[c]	cf. D1[e]
Ferret	17.5–21.0	10.0–12.5	19.0–22.5	12.0–15.5[a]	9.5–12.0	14.5–18.5	11.0–13.0[a]	21.0–23.0	16.0–18.5
Sci Cat	18.0–25.0[b]	–[d]	16.5–23.0[b]	14.0–19.0[b, c]	–[d]	24.0–30.0[c]	16.0–22.0[b, c]	20.0–25.0[c]	cf. D1[e]
Ferret	22.5–26.0	–[d]	19.0–25.0	15.0–19.0	–[d]	19.5–26.5	18.0–19.0	–[d]	20.00

[a] Only short-latency range given (cf. Fig. 1 and Table 1)
[b] Oscarsson and Sjölund 1977
[c] Ekerot and Larson 1979a; response latencies represent a range based on shortest response latencies in each experiment
[d] No input found
[e] The D2 zone in the ferret may correspond to the D1 zone in the cat

Note positive potentials attributable to mossy fibre input preceding some of the climbing fibre field potentials (Fig. 1, left panel).

Generally, taking into consideration the input from all nerves stimulated, responses in each zone were readily distinguished from those of its immediate medial and lateral neighbours, as outlined below (see Figs. 2–4). Response latencies in the four other experiments usually fell well within the ranges given for experiment I and experiment II (Table 2). When present, discrepancies were never more than 0.5 ms. Note that the sequence of field potentials from top to bottom in Fig. 1 (left panel) is schematic and should not be taken to represent single sites in a mediolateral sequence along a single folium, as input from forelimb and hindlimb nerves usually did not converge in this manner. Input from the contralateral SR nerve was found only in the C2 zone and will be commented upon in that context.

Spatial distribution of climbing fibre field potentials

The vermal A, X and B zones

The A zone (Fig. 2, top row), located in the medial-most part of the vermis, was usually positively identified in lobules IV and V, but no input was found in lobule VI. In two experiments, the zone was negatively defined as a non-responsive area medial to the X zone. Climbing fibre responses in the A zone evoked on Uln and SR nerve stimulation were characterized by intermediate to long latencies, small amplitudes and no obvious rostrocaudal or mediolateral bias in distribution within the zone. Input from the Sci nerve had long latencies and a somewhat biased distribution, with small amplitudes in lobule V and larger amplitudes in lobule IV.

The rather narrow X zone (Fig. 2, middle row), located lateral to the A zone, was positively identified in lobules IV, V and VI. Climbing fibre responses in the X zone evoked on Uln and SR nerve stimulation were characterized by very short latencies, rather small amplitudes and no obvious rostrocaudal or mediolateral bias in distribution within the zone. The X zone did not receive input from the Sci nerve.

The B zone (Fig. 2, bottom row), located lateral to the X zone, was positively identified in lobules IV, V and VI. Climbing fibre responses in the B zone evoked on Uln, SR and Sci nerve stimulation were all characterized by rather long latencies. Detailed inspection of the relative distribution of forelimb and hindlimb input along the longitudinal axis of the folia investigated revealed that, in general, input from the Uln and SR nerves dominated me-

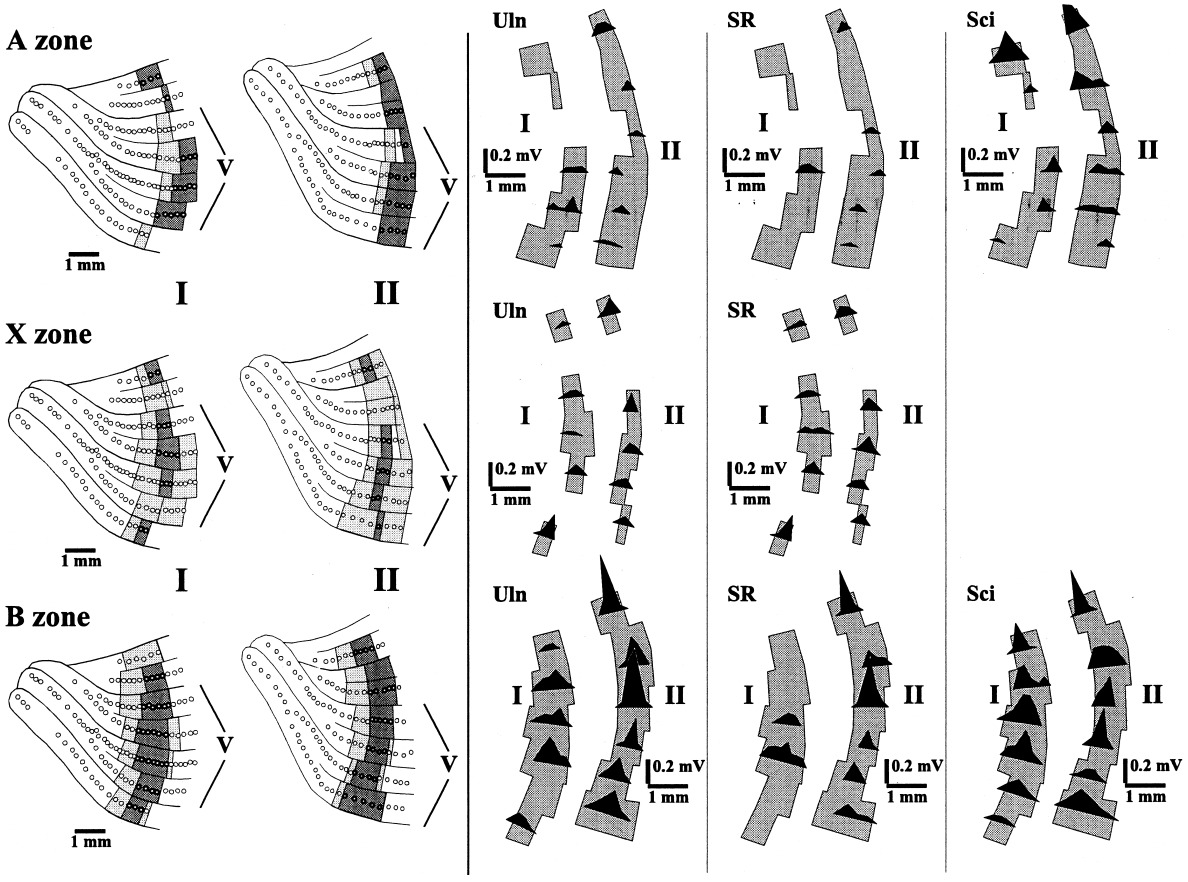

Fig. 2 Spatial distribution of climbing fibre field potentials in the vermal A, X and B zones. *Left panel:* Dorsal view of the left cerebellar anterior lobe for experiment I (*I*; *left column*) and experiment II (*II*; *right column*). *Open circles* indicate all recording sites from which averaged field potentials were obtained. *From top to bottom*, outlines of the A, X, and B zones (*dark grey*) shown superimposed on the outlines of the immediate medial and lateral neighbouring zones (*light grey*; *V* lobule V; cf. Voogd 1969). *Right panel: From top to bottom*, enlarged outlines of the A, X and B zones (cf. *left panel, dark grey*) shown in pairs for each peripheral nerve stimulated. Experiments I (*I*) and II (*II*) shown, respectively, to the left and to the right in each pair. Amplitudes of climbing fibre field potentials were plotted, as a mountain range along the zone, at right angles to a line connecting the recording sites in each folium

dially, while input from the Sci nerve dominated laterally in the zone. This was observed in five of six experiments.

Pars intermedia: the C zones

The C1 zone (Fig. 3, top row), located lateral to the B zone, was positively identified in lobules IV, V and VI. Although climbing fibre responses in the C1 zone evoked on Uln stimulation overall had a rather wide range of latencies, the data are suggestive of a bimodal distribution. Usually, responses within the short- or intermediate-latency range were found to be distributed caudal to responses within the long-latency range. By analogy, responses evoked on SR nerve stimulation had a bimodal distribution of latencies in experiment II and conformed, without exceptions, to the differential rostrocaudal distribution of short- or intermediate-latency compared with long-latency responses. The distribution of SR nerve response latencies in experiment I was unimodal. Climbing fibre responses in the C1 zone evoked on Sci nerve stimulation were characterized by short to intermediate latencies.

There was a clear difference in the spatial distribution of input from forelimb and hindlimb nerves within the C1 zone. Responses evoked on stimulation of the Sci nerve were found in lobule IV and rostral folia of lobule V, with decreasing amplitudes in the rostral-to-caudal direction. By contrast, responses evoked on stimulation of Uln and SR nerves were found mainly in lobule V (and to some extent also in lobule VI), with larger amplitudes in caudal than in rostral folia. The overall differential distribution of forelimb and hindlimb input was found in five of six experiments, while in one animal the caudal part of the C1 zone, and hence the forelimb input, appeared to be missing. The long-latency responses from the forelimb nerves in lobule IV of experiment II constitute an exception to the usual caudal localization of forelimb input. Note, however, that the forelimb input to these rostral parts of the C1 zone did not overlap entirely with the hindlimb input. Rather, the forelimb input consistently displayed maximal amplitudes lateral to the maximal amplitudes of the hindlimb input, also in experiments other than experiment II.

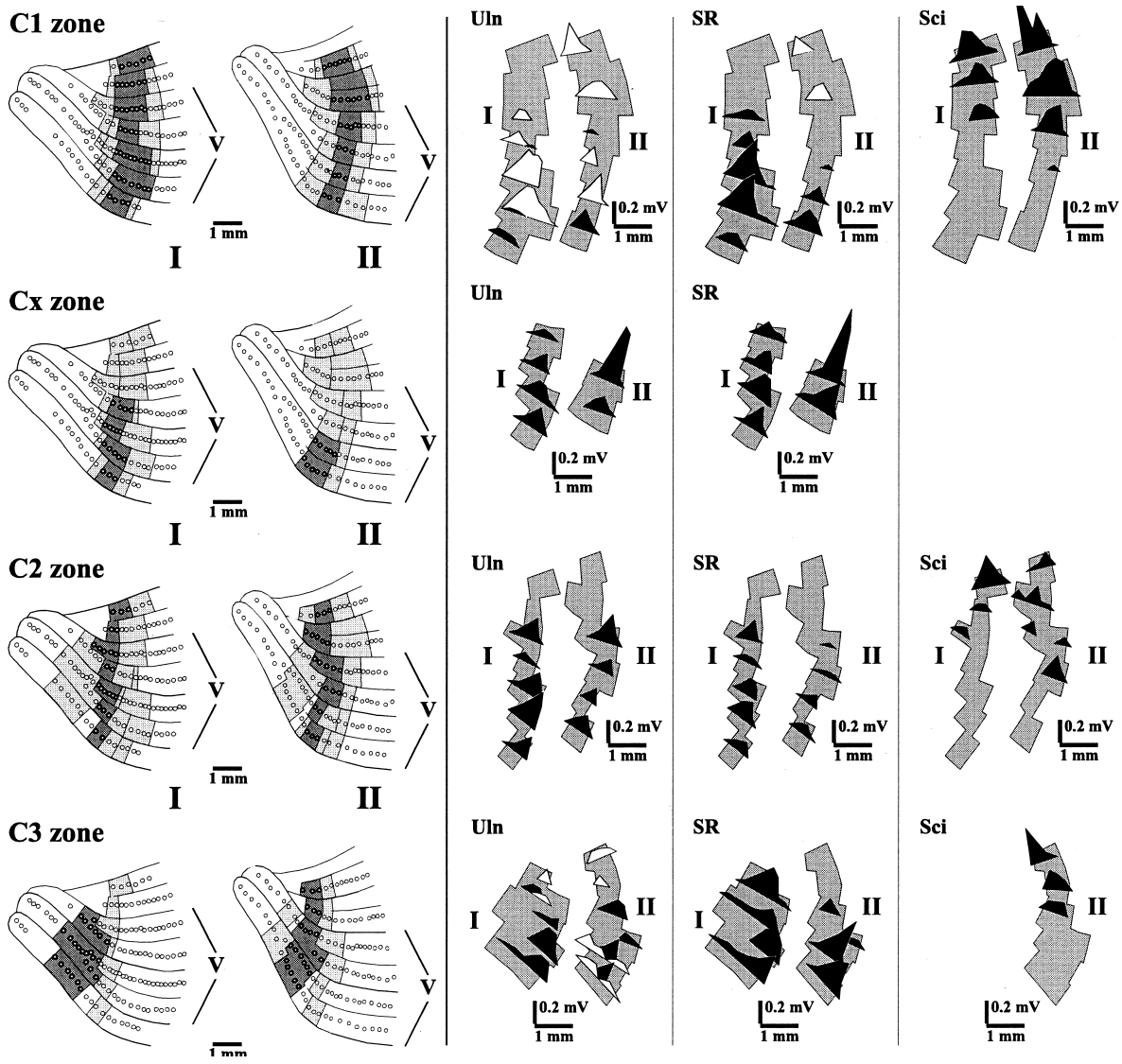

Fig. 3 Spatial distribution of climbing fibre field potentials in pars intermedia: the C zones. *From top to bottom:* The C1, Cx, C2 and C3 zones. Layout and conventions as in Fig. 2. In the response amplitude plots for forelimb input to the C1 and C3 zones (*right panel*), long-latency responses (see text and Fig. 1) are *white*

The Cx zone (Fig. 3, 2nd row), located lateral to the C1 zone, was positively identified in caudal folia of lobule V and in lobule VI. In one experiment, covering lobules IV and V, the Cx zone appeared to be missing. Climbing fibre responses in the Cx zone evoked on Uln and SR nerve stimulation were characterized by very short latencies and no obvious rostrocaudal or mediolateral bias in distribution within the zone. The Cx zone did not receive input from the Sci nerve, while in one experiment input from the Uln nerve was very weak.

The C2 zone (Fig. 3, 3rd row), located lateral to the Cx zone, was positively identified in lobules IV, V and VI. Climbing fibre responses in the C2 zone evoked on Uln and SR nerve stimulation were characterized by intermediate latencies. In addition to the input from ipsilateral forelimb nerves, the C2 zone received a unique and highly characteristic input from the contralateral SR nerve (not shown). The climbing fibre responses evoked from the contralateral peripheral stimulation had long latencies and in most experiments rather small amplitudes. Variations in both latencies and amplitudes on consecutive stimulus presentations were often observed, suggesting that this input is quite weak, at least in a barbiturate-anaesthetized preparation. Input from the Sci nerve to the C2 zone had long latencies.

There was a clear differential distribution of input from forelimb and hindlimb nerves within the C2 zone, except in one experiment in which no Sci nerve input to this zone was identified. Responses evoked on stimulation of the Sci nerve were found in lobule IV and rostral folia of lobule V, while responses evoked on stimulation of the Uln and SR nerves were found exclusively in lobules V and VI.

The C3 zone (Fig. 3, bottom row), located lateral to the C2 zone, was positively identified in lobules V and VI and, in three of five experiments, also in lobule IV. Although climbing fibre responses in the C3 zone evoked

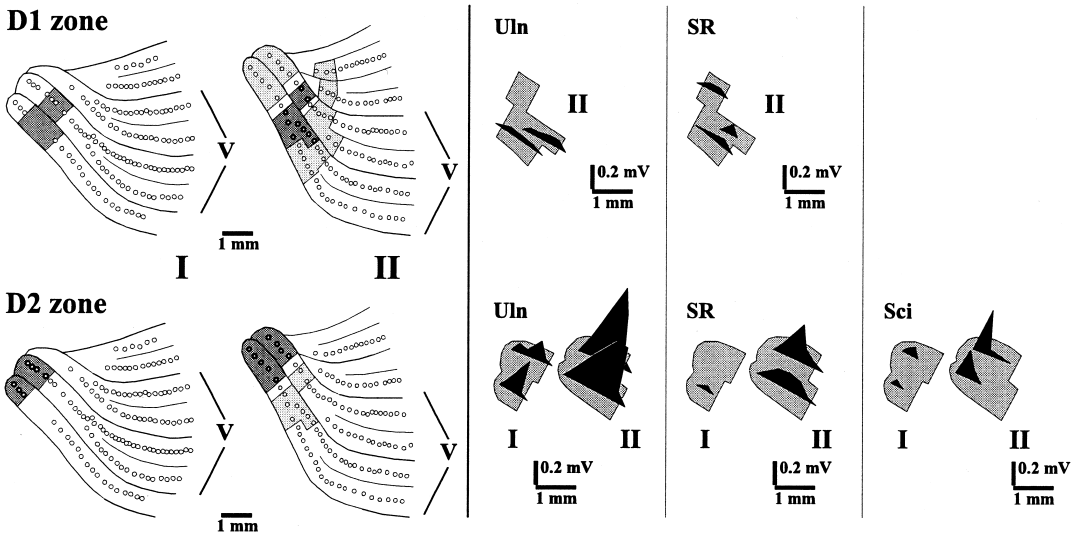

Fig. 4 Spatial distribution of climbing fibre field potentials in pars intermedia: the D zones. *Upper and lower row:* The D1 and D2 zones. Layout and conventions as in Fig. 2. In experiment I the D1 zone in lobule VI was mapped by inspection of single sweeps. No responses were found

on Uln stimulation overall had a rather wide range of latencies, there appeared to be a bimodal distribution, with only a few responses at intermediate latencies. Generally, responses within the short- or intermediate-latency range were found to be distributed caudal to responses within the long-latency range. The only exceptions to this rule were a few long-latency responses with small amplitudes found in caudal folia of the C3 zone in experiment II. These were consistently found close to the medial and lateral boundaries of the zone and never in its centre. By contrast, climbing fibre responses evoked on SR nerve stimulation had a unimodal distribution of latencies. Climbing fibre responses in the C3 zone evoked on Sci nerve stimulation were characterized by intermediate latencies and were found in two experiments.

In these two experiments, there was a clear differential distribution of input from forelimb and hindlimb nerves within the C3 zone (cf. experiment II in Fig. 3, right panel). Responses evoked on stimulation of the Sci nerve were found in lobule IV and rostral folia of lobule V, with decreasing amplitudes in the rostral-to-caudal direction. By contrast, responses evoked on stimulation of the Uln and SR nerves were found mainly in lobules V and VI. The long-latency responses from Uln in lobule IV of experiment II constitute an exception to the differential rostrocaudal distribution of forelimb and hindlimb input. Although there appeared to be a lateral bias for Sci nerve input and a medial bias for Uln input within rostral parts of the C3 zone, these findings were not consistent.

Pars intermedia: the D zones

The D1 zone (Fig. 4, top row) was located lateral to the C3 zone in lobules V and VI and was in most experiments defined as a non-responsive area between the C3 and D2 zones (e.g. experiment I; however, see experiment II). Input from the Sci nerve was never seen, while the occasional responses evoked on forelimb nerve stimulation were usually very small in amplitude and had long latencies both on Uln and SR nerve stimulation.

The D2 zone (Fig. 4, bottom row) was positively identified in the lateral-most part of lobules V and VI (and lobule IV in one experiment). Climbing fibre responses in the D2 zone evoked on Uln stimulation were characterized by short to intermediate latencies. SR nerve-evoked responses had longer latencies and were, within individual experiments, consistently smaller in amplitude than the Uln nerve-evoked responses. Field potentials evoked on Sci nerve stimulation were found in three of six experiments and had rather long latencies, with an inter-experiment variability not exceeding 0.5 ms. As seen in Fig 4, forelimb input dominated medially, while input from the hindlimb was restricted mainly to the lateral part of the zone.

In experiments with apparently weak or absent input from the Sci nerve to the D2 zone, the potentials in the extreme lateral part of the zone were often attenuated by shunting of currents at the recording electrode (see also experiment I, Fig. 4). This was probably due to a thickening of the pia mater commonly present at the junction between the anterior and posterior lobes, possibly also resulting in local accumulation of cerebrospinal fluid. On the other hand, whenever hindlimb input was observed, the differential distribution of forelimb and hindlimb input within the D2 zone was quite clear.

Discussion

This is the first systematic study of the organization of spino-olivary input to the cerebellar cortex of the ferret and also the first full electrophysiological description of the organization of the olivo-cerebellar system in any animal other than the cat. The main finding is that the char-

acteristics of climbing fibre field potentials evoked on peripheral stimulation appear to vary in an orderly fashion along the longitudinal axis of the cerebellar folia, responses with similar characteristics being distributed in sagittally oriented cortical zones. A comparison between experiments I and II suggests that the organization is remarkably constant in different animals, especially with regard to latencies of climbing fibre responses within different zones (Table 1).

Zonal organization of olivo-cortico-nuclear connections

It is likely that the electrophysiologically defined cortical zones reflect termination areas of different inferior olivary regions, as these receive input from specific sets of spino-olivary pathways and in turn terminate in a zonal pattern in the cerebellar cortex, at least in the cat (Oscarsson 1980). No evidence is provided that the zones are continuous throughout the cerebellar fissures, although this has been shown to be the case in the cat (Ekerot and Larson 1979a). A similar caveat pertains to the issue whether or not there is any mediolateral overlap between neighbouring zones. However, comparisons made in previous investigations between recordings from the cerebellar surface and intracortical recordings (Oscarsson and Sjölund 1977) suggest that the overlap in distribution of climbing fibre field potentials with different characteristics observed in the present study may have been mainly due to the limited spatial resolution of the recording technique used. Note also that in the present study even very small potentials close to the zonal boundaries have been included in the primary analysis, which thereby tends to exaggerate overlap between zones.

Overall, the zones in the present study were somewhat difficult to differentiate in rostral parts of the cortical regions investigated. This is particularly true concerning the distinction between the C2 and C3 zones in lobule IV, which should be regarded as tentative. Although consideration of both latencies and spatial distribution of field potentials evoked from all four nerves argues against it, one cannot conclusively exclude that some of the Sci nerve input identified as belonging to the C2 zone actually belongs to the C3 zone. If this is indeed the case, this could explain to some extent the inconsistency between experiments as regards the presence or absence of hindlimb input to the C3 zone.

Given the correspondence between characteristics of evoked climbing fibre field potentials and the compartmentalization of olivocerebellar and corticonuclear connections demonstrated in the cat (Oscarsson 1980; Voogd and Bigaré 1980), it is proposed that the cortical zones presently described reflect the zonal organization of the cortex evident from anatomical studies of the myeloarchitecture of corticonuclear fibres in the ferret (Voogd 1969). This is strongly supported by a comparison between the anatomical findings and a synthesis of Figs. 2–4 (see Materials and methods), shown schematically in Fig. 5. In addition to the originally identified A, B, C1, C2, C3, D1 and D2 zones, the present data demonstrate the existence of an X and a Cx zone (the latter often termed the lateral C1 zone; see Trott and Apps 1991), intercalated, respectively, between the A and B zones in the vermis and between caudal parts of the C1 and C2 zones in pars intermedia. In the cat, the X and Cx zones were first identified electrophysiologically (Ekerot and Larson 1979a, 1982) and only later confirmed by anatomical techniques. The X zone was shown to project to a region in the deep cerebellar nuclei close to the termination area of the adjacent A zone, while the Cx zone has a corticonuclear projection similar to that of the adjacent C1 zone (Voogd 1982; Trott and Apps 1991). Considering also the relative narrowness of the X zone, it therefore seems likely that these zones were not distinguishable in the original studies of the ferret (Voogd 1969).

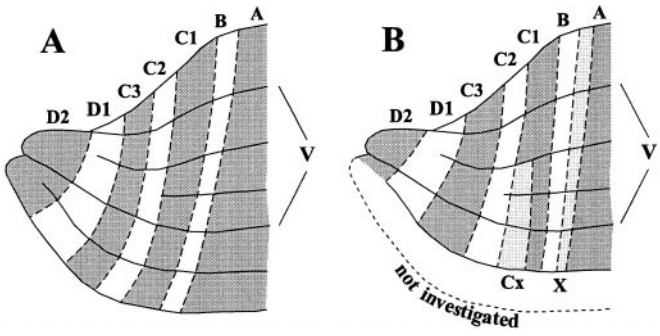

Fig. 5A, B Zonal organization of the ferret cerebellum as revealed by anatomical and electrophysiological techniques. The sagittal zones A to D2 (*shading* only for purposes of illustration), as defined by analysis of the myeloarchitecture of corticonuclear fibres (**A**; adapted from Voogd 1969) and characteristics of climbing fibre field potentials evoked on peripheral stimulation (**B**; present data). The location of zonal boundaries in **B** was based on measurements of the relative width of each zone in Figs. 2–4, separately for lobules IV, V and VI. Mean values for experiments I and II were used in lobules IV and V (see also Materials and methods). Due to lack of positive identification in lobule IV, the C3 zone in experiment I and the D1 zone (experiments I and II) were delineated by extrapolating zonal boundaries from lobule V. *Light shading* indicates zones previously not described in the ferret. (*V* lobule V; cf. Voogd 1969)

Comparative aspects of cerebellar zonal organization

The electrophysiological characteristics of the nine sagittal zones and their relative mediolateral localization indicate a strong similarity in cerebellar organization in ferret and cat, possibly with the exception of the more rostral extent of the X zone in the ferret and the arrangement of the D zones. Some comparative aspects are highlighted below.

Latencies of climbing fibre responses

In Table 2, the latencies of climbing fibre responses in the present study are compared with data obtained in cats (Oscarsson and Sjölund 1977; Ekerot and Larson

1979a). There was an essential overlap between the range of response latencies in cat and ferret for the Sci in the A zone, the Uln in the X zone, the Uln and Sci in the B zone and the Uln, SR and Sci in the C1 and C3 zones. Generally speaking, however, the differences between response latencies in the X (and Cx) zone on the one hand and the C1 and C3 zones on the other hand were distinctly clearer in the ferret as compared to the cat (see also Fig. 1). In the C2 zone, response latencies for all nerves were shorter in the ferret, with no overlap at all between ferret and cat for the range of Uln nerve responses. Response latencies on stimulation of the contralateral SR nerve (20.0–23.5 ms) were, on the other hand, similar to those in the cat (21.0–31.0 ms; Ekerot and Larson 1979a).

The D zones constitute an exception to the general similarity between the two species, since the electrophysiological characteristics of the D1 zone in the ferret did not appear equivalent to those of the D1 zone in the cat. For the range of Uln nerve response latencies, there was no overlap between cat and ferret and no input from Sci was found in the latter. More importantly, however, in the ferret the amplitudes of climbing fibre field potentials evoked in the D1 zone were at best very small, while the input to the D1 zone in the barbiturate-anaesthetized cat usually is characterized by large climbing fibre field potentials with a particularly fast rise time and no preceding mossy fibre field potential. In fact, these climbing fibre field potentials strongly resemble the D2 zone responses in the ferret (see Fig. 1), also in terms of response latencies (Table 2). Therefore, using electrophysiological criteria, it seems that the D1 zone in the cat has its counterpart in the D2 zone in the ferret.

Topographical organization within zones

A differential distribution between input from forelimb and hindlimb nerves was observed in the B, C1, C2, C3 and D2 zones, suggesting an intrazonal topographical organization. In the C zones, the forelimb input was more prevalent in caudal regions and the hindlimb input was more prevalent in rostral regions within each zone. A rostral bias of hindlimb input was seen also in the A zone, but these results were only tentative. In the D2 zone, forelimb input dominated medially in the zone, while hindlimb input was found laterally. A similar, less pronounced but clearly systematic bias was observed in the B zone. Most of these findings are entirely consistent with previous descriptions of intrazonal topographical organization in the cat cerebellum (Oscarsson and Sjölund 1977; Andersson and Oscarsson 1978; Ekerot and Larson 1979b), further emphasizing the similarities between the two species.

The topographical organization of the C2 zone in the cat is not known in detail. Note, however, that Ekerot and Larson (1979a) found forelimb nerve input in lobule V, while hindlimb nerve input was found both in lobule V and lobule IV. On the other hand, using natural stimulation of cutaneous receptors and recording from single Purkinje cells, Garwicz et al. (1992) reported that, while all cutaneously activated climbing fibres projecting to the C2 zone in lobule V had receptive fields on the ipsi- or bilateral forelimb(s), less than one-third of the receptive fields also included the hindlimb(s). The present results thus appear more compatible with the findings of the latter study.

The main exception with reference to intrazonal topographical organization is the D2 zone in the ferret. If this zone is equivalent to the D1 zone in the cat, there is a discrepancy between the medial-to-lateral topography described here and the caudal-to-rostral topography reported in the cat (Ekerot and Larson 1979a).

A detailed topographical microzonal organization has been demonstrated within the forelimb area of the C3 zone in the cat (Ekerot et al. 1991), and it is likely that also the forelimb area of the C1 zone is divided into microzones (cf. Ekerot and Larson 1979b). It is therefore an interesting possibility that the bimodal distributions of Uln and SR nerve responses latencies in the C1 and C3 zones in the ferret (Fig. 1) reflect differences in peripheral receptive fields of the climbing fibres activated. For example, electrical stimulation of the whole Uln nerve will inevitably activate nerve fibres innervating both distal ulnar receptive fields on the digits or paw and proximal receptive fields on the ulnar side of the forearm (cf. Ekerot et al. 1991). In the cat C3 zone, proximal forearm receptive fields are partly represented adjacent to the hindlimb area, and it is therefore noteworthy that the present data indicate a clear, differential rostrocaudal distribution of responses with different latencies, in the C1 and C3 zones.

The bimodal distribution of response latencies could also reflect the presence of input from different afferent modalities, in the case of the mixed Uln nerve potentially including muscle afferent input (cf. Jörntell et al. 1996). If this were the case, it would suggest a difference between the ferret and the cat, since in the latter species both cutaneous and muscle afferents converge onto the same olivary neurones and hence the two types of input do *not* have a differential distribution within the zone.

Links between zones with similar climbing fibre input

Although the zones are usually referred to as separate entities, there is ample evidence for links between some of the zones in the cat. Specifically, it has been demonstrated that some olivary axons branch to innervate two different zones, linking as pairs the X with the Cx zone (hence the ‘x’ in ‘Cx’), the C1 with the medial C3 zone and the lateral C3 with the Y zone (Ekerot and Larson 1982; Voogd 1982; see also Trott and Apps 1991. The Y zone is located in the lateral most part of lobules II to IV. It is defined by its projection to nucleus interpositus anterior and by the olivary input shared with the lateral C3 zone.) Furthermore, it appears that the zones within the two latter pairs also converge, at least to some extent, in their projections to the deep cerebellar nuclei (Voogd 1982; Trott and Armstrong 1987; see also Garwicz and Ekerot 1994; Garwicz et al. 1996). In the present study, the clearest

similarity in response latencies compatible with a branching olivo-cerebellar innervation within any of the three above-mentioned pairs of zones was found in the X and Cx zones (Fig. 1; Tables 1, 2). By contrast, the present findings for the C1 and C3 zones were rather mixed, with only partial overlap in Uln or SR nerve response latencies between the two zones in any given experiment (cf. Fig. 1). This does, of course, not exclude the possibility of some branching between the two zones. The Y zone, which is usually not accessible for cerebellar surface recordings in the cat (cf. Ekerot and Larson 1982), was not identified in the ferret.

Conclusions

The cerebellar cortex has a uniform cytoarchitecture throughout the cortical sheet, and it has therefore often been emphasized that the function carried out by any particular cortical region will be strongly determined by local afferent and efferent connections. The present study demonstrates that the electrophysiological method for precise identification of different cerebellar cortical zones, previously explored mainly in the cat, can be readily applied in the ferret. It is suggested that this methodological approach may prove a useful tool for defining recording sites and guiding the placement of neuronal tracer injections or lesions, thereby facilitating the systematization of data obtained in experiments concerned with different aspects of cerebellar function also in the ferret.

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