VESTIBULOCEREBELLAR AND VESTIBULO-OCULOMOTOR RELATIONS IN THE RABBIT

ISABEL THUNNISSEN

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VESTIBULOCEREBELLAIRE EN VESTIBULO-OCULOMOTOR RELATIES BIJ HET KONIJN

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PROMOTIECOMMISSIE

PROMOTOR:	Prof. Dr. J Voogd
COPROMOTOR:	Dr. N.M. Gerrits
OVERIGE LEDEN:	Prof. Dr. B. Hillen
	Prof. Dr. C.D.A. Verwoerd
	Dr. E. Marani

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1 INTRODUCTION

The vestibular nuclei

The vestibular complex constitutes the main termination of the vestibular nerve. Four main nuclei generally are distinguished, the superior (SV), the lateral (LV), the medial (MV) and the descending (DV) vestibular nuclei and a number of small cell groups in and around the vestibular nuclei (Brodal and Pompeiano, 1957; see Brodal, 1974 and Epema, 1990 for reviews). These nuclei differ in their relation towards the vestibular nerve, in their non-vestibular afferent connections, especially from the cerebellum, and in their efferent pathways.

Fibers of the vestibular nerve enter the vestibular complex from the lateral side, after passing between the spinal root of the trigeminal nerve and the restiform body. All fibers bifurcate in thin ascending and thicker descending branches in the ventrolateral region of the complex. This bifurcation was first observed with the Golgi method (Cajal, 1911) and later confirmed with intracellular tracing of electrophysiologically identified fibers (Mannen et al., 1982; Sato et al., 1989; Fig. 1.1) and immunocytochemistry (Morris et al., 1988). Early degeneration studies described a projection of the vestibular nerve to restricted areas in the vestibular complex (see Brodal 1974) for review). Later degeneration (Korte and Mugnaini, 1979) and tracer (Carleton and Carpenter, 1984; Kevetter and Perachio, 1986; Epema, 1990) studies showed, that terminal branches of the vestibular nerve terminate in all parts of the vestibular nuclei, with the exception of the LV.

The superior vestibular nucleus SV constitutes the rostral pole of the vestibular complex (Fig. 2.2). It is located ventral to the fibers of the superior cerebellar peduncle. It is traversed by ascending branches of the root fibers of the vestibular nerve, which give off collaterals to the entire nucleus. The distal branches of these ascending fibers continue in and along the superior cerebellar peduncle into the cerebellum. The SV usually is divided in a central portion, containing larger cells and a peripheral portion, with smaller neurons. Caudally the SV tapers off in a cell group located dorsolateral to the LV (corresponding to group l of Brodal and Pompeiano, 1957). At this point the SV is continuous with cell group y, which extends dorsal to the restiform body and ventral to the lateral cerebellar nucleus (see Epema, 1990 for review). The descending branches of the fibers of the vestibular nerve occupy a lateral position in the caudal part of the vestibular complex. This region, which also contains fiber bundles from other sources, mainly of cerebellar origin, is known as the descending or inferior vestibular nucleus (DV). It contains cells of different sizes among wich fairly large ones. Collaterals of the vestibular root fibers ramify among these cells. Cellgroup x of Brodal and Pompeiano (1957) consists of small neurons located at the border of the DV and the restiform body. The medial vestibular nucleus (MV) is located in the floor of the fourth ventricle and extends over the entire length of the vestibular complex. Medially directed collaterals from the descending branches of the root fibers enter it and ramify within its boundaries.The MV can be subdivided in a rostrolateral magnocellular part (MVmc), a rostromedial parvocellular part (MVpc) and a caudal part (MVc). The MVpc is separated from the MVpc by the stria acoustica dorsalis. Caudal to the genu of the facial nerve the MVmc and MVpc merge into a single MVc. The MVmc often was considered as the ventral part of the lateral vestibular nucleus (LV) in the cat (Brodal and Pompeiano, 1957) and pars alpha of the lateral vestibular nucleus in the rabbit (Meessen and Olszeweski, 1949). The MVmc differs from the LV by its content of large and medium sized neurons and the absence of giant Deiters' neurons. The inclusion of the MVmc as part of the medial vestibular nuclei is not only based on cyto- and myeloarchitecture but also on its connectivity (see Epema, 1990 for review). The LV is located dorsal to the MVmc, and does not share afferent projections from the vestibular nerve.

The vestibular nuclei project to different areas, including the cerebellum, the extraocular motor nuclei, the spinal cord and the ipsi- and contralateral vestibular nuclei. Cerebellar, commisural and intrinsic connections take their origin from all vestibular nuclei

with the exception of the LV (Ladpli and Brodal, 1968; Gacek, 1978; Pompeiano et al., 1978; Ito et al., 1985, Epema et al., 1988, 1990). The SV gives rise to ascending connections. Uncrossed projection from the SV to the extraoculomotor nuclei travel in the medial longitudinal fascicle (flm), crossed projections use the superior cerebellar peduncle (Yamamoto et al., 1978; Hirai and Uchino, 1984). The MV and DV give rise to ascending and descending connections. The vestibulospinal projections are both crossed and uncrossed and travel in the flm in the medial vestibulospinal tract (MVST). The MVST takes its origin from the MVc, the MVmc and the DV (Kuypers, 1981; Kuypers and Maisky, 1977). The ascending projections of these nuclei are almost entirely crossed (Busch, 1961). Vestibulooculomotor neurons, including those projecting to the abducens nucleus, are found in all subdivisions of the MV and in the DV (Carleton and Carpenter, 1983). Intra-axonal HRP injections into identified vestibular neurons showed branching of neurons in the MVmc to the spinal cord and the oculomotor area (Graf and Ezure, 1986). The LV gives rise to the uncrossed descending lateral vestibulospinal tract (LVST) only (Akaike et al., 1973; Akaike, 1983, Nyberg-Hanssen 1966).

The secondary vestibulocerebellar and the vestibulooculomotor projections will be the main subject of this thesis.

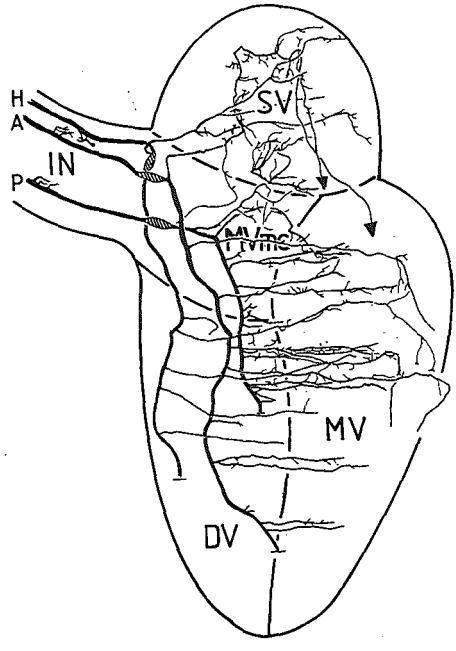


Fig. 1.1 Course and distribution of intra axonally stained fibers of the vestibular nerve of the cat. Redrawn from Mannen et al. (1982). The course of the horizontal (H), anterior (A), and posterior (P) canal fibers and their distribution to the four main nuclei of the vestibular complex is similar. Cerebellar collaterals arise from the ascending branches to the SV (arrows).

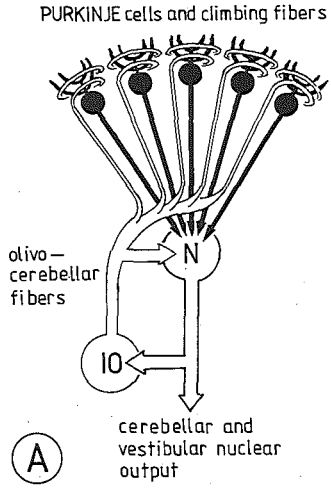
Structure and modular subdivisions of the cerebellar cortex

The two most prominent types of cerebellar afferents are the climbing and mossy fiber systems. All climbing fibers take their origin from the contralateral inferior olive (Desclin, 1974). They terminate on the Purkinje cell dendritic tree. The Purkinje cells constitute the only efferent system of the cortex. Numerous small granule cells are located in the granular layer under the layer of the Purkinje cells. Their axons ascend towards the surface, where they bifurcate and run as parallel fibers in the superficial molecular layer of the cortex, parallel to the transverse fissures and perpendicular to the Purkinje cell dendritic trees on which they terminate. Mossy fibers, the second type of afferent fiber, terminate on the granule cells. The synapses between mossy fibers and groups of granule cells are known as the cerebellar glomeruli. Glomeruli consist of a central mossy fiber terminal (the mossy fiber rosette), which may be located at the end or along the branches of a mossy fiber. Mossy fibers originate from many different nuclei in the brain stem and spinal cord.

The topographical arrangement of the corticonuclear connections and of the olivocerebellar climbing fiber projection is determined by the modular organization of the cerebellar cortex. Longitudinal strips of Purkinje cells, which send their axons to a particular central cerebellar, receive their climbing fibers from specific subdivisions of the inferior olive, which also sends collaterals to the target nucleus of these Purkinje cells (Groenewegen and Voogd, 1977; Groenewegen et al., 1979; Voogd and Bigaré, 1980). The Purkinje cell axons from a particular cortical zone and their olivocerebellar afferents do not intermingle with the efferents and afferents from neighbouring zones, but occupy a discrete compartment in the cerebellar white matter. A cerebellar module therefore consists of a longitudinal strip of Purkinje cells, their central cerebellar target nucleus and their climbing fiber afferent projection (Fig.1.2A). The output of the module is the efferent pathway from the central cerebellar nucleus, which contains also neurons that give rise to an inhibitory "nucleo-olivary" projection to the subnucleus of the olive, belonging to the module.

Such a modular organization has been well documented for the anterior lobe (see Voogd, 1989; Haines et al., 1982 for reviews). It has been known for a long time that the vermis of the anterior lobe is connected with the medial (i.e. fastigial) nucleus and the vestibular nuclei (Klimoff, 1899; Jansen and Brodal, 1940). In each hemivermis of the anterior lobe three zones can be disinguished. The medial A-zone projects to the fastigial nucleus and the vestibular nuclei (Bigaré, 1980; Voogd and Bigaré, 1980), and receives its olivocerebellar projection from the caudal half of the medial accessory olive. The-B zone which occupies a lateral position in the anterior vermis projects to the LV and receives its climbing fibers from the caudal pole of the dorsal accessory olive (Andersson and Oscarsson, 1978a,b; Trott and Armstrong, 1987). The x-zone (Ekerot and Larson, 1982) is situated in the dorsal part of the anterior lobe (lobules IV and V) between A and B. It projects to the junction of the fastigial and the interposed nucleus (Trott and Armstrong, 1987) and receives a projection from the central medial accessory olive (Campbell and Armstrong 1983).

For the flocculus and the caudal vermis the evidence for a possible modular organization is less complete. A zonal distribution of the Purkinje cells of the flocculus projecting to different vestibular or central cerebellar nuclei has been described for rabbit (Yamamoto, 1979, Fig 1.2B; Yamamota and Shimoyama, 1977; Balaban, 1984), cat (Bigaré, 1980; Voogd and Bigaré, 1980; Sato et al., 1982) and monkey (Balaban and Watanabe, 1984). Purkinje cell strips projecting to the MV and SV coincide with the climbing fiber projections from different parts of the olive (i.e. the dorsal cap and the ventrolateral outgrowth), which receive a visual projection from the nuclei of the accessory optic tract and the nucleus of the optic tract (Maekawa and Takeda, 1976; Walberg et al., 1981). The zonal distribution of floccular Purkinje cells is more difficult to reconcile whith results of anterograde axonal tracing studies in the cat, which showed a termination of the climbing fibers from the dorsal cap and the ventrolateral outgrowth in multiple strips (Fig. 1.2C, Gerrits and Voogd 1982).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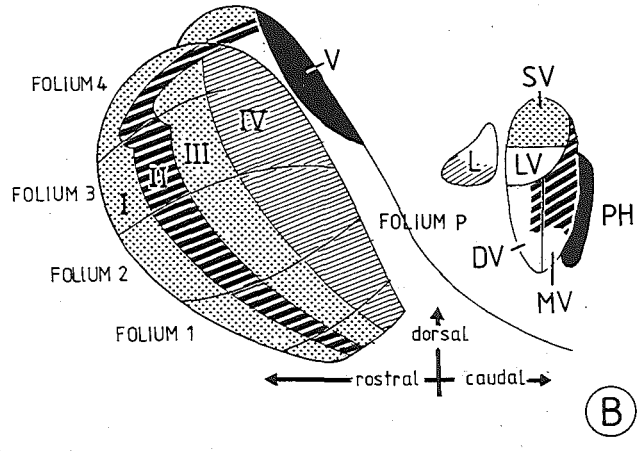
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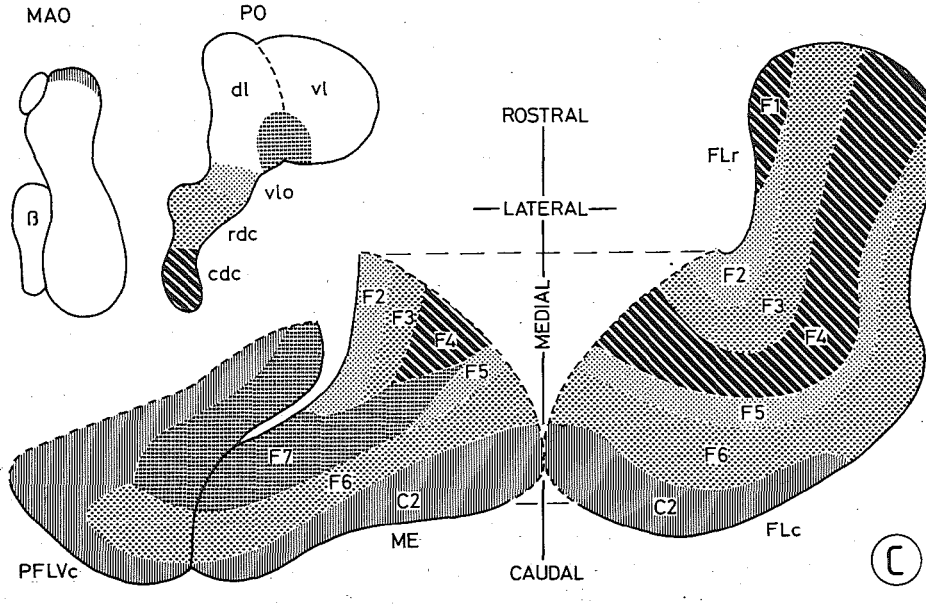
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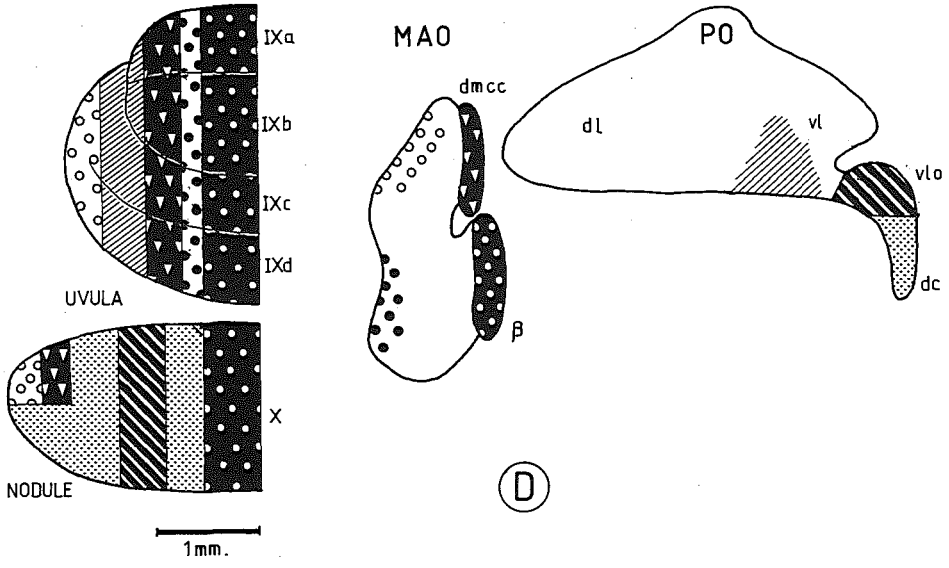


Fig. 1.2 A. Diagram of a cerebellar module. A longitudinal strip of Purkinje cells projects to a cerebellar or vestibular target nucleus (N). The Purkinje cells receive climbing fibers from a subdivision of the inferior olive (IO), which also projects to the target nucleus. The output of the target nucleus is reciprocally connected with the inferior olive.

B. Purkinje cells of the flocculus of the rabbit which project to the cerebellar or vestibular nuclei are arranged in zones, which are orientated perpendiculary to the longitudinal axis of the folia 1-4 of the flocculus and extend onto folium P of the paraflocculus. Left: diagram of a lateral view of the flocculus. Right: target nuclei are indicated in a diagram of a dorsal view of the vestibular nuclei and the lateral cerebellar nucleus(L). Corresponding areas in the flocculus and the target nuclei are indicated with the same symbols. Based on Yamamoto and Shimoyama (1977), redrawn from Ito (1984).

C. Patterns of climbing fiber projections of the dorsal cap and the ventrolateral outgrowth to the flocculus and the medial extension of the ventral paraflocculus (ME) in the cat. The origin of the climbing fiber zones F1-F6 and C2 in the inferior olive is indicated with similar symbols. Diagrams of the medial accessory olive (MAO) and the principal olive (PO) are drawn in left upper corner. Surface of the flocculus and paraflocculus is unfolded (from Gerrits and Voogd, 1982).

D. Diagram showing the topographical relationship between subdivisions of the inferior olive and parasagittal climbing fiber zones in the lobules IX (uvula) and X (nodulus) of the caudal vermis in the rabbit. Zones are indicated with different symbols. Left: diagram of the left side of the uvula and the nodulus. Right: diagrams of the right medial accessory olive (MAO) and the principal olive (PO). The origin of the climbing fiber zones is indicated with the same symbols. Notice the restriction of climbing fibers from the dorsal cap (DC) and the ventrolateral outgrowth (vlo) to the nodulus, and the presence of continuous projection zones in the nodulus and uvula from group beta (ß), the dorsomedial cell column (dmcc) and the rostral MAO. Redrawn from Katayama and Nisimaru (1988) and Sato and Barmack (1985).

Moreover, both these climbing fiber strips and the Purkinje cell zones of the flocculus extend beyond the flocculus into the adjoining part of the paraflocculus. This part of the paraflocculus is known as "folium P" of Yamamoto and Shimoyama (1977) in the rabbit, or the medial extension of the ventral paraflocculus (ME) of Gerrits and Voogd (1982) in the cat.

In the caudal vermis the medially located Purkinje cells of the lobules VIII-X project to the medial, ventral part of the fastigial nucleus (Buissert-Delmas and Angaut, 1989). Projections to the MV, DV, and SV are quite numerous. These Purkinje cells are found in medial as well as in lateral parts of the lobules VIII, IX and X. There is some evidence that they are arranged in longitudinal zones (Matsushita and Wang, 1986; Shojaku et al., 1987; Epema et al., 1985) but a correlation with the zonal arrangement of the olivocerebellar projection has not been attempted. The flocculus and the caudal vermis share the "visual" climbing fiber projections from the dorsal cap and the ventrolateral outgrowth. In addition to these visually dominated olivocerebellar projections, the caudal vermis receives climbing fibers from subdividions of the inferior olive (i.e. the group beta, the dorsomedial cell column and some parts of the medial accessory olive), which receive an input from the vestibular nuclei (Gerrits, 1985, Fig 1.2D). Two points are important in this respect. In the first place the "visual" and the "vestibular" climbing fiber projections to the lobules X and IX of the caudal vermis are arranged in parallel alternating longitudinal zones. Secondly the "visual" projections from the dorsal cap and the ventrolateral outgrowth are restricted to lobule X (Hoddevik and Brodal, 1977; Brodal and Kawamura, 1980; Karayama and Nisimaru, 1988; Eisenman, 1984; Bernard, 1987; Kanda et al., 1989) and the adjoining ventral aspect of lobule IX, bordering the posterolateral fissure (Balaban and Henry, 1988; Groenewegen and Voogd, 1977), which constitute the vermal portion of the vestibulocerebellum. The "vestibular" projections of the group beta and the dorsomedial cell column of the inferior olive on the other hand extend far beyond the vestibulocerebellum into the lobules IX, VIII and possibly lobule VII (Eisenman, 1981, 1984). Climbing fibers from the caudal dorsal accessory olive, the ventral leaf of the principal olive and the rostral part of the medial accessory olive have been located in the lateral most region of the lobules VIII and IX.

The mossy fiber activation of the Purkinje cells is mediated by the granule cells, which send their axon as parallel fiber into the molecular layer, where it synapses with the Purkinje cell dendrites. Although the mossy fibers in the cerebellar white matter are mostly directed transversely, the branching of their collaterals occurs in a parasagittal plane (Scheibel, 1977).The distribution of the terminal mossy fiber rosettes rarely matches the orderliness of the climbing fiber system. Instead, the mossy fibers are characterized by a wide variability in the degree of clustering of terminals in longitudinal strips. A zonal organization has been reported for afferents from the cuneate nuclei (Voogd et al., 1969, Gerrits et al., 1985; Jasmin and Courville, 1987b), the spinal cord (Van Rossum, 1969, Voogd et al., 1969; Ekerot and Larson, 1973,1980; Matsushita and Tanami, 1984; Matsushita et al., 1984; Matsushita and Yaginuma, 1989) and the lateral reticular nucleus (Kunzle, 1975; Russchen et al., 1976), the vestibular nuclei (Magras and Voogd, 1985; Matsushita and Wang, 1987) and in a less pronounced way for the nucleus reticularis tegmenti pontis (Gerrits and Voogd, 1978; Kawamura and Hashikawa, 1981). Although clustering of mossy fiber terminals in longitudinal strips is not uncommon, their overall distribution shows a basic difference as compared with the climbing fibers. Mossy fiber terminals from individual sources are usually present in a restricted number of lobules, in a wide mediolateral expanse of the cortex. While the mossy fiber projection is restricted in rostrocaudal direction, the climbing fiber projection is very much restricted in mediolateral direction. The longitudinal extent of the climbing fiber (and also the cortical) zones usually includes a large number of lobules, and sometimes even the entire cerebellum.

Vestibulocerebellar relations

Anatomical and physiological studies have demonstrated that the vestibular nerve and the vestibular nuclei are important sources of mossy fibers. Those parts of the cerebellar cortex in receipt of vestibular nerve fibers (the "primary" vestibulo-cerebellar fibers) are usually referred to as the vestibulocerebellum (Ingvar, 1918). However, the extent of this part of the cerebellum has never become clearly established, since the projection of the nerve fibers to certain lobules has been disputed continuously. Conversely,the vestibulocerebellum is an important, but not exclusive, source of the corticovestibular projection. Larsell (1934) introduced a division of the vertebrate cerebellum into two principal parts, the flocculo-nodular lobe, receiving primary vestibular fibers, and the corpus cerebelli, receiving somatosensory input (tecto-, trigemino-, and spinocerebellar systems). Later investigations confirmed the original opinion of Ingvar (1919), that primary mossy fibers terminate in the flocculus and the nodule (lobule X), as well as in the uvula (lobule IX) and the lingula (lobule I) (Ingvar, 1918; Dow, 1936; Carpenter, 1960; Brodal and Hoivik, 1964). A recent retrograde HRP study in the cat (Kotchabhakdi and Walberg (1978a) claimed that the primary vestibular fibers in addition reach the whole cerebellar vermis. In the rabbit, Gerrits et al. (1989) demonstrated with different anterograde tracers, that the primary fibers terminate in lobules I and II, in the cortex in the depth of the main fissures between lobules II and VI, in lobule X and in the ventral part of lobule IXd. Lisberger and Fuchs (1978) and Waespe et al. (1981) were puzzled by their failure in electrophysiological experiments to find substantial evidence for a direct vestibular nerve projection to the flocculus. The presence of a direct eighth nerve input in the flocculus was also questioned in anatomical studies in the monkey (Langer et al., 1985) and the cat (Korte and Mugnaini, 1979). Recently Gerrits et al. (1989) demonstrated its absence in the flocculus of the rabbit.

Secondary vestibulocerebellar afferents arise from various subdivisions of the vestibular nuclei. Anterograde axonal degeneration methods have shown that the major termination was in the vestibulocerebellum (Dow, 1936). Later the entire vermis has been included in the secondary vestibular projection area, on the basis of retrograde tracing with HRP in the cat (Kotchabhakdi and Walberg, 1978b) and the sheep (Saigal et al., 1982). The origin of the projection is bilateral and includes the superior (SV), medial (MV) and descending (DV) and interstitial (IN) vestibular nuclei and group y, as has been demonstrated in different mammals, using cellular degeneration (Brodal and Torvik, 1957; Carpenter, 1960), and retrograde axonal tracing with HRP or WGA-HRP (Blanks et al., 1983; Yamamoto, 1979; Kotchabhakdi and Walberg, 1978b; Sato et al., 1983; Rubertone and Haines, 1981; Brodal and Brodal, 1985; Langer et al., 1985; Frankfurter et al ., 1977; Saigal et al., 1982; Precht et al., 1977; Batini et al., 1978; Kimoto et al., 1978; Matsushita and Okado, 1981; Blanks et al., 1983; Sato et al., 1989). These studies suggest that the secondary vestibulo-cerebellar projection is topographically organized, with various lobules receiving their mossy fiber supply from different small groups of neurons in the vestibular nuclei. Epema et al. (1990) and Tan and Gerrits (in press), however, advocated the intermingling of the neurons in the vestibular nuclei of the rabbit, which project to the flocculus, the uvulo-nodular lobe or to both.

Anterograde axonal tracing of the secondary vestibulo-cerebellar projection is necessary to determine the exact projection area, the topographical organization with respect to other mossy fiber afferents and the modular organization of the cerebellar projection. In this respect the observation of Bower and Woolston (1983), that the granule cells, which receive the mossy fiber terminals, preferentially activate the Purkinje cells directly overlying them, is of particular interest. The distribution of the secondary vestibulocerebellar mossy fiber terminals found in this study, differs from the zonal (modular) organization of Purkinje cells and olivocerebellar climbing fibers and from the distribution over specific lobules as has been described thusfar (Epema et al., 1985; Magras and Voogd, 1985; Matsushita and Wang, 1987; Yamamota, 1979; Sato et al., 1989). The distribution of the secondary vestibulocerebellar mossy fiber terminals will be described for the anterior lobe

and simple lobule(Chapter 3), the posterior lobe (Chapter 4) and the flocculus (Chapter 5).

Vestibulo-oculomotor connections

Since the work of Lorente de No (1933), Szentagothai (1943) and Szentagothai and Schwab (1956) the relation between semicircular canals and extra-ocular muscles has been revealed in great detail. Excitatory connections link each canal with a set of two eye muscles, which move the eyes in approximately the same plane as the semicircular canal. Inhibitory connections link each canal with their antagonists (Highstein, 1971, 1973a,b; Ito et al., 1973a,b, 1976a,b). From electrophysiological data it could be inferred that the vestibulo-oculomotor projection is topographically organized (see Ito, 1984 for a review). Excitatory vestibulo-oculomotor connections are crossed and inhibitory vestibulo-oculomotor connections travel in uncrossed pathways. Inhibitory neurons mediating vertical canal information are located in the SV, those mediating horizontal canal information are found in the MV. Excitatory relay cells for the posterior and horizontal canals are located in the MV. Excitatory anterior canal cells are found in different locations, in group y and the dorsal SV in the rabbit (Highstein, 1971,1973a; Ito et al., 1976a,b; Yamamoto et al., 1978) and in the MV in the cat (Uchino et al., 1981). Recently anterior canal cells in SV and y which project to the oculomotor nucleus through the cerebellar peduncle, were shown to excist also in the cat (Hirai and Uchino, 1984). It is not known whether excitatory anterior canal neurons are present in the MV in the rabbit.

Anatomical studies using anterograde degeneration or tracing methods indicated that the termination of vestibular fibers in the oculomotor nuclei is in patches which are congruent with the motoneurons pools of individual eye muscles (McMasters et al., 1966; Carleton and Carpenter, 1983; Carpenter and Cowie, 1985).

There are large discrepancies between the vestibulo-oculomotor projections, as found in these studies, and the connections as they could predicted from the canal-2x2 eye muscle model. These differences can be explained by the presence of additional connections with other eyemuscles, which would correct for the small differences in the planes of the semicircular canals and the plane of action of the corresponding eye muscles (Baker et al., 1982; Ezure and Graf, 1984a,b). Some of these additional connections have indeed been found in intracellularly injected vestibulo-oculomotor relay cells in the cat (Graf et al., 1983). The vestibulo-oculomotor connections will be discussed in chapter 6.

The specificity of the control systems that relay in the vestibular nuclei suppose a high degree of topographical organization in the vestibulocerebellar, vestibulo-oculomotor and corticovestibular connections. Many studies of the role of the cerebellum in oculomotor control have focussed on the flocculus. It has been shown that Purkinje cells in the flocculus receive vestibular (Precht and Llinas, 1969; Lisberger and Fuchs, 1974; Ghelarducci et al., 1977) and visual information (Maekawa and Simpson, 1973; Maekawa and Takeda, 1975; Simpson and Alley, 1974) and propioceptive information from neck muscles (Wilson et al., 1975). According to Ito (1982) the inhibition by the flocculus in the rabbit is limited to the excitatory and inhibitory anterior canal cells in the SV and group y and the horizontal canal cells in the MV. Posterior canal cells and the presumed second population of excitatory anterior canal cells in the MV do not receive such a connection. Because the Purkinje cells projecting to the SV and the MV are located in different strips (Yamamoto, 1977), different modules of the flocculus may be engaged in the control of the vertical and horizontal eye movements. The possibility of the excistence of two spatially separated populations of excitatory anterior canal neurons, only one of which receives a projection from the flocculus, is of great interest for the interpretation of the experiments on the adaptation of the vestibuloocular reflex. Recently Lisberger (1988) and Lisberger and Pavelko (1986) showed that different populations of relay cells are intercalated in the modifiable and non-modifiable vestibulo-oculomotor reflex pathways; the former are inhibited by the flocculus (the floccular target neurons FTN's), the latter are not.

In contrast to the wealth of data on the functional synaptology of the flocculus

surprisingly little is known about the rest of the vestibulocerebellum. Similar to the flocculus, the cerebellar nodule receives vestibular (Precht and Llinas, 1969; Simpson et al., 1976; Precht et al., 1976; Marini et al., 1976) and visual information (Simpson et al., 1974), but more importantly neck and proximal forelimb input (Precht et al., 1976; Batini and Pompeiano, 1957). The distribution of nodular Purkinje cell axon terminals in the vestibular nuclei differs from that of the flocculus. It has been suggested that the nodule plays a role in the control of body posture and the vestibulocollic reflex (Precht et al., 1976,1979), but it also may be involved in the coordination of eye movements (Waespe et al., 1984).

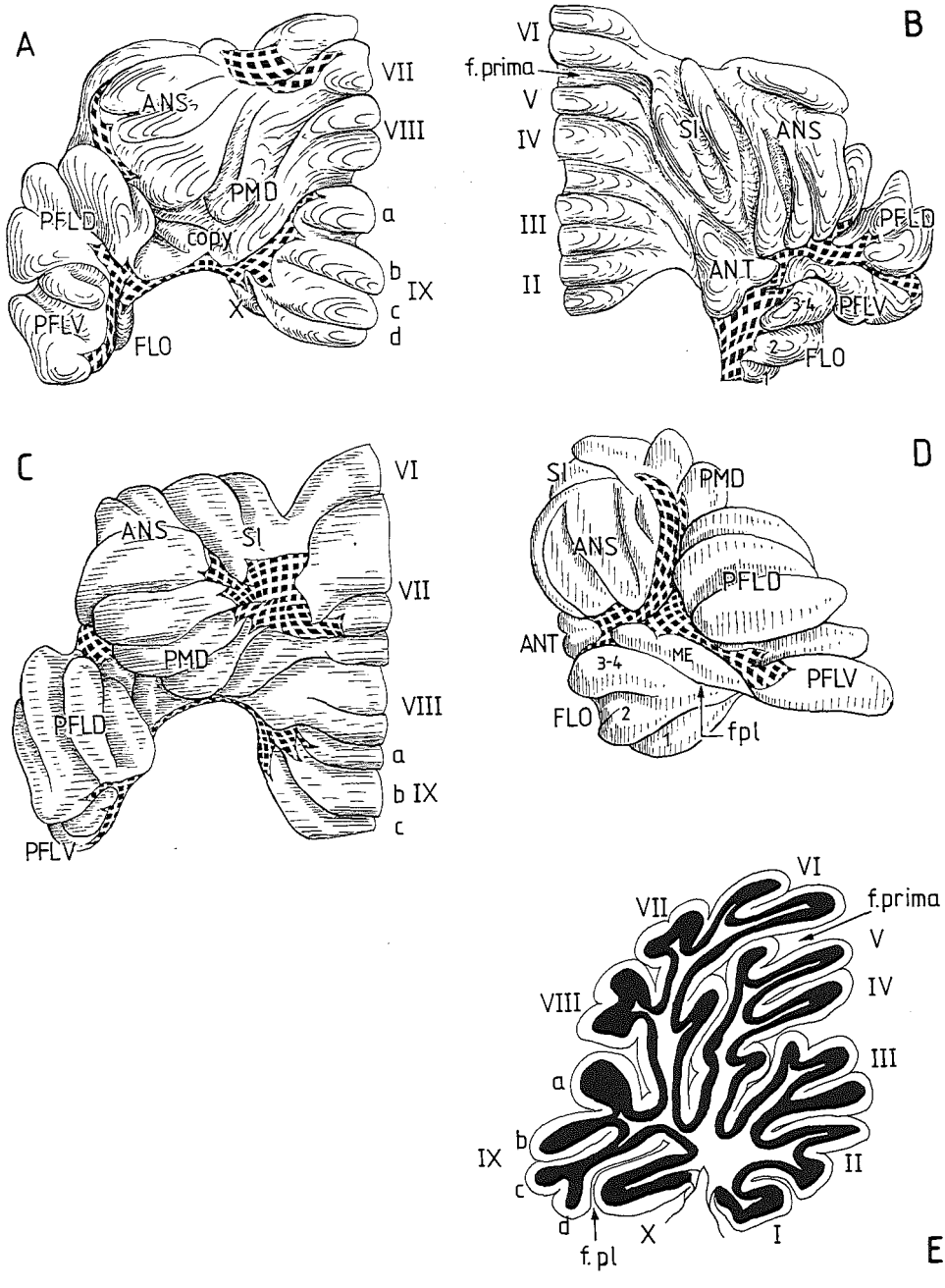


Fig. 2.1 The cerebellum of the rabbit, viewed from caudal (A), rostral (B), dorsal (C), and lateral (D). Black squares indicate areas with absence of (or) incomplete cortex. The lobules of the vermis are also illustrated in a midsagittal section (E).

2 MATERIAL AND METHODS

The experiments were carried out on pigmented Dutch belted rabbits (body weight 1.8 - 2.3 kg). The animals were premedicated with Hypnorm (0.3 ml/kg) and were placed in a David Kopf stereotaxic frame with a Wells rabbit head holder. During the operation general anaesthesia was maintained with a halothane-oxygen mixture (Fluotec Mark II) administered through an endotracheal tube (Portex 3.0). The vestibular nuclei and the cerebellum were reached following exposure of the brainstem through a dorsal approach and an enlargment of the occipital foramen.

The subdivision of the cerebellum

In Larsell's subdivision of the mammalian cerebellum the lobules of the vermis were indicated with roman numerals I-X and the corresponding lobules of the hemispheres with the prefix H. The primary fissure devides the cerebellum into the anterior and posterior lobes. The anterior lobe is subdivided into five lobules ((H)I-V). No sharp boundary can be drawn between vermis and hemisphere, and the inter- and intralobular fissures are continuous. In the posterior lobe the paramedian sulcus separates the vermis from the hemispheres. In this sulcus most transverse fissures and a part of the cortex are interrupted. The vermis of the posterior lobe is composed of the lobules VI-X. Besides the lateral part of the simple lobule the hemisphere consists of the ansiform lobule (crus I and crus II), the paramedian lobule, the dorsal and ventral paraflocculus and the flocculus. The flocculus of the rabbit is located in the rostrolateral part of the hemisphere. It consists of a rostrally directed rosette of three or four folia, which taper and fuse into a single folium more caudally. According to Yamamoto and Shimoyama (1977) these folia are indicated as the folia f1-f4. The cortex of the dorsal most folium f4 is continuous around the posterolateral fissure, with the cortex of the medial extension of the ventral paraflocculus (ME) (i.e. folium P of Yamamoto and Shimoyama, 1977) (Fig. 2.1).

The subdivision of the cerebellum used in the present study, is based on the work of van Rossum (1969) and Yamamoto and Shimoyama (1977) in the rabbit. Reconstructions of the midsagittal plane will be regulary used to indicate the cortical projection areas of the vestibular mossy fiber terminals. Its use facilitates the comparison between different experiments.

The vestibular nuclei of the rabbit

The vestibular nuclear complex includes four major divisions: the superior (SV), the lateral (LV), the medial (MV) and the descending (DV) vestibular nuclei, and a number of small cell groups. The subdivisions of the vestibular complex used in the present study is according to the one used by Voogd (1964) in the cat, and van Rossum (1969) and Epema (1990) in the rabbit (Fig 2.2).

To facilitate the comparison of different experiments the distribution of labeled neurons was plotted in horizontal diagrams of the vestibular nuclei. Following the method of Epema and Gerrits (1988) the diagrams were prepared from the charted sections by orthogonal projection of the labeled neurons and the boundaries of the nuclei.

The extraoculomotor nuclei

Each eyeball is equipped with six extraocular muscles. The motoneuron pool for the superior rectus (SR), medial rectus (MR), inferior rectus (IR) and inferior oblique (IO) muscles are found in the oculomotor nucleus. Those for the superior oblique muscle (SO) in the trochlear nucleus and for the lateral rectus muscle (LR) in the abducens nucleus.

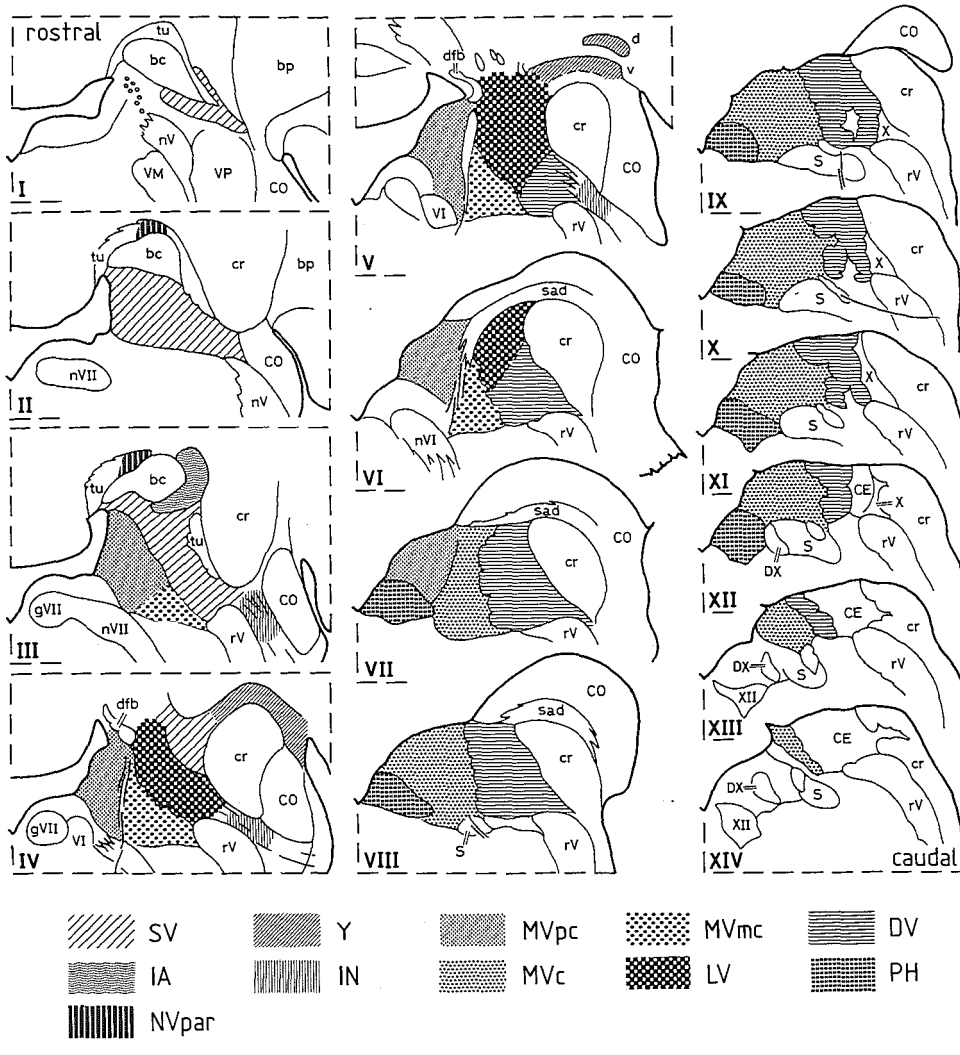


Fig 2.2 Diagrams of equally spaced, transverse sections through the vestibular complex. The left margin of the frames represents the midline. The interval between the frames is approximately 0.3 mm. Bar represents 0.1 mm

The localization of motoneurons for the different muscles in the oculomotor nucleus has been described in detail for monkeys (Warwick, 1953; Henn et al., 1982; Porter et al., 1983); cats (Tarlov and Tarlov, 1971; Gacek, 1974; Akagi, 1978; Miyazaki, 1985), rats (Glicksman, 1980) and rabbits (Akagi, 1978; Shaw and Alley, 1981; Murphy et al., 1986). Groups consisting of motorneurons, innervating an individual muscle, lay in the nucleus as elongated columns extending in a longitudinal direction. There are considerabely differences in the arrangement of the individual motor groups among different species, except for the group innervating the inferior rectus, which was generally found in ventral position running through the rostral twothirds of the oculomotor nucleus. The subdivision of the IIIrd nucleus used in the present study is according to the one proposed for the rabbit by Murphy et al. (1986)

HRP and WGA-HRP procedure

Pressure injections of horseradish peroxidase (HRP) (Sigma type VI, 33% in saline) or wheatgerm agglutinated HRP (WGA-HRP) (Sigma type VI, 5% in saline) were made using a 1 ul Hamilton syringe with a 25 gauge needle or with a micropipette (tip diameter 25-30 μm) connected to a hydraulic system. To reduce unwanted spread of WGA-HRP, delivery started 5 minutes after placing the needle or pipette in position. The injections were made at a rate of 0.1 ul per 10 min. After the injection the needle was left in place for an additional 15 minutes. Following a survival time of 2 - 4 days the animals were transcardially perfused under deep anaesthesia with 0.5 liters saline, followed by 1.5 liters citrate buffer (0.1 M, pH 7.2) containing 1% formaldehyde and 1.25% glutaraldehyde and washed with 0.5 liters citrate buffer (0.1 M, pH 7.2) containing 8% sucrose (Mesulam, 1978). The brains were embedded in 10% gelatin (Voogd and Feirabend, 1981) and transversely sectioned at 40 μm on a freezing microtome. Parallel series containing one out of every four sections were incubated with diaminobenzidine (DAB) (Graham and Karnovsky, 1966) and tetramethyl-benzidine (TMB) (Mesulam, 1978). Alternating sections of the DAB and TMB series were counterstained with respectively Cresyl Violet and Neutral Red. Sections were coverslipped with Permount. Retrogradely labeled vestibulocerebellar neurons and anterogradely labeled mossy fiber terminals were plotted with a x-y recorder coupled to the stage of a microscope.

Tritiated leucine procedure

The stock solution of L(4,5-^{3}H)-leucine (specific activity ±140 Ci/mM, concentration 1μCi/μl; Radiochemical Centre, Amersham, UK) was evaporated to dryness under a gentle flow of nitrogen at 40 ^{0}C, and redissolved in saline to a final concentration of 75 μCi/μl. The injections were performed in the same way as for HRP. Following a survival of 4-7 days the animal was transcardially perfused under deep anaesthesia with 0.5 liters saline and 2 liters 4% formaldehyde. The brains were embedded in 10% gelatin and trans-versely sectioned at 30 μm on a freezing microtome. The sections were mounted on slides prepared with chrome-alum gelatin solution, defatted in xylene, dipped in Ilford G5 emulsion and exposed for 6 weeks at 4 ^{0}C. The sections were then developed in Kodak D19 at 16-18 ^{0}C for 4 minutes, rinsed in distilled water, fixed in 24% sodiumthiosulphate for 8 minutes and stained with Cresyl Violet. For the delineation of the effective injection sites, criteria previously described by Groenewegen and Voogd (1977) were used.

Phaseolus lectine procedure

Phaseolus vulgaris leuco-agglutinin (PhaL, Gerfen and Sawchenko, 1984) (Vector, 2.5% in Tris-buffered saline (TBS) (0.05 M, pH 7.4) was delivered ionto-phoretically through glass micropipettes (tip diameter 10-20μm) with positive current pulses (4-8 μA DC; 7 seconds on, 7 seconds off) for 30 minutes. After a survival time of seven days the

animals were transcardially perfused under deep anaesthesia with 0.5 liters phosphate buffer (0.05 M pH 7.4), containing 0.8% NaCl, 0.8% sucrose and 0.4% d-Glucose, followed immediately by 2 l of a solution of 0.5% paraformaldehyde, 2.5% glutaraldehyde and 4% sucrose in phosphate buffer (0.05 M, pH 7.4) and rinsed with 10% sucrose in 0.05 M phosphate buffer (pH 7.4). The brains were embedded in 10% gelatin and sectioned at 40 µm on a freezing microtome. Every fourth section was collected in TBS (0.05 M pH 7.4) in vials. The sections were transferred to a solution of TBS (0.05 M, pH 8.6) with 0.4% Triton X- 100 (TBS-T), rinsed 3 times and incubated in TBS-T to which the primary antibody goat-anti-Phaseolus (Vector) was added (dilution 1 : 2000). Incubation was performed under continuous gentle agitation at room temperature for 18 hours. The sections were rinsed thoroughly in TBS-T (3 times) and incubated with rabbit-anti-goat whole serum (Sigma) in TBS-T (dilution 1 : 200) for 90-120 minutes, washed again in TBS-T and transferred to a goat-peroxidase- antiperoxidase (PAP, Nordic) in TBS-T (dilution 1 : 400) for 90-120 minutes. Hereafter the sections were washed with TBS (pH 7.6) and incubated for 45-60 minutes in 100 ml TBS (pH 7.6) containing 50 mg DAB and 33 µl 30% hydrogenperoxide. Alternating sections were counterstained with Cresyl Violet. Delineation of the injection sites was according to ter Horst et al. ['84].

There were no qualitative differences observed in the distribution of the labeled terminals between the cases in which ^{3}H-leucine, PhaL or WGA-HRP were used, lending support to the notion that the WGA-HRP is not transported anterogradely by passing fibers. The use of PhaL resulted in quantitative differences because the iontophoretic injections with this tracer were very small. The resulting labeling was distributed over approximately the same area as in cases with injection of WGA-HRP or ^{3}H-leucine.

3 ANTERIOR LOBE AND SIMPLE LOBULE

3.1 INTRODUCTION

Apart from the vestibulocerebellum several other cerebellar regions have been reported to receive vestibular information. Kotchabhakdi and Walberg (1978b) have shown with retrograde tracer methods a projection from the medial (MV) and descending (DV) vestibular nuclei to the entire vermal anterior lobe. With the exception of the lingula, they found no clear evidence for a projection to the anterior lobe from the superior (SV) and lateral (LV) vestibular nuclei. Matsushita and Okada (1981) demonstrated a projection from all the vestibular nuclei but the LV, to lobules I and II. Most other studies on the vestibulocerebellar afferents have been restricted to the vermis. Injections with HRP in the lobules VI and VII (Kristensson and Olson, 1971; Gould and Graybiel, 1976; Shinnar et al., 1975; Batini et al., 1978), and in lobules V and VI (Kristensson and Olson, 1971; Gould and Graybiel, 1976; Precht et al., 1977) have demonstrated a connection between these parts of the vermis and the MV and DV. In the Macaque monkey Brodal and Brodal (1985) found only a single labeled neuron in the DV after an HRP injection in the vermis of lobules V and IV.

Since most HRP studies focussed on the afferent input to certain areas or lobules of the cerebellum, the total extent of the vestibular projection area in the cerebellum is not known. There are only few data available obtained with anterograde tracing methods. Magras and Voogd (1985) described in an autoradiographic study a bilateral projection from the vestibular nuclei in the cat to the entire cerebellar vermis. In the anterior lobe vermis most mossy fiber terminals were observed ventrally in the lingula. The amount of mossy fiber terminals in lobules II-VIII was low. In the same animal, Matsushita and Wang (1987) have shown with the anterograde transport of WGA-HRP, that the MV and DV project to three bilateral longitudinal areas mainly in the basal one half of the lobules. Although the general outline of the secondary vestibular cerebellar projection has been set, various aspects of the projection such as the distribution of the mossy fiber terminals over the individual lobules and their degree of zonal organization still have to be clarified. The present study will investigate the origin and termination of the vestibular projection to the anterior and simple lobules in the rabbit with retograde and anterograde tracers.

Anterograde and retrograde tracing studies have demonstrated that the Purkinje cells of the vermis of the anterior lobe and the simple lobule (lobule VI) project to the LV and the MVmc. These Purkinje cells were located in two longitudinal strips. Purkinje cells of the medial A zone project to the MVmc , the B zone, which occupies the lateral part of the vermis projects to the LV. A wedge-shaped area (the x zone of Ekerot and Larson, 1982) containing Purkinje cells which do not project to the vestibular nuclei is located in between the A and B zone in the dorsal part of the anteror lobe (Voogd, 1964; van Rossum, 1969; Andersson and Oscarsson, 1978a,b; Corvaja and Pompeiano, 1979; Bigaré, 1980; Voogd and Bigaré, 1980; Haines et al., 1982; Dietrichs et al., 1983; Balaban, 1984; Trott and Armstrong ,1987; Epema, 1990).

Experiments with injections with WGA-HRP in the vestibular nuclei resulted both in retrogradely labeling of Purkinje cells and in anterograde labeling of the secondary vestibular mossy fiber terminals. In these cases it was possible to determine the lateral border of the vermis, which is otherwise quite obtuse. Moreover we will address the question whether cerebellum and vestibular nuclei are reciprocally connected. An assesment of the relation between vestibulocerebellar and corticovestibular connections is of great importance for the further evaluation of the functional role of the vermis in motor control.

3.2 RESULTS

Distribution of corticovestibular Purkinje cells.

The corticovestibular projection could be studied in those experiments in which WGA-HRP was injected in the VN (Table 3.1). The localization of the labeled Purkinje cells is best visualized when projected upon the reconstructed surface of the anterior lobe.

Large numbers of Purkinje cells were labeled when the injection involved the LV (C2143, Fig. 3.1) and/or areas directly adjacent to the LV such as the MVmc (2144, Fig. 3.1, Fig. 3.7) or the rostral DV (K109, Fig. 3.1, Fig. 3.6). In these experiments retrogradely labeled Purkinje cell axons could be followed in the vermal white matter into a medial and lateral bundle. In the lobules I-IV the labeled somata, which belong to these two bundles, were located in one broad strip. In lobule I the labeled Purkinje cells reached almost to the lateral margin of the lobule. In lobules IV and V these labeled Purkinje cells separated into two strips with a wedge-shaped empty area in between (Fig. 3.1).

In experiment K109 with an injection in the rostral DV the area immediately next to the midline remained free from label (Fig. 3.1). In case K216 with an injection restricted to the MVpc, labeled Purkinje cells were found in a single narrow medial strip. They are most numereous in lobules I-III (Fig. 3.1). Following injections in the caudal part of the VN (MVc: K108; DV: C2358) labeled Purkinje cells in the anterior lobe were never observed.

There are no cases available with injections restricted to the SV. In experiment K116 the injection site is located for most part in the SV, but there was some spread of tracer into the interposed nucleus. In this case labeled Purkinje cells were found throughout the vermis and parts of the hemisphere.

Table 3.1 Listing of experiments with injections in different vestibular nuclei. Injected volumes in µl, survival time in days. WGA-HRP: 5% wheatgerm agglutinated horseradish peroxidase in 0.01 M phosphate buffer, pH 7.8; PhaL: 2.5% in 0.05 M Tris-buffered saline, pH 7.4, delivered iontophoretically; ^{3}Hleu: 100 µCi/µl tritiated leucine in saline.

exp	tracer	vol	surv	Y	SV	LV	DV	MVc	MVpc	MVmc
K 116	WGA-HRP	0.3	4		++					
K 124	WGA-HRP	0.3	4		++	+				
K 82	^{3}H-leucine	0.3	4	+	++	+				
C 2143	WGA-HRP	0.3	3			++	+			
K 42	^{3}H-leucine	0.3	4			+	++	+		
K 128	PhaL	*	7				++			
K 109	WGA-HRP	0.3	4			+	++			
C 1922	WGA-HRP	0.3	3				++			
C 2059	^{3}H-leucine	0.3	7					++		
C 2098	^{3}H-leucine	0.3	7					++		
K 108	WGA-HRP	0.3	4					++		
K 127	PhaL	*	7						++	
K 157	WGA-HRP	0.3	3		+				++	
K 216	WGA-HRP	0.3	3						++	
K 138	PhaL	*	7			+	+			++
C 2144	WGA-HRP	0.3	3			+	+			++

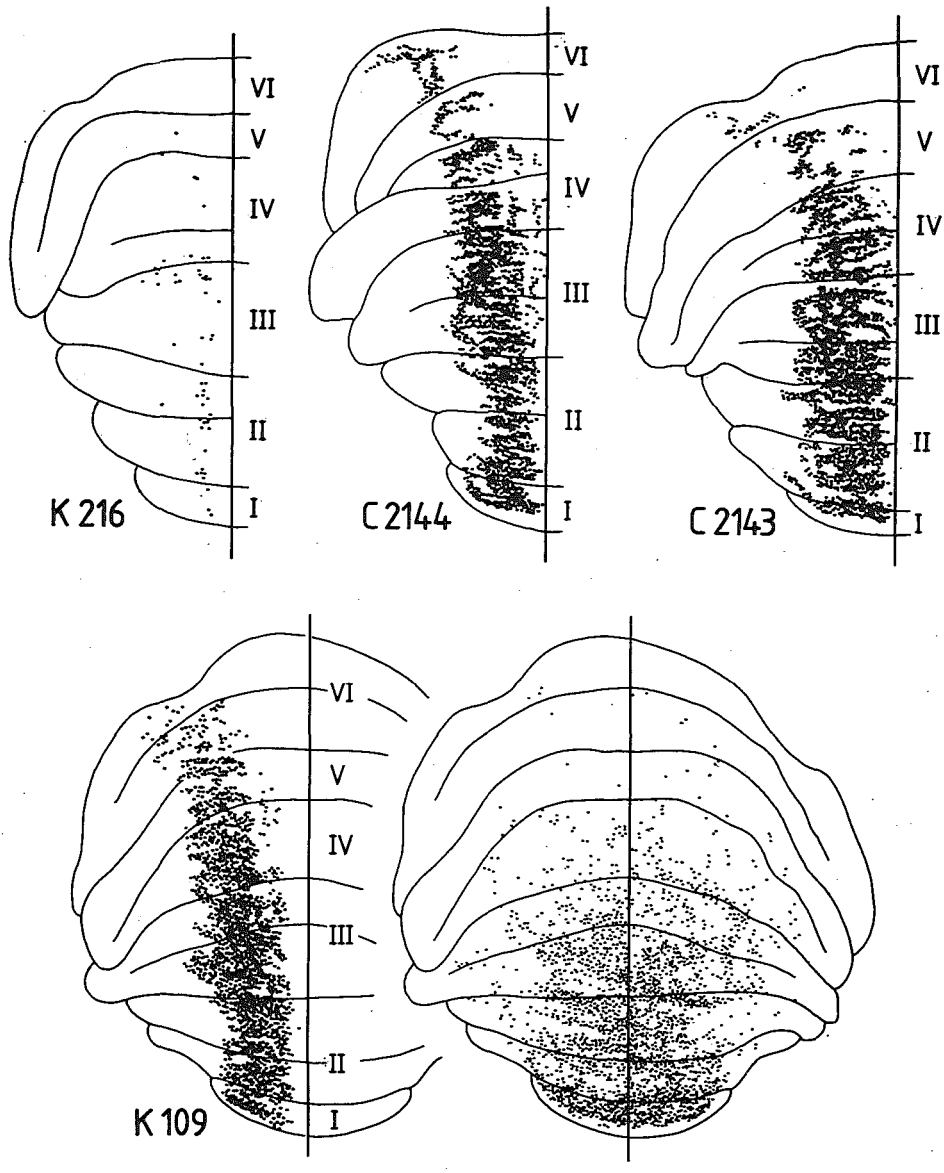


Fig 3.1 Distribution of labeled Purkinje cells (Exps. K216, C2144, C2143, K109) and mossy fiber terminals (bottem left, Exp. K109) as seen through the reconstructed surface of the anterior lobe and the simple lobule.

Distribution of neurons projecting to the anterior lobe vermis and the simple lobule

To assess the relevance of the injection sites used in the anterograde tracer experiments, the distribution of vestibulocerebellar neurons, projecting to the anterior lobe vermis and simple lobule was investigated by means of large injections of WGA-HRP. The experiments are listed in Table 3.2. The injections were placed near the midline and did not extend into the central white matter. The largest number of labeled neurons was found in experiments where the injection covered the proximal parts of the folia of the anterior lobe.

In experiment K186, with an injection comprising the lobules II and III almost completely, labeled neurons were found in all vestibular nuclei, with the exception of the LV. A large number of labeled neurons were found in group x, the cuneate nuclei (CN), while some were found in the prepositus hypoglossal nuclei (PH). Labeled vestibular neurons were concentrated in the DV, MVc and the central SV (Fig. 3.2). This distribution was also found in experiment K176, with an injection in lobule IV and the proximal parts of lobules V and VI. In this latter case labeled neurons were more numerous in the DV and the caudal aspect of the PH, than in case K186 (Fig. 3.2). Injection of WGA-HRP in the apical parts of the anterior lobe folia resulted in a small number of labeled neurons in the VN, while they were still abundantly present in the CN and the caudal PH. In case K264 labeled neurons were concentrated in the DV, few were present in the MVc and the SV. No labeled neurons were found in the magnocellular (MVmc) and the parvocellular (MVpc) medial vestibular nuclei (Fig. 3.2). In case K181, with an injection restricted to the most apical parts of lobules IV, V and VI only two labeled vestibular neurons were observed in the DV.

Table 3.2 Listing of experiments with injections of tracer in different lobules of the anterior lobe and simple lobule. The extent of the injection sites is illustrated in Fig. 1. Injected volume in µl, survival time in days. WGA-HRP: 5% wheatgerm agglutinated horseradish peroxidase in 0.01 M phosphate buffer, pH 7.8.

exp	tracer	vol	surv	VI	V	IV	III	II
K 264	WGA-HRP	0.3	2	+	+			
K 181	WGA-HRP	0.3	2	+	+	+		
K 176	WGA-HRP	0.3	2		+	+		
K 186	WGA-HRP	0.3	2				+	+

Distribution of vestibular mossy fiber terminals in the anterior lobe and simple lobule

The vestibulocerebellar projection was investigated with anterograde transport of tritiated leucine, WGA-HRP and PhaL. Injections were made in all subdivisions of the vestibular nuclei. The experiments are listed in Table 3.1. All experiments resulted in labeling of mossy fiber terminals (mft) in the cerebellar granular layer. The labeled parent fibers could not be followed in the white matter. The projection was bilateral and included the vermis of the anterior lobe and the cortex of the simple lobule facing the primary fissure. The greatest density of terminals was always found in the depth of the fissures, mostly in the precentral, intracentral, preculminate and primary fissures. Whereas lobule I was usually labeled completely, the apices of the other lobules were only sparsely labeled or remained free of labeled mft. Most of the labeled mft were confined to the vermis, as defined by the Purkinje cell labeling in some of the experiments, but labeled mft were also found more laterally.

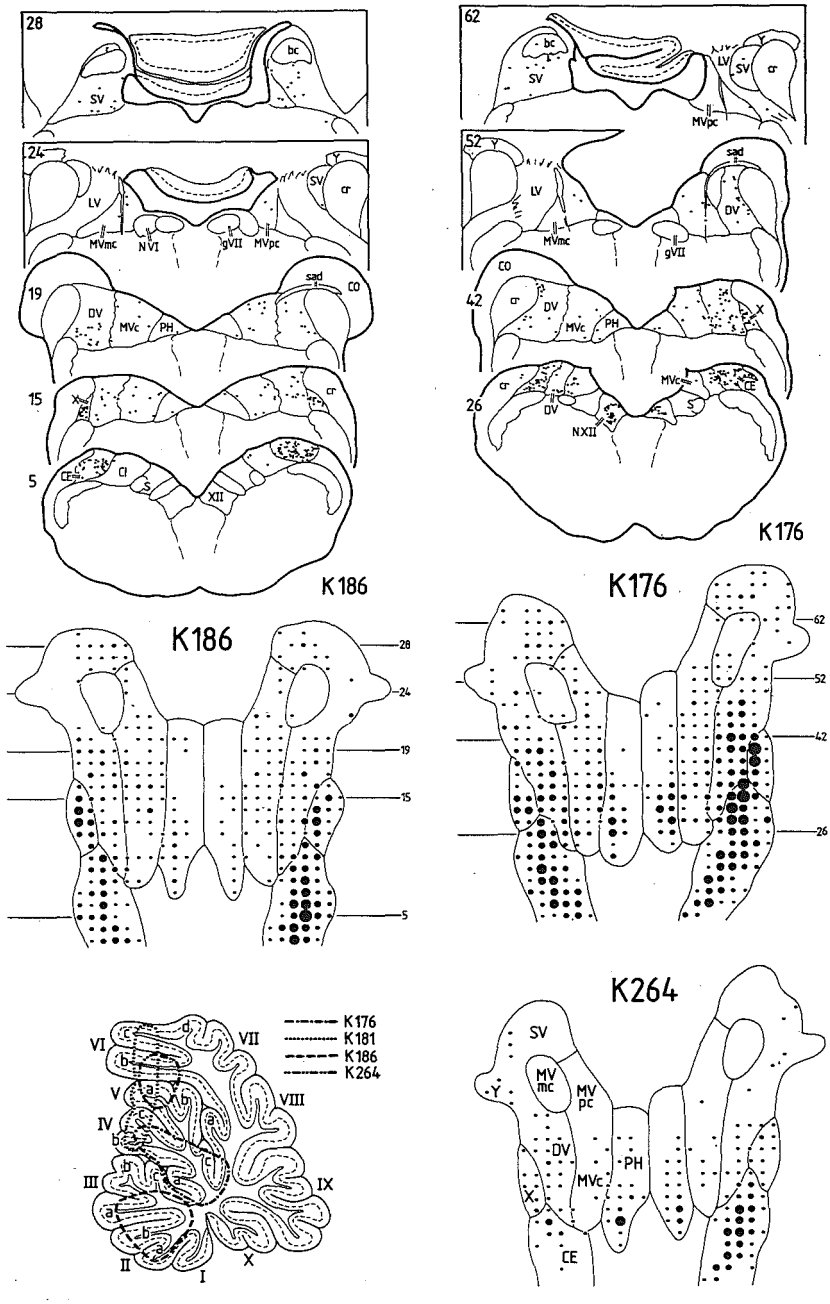


Fig 3.2 Extent of the injection sites of Exps. K176, K181, K186, K264 projected onto the midsagittal plane of the cerebellum (bottom left). Distribution of retrogradely labeled vestibular neurons in transverse sections (Exps. K186, K176). Distribution of labeled vestibulocerebellar neurons (Exps. K186, K176, K264) in horizontal diagrams of the vestibular complex.

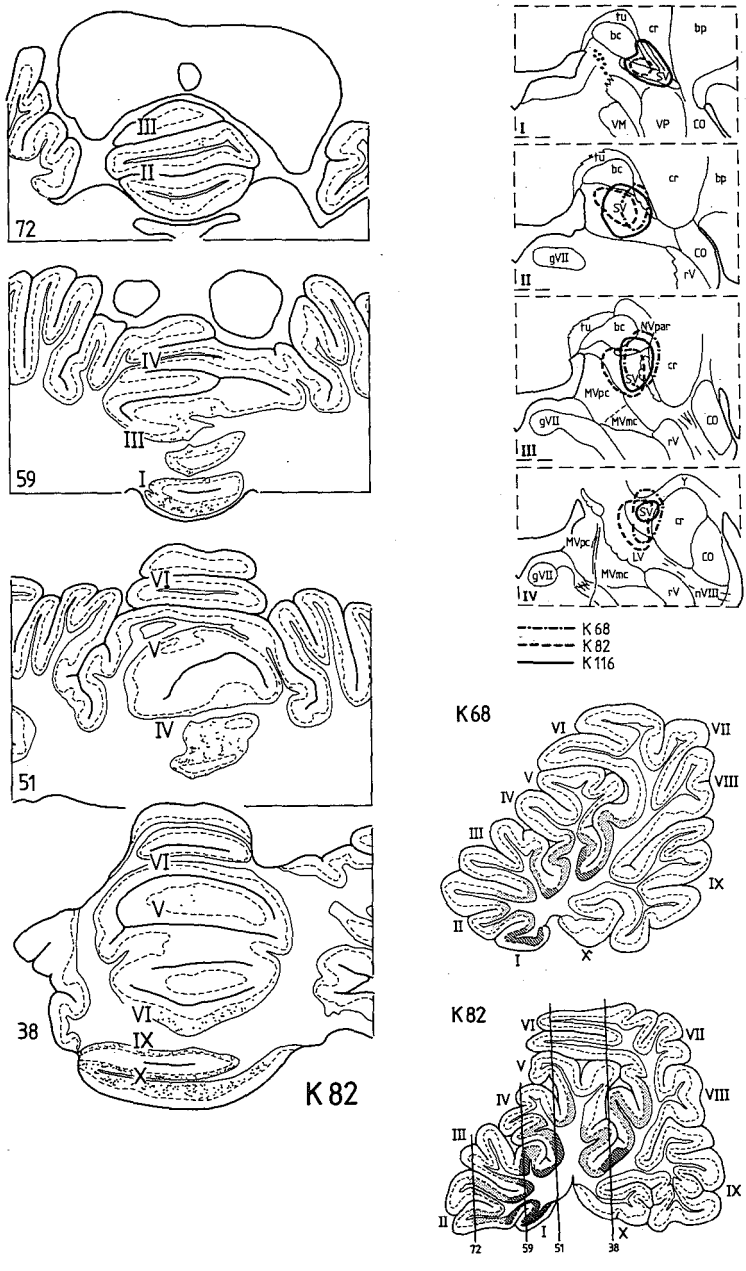


Fig 3.3 Injection sites in the SV (Exps. K68, K82, K116) and the consequent distribution of anterogradely labeled mossy fiber terminals in the anterior lobe (Exps. K82). The extent and intensity of the termination is projected onto the reconstructed midsagittal plane (Exps. K68, K82).

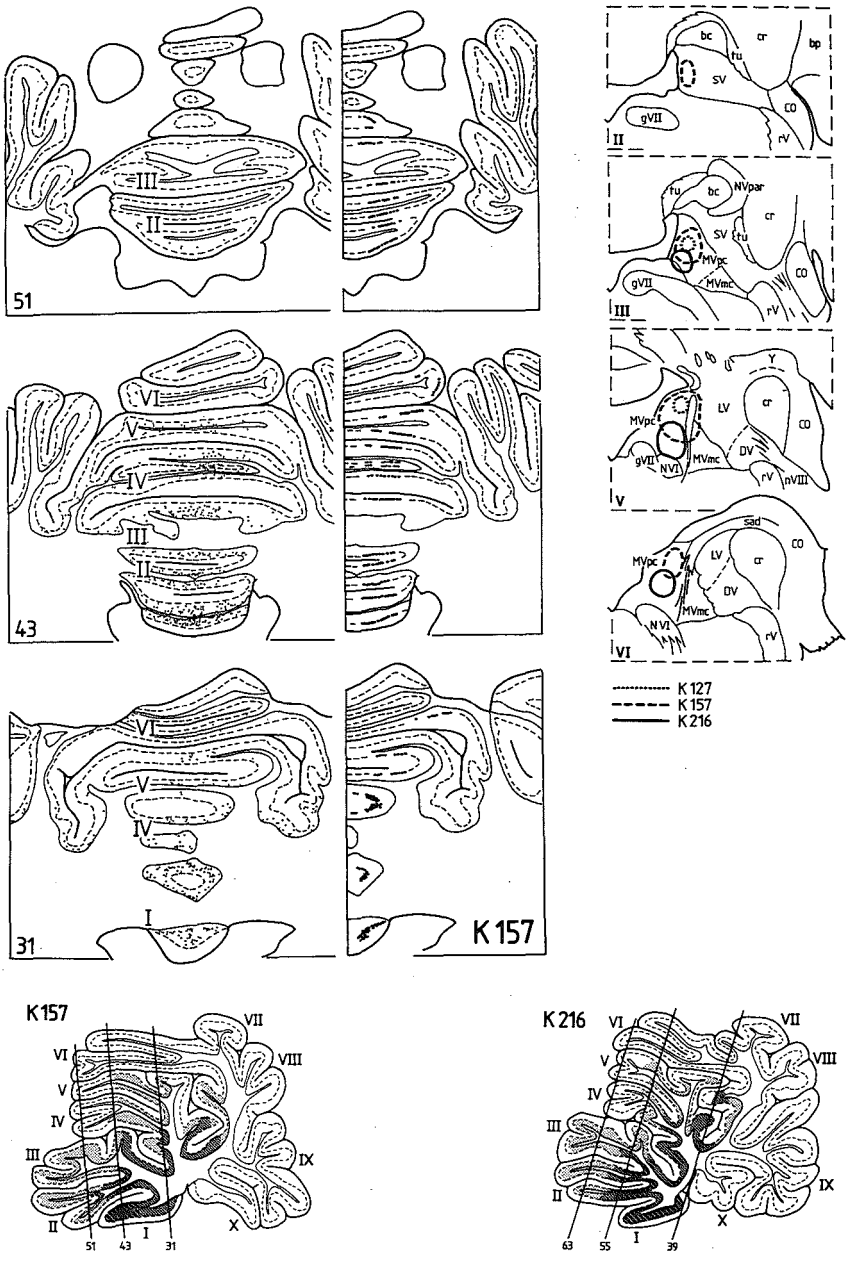


Fig 3.4 Injection sites in the MVpc (right column, Exps. K127, K157, K216) and the consequent distribution of anterogradely labeled mossy fiber terminals in the anterior lobe (left column, Exp. K157, WGA-HRP). The distribution of retrogradely Purkinje cells from the same sections is illustrated separately (middle column). The extent and intensity of the termination is projected onto the reconstructed midsagittal plane (bottom, Exps. K157, K216).

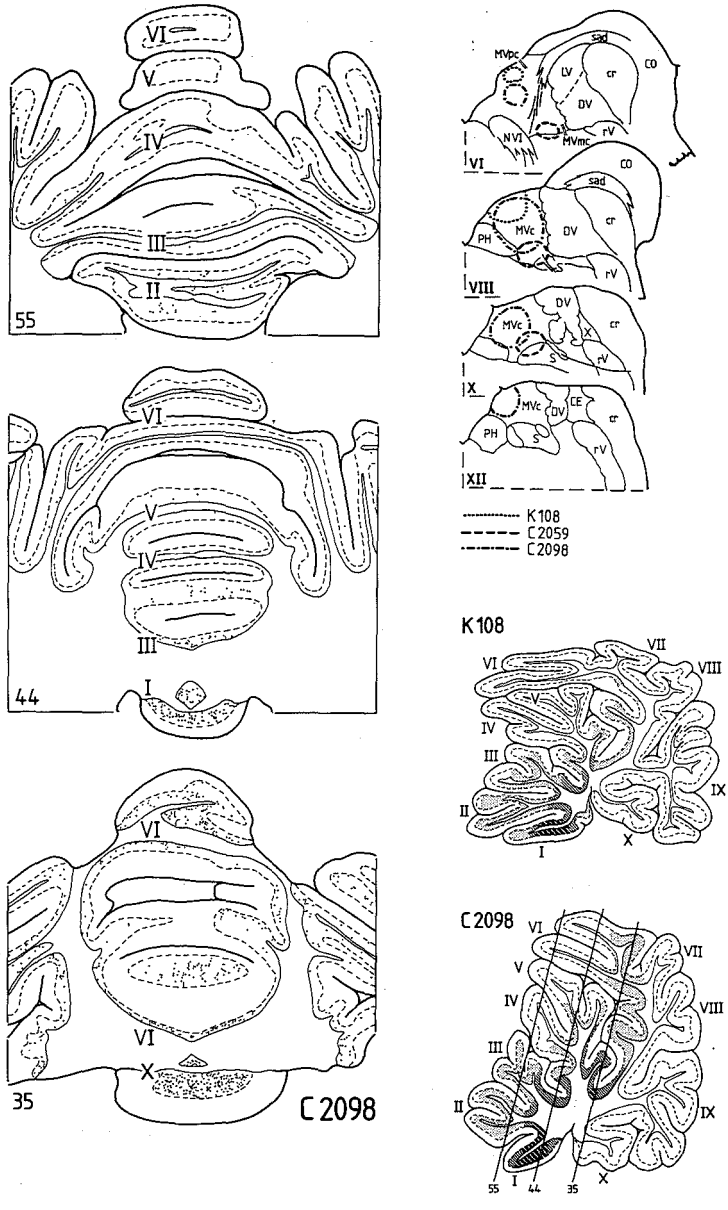


Fig 3.5 Injection sites in the MVc (Exps. K108, C2059, C2098) and the consequent distribution of anterogradely labeled mossy fiber terminals in the anterior lobe (Exps. C2098). The extent and intensity of the termination is projected onto the reconstructed midsagittal plane (Exps. K108, C2098).

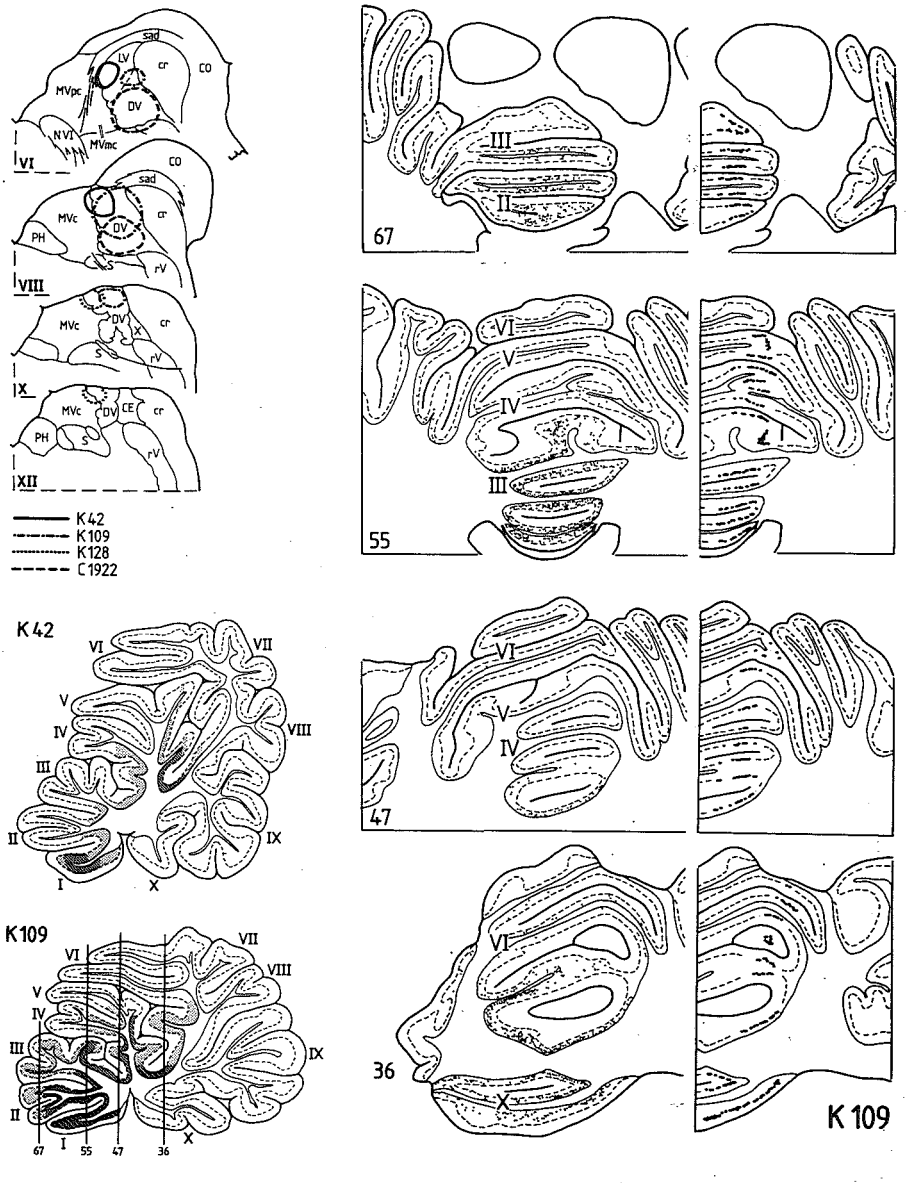


Fig 3.6 Injection sites in the DV (top left, Exps. K42, K109, K128, C1922) and the consequent distribution of anterogradely labeled mossy fiber terminals in the anterior lobe (middle column, Exp. K109, WGA-HRP). The distribution of retrogradely Purkinje cells from the same sections is illustrated separately (right column). The extent and intensity of the termination is projected onto the reconstructed midsagittal plane (bottom left, Exps. K42, K109).

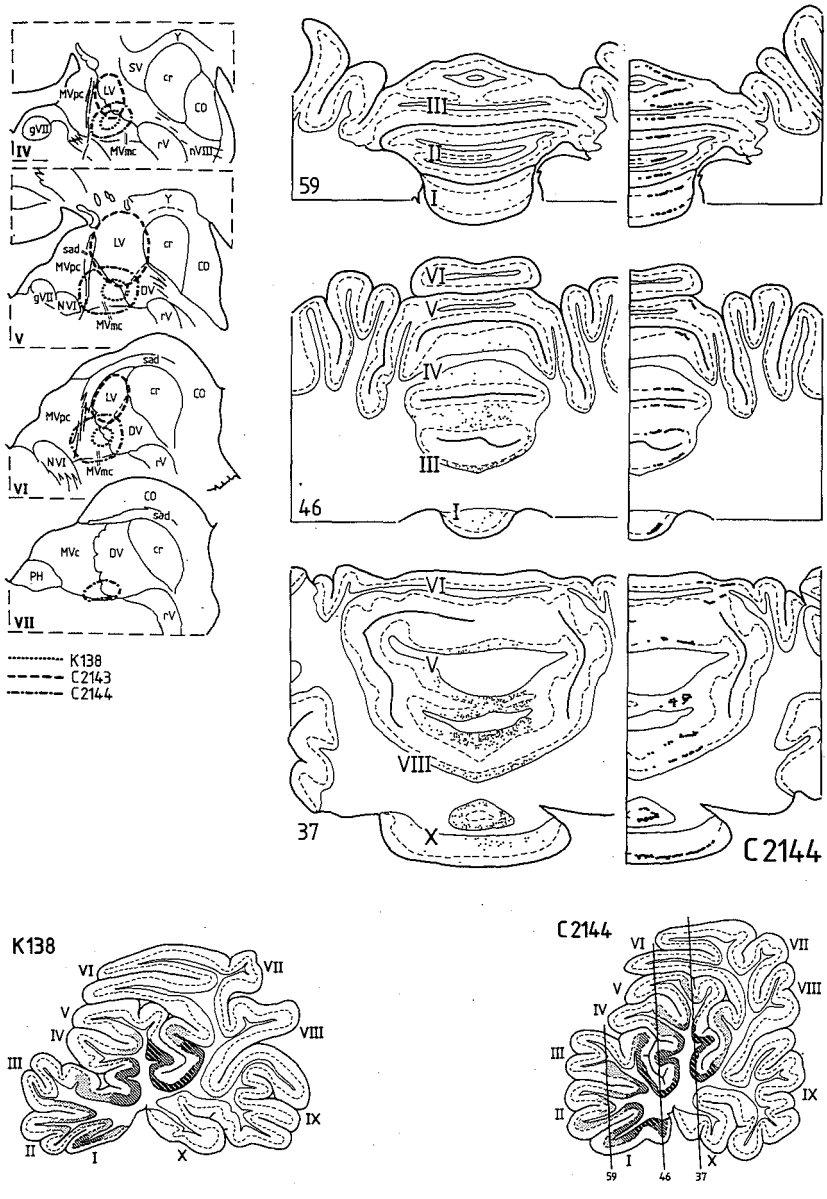


Fig 3.7 Injection sites in the LV (left column, Exp. C2143) and MVmc (left column, Exps. K138, C2144) and the consequent distribution of anterogradely labeled mossy fiber terminals in the anterior lobe following injection in the MVmc (middle column, Exp. C2144, WGA-HRP). The distribution of retrogradely Purkinje cells from the same sections is illustrated separately (right column). The extent and intensity of the termination is projected onto the reconstructed midsagittal plane (bottom, Exps. K138, C2144).

In case C2098 a fair amount of labeled mft were found in both hemispheres, including Crus I and II (Fig. 3.5). In cases K157 (Fig. 3.4) and K109 (Fig. 3.6) labeling of mft lateral to the the vermis is most prominent in the lobules I-III and in the primary fissure. In experiments K82 (Fig. 3.3) and C2144 (Fig. 3.7) the labeled mft were almost completely confined to the vermis. Vestibulocerebellar mft were not equally distributed but exhibited a certain degree of clustering. However, the clustering did not show a continuity in longitudinal zones over successive sections or lobules.

The amount of labeled terminals in the vermis varied with the location of the injection. The largest number of mft were found in cases with injections in the central SV (K82: ^{3}H-leucine, K116: WGA-HRP, Fig. 3.3), the MVpc (K127: PhaL, K157: WGA-HRP, K216: WGA-HRP, Fig. 3.4), the MVc (C2098: ^{3}H-leucine, K108: WGA-HRP, Fig. 3.5) and the central DV (C1922: WGA-HRP, K109: WGA-HRP, Fig. 3.6). In these experiments the largest concentration of mft was in lobules I and II. In case of injections in the MVmc (C2144: WGA-HRP, K138: PhaL, Fig. 3.7) the apices of lobules II-VI remained free of labeleling, while the largest concentration of mft is clearly in the depth of the primary fissure.

In experiments with injections in the lateral part of the SV (K68: ^{3}H-leucine, Fig. 3.2) and the dorsal DV (C2358: WGA-HRP, K42: ^{3}H-leucine, K128: PhaL, Fig. 3.6) the number of labeled mft were considerabely smaller than in the other cases.

The retrograde tracer experiments demonstrated that the LV does not contribute to the vestibulocerebellar projection. In experiment C2143 (WGA-HRP) with an injection almost completely restricted to the LV (Fig. 3.7) only very few labeled mft were found in the anterior lobe vermis. These terminals might be attributed to the slight involvement of the DV and the MVmc in the injection site.

3.3 DISCUSSION

Distribution of corticovestibular Purkinje cells and their relation to vestibulocerebellar mossy fibers

The distribution of the corticovestibular Purkinje cells is characterized by a high degree of order which is in strong contrast to the gradually changing densities observed in the termination of mossy fibers. The sagittal organization in the corticovestibular projection has been recognized for a long time (Voogd, 1964,1969,1989; Voogd and Bigaré, 1980; Corvaya and Pompeiano, 1979; Brown and Graybiel, 1983; Dietrichs et al., 1983). Retrograde tracer studies in the cat (Bigare, 1980; Voogd, 1989) have demonstrated that the corticovestibular Purkinje cells are restricted to the lateral part of the A-zone and the entire B-zone, separated in the dorsal part of the anterior lobe (lobule V) by the x-zone, which has only a limited rostrocaudal extent. The B-zone was found to be connected with the LV, the A-zone with the MVmc. The present material is not well suited to conclude on finer details of the corticovestibular projection. The distribution of Purkinje cells in our material confirms the findings of Bigaré (1980) and Voogd (1989) in the cat and of Epema (1990) in the rabbit. Moreover the present data suggest the possibility of a Purkinje cell projection to parts of the VN adjacent to the LV and MVmc, such as the rostral DV and the MVpc.

Distribution of vestibular neurons projecting to the anterior lobe vermis and the simple lobule

The present study demonstrated that injections in different parts of the anterior lobe vermis resulted in labeled neurons with an almost identical distribution in the vestibular nuclei. In accordance with previous retrograde tracer studies (Kristensson and Olsson,

1971; Gould and Graybiel, 1976; Shinnar et al., 1975; Precht et al., 1977; Kotchabhakdi and Walberg, 1978b; Batini et al., 1978; Matsushita and Okado, 1981) we have demonstrated labeled neurons in the DV, MV and group x, following injections with WGA-HRP in the anterior lobe and the simple lobule. In addition Kotchabhakdi and Walberg (1978) observed labeled cells in the SV, but only following large injections, which completely covered several lobules of the anterior lobe entirely. They suggested that the projection from the SV is rather diffuse and that large injections would be required for sufficient uptake of HRP in the terminals of the axons of those neurons to visualize their perikarya. However, Matsushita and Okado (1981) have found consistent labeling in the SV, following small injections covering most part of the lobules I or II. In the present study, discrete injections with WGA-HRP into the anterior lobe vermis and the simple lobule, including the cortex in the depth of the interlobular fissures, always labeled a considerable number of neurons in the SV. These findings indicated that the projection of the SV is more or less restricted to the proximal area of the cortex in the bottom of the fissures. Therefore the discrepancy with previous retrograde tracer studies (Kristensson and Olson, 1971; Gould and Graybiel, 1976; Shinnar et al., 1975; Batini et al., 1978; Precht et al., 1977; Kotchabhakdi and Walberg, 1978b) concerning the involvement of SV in the mossy fiber projection to the anterior lobe can be explained by the fact that most of their injection sites were restricted to the apical parts of the lobules, which are only minimally involved in the vestibulocerebellar projection of the SV.

Distribution of secondary vestibular mossy fiber terminals in the anterior and simple lobe

The most striking observation in our anterograde tracer material is that labeling was consistently present in the same parts of the anterior lobe, almost completely irrespective of the location of the injection site in the vestibular nuclei. In general our results obtained with anterograde and retrograde tracing are in fair agreement. The largest mossy fiber input originated from those nuclei containing the largest number of retrogradely labeled vestibular neurons. The area of termination of the vestibular mossy fibers which emerges from the present study differs from the results of previous retrograde tracer studies (Kristensson and Olson, 1971; Gould and Graybiel, 1976; Shinnar et al., 1975; Batini et al., 1978; Precht et al., 1977; Kotchabhakdi and Walberg, 1978b) with respect to its extent in the cerebellar cortex. Those studies described a much larger projection area. A carefull examination of the findings of these authors revealed that most of their injections were in superficial parts of the cortex on the outskirts of the termination area of the vestibular mossy fibers, as determined in the present study. The limited density of mft near the borders of the vestibular termination area is reflected in the small number of retrogradely labeled neurons in the VN in these earlier studies. Our findings confirm the results of Precht et al. (1977) that the vestibular projection is restricted to the cortex in the depth of the fissures. In their electrophysiological study the short latencies of field potentials recorded in these proximal parts of the folia of lobule V and VI were similar to those recorded in the vestibulocerebellum, which suggest that primary and/or secondary vestibular mossy fibers terminate in these deeper regions. In the apical parts of the lobules the latencies of vestibular evoked potentials are too long to represent mono- or disynaptic vestibular input. However, Precht did not made a distinction between primary and secondary vestibular mossy fibers. Hirai (1983) and Gerrits et al. (1989) demonstrated that the primary and secondary vestibulocerebellar afferents share a common termination area. It can be concluded, that the the projection areas of the central cervical nucleus (Matsushita and Tanami, 1987; Matsushita and Okado, 1981; Wiksten, 1979) and the cuneate nuclei (Gerrits et al., 1985; Jasmin and Courville, 1987a,b) resembled those of the vestibular nuclei revealed in the the present study, in terms of their location and extent within the lobules, i.e. in lobule I and the depth of the interlobular fissures. However, the termination area of the cuneate nuclei comprises a more extensive hemispheral part of the anterior lobe and the simple lobule.

The mode of distribution of the secondary vestibular mft over the cortex, like the primary ones (Gerrits et al., 1989), seems irregular rather than clustered in longitudinal strips. Whith respect to their termination in the deep parts of the folia of the vermis, our results with anterograde tracers in the rabbit are in agreement with previously published data in the cat (Magras and Voogd, 1985; Matshishta and Wang, 1987). In the cat the secondary vestibular projection seems to be zonally organized. A zonal organization is characteristic for many mossy fiber systems, both for those terminating in the depth of the fissures of the anterior lobe, including the projection from the central cervical nucleus (Matsushita and Tanami, 1987) and the cuneate nuclei (Gerrits et al., 1985; Jasmin and Courville, 1987a,b) and for those terminating more superficially, including spinocerebellar fibers from other sources (Matsushita and Tanami, 1984; Matsushita et al., 1984; van Rossum, 1969).

Secondary vestibulocerebellar mft are part of a transversely orientated fractionated system which conveys vestibular, and propioceptive information from neck receptors to the cerebellum. It should be noted, however, that the number of vestibulocerebellar mft is very low when compared to the afferents from the cuneate nuclei and the central cervical nucleus. This system of mft is oriented at right angles to the output of the A zone of the vermis to the fastigial nucleus and the MVmc and the B zone to the LV. Through its direct connections with the MVmc and its bilateral projection to this nucleus through the fastigial nucleus the A zone influences the activity in the medial vestibulospinal tract and the crossed vestibulomesencephalic tracts, which play a role in the execution of eye and head movements. The B zone influences the activity in the lateral vestibulospinal tract, which takes its origin from the LV and which descends to the medial part of the intermediate zone along the entier length of the spinal cord. There is a narrow area of overlap in the bottom of the fissures where vestibular and neck afferents dominate a small stretch of the A and B zones with their differential output to the vestibular nuclei, but over most of their extent these zones are dominated by information entering the cerebellum through other mossy fiber systems.

4 POSTERIOR LOBE

4.1 INTRODUCTION

Anatomical and physiological studies have demonstrated that the vestibular nuclei are an important source of secondary mossy fibers to the cerebellum (Brodal, 1974; Llinas and Precht, 1972). In experimental studies with the retrograde degeneration method in the cat (Brodal and Torvik, 1957), the vestibular projection to the caudal vermis has been shown to originate in all vestibular nuclei except for the lateral vestibular nucleus of Deiters (LV). This projection was subsequently confirmed with retrograde transport of horseradish peroxidase (HRP) in different mammals: cat (Gould, 1980; Kotchabhakdi and Walberg, 1978), sheep (Saigal et al., 1982), macaque (Brodal and Brodal, 1985), and Galago (Rubertone and Haines, 1981).

The anterograde degeneration method has also been used to map vestibulocerebellar projections (Dow, 1936). This study indicated that in the caudal vermis the majority of secondary vestibular mossy fibers terminate bilaterally in the nodulus and the adjacent folia of the uvula. The possibility that the lesions in the vestibular complex might have damaged passing fibers could not be excluded in these experiments, but the use of the anterograde transport of ^{3}H-leucine in the cat (Magras and Voogd, 1985) confirmed the degeneration study of Dow (1936). The distribution of the secondary vestibulocerebellar mossy fibers in the caudal vermis is very similar to the distribution of the primary vestibulocerebellar projection, which terminates as mossy fiber rosettes in the same lobules, with an ipsilateral predominance (see Gerrits et al., 1989 for review)

In the rabbit no data are available to show the localization of vestibular nucleus neurons projecting to the caudal vermis. From the species investigated sofar (cat, macaque, Galago), it is evident that vestibulocerebellar neurons are concentrated rostrally in the superior vestibular nucleus (SV) and caudally in the medial (MV) and descending (DV) vestibular nuclei. The present study includes a number of retrograde tracing experiments in order to localize these neurons in the rabbit and to assess the relevance of the injection sites of the anterograde tracer experiments.

Thusfar, the localization of mossy fiber terminals in the rabbit's caudal vermis has been illustrated by Epema et al. (1985) in two groups of injections: rostrally in the SV and caudally in the MV and DV. Unfortunately, the rostral part of the external cuneate nucleus was included in their caudal injections. A comparison of vestibular and external cuneate mossy fiber inputs to the caudal vermis has been the subject of a preliminary report (Gerrits and Thunnissen 1987), and indicated profound differences in the distribution of terminals from these nuclei. Mossy fibers from various sources display conspicious differences in distribution over individual lobules (Gerrits, 1985), and in the degree of clustering into sagittal zones (Gerrits et al., 1984; Gerrits et al., 1985; Jasmin and Courville 1987a,b; Kunzle, 1975; Matsushita, 1987; Voogd et al., 1969). The present study used different anterogradely transported tracers (^{3}H-leucine, WGA-HRP, PhaL) to illustrate and evaluate the vestibulocerebellar mossy fiber projection with respect to distribution and zonal organization.

Purkinje cells of the caudal vermis project to all vestibular nuclei except the LV (Angaut and Brodal, 1967; Dow, 1936; Haines, 1977; van Rossum, 1969; Walberg and Dietrichs, 1988), and are organized in longitudinal zones (Balaban, 1984; Epema et al., 1985; Epema, 1990; Matsushita and Wang, 1986; Shojaku et al , 1987; Voogd and Bigare, 1980). The present study also addresses the question whether the vestibular mossy fiber projection matches the distribution of the cortico-vestibular Purkinje cells.

4.2 RESULTS

Distribution of neurons projecting to the caudal vermis

In order to assess the relevance of the injection sites in the anterograde tracer experiments, the distribution of vestibulo-cerebellar neurons was investigated by means of injections with HRP and WGA-HRP in the uvulo-nodular lobule of the cerebellum. These experiments are listed in Table 4.1. The injection in experiment K79 was restricted to the nodular cortex (Fig. 4.1). In experiment C667 the injection involved the apical parts of the lobules IXd and IXc, while in experiment C1189 the injection was in the same lobules but included also their proximal parts (Fig. 1). The injection of experiment K220 involved lobule IXa and the proximal part of lobule IXb (Fig. 4.1).

In all the experiments labeled neurons were present bilaterally throughout the vestibular complex without clear differences in laterality. In the LV no labeled neurons could be found. The largest number of labeled neurons was found in experiment C1189 (Fig. 4.1), in which the injection site comprised the lobules IXc and IXd clearly including the cortex lining the bank of the postero-lateral fissure. In the SV labeled cells showed a preference for central and medial parts of the nucleus. A smaller number of labeled neurons was present in the parabrachial vestibular nucleus (NVpar) and group y. Labeling in the medial vestibular nucleus was concentrated in its caudal subdivision (MVc), and its parvocellular subdivision (MVpc), predominantly in their dorsal regions. The number of labeled cells in the magnocellular portion of the medial vestibular nucleus (MVmc) was very small. A fair amount of labeled cells was present throughout the DV. At caudal levels they are most abundant and not clearly separated from the labeling in either the MVc or group x. Within the the nucleus prepositus hypoglossi (PH) labeled cells were found throughout its rostrocaudal extent (Fig. 4.1).

In experiments with injections in other parts of the caudal vermis the number of labeled neurons was considerably smaller than in experiment C1189. In general, their distribution throughout the vestibular complex was similar. In case C667, with an injection in the apical parts of the lobules IXc and IXd the number of labeled neurons in the SV was relatively low, compared to the total number of labeled cells (Fig. 4.1). A strong reduction of labeled neurons was found in case of injection of WGA-HRP in the lobules IXa and IXb (Exp. K220, Figs. 4.1, 4.2). The number of labeled cells in case K79 (Figs. 4.1, 4.2) was small due to the size of the injection.

Except for the injection in lobule X (Exp. K79) labeled cells were found, with an ipsilateral preference, in the cuneate nuclei, directly adjacent to the DV. A moderate number was present in case C667, with an injection in the apical parts of the lobules IXc and IXd. A more substantial number was present following injections including the proximal parts of the lobules IXc and IXd (Exp. C1189) and the lobules IXa and IXb (Exp. K220).

Table 4.1 Listing of experiments with injections of tracer in different lobules of the caudal vermis. The extent of the injection sites is illustrated in Fig. 4.1. Injected volume in µl, survival time in days. HRP: 33% horseradish peroxidase in distilled water; WGA-HRP: 5% wheatgerm agglutinated horseradish peroxidase in 0.01 M phosphate buffer, pH 7.8.

exp	tracer	vol	surv	X	IXd	IXc	IXb	IXa
K 79	WGA-HRP	0.1	2	+				
C 667	HRP	0.1	2		+	+		
C 1189	HRP	0.2	2		+	+		
K 220	WGA-HRP	0.2	3				+	+

Distribution of vestibular mossy fiber terminals in the caudal vermis

The vestibulocerebellar projections was investigated with the anterograde transport of ^{3}H-leucine, WGA-HRP and PhaL. Injections were made in all vestibular nuclei. Photographic examples of injections with each tracer are illustrated in Figs. 4.3A-C. The experiments are listed in Table 4.2. All experiments resulted in labeling of mossy fiber terminals (mft) in the cerebellar granular layer (Figs. 4.3D-F).

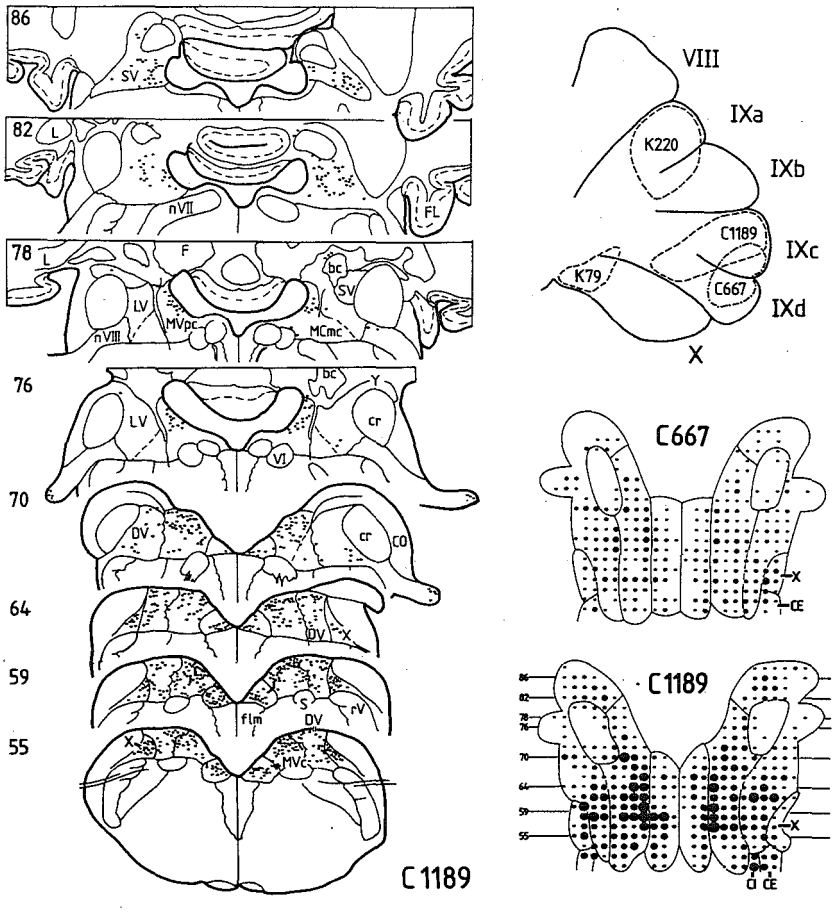


Fig 4.1 Extent of the injection sites of Exps. C667, C1189, K79, K220 projected onto the midsagittal plane of the caudal vermis (top right). Distribution of retrogradely labeled vestibular neurons in transverse sections (left side, Exp. C1189). Distribution of labeled vestibulocerebellar neurons (bottom right, Exps. C667, C1189) in horizontal diagrams of the vestibular complex.

Injections mainly restricted to the SV

The injection in experiment K82 (^{3}H-leucine) involved the central SV, group y and the rostral LV (Fig. 4.4). In the WGA-HRP experiments K116 and K124, the injections (Fig. 4.4) were restricted to the central SV with only limited involvement of the LV (K124). The distribution and the amount of labeled mft in the caudal vermis showed little variation with respect to the localization of the injection sites and the specific tracer used. The projection was bilateral with a clear preference for the lobules X and IXd (Exps. K82, K116; Fig. 4.5). Sparse labeling was found in the lobules VIII and IXa, b and c. The mft were concentrated in the proximal parts of the lobules and most abundant in the cortex lining the postero-lateral fissure. They were not equally distributed but exhibited in all experiments a certain degree of clustering.

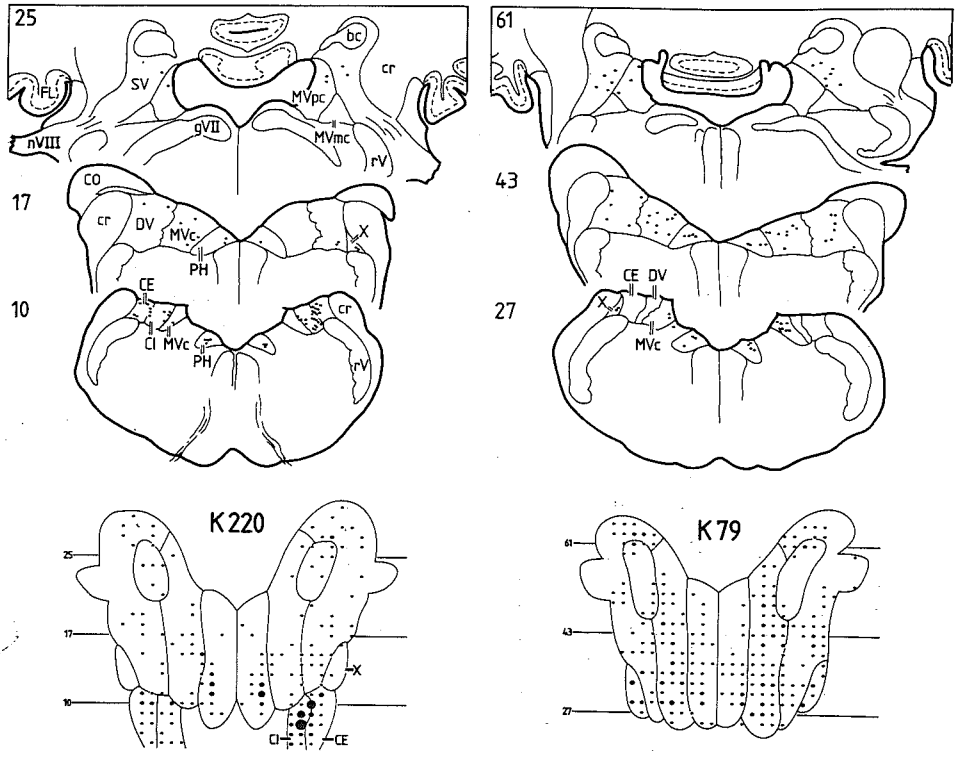


Fig 4.2 Distribution of retrogradely labeled vestibular neurons in transverse sections and horizontal diagrams following injection of HRP in lobules IXa and IXb (Exp. K220) and lobule X (Exp. K79).

Injections restricted to the LV

The retrograde tracer experiments demonstrated that the LV does not contribute to the vestibulocerebellar projection. The LV was included in a number of injections (Table 4.2). In experiment C2143 (WGA-HRP) the injection is almost completely restricted to the LV (Fig. 4.7). In this case only a few labeled mft in lobule X and lobule IXd were found, which could be attributed to a slight involvement of DV and MVmc in the injection site.

Injections restricted to the DV

The injection in experiment K42 (^{3}H-leucine) included the dorsorostral part of the DV, as well as a very limited number of cells in the MVc and the LV (Fig. 4.4). In experiment C1922 (WGA-HRP) the injection covers the ventrorostral part of the DV (Fig. 4.4), whereas the injection in K128 (PhaL) was restricted to the dorsocaudal DV, which usually contained many labeled neurons following injection of HRP in the caudal vermis (see Fig. 4.1) Experiment K109 (WGA-HRP) includes the entire dorsal aspect of the DV. The projection was bilateral and almost completely restricted to the lobules X and IXd and the ventral aspect of lobule IXc (Exps. K128, K109; Fig. 4.6). The mft showed a clear preference for the proximal parts of these lobules. The mode of distribution of mft over the cortex seemed irregular rather than clustered.

Injections restricted to the MVmc

In experiment C2144 (WGA-HRP) and experiment K138 (PhaL) the injections (Fig. 4.7) involved the MVmc, which nucleus showed only sparse labeling of vestibulocerebellar neurons in the retrograde tracer experiments. Experiment C2144 also included adjacent parts of the DV and MVpc. Both experiments resulted in a few labeled mft in lobule X and the cortex of lobule IXd, facing the postero-lateral fissure (Exp. C2144; Fig. 4.7). A few mft were found in the rest of lobule X and IX and in lobule VIII.

Table 4.2 Listing of experiments with injections in different vestibular nuclei. Injected volumes in µl, survival time in days. WGA-HRP: 5% wheatgerm agglutinated horseradish peroxidase in 0.01 M phosphate buffer, pH 7.8; PhaL: 2.5% in 0.05 M Tris-buffered saline, pH 7.4, delivered iontophoretically; ^{3}Hleu: 100 µCi/µl tritiated leucine in saline.

exp	tracer	vol	surv	Y	SV	LV	DV	MVc	MVpc	MVmc
K 116	WGA-HRP	0.3	4		++					
K 124	WGA-HRP	0.3	4		++	+				
K 82	^{3}H-leucine	0.3	4	+	++	+				
C 2143	WGA-HRP	0.3	3		-	++	+			
K 42	^{3}H-leucine	0.3	4			+	++	+		
K 128	PhaL	*	7				++			
K 109	WGA-HRP	0.3	4			+	++			
C 1922	WGA-HRP	0.3	3				++			
C 2059	^{3}H-leucine	0.3	7					++		
C 2098	^{3}H-leucine	0.3	7					++		
K 108	WGA-HRP	0.3	4					++		
K 127	PhaL	*	7						++	
K 157	WGA-HRP	0.3	3		+				++	
K 216	WGA-HRP	0.3	3						++	
K 138	PhaL	*	7			+	+			++
C 2144	WGA-HRP	0.3	3			+	+			++

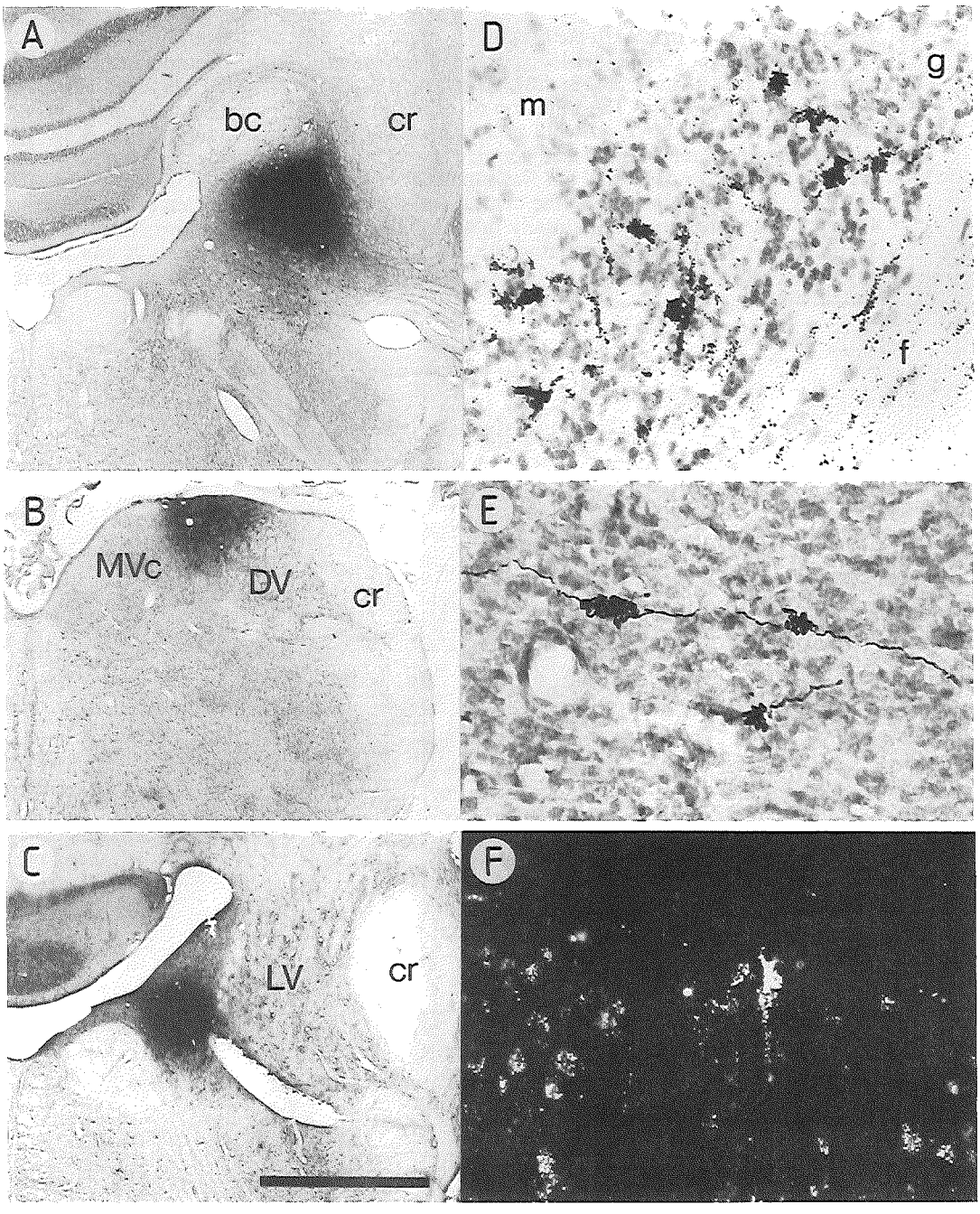


Fig 4.3 Example of injection sites with different tracers; A: Exp. K82, tritiated leucine in the SV; B: Exp. K128, PhaL in the dorsomedial DV; C: Exp. K216, WGA-HRP in the MVpc. Scale bar for A - C represents 1 mm. Example of mossy fiber terminals labeled with different tracers from the experiments illustrated in A - C; D: tritiated leucine; E: PhaL; F: WGA-HRP. Scale bar for D - E represents 50 µ.

Injections mainly restricted to the MVc

In experiment C2098 (^{3}H-leucine) the injection involved the central part of the MVc and a small part of the PH (Fig.4.8). Experiment K108 (WGA-HRP) is restricted to the dorsal part of the MVc. The injection of experiment C2059 (^{3}H-leucine) involved the ventral part of the MVc as well as a part of the reticular formation (Fig. 4.8). The distribution of the mft in the caudal vermis showed little variation between the experiments, whereas the number of labeled mft was smaller after injection in the ventral part of the MVc (C2059) than after injection in the central and dorsal parts of the MVc (C2098, K108). Labeled mft were present bilaterally in all lobules of the caudal vermis (Exps. C2098, K108; Fig. 4.9). The labeling showed a clear preference for the lobules X and IXd. A moderate number of mft were found in the lobules IXa, b, c, and VIII, especially in the proximal parts.

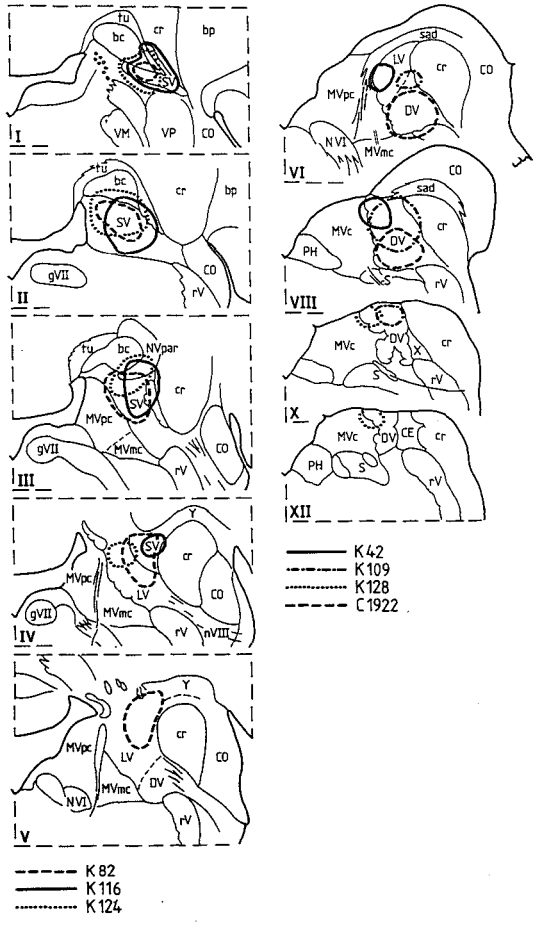


Fig 4.4 Injection sites in the SV (left column; Exps. K82, K116, K124) and the DV (right column; Exps. K42, K109, K128, C1922)

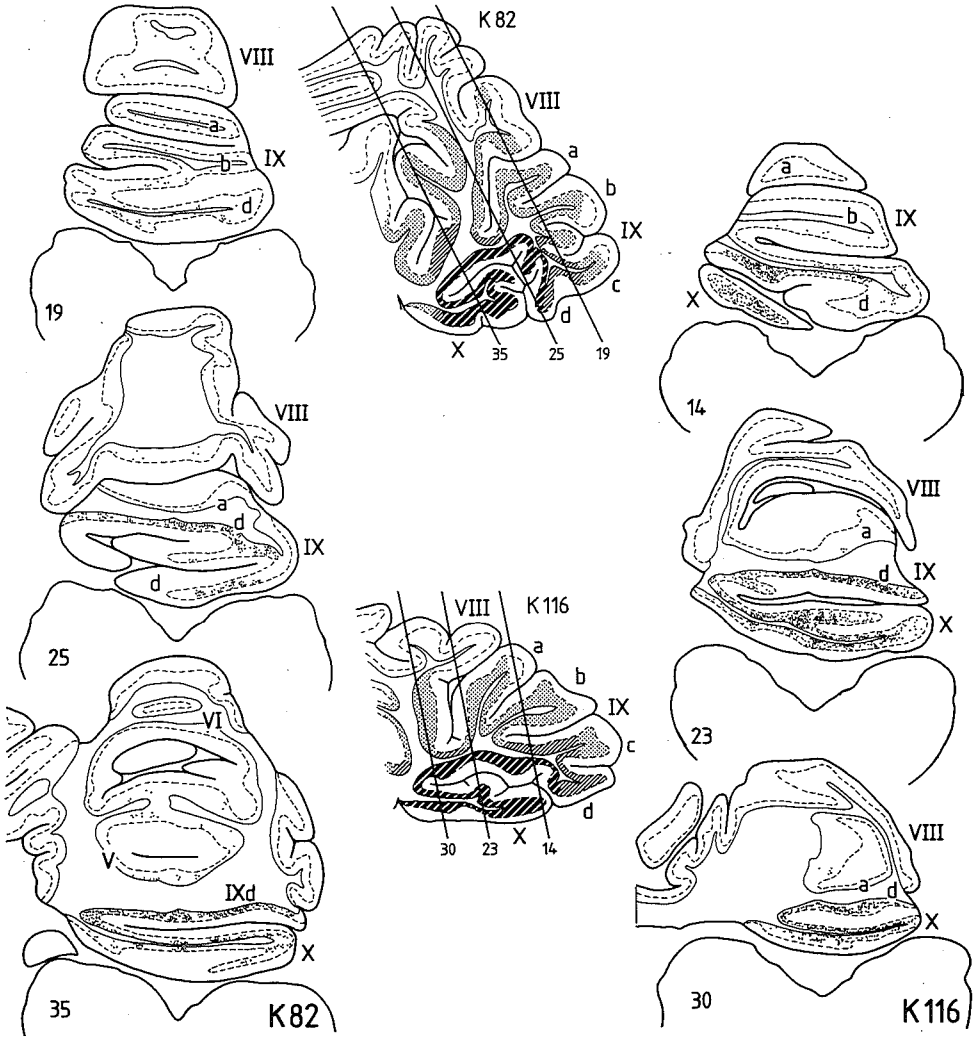


Fig 4.5 Distribution of anterogradely labeled mossy fiber terminals in transverse sections through the caudal vermis following injections in the SV (Exps. K82, K116). The extent and intensity of the termination is for each case projected onto the reconstructed midsagittal plane.

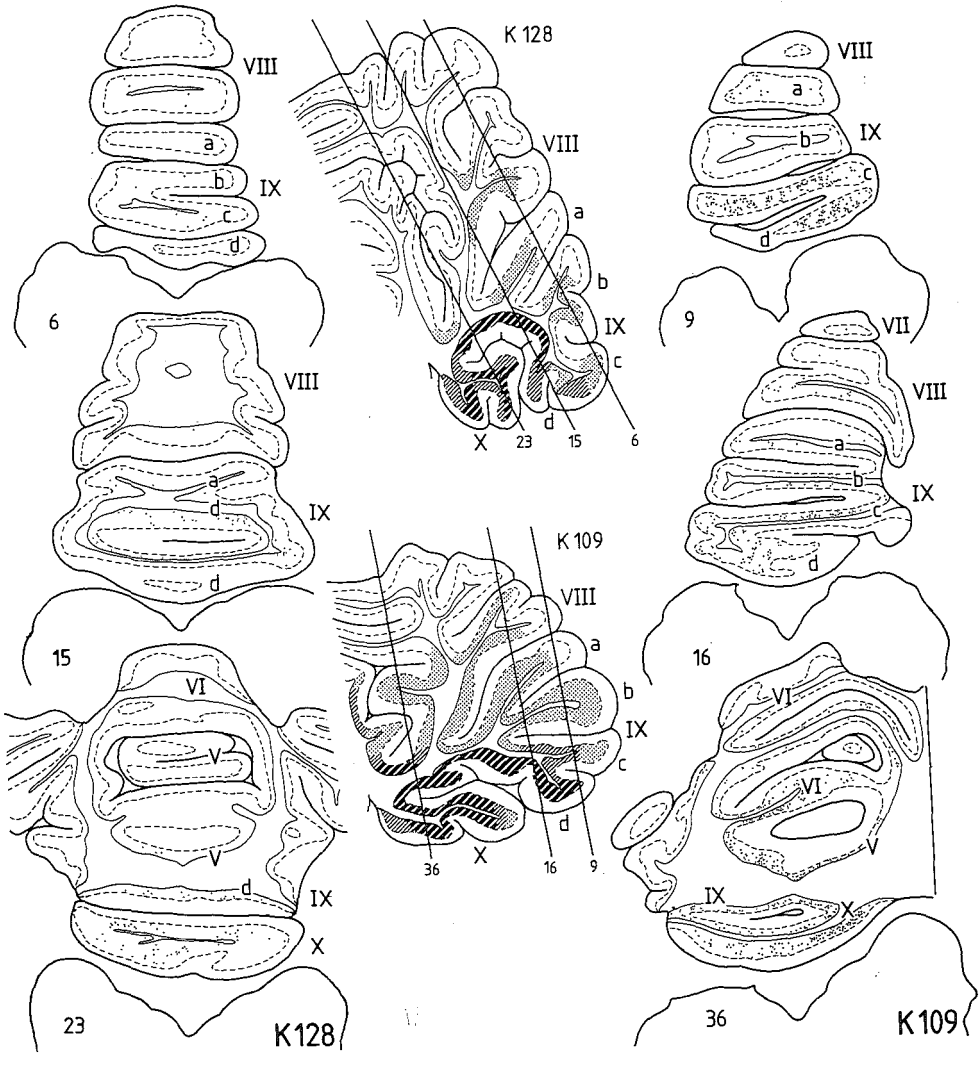


Fig 4.6 Distribution of anterogradely labeled mossy fiber terminals in transverse sections through the caudal vermis (Exps. K109, K128). The extent and intensity of the termination is for each case projected onto the reconstructed midsagittal plane.

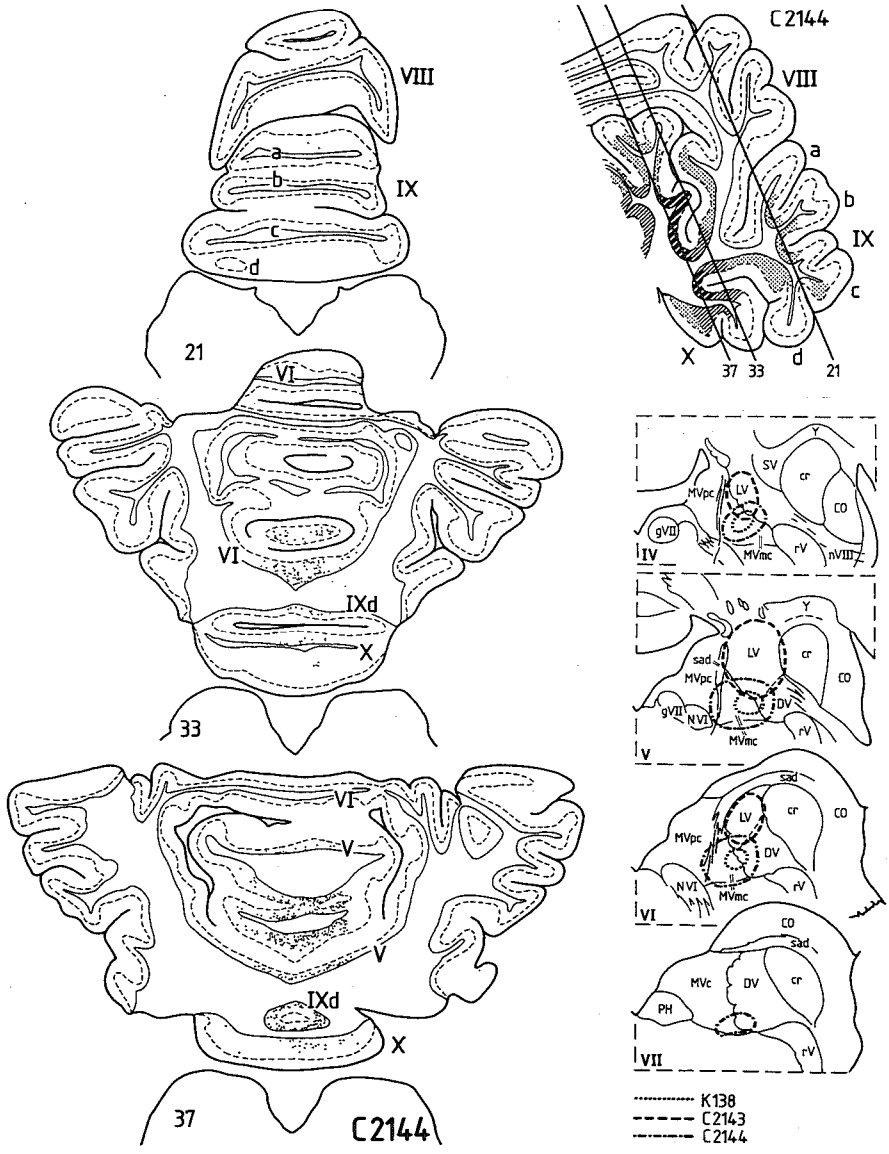


Fig 4.7 Injection sites in the LV (Exp. C2143) and MVmc (Exps. K138, C2144) and the distribution of anterogradely labeled mossy fiber terminals in transverse sections through the caudal vermis following injection in the MVmc (Exp. C2144). The extent and intensity of the termination is projected onto the

reconstructed midsagittal plane.
The mft were not equally dispersed but exhibited, in all experiments, a certain degree of clustering into longitudinal zones (Figs. 4.9, 4.10B, Exp. C2098). The most distinct clustering was found in the rostral folia of lobule IX and in lobule VIII, where the few labeled mft were arranged in three narrow strips, one in the midline and the others more laterally. This zonal arrangement was more distinct than after injections in the DV or SV.

Injections restricted to the MVpc

In the retrograde tracer experiments labeled neurons in the MVpc were most numerous after injections in the depth of the posterolateral fissure. In experiment K127 a very small injection with PhaL was placed in the MVpc (Fig. 4.11). This experiment resulted in only a few labeled terminals in the caudal vermis, concentrated in the bank of the posterolateral fissure. Of the other experiments, K216 (WGA-HRP) was restricted to the MVpc, while K157 (WGA-HRP) involved the MVpc and a small part of the SV (Fig. 4.11). Labeled mft were present bilaterally in all the lobules of the caudal vermis (Fig. 4.11, Exp. K216). The majority of labeled mft were present in the transition area between nodular and uvular cortices. Compared with terminal fields of the SV, DV, and MVc the amount of mft in the lobules X and IXd was smaller. A certain degree of mft clustering into longitudinal zones existed, marked by an increase and decrease in their density.

Distribution of cuneocerebellar fibers in the caudal vermis

Different anterogradely transported tracers (^{3}H-leucine, WGA-HRP, PhaL) have been used to analyse the mossy fiber projection from the cuneate nuclei to the posterior lobe vermis. The injection in experiment K36 (^{3}H-leucine) and K134 (WGA-HRP) involved the nucleus cuneatus externus (CE) and internus (CI). The injection sites in experiment C2053 (^{3}H-leucine) and K137 (PhaL) were restricted to the CE. (Table 4.3, Fig. 4.12) In all experiments labeled mossy fiber terminals were found bilaterally in the lobules VII-IX. The lobules VIIIb and IXa were the main recipients of the cuneate mossy fibers (Fig. 4.12). In lobule IXd only a small amount of labeled terminals was found. Apart from a rather strong ipsilateral predominance the projection from the cuneate nuclei was characterized by a very pronounced symmetrical clustering of the mossy fiber terminals in longitudinal zones. Each hemivermis contained two well-separated broad strips of cuneate mft, while the midline remained free. Rostralward, into lobule VIII the strips deviated in a lateral direction (Fig 4.12).

Table 4.3 Listing of experiments with injections in the cuneate nuclei. Injected volumes in µl, survival time in days. WGA-HRP: 5% in 0.01 M phosphate buffer (pH 7.8); PhaL: 2.5% in 0.05 M Tris-buffered saline (pH 7.4); 3Hleu: 100 µCi/µl ^{3}H-leucine in saline.

exp	tracer	vol	surv	CE	CI
C 2053	^{3}H-leucine	0.3	7	++	
K 137	PhaL	*	7	++	
K 36	^{3}H-leu	0.3	7	++	++
K 97	^{3}H-leucine	0.3	4	++	++
K 101	^{3}H-leucine	0.3	4	++	++
K 134	WGA-HRP	0.3	3	++	++

Distribution of corticovestibular Purkinje cells in the caudal vermis

The corticovestibular projection was investigated in the experiments with injections of WGA-HRP. The experiments are listed in Table 4.2. In all experiments retrogradely labeled Purkinje cells were found ipsilateral to the injection site. In experiments with injections in the MVpc, labeled Purkinje cells were observed only in the cortex of lobule X (Exp. K216, Fig. 4.13).Following injections in the MVc labeled Purkinje cells were found in lobule X and in the ventral part of lobule IXd lining the posterolateral fissure (Exp. K108, Fig. 4.13, Exps. K107, K105 not illustrated). Injections in the DV (Exp. K109, Fig 4.13, Exp. C1922), MVmc (Exp. C2144), LV (Exp. C2143) and the SV (Exp. K116 Fig. 4.10) resulted all in labeled Purkinje cells in the lobules X, IX and VIII. In lobule X and the ventral part of IXd labeled Purkinje cells were present over the entire mediolateral extent of the cortex. Clear borders, that would indicate the presence of a parasagittal zonation in these lobules were not found, although in some cases parasagittal or/and oblique interruptions in the Purkinje cell labeling could be distinquished (Fig. 4.13, Exps. K216, K108). From the caudal part of lobule IXd into lobule VIII labeled Purkinje cells were clearly arranged in two parasagittal strips extending over the entire apicobasal extent of the folia. One strip next to the midline and a lateral strip leaving the most lateral part of the lobules free from label. The medial strip started to broaden and shifts in lateral direction from the dorsal aspect of lobule IXb and onwards into lobule VIII (Exp. K109, Figs. 4.13, 4.14).

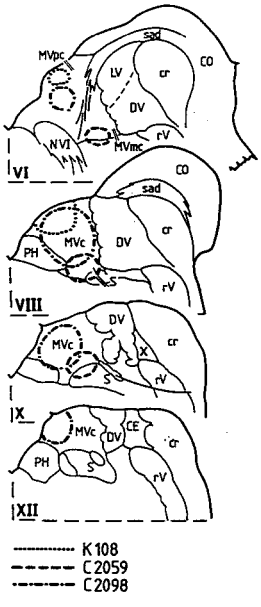


Fig 4.8 Injection sites in the MVc (Exps. K108, C2059, C2098).

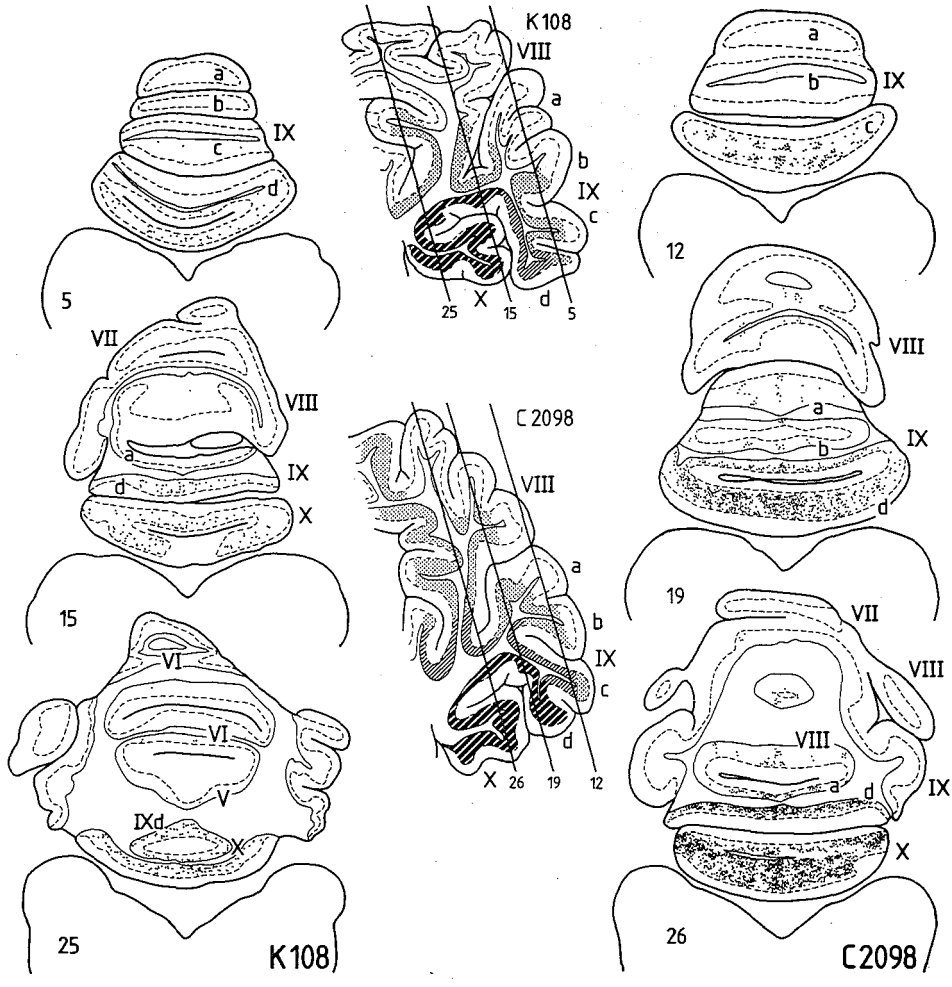


Fig 4.9 Distribution of anterogradely labeled mossy fiber terminals in transverse sections through the caudal vermis (Exps. K108, C2098). The extent and intensity of the termination is for each case projected onto the reconstructed midsagittal plane.

In two rabbits double labeling experiments have been conducted to label simultaneously the corticovestibular Purkinje cells and the cuneate mossy fibers. In these animals WGA-HRP was injected into the vestibular nuclei and tritiated leucine into the cuneate nuclei. In experiment K97 and K101 the injection with WGA-HRP involved the LV, MVmc and DV. In case K101 the SV was also included (Table 4.2). In both experiments the injections with 3H-leucine were restricted to the CE and the CI (Table 4.3). In these double labeling experiments a strong coincidence was found between the localization of strips of cuneate mft and corticovestibular Purkinje cells in the lobules VIII and IXa,b (Fig. 4.14).

4.3 DISCUSSION

Distribution of vestibular neurons projecting to the caudal vermis

The present experiments with HRP and WGA-HRP as a retrograde tracer have confirmed observations made in the cat (Gould, 1980; Kotchabhakdi and Walberg, 1978), the sheep (Saigal et al. 1982) the macaque (Brodal and Brodal, 1985), and the Galago (Rubertone and Haines, 1981), that the nodulus and uvula receive distinct bilateral projections, without clear differences in laterality, from all vestibular nuclei except the LV. The majority of labeled neurons were concentrated in the SV and in the MVc and DV.

In the rabbit, vestibulocerebellar neurons were not clearly restricted to the dorsal aspect of the DV as in the cat (Kotchabhakdi and Walberg, 1978b) or in Galago (Rubertone and Haines, 1981). Only few vestibular neurons were labeled when the injections were restricted to the dorsal folia of the uvula (lobules IXa-c). From the remarkable similarity in the distribution of retrogradely labeled vestibular neurons following widely separated injections, it can be concluded that a topological relation between individual nuclei and specific lobules of the caudal vermis is absent.

Distribution of secondary vestibular mossy fiber terminals in the caudal vermis

Most of the experimental data concerning the vestibulocerebellar projection have been obtained with retrograde degeneration or tracer methods (Brodal and Brodal, 1985; Gould, 1980; Kotchabhakdi and Walberg, 1978; Rubertone and Haines, 1981; Walberg and Dietrichs, 1988). Our experiments with antegrade tracing techniques are complementary to these studies and permit a precise localization of the vestibular mft in the caudal vermis, which confirms previous data in the cat (Magras and Voogd, 1985) and rabbit (Epema et al., 1985). In general, the results obtained with retrograde and anterograde tracing are in fair agreement. The largest mossy fiber input originated from the nuclei containing the largest number of retrogradely labeled vestibulocerebellar neurons: in descending order the MVc, SV, MVpc and DV. Labeled mft were most numerous in the lobules X and IXd. A small to moderate number of mft were found in lobules IXa, b, c, and VIII, where they showed a preference for the proximal parts of the lobules. The large vestibular projection to the lobules VI - VIII reported by Kotchabhakdi and Walberg (1978) could be explained if their HRP injection (B.St.L. 781) penetrated deeply into the central white matter, affecting the axons en route to lobules IXd and X.

There is a striking similarity in the qualitative distribution of mft over individual lobules following injections into different parts of the vestibular nuclei. Although in most cases the difference in the number of mft between the two areas in the caudal vermis (lobules X and IXd versus lobules IXa-c and VIII) were obvious, a sharp boundary was never found. The greatest change in density of mft usually occurred around the apex of lobule IXd and not in the depth of the posterolateral fissure.

Vestibulocerebellar mft were not equally distributed but exhibited a certain degree of clustering. The most distinct clustering was found in the rostral folia of lobule IX and in lobule VIII. However, the clustering in the caudal vermis did not show a continuity in longitudinal zones over successive sections or lobules.

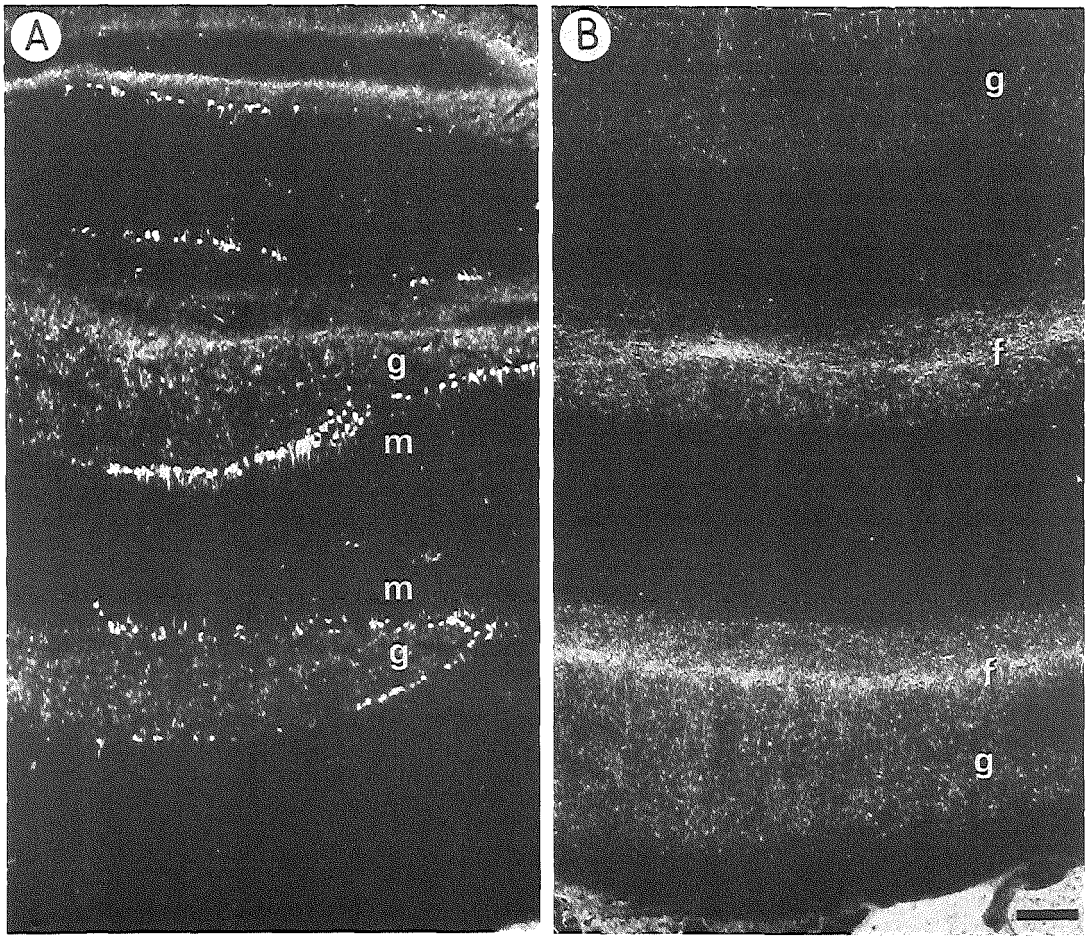


Fig 4.10 A: Retrogradely labeled Purkinje cells with their dendrites extending into the molecular layer (m), and anterogradely labeled mossy fiber terminals in the granular layer (g) of the lobules IX and X of the caudal vermis, following injection of WGA-HRP in the SV (Exp. K116, section 16).
B: Labeled mossy fibers in the fiber layer (f) and mossy fiber terminals in the granular layer (g) of the lobules IX and X of the posterior vermis following injection of tritiated leucine in the MVc (Exp. C2098, section 27).
Scale bars in A and B represent 0.4 mm.

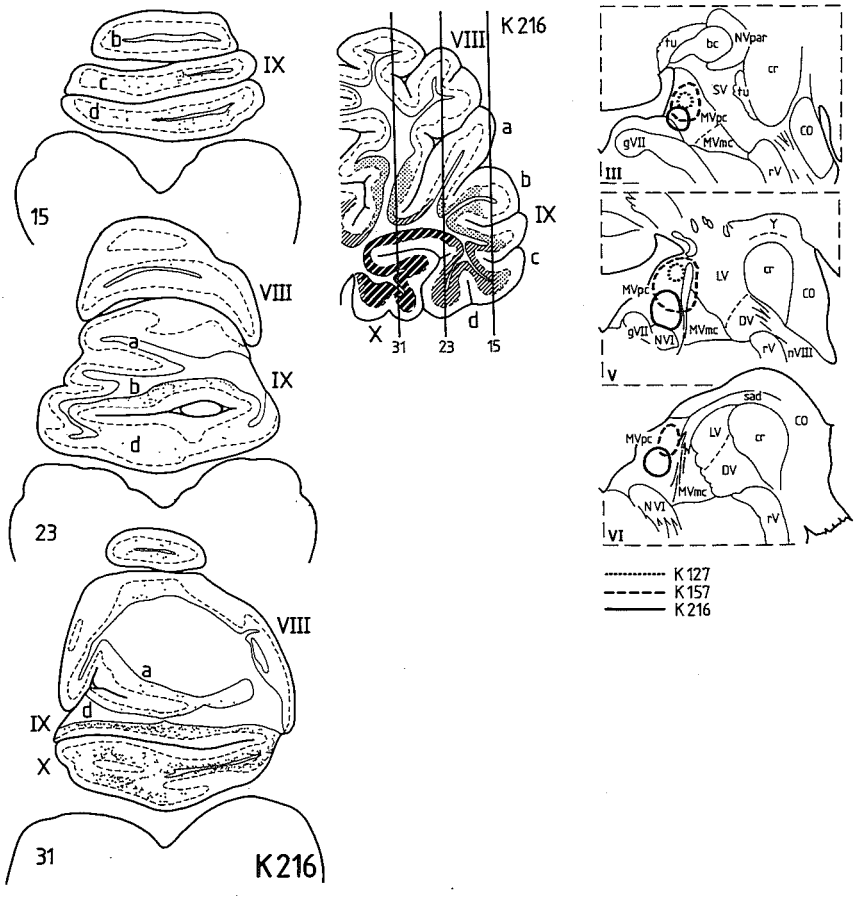


Fig 4.11 Injection sites in the MVpc (Exps. K127, K157, K216) and the consequent distribution of labeled mossy fiber terminals in transverse sections through the caudal vermis (Exp. K216). The extent and intensity of the termination is projected onto the reconstructed midsagittal plane.

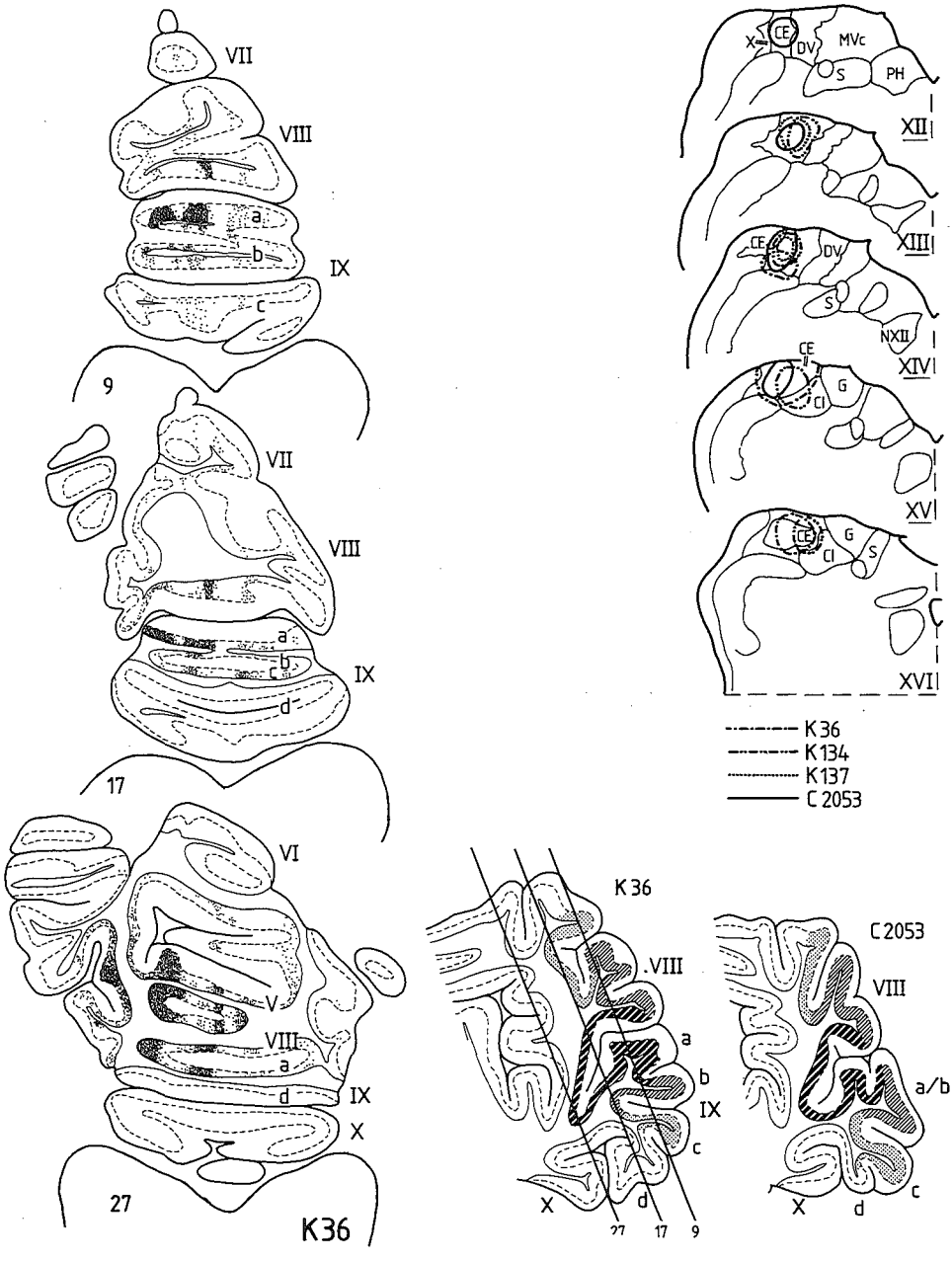


Fig 4.12 Injection sites in the cuneate nuclei (Exps. C2053, K36, K134, K137) and the consequent distribution of labeled mossy fiber terminals in transverse sections through the caudal vermis (Exp. K36). The extent and intensity of the termination is projected onto the reconstructed midsagittal plane (Exps. K36, C2053).

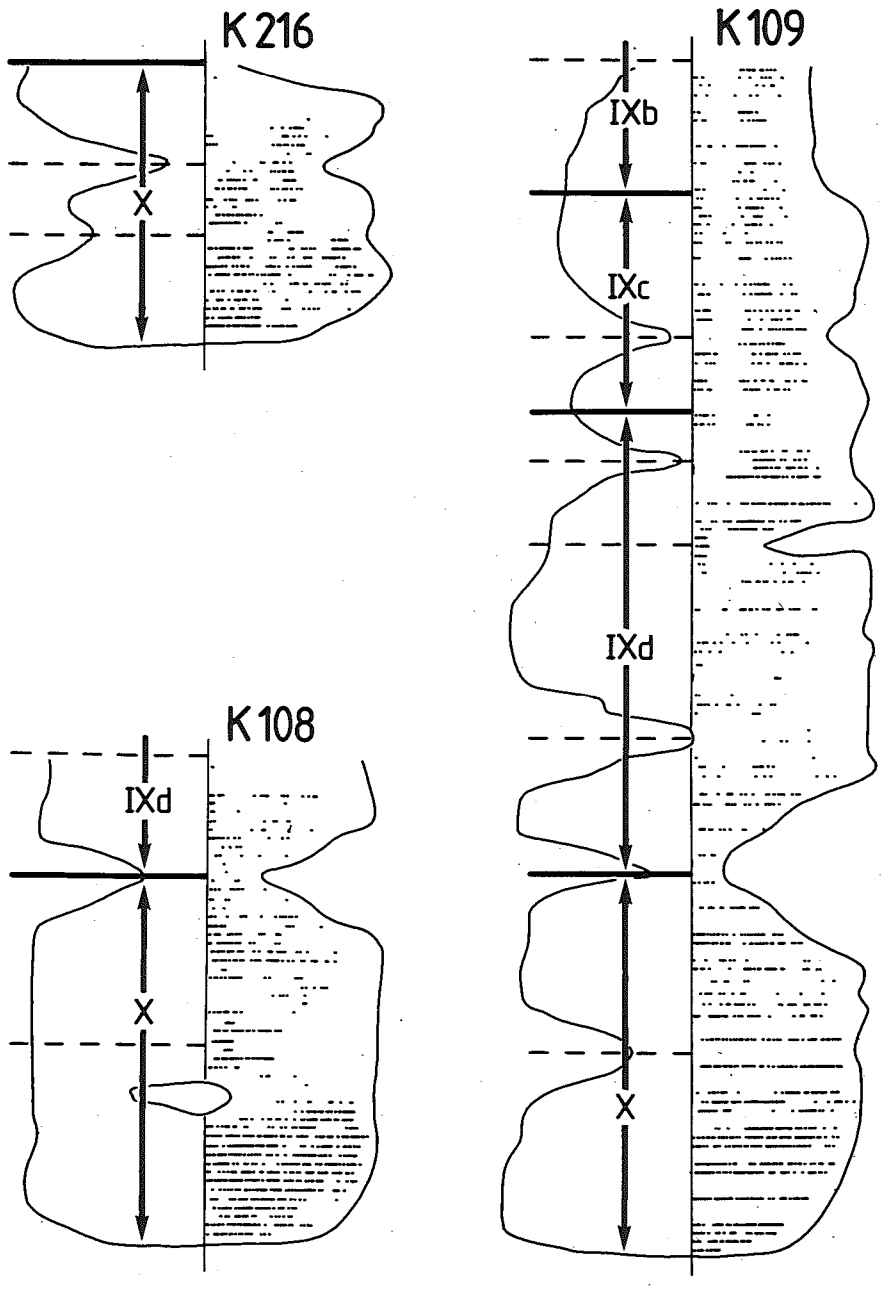
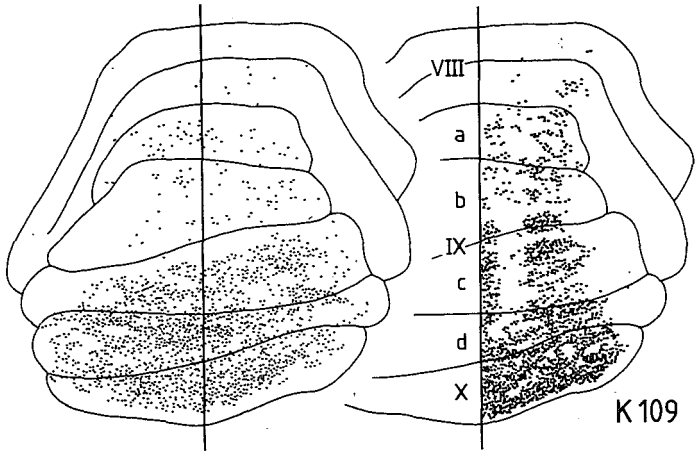


Fig 4.13 Distribution of labeled Purkinje cells in the nodulus and uvula depicted in the unfolded cerebellum (exps K216, K108, K109). Dotted lines represent the apices of the folia.



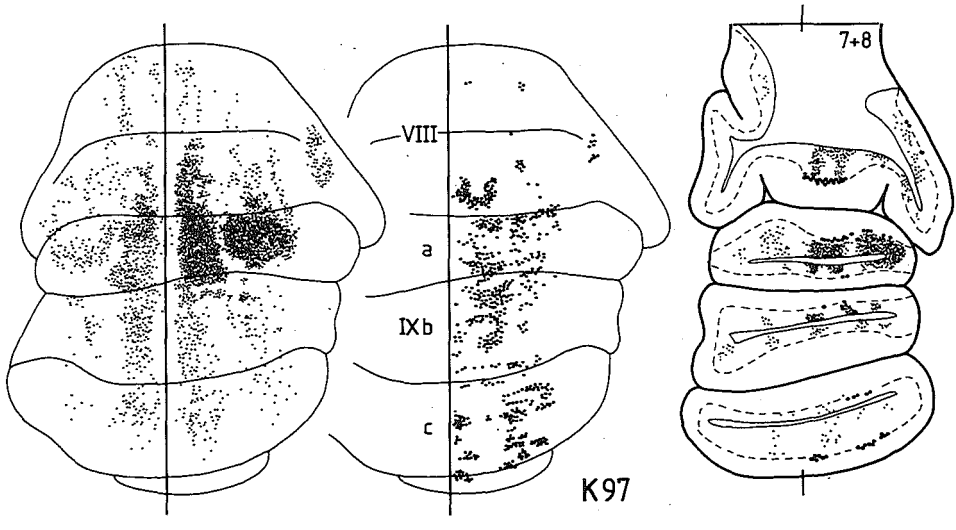


Fig 4.14 Distribution of mossy fiber terminals (left) and Purkinje cells (right), labeled following injection of WGA-HRP in the DV (see Fig 4.4; exp K109), projected onto the reconstructed surface of the caudal vermis (top). Distribution of cuneate mossy fibers and corticovestibular Purkinje cells, projected onto the reconstructed surface of the caudal vermis and as two overprojected successive sections. Mossy fibers labeled following injection with tritiated leucine in the CE, Purkinje cells following injection with WGA-HRP in the VN.

In the caudal vermis, the primary and secondary vestibulo-cerebellar mossy fibers share a common termination area in the lobules IXd and X (Carleton and Carpenter, 1984; Gerrits and Thunnissen, 1987; Gerrits et al., 1989; Kevetter and Perachio, 1986; Korte and Mugnaini, 1979). Both types of mossy fibers are present bilaterally but the primary terminals are absent from the lateral two-third of the cortex contralateral to the injected ganglion (Gerrits and Thunnissen, 1987; Gerrits et al., 1989). The vestibular root fibers pass through the LV and SV on their way to the cerebellum. Uptake of tracer in our WGA-HRP injections in these fibers cannot be excluded, but no obvious laterality differences were observed in a comparison with injections of ^{3}H-leucine (which is not taken up by fibers) in the same vestibular region.

In line with previous retrograde and anterograde tracer studies we have demonstrated that the cuneate mossy fiber terminals are present in a cortical region extending from lobule VIIb into lobule IX (Somana and Walberg, 1980; Grant, 1962; Rubertone and Haines, 1981; Sato et al., 1989; Gerrits et al., 1985; Gerrits and Thunnissen, 1987; Jasmin and Courville, 1987a,b). In addition Rubertone and Haines (1981) demonstrated a cuneocerebellar projection to the transition zone of the nodular and uvular cortices. Our findings, however, show, that the nodule and the ventral uvula (IXd) are not involved in the cuneocerebellar projection. The majority of cuneate and vestibular mossy fibers terminate in complementary areas in the caudal vermis and only coexist in a limited part of the uvula (Gerrits and Thunnissen, 1987). Evidence for this complementarity is also provided by the present study, since retrogradely labeled cells were found in the cuneate nuclei only after injections involving the lobules IXa-c.

The distribution of the cuneocerebellar mossy fiber terminals is characterized by a high degree of order, which is in strong contrast to the gradual changes in densities of the vestibular mossy fiber terminals. The cuneocerebellar mft are arranged in two longitudinal strips in each hemivermis. Apart from the previous observations of a sagittal organization of afferents from the cuneate nuclei (Voogd et al., 1969; Gerrits et al., 1985; Jasmin and Courville, 1987), a similar periodicity has also been described for the spinocerebellar system (Van Rossum, 1969; Voogd et al., 1969; Ekerot and Larsson, 1973, 1980; Matsushita and Tanami, 1984; Matsushita et al, 1984, 1985; Matsushita and Yaginuma, 1989) and reticulocerebellar fibers of the lateral reticular nucleus (Kunzle, 1975; Russchen et al., 1976) and in a less pronounced way for the projection of the nucleus reticularis tegmenti pontis (Gerrits and Voogd, 1978; Kawamura and Hashikawa, 1981).

Distribution of corticovestibular Purkinje cells and their relation to vestibulocerebellar mossy fibers

Our findings concerning the distribution of the retrogradely labeled Purkinje cells confirm earlier investigations which concluded that the presence of a parasagittal organization in lobule X and the ventral folia of lobule IXd must be questioned (Epema et al., 1985; Dietrichs et al., 1983). In addition our data support the existence of two longitudinal zones of Purkinje cells in the dorsal folia of of the uvula as has been described by Matsushita (1987) and Shojaku et al (1987) for the projection to the DV. Epema et al (1985) described three longitudinal zones of Purkinje cells after injections in the DV and MVc, which are most distinct in the dorsal folia of lobule IX. The discrepancy may be explained by assuming that they divided the medial strip, which widens in the dorsal folia of lobule IX, into two strips, which continue in lobule VIII.

The involvement of the LV in the corticovestibular projection from posterior lobe has been denied by a number of authors (Ito et al., 1982; Shjojaku, 1986; Voogd, 1964; Haines, 1979). The existence of a Purkinje cell projection to the LV is supported by the findings of Haines (1975) and Sreesai (1974) using anterograde degeneration. After lesions in the pyramis and the dorsal folia of lobule IX degenerating fibers could be followed to the vestibular complex where they terminate within the LV.

However, after lesioning lobule X (Haines 1979) and the ventral folia of lobule IX (Haines 1977), the LV contains few, if any, terminals only at its border with the SV, MVmc and DV. Thus, retrograde labeling of Purkinje cells in the lobules X and IXd following injections with WGA-HRP in the LV in the present study, might be attributed to uptake by passing fibers.

The present data, obtained with different anterogradely transported tracers, showed no evidence for a similar longitudinal organization of mft. Our experiments with WGA-HRP, which is transported in both anterograde and retrograde direction, permitted the simultaneous visualization of labeled secondary vestibular mft and Purkinje cells. Only in the lobules X and IXd a co-localization between labeled mft and labeled Purkinje cells was found. Implantation of WGA-HRP cristalls in lobule X (Walberg and Dietrichs, 1988) has revealed a high degree of overlap between the distribution of Purkinje cell terminal axons and labeled neurons in the vestibular nuclei of the cat, lending support to a reciprocal relation with this part of the cerebellum. More than half of the Purkinje cells that, could be labeled retrogradely with injections in the vestibular nuclei, were located in lobules VIII and IXa-c, where only a small number of vestibular mft was found and proprioceptive information from cervical axial and proximal forelimb muscles might be the dominant mossy fiber input relayed in the rostral external cuneate nucleus (Bakker et al., 1985). The precise matching of the cuneate mossy fiber strips with the Purkinje cells, projecting to the vestibular nuclei derives its significance from the observation of Bower and Woolston (1983) that granule cells preferentially modulate the simple spike activity of the Purkinje cells, which are located directly above them.

The present data support the view of Precht et al. (1979) that this part of the cerebellum is mainly concerned with the control of spino-vestibulo-spinal interactions. The targets of the Purkinje cells of the pyramis and dorsal uvula are the DV, MVmc and LV, which are characterized by a large proportion of premotoneurons of the vestibulospinal pathways (Kuypers, 1981; Kuypers and Maisky, 1975). In an electrophysiological study, Akaike and coworkers (1973) demonstrated that both LVST and MVST neurons could be inhibited by stimulation of the cortex of the pyramis and the dorsal uvula.

5 FLOCCULUS

5.1 INTRODUCTION

The flocculus is the last lobule of the folial chain of the hemisphere. It is usually included in the vestibulocerebellum, together with the nodule and the ventral part of the uvula. However, the flocculus lacks the primary vestibulocerebellar projection from the vestibular nerve, which terminates profusely in the lobules X and IXd (Gerrits et al., 1989) and which often was considered as the main criterion to delimit the vestibulocerebellum (see Larsell and Jansen 1972, for a review). Both the output of the flocculus and the lobules X and IX of the caudal vermis is directed at the vestibular nuclei (Dow, 1936; Voogd,1964; Angaut and Brodal, 1967; Haines, 1977; see Epema, 1990 for a review). The presence of Purkinje cells with projections to the vestibular nuclei, therefore, may offer a more reliable criterion to distinguish the vestibulocerebellum. In the hemisphere, however, Purkinje cells with projections to the vestibular complex were found to be present both in the flocculus and the adjoining cortex of the ventral paraflocculus in the rabbit (Yamamoto and Shimoyama, 1977) and in the cat (Bigaré, 1980). Similarly the climbing fibers from the dorsal cap and the ventrolateral outgrowth of the inferior olive, which supposedly are confined to the vestibulocerebellum (see Brodal and Kawamura, 1980, for a review) extend into the ventral paraflocculus in the cat (i.e. the medial extension of the ventral paraflocculus (ME) of Gerrits and Voogd, 1982).

The secondary vestibulocerebellar projection has been studied repeatedly with retrograde and anterograde tracing techniques. Early studies using retrograde cellular degeneration have described the origin of the secondary vestibulo-floccular projection in the medial (MV) and the descending (DV) vestibular nucleus. Absence of retrograde changes was reported for the lateral vestibular nucleus (LV) (Brodal and Torvik, 1957; Carpenter, 1960) and the superior vestibular nucleus (SV) (Brodal and Torvik, 1957). According to more recent studies, using retrograde transport of HRP, the SV also contributes to this projection (Langer et al.,1985; Brodal and Brodal, 1985; Rubertone and Haines, 1981; Kotchabhakdi and Walberg, 1978b; Sato, 1983; Blanks et al., 1983; Epema et al., in press; Yamamoto et al., 1979). In these studies labeled neurons were found throughout all vestibular nuclei on both sides, with a preference for the MV and SV. Anterograde degeneration (Ingvar, 1918; Dow, 1936) and anterograde tracing studies (Magras and Voogd, 1985) have mentioned a mossy fiber projection to the flocculus with an origin in the DV, MV and SV. A systematic analysis of the distribution of the mft within the flocculus has never been made, although the anterograde tracer technique does have, in contrast to the retrograde HRP method, the necessary resolving power. The present study will extend the previous investigations with an evaluation of the mode of termination of the mft in the flocculus, employing anterograde axonal transport of WGA-HRP, tritiated leucine and Phal.

Several studies have shown that there is a conspicuous reciprocal connection between the vestibular complex and the cerebellar nodulus (Epema et al., 1985; Walberg and Dietrichs, 1988; Thunnissen et al. 1989): secondary vestibular mossy fibers terminate in the area that contains Purkinje cells which project to the same vestibular nucleus. In the Galago, Rubertone and Haines (1978) claimed the presence of a similar reciprocal connection between the VN and the flocculus. In the present study this reciprocity will be investigated in the experiments with injections of WGA-HRP in the VN.

5.2 RESULTS

Distribution of vestibular neurons projecting to the flocculus

In order to assess the relevance of the injection sites in the anterograde tracer experiments the distribution of vestibulo-cerebellar neurons was investigated by means of injections with HRP in the flocculus. The injection sites of the HRP experiments K26 and C562 involved the rostral flocculus and included folia f2, f3, and f4 (Fig. 5.1, modified from Epema, 1990).

In both cases labeled neurons were present bilaterally, throughout the vestibular complex, without clear differences in laterality (Fig. 5.1). In the LV, group x and the vestibular ganglion no labeled neurons could be found. In the SV, labeled cells were most abundant in the center and scarce in the periphery of the nucleus. There is no clear interruption between the areas with labeled neurons in the SV and the labeling in surrounding subnuclei, such as the NVpar, the interstitial nucleus of the eighth nerve (IN) and group y. A small amount of labeled cells was always found in the center and dorsal parts of the MVpc. Very few cells were observed in the MVmc. In the caudal aspect of the vestibular complex, labeled neurons are distributed throughout the rostrocaudal extent of the MVc and the DV. Rostrally the area with labeling seems continuous with that of the MVpc. In the MVc most labeled cells are present in the central part of the nucleus. Most labeled neurons in the DV were located laterally in the nucleus and clearly separated from the labeling in the MVc. The majority of labeled neurons in the PH is present ventrally in its caudal half (Fig. 5.1)

TABLE 5.1 Listing of experiments with injections in different vestibular nuclei. Injected volumes in µl, survival time in days. HRP: 33% horseradish peroxidase in distilled water; WGA-HRP: 5% wheatgerm agglutinated horseradish peroxidase in 0.01 M phosphate buffer, pH 7.8; PhaL: 2.5% in 0.05 M Tris-buffered saline, pH 7.4, delivered iontophoretically; 3Hleu: 100 µCi/µl tritiated leucine in saline.

exp	tracer	vol	surv	Y	SV	LV	DV	MVc	MVpc	MVmc
K 116	WGA-HRP	0.3	4		++					
K 124	WGA-HRP	0.3	4		++	+				
K 82	^{3}H-leucine	0.3	4	+	++	+				
K 68	^{3}H-leucine	0.3	4	+	++	+				
C 2143	WGA-HRP	0.3	3			++	+			
K 42	^{3}H-leucine	0.3	4			+	++	+		
K 128	PhaL	*	7				++			
K 109	WGA-HRP	0.3	4			+	++			
C 1922	WGA-HRP	0.3	3				++			
C 2059	^{3}H-leucine	0.3	7					++		
C 2098	^{3}H-leucine	0.3	7					++		
K 105	WGA-HRP	0.3	4					++		
K 108	WGA-HRP	0.3	4					++		
C 496	HRP	0.3	3					++	++	
K 127	PhaL	*	7						++	
K 157	WGA-HRP	0.3	3		+				++	
K 216	WGA-HRP	0.3	3						++	
K 138	PhaL	*	7			+	+			++
C 2144	WGA-HRP	0.3	3			+	+			++

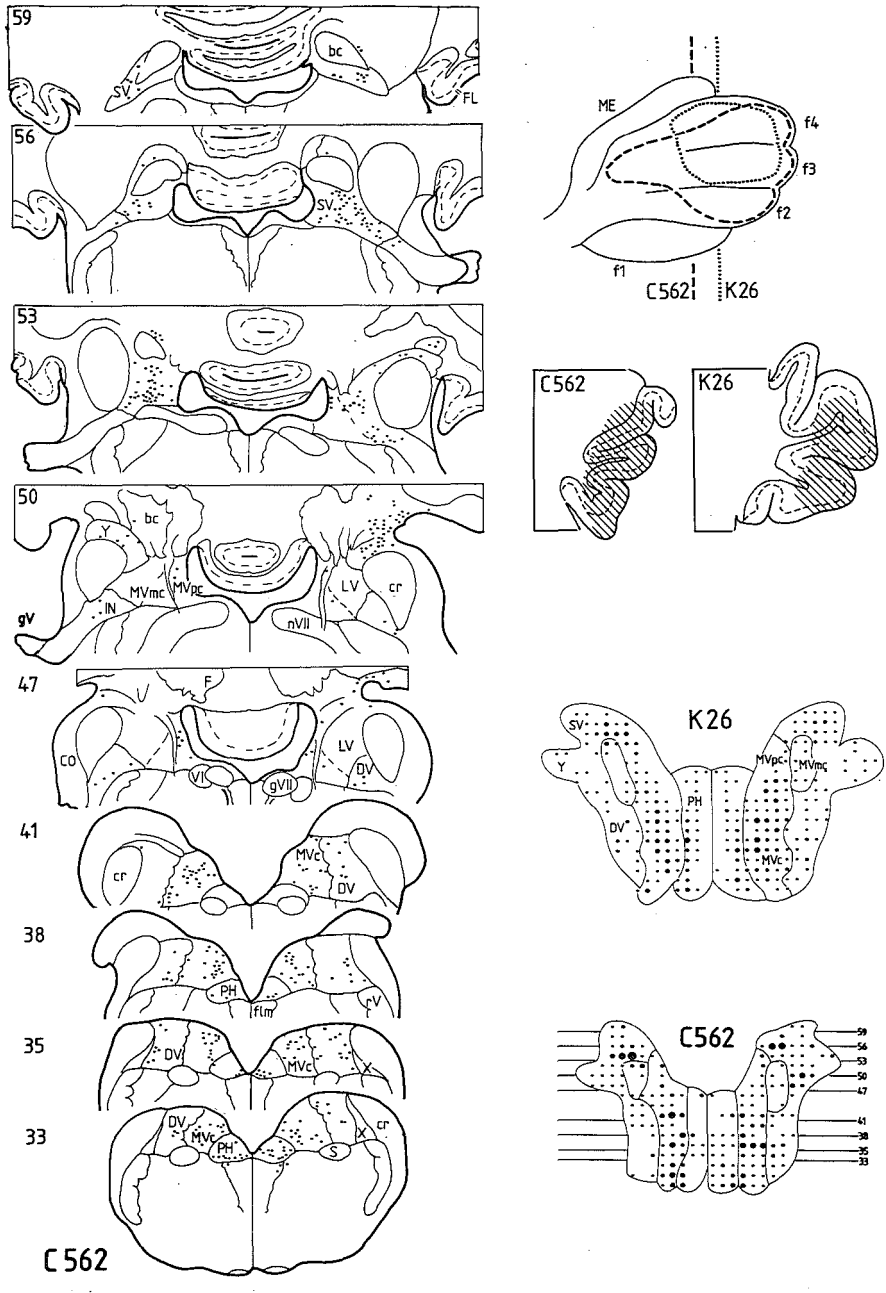


Fig . 5.1 Injections in the right flocculus. Extent of the injection sites Exps C562 (HRP) and K26 (HRP) in transverse sections through their largest extend and projected onto the floccular surface (according to Yamamoto 1979) (top right). Distribution of labeled neurons in Exp. C562 in transverse sections (left). Diagram of the vestibular nuclei showing the overall distribution of labeled neurons in Exps C562 and K26 (bottom right) (modified from Epema, 1990)

Distribution of vestibular mossy fiber terminals in the flocculus

The vestibulofloccular projection was investigated with anterograde transport of WGA-HRP, 3H-leucine and PhaL. Injections were made in all vestibular nuclei. The experiments are listed in Table 5.1.
In all experiments the labeled mft were distributed bilaterally throughout the rostrocaudal extent of the floccular cortex. The distribution of the terminals over the different folia is approximately equal. Terminal labeling in the medial extension of the ventral paraflocculus (ME) was found in all cases.The amount of labeled terminals in the flocculus varied with injection locus. The vestibulocerebellar mft were distributed irregulary over the floccular cortex, but no indication of a zonal organization was observed. The contribution of the different vestibular nuclei to the vestibulofloccular projection is illustrated in a semiquantitative way in Fig. 5.2. The largest number of labeled mft were found in cases with injections involving the SV and the MVc. The LV does not contribute to the vestibulofloccular projection. In case C2143 (WGA-HRP) with an injection almost completely restricted to the LV, only 3 mft were found in the ipsilateral flocculus.

Injections in SV

The largest number of labeled terminals was found in experiment K116 (WGA-HRP) in which the injection was restricted to the central SV (Table 5.2, Figs 5.2, 5.3). A smaller number of labeled mft was present in experiment K82 (^{3}H-leucine) with an injection in the caudal aspect of the central SV, group y and the rostral LV and in experiment K68 (^{3}H-leucine), in which the injection site comprised the lateral SV, group y and the rostral LV (table 5.2). Labeled terminals were most numerous in the rostral flocculus. A preference for the contralateral side was observed in case K116, while in case K68 a preference for the ipsilateral sidewas found (Table 5.2). The ME only contained labeled mft in its medial and rostral parts.

Injections in the MV

The largest amount of labeled mft in the flocculus and the adjacent ME were found in experiment C2098 with an injection in the central MVc (Table 5.2, Figs 5.2, 5.4). Smaller amounts of labeled mft were present following injections restricted to the dorsal (Exps. K108 WGA-HRP, K105 WGA-HRP) and ventral (Exp. C2059, ^{3}H-leucine) MVc, the MVmc (Exps. C2144 WGA-HRP, K138 PhaL) and the MVpc (Exps. K127 PhaL, K157 WGA-HRP, K216 WGA-HRP) (Table 5.2). Except for case K157, in which the labeled mft showed a clear preference for the contralateral side, no differences were observed in laterality. (Table 5.2). Labeled mft were present in all folia of the flocculus and extended into the ME, where they were quite numerous at rostral levels. They were absent from more caudal parts of the ventral paraflocculus.

Injections in the DV

The number of labeled terminals in the floccular complex resulting from injections in the DV is small but their distribution is similar to that of the MV injections. In cases with injections in the rostral (Exps. C1922 WGA-HRP, K42 ^{3}H-leucine) and central DV (Exp. K109 WGA-HRP) the labeled terminals were present bilaterally without a clear difference in laterality (Table 5.2, Figs 5.2, 5.5). In experiment K128 (PhaL) with an injection restricted to the dorsocaudal part of the DV, the labeled mft have a preference for the ipsilateral side.

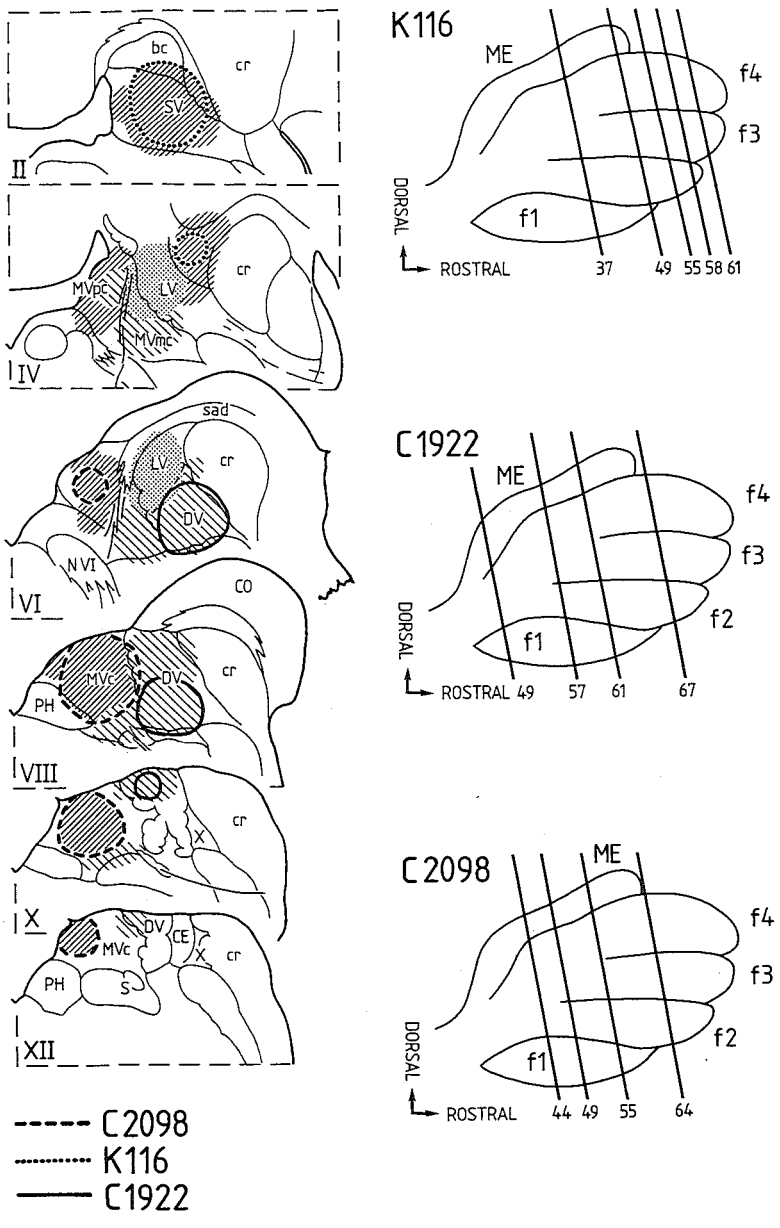


Fig 5.2 Diagram of transverse sections through the vestibular complex, showing the cumulative extent of the injection sites in the vestibular nuclei. The injection sites of experiments K116 (dotted line) and C2098 (broken line) and C1922 (solid line) are indicated individually. The contribution to the vestibulofloccular projection is illustrated in a semiquantitative way (stippled: no projection; hatching: moderate projection; dense hatching: dense projection). The position of the sections in the floccular complex illustrated in Fig. 5.3, Exp. K116; Fig. 5.4, Exp. C2098; Fig. 5.5, Exp. C1922.

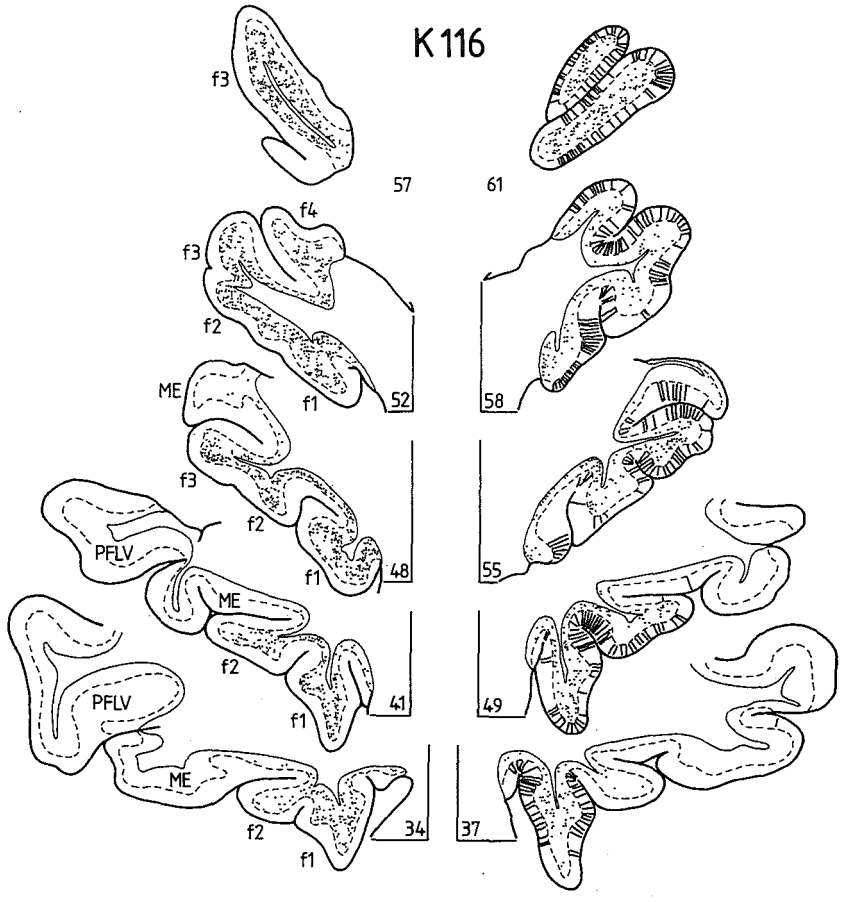


Fig 5.3 Distribution of anterogradely labeled mossy fiber terminals and retrogradely labeled Purkinje cells in transverse sections through the flocculus following injection in the right SV (Exp. K116).

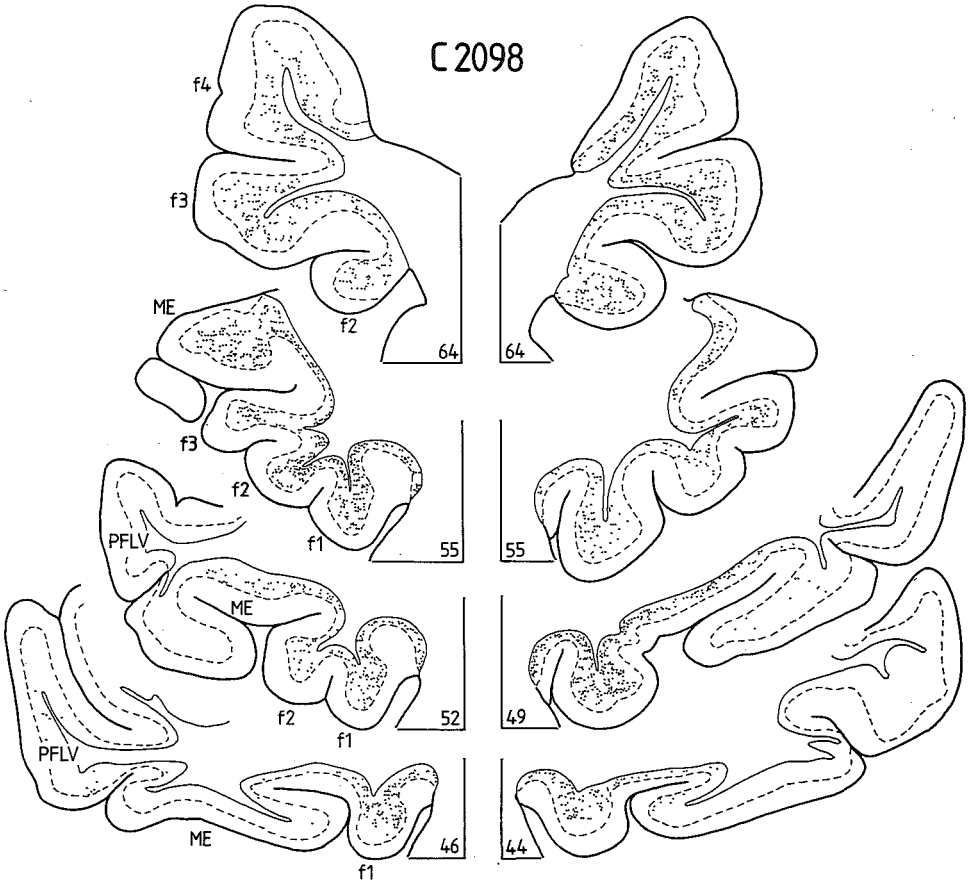


Fig 5.3 Distribution of anterogradely labeled mossy fiber terminals in transverse sections through the flocculus following injection in the right MVc (Exp. C2098).

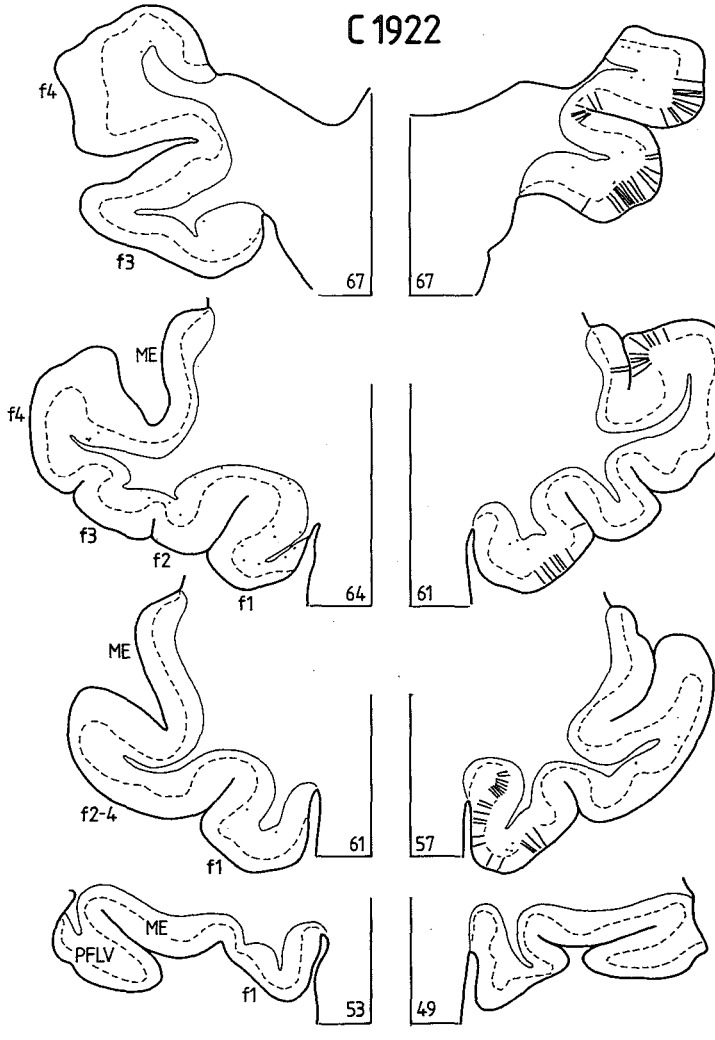


Fig 5.5 Distribution of anterogradely labeled mossy fiber terminals and retrogradely labeled Purkinje cells in transverse sections through the flocculus following injection in the right DV (Exp. C1922).

Table 5.2 Number of anterogradely labeled mossy fiber terminals following injection of WGA-HRP, 3H-leucine and Phal in the vestibular nuclei, and number of retrogradely labeled Purkinje cells following in the experiments with WGA-HRP.
contralateral/ipsilateral

		mossy fiber terminals		Purkinje cells	
site	exp	FL	ME	FL	ME
SV	K 116	1490/575	103/160	-/587	-/122
	K 82	203/225	68/42		
	K 68	235/759	75/159		
MVc	C 2098	1260/1214	474/459		
	C 105	55/47	11/6	-/102	-/4
	C 2059	30/41	9/10		
	K 108	106/114	31/27		
MVpc	K 157	832/465	117/107	-/639	-/169
	K 127	3/8	0/0		
	K 216	301/286	42/37		
MVmc	C 2144	29/42	3/6	-/211	-/81
	K 138	0/9	0/5		
DV	C 1922	40/40	5/6	-/127	-/19
	K 42	95/85	29/31		
	K 109	47/39	13/10	-/99	-/7
	K 128	17/66	7/18		

Distribution of corticovestibular Purkinje cells

In all WGA-HRP experiments retrogradely labeled Purkinje cells were found in the ipsilateral flocculus and in the ME. The largest number of labeled Purkinje cells were found in cases with injections involving the SV. In individual sections distinct sets of labeled Purkinje cells alternate with unlabeled areas. Single Purkinje cells were observed throughout the floccular cortex (Exp. K116, Fig. 5.3; Exp. C1922, Fig. 5.5). The localization of the labeled Purkinje cells is best visualized when projected upon the reconstructed surface of the flocculus. Following injections in the SV (Exp. K116, Table 5.1, Fig 5.6A) labeled Purkinje cells were organized in at least two but probably three zones, a ventral zone and a dorsal zone. The dorsal zone seems to be further subdivided in two zones. These zones exhibited a oblique orientation with respect to the longitudinal axis of the folia, and shift in mediolateral direction from caudal to rostral. Both zones run over all folia of the flocculus, the dorsal zone continues in the cortex of the ME.
Following injections in the MV or DV (Exp C496, Fig. 5.6, Table 5.1, injection site not illustrated) labeled Purkinje cells were distributed in two longitudinal zones. One runs slightly oblique over all folia of the cortex and continues in the ME. The other one is present in the ME and the medial aspect of the caudal end of folium f3. Folium f4 is lacking in this animal.

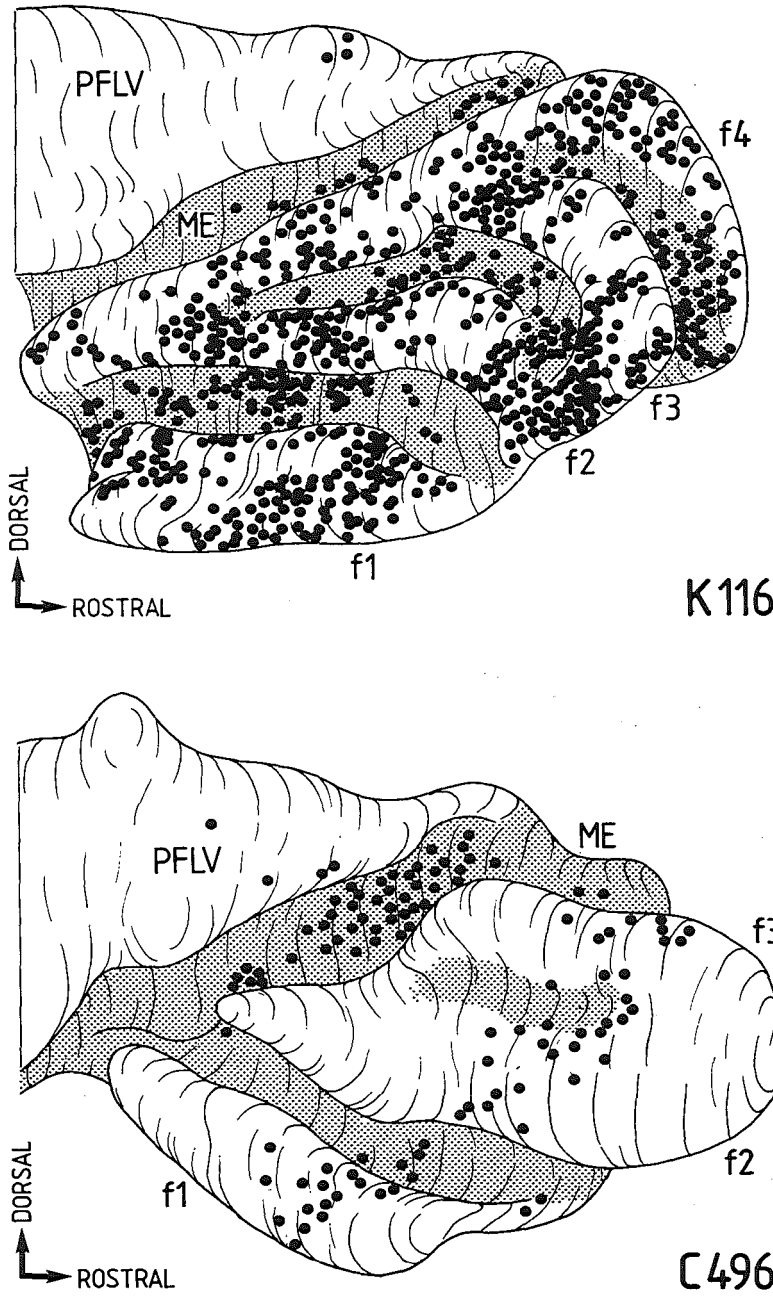


Fig 5.6 Distribution of retrogradely labeled Purkinje cells as seen through the reconstructed surface of the flocculus A: Following injections in the SV (Exp. K116). B: Following injection in the MVc and the caudal MVpc (Exp. C496)

5.3 DISCUSSION

Distribution of vestibular neurons projecting to the flocculus

The origin of the secondary vestibular projection to the flocculus has been determined in a number of studies in various mammals using HRP as a retrograde tracer. In general, the retrograde tracer results in the rabbit (from Epema, 1990) are similar to the data obtained in the rat (Blanks et al. 1983), the cat (Gould, 1980; Kotchabhakdi and Walberg, 1978b Sato et al., 1983), the bushbaby (Rubertone and Haines, 1981) and the macaque (Brodal and Brodal, 1985; Langer et al., 1985). Labeled neurons are present bilaterally throughout all the subdivisions of the vestibular nuclei, except for the LV. The neurons are most numerous in the MVc, the SV and the PH Labeled neurons showed a tendency to cluster, although not as strongly as demonstrated by Yamamoto (1979).

Distribution of secondary vestibular mossy fiber terminals in the flocculus

Retrograde and anterograde tracer studies (Yamamoto, 1979; Epema et al., 1990; Brodal, 1985; Kotchabhakdi and Walberg, 1978b; Langer et al., 1985; Blanks, 1983; Rubertone and Haines, 1981; Magras and Voogd, 1985) have shown that the vestibular nuclei project to the flocculus, but these studies did not give information about the precise distribution of the vestibular mft. The present findings, obtained with different anterograde tracing techniques are generally consistent with the previous observations. The vestibular afferents arose from each of the vestibular nuclei bilaterally, but most heavily from the SV and MVc. Quantitative differences in laterality were found for the projection of the SV, but these were not consistent. Similarly, Tan and Gerrits (in press) have demonstrated with retrograde tracing from the flocculus preferences in laterality which were not statistically significant when tested.

There is a striking similarity in the qualitative distribution of the mft over the individual folia following injections into different parts of the vestibular nuclei. We agree that the mossy fiber input of the SV shows a preference for the rostral flocculus (Epema et al., 1990; Kotchabhakdi and Walberg, 1978b; Gerrits, 1985). Secondary vestibular mft are also present in rostral and medial parts of the ME, but are absent from more caudal parts of the ventral paraflocculus. .

The vestibulocerebellar mft were distributed irregulary over the floccular cortex, but no indication of a zonal organization was observed. The absence of a zonal organization was also suggested earlier by Yamamoto (1979).

The LV does not contribute to the floccular projection. The apparent difference with reports claiming involvement of the LV in the vestibulocerebellar projection (Blanks et al., 1983; Sato et al., 1983; Langer et al., 1985; Kotchabhakdi and Walberg, 1978b) may be due to our more restricted definition of the LV borders and the re-introduction of the MVmc (Epema et al., 1989).

In most models that include the flocculus in the circuitry underlying the modification of the vestibulo-ocular reflex (VOR), it is assumed that the required vestibular input, related to head angular velocity, comes directly from the eighth nerve. Mossy fibers in the flocculus exhibit a variety of dicharge patterns (Lisberger and Fuchs, 1978; Miles and Lisberger, 1981), which resemble those recorded in the vestibular nuclei. In the rabbit Gerrits et al. (1989) have demonstrated that the flocculus does not receive vestibular nerve fibers. Therefore, the vestibular input in the flocculus must have its origin in the vestibular nuclei.

The main sources supplying mossy fibers to the flocculus are the VN (35% of the total number of labeled brainstem neurons), the nucleus prepositus hypoglossus (30%), the nucleus reticularis tegmenti pontis (NRTP) (15%) and the nucleus reticularis lateralis (10%) (Gerrits, 1985). Thus a large number of mossy fiber systems converge upon the flocculus. The nodule and the ventral uvula receive a much stronger vestibular input which includes

both primary and secondary vestibulocerebellar fibers. Mossy fibers from the NRTP are absent from these lobules, but projections from the nucleus prepositus hypoglossi have been described (see Belknap and McCrea, 1988 for review). Following injections with WGA-HRP in the nodulus and the ventral uvula, 85% of the retrogradely labeled cells in the brainstem were found in the vestibular nuclei (Thunnissen et al. 1990). Moreover, the nodulus and ventral uvula are in receipt of a dense direct eighth nerve input, which is absent in the flocculus. Our data support the conclusions, that vestibular neurons projecting to the nodulus outnumber those projecting to the flocculus in a ratio of approximately 2:1 (Epema et al., 1990; Tan and Gerrits, in press). These observations put some strain on the relative importance attributed to the vestibular input in the flocculus.

The absence of a zonal organization of mossy fibers in the flocculus has been reported earlier in the cat for vestibular (Gerrits, 1985) and NRTP afferents (Gerrits et al., 1984).

Distribution of corticovestibular Purkinje cells in the flocculus and their relation with vestibulocerebellar mossy fibers

Reports of floccular efferents based on experiments with anterograde degeneration (Angaut and Brodal, 1967; Voogd, 1964; Sreesai, 1974; Haines, 1977; Dow, 1936) and tracer (Langer, 1985) methods have established projections to the SV, MV, DV and group y. These studies do not provide evidence for a zonal organization in the floccular complex. With the HRP method retrogradely labeled corticovestibular Purkinje cells were found in the FL and the ME. Yamamoto and Shimoyama (1977) identified four longitudinal zones in the flocculus on the basis of differential efferent projections with two Purkinje cell strips projecting to the SV. This organization later has been confirmed, again in the rabbit (Balaban, 1984) and also in the monkey (Balaban et al., 1981) and the cat (Bigaré, 1980). A subdivision of the white matter of the flocculus into compartments, which may correspond to a longitudinal zonation of the cortex, was discovered using acetylcholinesterase in the monkey (Hess and Voogd, 1986) and the rabbit (Tan et al., 1989).

The present data support such projection from the flocculus to the SV and the MV and DV. The ventral MV zone seems to fit in the "empty" area between the two SV zones. But strictly organized cortical zones, as have been suggested in the previous flocculus studies were not found. A major problem in the analysis is that the zones in the flocculus have an oblique orientation with respect to the long-axis of the folia. In rostrocaudal direction the zones shift gradually medialward, and every few hundred microns the "apex" of the folia contains another zone. In addition, the individual variations in the floccular folia (as in most species) are so large, that different experiments cannot be compared. At present the results are difficult to interprete, without marking the compartments in the white matter in the flocculus and the zones in the cortex simultaneously.

The present data, obtained with different anterogradely transported tracers, showed no evidence for a similar longitudinal organization of the vestibulocerebellar mft. Our experiments with WGA-HRP, which is transported in both retrograde and anterograde direction, permitted the simultaneous visualization of labeled secondary vestibular mft and corticovestibular Purkinje cells. A 'strict' co-localization between the labeled mft and the labeled Purkinje cells, as has been observed for the nodulus and ventral uvula (Epema et al., 1985; Walberg and Dietrichs, 1988; Thunnissen et al., 1989), was not found in the flocculus.

Physiological investigations have provided evidence that floccular Purkinje cells can be divided into distinct subgroups (Dufossé et al., 1977, 1978; Maekawa and Takeda, 1976). Each of these subgroups establishes inhibitory contact with the VOR relay neurons which mediate a specific canal-ocular reflex. Whereas floccular climbing fibers, and to a lesser extent the flocculovestibular Purkinje cells are organized in longitudinal zones it seems paradoxical that the important vestibular input in the flocculus does not display such a clear topographical organization.This raises question as to how the vestibular information is used in maintaining the high specificity of the VOR.

6 VESTIBULO-OCULOMOTOR RELATIONS

INTRODUCTION

In antegrade degeneration and transport studies two main pathways were distinguished which carry the fibers from the vestibular nuclei to the oculomotor nuclei (OMN). Fibers from the SV generally were found to ascend in the (lateral wing of the), ipsilateral medial longitudinal fascicle (flm), whereas fibers from the lateral vestibular nucleus (i.e. the ventral part of the lateral vestibular nucleus of Brodal and Pompeiano 1957, corresponding to the MVmc from our nomenclature), and the medial and descending vestibular nuclei ascend predominantly in the contralateral flm (in the cat: Tarlov, 1970; Gacek 1971; Carleton and Carpenter 1983; Carpenter and Cowie 1985; the monkey: McMasters et al., 1968; Carleton and Carpenter 1983; the opossum: Henkel and Martin 1977).

According to the electrophysiological studies in the rabbit (Highstein and Ito, 1971; Highstein, 1971,1973a; Precht and Baker, 1972; Highstein and Reisine, 1979) the crossed ascending pathway in the flm is excitatory, whereas the ipsilateral component of the flm from the SV is an inhibitory path. This also holds for the vestibular projections to the abducens nucleus (AN), which consist of the uncrossed inhibitory and crossed excitatory components both arising from MV and DV (in the cat: Baker et al., 1969; Baker and Highstein, 1975; Uchino et al., 1982; Nakao et al., 1982; in the rabbit: Highstein, 1973b).

The connections of the semicircular canals with the extrinsic eye muscles are organized in such a way that each canal is connected with the two pairs of muscles which move the eyeballs in approximately the same plane as the same circular canal. The muscles, which are responsible for compensatory movement of the eyeball to rotation of the head receive an excitatory connection, the antagonists are inhibited from the same canal. In the most simple model the vestibulo-ocular reflex makes use of the crossed, excitatory and the uncrossed inhibitory components of the flm. This is possible because the axons of the superior rectus component of the IIIrd nerve and the innervation of the superior oblique by the IVth nerve both cross within the brain stem. Several anatomical and physiological observations on the vestibulo-ocular system, however, cannot be explained by this simple scheme.

Two additional pathways have been described which also differ in their functional properties. One is a crossed, excitatory connection of the group y and the dorsal SV with the OMN, which passes through the brachium conjunctivum (bc). Originally described in the rabbit (Highstein and Ito, 1971; Highstein, 1971,1973a; Ito et al., 1976a,b; Yamamoto et al., 1978; Highstein and Reisine, 1979), it also seems to exist in the cat (Gacek, 1971; Carpenter and Cowi, 1985; Hirai and Uchino, 1984) and the monkey (Carleton and Carpenter, 1983; Stanton, 1980). This pathway contains the axons of excitatory vestibulo-ocular relay cells of the anterior canal which terminate on OMN moto-neurons innervating the ipsilateral superior rectus and contralateral inferior oblique muscles. Excitatory anterior canal relay cells are also found as neurons in the MV and DV with axons passing in the contralateral flm (rabbit: Highstein, 1973a; cat: Uchino et al., 1981; Graf and Ezure 1986; Hirai and Uchino 1984).

A second additional pathway is known as the ascending tract of Deiters. It is located lateral in the flm and forms an uncrossed excitatory connection between the vestibular nuclei and the medial rectus subdivision of the OMN (rabbit: Highstein and Reisine 1979; cat: Gacek, 1971; Furaya and Markham, 1981; Carleton and Carpenter, 1983). A similar pathway is absent in the monkey. The disynaptic inhibitory pathway from the horizontal canal to the contralateral MR motoneurons, seems to be absent in the cat (Highstein and Reisine, 1979).

With antegrade tracing cells in the SV and in the MV and DV were found to project bilaterally to motoneurons in the trochlear nucleus (TN) and the OMN (cat: Tarlov, 1970 ; Carleton and Carpenter, 1983; Carpenter and Cowie, 1985; Gacek, 1971; monkey: McMasters et al., 1966; Carleton and Carpenter, 1983). Some of these "aberrant" connections (contralateral in the case of SV and ipsilateral in the case of MV and DV) can be

explained by the existence of vestibulo-ocular fibers in the brachium conjunctivum and the ascending tract of Deiters. Others, however, represent connections with other eye muscles than the prime movers of the eye in the plane of the semicircular canal. These additional motoneuronal connections, which cross in the level of the TN and the OMN, have been observed for the axons of intracellularly injected vestibulo-ocular neurons in the cat (Graf and Ezure, 1986; Graf et al., 1983; Furaya and Markham, 1981; McCrea et al., 1981). Several explanations have been advanced for these additional connections whith extra-ocular muscles. The most likely one seems to be that these muscles correct for the deviation between the planes of the semi-circular canal and the corresponding extra-ocular muscle pairs (Baker et al., 1982). Different combinations of extra-ocular muscles are needed to achieve a co-planar action in frontal eyed mammals, like the cat, and lateral eyed mammals like the rabbit. These additional muscles have been enumerated for each canal in a model study by Ezure and Graf (1984a,b) for the cat and the rabbit.

The vestibulo-ocular projection has been studied in many species with antegrade and retrograde methods; but no detailed analysis with antegrade tracers has been made in the rabbit, a species which has become increasingly important for the study of ocular motility. Projections from the OMN and TN to the VN have been demonstrated with retrograde axonal transport of WGA-HRP in the rabbit (Thunnissen and Gerrits, 1985) and the cat (Carpenter and Cowie, 1985; Spence and Saint-Cyr, 1988). However, neither the precise localization of these neurons within the TN and OMN nor their axonal termination within the VN have been described in sufficient detail.The present report examines the sites of origin and termination of these connections by means of different tracer techniques for retrograde and anterograde transport. They will be discussed in the light of our present knowledge of the vestibulo-ocular connections.

In addition to their projection to the extra-ocular motor nuclei, ascending vestibular axons also terminate in and around the interstitial nucleus of Cajal (INC), located rostrolaterally to the OMN. The INC presumably plays a role in the coordination of head and eye movements by virtue of its monosynaptic projection to both extra-ocular and spinal motoneurons (King et al., 1980). In degeneration studies the INC was first identified as source of afferents to the MV and DV (Pompeiano and Walberg, 1957, Mabuchi and Kusama, 1970, Henkel and Martin, 1977). Studies with retrograde transport of HRP have confirmed this projection and in addition showed a projection to the SV. (Magnin et al., 1983; Carpenter and Cowie, 1985; Carpenter and Carleton, 1983; Barmack et al., 1979; Thunnissen and Gerrits, 1985). In turn, the VN project to the INC bilaterally. The projection from the SV is predominantly ipsilateral, that from the MV and DV is mainly contralateral (Carleton and Carpenter, 1983; Carpenter and Cowie, 1985; Thunnissen and Gerrits, 1985; Gacek, 1971; Tarlov, 1970).

The subdivision of the IIIrd nucleus used in the present study is according to the one proposed for the rabbit by Murphy et al (1986). The boundaries between the different motoneurton groups in the figures in the present study are drawn as correctly as possible, purely on morphological criteria.

6.2 RESULTS

I VESTIBULO-OCULOMOTOR PROJECTIONS

Distribution of vestibular nucleus neurons projecting to the oculomotor region

The distribution of the vestibulo-oculomotor neurons was investigated by means of injections of WGA-HRP aimed at the OMN (Table 6.1). The TN was included in one experiment, the projections to the AN were not studied.

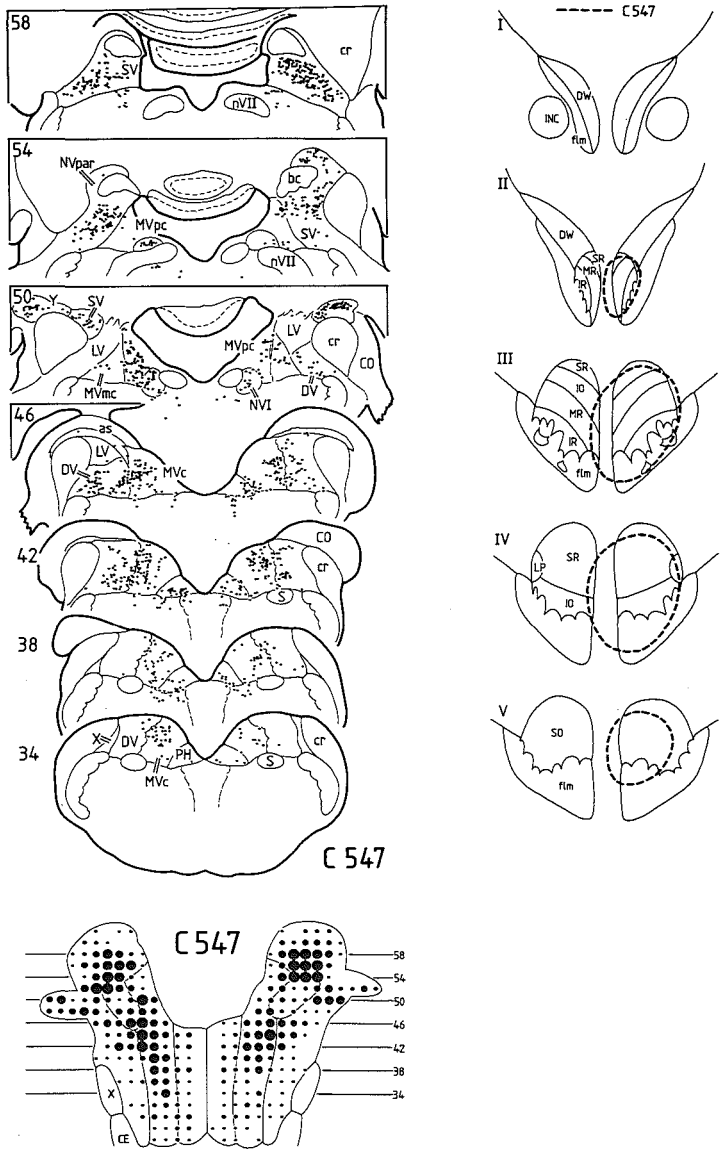


Fig 6.1 Experiment C547. Distribution of retrogradely labeled neurons in transverse sections through the vestibular nuclei (left) and in a horizontal diagram (bottom), following injection of WGA-HRP in the OMN (right).

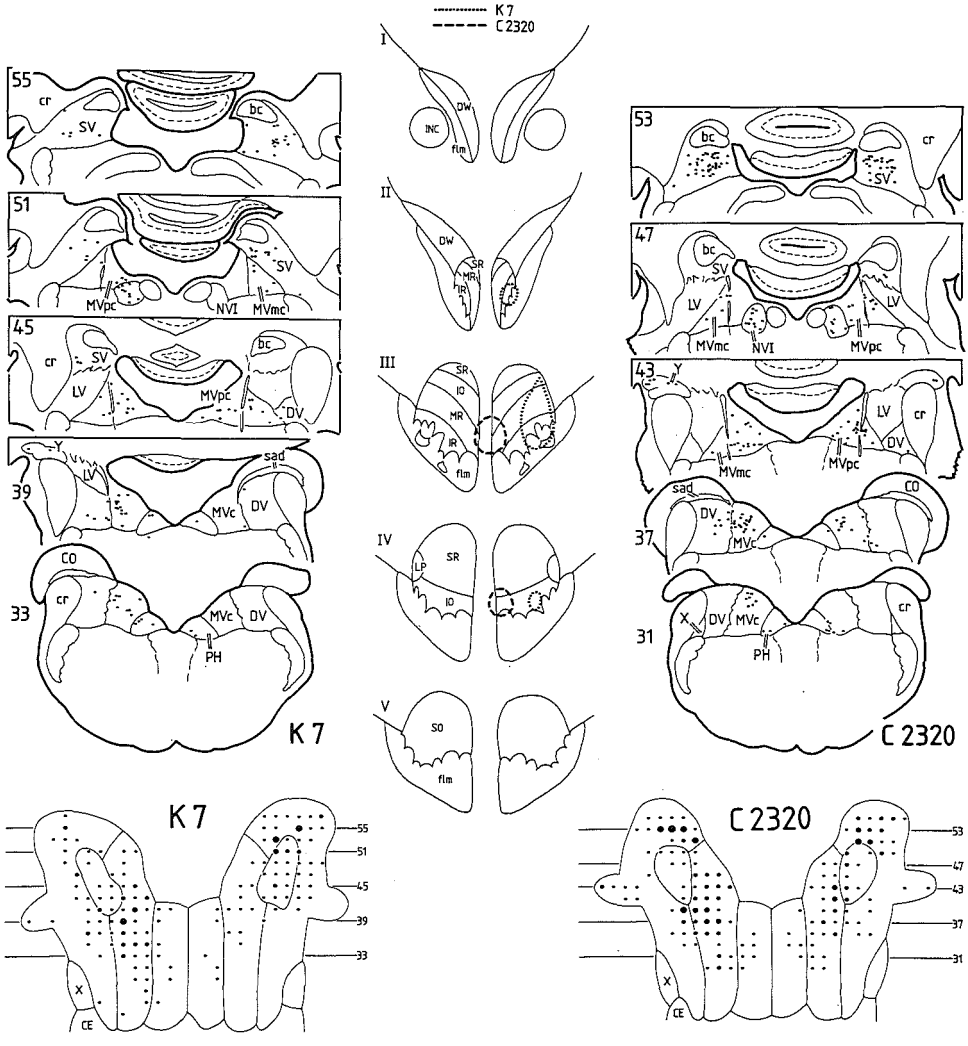


Fig 6.2 Experiments K7 and C2320. Distribution of retrogradely labeled neurons in transverse sections and horizontal diagrams of the vestibular nuclei, following injection of WGA-HRP in the OMN.

Table 6.1 Listing of experiments with injections in the oculomotor nuclei. Injected volumes in uμ, survival time in days. WGA-HRP: 5% in 0.01 M phosphate buffer (pH 7.8); PhaL: 2.5% in 0.05 M Tris-buffered saline (pH 7.4); WGA-HRP: 5% WGA-HRP + 100 μCi/μl ^{3}H-leucine in 0.01 M phosphate buffer (pH 7.8).

				TN	OMN			
exp	tracer	vol	surv	SO	SR	IO	MR	IR
C547	WGA-HRP	0.4	3	+	+	+	+	+
C2320	WGA-HRP	0.2	3		+	+	+	
K178	PhaL	*	7		+	+	+	
K7	WGA-HRP +^{3}H-leu	0.2	4			+	+	+

In experiment C547 the injection involved unilaterally the entire TN and OMN with a small inclusion of the medial aspect of the contralateral OMN (Fig 6.1).
The injection of experiment K7 included the lateral parts of the IO, MR and IR subdivisions of the OMN. In experiment C2320 the injection was restricted to the medial parts of the IO and MR subdivisions with a probable involvement of the SR cell group (Fig. 6.2).In experiment C547 labeled neurons were found throughout the VN, except for the LV. Contralateral to the injection site, the number of labeled neurons was 10% higher than ipsilaterally. Labeled neurons showed a preference for the central and medial parts of the SV, their number ipsilateral to the injection site was higher by 15%. A fair amount of labeling was found in group y. Labeling in the MV was concentrated in the rostral half of the caudal medial vestibular nucleus (MVc) and the adjacent caudal half of the parvocellular medial vestibular nucleus (MVpc), while a small number of neurons was present in the magnocellular medial vestibular nucleus (MVmc). Labeled cells were present throughout the DV, most abundant at rostral levels and in clear continuity with the labeling in the MVc. In the MV and DV the number of labeled neurons on the contralateral side was 22% higher than on the ipsilateral side. Throughout the rostrocaudal extent of the nucleus prepositus hypoglossi (PH), labeled cells were found with a preference for the periphery of the nucleus.

In experiments with small injections involving only part of the OMN subdivisions the total number of neurons was considerably smaller than in experiment C547, but their distribution seemed rather similar. In case C2320, where the injection was restricted to the medial parts of the IO and MR subdivisions unilaterally, labeled neurons were most abundantly in the central SV, the MVpc and the rostral half of the MVc (Fig 6.2). Contralateral to the injection the total number of labeled neurons was 35% higher than on the ipsilateral side.

In case K7 with an unilateral injection in the lateral parts of the MR and IR subdivisions, there is a clear difference in laterality within the VN (Fig 6.2). Ipsilateral to the injection the largest number of labeled neurons were found in the SV, contralaterally the labeled neurons were concentrated in the MV.

Distribution of vestibular axon termination in the oculomotor region

The termination of the vestibulo-oculomotor projection was investigated with anterograde transport of tritiated leucine, WGA-HRP and Phal. Injections were made in all subdivisions of the vestibular complex (Table 6.2). All experiments resulted in labeling of terminals in the AN, TN, OMN, and INC. A semi-quantitative estimation of the amount of terminals in the different motoneuron groups innervating the individual eye muscles is given in Table 6.3. The labeling always invaded areas neighbouring these nuclei such as the periaquaductal grey and the nucleus of Darkschewitsch (DW). In some experiments, most notably in case of PhaL injections, labeled fibers were observed crossing the midline at the level of the OMN. The distribution of terminals obtained with injections of different tracers showed a remarkable similarity. With the Phal technique it was possible to study the terminal arborizations in more detail than with the other techniques (Fig. 6.3).

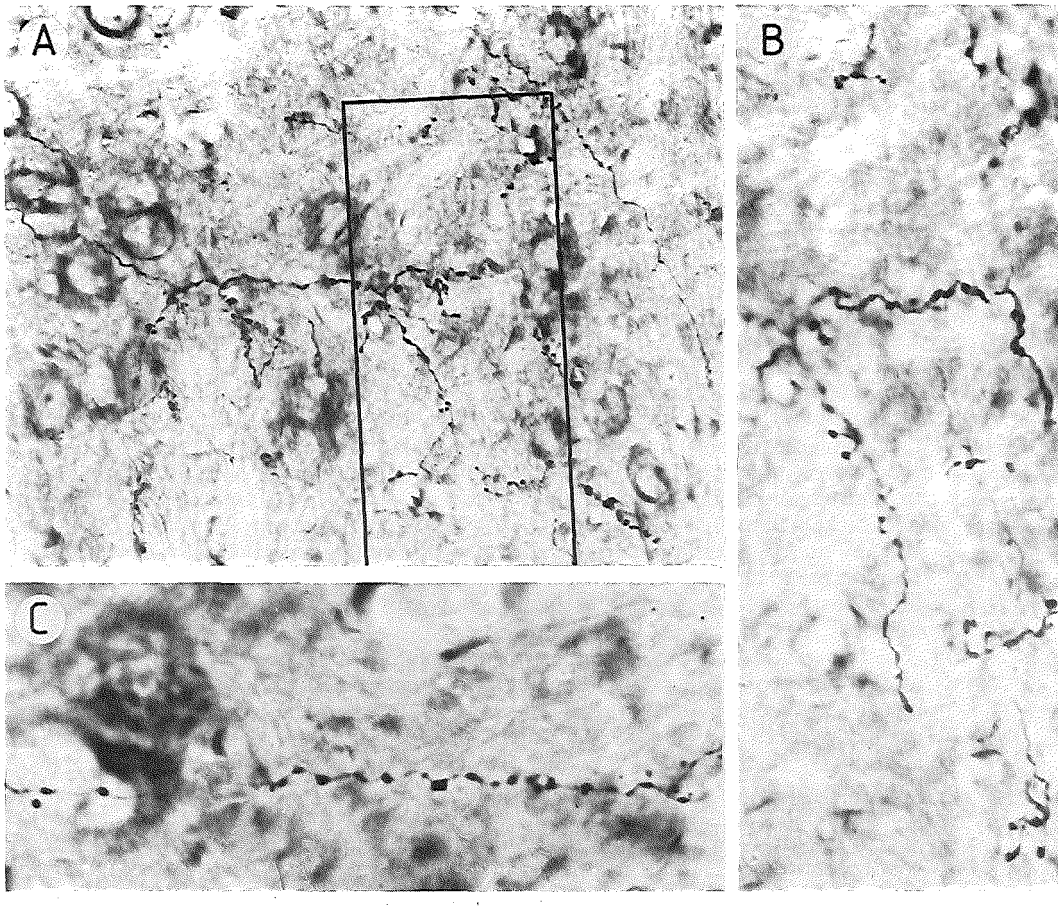


Fig 6.3 Labeled terminal arborization in the contralateral IO and MR motoneuron groups, following injection of PHAL in the MVmc (Exp. K138, section 90). B: High magnification of terminal arborization from the framed area. C: Labeled terminal axon with boutons in the IO motoneuron group following injection with PHAL in the MVpc (Exp. K127, section 62).

Table 6.2 Listing of experiments with injections in different vestibular nuclei. Injected volumes in µl, survival time in days. HRP: 33% horseradish peroxidase in distilled water; WGA-HRP: 5% in 0.01 M phosphate buffer (pH 7.8); PhaL: 2.5% in 0.05 M Tris-buffered saline (pH 7.4); ^{3}Hleu: 100 µCi/µl ^{3}H-leucine in saline.

exp	tracer	vol	surv	Y	SV	LV	DV	MVc	MVpc	MVmc
K 116	WGA-HRP	0.3	4		++					
K 124	WGA-HRP	0.3	4		++	+				
K 68	^{3}H-leucine	0.3	4	+	++	+				
K 82	^{3}H-leucine	0.3	4	+	++	+				
C 2143	WGA-HRP	0.3	3			++	+			
K 42	^{3}H-leucine	0.3	4			+	++	+		
K 128	PhaL	*	7				++			
K 109	WGA-HRP	0.3	4			+	++			
C 1922	WGA-HRP	0.3	3				++			
C 2059	^{3}H-leucine	0.3	7					++		
C 2098	^{3}H-leucine	0.3	7					++		
K 108	WGA-HRP	0.3	4					++		
K 127	PhaL	*	7						++	
K 157	WGA-HRP	0.3	3		+				++	
K 216	WGA-HRP	0.3	3						++	
K 138	PhaL	*	7			+	+			++
C 2144	WGA-HRP	0.3	3			+	+			++

Injections mainly restricted to the SV

In experiments K68 and K82 (^{3}H-leucine) the injections included the lateral (K68) and central (K82) SV, group y and the rostral LV. In experiments K116 and K124 (WGA-HRP) the injections were restricted to the central SV with only limited involvement of the LV (K124). In the cases K68 and K82 a small amount of labeled terminals were found in the AN on both sides. In the WGA-HRP injections K116 and K124, a high background labeling on the injected side prevented an accurate estimation of the amount of termination (Table 6.3). Labeled axons to the TN and OMN ascended through the ipsilateral medial longitudinal fascicle (flm) and the contralateral brachium conjunctivum (bc) (Fig6.5A Exp. K82). Within the flm labeled fibers were mainly present in the dorsal aspect of its lateral wing. From the level of the caudal OMN they leave the flm and proceed within the nucleus itself. Labeled fibers reach the brachium conjunctivum from ventrolaterally, cross in the tegmentum pontis and enter the OMN on the lateral side.Termination was observed in the TN, OMN, INC and DW with clear ipsilateral predominance. Terminal labeling in the OMN showed a strong preference for the ipsilateral IR and rostral MR subdivisions and the contralateral IO and SR subdivisions. This was most obvious when the injection was located in the lateral part of the SV (K68, Fig. 6.4, A). Here, labeling was almost absent in the other subdivisions. When the central SV was included in the injection, the ipsilateral IO, SR and caudal MR subdivisions were also clearly labeled (K116, Fig. 6.4).

Injections restricted to the DV

In experiment K42 (^{3}H-leucine) the injection included a small dorsorostral part of the DV, as well as a limited area in the adjacent MVc and LV (Fig. 6.6). Two WGA-HRP injections covered the ventrorostral DV (C1922) and its entire dorsal aspect (K109).

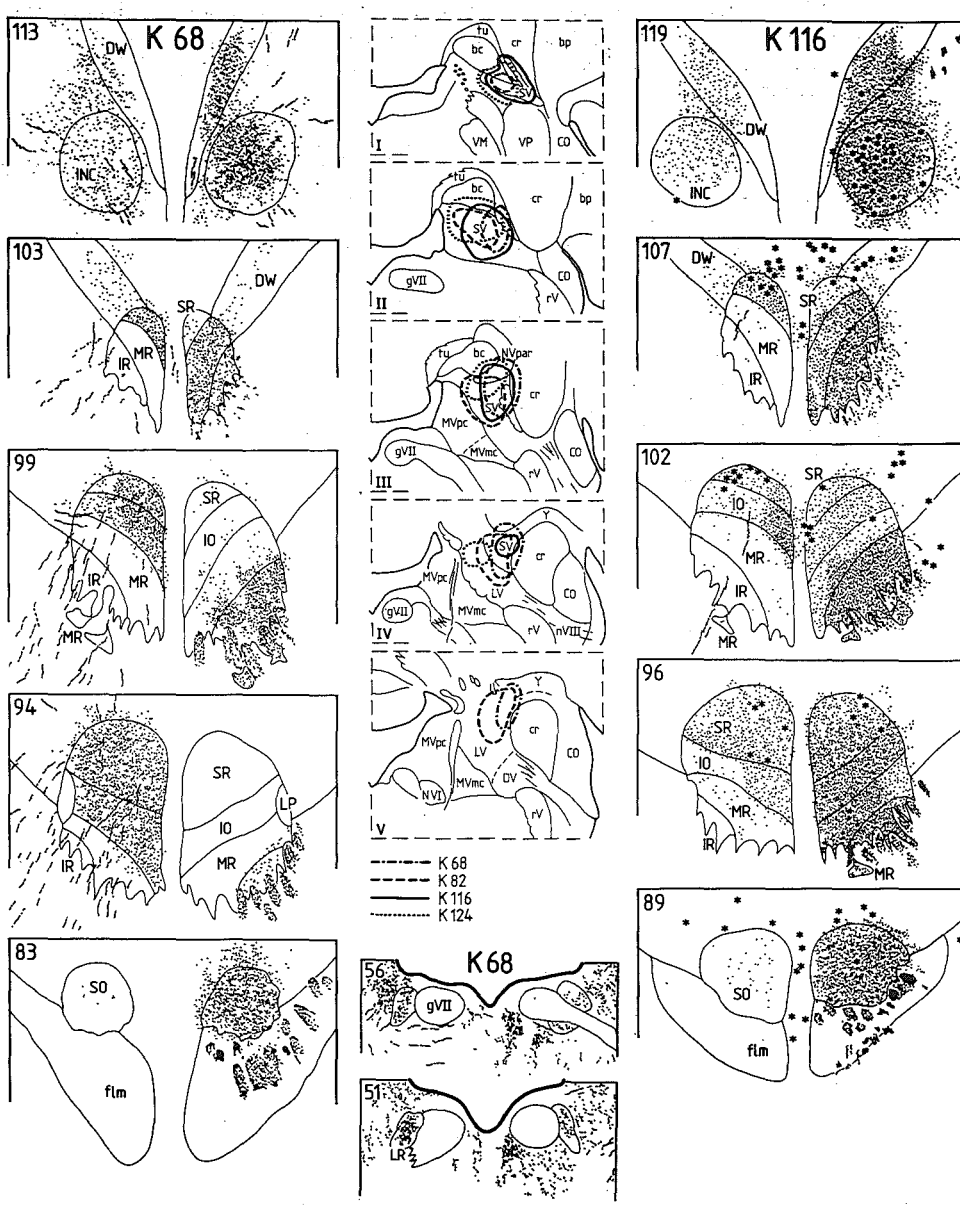


Fig 6.4 Injections mainly restricted to the SV (middle column top, Exps K68, ^{3}H-leu; K82, ^{3}H-leu; K116, WGA-HRP; K124, WGA-HRP) and the resulting distribution of anterogradely labeled terminals in the OMN and TN (left column, Exp. K68, right column, Exp. K116) and in the AN (middle column bottom, Exp. K68).

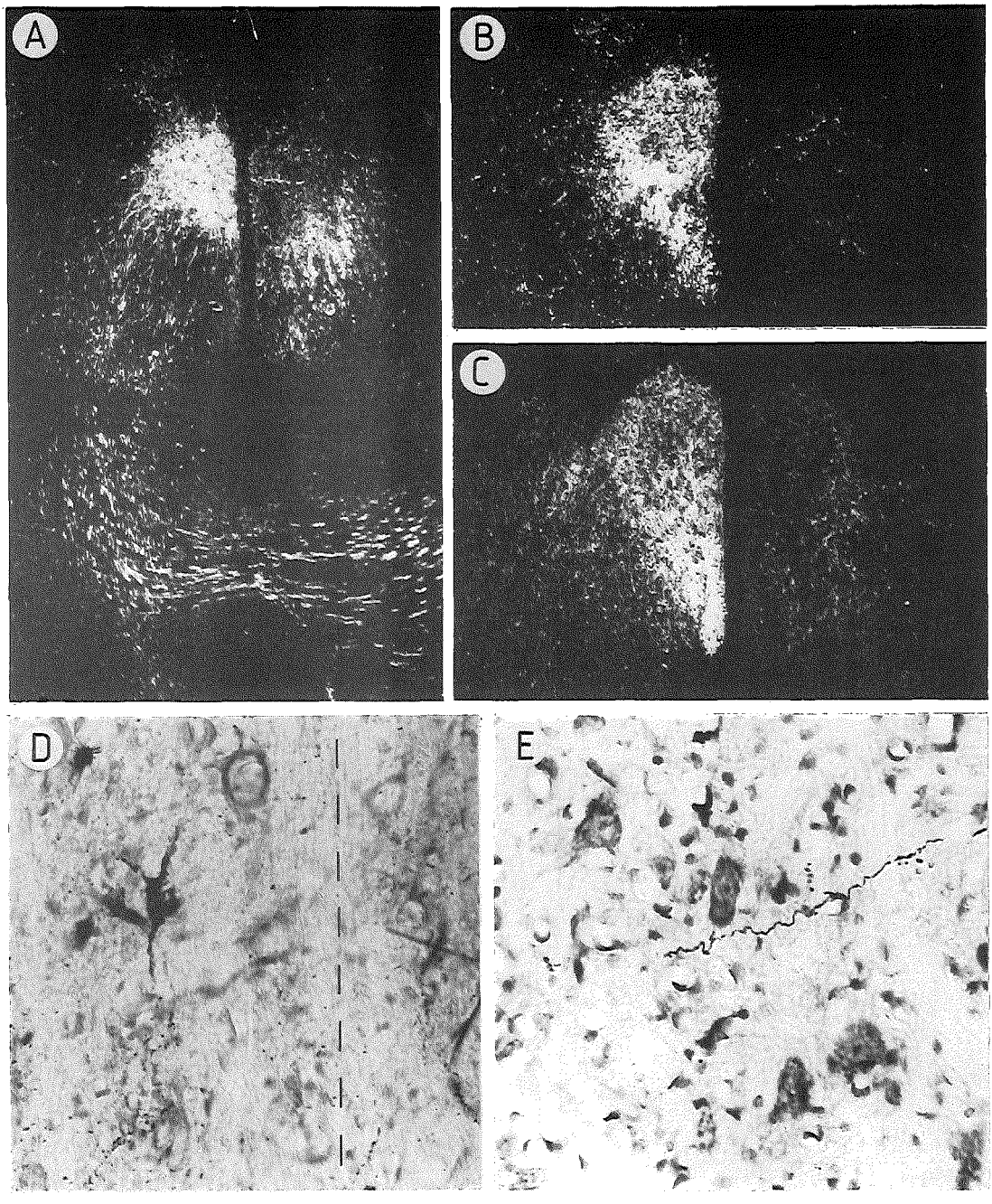


Fig 6.5 A, B and C: Distribution of terminal labeling in the oculomotor nuclei. A: Injection with ^{3}H-leucine in the SV (Exp K82). Ipsilateral terminal labeling predominantly present in the MR and IR motoneuron groups. Contralateral terminal labeling mainly in the IO and SR motoneuron groups. B: Injection with ^{3}H-leucine in the DV (Exp. K42) and C: injection with ^{3}H-leucine in the MVc (Exp. C2098). Terminal labeling with a strong contralateral predominance in the IO, SR, MR and IR motoneurongroups, with an emphasis on the latter two. D: Retrogradely labeled neuron in the contralateral SR motoneuron group, following injection with WGA-HRP in the MVpc (Exp. K216). E: Anterogradely labeled axon with terminal boutons in the SV following injection with PhaL in the OMN (Exp. K178).

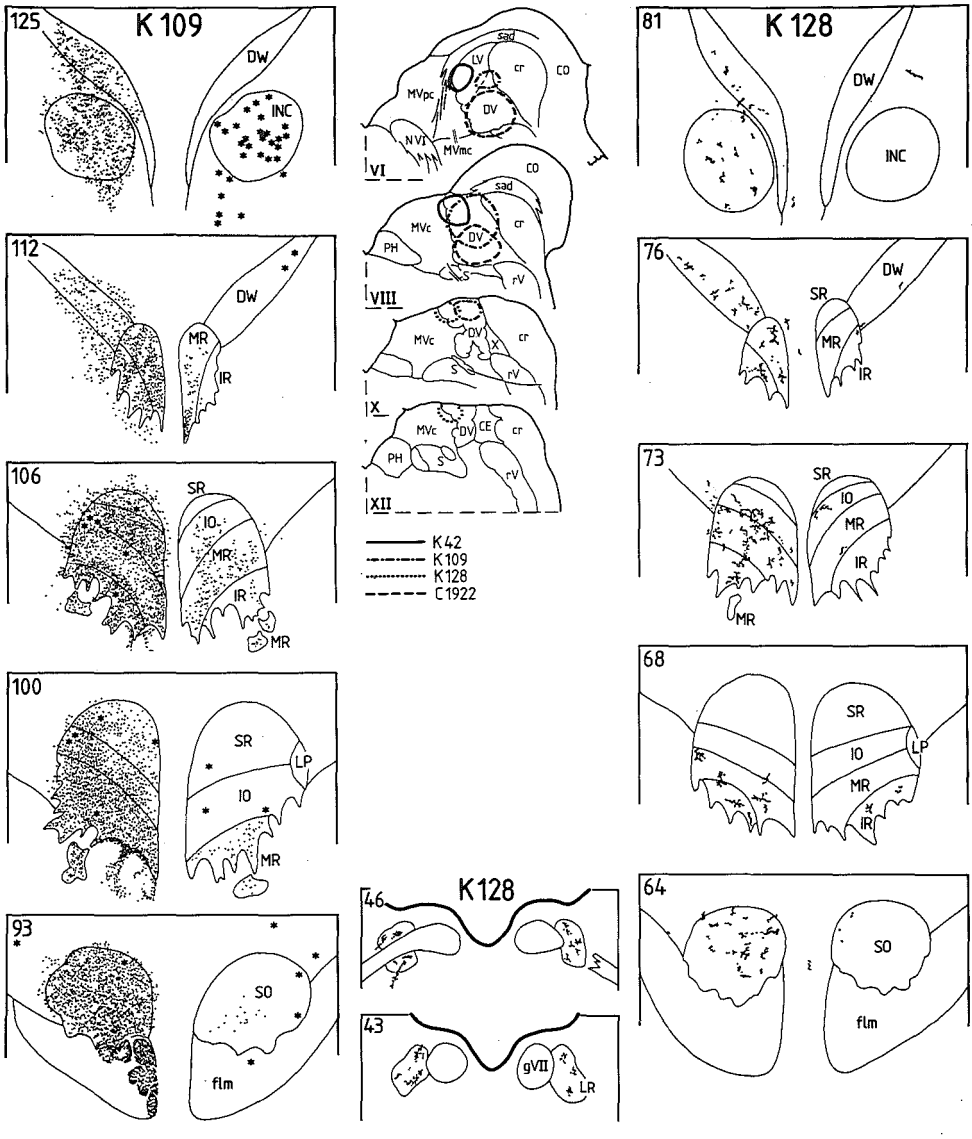


Fig 6.6 Injections mainly restricted to the DV (middle column top, Exps K42, 3H-leu; K109, WGA-HRP; K128, PhaL; C1922, WGA-HRP) and the resulting distribution of anterogradely labeled terminals in the OMN and TN (left column, Exp. K109, right column, Exp. K128) and in the AN (middle column bottom, Exp. K128)

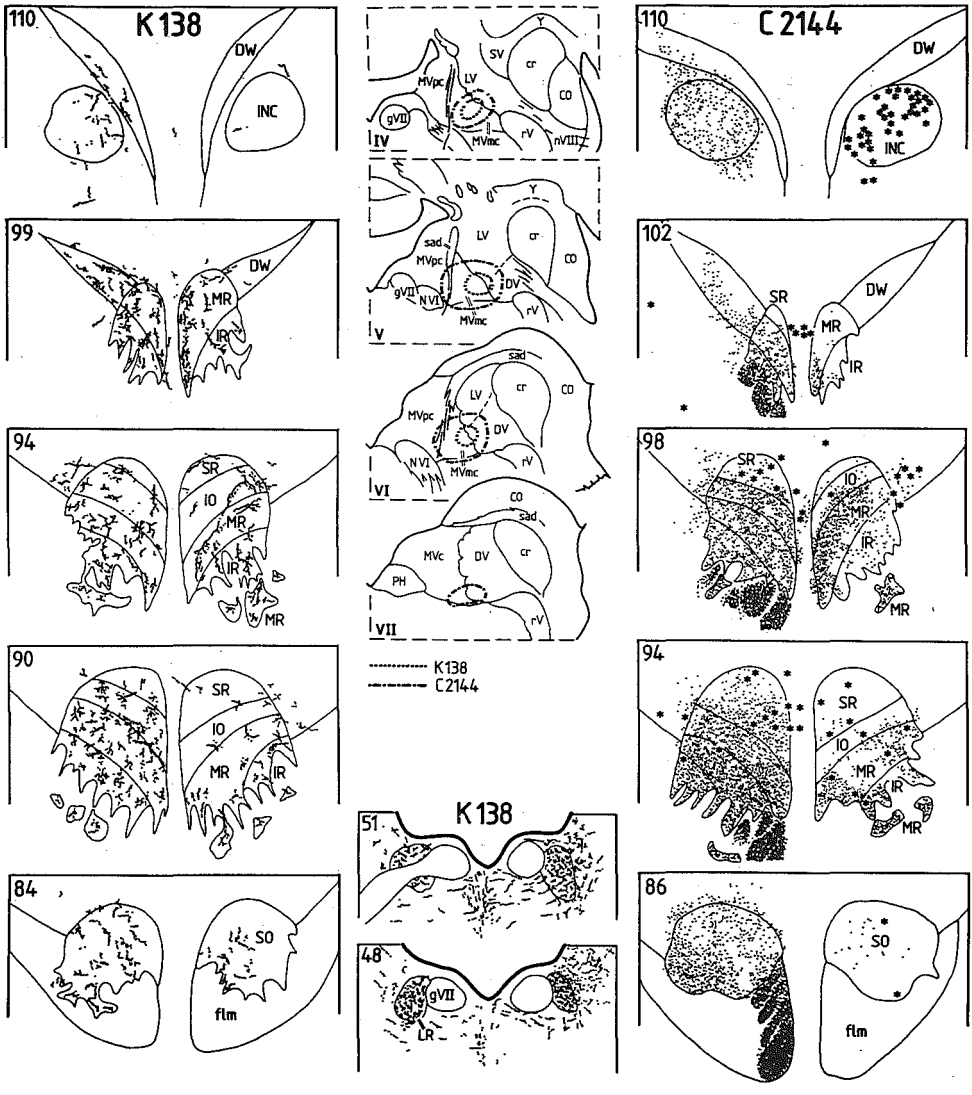


Fig 6.7 Injections mainly restricted to the MVmc (middle column top, Exps. K138, PhaL; C2144, WGA-HRP) and the resulting distribution of anterogradely labeled terminals in the OMN and TN (left column Exp. K138, right column Exp. C2144) and in the AN (middle column bottom, Exp. K138).

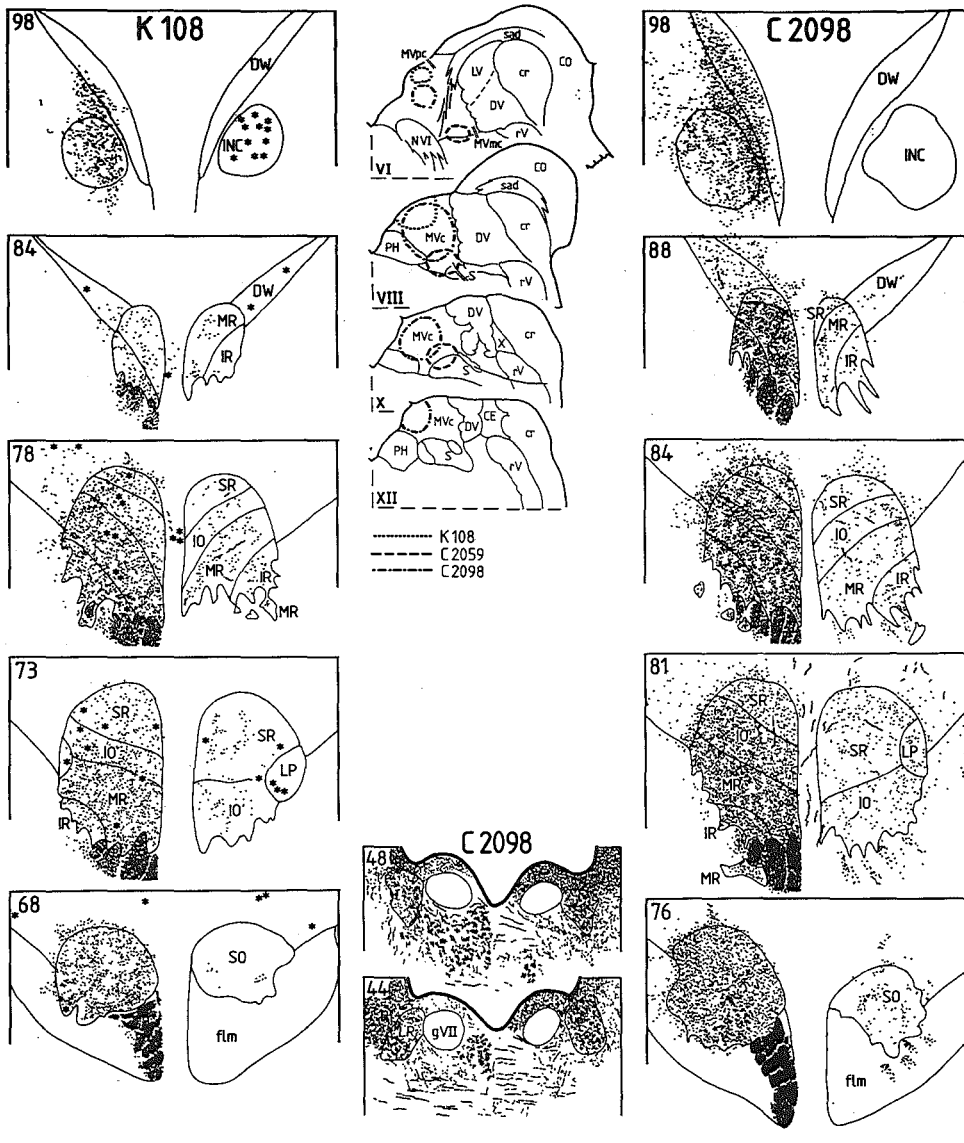


Fig 6.8 Injections mainly restricted to the MVc (middle column, Exps K108, WGA-HRP; C2059, ^{3}H-leu; C2098, ^{3}H-leu) and the resulting distribution of anterogradely labeled terminals in the OMN and TN (left column, Exp. K108, right column, Exp. C2098) and in the AN (middle column bottom, Exp. C2098).

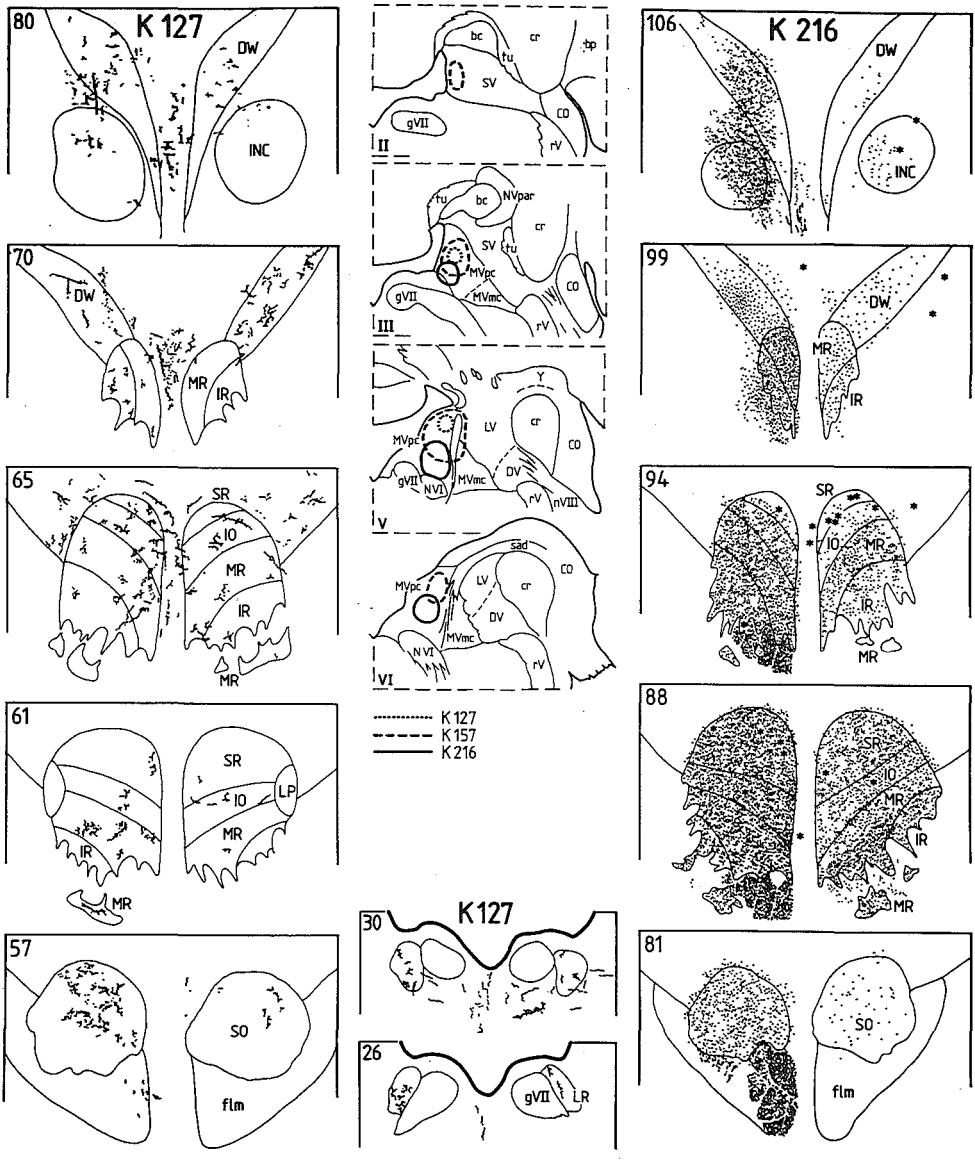


Fig 6.9 Injections mainly restricted to the MVpc (middle column top, Exps K127, PhaL; K157, WGA-HRP; K216, WGA-HRP) and the resulting distribution of labeled terminals in the OMN and TN (left column Exp. K127, right column, Exp. K216) and in the AN (middle column bottom, ExpK127).

The injection in experiment K128 (PhaL) was restricted to the dorsocaudal DV, with some involvement of the MVc. In all cases labeled terminals were found bilaterally in the AN (Fig. 6.6, Table 6.3). Labeled ascending fibers were only observed in the dorsomedial aspect of the contralateral flm (Fig. 6.5B Exp K42, Fig. 6.6 Exps K109, K128). At caudal levels of the OMN they leave the flm and proceed within the nucleus itself. The distribution and the amount of the labeled terminals showed little variation with respect to the localization of the injections or the specific tracer used. In the oculomotor region the majority of labeling was present contralaterally in the TN, INC and DW, and throughout the OMN with the weakest input in the SR subdivision (Fig. 6.6). On the ipsilateral side sparse termination was found in the MR cell group. Ramifications of PhaL labeled axons were found in areas that received the densest input in the other cases (Fig. 6.6, Exp. K128).

Injections restricted to the MVmc

In two experiments (C2144, WGA-HRP; K138, PhaL) the injections were mainly restricted to the MVmc, but included small parts of the LV, DV and MV (Fig. 6.7). In case K138 many labeled terminals were found in the AN on both sides. In case C2144 termination in the AN ipsilateral to the injection site could not be evaluated due to a high background of TMB reaction product (Table 6.3). Labeled fibers were only observed in the contralateral flm. They occupied a medial position at the level of the TN from where they shifted lateralward in rostral direction, still concentrated in bundles (Fig. 6.7). Most terminals were concentrated contralaterally in the TN and the MR, IR, and IO subdivisions of the OMN. The SR cell group, the INC and the DW received the smallest number.

Table 6.3 Semiquantitative estimation of the amount of terminals in the different motoneuron groups innervating the different eye muscles.
contralateral/ipsilateral
-: absent, o: trace, +: moderate, ++: heavy, * could not be estimated, due to a high background

site	exp	LR	SO	SR	IO	MR	IR	INC
SV	K68	+/+	o/++	++/o	++/o	o/+	-/++	+/++
	K82	+/+	o/++	++/+	++/+	o/++	-/++	+/++
	K116	+/*	o/++	++/++	++/++	o/++	o/++	+/++
	K124	+/*	o/++	++/++	++/++	o/++	o/++	+/++
MVpc	K157	++/*	++/+	++/+	++/+	++/++	++/+	++/+
	K216	++/*	++/o	++/++	++/++	++/++	++/++	++/o
	K127	++/++	++/+	++/+	++/++	++/++	++/+	++/+
MVmc	K138	++/++	++/+	+/o	++/+	++/++	++/+	++/-
	C2144	++/*	++/o	+/o	++/+	++/++	++/+	++/-
MVc	C2098	++/++	++/+	++/+	++/+	++/+	++/+	++/-
	K108	++/++	++/o	++/+	++/+	++/+	++/+	++/-
	C2059	+/+	++/o	++/o	++/o	++/+	++/+	++/-
DV	K109	++/++	++/o	+/-	++/o	++/+	++/o	++/-
	K128	++/++	++/o	o/-	++/o	++/o	++/o	++/-
	K42	++/++	++/o	++/-	++/o	++/+	++/o	++/-
	C1922	+/+	++/o	++/-	++/o	++/+	++/o	

Ipsilaterally, the termination was concentrated in the MR subdivision with some spread into the adjacent IO and IR cell groups (Table 6.3). In exp. K138 the ipsilateral projection was more extensive than in case C2144 (Fig. 6.7).

Injections restricted to the MVc

In experiment C2098 (3H-leucine) the injection included most of the central MVc and a small part of the PH. Two smaller injections were limited to the dorsorostral (exp. K108, WGA-HRP) and ventral MVc (C2059, 3H-leucine), in the latter case with spread of labeling into the MVmc and the solitary nucleus (Fig. 6.8). Labeled fibers ascended in the contralateral flm (Figs 6.5C, 6.8), their localization was similar to the cases with injections in the MVmc. However, in experiment C2098 few labeled fibers were also present in the ipsilateral flm, in a position like the SV axons (Fig. 6.8). Labeled terminals in the AN were present on both sides with equal density. In the oculomotor region terminal labeling was present contralaterally in the TN, OMN, INC and DW (Table 6.3). In both small injections (K108, C2059) the amount of labeling decreased towards rostral levels of the OMN. At the ipsilateral side, a small number of terminals were present in all experiments in the TN and OMN, without preference for any of the motoneuron subdivisions (Fig. 6.8).

Injections restricted to the MVpc

In experiment K127 a very small injection with PhaL was made in the MVpc (Fig. 6.9). Two WGA-HRP injections involved more extensively the ventral (K216) and dorsal aspect (K157) of the MVpc, the latter including a small part of the SV (Fig. 6.9). Only in case K127 the background labeling around the injection was low enough to show a bilateral symmetry in the AN projection (Table 6.3). The course of the labeled fibers to the oculomotor region and their termination on the contralateral side were similar to that observed following MVc injections. Ipsilateral to the injection site very few fibers were present in the flm. The contribution to the caudal contralateral OMN was weak in case K127, but nevertheless this very small PhaL injection had an extensive termination area. The differences with the MVc projection concerned the ipsilateral side, where in the cases of MVpc injections the OMN received a rather dense input, whereas a few terminals were present in the INC and DW (compare Figs. 6.8 and 6.9).

II OCULOMOTOR-VESTIBULAR PROJECTION

Distribution of neurons in the region of the oculomotor nuclei, projecting to the vestibular nuclei

In all experiments with injections of WGA-HRP in the vestibular nuclei retrogradely labeled neurons were observed in the AN, TN, OMN, and INC (Table 6.4; Figs. 6.4 - 6.9). Labeled neurons were also found in neighbouring regions such as the DW and the periaquaductal grey. The data in Table 6.4 show a surprisingly uniform pattern in the distribution of labeled neurons, regardless what nucleus was injected. In the oculomotor nuclei few labeled neurons were scattered throughout most subdivisions. However, a substantial number was concentrated in the SR and the directly adjacent parts of the IO and MR cell groups. The number of labeled neurons in the INC was approximately 60% of that in all oculomotor neuron groups. But for an odd neuron, they were found exclusively ipsilateral to the vestibular injections.

Table 6.4 Number of labeled neurons in different extra-ocular motoneuron groups and the INC following injection of WGA-HRP in the vestibular nuclei, counted in N 40 µm sections, spaced 160 µm apart. contralateral/ipsilateral

site	exp	(N)	LR	SO	SR	IO	MR	IR	INC
SV	K116	44	6/8	3/2	39/22	6/4	4/8	2/2	1/62
	K124	37	6/7	0/1	31/14	3/3	3/4	1/3	0/57
MVpc	K157	39	4/2	1/0	7/5	3/3	1/3	0/0	1/106
	K216	36	1/5	0/1	23/19	9/11	6/7	0/2	0/31
MVmc	C2144	38	5/4	2/3	26/17	8/8	8/13	2/2	0/74
MVc	K108	40	6/4	2/3	23/15	12/7	16/8	3/2	0/45
DV	K109	37	0/2	2/3	13/6	10/6	7/3	1/0	0/34

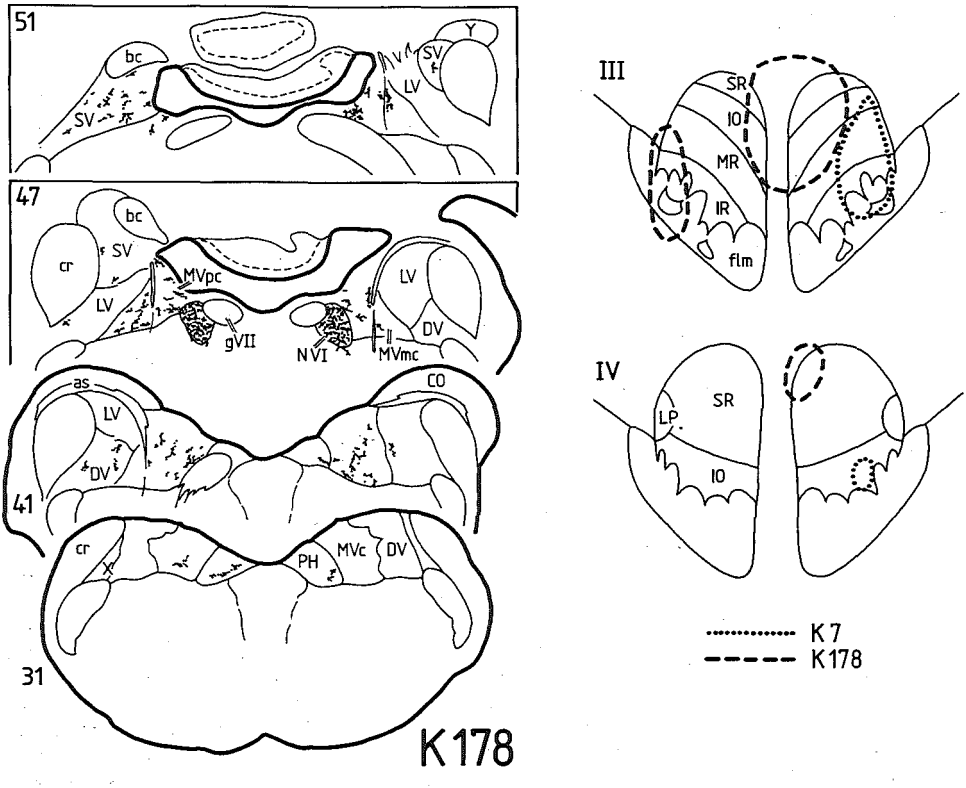


Fig 6.10 Left: Distribution of anterogradely labeled terminals in transverse sections following injection with PhaL in the OMN (Exp. K178). Right: Extent of the injection sites of Exps K7 (^{3}H-leucine), K178 (PhaL)

Distribution of projections from the region of oculomotor nucleus to the vestibular nuclei

Descending input in the VN was studied in two experiments with injections in the OMN. In experiment K178 (PhaL) two injections at either side of the midline cover together all subdivisions of the OMN (Fig. 6.10). The injection of experiment K7 (^{3}H-leucine) is unilateral and located in the IO, MR and IR subdivisions (Fig. 6.10). In both experiments a small amount of labeled terminals were found bilaterally in all VN except the LV. From Fig. 6.10 it is clear that the intensity of the labeling matches the quantitative distribution of labeled vestibulo-oculomotor neurons (c.f. Figs. 6.1, 6.2). In the PH labeled terminals were found throughout its rostrocaudal extent. Large amounts of labeled terminals were present with bilateral symmetry in the AN (Fig. 6.10).

6.3 DISCUSSION

Distribution of vestibular neurons projecting to the oculomotor nuclei

The present WGA-HRP experiments confirm previous findings in the cat (Graybiel and Hartwieg, 1974; Gacek, 1977; Carpenter and Cowie, 1985), the monkey (Graybiel, 1977; Steiger and Buttner-Ennever, 1979), the rabbit (Yamamoto et al., 1978), the gerbil (Coffey et al., 1989) and the opossum (Henkel and Martin, 1977), that the projection to the oculomotor and trochlear nuclei originates in all the vestibular nuclei, except in the LV. Retrogradely labeled pre-oculomotor neurons form two large clusters within the VN: one in the SV with a predominant ipsilateral ascending connection, the other in the rostral MV, predominantly connected with the contralateral side (Graybiel and Hartwieg, 1974; Steiger and Buttner-Ennever, 1979; Yamamoto et al., 1978). The present data, obtained with the highly sensitive TMB reaction, also show that labeled neurons were most numerous in the SV, and in the MVpc and rostral MVc, but that these "clusters" are mere concentrations in a column of neurons streching from the SV into the MVc. Even following small injections in the OMN (exps. K7, C2320) labeled neurons were found throughout this column.

Course of ascending vestibular axons

The present study is the first to demonstrate with anterograde axonal transport connections from the SV and possibly the group y through the superior cerebellar peduncle and the flm to the oculomotor nuclei in the rabbit. Similar connections from SV and y were traced by Henkel and Martin (1977) in the opossum, by Stanton (1980) in the monkey and by Carpenter and Cowie (1985) in the cat. Other authors did not consider the superior peduncle to be an efferent pathway from these nuclei (Gacek, 1971; Tarlov, 1970). Our observations are in compatible with the conclusions of Highstein and Ito (1971), Highstein (1971, 1973a) and Yamamoto et al. (1978), all in the rabbit, and Hirai and Uchino (1984) in the cat, that the excitatory (presumably anterior canal) neurons from the dorsal SV and group y project through the superior cerebellar peduncle, and that the axons of inhibitory neurons from the SV ascend in the ipsilateral flm.

According to the literature the main ascending projection of the DV, MV and the ventral part of the LV (the MVmc in our study) is crossed and passes through the medial part of the flm (Busch, 1961; Gacek, 1971; Tarlov, 1970, cat; Henkel and Martin, 1977, opossum; Carleton and Carpenter, 1983, cat and monkey). Our experiments are in accordance with these conclusions. Ipsilaterally ascending fibers were only observed in small numbers, following our largest injections in the MVpc and the MVc. This was unexspected since the termination in the ipsilateral oculomotor nucleus was substantial, and some fibers also were present in the trochlear nucleus in these cases. In the literature this discrepancy has been explained by the presence of an ipsilateral ascending tract of Deiters. Originally this tract was described by Winkler and by Muskens in the early part of this

century, after large lesions including Deiter's nucleus, but its origin seems to be located more ventrally in the MVc or the MVmc (see Baker and Highstein, 1978 for a review). According to Busch (1961) it may have been confused with the ascending fibers of the uncinate tract. More recently the tract was described in the cat by Gacek (1971), in a position lateral to the flm and avoiding the nucleus of the trochlear nerve, and in the monkey by Lang et al. (1979). Baker and Highstein (1978) demonstrated that the axons of vestibulo-ocular relay cells for the horizontal canal, which are implicated in the monosynaptic excitation of the ipsilateral medial rectus motoneurons in the OMN, travel in the ascending tract of Deiters. In our experiments in rabbits we never obseved ipsilaterally ascending fibers in the position of the ascending tract of Deiters. An alternative explanation for the presence of ipsilateral termination in the OMN after injections of parts of the MV or DV would be recrossing of the vestibulo-oculomotor fibers at the level of these nuclei (Graf et al., 1983; Graf and Ezure, 1986 cat; Carleton and Carpenter 1983, monkey). In our experiments these recrossing fibers were relatively scarce and best visualized with PhaL.

Termination of vestibular axons in the nuclei of the extra-ocular muscles

The anterograde axonal tracing technique permitted a detailed analysis of the localization of the vestibulo-oculomotor terminals. Qualitative differences between the different tracers were not observed. To facilitate comparison, data from the literature on the termination in individual motoneuron groups were combined with a summary of the present results (Table 6.5). Our results in the rabbit confirm the outcome of earlier investigations using retrograde axonal transport in other mammals (Langer et al., 1986; Belknap and McCrea, 1988, monkey: Gacek, 1979a; Maciewicz et al., 1977, cat), which demonstrated a bilateral projection from the vestibular nuclei to the AN. In accordance with electrophysiological studies (Highstein, 1973b, rabbit; Isu and Yokota, 1983; Nakao et al., 1982; Uchino et al., 1982, cat) and intracellular injections of the vestibulo-oculomotor neurons projecting to the AN (McCrea et al., 1980) most of these cell were found to be located bilaterally in the MVmc. In our experiments the MVmc provided the largest number of afferents to the AN but bilateral labeling was also observed after injections of the other vestibular nuclei including the SV. A projection of the SV to the AN was denied by Gacek (1971, cat) but was documented by Tarlov (1971, cat), Langer et al. (1986, cat and monkey) and Stanton (1980, monkey) for the SV and/or group y. The wide origin of these projections may indicate the presence of neurons with principal projections to other targets and collateral projections to the AN. Such connections were postulated by Ezure and Graf (1984a,b) for excitatory and inhibitory neurons of the anterior and posterior canals both in the rabbit and in the cat. Such collaterals to the AN indeed were demonstrated by the intracellular injection of excitatory posterior canal neurons in the rabbit (Graf et al., 1983). Anterior and posterior canal neurons with collateral projections to the AN could not be demonstrated in the cat, either with physiological methods (Uchino et al., 1980) or intracellular injection (Graf et al., 1983; Graf and Ezure, 1986).

In agreement with previous studies (Carleton and Carpenter, 1983; Carpenter and Cowie, 1985; Henkel and Martin, 1977; McCrea et al. 1981; Graf et al., 1983; Graf and Ezure, 1986; Coffey and Kevetter, 1989), the vestibular nuclei were found to have a bilateral projection to the trochlear nucleus. The projection from the SV is predominantly ipsilateral, that from the MV and DV is predominantly contralateral (Table 6.5). This is in agreement with the expectation that the contralateral superior oblique muscle is inhibited by anterior canal neurons located in the central SV and that the ipsilateral superior oblique muscle is excited by posterior canal neurons located in the MV and the DV (Highstein, 1973a). According to Gacek (1979b) crossed projections to the TN from the SV in the cat use the brachium conjuctivum. The weak projections in the rabbit to the contralateral TN from the SV and to the ipsilateral TN from the MV and DV are in accordance with Ezure and Graf's (1984a,b) postulate for the rabbit of the accessory inhibitory connections to motoneurons innervating the ipsilateral SO muscle from the anterior canal and an excitation

of the contralateral SO from the posterior canal. These accessory connections were not verified in intracellularly injected relay cells.

A number of studies claim that the termination sites of the vestibular fibers in the OMN are restricted to a few individual motoneuron groups (McMasters et al., '66; Carleton and Carpenter '83; Carpenter and Cowie '85). Our observations do not support these findings but give evidence for a more diffuse termination comprising many subdivisions of the OMN. However, within their termination area the vestibulo-oculomotor terminals show a clear preference for some of the motoneuronal groups.

The projections to the OMN from the DV and from the subdivisions of the MV were bilateral. Contralateral projections from the MVc, MVmc and MVpc terminated in the MR, IR and IO motoneuronal groups. Contralateral projections from the DV were restricted to the MR subdivision. The MVpc and MVc in addition projected to the contralateral SR motoneuronal group. Ipsilateral projections from the DV and MV and its subdivisions were distributed to all subdivisions of the OMN. The MV in the rabbit contains the excitatory relay cells for the posterior canal, which innervate the motoneurons for the contralateral IR and SO muscles and both excitatory and inhibitory horizontal canal cells, which project to the ipsilateral and contralateral MR subdivision of the OMN respectively (Graf et al., 1983; Highstein, 1973a; Ito et al., 1976a,b). These observations are in accordance with the presence of labeled terminals contralaterally in the IR motoneuronal pool and bilaterally in the MR motorneuronal pool of the OMN in our experiments,with injections of the MV and DV. However, the labeling in other subdivisions of the OMN remains unexplained, and the pathways to the MR motoneurons, mediating the ipsilateral excitation (the ascending tract of Deiters, see above) and the crossed inhibition have not yet been identified in the rabbit.

The presence of labeling in the contralateral SR and IO motoneuron pools after injections of the MVpc and MVc suggests that excitatory anterior canal cells may be present in these nuclei. Until now all excitatory anterior canal cells in the rabbit, were located in the dorsal SV and /or the group y (Highstein and Ito, 1971; Highstein, 1973a; Yamamoto et al., 1978). Excitatory relay cells for both the anterior and the posterior canal have been found in the MV and DV nucleus in the cat (Uchino et al., 1982). A second population of excitatory anterior canal cells is present in the cat in the dorsal SV and/or group y, in the same position as in the rabbit (Hirai and Uchino, 1984). It seems likely from our experiments that a second population of excitatory canal cells is also present in the rabbit.

Labeling in this group of experiments in the ipsilateral SR, IR and IO sudivisions of the OMN may be related to the secondary projections of the excitatory anterior and posterior canal cells to other muscles than the pair of prime movers. Ezure and Graf (1984a,b) postulated projectioins from the anterior canal cells to the ipsilateral SR and IO, and from the posterior canal cells to the ipsilateral IR motoneuronal pool. Graf and Ezure (1986) confirmed some of these collateral projections in the intracellularly injected neurons of the rabbit (Graf et al., 1983).

The localization of the terminals after injections in the SV in the ipsilateral OMN in the subdivisions containing motoneurons innervating the SR, IR and IO muscles is in accordance with the presence of inhibitory anterior and posterior canal cells in this nucleus (Highstein, 1973a; Ito et al., 1976b; Yamamoto et al., 1978). In experiment K68, with a more laterally located injection site, labeling was confined to the MR and IR motoneuronal pools. This case indicates that the anterior canal cells, which inhibit ipsilateral IR motoneurons, extend laterally to the posterior canal cells, which inhibit neurons in the ipsilateral OMN, innervating the SR and the IO. A similar localization of inhibitory anterior and posterior canal cells in the lateral and medial part of the SV, formerly was demonstrated by Abend (1977) in the squirrel monkey. Such a localization is in accordance with the topical projection of the root fibers of the vestibular nerve innervating the christae of the anterior and posterior canals to the lateral and medial parts of the SV respectively (Gacek, 1969, cat; Carleton and Carpenter, 1984, monkey; Kevetter and Perachio, 1986, gerbil).

Crossed projections to the SR and IO subdivisions were derived from ascending fibers in the superior cerebellar peduncle and therefore represent terminations from the second

group of excitatory anterior canal neurons, located in the dorsal SV, which were discussed in the previous paragraphs. A secondary action of these neurons on the ipsilateral MR was observed by Ito et al. (1976a), and is in accordance with the presence of an ipsilateral projection of the SV to these cells in our experiments.
The secondary connection of the inhibitory canal neurons to the contralateral IO was not present in our experiments.
It can be concluded from our analysis of the termination of the vestibular axons in the nuclei of the extraocular muscles that:
(1) the connections of the SV neurons through the superior cerebellar peduncle correspond to those of the excitatory anterior canal neurons in this region
(2) the connections of the SV neurons through the ipsilateral flm correspond to those of the inhibitory anterior and posterior canal neurons of this nucleus.
(3) there is a differential localization of neurons corresponding to the inhibitory vertical canal cells, with anterior canal cells, which extend into more lateral parts of the SV than posterior canal cells.
(4) neurons corresponding to excitatory neurons of both the anterior and posterior canal occur in the MV, they project through the contralateral flm. Two separate populations of excitatory anterior canal cells, therefire can be distinguished in the rabbit. One is located in the dorsal SV, the other in the MVpc or MVc.
(5) neurons in the MV and DV with projections to the ipsilateral and /or contralateral MR motoneurons are located in the DV and in all subdivisions of the MV. The pathways of these presumed excitatory and inhibitory horizontal canal cells are not known. An ascending tract of Deiters was not observed in our material.
(6) we were able to confirm several of the accessory connections of vestibulo-ocular relay cells to motoneurons innervating other eyemuscles, which move the eye in the plane of the corresponding semicircular canal, reported in the litterature (Cohen and Suzuki, 1963; Ezure and Graf, 1984a,b; Ito et al., 1973a,b; Graf and Simpson, 1981).

In line with the findings of Graf et al. (1983) and Graf and Ezure (1986) labeled terminals were also demonstrated in areas neighbouring the TN and the OMN such as the adjacent central grey and the supraoculomotor area. Since Edwards and Henkel (1978) have demonstrated that oculomotor neurons extend their dendrites into the central gray matter directly overlying the oculomotor nucleus, the terminals found in there may contact the dendrites of the motoneurons directly.

Oculomotor projections to the vestibular nuclei

Projections from the OMN to the vestibular nuclei have been mentioned by Carpenter and Cowie (1985), Thunnissen and Gerrits (1985) and Spence and Saint-Cyr (1988).These authors found retrogradely labeled OMN neurons after injections in the VN, but gave no details concerning their localization. Our retrograde tracing experiments showed that the pattern of distribution and the clustering of labeled OMN neurons in the SO subdivision was a constant phenomenon and not affected by the localization of the injections. The anterograde tracing experiments showed a diffuse termination of oculomotor axons comprising the entire VN. The nature of the OMN projection to the VN seems enigmatic; it has a diffuse character rather than showing topographic reciprocity between specific subnuclei in the OMN and VN.

Connections between the vestibular nuclei and the INC

The present data show that the ascending vestibular axons not only terminate in the OMN but also more rostrally in the INC. They confirm earlier observations describing a crossed projection from the MV and DV (Carleton and Carpenter, 1983; Carpenter and Cowie, 1985; Gacek, 1971; Tarlov, 1970; Thunnissen and Gerrits, 1985), and a bilateral projection from the SV (Carpenter and Cowie, 1985).

Table 6.5 Semiquantitative estimation of the amount of terminals in the different motoneuron groups innervating the different eye muscles. Mean values of the present data compared with data from the literature. contralateral/ipsilateral
-: absent, o: trace, +: moderate, ++: heavy

	LR	SO	SR	IO	MR	IR	INC
SV	+/+	o/++	++/++	++/++	o/++	o/++	+/++
McMasters et al. '66			-/-	-/+	-/-	-/+	
Tarlov '70	-/+	o/+	+/+	+/+	+/+	+/+	+/+
Carpenter & Cowie '85	-/-	-/+	++/-	++/-	-/-	-/++	o/++
MVpc	++/++	++/+	++/+	++/++	++/++	++/+	++/+
MVmc	++/++	++/o	+/o	++/+	++/++	++/+	++/-
MVc	++/++	++/o	++/+	++/+	++/+	++/+	++/-
MV	++/++	++/+	++/+	++/+	++/++	++/+	++/-
McMasters et al. '66			-/-	+/+	+/+	+/+	
Tarlov '70	+/+	+/-	+/+	+/+	+/+	+/+	+/o
Carleton & Carp. '83	++/++	+/-	++/+	++/+	++/+	++/+	++/-
DV	++/++	++/o	++/-	++/o	++/+	++/o	++/-
McMasters et al. '66			-/-	+/+	+/+	+/+	
Tarlov '70							
Carleton & Carp. '83	-/-	+/-	-/-	-/-	+/-	+/-	+/-

Our experiments show that the INC is not the specific target of these vestibular axons, but that this nucleus is embedded in a termination area which also includes the dorsal reticular formation, Darkschewitsch' nucleus, and to a lesser extent the periaquaductal grey matter. A significant proportion of neurons that project to the VN is concentrated in the INC. This ipsilateral projection has been demonstrated in many previous studies (Pompeiano and Walberg, 1957; Mabuchi and Kusama, 1970; Barmarck et al., 1979; Carleton and Carpenter, 1983; Carpenter and Cowie, 1985; Magnin et al., 1983; Henkel and Martin, 1977; Thunnissen and Gerrits, 1985; Spence and Saint-Cyr, 1988). Functionally, the INC has been implicated in the control of vertical and rotational eye and head movements (Hassler and Hess, 1954; Hyde and Eliasson, 1957; Hyde and Toczek, 1962; Carpenter et al., 1970; King et al., 1980). Specific projections to SR and IR subserving vertical eye movements (Graybiel and Hartwieg, 1974; Graybiel, 1977; Steiger and Buttner-Ennever, 1979; Labandeira-Garcia et al., 1989), as well as a projection to neckmuscle motoneurons (Kuypers and Maisky, 1975; Nyberg and Hansen, 1966; Zuk, 1983; Fukushima et al., 1979) support this view.

Fukishima et al. (1983) suggested that the vestibular complex may be the site of interaction of labyrinthine and interstitional inputs which influence head posture. Our experimental data does not support this view, since no specific relationship between the INC and the MVST and LVST neurons could be demonstrated. No topological localization of the INC axons excist in the vestibular complex.

7 FINAL REMARKS

A SIMPLIFICATION

Vestibulocerebellar mossy fibers and corticovestibular Purkinje cells are distributed in three separate regions of the cerebellar cortex: the flocculus, the nodulus and uvula of the caudal vermis, and the vermis of the anterior lobe. Although these regions share a relation with the vestibular nuclei they are implicated in rather different functions. Nevertheless it seems possible to find a common functional denominator.

In a highly simplified manner, direction sensitive ganglion cells within the retina can be considered as the final element of a kinematic chain, which consists of 4 elements, connected by 3 joints: the world, the body and limbs, the head, and the eyes. The objective of this chain is to maintain a stationary retinal image thus consequently, a stable image of the world. Information about the static and dynamic states of each of the three joints is monitored and processed in the cerebellum. The postulate is that the three joints of the kinematic chain have their representation in the afferent and efferent connections of the three regions of the cerebellum; the world-body joint in the anterior lobe vermis, the body-head joint in the caudal vermis, and the head-eye joint in the flocculus.

The reason why the information about the state of this chain is not concentrated in a single region of the cerebellum is that an organism as a whole is not retina-centered. All its elements must be able to function in many different situations and consequently its various functional states must be subject to the appropriate correction mechanisms for learning and adaptation in case a mismatch occurs between intended and executed movement.

A complete separation of the afferent input from the different joints is also not to be expected since this would circumvent the special integrative capacities alleged to the cerebellar cortex. In other words, the performance of the neural circuitry is likely to be enhanced by feeding information concerning events in the distal part of the chain into the region which controls its proximal parts.

In the next paragraphs the relation of the three cerebellar regions and the vestibular nuclei will be discussed. Emphasis will be put on two aspects of connectivity. Firstly, an evaluation of the vestibulo-cerebellar mossy fiber system in relation to the various inputs from other sources that converge in each specific region. Secondly, the organization of the cortico-vestibular Purkinje cell projection will be discussed in relation to the climbing fiber innervation and in terms of the final common pathway that influences the motoneurons of the kinematic chain.

FLOCCULUS

As has been discussed already in chapter 5, the vestibular input in the flocculus is one out of many and quantitatively of the same order of magnitude as the other mossy fiber inputs from the NRTP, PH and the rest of the brain stem. These mossy fibers mediate vestibular, optokinetic (Lisberger and Fuchs, 1978; Blanks and Fuchs, 1983; Graf et al., 1988), neck afferent (Wilson et al., 1975) and extraocular muscle afferent information (Maekawa and Kimura, 1980). All mossy fiber terminals are distributed throughout the rostro-caudal extent of the floccular granular layer. Although preferences of different afferents for certain lobules have been observed, clustering in longitudinal strips does not occur in the rabbit nor in the cat (Gerrits, 1985). Through the intermingling of mossy fiber terminals all floccular Purkinje cells seem influenced by a homogenous and more or less identical input.

There are two important facts that should be considered with respect to the connections

and the possible function of the flocculus. Firstly, the climbing fiber input in the flocculus is completely dominated by information about retinal movement, relayed by the NOT, DTN, MTN (Alley et al., 1975; Barmarck and Hess, 1980; Maekawa and Simpson, 1973; Maekawa and Takeda, 1976; Takeda and Maekawa, 1980) and PH (Gerrits and Voogd, 1986) to the dorsal cap (dc) and ventro-lateral outgrowth (vlo) of the inferior olive. These parts of the inferior olive are devoid of a direct input from the vestibular nuclei (Gerrits et al., 1985). The floccular climbing fibers are organized in longitudinal zones (Yamamoto, 1979; Gerrits and Voogd, 1982; Groenewegen and Voogd, 1977; Sato et al., 1983). Secondly, the floccular Purkinje cells are clustered into longitudinal strips, which project to the vestibular nuclei (Yamamoto and Shimoyama, 1977; Bigaré, 1980; Sato et al., 1982). A clearcut topological relation between individual Purkinje cell zones and specific regions of the vestibular nuclei is still enigmatic. The termination area as a whole comprises group y, the SV, the MVpc and the rostral MVc, and is clearly more restricted than the area containing the vestibulo-oculomotor neurons (Epema, 1990; chapter 6). Physiological investigations have provided evidence that floccular Purkinje cells can be divided into distinct subgroups. Each of these subgroups establishes inhibitory contact with the VOR relay neurons for the horizontal and anterior canals (Dufossé et al., 1977, 1978; Ito et al., 1982; Nagao et al., 1985).

The flocculus participates in optokinetic responses and the flocculus has a crucial role in smooth pursuit and in visual suppression of the VOR (Waespe et al., 1981; Miles and Lisberger, 1981; Ito, 1982; Blanks et al., 1983). A number of models include the flocculus in the circuitry underlying the modification of the VOR. The required head acceleraion signal in these models is thought to be mediated by primary vestibular afferents (Ito, 1984; Lisberger, 1985). The absence of a direct eight nerve input in the flocculus (Gerrits et al., 1990) stresses the importance of the secondary vestibulocerebellar mossy fiber projection. It seem paradoxical that the all-important vestibular input in the flocculus does not display a clear topographical organization, neither in the vestibular nuclei nor in the flocculus (chapter 5). The major question that arises is how canal-specificity is maintained in vestibulocerebellar fibers from at least three different, but not topographically separated, populations of vestibular neurons providing inputs from the contralateral and ipsilateral sides (Tan and Gerrits, 1990) and from one which, collateralizes between flocculus and caudal vermis (Epema et al. 1990). Perhaps one should consider the vestibular nuclear input into the flocculus, which contains information about the second joint in the chain, as an open loop additional input (Gerrits 1987) that enhances the performance of the floccular, visually driven closed loop circuitry.

Oddly enough, the circuitry that the models consider a prerequisite is absent from the flocculus, but completely present in the caudal vermis.

CAUDAL VERMIS

Signals proportional to head position and angular and linear acceleration originate in the vestibular receptors and generate reflexes to compensate for deviation of the head from the preferred upright position.Signals arising from neck propioceptors interact with vestibular input during stabilization of posture and gaze. The nodulus and uvula of the caudal vermis are in receipt of these inputs and have been implicated in the control of the vestibulocollic reflex (Precht et al., 1979). However, judged from the literature, these lobules are grossly ignored when it comes to their possible influence on the VOR and ocular motility in general.

Whereas in the flocculus, vestibulo-cerebellar mossy fibers and cortico-vestibular Purkinje cells share the same cortical area, the caudal vermis is characterized by a partial dissociation of these elements. There is overlap only in the lobules IXd and X. In the rostral folia of the uvula vestibular mossy fiber are nearly absent and here the corticovestibular Purkinje cells coincide with the distribution of mossy fibers from the external cuneate nucleus (CE) (Gerrits and Thunnissen, 1987), which relay neck and proximal forelimb information (Precht et al., 1976; Batini and Pompeiano, 1957). The separation of the two

input qualities along the long-axis of the cerebellar cortex excludes their integration at this level: the anatomical dissociation has important consequences for the functional role of the two cortical areas and their efferent projection to the vestibular nuclei.

NODULUS AND VENTRAL UVULA

In the lobules X and IXd the co-localization between the vestibular mft and the corticovestibular Purkinje cells has been unequivocally demonstrated with the anterograde and retrograde transport properties of WGA-HRP (chapter 4). In these lobules the labeled Purkinje cells as well as mft are present over the entire mediolateral extent of the cortex. Clear borders that would indicate the presence of a parasagittal zonation were not found. Thusfar, only two other afferent system have been shown to terminate in this area: the primary mossy fibers from the vestibular nerve, which have their highest density of terminals in the cortex lining the posterolateral fissure (Gerrits et al., 1989) and mossy fiber from the PH (Belknap and McCrea, 1988).

The organization of the secondary fibers is reminescent of those providing the flocculus. Each hemivermis is innervated from two large population of vestibular neurons at the ipsi- and contralateral sides and a very small population projects bilaterally (Tan and Gerrits, 1990). The neurons of these different populations are completely intermingled. About 10% of the vestibular neurons that project to the caudal vermis send a collateral axon to the flocculus (Epema et al., 1990).

The nodulus and ventral uvula are innervated by a set of climbing fiber strips. From medial to lateral they have their origin in subnucleus beta and the dorsal medial cell column (dmcc) (Balaban and Henry, 1988; Groenewegen and Voogd, 1977; Eisenman, 1981,1984), the ventolateral outgrowth and the dorsal cap (Hoddevik and Brodal, 1977; Kawamura and Brodal, 1980; Sato and Barmarck, 1985; Karayama and Nisimatu, 1988; Eisenman, 1984; Bernard, 1987; Kanda et al., 1989; Balaban and Henry, 1988; Groenewegen and Voogd, 1977). Some aspects of this projection are still disputed, especially the exact rostrocaudal extent of the zones. Subnucleus beta and the dmcc receive afferents from the vestibular nuclei (Gerrits, Voogd and Magras 1985) and to a lesser extent from the CE (Gerrits, Voogd and Nas 1985). The vlo and dc relay retinal slip information.

One may conclude that this part of the cerebellum controls the head position in the sense of stabilization of "head-gaze", on the basis of information derived from the state of the second joint of the kinematic chain. The VOR is a response to head movements in a dual sense. It must counteract movements of the head relative to the body and movements of the head relative to the world with second joint of the chain in a fixed position. Therefore it seems logical that it operates on the basis of vestibular information rather than on direct neck proprioception, and visual control or modification and adaptation should be installed at this level.

THE DORSAL UVULA AND THE PYRAMIS

Just as there are some cuneate mossy fibers in the ventral uvula, the dorsal uvula contains a small amount of vestibular mossy fibers. Nevertheless the separation between the two afferent systems is very evident (chapter 4).

More than half of the Purkinje cells that could be labeled retrogradely following WGA-HRP injections in the vestibular nuclei, are located in lobules VIII and IXa,b,c. Their precise matching with the CE mossy fiber strips deserves particular interest. Bower and Woolston (1983) have noted that simple spike activity of Purkinje cells could only be modulated through activation of granular cells directly beneath them. The importance of the different roles for the ascending and parallel branches of granule cell axons have also been stressed by Llinas (1982). Other mossy fiber inputs in the uvula, with an origin in the rostral pons (Gerrits et al., 1984), the PH (McCrea and Baker, 1985; Belknap and McCrea, 1988), and the trigeminal nuclei (Somana et al., 1983) may change our view of the functional role(s) of the uvula as a whole, but will leave unchanged the inhibitory effect

exerted on the vestibular nuclei by this neck-afferent system, that is isolated from direct vestibular mossy fiber influence at the cerebellar cortical level.

In the dorsal uvula and pyramis climbing fiber strips are present with an origin in different parts of the inferior olive. Subnucleus beta projects to a medial strip (Balaban and Henry, 1988; Groenewegen and Voogd, 1977; Eisenman, 1981,1984), which is flanked by an A zone from the caudal medial accessory olive (MAO), and a C2 zone from the rostral MAO. The lateral most region of these lobules is innervated by the dorsal accessory olive and the ventral lamina of the principal olive (Groenewegen and Voogd 1977; Kawamura and Brodal, 1980).

The precize coincidence between strips of vestibular Purkinje cells and climbing fibers in the dorsal uvula and pyramis is not known. The medial Purkinje cell strip is most likely influenced by subnucleus beta, which receives vestibular (Gerrits Voogd and Magras 1985) and cuneate Gerrits Voogd and Nas, 1985) information. The lateral strip by bilateral limb information relayed in the rostral MAO (Groenewegen and Voogd, 1977).

The present data support the view of Precht et al. (1979) that this part of the cerebellum is mainly concerned with the control of spino-vestibulo-spinal interactions. The targets of the Purkinje cells of the pyramis and dorsal uvula are the DV, MVmc and LV, which are characterized by a large proportion of premotoneurons of the vestibulospinal pathways (Kuypers, 1981; Kuypers and Maisky, 1975; Epema et al., 1990). In an electrophysiological study, Akaike and coworkers (1973) demonstrated that both LVST and MVST neurons could be inhibited by stimulation of the cortex of the pyramis and the dorsal uvula.

ANTERIOR LOBE VERMIS

In the anterior lobe, secondary vestibulocerebellar mossy fibers share a territory with input from the vestibular nerve (Gerrits et al. 1989), the central cervical nucleus (Wiksten, 1979; Matsuhita and Tanami, 1987; Matsushita and Okado, 1981)), and the cuneate nuclei (Gerrits, Voogd and Nas, 1985). When the body and head elements of the kinematic chain undergo a positional change, the labyrinthine receptors will signal movement or displacement of the direction of gravity. The information is thought to be transmitted in the primary and secondary vestibulo-cerebellar mossy fibers, and is available to Purkinje cells of different cortical zones that control the axial (zone A) and limb musculature (zones B, C1 and C2).

As it is the case in the caudal vermis, the anterior lobe contains Purkinje cells that project directly to the vestibular nuclei. Again with a partial dissociate nature with respect to the distribution of vestibular mossy fibers: the Purkinje cells have a far greater rostrocaudal distribution. It has been demonstrated that most of the LV neurons receive a projection from the B-zone. The LV give rise to the LVST, which descends to the spinal cord and completely by-passes the vestibular brainstem network. Moreover, the LV does not receive vestibular root fibers (Gerrits et al. 1989) and its neurons are not engaged in the intrinsic or commisural connections with other vestibular nuclei or in the vestibulocerebellar projection.

The Purkinje cells of the A-zone influence neurons in the MVmc and rostral DV which receive an input from intrinsic connections from the surrounding SV, MVc, and DV (Epema et al., 1988).

8 ABBREVIATIONS

AN	abducens nucleus
bc	brachium conjunctivum
cdc	caudal dorsal cap
CE	external cuneate nucleus
CI	internal cuneate nucleus
cr	restiform body
CO	cochlear nuclei
dl	dorsal leaf of the principal olive
DV	descending vestibular nucleus
DW	nucleus of Darkschwits
EW	Edinger-Westphal nucleus
F	fastigial nucleus
FLc	caudal flocculus
FLr	rostral flocculus
flm	medial longitudinal fascicle
gVII	facial genu
INC	nucleus interstitialis of Cajal
IO	inferior oblique division of OMN
IR	inferior rectus division of OMN
LP	levator palpabrae division of OMN
LR	motoneurons for lateral rectus eye muscle
LV	lateral vestibular nucleus
ME	medial extension of the ventral paraflocculus
MR	medial rectus division of OMN
MVc	caudal medial vestibular nucleus
MVmc	magnocellular medial vestibular nucleus
MVpc	parvocellular medial vestibular nucleus
NVpar	parabrachial vestibular nucleus
NVI	abducens nucleus
nVII	facial nerve
nVIII	vestibular nerve
OMN	oculomotor nucleus
PFLVc	ventral paraflocculus
PH	prepositus hypoglossal nucleus
rdc	rostral dorsal cap
rV	descending root of the trigeminal nerve
S	solitary tract and nucleus
sad	dorsal acustic striae
SO	motoneurons for superior oblique eye muscle
SR	superior rectus division of OMN
SV	superior vestibular nucleus
TN	trochlear nucleus
tu	uncinate tract
vl	ventral leaf of the principal olive
VM	trigeminal motor nucleus
VN	vestibular nuclei
VP	trigeminal principal sensory nucleus
x,y	groups x and y of Brodal

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10 SAMENVATTING

In dit proefschrift wordt een beschrijving gegeven van de verbindingen van de vestibulaire kernen met het cerebellum en de oogspierkernen. De projectie vanuit de vestibulaire kernen werd zowel met retrograad als anterograad getranporteerde tracers onderzocht. Het inleidende hoofdstuk geeft een indruk van de verschillende topografische aspecten van de vestibulocerebellaire, vestibulo-oculomotor en corticovestibulaire verbindingen.
In hoofdstuk 3, 4 en 5 wordt de vestibulocerebellaire projecties beschreven. Na injectie van mierikswortel peroxidase gekoppeld aan wheatgerm agglutinine (WGA-HRP) in het cerebellum werden retrograad gelabelde neuronen gevonden in alle vestibulaire kernen met uitzondering van de laterale vestibulaire kern. Injecties in verschillende delen van het cerebellum leverde steeds een vrijwel identiek verdelingspatroon van gelabelde neuronen in de vestibulaire kernen op.

Vervolgens werd de distributie van de mosvezels in het cerebellum bestudeerd met behulp van antegrade tracer technieken. In de afzonderlijke vestibulaire kernen werden injecties met verschillende tracers geplaatst nml getritieerd leucine, WGA-HRP en Phaseolus vulgaris lectine. De anterograad gelabelde mosvezels zijn bilateraal aanwezig zonder grote verschillen in lateraliteit. De gelabelde mosvezels waren zonder uitzondering steeds in dezelfde delen van het cerebellum te vinden. In de lobjes X en IXd van de lobus posterior, in lobje I van de lobus anterior en in de flocculus werden het grootst aantal gelabelde mosvezel eindigingen aangetroffen. De eindigingen zijn niet gelijkmatig over de cortex verdeeld maar tonen in de vermis van het cerebellum een voorkeur voor de proximale delen van de afzonderlijke lobjes. Het duidelijkst kwam dit tot uiting in de lobus anterior. Hier zijn de mosvezeleindigingen met name te vinden in de diepte van de cortex rond de interlobulaire fissuren. De mosvezel eindigingen vertoonden in alle experimenten een zekere mate van clustering in longitudinaal georienteerde strips. Deze clusters zijn echter niet continue over de opeenvolgende coupes.

In experimenten met WGA-HRP, welke tracer zowel anterograad als retrograad getransporteerd wordt, kan de relatie tussen de vestibulaire mosvezeleindigingen en de Purkinje cellen die op de vestibulaire kernen projecteren, bestudeerd worden. In tegenstelling tot de organisatie van de mosvezeleindigingen in de cerebellaire cortex, wordt de verdeling van de corticovestibulaire Purkinje cellen gekenmerkt door een hoge mate van ordening. De Purkinje cel projectie is georganiseerd in een aantal parasagittaal gerangschikte longitudinale zones. In de vermis van de lobus anterior zijn twee projectie zones te onderscheiden. Een tegen de mediaan lijn gelegen A-zone welke verbonden is met de grootcellige mediale vestibulaire kern en een lateraal gelegen B-zone welke verbonden is met de laterale vestibulaire kern. Corticovestibulaire Purkinje cellen in de lobus posterior zijn in de lobjes VIII en IX eveneens in twee zones gerangschikt en projecteren naar de laterale, de grootcellige mediale en de descenderende vestibulaire kernen. In lobje X en het ventrale gedeelte van lobje IXd zijn de Purkinje cellen te vinden in een min of meer continue strook over de gehele mediolaterale breedte van het lobje. Deze Purkinje cellen projecteren op alle vestibulaire kernen uitgezonderd de laterale vestibulaire kern. In de flocculus werd geen duidelijke longitudinale organisatie gevonden in onze experimenten.

Ofschoon er enige overlap aanwezig is tussen de distributie van corticovestibulaire Purkinje cellen en van vestibulocerebellaire mosvezel eindigingen komt uit ons onderzoek naar voren dat het organisatiepatroon van de twee systemen geheel verschillend is. Alleen in lobje X en IXd is er een colocalisatie van de vestibulaire mosvezeleindigingen en de corticovestibulaire Purkinje cellen, en kan men spreken over reciprociteit tussen de vestibulaire kernen en het cerebellum.

In hoofdstuk 6 wordt de vestibulo-oculaire verbindingen beschreven. Retrograde

labeling experimenten toonden aan dat alle vestibulaire kernen, met uitzondering van de laterale vestibulaire kern, op de oculomotor kernen projecteren. Het grootste aantal gelabelde neuronen werd aangetroffen in de superieure, de kleincellige mediale en het rostrale gedeelte van de caudale mediale vestibulaire kernen. In de anterograde labeling experimenten is het verloop van de vestibulo-oculomotor vezels goed te vervolgen, vooral in de experimenten waarin de tracer Phaseolus vulgaris lectine gebruikt werd. Axonen vanuit de superieure vestibulaire kern stijgen ipsilateraal op in het laterale deel van de fasciculus longitudinalis medialis en contralateraal in het brachium conjunctivum. Axonen van de mediale en descenderende vestibulaire kernen stijgen bilateraal op in het mediale gedeelte van de fasciculus longitudinalis medialis, ipsilateraal is het aantal axonen echter zeer gering. Het eindigingsgebied van de vestibulaire axonen in de oogspierkernen is niet beperkt tot enkele motoneuron groepen maar beslaan over het algemeen alle subdivisies van de oogspierkernen. De vestibulo-oculaire eindigingen vertonen echter een voorkeur voor enkele motoneuron groepen. Eindigingen in de oculomotor kern, afkomstig uit de caudale en grootcellige mediale en uit de descenderende vestibulaire kernen, waren met name in de motoneuron groepen te vinden die de medial rectus, inferior rectus en inferior oblique spieren innerveren. Eindigingen afkomstig uit de superieure vestibulaire kern zijn geconcentreerd in de ipsilaterale inferior rectus, medial rectus en superior oblique motoneuron groepen en de contralaterale superior rectus en inferior oblique motoneuron groepen. Dit wijst erop dat naast de primaire vestibulo-oculomotor verbindingen, andere ofschoon minder efficiente verbindingen bestaan die elk semicirculair kanaal met meer dan twee extraoculaire spieren verbinden.

CURRICULUM VITAE

Isabel Elisabeth Thunnissen werd geboren op 20 september 1955 te 's Gravenhage. Na het eindexamen Gymnasium B in 1975 werd een begin gemaakt met de studie Biologie aan de Rijksuniversiteit te Leiden. Het doctoraalexamen werd in 1983 afgelegd. Van juli 1983 tot mei 1984 was zij werkzaam bij de vakgroep Fysiologie van de Rijksuniversiteit Leiden . Van mei 1984 tot maart 1989 (tot augustus 1987 aanstelling via ZWO/Fungo, resterende periode in dienst van de Erasmus Universiteit te Rotterdam) was zij werkzaam als wetenschappelijk medewerker op de afdeling Neuroanatomie. Sinds maart 1989 is de promovenda verbonden aan de vakgroep Functionele Anatomie van de Rijksuniversiteit Utrecht.