CONNECTIONS OF THE VESTIBULAR NUCLEI IN THE RABBIT

Verbindingen van de vestibulaire kernen in het konijn

PROEFSCHRIFT

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Promotor:	Prof. Dr. J. Voogd
Co-promotor:	Dr. N.M. Gerrits
Overige leden:	Prof. Dr. H.F.M. Busch
	Prof. Dr. H. Collewijn
	Dr. E. Marani

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Cover: acetylcholinesterase stained section through the lateral and the magnocellular part of the medial vestibular nucleus. See chapter 2 for explanation

Aan Monique

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CONTENTS

1 INTRODUCTION

The vestibular nuclear complex forms a relay in the pathways that subserve the bodily functions of equilibrium. Information concerning the parameters of head movement and head position reaches the brainstem through the vestibular nerve. This cranial nerve is one of the main sources of input to the vestibular complex. Other major afferents include the contralateral vestibular nuclei and the cerebellum. Vestibular efferents reach the oculomotor complex, the spinal cord, the contralateral vestibular complex and the cerebellum.

Afferent, efferent and intrinsic connections of the vestibular nuclei form the subject of this experimental neuroanatomical study in the Dutch belted rabbit.

Subdivision of the vestibular nuclei

The vestibular nuclei are a group of neurons in the wall of the fourth ventricle, medial to the inferior cerebellar peduncle. The first description of the vestibular complex comes from Clarke (1861), who distinguished a medial and a lateral nucleus. Deiters (1865) described the large neurons in the lateral nucleus of Clarke. The term vestibular was introduced by Cajal (1896). He observed the bifurcation of the fibers of the vestibular nerve and traced their descending branches to a part which became known as descending vestibular nucleus. Bechterew (1885) studied the most rostral subdivision of Clarke's lateral nucleus (the superior vestibular nucleus) to which he traced the ascending part of the vestibular nerve (NVIII).

Nowadays most authors recognize four subnuclei in the vestibular nuclear complex, the superior (SV), the lateral (LV), the medial (MV) and the descending vestibular nucleus. For a review of the literature see Brodal (1984), who in addition described several subgroups in and around the vestibular nuclei (VN) (Brodal and Pompeiano, 1957).

There are different opinions in the literature on the extent of LV. Brodal and Pompeiano (1957) used the term lateral vestibular nucleus for the entire area of large neurons in the vestibular nuclei. Some authors restrict this term to the dorsal group of giant neurons, which do not receive a direct input from the 12vestibular nerve (Voogd, 1964; Van Rossum, 1969; Langer et al, 1985). The ventral part of Brodal's LV, which receives dense innervation from the vestibular root, Is included in the MV. It is called magnocellular medial vestibular nucleus (MVmc) (Voogd, 1964).

A detailed description of the vestibular nuclei of the rabbit using enzymhistochemistry of acetyl cholinesterase and cytochromoxidase will be provided in chapter 2.

The cerebellum and the flocculo-nodular lobe

Two parts can be distinguished in the paired primordia of the cerebellum. The parts are separated by the fissure that first appears during phylogeny and ontogeny of the cerebellum, the posterolateral fissure of Larsell (1970). The region caudal to this fissure has an intimate relation to the vestibular complex and is called flocculonodular lobe. The corpus cerebelli develops rostrally to the posterolateral fissure and receives several types of somatosensory input (tecto,

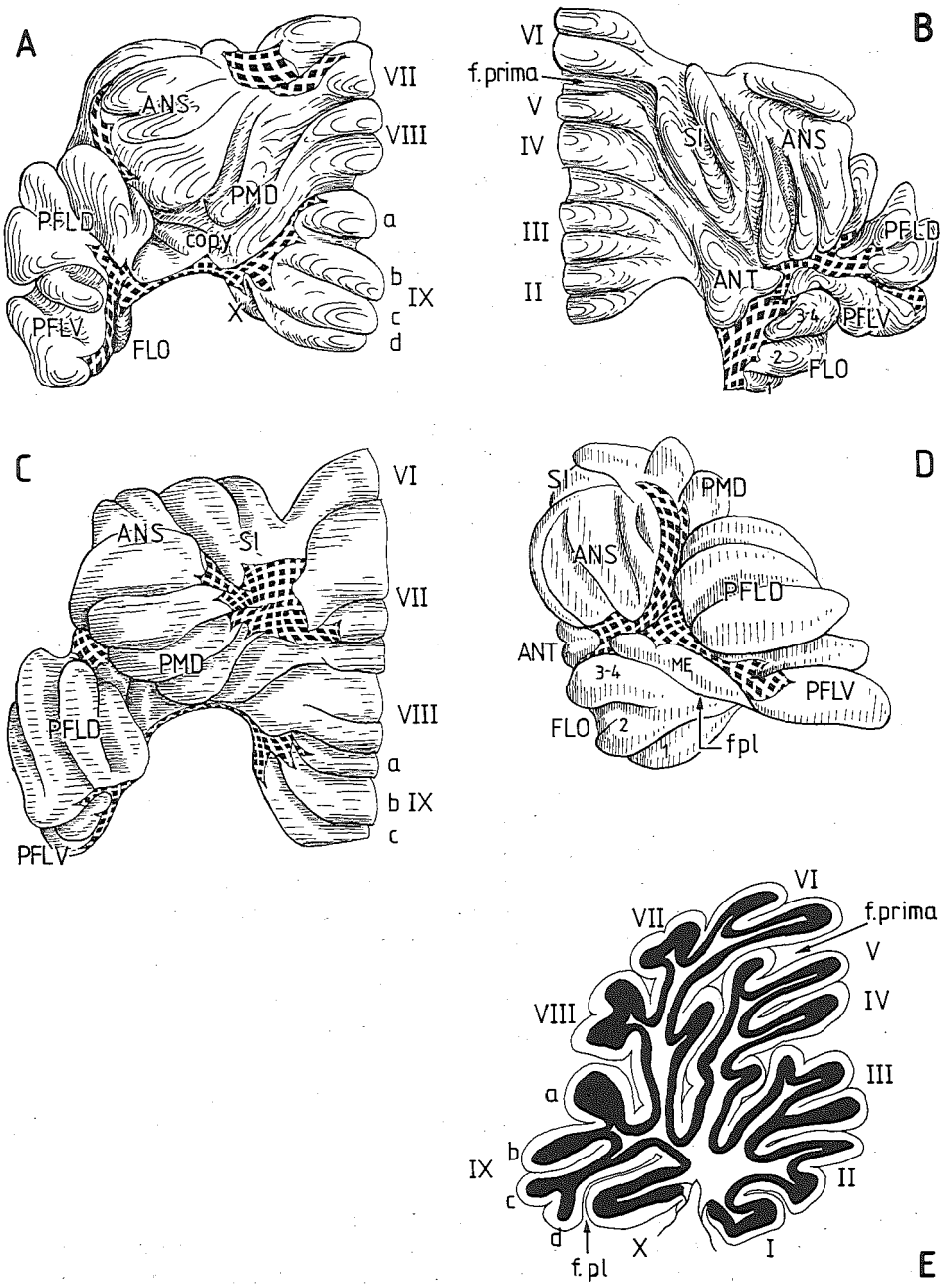

Fig 1.1 The cerebellum of the rabbit, viewed from caudal (A), rostral (B), dorsal (C), and lateral (D). The folia of the flocculus are labeled according to Yamamoto (1979). The lobules of the vermis are labeled I-X according to Larsell (1953) and also illustrated in a midsagittal section (E). Black squares indicate areas with absence of (or incomplete) cortex.

trigemino-, and spinocerebellar systems) (Larsell, 1934). Larsell used roman numbers I- X in his subdivision of the various lobules of the vermis of the cerebellum. The primary
fissure separates the anterior lobe from the posterior lobe. Lobules I-V constitute in the anterior lobe. The posterior lobe consists of lobules VI-X (Larsell, 1952, 1953). Corresponding lobules of the hemisphere were indicated with the prefix H(emispheric) to the roman numbered vermal lobules. In Larsell's terminology the most caudal lobule X is the nodule, and consequently the flocculus is HX. Lobule IX is the uvula and the paraflocculus is HIX. Flocculus and nodule, and paraflocculus and uvula are separated in most mammals (Voogd, 1967) by an area of white matter in the paramedian sulcus. The cortex of the vermis and hemisphere is interrupted at the base of lobules VI-VII to HVI-HVII (Fig 1.1).

Often the terms flocculonodular lobule and vestibulocerebellum are used as if they are similar entities. In its early definition that part of the cerebellum which receives primary vestibular root input is designated as vestibulocerebellum (Ingvar, 1918). However, the extent of the vestibular nerve projection to the cerebellum has been disputed. Some authors using anatomical methods doubt whether primary vestibular projections to the flocculus exist (Langer et al, 1985; Korte, 1979). Others, using the method of retrograde transport of HRP (Kotchabakdi and Walberg, 1978), found a projection from the vestibular ganglion to almost the entire cerebellar cortex. Electrophysiological studies of Lisberger and Fuchs (1978) and Waespe et al (1981) were unable to detect direct vestibular projections to the flocculus. In chapter 3 the cerebellar projection of the vestibular nerve will be described followed by a more detailed discussion on the extent of this primary vestibular mossy fiber projection.

Afferents of the vestibular nuclei

Vestibular nerve projections

The vestibular nerve enters the VN between the restiform body and the spinal root of the trigeminal nucleus. Primary vestibular fibers bifurcate in an ascending and a descending branch. Some of the ascending fibers leave the SV to enter the cerebellum. This was first observed by Cajal (1911) and later confirmed in intraaxonal studies of identified root fibers (Sato, et al, 1989). 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, 1979) and tracer studies (Carleton and Carpenter, 1984; Kevetter and Perachio, 1986) extended this projection to the entire vestibular complex except for the LV. There are no data available on the vestibular nerve projection to the brainstem of the rabbit. In chapter 3 the results will be presented of tracer injections into the vestibular ganglion.

Commissural and intrinsic vestibular projections

The vestibular commissure was first mentioned by Cajal (1909). It was considered however, as the crossing root of the vestibular nerve projection. Several decades later degeneration studies disproved the existence of such a

connection (Rasmussen, 1932; Walberg et al, 1958). The importance and the extent of the commissural vestibular connection was demonstrated in the late sixties (Ladpli and Brodal, 1968). Two views emerged from the literature. In one a topical connection is proposed between the SV and DV of both sides (Carpenter, 1960; Ladpli and Brodal, 1968; Ito et al, 1985). The other favors a more diffuse organization of this projection, which would interconnect all vestibular subnuclei with the exception of LV (Carleton and Carpenter, 1983; Gacek, 1978; Pompeiano et al, 1978).

Early Golgi studies suggested the presence of intrinsic vestibular connections (Cajal, 1909; Lorente de Nó, 1933). Later Golgi (Haugli-Hanssen, 1968) and tracer studies proved the existence of an extensive system of intrinsic connections (Rubertone et al, 1983; Ito et al, 1985; Carleton and Carpenter, 1983). Prior to our studies (Epema et al, 1988), data on this projection in the rabbit were lacking.

Chapter 4 contains a paper on the commissural vestibular and intrinsic connections using retrograde transport of horseradish peroxidase (HRP).

Cerebellovestibular projections

Older investigations of cerebellar morphology mainly focussed on its subdivision into lobes and lobules (Bolk, 1906; Larsell, 1952) and on the relation between vermis and hemivermis (Voogd, 1975). More recent anatomical and physiological studies clearly demonstrated that cerebellar afferents and efferents are organized in longitudinal patterns (see Voogd, 1964; Voogd and Bigaré, 1980; Oscarsson, 1980; Ito, 1984 for reviews on this subject).

The cerebellar cortex consists of three layers and is uniformly built. The outer molecular layer contains relatively few cells. It is the termination area of climbing fibers ,one of the two major types of cerebellar input, from the contralateral inferior olive. Climbing fibers terminate on the smooth surface of the dendritic tree of Purkinje cells, which are located in a monolayer at the basis of the molecular layer. The dendritic tree of the Purkinje cells and the climbing fiber termination show an orientation perpendicular to the transverse fissures. Purkinje cell axons project to the ipsilateral cerebellar and vestibular nuclei. The inner cell rich granular layer is the target of the second input system. This system consists of mossy fibers from different sources in the brainstem and the spinal cord. Primary and secondary vestibulocerebellar mossy fibers take their origin respectively from the vestibular nerve and the vestibular nuclei. Granule cells in the inner cortical layer have axons which bifurcate in the molecular layer and run parallel to the transverse fissures. Parallel fibers extend over the entire width of a lobule and thus form bilateral connections to the dendrites of Purkinje cells on which they synapse. The projections of the Purkinje cells and the climbing fibers are organized in identical longitudinal patterns. Purkinje cell strips that send their axon to a specific cerebellar or vestibular nucleus, receive climbing fibers from specific parts of the contralateral inferior olive (see Voogd, 1989 and Ito, 1984 for reviews).

Corticovestibular projection (=Purkinje cell projections) of the anterior lobe vermis are organized in longitudinal zones as has been demonstrated in degeneration (Van Rossum, 1969; Haines, 1976, 82), anterograde transport of WGA-HRP (Bernard, 1987) and retrograde transport of HRP studies (Corvaja

and Pompeiano, 1979, Voogd and Bigaré, 1980; Balaban, 1984). The middle A zone projected to the medial vestibular nucleus (MV) and the lateral B zone to the lateral vestibular nucleus.

The organization of the corticovestibular projection from the caudal vermis (lobules IX and X) is less clear. Axonal degeneration studies showed projections of lobules IX and X to SV, MV and DV (Dow, 1936; Angaut and Brodal, 1967; van Rossum, 1969). Some authors described a zonal organization of the Purkinje cells projecting to the vestibular complex (Epema et al, 1985; Matsushita and Wang, 1986; Shojaku et al, 1987). Other students found a great deal of in overlap in these projections (Bernard, 1987; Tabuchi, 1989).

Axonal degeneration (Voogd, 1964; Haines, 1977) and tracer studies (Langer et al, 1985) demonstrated flocculo vestibular fibers to all vestibular nuclei. A zonal origin of these Purkinje cell projections was advocated in the rabbit using HRP (Yamamoto and Shimoyama, 1977). Three or four zones were distinguished, which projected to the SV, the MV, the SV and the group y. Injection of tritiated leucine were included in the present study to determine the termination of the flocculus projection in the vestibular nuclei. These data will be compared with the results of similar injections into the caudal vermis and the fastigial nucleus. Injections of HRP and WGA-HRP into the vestibular nuclei were used to test the concept of a zonal origin of the Purkinje cell projections from the anterior lobe and the caudal vermis. The same experiments were used to map the extent of the fastigiovestibular projection.

Crossed and uncrossed pathways from the fastigial nucleus have been demonstrated to the LV, DV and MV (Carpenter, 1959, Walberg et al., 1962). Later axoplasmic transport studies proved that the projection to LV was due to interruption of passing fibers (Batton III et al, 1977).

Chapter 8 contains a study of cortico and nucleo vestibular projections in the rabbit.

Efferents of the vestibular nuclei

Secondary vestibulocerebellar mossy fiber projection

The vestibular nuclei are not only a target of the cerebellum, but also a source of mossy fibers to different parts of the cortex. Degeneration studies demonstrated major termination in the flocculus and caudal vermis (Dow, 1936; Ingvar,1918). Retrograde cell lysis studies found the origin in MV and DV (Brodal and Torvik, 1957; Carpenter, 1960). Later HRP studies demonstrated a contribution of the entire bilateral vestibular complex except for the LV (Kotchabhakdi and Walberg, 1978; Sato et al, 1983; Langer et al, 1985; Rubertone and Haines, 1981). It was not clear whether the neurons to the flocculus and the caudal vermis constitute separate populations or whether axonal branching between both targets occurred. The double fluorescent tracer technique (Kuypers et al, 1980) is a unique tool to solve this problem. A brief description of the afferent projection to the flocculus was provided by Alley et al (1975). Projections to the caudal vermis have not been studied in the rabbit. HRP injections in the caudal vermis are included in this study to map the origin of the secondary vestibulocerebellar projection. For more details regarding the

extent and the distribution of secondary mossy fiber projections in the cerebellum the reader is referred to Thunnissen (1990). She studied these connections with anterograde techniques. Branching of secondary vestibulo cerebellar mossy fibers to the caudal vermis and the flocculus is analysed in chapter 5.

Oculomotor projections

Since the description of the vestibulo ocular reflex (Lorente de Nó, 1933), which in its basic form consists a three-neuronal reflex arc, many studies were devoted to clarify this elegant model. The ganglion cells of the vestibular nerve are the first link, and the actual motor neuron in the extraoculomotor nuclei constitutes the final leg of this pathway. The middle leg is located in the vestibular nuclei. Electrophysiological studies demonstrated a topographical projection from the vestibular complex to the oculomotor nuclei (see Ito, 1984 for review). Injections of HRP into the oculomotor nuclei showed a contribution of SV, MV, MVmc and DV. A more detailed account is provided by Thunnissen (1990).

Spinal cord projections

Vestibulospinal projections are found in separate pathways, the lateral vestibulospinal tract (LVST) and the medial vestibulospinal tract (MVST). The LVST takes its origin from LV (Nyberg-Hanssen, 1964) neurons and projects ipsilaterally, the MVST is a bilateral descending pathway (Busch, 1961). The MVST takes its origin from MV (Nyberg-Hanssen, 1964) SV and DV (Kuypers and Maisky, 1977; Peterson and Coulter, 1977; Leong et al, 1984). Intraaxonal HRP injections into identified vestibular neurons showed branching of neurons in the MVmc to the spinal cord and the oculomotor area, and of neurons to the oculomotor area and the contralateral vestibular nuclei (Graf and Ezure, 1986; Highstein and McCrae, 1988).

The overlap, as described above, between the distributions of vestibulo oculomotor, vestibulo spinal, vestibulo cerebellar and vestibulo commissural projections prompted a double labeling fluorescent tracer study to determine the relation between these groups of neurons. The results of this part of the study are described in chapter 7.

2 EXPERIMENTAL PROCEDURES

Anesthesia, surgery, injections.

The experiments were carried out on pigmented Dutch belted rabbits (body weight 2 - 2.5 kg). They received a premedication with Hypnorm (0.3 ml/kg) and atropine (0.05 mg/kg), and were placed in a stereotaxic frame with a rabbit head holder. General anaesthesia was maintained with a halothane-oxygen mixture administered through an endotracheal tube.

The cerebellar lobules of the caudal vermis and the vestibular nuclei were approached dorsally via an enlargement of the occipital foramen. In several cases the brainstem was injected using a ventral parapharyngeal approach.

The vestibular ganglion was injected or transected unilaterally using a ventral approach of the internal acoustic meatus. These injections and lesions were made under direct visual guidance.

Injections into the flocculus followed its exposure from the middle cranial fossa by removing the occipital lobe and an incision of the tentorium cerebelli. The oculomotor nuclei were injected after trepanation of the calvarium.

Tracers were injected into the fastigial nucleus from a dorsal direction passing through either the anterior lobe or the caudal vermis. For stereotactic procedures the coordinate system of Matricali (1961) was used (a lambda-bregma horizontal distance of 10.5mm).

HRP and WGA-HRP procedure

Pressure injections of HRP (Sigma type VI or Boeringer grade II; 33% in phosphate buffered saline) or 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 um) connected to a hydraulic system. To reduce unwanted spread of WGA-HRP, delivery started 5 minutes after placing the needle or pipette in position. Injections were made at a rate of 0.1 ul per 10 min. Iontophoretic delivery of HRP (2% in 0.01M phosphate buffer pH8.6; Graybiel and Devor, 1974) was performed with micropipettes (tip diameter 10-20 u) and a discontinuous current of 3-5 uA (Midgard current source) for 15-20 minutes. After the injection, the needle was left in place for an additional 15 minutes. Following a survival time of 2 days the animals were heparinized and transcardially perfused under deep anaesthesia with 0.5 l saline, followed by 1.5 l citrate buffer (0.1 M, pH 7.2) containing 1% formaldehyde and 1.25% glutaraldehyde and washed with 0.5 l citrate buffer (0.1 M, pH 7.4) containing 8% sucrose (Mesulam, 1978). The brains were embedded in 10% gelatin (Voogd and Feirabend, 1981) and transversely sectioned at 40 um 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. The WGA-HRP sections were processed in the same way as the HRP material (Epema et al., 1984).

The brain of a control animal was tested for endogenous peroxidase activity (Wong-Riley et al, 1976; Marani, 1981).

The sections were examined under bright and dark field illumination. Cells

were considered as labeled when they met the criteria described by Nauta et al (1974). Irregularly shaped neurons and those with diffusely stained somata without a granular reaction product were excluded.

Retrogradely labeled neurons and anterogradely labeled axon terminals were plotted with a x-y recorder coupled to the stage of a microscope or with camera lucida. The nomenclature of the vestibular and the oculomotor nuclei follows the description of Epema et al. (1988) and Murphy et al. (1986). The nomenclature of the cerebellum follows the description of Van Rossum (1969), Larsell (1970) and Yamamoto (1979; for subdivision of the flocculus).

To facilitate comparison of the distribution of labeled neurons in the different experiments they were plotted in horizontal diagrams of the vestibular nuclei. The diagrams (Fig 2.3) were prepared from the charted sections by orthogonal projection of the boundaries of the nuclei (Epema et al. 1988). For acetylcholinesterase (AChE) and cytochrom oxidase (CO) histochemistry see Marani (1986).

Fluorescent tracer procedure

Pressure injections of Fast Blue (FB) (Dr. Illing, KG; 3% in distilled water; Bentivoglio et al, 1980b), Nuclear Yellow (NY) (Hoechst; 1% in distilled water containing 2% dimethylsulfoxide; Huisman et al, 1982) and Diamidino Yellow (DY) (Dr. Illing, KG; 2% in distilled water; Keizer et al, 1983) were made with a micropipette (tipdiameter 25-50 um) attached to a hydraulic system.

Migration of NY out of the retrogradely labeled neurons into glial cells (Bentivoglio et al, 1980a) could be avoided by using appropriate survival times about one day (see tables). This was established in preliminary experiments. Animals receiving fluorescent tracer injections were operated twice because the optimal survival time for the two tracers differed.

The animals were killed with an overdose of Nembutal, heparinized and transcardially perfused with 1.5 l (2.7% NaCl) followed by 2 l citrate buffer (0.1 M, pH 7.2), containing 30% formaldehyde and washed with 1 l citrate buffer (0.1 M, pH 7.2) containing 8% sucrose. Except for the experiments in which NY was injected, the brains were embedded in 10% gelatin (Voogd and Feirabend 1981), and transversely sectioned at 30 um on a freezing microtome. One out of four sections was immediately mounted from distilled water and air-dried at room temperature in the dark. Sections were stored in light tight boxes at 4 °C. The presence of fluorescent cells was studied with a Zeiss Ploemopack fluorescence microscope equipped with a filter-mirror system providing an excitation wave length of 360 nm. Single and double labeled neurons (Fig 5.1) showed characteristics matching previously published data (Bentivoglio et al, 1980a; Keizer et al, 1983). The differentiation of the injection sites into zones I - III was according to the same authors. Overlap of the zones III of injections in the flocculus and caudal vermis did not occur in the central white matter of the cerebellum. Experiments with leakage of tracer into the ventricular system were excluded. The distribution of labeled neurons was plotted on a X-Y recorder (Hewlett Packard) connected to displacement transducers attached to the stage of the Zeiss fluorescence microscope. Fluorescent tracer sections were counterstained with Cresyl Violet after the mapping, to study the localization of labeled neurons and the architecture of the vestibular nuclei and the cerebellum.

To facilitate comparison of the distribution of labeled neurons in the different experiments they were plotted in horizontal diagrams of the vestibular nuclei. These horizontal diagrams (Fig 2.3) were prepared from the charted sections by orthogonal projection of the boundaries of the nuclei (Epema et al, 1988).

Tritiated leucine procedure

The stock solution of L(4,5-^{3}H)-leucine (specific activity $\pm$140 Ci/mM, concentration 1uCi/ul; Radiochemical Centre, Amersham, UK) was evaporated to dryness under a gentle flow of nitrogen at 37 °C, and redissolved in saline to a final concentration of $\pm$100 uCi/ul. Injections were performed in the same way as described for HRP. Following a survival of 7 days the animals were perfused transcardially under deep anaesthesia with 0.5 l saline followed by 2 l 4% formaldehyde. The brains were embedded in 10% gelatin and transversely sectioned at 24u on a freezing microtome. The sections were mounted on chrom-alum subbed slides, defatted in xylene, dipped in Ilford G5 emulsion and exposed for 8-12 weeks at 4 °C. The sections were then developed in Kodak D19 at 16-18 °C for 4 minutes, rinsed in aqua dest, fixed in 24% sodiumthiosulphate for 5 minutes, washed in running tap water for at least 30 minutes, and stained with Cresyl Violet. For the delineation of the effective injection sites, criteria previously described by Groenewegen and Voogd (1977) were used. The sections were studied under both bright and dark field illumination. Drawings of the sections were prepared using camera lucida or photographic techniques.

Lesion procedure

The vestibular nerve was transected using a ventral approach. After a survival of time of 4 days (C 1384) or 7 days (C 1371) the animals were killed by intracardial perfusion under deep nembutal anesthesia with 10% formalin. The brains were blocked, embedded in 10% gelatin, hardened in 10% formalin and sectioned in 24 m sections on a freezing microtome. The sections were impregnated with Nauta's method for degenerated axons (Nauta and Gygax, 1954) using an ammoniacal silver solution (Nauta and Gygax, 1951) and counterstained with Cresyl violet.

Construction of horizontal diagrams

To facilitate the comparison of the distribution of labeled neurons in the different experiments they were plotted in horizontal diagrams of the vestibular nuclei. Following the method described by Gerrits et al. (1984) the diagrams were prepared from the charted sections by orthogonal projection of the boundaries of the nuclei (Fig 2.3). On the charts of the transverse sections, lines were superimposed which were parallel to the midline raphe. The distance between the lines was equal to the distance between two consecutive plotted sections. The compartments were classed according to the number of labeled neurons in multiples of three, including a ninth class of all compartments containing 25 or more labeled neurons. The distribution of the classes in the horizontal diagrams is visualized by dots of increasing size. No correction was made to express the counts as the number of labeled neurons per unit of volume. Question marks in the diagrams indicate areas where labeling in neurons was

obscured by the fringe of the injection sites (see for instance figures of chapter 4).

Subdivision of the vestibular complex.

A reliable subdivision of the vestibular nuclei in the rabbit is a prerequisite for an accurate description of retrogradely labeled cells and the termination of vestibular afferents within the vestibular complex.

The first description of the vestibular nuclei comes from Clarke (1861), who distinguished a lateral nucleus (Äussere Kern) located within the juxtarestiform body (Stilling, 1843) and a medial nucleus (Innere Kern; nucleus triangularis) located medial to it. Large cells belonging to Clarke's lateral nucleus in the rostral part of the juxtarestiform body were described by Deiters (1865). The caudal part of Clarke's lateral nucleus contains the descending root of the vestibular nerve. In Roller's (1880) description of this nucleus these fibers are still considered as ascending and were supposed to originate from the dorsal column nuclei. Only after the origin of sensory components of cranial nerves from peripheral ganglia was established (Koelliker, 1891) the nucleus containing the descending bundles of the vestibular nerve became known as the descending vestibular nucleus (Cajal, 1896). Bechterew (1885) traced the ascending branch of the vestibular nerve to a small celled nucleus located ventral to the superior cerebellar peduncle and dorsal to Deiters' nucleus. Bechterew's nucleus constitutes the rostral pole of Clarke's medial nucleus, but its exact borders have since been disputed. According to Kohnstamm (1908, 1910/11) and Fuse (1912) the parvocellular part of the medial nucleus, located medial to the acoustic striae in the floor of the fourth ventricle should be included in Bechterew's nucleus. The portion of the medial nucleus located lateral to the acoustic striae contains a portion of large cells and therefore is often included in Deiters' nucleus. Most older authors recognized that its location outside the juxtarestiform body and its content of small-and medium sized cells set it apart from the large cells of Deiters' nucleus proper. It became known as the "y-nucleus" of Sabin (1897), the parvocellular part of Deiters' nucleus (Kohnstamm, 1908,1910/11) or the "Triangularis Anteil" of Deiters' nucleus of Fuse (1912). Vander Schueren (1914), who gave an accurate description of the vestibular nuclei of the rabbit, called it the magno cellular part of the medial nucleus (MVmc), a name we adopted for the present study. The rostromedial parvocellular part of the medial nucleus, which Vander Schueren incorporated in the superior vestibular nucleus of Bechterew, shall be considered as a separate subnucleus.

Most of these features were recognized by Brodal and Pompeiano (1957) in their subdivision of the vestibular nuclei of the cat. In addition they described several cell groups, which are associated with the vestibular nuclei, but do not receive fibers of the vestibular nerve (Walberg et al, 1958). The main part of the vestibular nuclei which does not receive vestibular nerve fibers is Deiters' nucleus. This fact was already recognized by earlier authors (Probst, 1902; Fuse, 1912 in dog and cat; Leidler, 1916; and Van Gehuchten, 1927 rabbit) and served as one of the main criteria to distinguish Deiters' nucleus from the MVmc, which contains a rich plexus of vestibular root fibers.

Brodal and Pompeiano (1957) distinguished several small cell groups in

addition to the classical subdivision of the vestibular complex (medial vestibular nucleus, MV; superior vestibular nucleus, SV; descending vestibular nucleus, DV; lateral vestibular nucleus of Deiters, LV). The group x is located at the border of DV and the restiform body. This caudal cell group receives ascending fibers from the lateral funiculus of the cord (Brodal and Pompeiano, 1957). The group f is a collection of medium sized neurons ventrocaudally in the DV. Group z is found more caudally, dorsal to the caudal pole of DV at its transition in the parasolitary nucleus. It is a relay in a proprioceptive spino thalamic pathway for the lower extremity (Landgren and Silfvenius, 1971).

Other small cell groups mentioned by Brodal and Pompeiano (1957) are the interstitial nucleus of the vestibular nerve (IN VIII) and the groups l and y. IN VIII is located among the fibers of the incoming vestibular root and was first identified by Meessen and Olzsewski in the rabbit (1949). The group l consists of small cells and is located dorsolateral to Deiters' nucleus. The group y is found at a slightly more caudal level dorsal to the restiform body and ventral to the dentate nucleus. Recently Mehler and Rubertone (1985) and Voogd et al (1985) pointed out that the term group y has been used to indicate different cell groups.

A compact group of small cells, closely applied to the dorsal surface of the restiform body, which is laterally continuous with the cochlear nuclei, is included or even identified with the group y (Gacek, 1977). According to Gacek (1969), Kevetter and Perachio (1986; see also Kuruvilla et al,1985) this cell group receives a projection from the sacculus. It gives rise to commissural connections to the contralateral vestibular nuclei (Gacek, 1978), but is not a major source for the ascending projections to the oculomotor nuclei (Gacek, 1977). It is sometimes referred to as the "ventral group y" (Carpenter and Cowie, 1985).

In some mammals, such as the cat and the rabbit, medium-sized fusiform cells of the y group are found among the fibers of the floccular peduncle, where this arches over to the restiform body. Ventrally these cells merge with the ventral group y, dorsally they may be difficult to distinguish from the lateral cerebellar nucleus. These cells were referred to as the "infracerebellar nucleus" by Gacek (1977) and as the "dorsal group y" by Carpenter and Cowie (1985). Mehler (1985) considered these nuclei as the bed nuclei of the floccular peduncle. According to Gacek (1969) the infracerebellar nucleus does not receive a projection from the vestibular nerve, but this conclusion has been contested (Carleton and Carpenter, 1984; Gerrits et al, 1989). The infracerebellar nucleus gives rise to a crossed ascending projection to the oculomotor nuclei (Carpenter and Cowie, 1985; Thunnissen, 1990) and to intrinsic vestibular pathways, but not to a commissural connection (Epema et al., 1988).

When the group y cannot be distinguished from the lateral cerebellar nucleus part of this nucleus may be included in the infracerebellar nucleus (Epema et al., 1988).

The group y constitutes the caudal border of a sheet of cells which is located dorsal to Deiters' nucleus, extending caudally and laterally from SV to the floccular peduncle where it is located in the rostral wall of the lateral recess. Formerly this sheet was indicated by Lorente de Nó (1933, nucleus vestibo-cerebellosus) as the main route of entry of primary vestibulo-cerebellar fibers

from the superior vestibular nucleus.

Group l of Brodal and Pompeiano (1957) which is located dorsolateral to Deiters' nucleus, corresponds to the compact, caudal pole of SV. It is part of this sheet, which then continues in the more loosely structured infracerebellar nucleus and the ventral group y. In monkeys a group l cannot be identified (Langer, 1985) but the caudal pole of SV protrudes laterally as a massive nucleus in the floccular peduncle, where it is usually indicated as the group y (Carpenter and Cowie, 1985; Carleton and Carpenter, 1984; Langer, 1985).

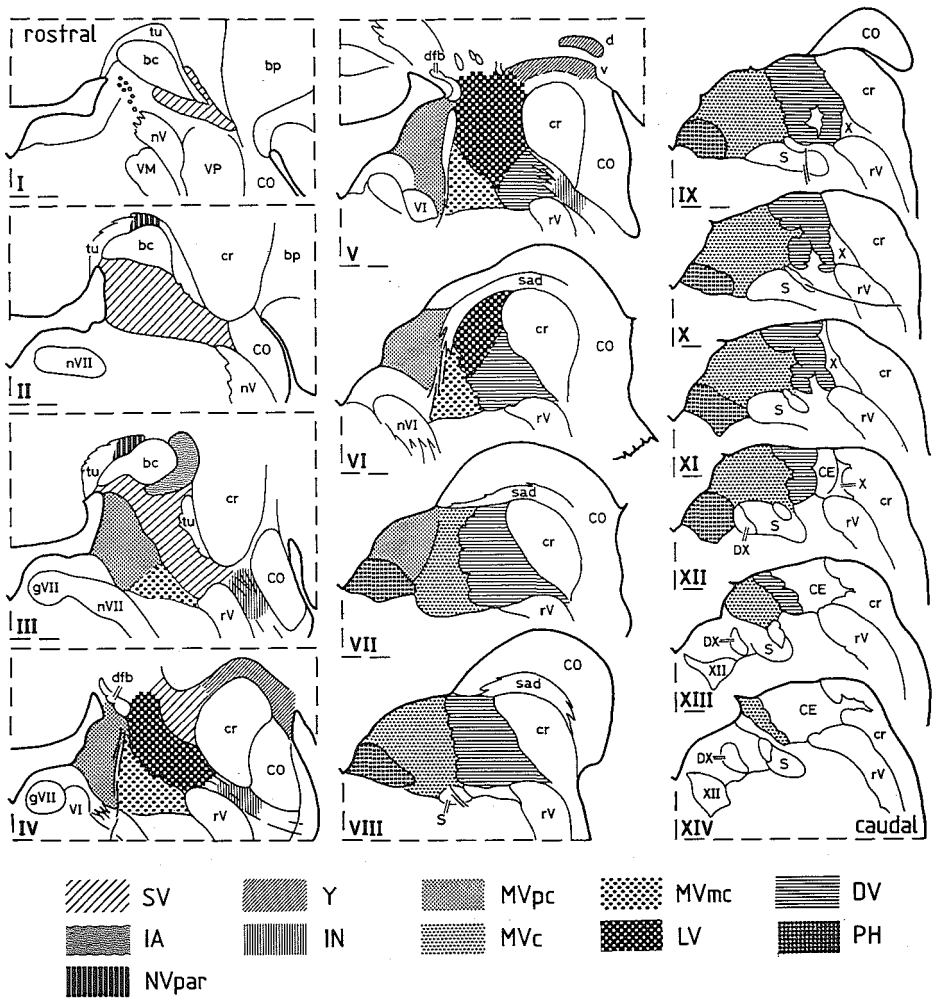

Fig 2.1 Diagrams of equally spaced, transverse sections through the vestibular complex. The left margin of the frames represents the midline. The interval between the frames is approximately 0.3 mm. Bar represents 0.1 mm.

The infracerebellar nucleus may be difficult to separate from the basal interstitial nucleus of Langer (1985). This term refers to a diffusely organized collection of cells in the monkey, which extends still more caudally, ventral to and among the cerebellar nuclei and between the white matter of the flocculus and the nodule.

The subdivision of the vestibular nuclei as used in this paper corresponds to the one used by Voogd (1964) for the cat and Van Rossum (1969) for the rabbit. It diverges from the subdivision of Brodal and Pompeiano (1957) in the use of the term lateral vestibular nucleus (LV) for the dorsal magnocellular portion of Deiters' nucleus only. The term magnocellular part of the medial nucleus (MVmc) as introduced by Vander Schueren (1914) is employed for the ventral part of the lateral vestibular nucleus of Brodal and Pompeiano's nomenclature. A comparison of alternate sections stained for myelin (Häggqvist method), Nissl, and for the enzymes acetylcholinesterase (AChE) and cytochrome-oxidase (CO) facilitates the identification of the subdivisions. The distribution of the commissural connections, and of the vestibular root projections as described in later chapters, provides additional evidence for this subdivision of the vestibular nuclei.

Subdivision of the vestibular nuclei in the rabbit: a comparison of cytoarchitecture with enzyme histochemistry of acetylcholinesterase and cytochromoxidase.

The lateral vestibular nucleus (LV) of Deiters is located in the center of the vestibular complex (Fig 2.1 levels IV-VI). Rostrally it is bordered by the superior vestibular nucleus (SV), caudally by the descending vestibular nucleus (DV). Fibers of the stria acoustica make up a part of its medial border. The juxtarestiform body, which contains a major contingent of Purkinje cell fibers from the vermis and the flocculus, enters LV at its dorsal side. Part of these fibers pass between the giant cells of LV and descend towards DV, where they descend in bundles located in its dorsomedial part. Ventrally the LV is bordered by the magnocellular part of the medial vestibular nucleus (MVmc) (Fig 2.1), which contains cells of different sizes, among which giant cells of the same size as in LV. In Nissl material it is difficult to draw the borders between LV and MVmc, and between LV and DV, which also contains some large cells in its rostral part. Neurons of LV and MVmc both react with AChE (enzymhistochemistry of acetylcholinesterase and cytochromoxidase is according to Marani, 1986), but the neurons in MVmc stain less intensely (medium) than the giant cells in LV (high AChE activity). In contrast the large neurons of DV are only slightly reactive for AChE (low) (see table 2.1). The neuropil of MVmc, which lacks the bundles of Purkinje cell fibers which traverse LV, shows a medium activity of AChE (Fig 2.2), whereas the activity in LV is absent. The neuropil in DV shows low amounts of AChE reaction product.

The vestibular nerve enters the vestibular complex between the restiform body and the spinal root of the trigeminal nerve, at the level of the junction of LV, MVmc and DV (Fig 2.1 level III). The fusiform shaped neurons of the interstitial nucleus (IN VIII), which are clearly visible in CO (Marani, 1986) preparations of the same level, are located between the incoming fibers of the vestibular root.

The superior vestibular nucleus of Bechterew (SV) (Fig 2.1) can be subdivided in three parts (Korte, 1979). A central region contains bundles of obliquely oriented fibers of the ascending root of the vestibular nerve and large neurons, which are larger than its medial and lateral parts.

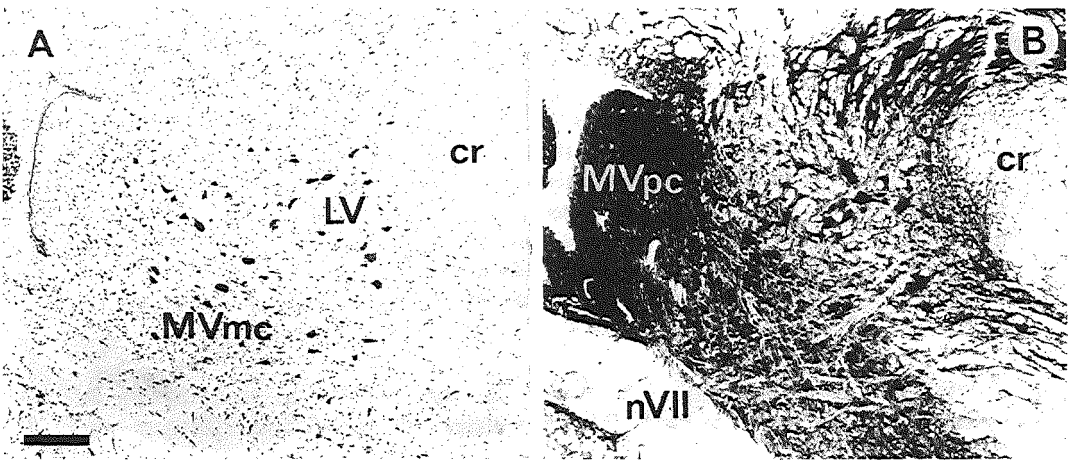

Fig 2.2A: Nissl stained section through the LV and MVmc illustrating the gradual cytoarchitectonic transition between the two nuclei. Bar represents 0.3 mm. B: Differential distribution of acetylcholinesterase (AChE) in the vestibular complex in a section adjacent to the one in A. The LV neurons are strongly positive in a negative neuropil. The entire MVmc is positive following AChE histochemistry.

The brachium conjunctivum (bc) and the anterior interposed cerebellar nucleus (IA), which folds laterally around it, form the dorsal border of SV. SV contains a high amount of AChE reaction product, which distinguishes it from the faintly staining IA. Dorsal to the bc and medial to the IA a densely AChE stained group of neurons is present, which is continuous with SV through and along the bc (Fig 2.1, level III). This group and the adjoining IA nucleus were incorporated in the VN in our recent study of commissural and intrinsic vestibular connections as the parabrachial vestibular nucleus (NVpar, Epema et al, 1988) (Fig 2.1 levels II and III). But it now appears that this term should be limited to the densely stained medial dorsal portion, which receives root fibers of the vestibular nerve (see below). SV and MV both contain AChE-positive cells in a darkly staining neuropil and are difficult to delimit. Large cells of MVmc which extend rather far rostrally and invade the ventral border of SV, are conspicuous in CO reacted material.

Group y consists of small cells located in the triangle between rostral LV, restiform body and cerebellar nuclei. They are continuous with SV rostrally and arch over the restiform body caudally (Fig 2.1 level IV-V). The neurons of group y stain heavily with CO, which distinguishes them from the central cerebellar

nuclei at their dorsal border. Ventral group y consists of a narrow, intensely AChE positive rim of cells, at the dorsal border of the restiform body. Fusiform neurons, belonging to the infracerebellar nucleus (Inf, i.e. dorsal group y) are located between the fibers of the pedunculus flocculi. Inf is located dorsally to the floccular peduncle and stains clearly in CO (see also table 2.1). In AChE stained sections Inf merges with the lateral cerebellar nucleus. Inf is compact and stains more homogeneously than ventral group y (Fig 2.1 level V, see also footnote for differences in AChE and CO within the group y area).

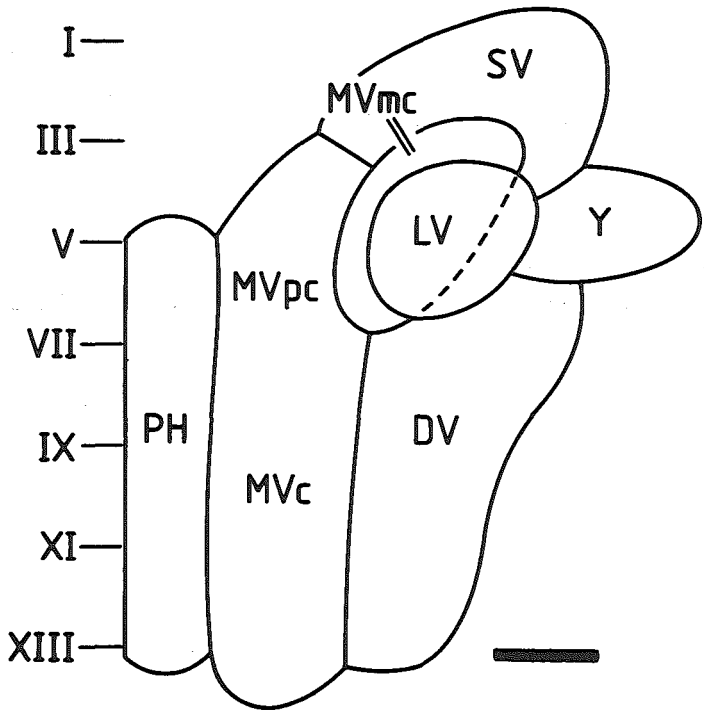

Fig 2.3 Horizontal diagram of the vestibular complex as a result of orthogonal projection of the boundaries between the individual nuclei. The IN, the INF and the NVpar are not indicated. Roman numbers refer to the sections of Fig 2.1. Bar represents 0.6 mm.

The medial vestibular nucleus (MV) extends over the entire length of the vestibular complex. It consists of the magnocellular (MVmc), parvocellular

(MVpc) and caudal (MVc) parts. MVmc extends, as a ventrally located column in LV, over a longer rostrocaudal distance than LV (fig 2.1). The stria acoustica divide the MVmc from the medially located MVpc. The medial border of MVpc lies beneath the ependyma of the fourth ventricle. The perikarya of MVpc are less reactive for AChE than those of the Mvmc. Rostrally MVpc and MVmc become located medial and ventral to the SV, respectively. The rostroventral border of MVpc is formed by the nucleus supragenualis and the abducens nucleus. MVpc neuropil reacts strongly for AChE and continues uninterruptedly into the neuropil of the abducens nucleus. Caudal to the genu of the facial nerve, where the large neurons of the MVmc have disappeared, the MVmc and MVpc merge into a single caudal portion of the medial vestibular nucleus (MVc).

At the same level, caudal to the genu of the facial nerve, the nucleus prepositus hypoglossi (PH) makes its appearance. In the Nissl stained sections a distinct border, consisting of an area containing relatively few, small cells, is present between MVc and PH. However, in AChE material this border cannot be identified. Within rostral PH a ventral region is more positive for AChE than its dorsal part, which shows a medium activity of both cells and neuropil.

The descending vestibular nucleus (DV) is characterized by a reticular neuropil, containing fiber bundles of the juxtarestiform body (Fig 2.1 level V-XIII), which are AChE negative. Small cells of group x (Fig 2.1 level X) are located between caudal DV, the rostral pole of the external cuneate nucleus and the restiform body. Group f forms a condensation of cells in the ventrocaudal part of DV. In CO preparations these cells, like group x, are heavier stained than the surrounding neurons of DV (group f is the white irregularly shaped spot in DV at level IX of Fig 2.1).

Table 2.1

Table	SV	MV	DV	LV	MVmc	x	y[1]	IN	N6	Cuex	PH
AChE											
Soma	+/-	+	+	+++	++	++	++	+++	++	++	+/-
Neuropil	+++	+++	+	-	++	+	-	+	++	-	++
CO	*	***	***								**
Soma	+,++	+,++	+,++	+++	++(+)	++	++	++	++	++	+
Neuropil	++	++	-	+/-	+	+/-	+/-	+/-	+	++	+

* SV: central medium sized neurons ++, rest and peripheral neurons +
** PH: ventral neurons ++, dorsal +
*** MV and DV: scattered in these nuclei some neurons were more heavily stained

Legend to table 2.1
Distribution of reaction product of acetylcholinesterase (AChE) and cytochro-oxidase histochemistry in the somatas (soma) and neuropil of the vestibular subnuclei, the cell groups x and f, the interstititial nucleus of the vestibular nerve (IN), the abducens nucleus (N6), the external cuneate (Cuex) and the prepositus hypoglossal nucleus (PH). The amount of reaction product is subdivided in high (+++), medium (++), low (+) and negative (-).

The groups of neurons dorsal and ventral to the floccular peduncle are sometimes referred to as infracerebellar nucleus (ICN; Gacek, 1977) and Y group. Mehler (1985) considers them as ventral and dorsal bed nucleus of the floccular peduncle and thus ventral and dorsal y. Differences in AChE and CO reacted sections support this subdivision.

[1]

	AChE	CO
y ventral soma	++	+
neuropil	-	+/-
y dorsal soma	+	+/++
(Inf) neuropil	+	+
in ped. flocculi soma	+	++
neuropil	-	-

3 THE PRIMARY VESTIBULAR PROJECTION TO THE BRAINSTEM AND THE CEREBELLUM IN THE RABBIT.[2]

INTRODUCTION

Brainstem projection

Golgi studies demonstrated that primary vestibular root fibers divide into ascending and descending branches. Ascending branches pass through ventral LV to SV, some continue into the cerebellum. The descending branches passed through DV and give off collaterals to MV. Primary vestibular afferents were present in all vestibular subnuclei (Cajal, 1909; Lorente de Nó, 1933; Hauglie-Hanssen, 1968). These observations were confirmed by intraaxonal HRP injections of individual vestibular fibers (Mannen, 1982; Sato, 1989)

Early degeneration studies showed distributions of the vestibular nerve within all subnuclei, except dorsal LV, peripheral portions of SV and caudal parts of MV (see Brodal, 1974 for review). The projection included cell group y and INT. In contrast a later lesion study by Korte (1979) demonstrated terminals in the entire SV and MV. Similar results were obtained in studies employing implants of tritiated amino acids into the ampulla (Carleton and Carpenter, 1984) or HRP injections into the receptor organs of the sacculus and posterior canal (Kevetter and Perachio, 1986).

Cerebellar projection

Silver impregnation of degenerated vestibular root fibers unequivocally demonstrated terminal degeneration of mossy fibers (mf) and mf terminals in the nodulus and uvula of the caudal vermis (Brodal and Hoivik, 1964; Carpenter et al, 1972; Korte and Mugnaini, 1979) in cat and monkey. However, in none of the cerebellar studies was a significant number of degenerated mf found in the flocculus (FL) and some authors (Korte and Mugnaini, 1979) were not convinced of the primary nature of the degeneration they observed in this lobule.

Retrograde tracing from the FL with HRP has yielded ambiguous results. Labeled vestibular ganglion cells were found in rabbit (Alley et al, 1975; Barmack et al,1986) and rat (Blanks et al, 1983), but absent in the monkey (Langer, 1985). In the rabbit only 3% of the ganglion cells could be labeled from the FL, against 70% from the caudal vermis (Barmack et al., 1986). Two recent studies employing anterogradely transported axonal tracers have not elucidated the question. Carleton and Carpenter (1984) described sparse to moderate termination in the FL in association with abundant termination in nodulus and uvula. As a tracer, they used a mixture of leucine and proline, the latter known to diffuse very rapidly from injection sites (Groenewegen and Voogd, 1977). Kevetter and Perachio (1986) used transganglionic transport of HRP in the gerbil and showed labeled mf in nodulus, uvula and lingula but did not mention presence or absence of termination in the FL. Examination of vestibular ganglia

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[2] Based on the paper The primary vestibulocerebellar projection in the rabbit: absence of primary afferents in the flocculus. N.M.Gerrits, A.H.Epema, A van Linge and E.Dalm; Neuroscience Letters, 105 (1989)27-33.

in rabbits which in the course of other studies in our laboratory received injections of HRP, wheatgerm agglutinin-HRP (WGA-HRP), or fluorescent tracers in the FL (16 cases), and the uvulo-nodular part of the caudal vermis (25 cases), revealed labeling of vestibular ganglion cells in the latter cases only.

The aim of the present study was to verify this observation with anterograde tracing.

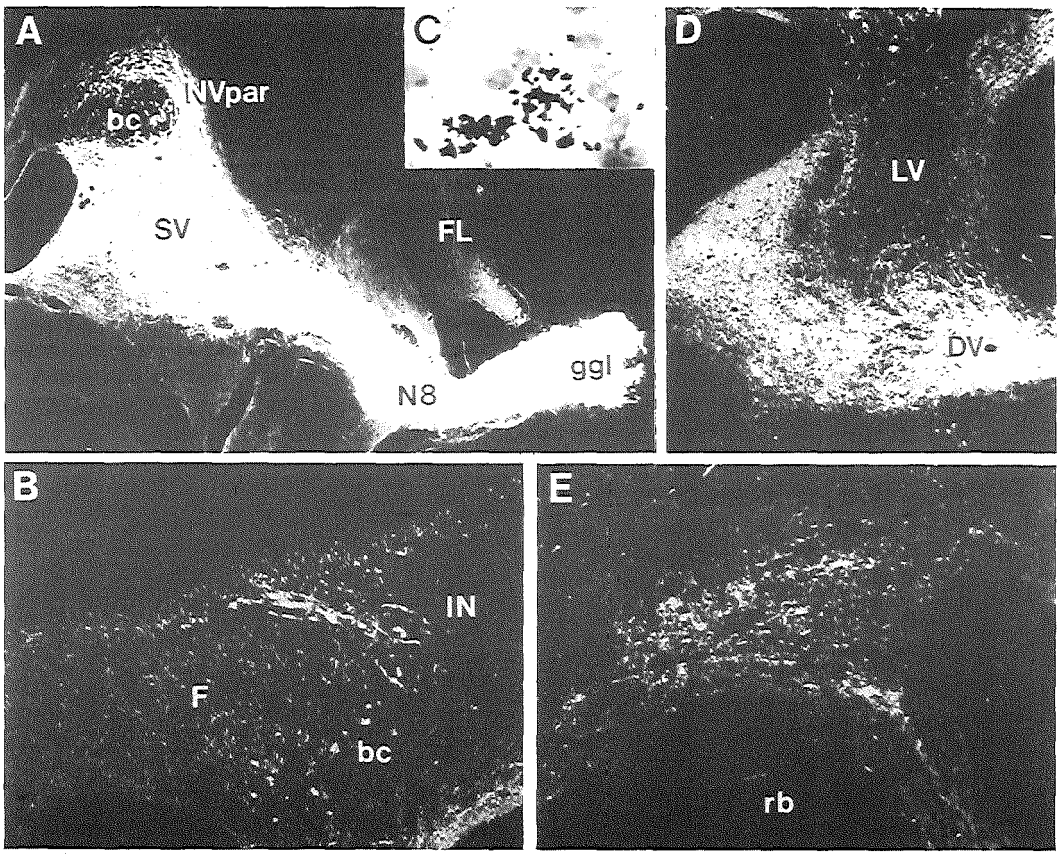


Fig 3.1 Labeling in the vestibular nuclei and the cerebellum ipsilateral to injection of ^{3}H-leucine in the right vestibular ganglion. A: Labeling in the vestibular ganglion (ggl), the vestibular nerve (N8), and the superior vestibular nucleus (SV) including its parabrachial subdivision (NVpar). Note the fibers running across and dorsal to the brachium conjunctivum (bc). The molecular layer of the flocculus (FL) shows a small herniation which has taken up some label, but neither labeled Purkinje cells nor other labeled structures were found in the FL (x 37.5). B: Labeled fibers ascending into the cerebellum running through the bc, and the fastigial (F) and interposed (IN) nuclei, spreading out widely dorsal to these nuclei (x 55). C: Labeled mossy fiber terminal in the granular layer (x 325). D: Labeled fibers and terminal ramifications in the descending (DV), and magnocellular (MVmc) and parvocellular (MVpc) medial vestibular nuclei. Note the absence of labeling in the lateral vestibular nucleus (LV) (x 55). E: Labeled fibers and terminal ramfications in group Y dorsal to the restiform body (rb) (x 90).

Tracer studies of the vestibular nerve projection used transganglionic transport techniques (Carleton and Carpenter, 1984; Kevetter and Perachio, 1986). We injected the tracers directly into the vestibular ganglion. Thus we tried to combine the high resolution power of the modern tracer techniques with an uncontaminated approach of the primary vestibular neurons. Although the rabbit is extensively used in vestibuloocular research, there is no complete description of the brainstem projection of the vestibular nerve in the rabbit. This will be included in the present study.

RESULTS

Brainstem projection

Seven cases with axonal labeling in the vestibular nerve and no leakage of tracer into the fourth ventricle are listed in Table 3.1. In addition two experiments with lesions of the vestibular nerve were available (Table 3.1).

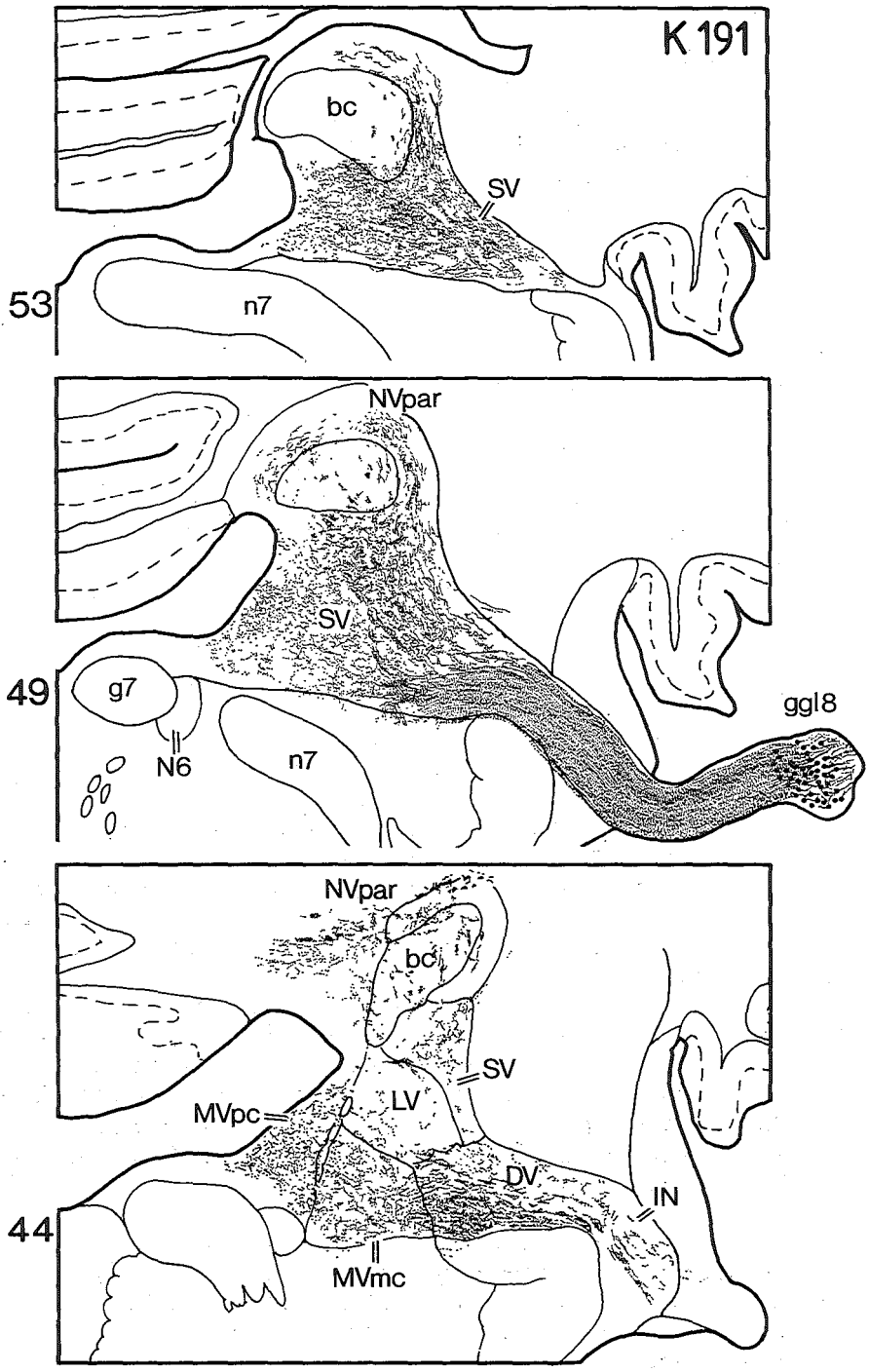
The distribution of vestibular root fibers in the brain stem was similar in cases with lesions of the vestibular nerve and in those with injections of either WGA-HRP or 3H-leucine injected into the vestibular ganglion. Labeling and degeneration in the brainstem were strictly ipsilateral.

Upon entering the VN between the restiform body and the trigeminal nucleus the vestibular nerve divided in ascending and a descending branches. This occurred in and lateral to the MVmc. Neurons belonging to IN VIII located between the entering root fibers (fig 3.2 section 44) were surrounded by densely labeled or degenerated neuropil.

Ascending fibers passed diagonally from lateroventral to dorsomedial through SV. From SV small and thick root fibers proceed in bundles throughout and around the brachium conjunctivum (bc) to enter the cerebellum. Dense labeling or fiber degeneration was present among the cells of NVpar located dorsomedially to bc (Fig 3.1 A; 3.2, 49). No signs of termination were found in IA. Both the central area of SV containing large cells and the peripheral small celled parts of this nucleus contained dense patterns of termination (Fig 3.1, A) (Korte, 1979), except for the most medial ventricular part of SV, which contained sparse labeling.

At the level of the division of the vestibular root numerous labeled fibers and terminals could be observed in MVmc (Fig 3.1 D). Some could be traced through this nucleus to enter the adjoining MVpc from medially. Most coursed ventral to the stria acoustica (sad). Some passed through this fiber bundle, sometimes even travelling in ventral parts of LV (Fig 3.2 section 40). We never observed termination in the LV. Labeling was present in the entire MVpc. From its dorsal tip a bundle of labeled fibers passed dorsal to LV to the caudal part of SV and the group y.

Labeling was clearly present in the ventral group y (Fig 3.1 E). Some fibers entered the floccular peduncle. They probably terminated on the typical fusiform neurons located between its fibers and in Inf. Fibers could not be traced towards the flocculus. Labeled fibers even penetrate in the area of the cochlear nucleus (see fig 3.2 37 and 32). It was not clear whether this area corresponds to the most lateral and caudal position of ventral group y or whether it belongs to the


K 191
bc
SV
53
n7
NVpar
SV
49
g7
N6
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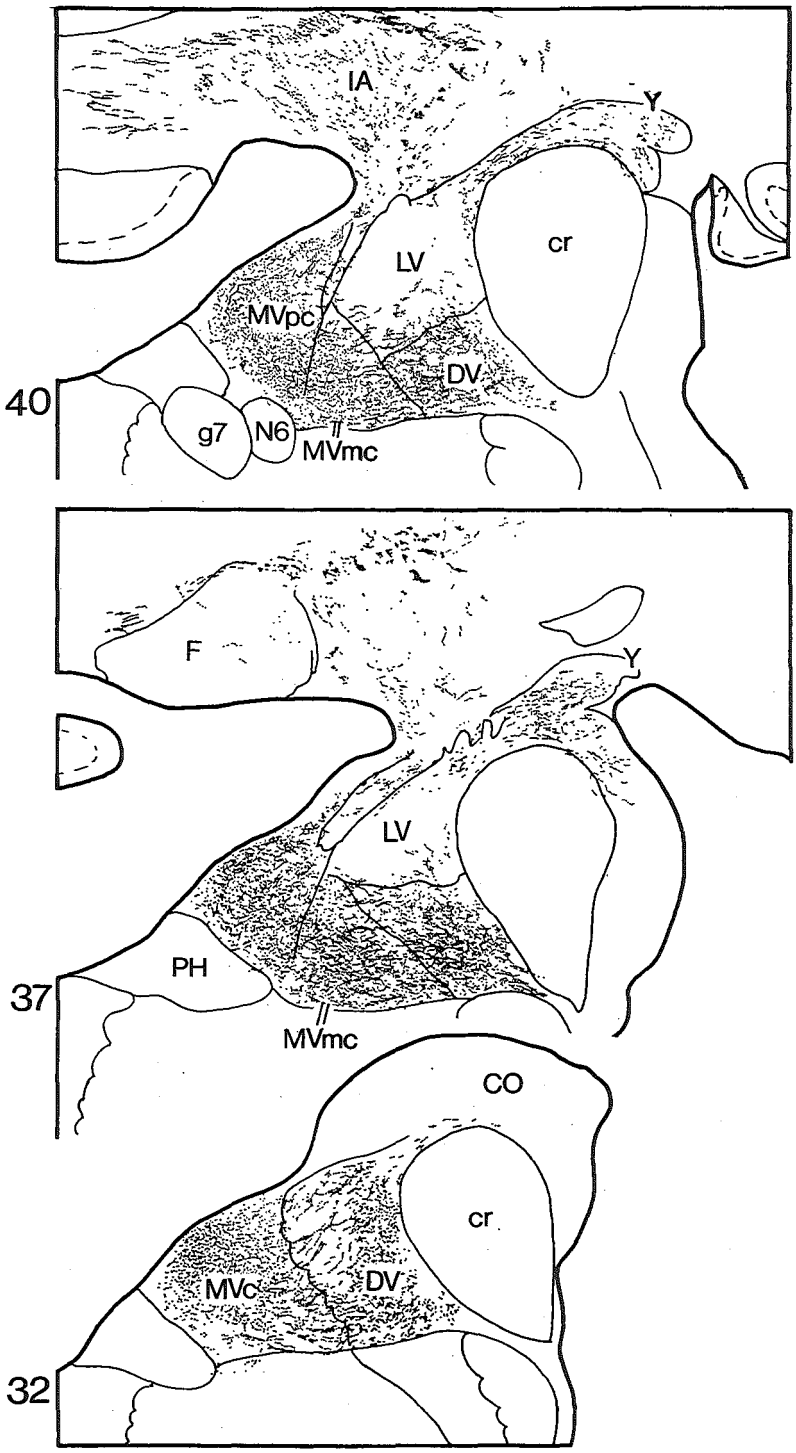
IA
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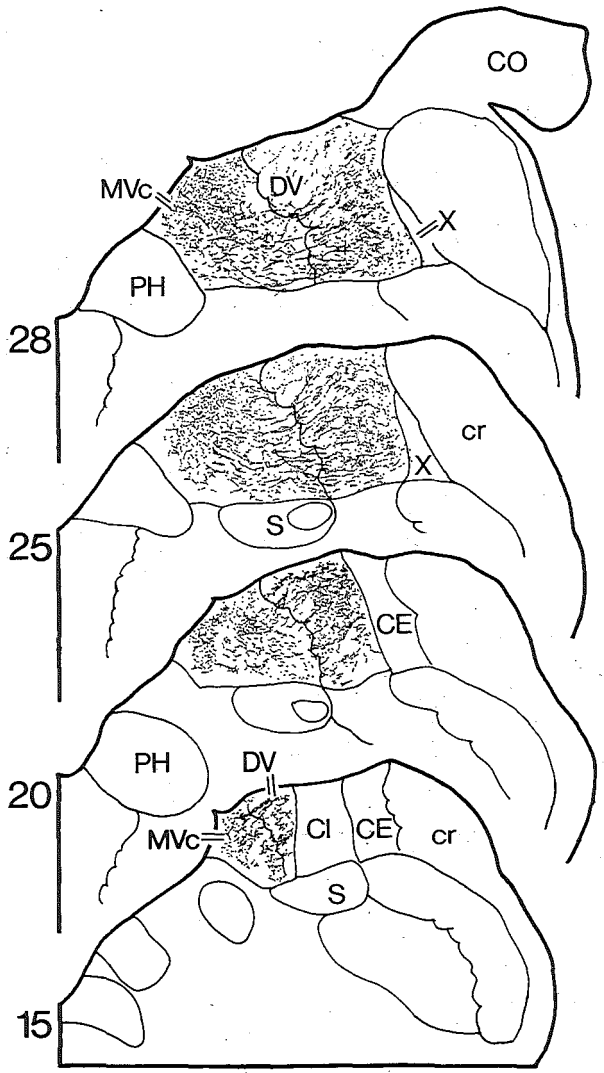

Fig 3.2 Labeled fibers and termination following injection of tritiated leucine in the right vestibular ganglion (K191), illustrated in transverse sections through the vestibular nuclei (3 pages).

cochlear nuclei.

The fibers of the descending root of the vestibular nerve were located in ventromedial DV but labeling was found over the entire neuropil between the fiber bundles of the nucleus. The fiber bundles within dorsal DV clearly remained unlabeled (Fig 3.2 section 28). At all levels labeled and degenerated fibers emerged and took a straight course to enter MV at its medial border. Massive termination was observed over the entire MVc. Labeling was absent from the prepositus hypoglossal nucleus and group x in all cases, nor could we confirm a primary vestibular input into the abducens nucleus.

Termination in all labeled subnuclei extended to their outer borders. In contrast the border zone of MVpc and MVc, and the PH was free from label. Cytoarchitectonically this zone corresponded to the small, relatively cell poor area between the PH and MV (see Fig 3.2 sections 20 and 25).

Termination in the brainstem outside the vestibular complex was only observed in WGA-HRP injection K58, in which the facial nerve was also damaged and had taken up a considerable amount of tracer. Here, labeled terminal axons were found in the subtrigeminal part of the lateral reticular nucleus and in the transition area between the DV and the rostral external cuneate nucleus (Korte and Mugnaini, 1979; Waespe et al, 1981).

Cerebellar projection

Most fibers that enter the cerebellum pass along the rostral border of the LV, ascend through the SV, and collect in thick bundles within and around the brachium conjunctivum (Fig 3.1 A). From here, the majority run in caudal direction and fan out as a thin sheet dorsal to the central cerebellar nuclei (Fig 3.1B). A minority of fibers enter through the caudal part of the LV and ascend through the fastigial and interposed nuclei (Fig 3.1B) without evidence of terminal branching (Korte and Mugnaini, 1979). Labeled fibers could be traced into the anterior and posterior lobe vermis bilaterally (Fig 3.3). In the anterior lobe small numbers of mf terminals (Fig 1C) were found close to the midline throughout lobules I and II and furthermore in the cortex lining the depth of the main fissures between lobules I - VI, largely concentrated in the primary fissure. In the caudal vermis many mf terminals were found ipsilaterally throughout lobule X and the cortex of lobule IXd lining the posterolateral fissure. Contralaterally smaller numbers were present in the medial one-third of these lobules.

DISCUSSION

Projection to the vestibular nuclei

The present study of brainstem projections of the vestibular nerve provides the first complete description of the primary vestibular projection in the rabbit. It confirms former degeneration (Korte and Mugnaini, 1979) and tracer studies (Carleton and Carpenter, 1984; Kevetter and Perachio, 1986) in other mammalian species. In agreement with former studies termination of the vestibular nerve was present throughout all vestibular subnuclei except LV. The groups x and f, the PH and the abducens nucleus also lacked silver grains. Dense

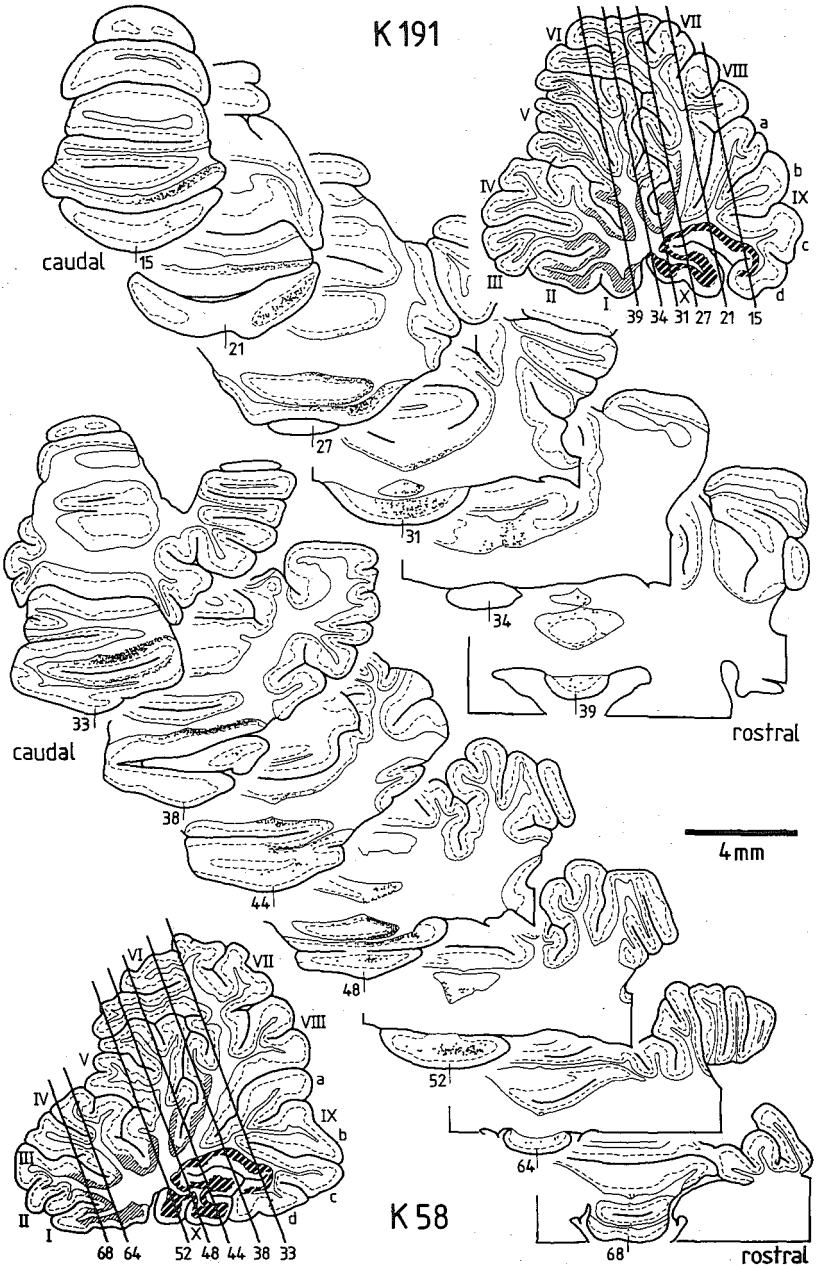

Fig 3.3 Labeled mossy fiber terminals in the cerebellar vermis following injection (right side) of ^{3}H-leucine (K 191) and WGA-HRP (K 58) in the vestibular ganglion. The distribution is shown in transverse sections (dots) and projected onto the reconstructed midsagittal planes (dark hatching: heavy labeling, light hatching: weak labeling). The position of the individual sections is indicated in the reconstructions. The Purkinje cell layer is shown as a broken line.

termination was found in the MVmc. The observed difference in vestibular nerve projection justifies in our opinion the distinction of LV and MVmc as separate nuclei (Voogd, 1964). Differences in AChE staining, in commissural and intrinsic vestibular connections,
and in secondary vestibulocerebellar projections (Epema, 1988 and 1990) provide additional data to support this subdivision.

The NVpar which we included in the VN in a study of commissural vestibular connections, clearly received vestibular nerve termination (Epema et al, 1988). In a double label fluorescent tracer study we demonstrated that some of the cells of the NVpar project both to the caudal vermis and the flocculus (Epema et al, 1990). It also contains neurons which project to the anterior lobe (Thunnissen, 1990). Thus in our opinion it is appropriate to include NVpar with the vestibular cell groups as described by Brodal and Pompeiano (1957).

The course of the vestibular root fibers within the VN is similar to that observed in former Golgi (Cajal, 1909), degeneration (see Brodal, 1974 for review older literature; Korte, 1979) and tracer (Carleton, 1984) studies. A striking feature is the course of the descending root through DV. Its localization in ventromedial DV is complementary to the trajectory of the Purkinje cell axons of the juxtarestiform body in the dorso medial parts of DV. Intraaxonal staining of electrophysiologically identified vestibular nerve fibers clearly showed the ascending and descending branches in the vestibular nuclei (Sato et al, 1989). Some collaterals of the ascending branch entered the cerebellum and could be traced into the fastigial nucleus without sign of termination (Sato ta al, 1989). The fiber trajectory to the caudal vermis in this study has a similar course. Thus part of the termination in the caudal vermis is a collateral of the vestibular nuclei branches. This pattern of primary vestibular fibers and their collaterals to the vestibular nuclei and the cerebellum is already present early in their development (Morris et al, 1988).

Cerebellar projection

This distribution in the vermis is in agreement with most of the previous data on the termination of primary vestibular afferents (Brodal and Brodal, 1985; Carleton and Carpenter, 1984; Carpenter et al, 1972; Kevetter and Perachio, 1986; Korte and Mugnaini, 1979; Kotchabhakdi and Walberg, 1978). The most notable discrepancy is that the contribution to the anterior and posterior lobe vermis is smaller in quantity and extent than suggested by retrograde HRP tracing (Kotchabhakdi and Walberg, 1978).

We have not been able to find labeled mf in the FL or the adjacent ventral paraflocculus as has been reported previously (Brodal and Hoivik, 1964; Carpenter et al, 1972), while termination in the vestibular nuclei and cerebellar vermis was always present. We therefore conclude that the FL region in the rabbit does not receive a primary vestibular projection. A similar conclusion was reached for the rabbit (Barmack et al, 1986), cat (Korte 1979), and monkey (Langer et al, 1985) using other anatomical methods.

The fact that degenerated mf terminals have been observed in the FL may very well be explained by non-specific trauma inflicted during surgery. Most significant in this respect is the description of concomitant degeneration around Purkinje cells and in the molecular layer of the FL (Carpenter et al, 1972). The

origin of FL climbing fibers however, is in the inferior olive (Alley et al, 1975; Gerrits and Voogd, 1982; Langer et al, 1985). Involvement of the vestibular nuclei, which were demonstrated to project to the FL bilaterally (Alley et al, 1975; Blanks et al, 1983; Brodal and Brodal, 1985; Epema et al, 1990), more specifically of the interstitial nucleus of the eighth nerve (Brodal and Brodal, 1985; Epema et al, 1990), could have led to degeneration (Korte and Mugnaini, 1979) or labeling (Carleton and Carpenter, 1984) of mf terminals or even to a bilateral projection (Brodal and Hoivik, 1964) to the FL. Spread of tracer into group y, which receives a distinct primary input (Fig 1E), can account for the observation of labeled ganglion cells in HRP studies of the FL in rabbit (Alley et al, 1975; Barmack et al, 1986) and rat (Blanks et al, 1988).

Electrophysiological studies of the vestibular input in the mammalian FL relied on anatomical connections that should have been subjected to a critical evaluation. As a result, an apparently inadequate paradigm for discrimination between primary and secondary vestibulocerebellar fibers was used, and it was concluded that both type of fibers terminated in FL and caudal vermis bilaterally (Precht and Llinas, 1969; Shinoda and Yoshida, 1975). Specific reference to the different characteristics of primary and secondary fibers is treated explicitly in only a few reports. Lisberger and Fuchs (1978) and Miles et al. (1980) described "vestibular-only" mf in the FL with a much lower resting discharge rate than might have been expected if they had been primary afferents. Waespe et al. (1981) stated that they did not record fibers which could clearly have been identified as primary vestibular afferents.

In many models that describe the influence of the FL on the modification of the vestibulo-ocular reflex (VOR), the required head velocity input is thought to be mediated by primary vestibular afferents (for a review see Ito (1984). The absence of such afferents in different mammals does not necessarily challenge the basic concept of the VOR hypothesis. However, it stresses the importance of the secondary mf projection (Epema et al, 1990) and the neuronal circuitry in which the vestibulocerebellar neurons participated.

TABLE 3.1

WGA-HRP			[^{3}H]Leucine			Lesion	
exp	vol	surv	exp	vol	surv	exp	surv
C1367	0.8	2	K131	0.2	7	C1384	4
K58	1.0	4	K190	0.4	2	C1371	7
K77	0.4	2	K191	0.5	7		
K87	0.4	3					

Legend to table 3.1
Listing of experiments (exp) with either injections of WGA-HRP or tritiated leucine into the vestibular ganglion, or lesions of the vestibular ganglion. Injected volumes in ul (vol) and survival times in days (surv).

4 COMMISSURAL AND INTRINSIC CONNECTIONS OF THE VESTIBULAR NUCLEI IN THE RABBIT: A RETROGRADE LABELING STUDY[3]

INTRODUCTION

The first mention of a vestibular commissure was by Ramón y Cajal (1909) who considered it as consisting of crossed vestibular root fibers. The existence of such fibers in the commissure was subsequently disproved in a number of studies using axonal degeneration techniques (Rasmussen 1932; Walberg et al. 1958; Stein and Carpenter 1967; Korte 1979). Ladpli and Brodal (1968) were the first to demonstrate the topology and the importance of the vestibular commissure as one of the major contingents of vestibular efferents. Their study in the cat was later confirmed and extended in experimental work using retrograde and anterograde tracers.

Lesion studies (Carpenter 1960; Ladpli and Brodal 1968; Tarlov 1969) and a recent retrograde transport study, employing HRP as a tracer (Ito et al. 1985) showed a large number of interconnections of the descending (DV) and the superior vestibular nucleus (SV) with their contralateral homonyms. Other HRP and tritiated amino acid studies observed a much more divergent projection of individual vestibular nuclei to all vestibular nuclei on the contralateral side (Gacek 1978; Pompeiano et al. 1978; Carleton and Carpenter 1983; Carpenter and Cowie 1985).

The investigation of the contribution of the lateral vestibular nucleus (LV) to the commissural connection has yielded ambiguous results. Some of the studies with lesions of the LV (Carpenter 1960; McMasters et al. 1966; Ladpli and Brodal 1968) showed axonal degeneration in the contralateral LV and DV. Later studies with HRP injections into various parts of the vestibular nuclei including the LV, failed to demonstrate HRP-labeled somatas in the LV in the cat (Gacek 1978; Pompeiano et al. 1978; Carleton and Carpenter 1983; Carpenter and Cowie 1985) and the monkey (Carleton and Carpenter 1983).

The Golgi studies of Ramón y Cajal (1909) and Lorente de Nó (1933) suggested the presence of ipsilateral intrinsic vestibular connections, which were later experimentally demonstrated by use of the axonal degeneration technique (Ladpli and Brodal 1968). Recent studies using retrograde and anterograde axoplasmatic transport techniques have confirmed this type of connections (Rubertone et al. 1983; Carleton and Carpenter 1983; Carpenter and Cowie 1985; Ito et al. 1985). Observations on intracellularly stained vestibular neurons support the earlier demonstration of intrinsic and commissural vestibular projections (Ishizuka et al. 1980; McCrea et al. 1980; Mitsacos et al. 1983).

Electrophysiological and pharmacological studies have mainly focussed on the role of the commissural vestibular system in the bilateral coupling of semicircular canal information in the control of extra-ocular muscles (see for a review: Precht 1978; Ito 1984). In addition a number of studies have been conducted in the rabbit on vestibular nuclear control of posture and movement

[3]Based on the paper Commissural and intrinsic connections of the vestibular nuclei in the rabbit. AH Epema, NM Gerrits and J Voogd: a retrograde labeling study. Exp Brain Res (1988) 71: 129-146

(Akaike et al. 1973; Highstein 1973a, b). There is, however, a paucity of anatomical data on the commissural vestibular connections in this species. The present study reports on the efferent vestibular connection to the contralateral vestibular nuclei in the rabbit using the HRP and the wheat germ agglutinated-HRP (WGA-HRP) techniques. The intrinsic vestibular projections were also studied in this material.

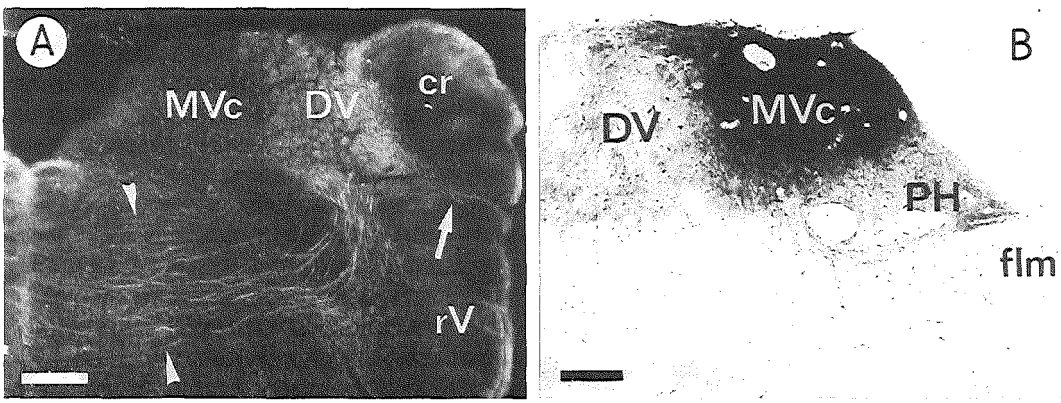

Fig 4.1A: experiment C1192, section 54 (DAB, neutral red counterstain); Darkfield photograph illustrating the course of HRP labeled commissural fibers. Internal arcuate fibers, originating in the DV and MVmc) run in parallel bundles in the dorsal reticular formation (between arrowheads). External arcuate fibers run through the ventral aspect of the restiform body (arrow) and continue their course along the lateral and ventral surface of the brain stem. Bar represent 0.5 mm. B: experiment C333, section 33; bright field photograph of a DAB stained section through the caudal part of the vestibular complex, illustrating the extent of the injection site in the medial vestibular nucleus. Bar represents 0.3 mm.

RESULTS

General comments

The various HRP and WGA-HRP injections cover all vestibular subnuclei. The material studied does not include injections exclusively restricted to the interstitial nucleus of the VIIIth nerve (IN) and the groups x, y and f.

All experiments involving injections in the subdivisions of the vestibular nuclei with the exception of those into the LV, resulted in labeled cells in the

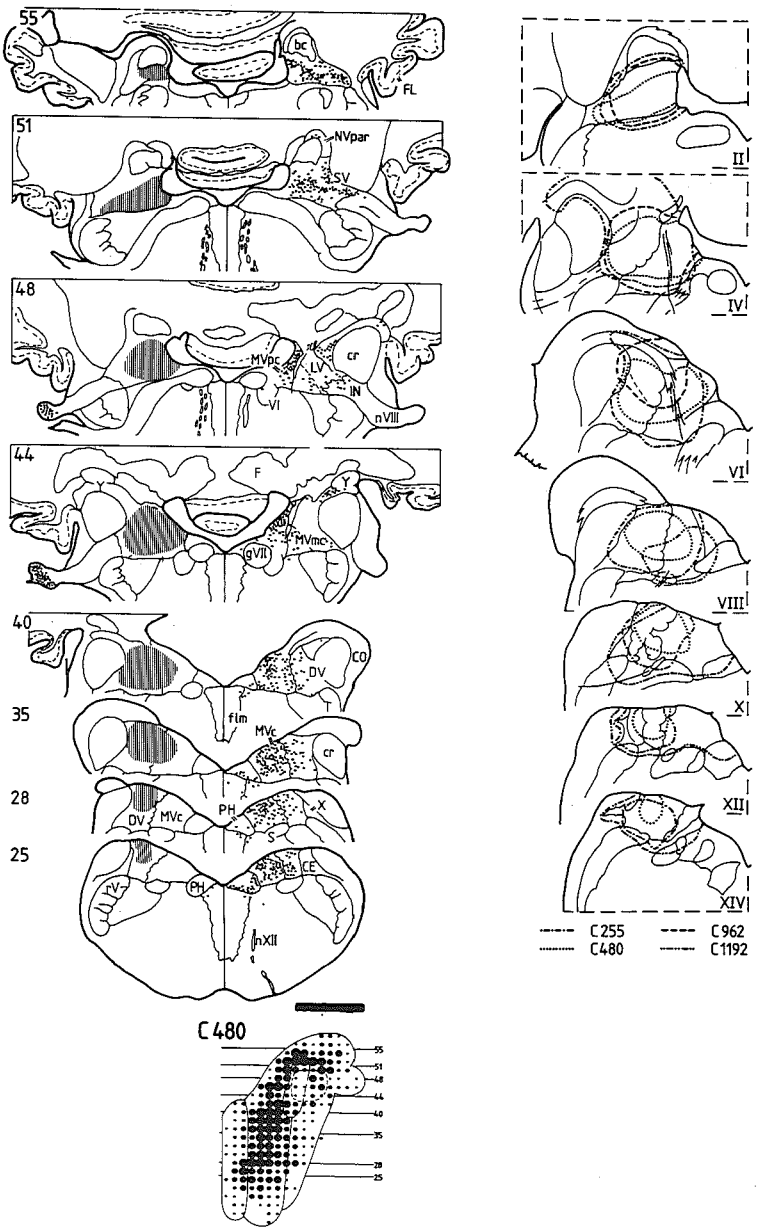

Fig 4.2 Experiment C480; transverse sections (left column) and horizontal diagram illustrating the extent of the HRP injection site in the SV, LV, MVpc, MVmc, MVc and DV and the distribution of HRP labeled cells in the contralateral vestibular complex (labeled cells on the ipsilateral were not plotted). Bar represents 2 mm. The right column shows the extent of the injection sites C255, C480, C962 and C1192 redrawn in semidiagrammatic sections taken from Fig 2.1.

ipsilateral and contralateral vestibular nuclei.

HRP is transported in both anterograde and retrograde direction. Retrogradely
labeled Purkinje cell axons located in the juxtarestiform body were frequently observed. Anterogradely labeled fibers were observed in the lateral vestibulospinal tract and the medial longitudinal fascicle (flm).

Anterograde transport and termination was clearly present after the WGA-HRP injections and occupied approximately the same area as the retrogradely labeled neurons. Retrograde labeling with WGA-HRP was not only found in somata but also in dendrites.

Course of commissural fibers

In general, commissural fibers of different calibre cross at the level of the injection site, but the crossing fibers follow different routes. Fibers emerging from the DV and the MV collect in the ventral border region of these two nuclei, and proceed medialward as parallel bundles in the dorsal reticular formation (Fig 4.1 C). Some fibers about to cross run through the ipsilateral flm. Most fibers enter and leave the midline raphe with a perpendicular deflection. In the raphe they run in a dorsoventral direction and reach the contralateral vestibular nuclei in a region similar to their point of origin. Fibers of the MVc take a more dorsal course than the fibers from the DV. Some of them leave the MVc dorsomedially and pass through the PH. Fibers from the medial SV cross in a similar direct manner in the dorsal reticular formation.

Other fibers from the caudal parts of the vestibular nuclei and the lateral part of the SV run in a ventral direction through the medial aspect of the restiform body (Fig 3.1 C), and cross as external arcuate fibers near the surface of the medulla. While some of them even cross through the nucleus reticularis tegmenti pontis, the majority passes between the inferior olive and the pyramidal tract. They reach the contralateral vestibular nuclei through the restiform body dorsal to the spinal tract of the trigeminal nerve and caudal to the entry of the vestibular nerve.

Following injections in the rostral half of the vestibular complex, labeled fibers were found to enter the contralateral SV via the cerebellum. They could be traced dorsal to the contralateral brachium conjunctivum.

Distribution of labeled neurons in the vestibular complex

Total distribution

Following injections of either HRP or WGA-HRP, covering a number of subdivisions of the vestibular nuclei (Table 4.1), retrogradely labeled cells were found in all contralateral subdivisions except the LV and the groups x and f. The WGA-HRP injections resulted in a dense pattern of anterogradely labeled fibers which occupied the same areas in the contralateral nuclei as the retrogradely labeled cells. Thus the contralateral LV is also devoid of anterograde labeling.

Representative of this group is experiment C480, in which the injection (Fig 4) covers the MVpc, the MVmc, lateral parts of the MVc, medial parts of the

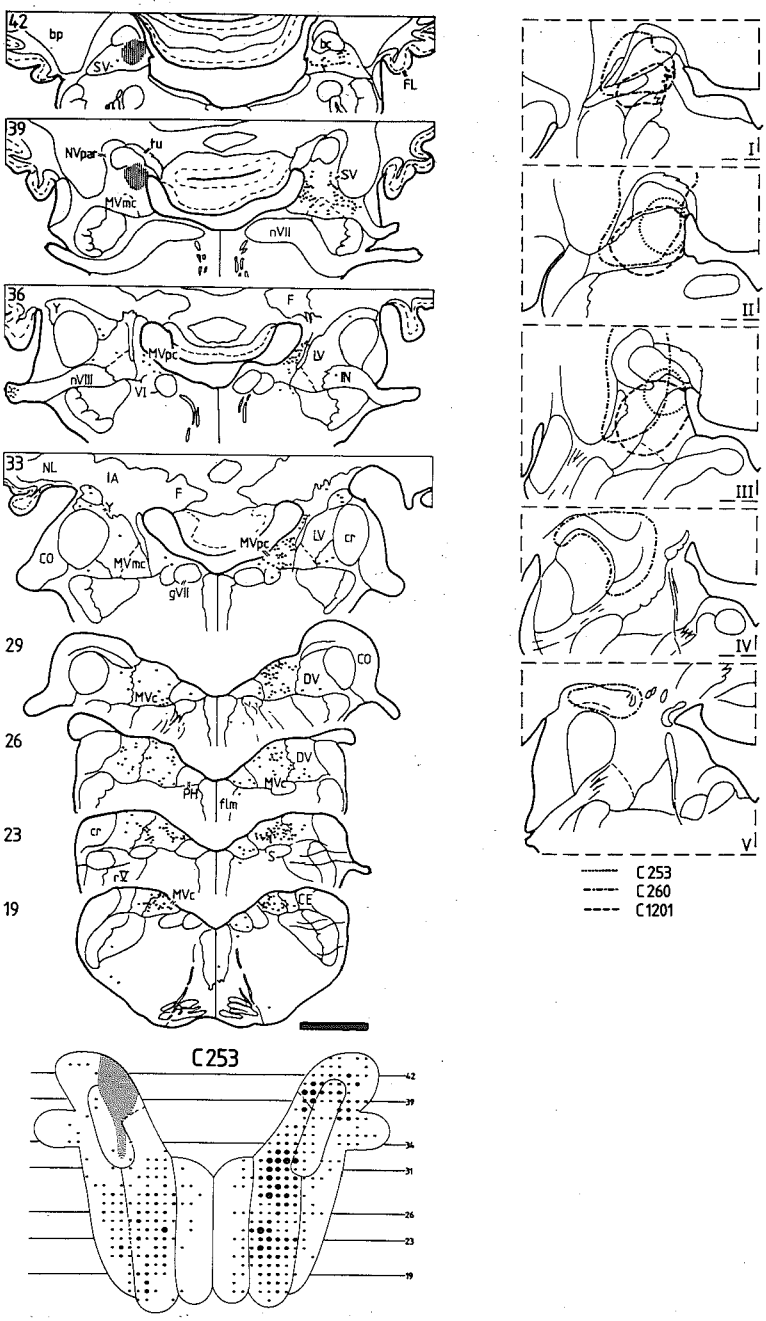

Fig 4.3 Experiment C253; transverse sections (left column) and horizontal diagram illustrating the extent of the HRP injection site in the SV and the distribution of labeled cells. Bar represents 2 mm. The right column shows the extent of the injection sites of the experiments C253, C260 and C1201 redrawn in semi-diagrammatic sections taken from Fig 2.1.

DV, most of the LV, and a medial portion of the SV. Ipsilateral labeled cells were not illustrated, because the extent of the injection site made accurate plotting difficult.

HRP reaction product at rostral levels (Fig 4.2, section 55) showed a clear preference for the small cells in the periphery of the contralateral SV. At intermediate[4] levels of the SV two clusters of HRP positive cells were present (Fig 4.2, section 51). One cluster is located dorsolateral within the SV; the other

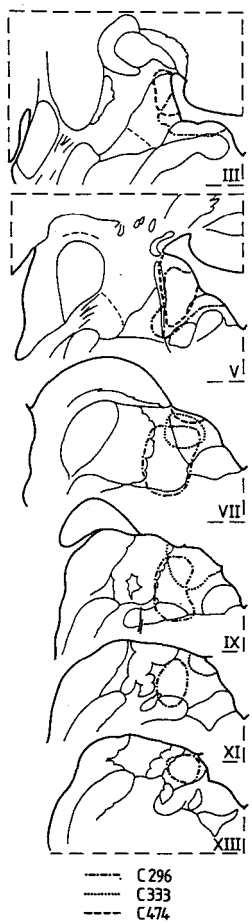

Fig 4.4 Injection sites of experiments C296, C333 and C474 redrawn in semidiagrammatic sections taken from Fig 2.1.

[4]The word intermediate is used to describe the localization of cells with respect to the rostrocaudal axis of a specific nucleus; e.g. intermediate between its rostral and caudal parts.

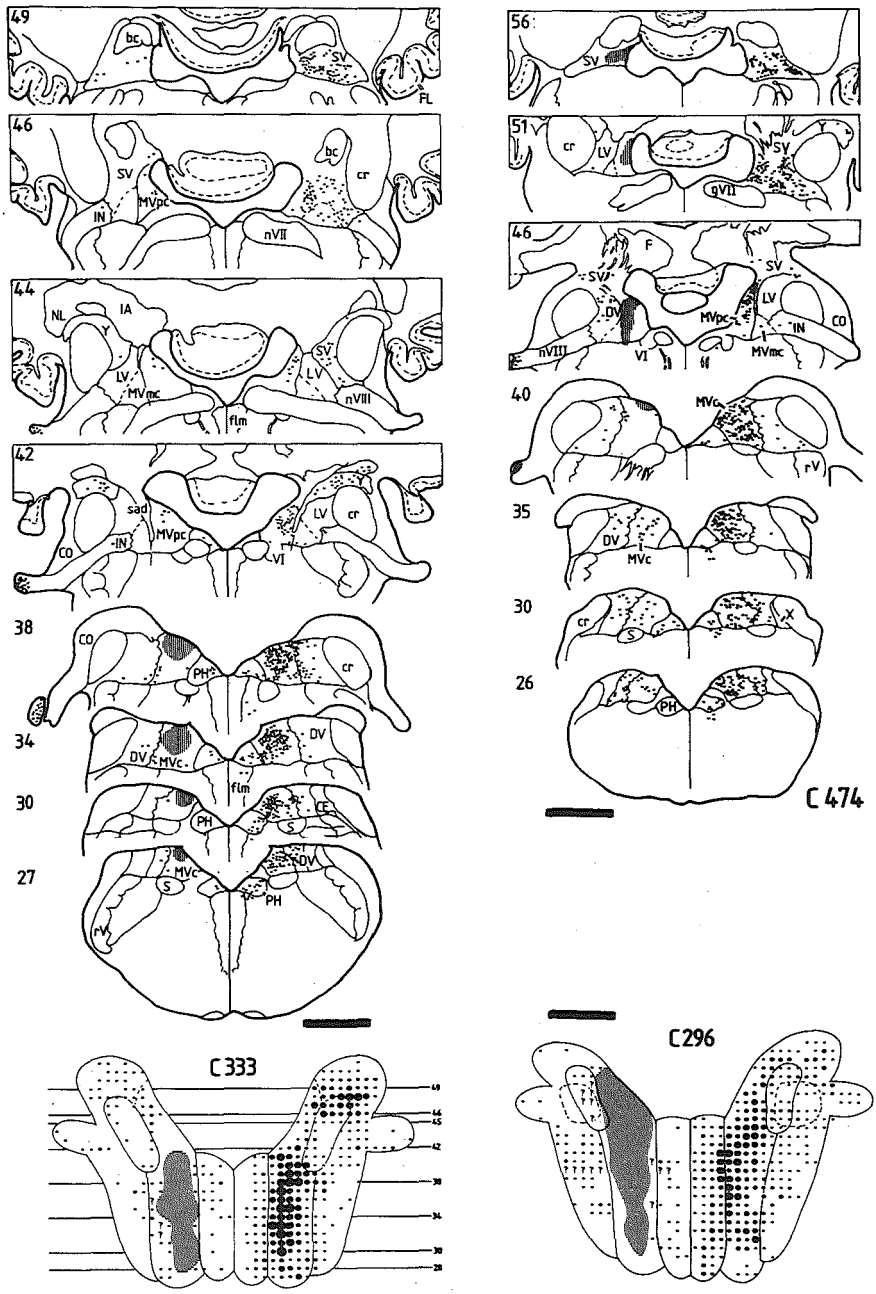

Fig 4.5 Experiment C474; transverse sections illustrating the extent of the HRP injection site in the MVpc and the bilateral distribution of labeled cells. Experiment C296; horizontal diagram illustrating the extent of the HRP injection site in the MVpc and MVc and the distribution of labeled cells. Experiment C333; transverse sections and horizontal diagram illustrating the extent of the HRP injection site in the MVc and the distribution of labeled cells. Bars represent 2 mm.

is located medioventrally and extends along the ventricle into the MVpc. Labeled neurons were more numerous in the dorsal part of the MVpc than in the ventral part. In the MVc they are more evenly distributed. HRP containing cells in MVmc were present throughout this nucleus and are small to medium-sized, the large cells were never labeled. Labeled cells in DV are located medially and ventrally, along
the border with the MVc.

The area between group y and the lateral cerebellar nucleus contains labeled neurons which may represent the INF. The ventral part of group y contains numerous labeled cells. Together with the cells in the adjacent part of the dorsolateral SV they form the rostral end of an area with labeled cells that extends, lateral to the LV, into the vestibular root (Fig 4.2, section 48). The IN contains fusiform HRP-positive cells. The groups x and f are completely devoid of labeled commissural cells. Labeling in the contralateral PH was mainly restricted to its ventrolateral periphery. However, in the most caudal levels of the PH labeled cells were also found in the central part of this nucleus. Moreover, labeled neurons were always present in the dorsomedial reticular formation and within the flm just outside the cytoarchitectonic borders of PH. These neurons had the same appearance as the HRP-filled neurons in the PH and were small to medium-sized. Labeled cells were not seen in the abducens nuclei. Dorsal to the brachium conjunctivum, labeled cells were present in the NVpar.

In the semiquantitative diagram (Fig 4.2) the labeled neurons in the contralateral vestibular complex appear to be concentrated in a rostrocaudal column which crosses the borders of the classical subdivisions. Rostrally this column comprises the MVpc and the adjacent caudomedial part of the SV. Caudalward, the column shifts in medial direction and includes the lateral PH.

Injections in the superior vestibular nucleus

The injection sites of experiments C1201 and C253 were restricted to the SV, with a slight involvement of the rostral MVpc. The injection site of C260 comprised the SV and group y (Table 4.1, Fig 4.3).

The distribution of labeled neurons was similar in the three experiments and will be described for case C253. The injection site in this case covers the rostromedial SV. Some reaction product was found along the needle track, which passed through the sad, but neurons close to the track were not labeled.

Retrogradely labeled cells were present throughout the contralateral vestibular complex, included the PH, but were absent from the LV and group x (Fig 4.3). Their distribution is roughly similar to that observed after complete injection of the vestibular complex. However, the labeled cells no longer form a single column, as was the case for the injections covering more than one subdivision. They occurred in three concentrations located at rostral, intermediate and caudal levels of the MV.

Numerous labeled small neurons were present along the periphery of the contralateral SV, concentrated in a ventromedial and a lateral cluster. A few medium-sized neurons were labeled in the most rostral part of the MVmc (Fig 4.3, section 39). A dorsally located column of labeled cells (Fig 4.3, sections 34-39) is present along the entire rostrocaudal extent of the MVpc. At intermediate

and caudal levels HRP labeled cells were also found in the ventral part of this subnucleus. In the DV labeled neurons are only present ventrally. The IN, group y and the INF contain some labeled neurons. Labeled cells in the PH are scarce and accompanied by a few cells in the adjacent reticular formation.

On the ipsilateral side almost no label was found in the SV and group y. In the MVpc and rostral parts of the MVc a few cells were present ventrally. The majority were located in the periphery of the MVc, including the border area with the DV. Labeled cells in the PH were few in number and confined to its rostral part.

As becomes evident from the horizontal diagrams, the distribution of labeled neurons on the ipsilateral and contralateral sides has a reversed pattern (Fig 4.3).

Injections in the parvocellular and the caudal medial vestibular nucleus

Three injections which were restricted to the MVpc and the MVc resulted in HRP positive cells in all contralateral vestibular subnuclei, again with the exception of the LV and group x.

The HRP injection in case C474 was made via a ventral approach to avoid damage of the caudal parts of the MV and covered the most rostral part of the MVpc with a slight involvement of the caudomedial SV. The injections in the cases C333 (MVc) and C296 (MVpc and MVc) were made through a dorsal approach and thus avoided all other brain stem structures (Table 4.1, Figs 4.4 and 4.5). The number and distribution of contralaterally labeled neurons in the cases C474 and C333 is remarkably similar, although there is only limited overlap between the injection sites. The majority of labeled cells are present in the contralateral MV and SV. In experiment C296 the labeled cells occupied a somewhat more medial position in the rostral MVc than in the other two cases. The number of cells in the SV was smaller. Labeling in the SV is largely absent from the center of this nucleus and concentrated in two groups, a lateral and a ventral one. Due to overprojection the groups are not visible in the diagram of experiment C296, but clearly illustrated in case C474 (Fig 4.5). Labeled cells in the MVpc are restricted to its dorsal part, those in the caudal MV are distributed throughout the nucleus. Labeling in the DV is limited to a few scattered cells. Labeled neurons are present in the ventral PH as well as in the bordering reticular formation.

Labeled neurons in group y in case C474 are less in number and occupy a more lateral position as compared to those in case C333. A few labeled cells were found dorsal to the group y in the latter case (Fig 4.5, section 42) in a region corresponding to the INF.

Labeling on the ipsilateral side is similar in the cases C296 and C474, although more extensive in the latter. Labeled cells were present in the MV, the DV and the PH and absent from rostral levels of the vestibular complex. The distribution of ipsilateral labeled cells in case C333 is almost opposite to the other cases and restricted to the rostral PH and the SV. This is clearly illustrated in the horizontal diagrams (Fig 4.5).

Injections in the descending vestibular nucleus

Three injections were restricted to the DV (C371, C372, C813). Although the

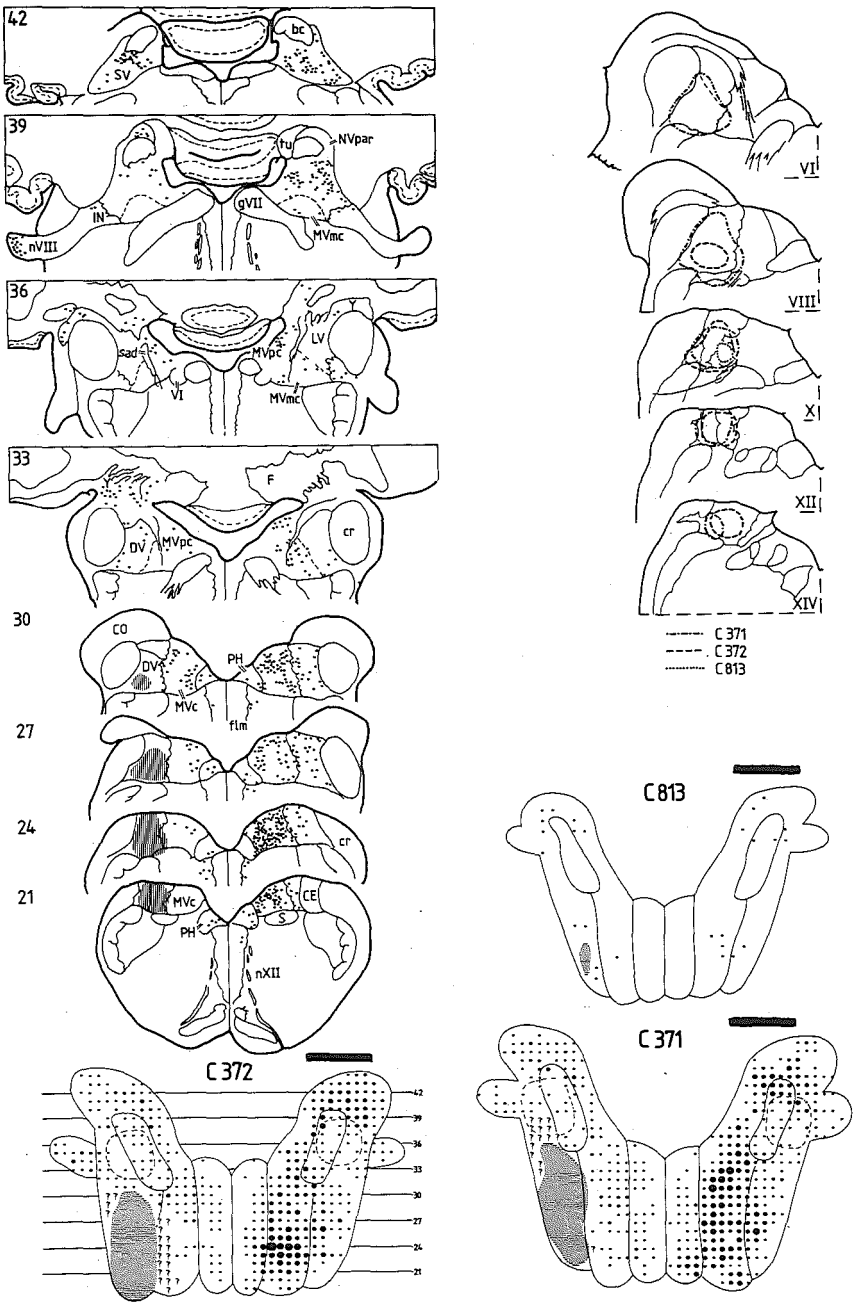

Fig 4.6 Experiments C371, C372 and C813; transverse sections and horizontal diagrams illustrating the extent of the HRP injection sites in the DV and the distribution of labeled cells. Bars represent 2 mm. The right column shows the injection sites of experiments redrawn in semidiagrammatic sections taken from Fig 2.1.

size and the rostrocaudal extent of the injection sites differed considerably (Fig 4.6) the distribution of labeled neurons in these cases was remarkably similar. In the experiments C371 and C372 all contralateral nuclei, except the LV and the groups x and f, contained HRP labeled cells. Many labeled neurons were present in the periphery of the SV, the NVpar and the MVc. A few cells were found in the ventral part of the MVpc at more rostral levels. The majority of labeled cells in the MVmc were located in its caudal part. Most of them were medium-sized; large cells were never found labeled. The distribution of the cells in the MV differs in the experiments C371 and C372 (Fig 4.6). The presence of labeled cells at more rostral levels coincides with the more rostral location of the injection site in case C371. Surprisingly few cells were found in the DV itself, but they were present throughout its entire extent. Labeling was distinct in the IN and in group y. The PH was almost devoid of labeled cells, but a considerable number was present bilaterally in the adjacent reticular formation and the flm. In experiments C813 with a very small iontophoretic injection in the ventral DV close to the border with the trigeminal nucleus few labeled cells were found, but the overall distribution was not different from the other cases (Fig 4.6).

Ipsilaterally the distribution of labeled cells in cases C371 and C372 differs considerably from that of the commissural neurons. Neurons in the SV were scattered throughout this nucleus with a concentration in a dorsal group. Labeled cells are also present in the NVpar. Labeling in the entire MV was far less pronounced than on the contralateral side. Labeled cells were observed in group y, in a more lateral position than the commissural ones. The distribution pattern on the ipsilateral and contralateral sides is more or less complementary.

Injections in the magnocellular medial and the lateral vestibular nuclei

The injections in a number of experiments involved both the MVmc and the LV to varying extends (C255, C501, C962; Table 4.1, Fig 4.7). In experiment C501, with an injection comprising the MVmc, the MVc and the DV, the distribution of HRP labeled neurons is very similar to the pattern described for large injections covering almost the entire vestibular complex (C480, Fig 4). Two small iontophoretic injections were almost completely restricted to the LV (C789) and the MVmc (C707). Experiment C789 resulted in very few labeled neurons on the ipsi- and contralateral sides (Fig 4.7). However, a large number of labeled Purkinje cells were present in a region corresponding to Voogd's (1964) B-zone of the anterior lobe of the cerebellum. Thus the scarcity of labeled neurons in this case cannot be due to a failure in the uptake of HRP. The very few labeled cells may be a result of a small leakage of HRP along the pipet track through the DV.

The injection in experiment C707 (Fig 4.7), which may include a number of cells in the ventral margin of the LV, resulted in bilateral labeling in most nuclei. Contralaterally, labeled neurons in the SV were restricted to its medial and lateral parts. HRP labeled somata were found scattered throughout all the subdivisions of the MV. Some neurons were present in group y and the IN. The labeling in the PH was located ventrolaterally along its entire rostrocaudal extent. On the ipsilateral side, labeled neurons are less in number and concentrated in a rostral and a caudal area. Rostrally, the SV contained a few cells in its medial and lateral periphery. Caudally, most of the labeled neurons

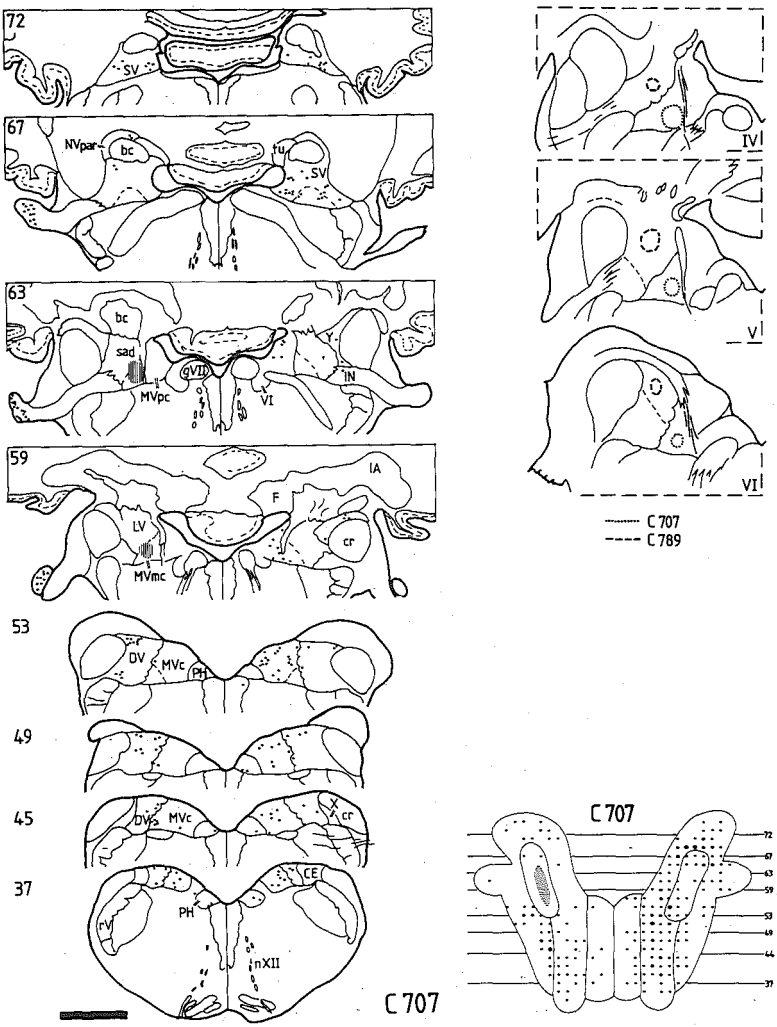

Fig 4.7 Experiment C707; transverse sections and a horizontal diagram illustrate the extent of the HRP injection in the MVmc and the distribution of labeled cells in the vestibular complex. Bar represents 2 mm. The right column shows the injection sites of experiments C707 and C789 redrawn in semidiagrammatic sections taken from Fig 2.1.

were found in the MVc. The labeling in the ipsilateral DV was situated close to the pipet track and could be the result of local uptake of HRP. Only one labeled cell was found in group y.

DISCUSSION

Course of the commissural fibers

The present material confirms the findings in previous investigations on the course of the commissural fibers in the cat and the monkey (Ladpli and Brodal 1968; Gacek 1978; Pompeiano et al. 1978; Carleton and Carpenter 1983; Carpenter and Cowie 1985), that these fibers tend to cross at the level of their origin.

It was also noted that the majority of fibers leave the vestibular complex along a short centrifugal course and do not pass massively through the medial vestibular nucleus. This decreases the risk of contamination by uptake of HRP in inadvertedly interrupted fibers. Of course such uptake must be envisaged and it could be responsible for part of the observed labeling in heteronymous contralateral subdivisions. While an accurate estimate would require anterograde tracing, the role of fibers of passage in the present results is probably small, since the distribution of labeled neurons did not differ significantly as a result of either small or large injections.

In addition to the external arcuate fibers which have been described in an anterograde tracing study in the cat (Gerrits et al. 1985), the transcerebellar route is of interest. These fibers offer an explanation for the persistent inhibition of contralateral type I neurons after midline lesions in the brainstem (Furuya et al. 1976). Similar brainstem lesions, followed by injection of HRP into the vestibular complex of the cat (Gacek 1978) resulted in labeling of contralateral vestibular neurons and could be accounted for by the transcerebellar or external arcuate paths.

Intrinsic vestibular connections

The pattern of intrinsic connections in the vestibular nuclei which emerges from the present study differs from the results of previous investigators with respect to its extent and the high degree of reciprocity between the SV, the MV and the DV (Fig 4.8).

The HRP study of Rubertone et al. (1983) in the rat already stressed the extent of the intrinsic network and its reciprocity by describing connections between SV and MV, SV and DV, and MV and LV. Other authors, using retrograde and anterograde tracer techniques described the predominant unidirectional character of the connections between the different nuclei in the cat (Pompeiano et al. 1978; Ito et al. 1985; Carleton and Carpenter 1983; Carpenter and Cowie 1985) and the monkey (Carleton and Carpenter 1983). Together with the present data, the findings of these previous studies are summarized in Fig 4.8.

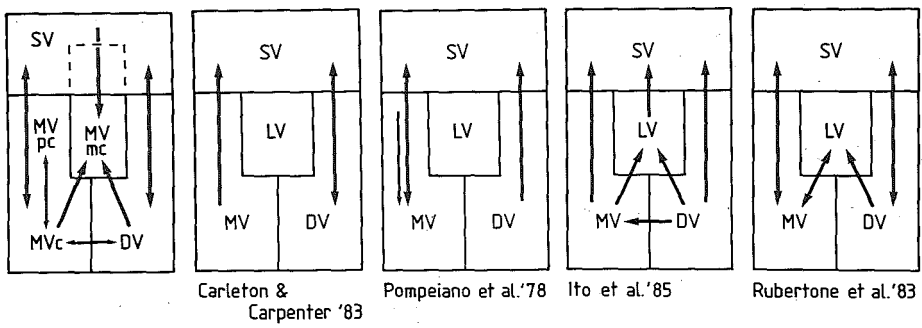

Fig 4.8 Diagrams of the vestibular nuclei summarizing the pattern of intrinsic connections from the present (left) and previously published investigations.

The present study supports a subdivision of the MV into the MVc and the MVpc, and the separation of the MVmc from the LV. The MVmc occupies a unique position because it receives input from all ipsilateral vestibular nuclei which it does not reciprocate. In line with previous studies (Rubertone et al. 1983; Pompeiano et al. 1978; Carleton and Carpenter 1983; Carpenter and Cowie 1985; Ito et al. 1985) we did not observe labeled Deiters' neurons. In one of their experiments with an injection into the DV Rubertone et al. (1983: their figure 2b) observed anterogradely labeled axons to the LV which terminated exclusively in its ventral part. This indicates that a distinction between a dorsal Deiters' nucleus and a MVmc could also be made in the rat. Ito et al.'s (1985) description of a projection from the MV and DV to the LV in the cat also support the distinction. All his HRP injections which resulted in labeling of neurons in the ipsilateral MV and DV, were located in Voogd's (1964) MVmc (Ito et al. 1985: injection sites 21, 22, 24, 26, 28).

Apart from the demonstration of connections between the individual subnuclei, Pompeiano et al. (1978) pointed towards intrinsic fibers connecting the rostral with the caudal MV. The present results demonstrate such a projection in the rabbit but show in addition that it is reciprocal.

Intrinsic connections from the MV to the DV have not been described before. Labeling in the MV in our cases with injections into the DV could have been caused by uptake of tracer in interrupted secondary vestibulocerebellar mossy fibers passing through the DV. However, direct projections from the MV to the DV were observed following intracellular HRP injection of MV neurons (Grant et al. 1980), and in Golgi studies (Lorente de Nó 1933).

Labeled cells were found in group y following HRP injections in the ipsilateral DV, MVc, MVpc, MVmc and SV. This confirms reports on connections of group y with the SV (Carpenter and Cowie 1985) the central MV (Pompeiano et al. 1978; Rubertone and Mehler 1980) and the MV and DV (Carleton and Carpenter 1983). No specific information is available in the

literature concerning the ipsilateral projections of the INF, the IN and the NVpar.

Commissural connections

The wide-spread distribution of SV efferents, with their origin in the ventral and lateral periphery of this nucleus, and with a principal projection to the contralateral MV is supported by results of previous HRP studies in the cat (Pompeiano et al.; 1978; Gacek 1978) and the opossum (Henkel and Martin 1977). However, a discrepancy exists with other studies in the cat and the monkey (Carleton and Carpenter 1983; Carpenter and Cowie 1985; Ito et al. 1985), which indicated a more restricted projection to the contralateral SV and adjacent MVpc.

Although small in number in any of our cases, the distribution of labeled DV neurons is comparable to the results of Pompeiano et al. (1978) and Carleton and Carpenter (1983). Gacek (1978) proposed a reciprocal relation between the DV's, although his illustrations show a much larger projection area. The limited participation of the DV may explain why Ladpli and Brodal (1968) with the degeneration technique and Ito et al. (1985) with HRP labeling reported only DV efferents to its contralateral homonym.

In a number of studies the commissural connections of the LV have been described as originating within the ventral part of this nucleus (Gacek 1978; Pompeiano et al. 1978; Carleton and Carpenter 1983; Ito et al. 1985). As in the present study, HRP reaction product was never found in the typical giant cells of Deiters, but only in medium-sized and small cells, ventrally and medially in the nucleus. A careful examination of the findings of these authors revealed that this part of the lateral vestibular nucleus is the equivalent of the MVmc in both cat and rabbit.

Throughout the dorsolateral part of the MVpc and the entire MVc, a continuous area of neurons is present which is responsible for the majority of all commissural fibers. The cells project heavily to the periphery of the SV, the dorsolateral MVpc, the MVc and to a lesser extent to the DV and MVmc. These findings are roughly comparable with the results of previous studies by Gacek (1978) and Pompeiano et al. (1978). Considerable differences exist with the work of Carleton and Carpenter (1983) and Carpenter and Cowie (1985) who only described projections from the rostral MV to the DV.

Two models emerge from the different studies of the vestibular commissure. According to one, the system is thought to consist mainly of reciprocal connections between homonymous nuclei. The second emphasizes the divergence of axons to more than one contralateral subnucleus. The degeneration study of Ladpli and Brodal (1968) and the recent HRP study of Ito et al. (1985) represent the first model. They describe major connections between both the SV and DV and their contralateral homonyms. Unfortunately, Ladpli and Brodal (1968) gave no conclusion with respect to the MV. The second model is most clearly represented by the HRP study of Pompeiano et al. (1978) who found the distribution of labeled commissural cells to be largely independent of

the site of their injections in the vestibular complex. Labeled cells in their experiments were always present in the SV, the MV and in group y. The present results in the rabbit favor the latter model of divergent contralateral projections.

Functional considerations

Electrophysiological studies demonstrated that vestibular canal and otolith information which subserves the vestibulo-ocular reflex is bilaterally coupled via the commissural vestibular connection (Shimazu and Precht 1966; Shimazu 1972; Kasahara and Uchino 1971; Precht 1978; Uchino et al. 1979, 1980). The organization of the vestibular commissure is specific in the sense that neurons receiving input from the horizontal canal influence contralateral vestibular neurons that are driven by the contralateral horizontal canal. Pre-oculomotor neurons in the rabbit, which are driven from different canals, are located in specific parts of the vestibular complex. Neurons which mediate horizontal canal information were found in the rostral MV (Highstein 1973b; Ito et al 1976) and in the MVmc (the ventral part of the lateral nucleus: Kawaguchi 1985). Vertical relay neurons occupy the central and probably also the dorsolateral regions of the SV (Highstein 1973a; Abend 1977; Ghelarducci et al. 1977; Yamamoto et al 1978). Similar localizations have been described for the vestibulo-ocular relay cells in the cat (Graybiel and Harting 1974; Gacek 1977).

The functional specificity which was attributed to the commissural system is not in accordance with the anatomical organization of these connections. In the present study it was found that commissural neurons avoid the central SV, the MVmc and the ventral MVpc and MVc. This part of the vestibular complex is characterized by the presence of a large proportion of premotor neurons of the vestibulo-ocular (Graybiel and Hartwieg 1974; Gacek 1977) and vestibulospinal (Busch 1961; Kuypers and Maisky 1975; Huerta and Harting 1982) pathways.

Neurons in the peripheral parts of the SV as well as those in the DV, the MVpc and the MVc, which are the origin of the majority of commissural connections, are also linked through reciprocal intrinsic connections. Moreover, these peripheral areas are the origin of the bulk of the secondary vestibulocerebellar mossy fibers (Rubertone et al. 1983; Gould 1980). The differential localization of vestibulo-ocular and vestibulocerebellar relay cells and the termination of the vestibular commissure on the latter and not on the former, has been shown for the SV in the cat (Mitsacos et al. 1983). These authors demonstrated that pre-oculomotor neurons which received excitatory input from the ipsilateral vestibular nerve could only be weakly inhibited through the commissure. This in contrast to vestibulocerebellar neurons which could be monosynaptically inhibited from the contralateral side.

Thus, an influence of the vestibular commissure on centrally located premotor neurons can only be mediated indirectly via the intrinsic connections of the contralateral side.

TABLE 4.1

exp	tracer	vol ul	surv days	SV	MVpc	MVmc	LV	MVc	DV	PH
C962	5% WGA-HRP	0.3	1	+	+	+	+			
C255	33% HRP	0.8	3	+	+	+	+		+	
C480	33% HRP	1.0	2	+	+	+	+	+	+	
C1192	5% WGA-HRP	0.25	2					+	+	+
C260	33% HRP	0.2	2	+						
C253	33% HRP	0.1	3	+						
C1201	5% WGA-HRP	0.15	1.5	+						
C474*	33% HRP	0.2	2		+					
C296	33% HRP	0.5	2		+			+		
C707	HRP #	2			+					
C501	20% HRP	0.15	2			+	+		+	
C789	HRP #		2				+			
C333	33% HRP	0.3	2.5					+		
C371	33% HRP	0.2	2						+	
C372	33% HRP	0.15	2						+	
C813	HRP #		2						+	

LEGEND TO TABLE 4.1

Listing of the experiments, with type of tracer, injected volume and survival time. The plus signs indicate which vestibular subnuclei are included in the different injection sites. Symbols; *: parapharyngeal approach of the brain stem; #: iontophoresis of 5% HRP in 0.01M Trisbuffer, pH 8.6 (Graybiel and Devor 1974)

5 SECONDARY VESTIBULOCEREBELLAR PROJECTIONS TO THE FLOCCULUS AND UVULO-NODULAR LOBULE OF THE RABBIT: A STUDY USING HRP AND DOUBLE FLUORESCENT TRACER TECHNIQUES[5]

INTRODUCTION

The vestibular nuclei are among the many sources of cerebellar mossy fibers (Brodal 1974). This "secondary vestibulocerebellar" connection (Dow 1936), as opposed to the primary vestibulocerebellar afferents from Scarpa's ganglion, has been subjected to a number of investigations employing the method of retrograde transport of horseradish peroxidase (HRP). These studies included a variety of species such as the rat (Blanks et al. 1983), the rabbit (Alley et al. 1975; Yamamoto 1979), the cat (Gould 1980; Kotchabhakdi and Walberg 1978; Sato et al. 1983), and the macaque monkey (Brodal and Brodal 1985; Langer et al. 1985). Together with studies using anterograde axonal tracing (Magras and Voogd 1985; Thunnissen et al. 1989), they have demonstrated that the flocculus and the uvulo-nodular lobule are the main recipients of secondary vestibulocerebellar mossy fibers. The data obtained with the anterograde and retrograde techniques show that the neurons projecting to these lobules are not arranged in topographically separated populations within the vestibular complex.

Flocculus, uvula and nodulus, the constituents of the "classical" vestibulocerebellum, have been implicated in different functions. According to Ito (1984) the flocculus has a modulatory effect on the vestibulo-ocular reflex and its adaptive properties. The nodulus and the uvula of the posterior vermis influence the vestibulo-collic and postural reflexes (Precht 1979).

In view of this functional specialization, the question arises whether vestibular mossy fibers to the flocculus and the uvulo-nodular lobule are collaterals from single axons or originate from separate neurons. In the present study this problem was investigated using simultaneous injection of different retrogradely transported fluorescent tracers: Fast Blue and Nuclear Yellow or Diamidino Yellow (Kuypers et al. 1980). Since knowledge of the secondary vestibulocerebellar projection in the rabbit is limited, and restricted to the connection with the flocculus (Alley et al. 1975; Yamamoto 1979), the distribution of vestibulocerebellar neurons was also determined in separate experiments with injections of HRP in the flocculus and uvulo-nodular lobule.

RESULTS

By creating two groups of experiments in which the specific fluorescent tracers were exchanged with respect to the injection site, an attempt was made to

[5]Based on the paper Secondary vestibulocerebellar projections to the flocculus and uvulo-nodular lobule of the rabbit: a study using HRP and double fluorescent tracer techniques. AH Epema, NM Gerrits and J Voogd; Exp Brain Res (1990) in press.

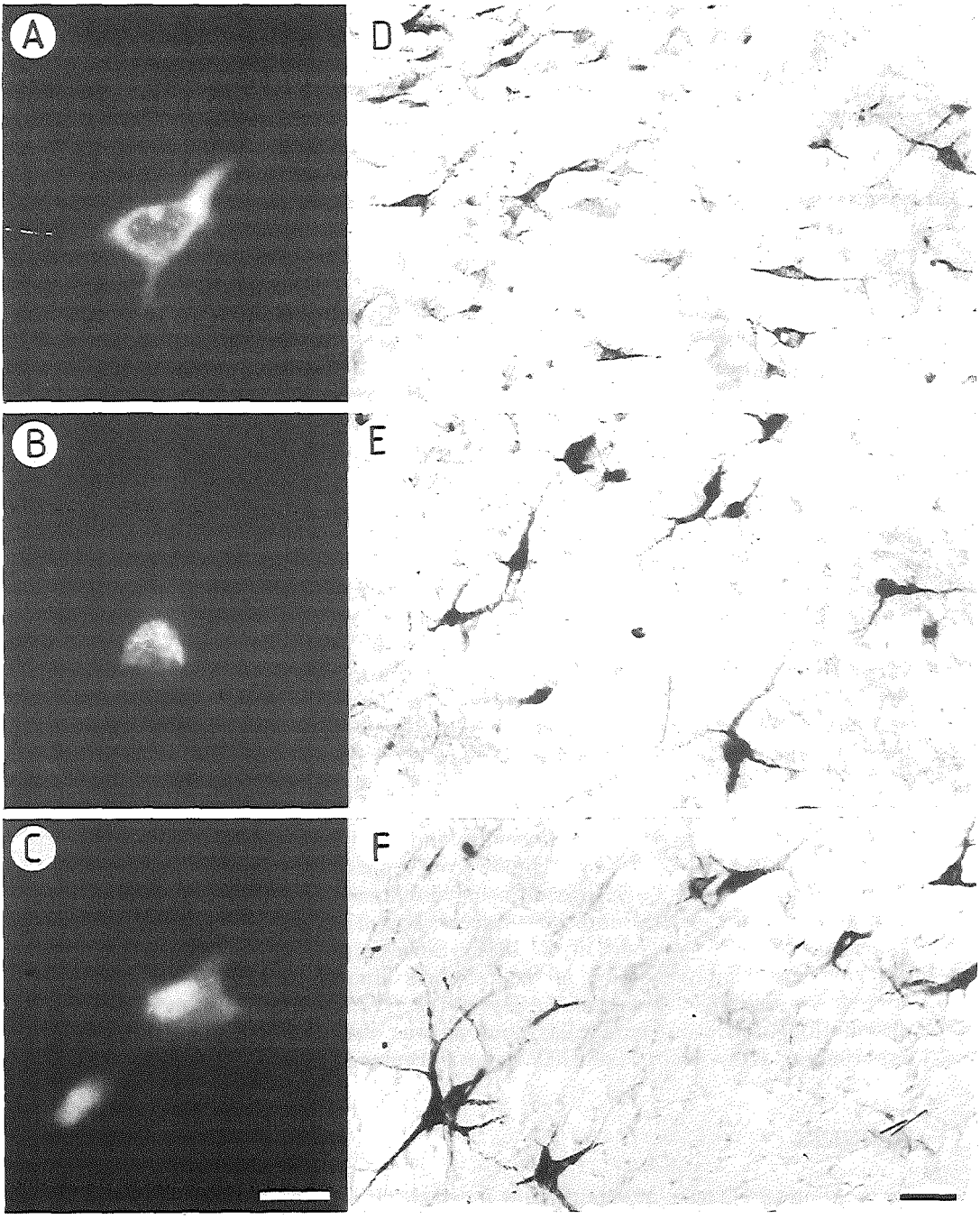

Fig 5.1 Neurons retrogradely labeled with fluorescent tracer; A: Fast Blue (FB); B: Diamidino Yellow (DY), double labeled with FB + DY. Scale bar for A - C represents 20 u.
Vestibulocerebellar neurons labeled retrogradely with HRP; D: superior vestibular nucleus; E: caudal medial vestibular nucleus; F: descending vestibular nucleus. Scale bar for D - F represents 50 u.

counteract possible effects of differences in transport capacity of the dyes. From either group one experiment was choosen for illustration. To obtain maximum uptake of the fluorescent tracers, the injections were intentionally large and in case of the uvulo-nodular lobule mostly bilateral. The distribution of vestibulocerebellar neurons labeled with fluorescent tracers was also compared with a number of cases with injection of HRP into the flocculus and uvulo-nodular lobule to detect tracer-specific differences in uptake or transport.

Injections in the flocculus

The injection sites of the HRP experiments K26 and C562 involved the rostral flocculus and included to differing degrees Yamamoto's (1979) folia f2, f3 and f4 (Fig. 5.2). The injections did not involve the brachium pontis or the central cerebellar white matter. In experiment C825 the zones I and II of the NY injection site in the flocculus covered part of folia f1 and f4 and most of folia f2 and f3. In experiment K78 the FB injection site was smaller and the zones I and II included folia f3, f4 and p, leaving the rostral part of the folia free of tracer. The FB injection site of experiment K275 had a similar rostrocaudal position as K78, but included folia f1 - f4. Zones I and II of the DY injection site of experiment C1275 (not illustrated) included the rostral three-fourth of folium f4. In all cases zone III extended up to the pial surface and included the medially located floccular white matter (Fig. 5.2). Further medialward diffusion of tracer seemed effectively blocked by the brachium pontis. Thus, the central cerebellar nuclei and group y were well outside the labeled area.

In all cases labeled neurons were present bilaterally, throughout the vestibular subnuclei, but absent from the lateral vestibular nucleus (LV), group x and the vestibular ganglion (Figs. 5.1, 5.2, 5.4, 5.5). In the HRP experiments differences in laterality were small; not more than a few percent. In the fluorescent tracer experiments the number of labeled cells at the ipsilateral side was higher in two cases (C825, K78, 25%) and lower in the other two cases (C1275, 8%; K275, 25%) (Table 5.2).

In the superior vestibular nucleus (SV), labeled cells are most abundant in the centre 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 parabrachial vestibular nucleus (NVpar), the interstitial nucleus of the eighth nerve (IN VIII) and group y. A small amount of labeled cells was always found in the centre and dorsal parts of the parvocellular medial vestibular nucleus (MVpc). Very few cells were observed in the magnocellular medial vestibular nucleus (MVmc).

In the caudal aspect of the vestibular complex, labeled neurons are distributed throughout the rostrocaudal extent of the caudal medial vestibular (MVc) and the descending vestibular (DV) nuclei. Rostrally, the area with labeling seems continuous with that in 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 prepositus hypoglossal nucleus (PH) is present ventrally in its caudal half. A substantial number of labeled cells is present in the adjacent dorsal aspect of the medial longitudinal fascicle (flm) (Fig. 5.2).

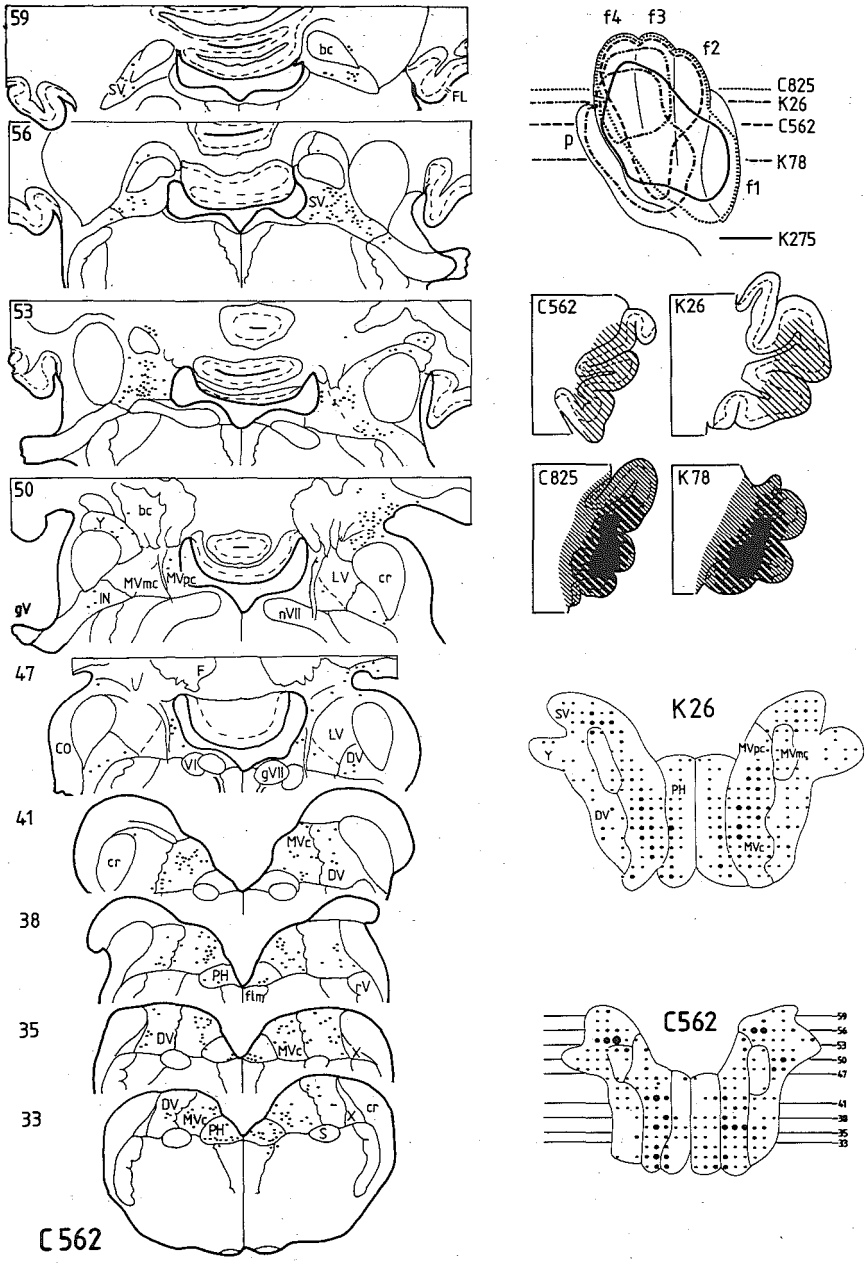

Fig 5.2 Injections in the right flocculus. Extent of injection sites C562 (HRP), K26 (HRP) (DAB reaction), C825 (NY), K78 (FB) (zone I: black, zone II: dark hatching; zone III: light hatching) in transverse sections through their largest extent and projected onto the floccular surface (according to Yamamoto 1979) (top right). Distribution of labeled neurons in C562 in transverse sections (left). Diagram of the vestibular nuclei showing the overall distribution of labeled neurons in C562 and K26 (bottom right). Left and right side of the brain as illustrated.

Injections in the uvulo-nodular lobule

In experiment C588, the HRP injection site covers most of the sublobules IXc-d on the right side as well as the medial one-third of these lobules on the left side (Fig. 5.3). The injection in case C1031 is bilateral and includes most of the white matter at the base of lobules IX and X (Fig. 5.3), thereby interrupting the connections of the central two-third of their expanse of cortex. In experiment C825 the FB fluorescent zones I-III covered bilaterally the medial part of the lobules X and IXd and the ventral aspect of lobule IXc. The injection site in the FB experiment C1275 (not illustrated) matched the extent of case C825 in the right hemivermis, but on the left side included only the medial one-third of folium IXd. The zones I to III in the DY injection site of experiment K78 mainly included the medial part of the folia IXc-d and the ventral part of IXb, but zone II penetrated more deeply into the white matter at the base of the lobules, including the basis of lobule X. The DY injection site of experiment K275 is unilateral (Fig. 5.3). It only involves the centre of lobule IXd and the apical half of lobule X (Fig. 5.3).
The injection sites all include a substantial proportion of the lobules IXd and X, which were shown by Thunnissen et al. (1989) to contain the majority of vestibulocerebellar mossy fiber terminals.

In all experiments, labeled neurons were found bilaterally throughout the vestibular complex, but were not observed in the LV or the infracerebellar nucleus (Figs. 5.3 - 5.5). A large number of labeled neurons was always present in the vestibular ganglion and in group X, lateral to the caudal part of the DV. Differences in laterality were smaller than in the cases with injections in the flocculus. The number of labeled cells was higher in the ipsilateral (= side of the flocculus injection) vestibular nuclei in two cases (K78, 4%; C1275, 17%) and lower in two cases (C825, 3%; K275, 19%) (Table 5.2). The number of uvulo-nodular projection cells in C825 and K78 (Table 5.2) differs by a factor 2 and represent the extremes of the series of experiments. However, they are in the same range as the data obtained with HRP. From the present material and also from fluorescent tracer injections elsewhere in the cerebellum we have the impression that the number of labeled precerebellar neurons strongly depends on the extent of damage to the white matter inflicted in zone I.

In the SV, labeled cells showed a tendency to be located in the central and medial parts of the nucleus. This area with labeled neurons continues into the NVpar and group y, but their number is considerably smaller there. Labeling in the medial vestibular nucleus is concentrated in the dorsal part of the MVpc and shifts to a more central position in the MVc, forming a continuous area of labeled cells. The number of labeled neurons in the MVmc was small. The rostral part of the DV contained a limited number of labeled cells. At caudal levels of the DV they were more abundant and not clearly separated from the labeling in MVc and group x. The high number of labeled neurons caudally in the DV, the MVc and group x in the experiments with fluorescent tracer injections as compared to the HRP experiments may be due to the greater mediolateral extent of the injection sites in these cases. Within the PH, labeled cells occupied a central position throughout its rostrocaudal extent.

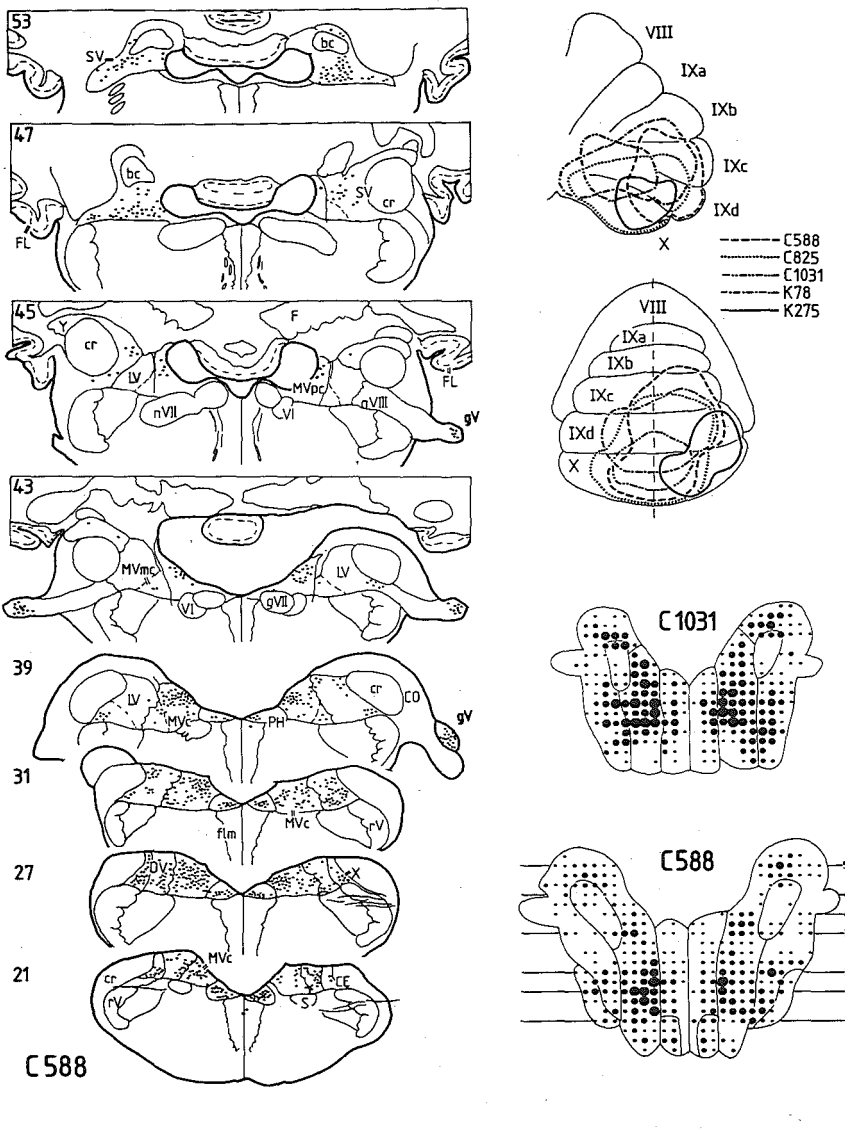

Fig 5.3 Injections in the uvulo-nodular lobule. Extent of injection sites C588 (HRP), C1031 (HRP) (DAB reaction), C825 (FB), K78 (DY) (boundary of zone II) projected onto the cerebellar posterior surface and onto the midsagittal plane (top right). Distribution of labeled neurons in C588 in transverse sections (left). Diagrams of the vestibular nuclei showing the overall distribution of labeled neurons in C588 and C1031 (bottom right). Left and right side of the brain as illustrated.

Double fluorescent labeled neurons

Double labeled neurons were found in nearly all subdivisions of the vestibular nuclei as well as in the PH, but were absent from the LV. Their distribution is very similar to that of neurons projecting to the flocculus (Figs. 5.2, 5.4, 5.5). Consequently they are absent from group x, almost absent from the MVmc and not numerous in the caudal aspect of the vestibular complex where the majority of uvulo-nodular projection neurons are located (Figs. 5.3 - 5.5).

Similar to what was observed with single labeled neurons, the difference in laterality was not pronounced (Table 5.2). The quantitative differences between C825 on the one hand and C1275, K78 and K275 on the other are mainly caused by a different number of labeled neurons in the SV that might be related to the extensive coverage of the rostral folia of the FL with tracer in case C825. The unilateral nature of the caudal vermis injection in K275 may explain the very low number of double labeled neurons in that case.

DISCUSSION

This study is the first to show the distribution of vestibulocerebellar neurons projecting to the uvula and nodulus of the caudal vermis in the rabbit. It also confirms and extends previous data for vestibular neurons projecting to the flocculus (Alley et al. 1975; Yamamoto 1979). The differences which were observed in the amount of single and double labeling, as well as the distribution of the neurons did not exceed the expectations due to differences in the size and localization of the injection sites. There were no consistent differences between the two groups in which the specific tracers were switched with respect to the injection sites. The different types of neurons were not found in spatially segregated subpopulations, but were intermingled without apparent organization.

Double labeling of neurons can be caused by leakage of tracer out of retrogradely labeled somata followed by successive uptake in glial cells and transfer to other neurons (Bentivoglio et al. 1980a). The probability of leakage of NY in our experiments was reduced by determining optimal survival times in a preliminary series of experiments, which confirmed earlier findings (Bentivoglio et al. 1980a; Kuypers et al. 1980). Following injection with DY no evidence for leakage into glia was found. Since in all experiments single labeled FB and DY or NY neurons were present in (sometimes very) close proximity, leakage is thought to be practically absent.

Neurons projecting to the flocculus

The origin of the secondary vestibulocerebellar projection to the flocculus has been determined in a number of studies in various mammals using HRP as a retrograde tracer. The two reports on this projection in the rabbit (Alley et al. 1975; Yamamoto 1979) provide little detail about the distribution of labeled neurons. In general, our results are similar to previous data obtained in the rat (Blanks et al. 1983), the cat (Gould 1980; Kotchabhakdi and Walberg 1978; 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

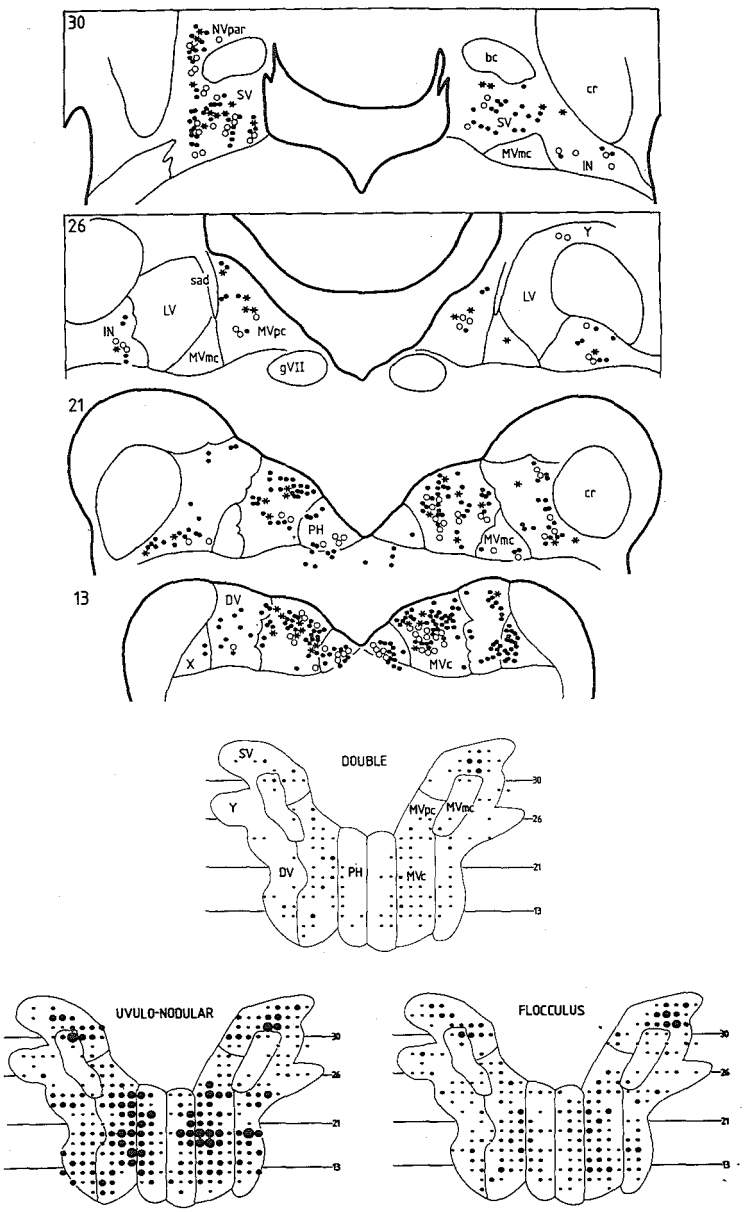

Fig 5.4 Experiment C825: injection of NY into the right flocculus and FB into the uvulo-nodular lobule. Transverse sections (top) through the vestibular complex. Symbols represent labeled neurons; dots: FB, circles: NY, asterisks: double labeled. Diagrams (bottom) illustrate the distribution of single and double labeled neurons projecting to the flocculus, the uvulo-nodular lobule and both areas simultaneously. Left and right side of the brain as illustrated.

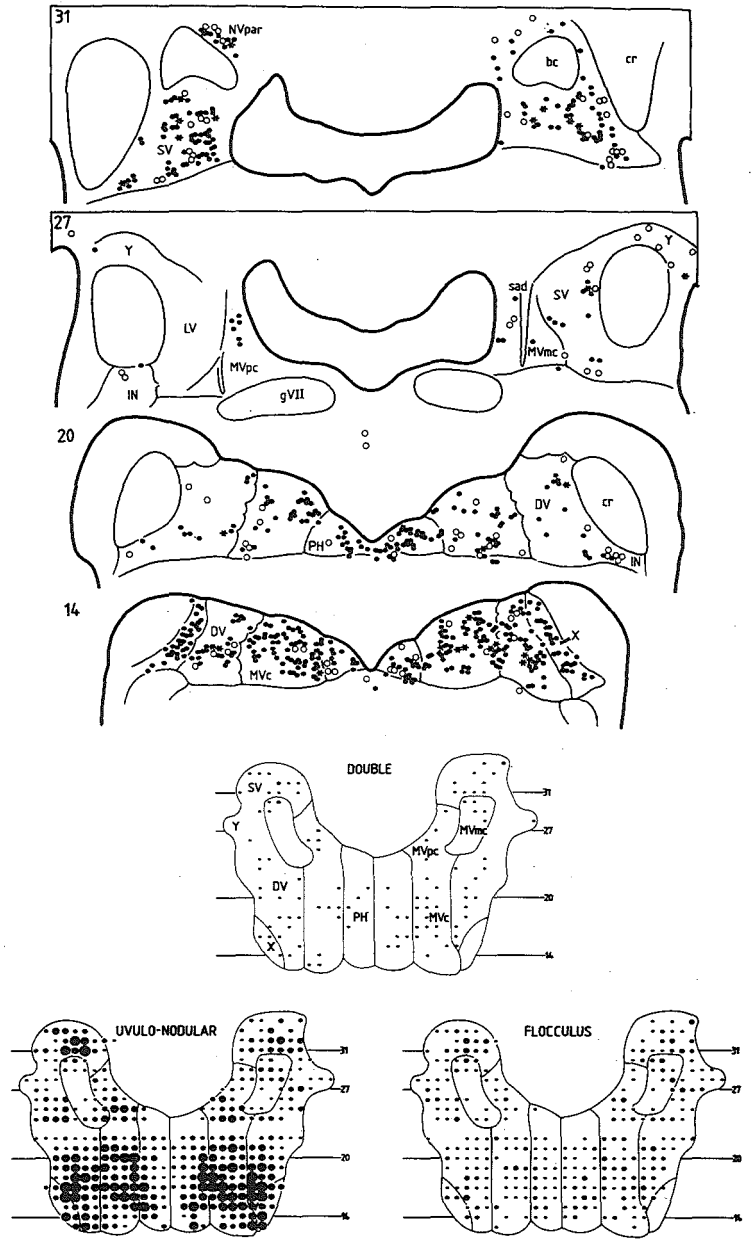

Fig 5.5 Experiment K78: injection of FB into the right flocculus and DY into the uvulo-nodular lobule. Transverse sections (top) through the vestibular complex. Symbols represent labeled neurons; dots: DY, circles: FB, asterisks: double labeled. Diagrams (bottom) illustrate the distribution of single and double labeled neurons projecting to the flocculus, the uvulo-nodular lobule and both areas simultaneously. Left and right side of the brain as illustrated.

bilaterally throughout the PH and all subdivisions of the vestibular complex, except for the LV. The neurons are most numerous in the MVc, the SV and the PH. According to Gerrits (1985) the SV projects most heavily to the rostral FL. This may explain the high number of retrogradely labeled neurons in this nucleus in case C825. Labeled neurons in the NVpar were not mentioned by previous authors, since this subdivision of the SV was introduced recently (Epema et al. 1988). Labeled vestibulo-cerebellar neurons showed a tendency to cluster, although not as strongly as illustrated by Yamamoto (1979). The difference with reports claiming labeling in the LV can be explained either by the fact that the borders of the LV with the DV and SV are difficult to establish (Kotchabhakdi and Walberg 1978; Langer et al. 1985) or that the MVmc, which only contains small labeled neurons, is sometimes included in the LV (Blanks et al. 1983; Sato et al. 1983). A most striking difference seems to exist between the rat and other animals (rabbit, cat, monkey) with respect to group x, which in the rat was found to be densely labeled following injections in the flocculus (Blanks et al. 1983). A species difference cannot be excluded, but another possibility is that the labeled neurons instead of belonging to group x are located in the caudolateral part of the DV, an area which was reported to contain vestibulothalamic projection neurons (Kevetter et al. 1982). A further difference is the exclusively ipsilateral labeling of group y neurons in the rat (Blanks et al. 1983), which were consistently labeled bilaterally in the other species.

The location of labeled neurons in the periphery (mainly ventrally) of the PH is in agreement with previous reports (Kotchabhakdi et al. 1978; Sato et al. 1983; Yamamoto 1979). An explanation for the markedly lower amount of labeled neurons in the PH in our study as compared to other studies may be due to the fact that in these cases the injection sites included parts of the ventral paraflocculus (Blanks et al. 1983; Sato et al. 1983).

Neurons projecting to the uvulo-nodular lobule

The secondary vestibular projections to the lobules IX and X of the posterior vermis has its origin in all subdivisions of the vestibular nuclei except for the LV, and in addition in the PH and group x. Labeled neurons were present bilaterally in equal numbers, which was also observed following unilateral HRP injections in lobules IX and X (Thunnissen et al. 1989). The greatest density of neurons was observed in the caudal half of the vestibular complex in the MVc, the neighbouring DV and in group x, and rostrally in the SV. Our data are in agreement with the results of previous studies in the cat (Gould 1980; Kotchabhakdi and Walberg 1978; Walberg and Dietrichs, 1988) and the macaque (Brodal and Brodal 1985). Apart from the difference with respect to group x, the neurons which project to either the flocculus or the uvulo-nodular lobule do not occupy separate territories in the vestibular complex.

The projection of the PH to the uvulo-nodular lobule originates from neurons clustered in the centre of this nucleus. This is in agreement with previous data in cat and monkey (Brodal and Brodal 1985; Kotchabhakdi et al. 1978).

Double fluorescent labeled neurons

Until now, collateral branches heading in the direction of the flocculus and uvulo-nodular lobule have only been reported for some vestibulocerebellar axons (Highstein et al. 1987; Mitsacos et al. 1983). However, these authors were not able to trace the axons to their final destination, due to the limitations of the intra-axonal HRP staining technique.

Our study establishes the presence of at least three types of secondary vestibulocerebellar neurons in the vestibular nuclei, projecting respectively to the uvulo-nodular lobule, the flocculus and to both parts of the cerebellum. The number of labeled neurons and their distribution following injections of either fluorescent tracers or HRP (Table 5.2) are similar. Therefore, the populations of single and double labeled cells may be considered unbiased.

The greatest difference in the number of single fluorescent labeled neurons between individual cases was a factor 2.1 for the uvulo-nodular lobule (exps. K78 and K275) and a factor 1.6 for the flocculus (exps. C825 and K275). Taking into account the different extent of the injection sites and the degree of involvement of the white matter in their central zones, these differences are not excessively large. Naturally, the effect of variation in the individual injections multiplies with respect to the amount of double labeled neurons, in absolute number as well as in percentage. This may be the explanation for the high number of double labeled neurons in case C825 as well as for the large differences in double labeling between the experiments: e.g. a factor of 6 between experiments C825 and K275.

Thus, it is difficult to conclude what is the precise ratio between the three types of vestibulocerebellar neurons. The amount of material is too small to do so and we refrain from a strict interpretation of the cell counts. If the highest number of neurons of each of the three types is taken from Table 5.2 (regardless of the experiment it comes from) one arrives at a ratio of approximately 12:4:1 (uvulo-nodular lobule single labeled : flocculus single labeled : double labeled).

It remains to be determined how the number of cell types and their ratio will change when other combinations of injection sites are tested (e.g. left FL - right FL). If laterality is taken into account, even with our limited experimental paradigm, the number of possible types increases to five, projecting to: (1) ipsilateral FL, (2) contralateral FL, (3) ipsilateral FL and uvulo-nodular lobule, (4) contralateral FL and uvulo-nodular lobule, and (5) uvulo-nodular lobule.

The distribution of double labeled neurons is very similar to that of neurons projecting to the flocculus. As long as there is no specific knowledge about input on vestibulocerebellar neurons it will be premature to attribute specific functions to the different types. Nevertheless, it should be noted that in the flocculus and the uvulo-nodular lobule the influence of double labeled neurons may differ considerably, since the ratio with single labeled ones may be 1:4 for the flocculus and 1:12 for the uvulo-nodular lobule. The diversity of responses observed in electrophysiological studies of vestibulocerebellar mossy fibers (Lisberger and Fuchs 1978; Waespe et al. 1981) could find their anatomical substrate in the types of neurons demonstrated here.

TABLE 5.1

	FL		IX + X	
exp	vol	surv	vol	surv
K26	0.5 HRP	2.0		
C562	0.8 HRP	1.1		
C588			0.8 HRP	2.0
C1031			0.5 HRP	2.0
K74	0.7 FB	8	0.7 DY	6
K75	0.9 FB	7	0.9 DY	4
K78	0.9 FB	10	0.9 DY	6
K275	0.8 FB	19	0.8 DY	11
C825	0.5 NY	0.8	0.9 FB	12
C858	0.5 NY	0.9	0.7 FB	12
C1275	0.2 DY	6	0.4 FB	14

LEGEND TO TABLE 1
Listing of experiments with injections in the flocculus (FL) and uvulo-nodular lobule (IX + X). HRP: 33% horseradish peroxidase; DY: 2% Diamidino Yellow; FB: 3% Fast blue; NY: 1% Nuclear Yellow. Injected volumes in ul, survival time in days.

TABLE 5.2

		U-NL single			double			FL single		
Exp	N	contra	ipsi	%	contra	ipsi	%	contra	ipsi	%
C825	(20)	608	590	62	103	124	12	214	284	26
K78	(21)	1220	1258	79	65	64	4	224	288	17
K275	(24)	632	516	58	26	11	2	460	347	40
C1275	(7)	248	296	62	24	20	5	148	136	33
K26	(19)							374	394	
C562	(17)							300	310	
C588	(21)	897	831							
C1031	(17)	1020	1083							

LEGEND TO TABLE 2
Number of labeled neurons following injection of tracer in uvulo-nodular lobule and/or flocculus, counted in N equally spaced sections. In experiments C825, C1275, K78 and K275 the section thickness was 30 um and ipsi- and contralateral refers to the right (flocculus injection site) and left sides of the brain respectively. In experiments K26, C562, C588 and C1031 the section thickness was 40 mu and ipsi- and contralateral refers to respectively the right (flocculus injection site) and left sides of the brain. The percentages give the sum of ipsi- and contralateral sides as a proportion of the total number of labeled neurons. U-NL: uvulo-nodular; Fl: flocculus.

6 COLLATERALIZATION OF VESTIBULO-MESENCEPHALIC AXONS TO SPINAL CORD, CEREBELLUM AND CONTRALATERAL VESTIBULAR NUCLEI IN THE RABBIT. A STUDY USING THE DOUBLE FLUORESCENT TRACER TECHNIQUE

INTRODUCTION

The vestibular nuclei contain the elements that link the sensory neurons of the vestibular ganglion and the motoneurons of the eye muscles into Lorente de Nó's (1933) three-neuronal vestibulo-ocular reflex arc. Other premotoneurons project to the spinal cord, but the majority of vestibular nuclear cells send their efferents to other targets: the cerebellum and the contralateral vestibular complex. Data collected in different species, particularly with the method of retrograde axonal transport of horseradish peroxidase (HRP) have revealed that each of these populations of vestibular neurons occupies an extensive territory in the vestibular nuclei.

Injections with HRP in the oculomotor nuclei in the cat (Graybiel and Hartwieg, 1974, Graybiel 1975; Gacek, 1977), the monkey (Graybiel, 1977), and the rabbit (Ghelarducci et al, 1977; Thunnissen, 1990) demonstrated the presence of labeled cells in the superior (SV), the medial (MV) and the descending (DV) vestibular nuclei, group Y and also in the prepositus hypoglossal nucleus (PH). Electrophysiological studies have led to the characterization of several populations of vestibular neurons connected with motoneurons that innervate individual eye muscles. For a review of this literature the reader is referred to Ito (1984).

Vestibulospinal connections are organized in two separate pathways. The lateral vestibulospinal tract (LVST) has its origin in the giant Deiters' neurons in the lateral vestibular nucleus (LV) (Nyberg-Hansen, 1964) and runs through the ipsilateral ventral reticular formation into the ventrolateral funiculus of the cord (Busch, 1961). The medial vestibulospinal tract (MVST) descends bilaterally in the flm with a dominant crossed component (Busch, 1961; Nyberg-Hansen, 1964). Retrograde transport of HRP has demonstrated that the MV, the DV (Peterson and Coulter, 1977; Wold, 1978; Leong et al., 1984), and to a lesser extent the SV contribute to the MVST (Tohyama et al, 1979; Leong et al, 1984).

Vestibulocerebellar neurons have been found in the SV, MVpc, MVc and DV, whereas they were absent from the LV (Brodal, 1974; Kotchabhakdi and Walberg, 1978; Blanks et al., 1983; Sato et al., 1983; Epema et al., 1990; Thunnissen et al, 1989). The magnocellular part of the MV, the MVmc contributed with only few neurons to the projection. A study in the rabbit with different retrogradely transported fluorescent tracers (Epema et al, 1988) showed that the projection to the flocculus and the caudal vermis originates from different sets of neurons. These neurons however, share the same territory. Between 5 and 10% of these vestibulo-cerebellar neurons showed axonal branching.

Vestibular neurons projecting to the contralateral vestibular complex seem even more abundant than vestibulo-cerebellar neurons but have a similar distribution. They were found in the periphery of the SV, and in the entire MV and DV, but they are absent from the LV and the MVmc. There is a

disagreement in the literature on the precise topographical relations between the vestibular nuclei of both sides (Ladpli and Brodal, 1968; Pompeiano et al., 1978; Carleton and Carpenter, 1983; Epema et al., 1988).

Many neurons in the vestibular nuclei could be related electrophysiologically to eye as well as head movements (Highstein, 1973b; Wilson et al, 1968; Isu and Yokota, 1983), but anatomical data on collateralization is sparse. McCrea et al. (1986) injected HRP intraaxonally into identified secondary vestibular pre-oculomotor neurons (i.e., neurons receiving monosynaptic vestibular nerve input and related to a certain type of eye movement), mainly located in MV. One type that projected to the oculomotor nuclei showed axonal branches to the spinal cord. Other authors described similar branching patterns (Graf and Ezure, 1986; Highstein and McCrea, 1988).

The aim of the present study is to determine the relation between the distribution of vestibulo-mesencephalic neurons and that of other projection neurons by means of injection of different fluorescent tracers in the same animal. This approach not only enables the study of different vestibular efferent neurons, but more importantly a study of the collateralization of vestibulo-oculomotor axons to other targets. Although several studies using HRP as a retrograde tracer in the rabbit described the projection of vestibular neurons to the mesencephalon, the cerebellum and the contralateral vestibular nuclei, similar data on the vestibulospinal projection in this species are absent. To facilitate the comparison with the other HRP studies, experiments with injection of HRP in the spinal cord are included in the present material.

RESULTS

The description of the results is divided into four paragraphs which deal with the comparison of the distribution of vestibular neurons projecting to the oculomotor nucleus (V-O neurons), the spinal cord (V-S neurons), the caudal vermis (V-C neurons), the flocculus (V-F neurons), and the contralateral vestibular nuclei (V-V neurons), and neurons with collateralization of axons to dual targets. The description of the results starts with a description of HRP injections into the spinal cord.

I HRP injections

HRP and WGA-HRP injections into the spinal cord

Large injections of HRP and WGA-HRP (Table 6.1) into the cervical- (C571) and lumbosacral spinal cord (C1829) resulted in retrogradely labeled neurons in SV, MV, LV, MVmc and DV.

Case 571 (Fig 6.1) is a unilateral injection located at C3-C5, which extended slightly into the grey matter of the contralateral side (not illustrated). Throughout their extent the ipsilateral LV and MVmc contained labeled large and giant neurons, characteristic for these subnuclei. The labeled neurons in the MVmc were somewhat smaller and rounder than those in the LV. Medium-sized neurons were scattered in the dorsal aspect of LV and throughout the entire rostro-caudal extent of MVmc. The ventral part of the caudal SV and the MVpc

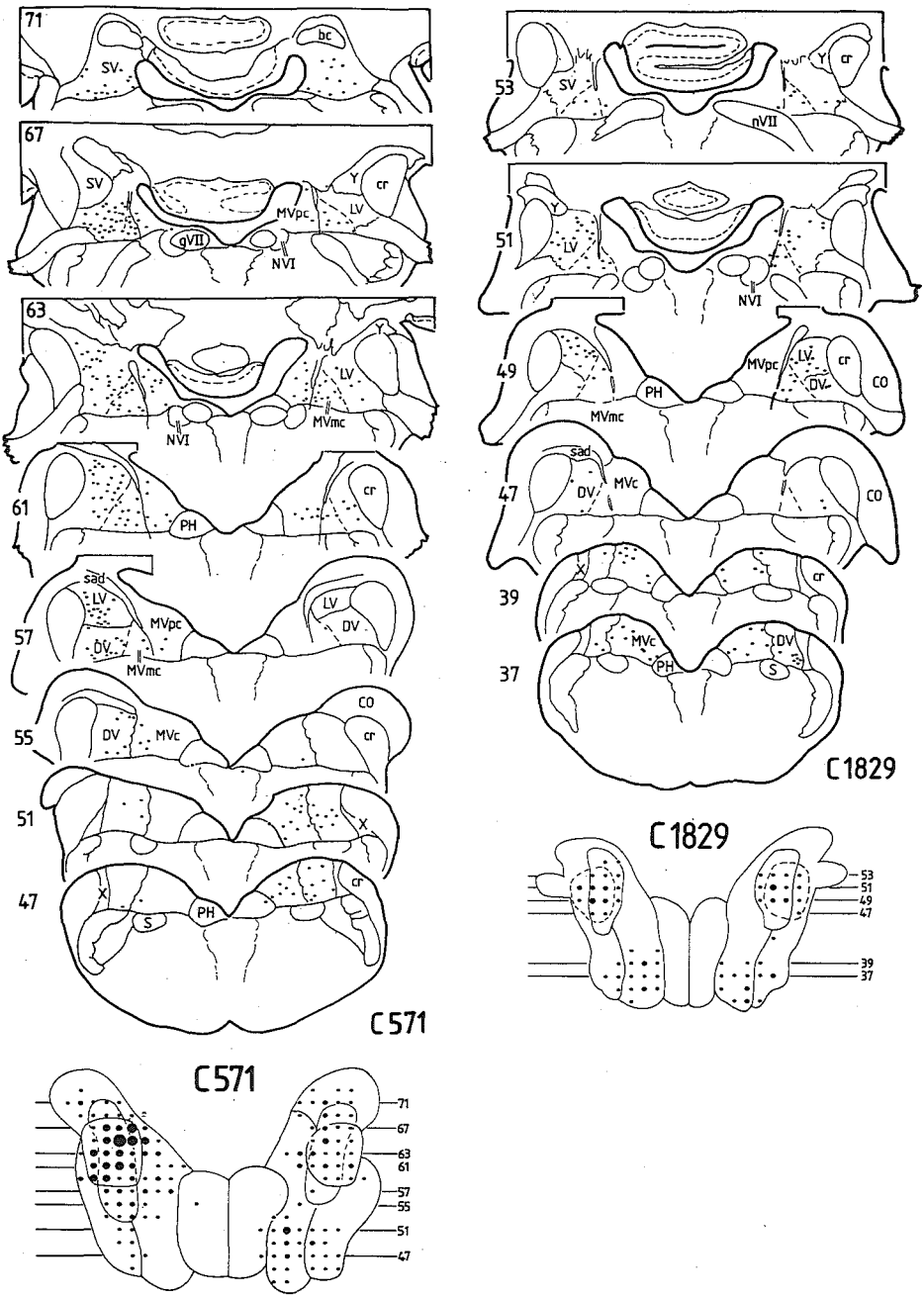

Fig 6.1 Transverse sections and horizontal diagrams through the vestibular nuclei illustrating the distribution of retrogradely labeled neurons following injection of HRP in cervical (C571) and lumbar (C1829) levels of the spinal cord. Injection sites on the left and in case C1829 bilateral.

contained a few labeled neurons. Some labeled neurons were found in the IN, between the entering vestibular nerve fibers. In the caudal part of the vestibular nuclei few labeled neurons were located near the border between the MV and DV.

Contralaterally, a few labeled large cells were present in the LV. The labeled cells in MVmc were smaller than on the ipsilateral side and distributed throughout this nucleus. Few labeled neurons were located in the central SV, and the MVpc. Labeled neurons were restricted to the caudal halves of the MVc and DV and were scattered more widely than on the ipsilateral side. The distribution of labeled neurons observed in exp. C571 was replicated by injections with fluorescent tracers (exps. C1263, C1272), which will be described in subsequent paragraphs.

Following a large bilateral injection of HRP into the lumbosacral region of the spinal cord (C1829), labeled vestibular neurons had a distribution not unlike that following injections in the cervical cord (Fig 6.1). The differences were the absence of labeling in the SV and a more distinct separation of the rostral and caudal populations in the LV and MVmc, and the MVc and DV respectively. The retrogradely labeled large and giant neurons showed a slight preference for the border area between the LV and MVmc. In smaller injections into the lumbosacral cord (Table 6.1, not illustrated) with unilateral (C2047) and bilateral (C2094) involvement of the dorsal horn and the intermediate zone labeled neurons were almost completely restricted to the LV and MVmc, in exp. C2047 ipsilaterally, in exp. C2094 bilaterally.

II Fluorescent tracer injections

Injections in mesencephalon and spinal cord

In two cases with injections of FB and DY in the mesencephalon and the spinal cord (C1263, C1272; Table 6.2) the vestibular nuclei contained single and double labeled neurons. FB labeled neurons displayed a blue fluorescence of the cytoplasm. DY labeling resulted in a yellow labeling of the nucleus. Leakage of tracer in surrounding glial cells was not observed. In exp. C1263, the zones I and II of the DY injection in the mesencephalon covered unilaterally most of the oculomotor nucleus, and parts of the flm and central grey (Fig 6.2). Zone III included parts of the contralateral oculomotor nucleus, as well as the flm, the central grey, and the tegmentum bilaterally. The effective injection site was judged to be unilateral because labeled neurons in the abducens nucleus were only present contralaterally to the injection. The FB injection in the cervical spinal cord was made by multiple penetrations and reached from C3 to C7. The zones I and II covered the ventral and ventrolateral funiculi and part of the grey matter bilaterally (Fig 6.2). Zone III extended over the entire cross-section of the cord.

In case C1272 the zones I and II of the FB injection in the mesencephalon covered a part of the oculomotor nucleus unilaterally, the flm, and parts of the adjacent central grey and reticular formation (Fig 6.2), while zone III extended

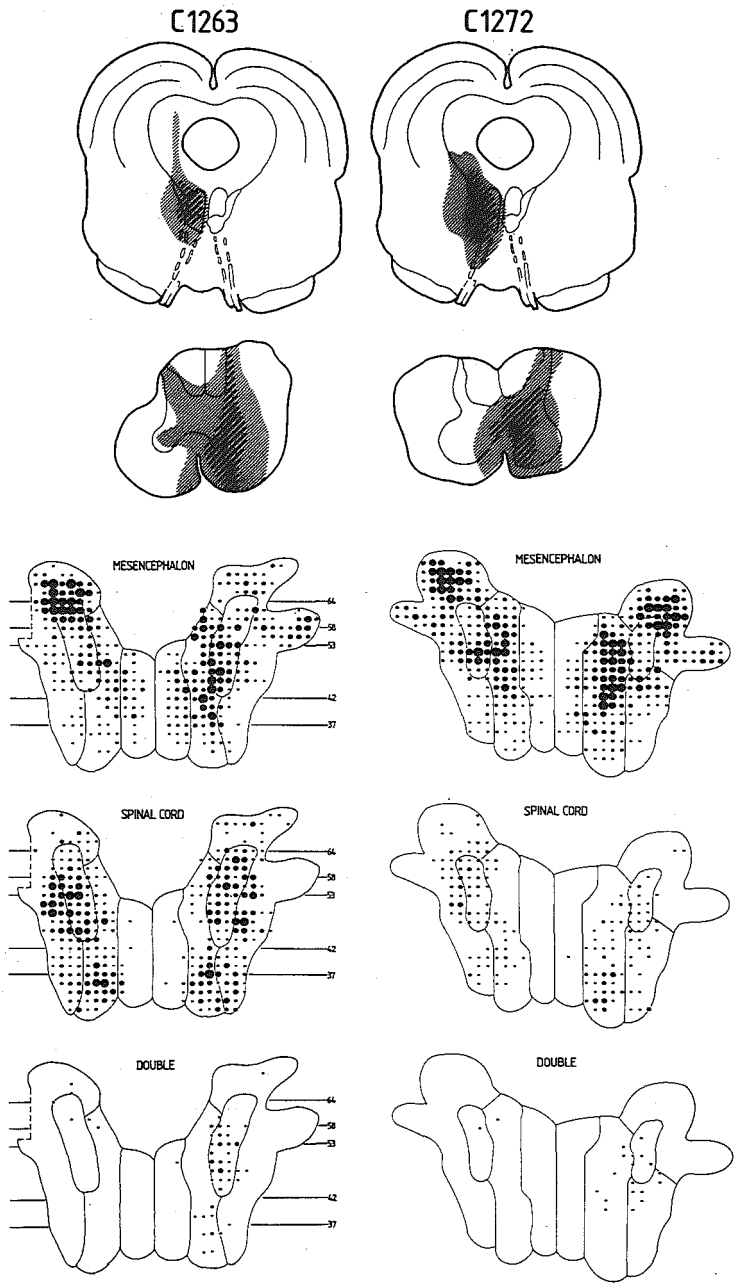

Fig 6.2 Experiments with combined injections in the mesencephalon (C1263: DY, C1272: FB) and the spinal cord (C1263: FB, C1272: DY). Zone I: dark hatching, zone II: light hatching. The distribution of single labeled vestibulomesencephalic, vestibulospinal, and double labeled neurons is indicated in horizontal diagrams (left column C1263, right column C1272).

into the central gray and tegmentum. The DY injection into the cervical spinal cord was made by multiple penetrations. The zones I and II covered the ventral and ventromedial funiculi bilaterally (Fig 6.2). Zone III extended over the entire cross section of the cord, but for the lateral funiculus of the left side.

Distribution of single labeled V-O neurons

The largest number of neurons, labeled retrogradely from the mesencephalon, was present in case C1272, but the distribution was similar in both cases.

Contralateral to the injection, DY labeled neurons (exp C1263: Figs 6.2, 6.3 dots; exp C1272: Fig 6.2) were found in the central and dorsolateral SV. Labeling in the caudal parts of the nucleus is continuous with the label in group Y, which contained a relatively large number of labeled cells. Modest numbers of DY neurons were located in the central and ventral parts of the MVpc and the rostral MVc. In the caudal part of the MVc the number of labeled neurons clearly decreases. More DY labeled neurons were present in MVmc, DV, ventral and lateral PH, and the NVI.

A large number of cells was labeled in the ipsilateral SV. They were found both in its central and peripheral parts. A few DY neurons occurred in the ventral aspect of group Y. MV labeling was mainly confined to the MVpc centrally and ventrally, and was continuous with scarce labeling in the MVmc. Some scattered DY cells were present in the border region of the MVc and DV, and in the ventrolateral PH. The giant neurons of the LV and MVmc were never found labeled. The ipsilateral abducens nucleus did not contain labeled neurons.

Distribution of single labeled V-S neurons

The distribution of single labeled cells with either fluorescent tracer (exp C1263: Figs 6.2, 6.3 circles; exp C1272: Fig 6.2) was similar to that of the cases with injections of HRP into the spinal cord as reported in the previous paragraph (Fig 6.2).

In experiment C1263, large numbers of giant cells were labeled within the LV and MVmc, bilaterally. Moreover, the MVmc contained a limited number of medium-sized labeled neurons. Few FB labeled cells were present in the SV, but none in the IN. The MV contained labeled neurons throughout its extent; some in the central and ventral parts of the MVpc, the majority more caudally in central parts of the MVc, bordering on the DV. A considerable number of labeled neurons was found clustered in the DV and in the adjacent group X. There were no V-S neurons in group Y, the PH and the NVI.

Distribution of double labeled V-OS neurons

A fair number of double labeled neurons (exp C1263: Fig 6.2, 6.3 asterisks; exp C1272: Fig 6.2) was present contralateral to the injection site in the mesencephalon. They were intermingled with single labeled cells. This double labeling occurred in central parts of the MVpc, in the adjacent parts of the MVmc, and clustered centrally at the border between the MVc and the DV. Double labeled neurons thus form a rostrocaudally extending column in the center of the vestibular complex, which begins caudal of the SV. The labeling in

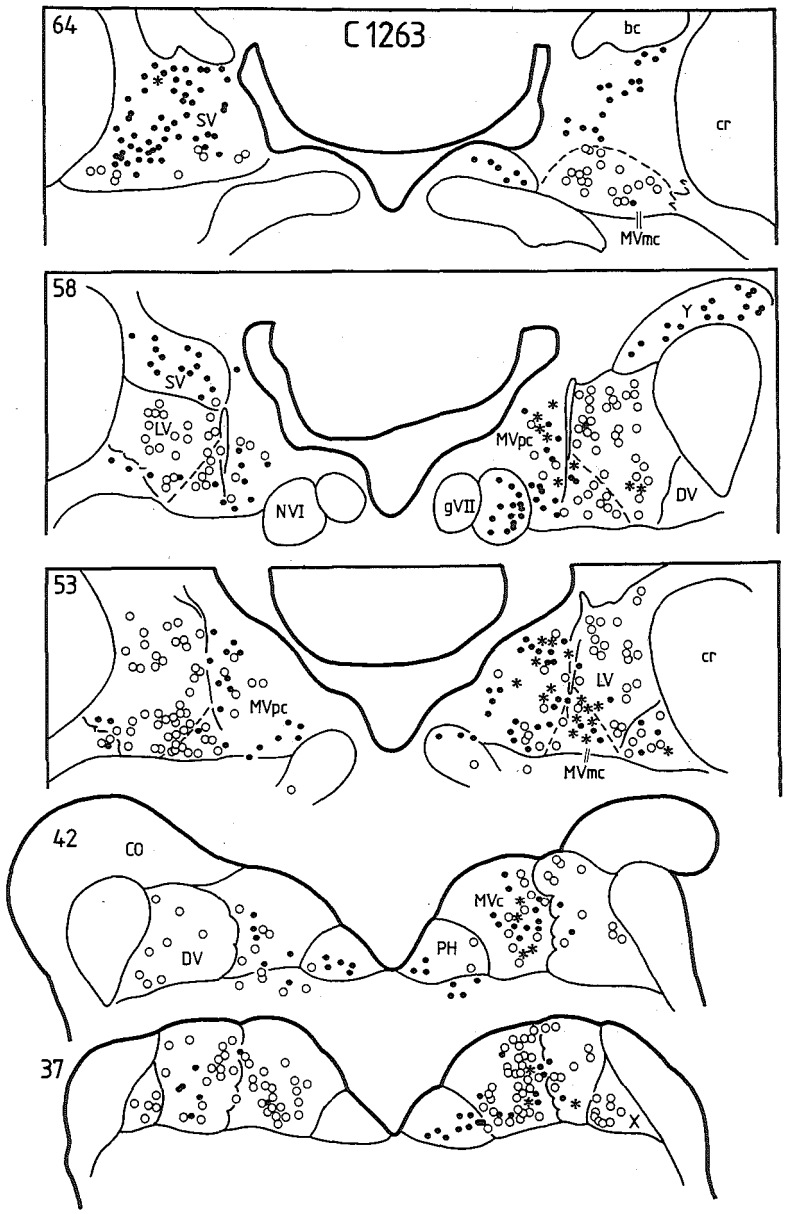

Fig 6.3 Transverse sections through the vestibular nuclei (experiment C1263) showing the distribution of neurons projecting to the mesencephalon (dots), to the spinal cord (circles), and to both targets (asterisks).

MVmc always concerned medium-sized neurons and did not involve giant cells. There were no double labeled cells in SV, group Y and PH. Double labeling was found in 2-3% of the total number of labeled neurons. In the MVmc, where the largest number of double labeled neurons was present, they constituted 40% of the total number of vestibulospinal neurons and 9% of the total number of vestibulomesencephalic neurons.

A few double labeled neurons were present in the SV and MVpc, ipsilateral to the injection in the mesencephalon.

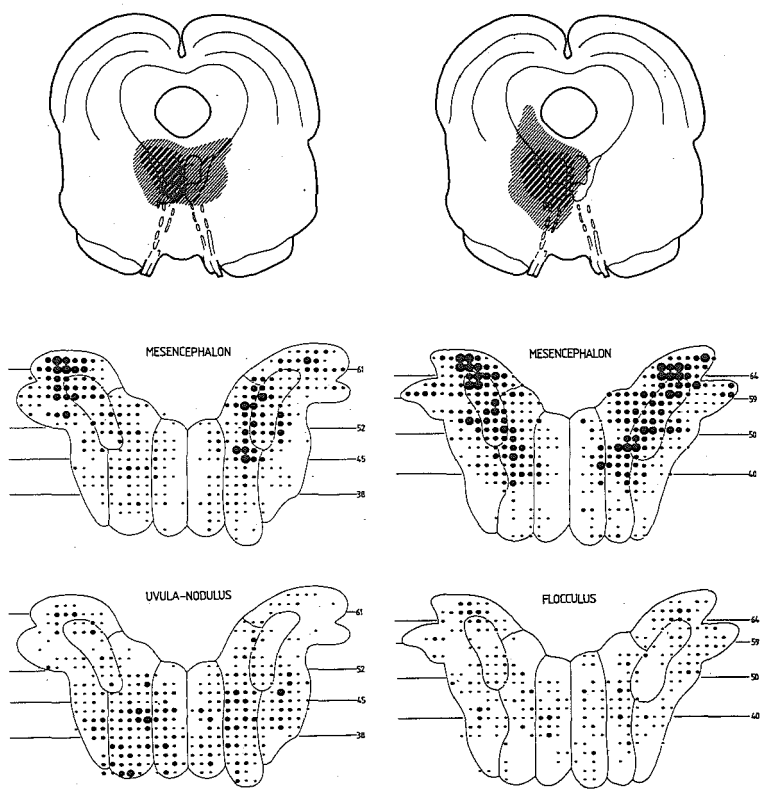

Fig 6.4 Experiments with combined injections of fluorescent tracers in the mesencephalon (C788: NY, C808: FB) and the uvula and nodulus (C788: FB) and flocculus (C808: NY). Zone I: dark hatching, zone II: light hatching. The distribution of vestibulomesencephalic and vestibulocerebellar neurons is indicated in horizontal diagrams (left column C788, right column C808).

Injections in mesencephalon and caudal vermis

In four rabbits fluorescent tracer injections were placed in the oculomotor region and the lobules IX and X of the caudal vermis (Table 6.2). Injections with FB were combined with either DY or NY. All injections in the caudal vermis were bilateral. The optimal survival time for injections with NY were determined in separate set of experiments (see Chapter 2). The NY fluorescence sometimes extended into the cytoplasm of retrogradely labeled neurons, but involvement of surrounding glial cells was not observed. Despite the different tracers and survival times applied, all injections in the mesencephalon and the cerebellum resulted in similar patterns of single labeled blue fluorescent somata and yellow fluorescent nuclei throughout the entire vestibular complex, except the LV, and in the PH. Double labeled neurons were never found in these experiments.

The zones I-III of the FB injection in case C788 covered the folia b, c and d of lobule IX and extended across the fissura posterolateralis into the dorsal part of lobule X (not illustrated). The zones I and II of the NY injection in the mesencephalon covered bilaterally the oculomotor nuclei and the flm, and some of the surrounding central grey and reticular formation (Fig 6.4, left column), while zone III slightly extended beyond this area.

Distribution of single labeled V-O neurons

Large numbers of NY labeled neurons were found bilaterally in the SV, group Y, the MV, the DV, the IN, and the PH (Figs 6.4, 6.5 dots). Their distribution was similar to that observed in other cases with injections fluorescent tracers in the mesencephalon (exps C1263, C1272, C808, C1317). Contralateral to the injection in the mesencephalon, NY labeled nuclei were observed throughout the entire SV, and more dorsally, lateral to the brachium conjunctivum in the NVpar, and in the ventral part of group Y. The majority of labeled cells in the MV were present ventrally and centrally in the MVpc, and centrally in the rostral MVc. Few labeled nuclei were present in the caudal part of the MVc and adjacently in the DV. Ipsilateral to the injection in the mesencephalon, the area containing labeled neurons was similar, but the majority of labeled neurons was found in the central SV, whereas the rostral MVc contained only few labeled neurons. The PH contained retrogradely labeled neurons throughout its entire rostrocaudal extent bilaterally, but preferentially in its caudal half. In addition, fluorescent nuclei were located bilaterally in the NVI.

Distribution of single labeled V-C neurons

The distribution of FB neurons labeled from the multiple injections in the caudal vermis was similar to that reported for HRP and fluorescent tracer labeled neurons after injections of this part of the cerebellum as described in chapter 5. The FB labeled neurons (Fig 6.4, 6.5 circles) were present in the SV, the entire MV, and in rostral levels of the DV. The PH contained many FB labeled cells throughout its rostrocaudal extent. In contrast to the injections in the mesencephalon, the injection in the caudal vermis resulted in labeled neurons in group X.

It was found that vestibulocerebellar neurons were distributed diffusely

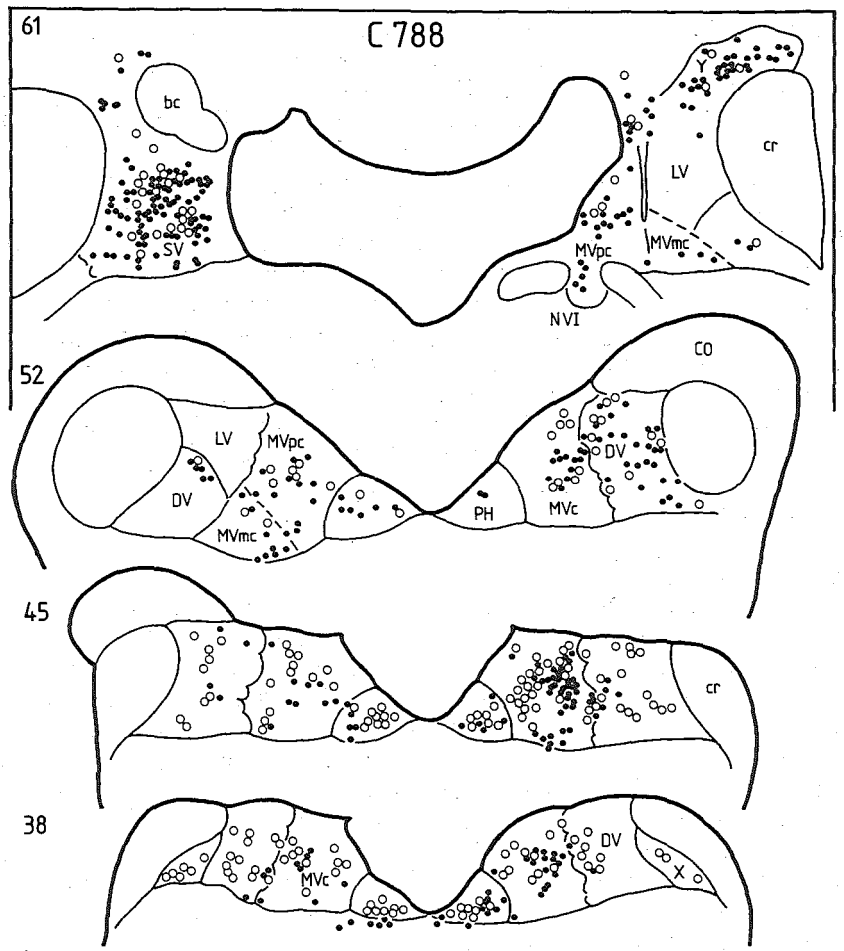

Fig 6.5 Transverse sections through the vestibular nuclei (experiment C788) showing the distribution of neurons projecting to the mesencephalon (dots), and to the uvula and nodulus (circles).

throughout the vestibular nuclei, especially in the MVc and DV, whereas vestibulomesencephalic neurons were more clustered and more concentrated in the rostral parts of the nuclei. Moreover, vestibulomesencephalic neurons were often larger, especially those in the central SV and, the ventral MVpc, the MVmc and the central MVc, where in addition the number of vestibulocerebellar neurons was low. Very often, neurons projecting to either of the injected targets, and thus labeled with a yellow nucleus or blue cytoplasm, were closely apposed as if representing a sort of units. This phenomenon was observed in all subnuclei, but particularly in the SV and the MV.

Injections in mesencephalon and flocculus

In two rabbits injections were made in the mesencephalon and the flocculus (Table 6.2). Single labeled neurons were found throughout the vestibular nuclei, except in the LV, and the PH. Double labeled neurons were not observed in these experiments.

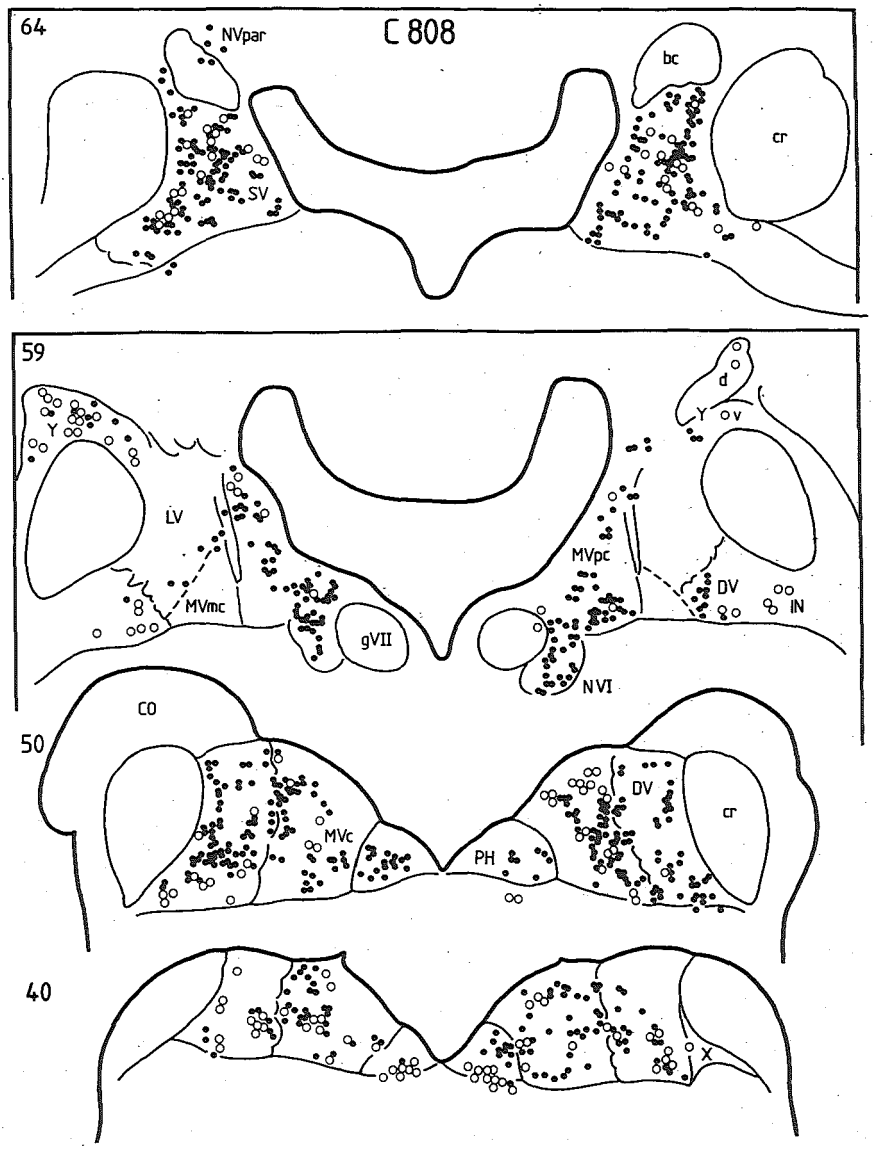

Fig 6.6 Transverse sections through the vestibular nuclei (experiment C808) showing the distribution of neurons projecting to the mesencephalon (dots), and to the flocculus (circles).

The zones I and II of the NY injection in the flocculus in experiment C808 covered most of its folia (not illustrated). Zone III extended into the white matter of the pedunculus flocculi, but did not involve the central cerebellar nuclei or group Y. The FB injection covered with its zones I and II the oculomotor nucleus bilaterally, and the flm, the central grey and the reticular formation unilaterally (Fig 6.4 right column). Zone III extended somewhat further into the reticular formation of both sides.

Distribution of single labeled V-O neurons

The total distribution area of FB labeled neurons was similar to that described in the previous experiments, but showed a clear bilateral symmetry (Fig 6.4, 6.6 dots). The labeled neurons were found throughout in the entire SV, in group Y, in the MVpc, MVc, DV, and the IN. There was a remarkable scarcity of labeling in the MVmc. The PH and the NVI contained FB labeled neurons bilaterally.

Distribution of single labeled V-F neurons

The distribution of NY fluorescent nuclei labeled from the flocculus (Fig 6.4, 6.6 circles) is similar to that reported in chapter 5. They were located bilaterally in the central SV, in the dorsal MVpc, the IN, and in clusters throughout the MVc and DV. Group Y contained ipsilaterally more NY labeled nuclei than contralaterally. NY fluorescence in PH was confined to its ventral and caudal parts, and extended into the adjacent flm.

Injections in mesencephalon and vestibular nuclei

In 4 rabbits injections of FB in the mesencephalon were combined with injections of either NY or DY in the vestibular nuclei of the left side (Table 6.2). The distribution of single and double labeled neurons within the vestibular complex was similar in all cases. Experiment C1317 is representative for this group. In this case the zones I and II of the FB injection in the mesencephalon covered the central part of the oculomotor nucleus, the medial aspect of the flm, and the adjacent central grey (Fig 6.7). Zone III encroached upon the contralateral oculomotor nucleus and the ipsilateral flm and reticular formation. DY was injected in the vestibular nuclei on the same side. The zones I and II covered large parts of all vestibular nuclei (Fig 6.7) and extended into the rostral aspect of the dorsal column nuclei. Zone III reached somewhat ventralward into the reticular formation. The PH was not included in the injection site.

Distribution of single labeled V-O neurons

In accordance with previously described experiments, most FB labeled neurons (Fig 6.7 dots) were located across the central SV, extending into the NVpar and group Y, in the central MVpc and rostral MVc, and in the rostromedial DV. Labeling was also present in the IN, the PH and NVI. Few labeled neurons were found in the MVmc, while they were absent from the LV. Within PH blue fluorescent somata were located centrally. Some FB neurons were present in the reticular formation bordering on the PH, a region which also

contained numerous DY labeled commissural neurons.

Distribution of single labeled V-V neurons

DY labeled nuclei of commissural vestibular neurons (Fig 6.7 circles) were concentrated in the lateral and medial SV, in the dorsal MVpc, throughout the entire MVc, in the medial DV, and in group Y. Few labeled neurons were found in the peripheral parts of the SV, the ventral MVpc, the MVmc, and the IN. Labeling was absent from the LV and group X. The PH and ventral to it the flm and the reticular formation also contained retrogradely DY labeled neurons. The abducens nucleus, contralateral to the injection did not contain labeled neurons. The distribution matches the commissural projection described in chapter 4.

Distribution of double labeled V-OV neurons

Double labeled neurons (Fig 6.7 asterisks) were only present in quantity in the central parts of the MVpc and MVc. A few double labeled cells were found around the border region of the rostral DV and MVc, but predominantly in the latter nucleus. The PH and the adjacent reticular formation also contained some double labeled neurons.

There is a large difference between the number of neurons that was labeled from the vestibular nuclei and from the mesencephalon. The ratio of V-V neurons over V-O neurons differed from about 2:1 or 3:1 for rostral and intermediate levels of the vestibular complex, to 10:1 for the most caudal levels.

The number of double labeled neurons in the MVpc, MVc, and DV is 2.5-4% of the total number of commissural neurons and 10-15% of the total number of vestibulo-oculomotor neurons. However, the overall percentage obscures a rostrocaudal gradient, due to the distribution of vestibulo-oculomotor neurons. If, for instance, the MVc is divided in rostral, intermediate and caudal parts, the percentage double labeling in the latter population is 9, 20 and 36 respectively.

DISCUSSION

This study is the first to show the distribution of neurons from which the vestibulo-spinal projection in the rabbit originates. It confirms and extends our former studies on the commissural vestibular projection (Epema et al, 1988), the projection of secondary vestibulo-cerebellar mossy fibers to the flocculus (Epema et al, 1990) and to the caudal vermis (Thunnissen et al, 1989; Epema et al, 1990), and the vestibulo-oculomotor projection (Thunnissen, 1990).

The fluorescent tracer method demonstrated collateral branches of vestibulomesencephalic axons to the spinal cord and the contralateral vestibular nuclei. In addition, it showed that neurons projecting to the mesencephalon and the cerebellum constitute different populations in the vestibular nuclei. Moreover, the fluorescent tracer technique showed clearly that neurons projecting to different targets share the same territory and are often located in close proximity to each other.

Switching the tracers between the injection sites did not result in consistent dissimilarities in the different groups of experiments. Some problems have been

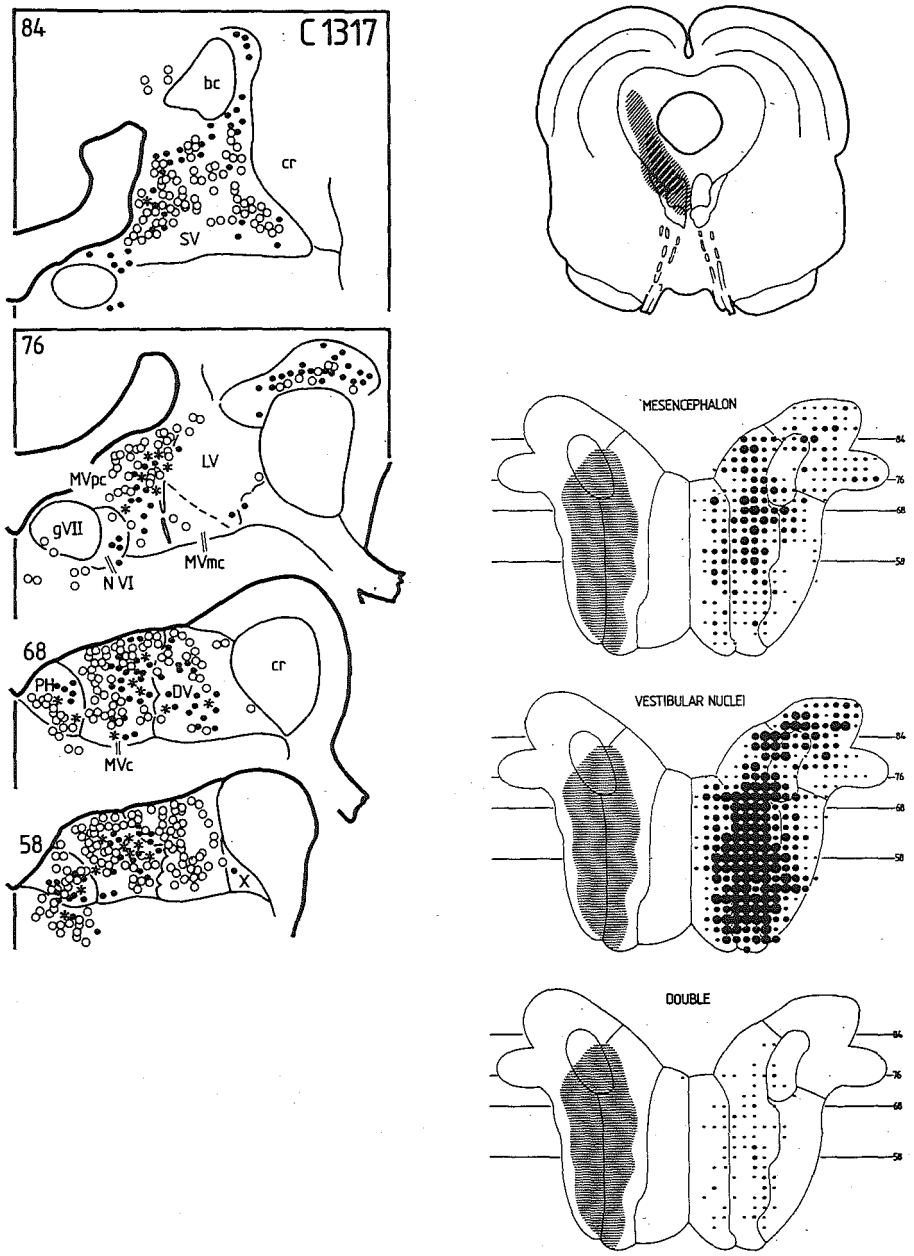

Fig 6.7 Experiments with combined injections of fluorescent tracers in the mesencephalon (C1317: FB; zone I: dark hatching; zone II: light hatching) and the vestibular nuclei (DY). The distribution of vestibulomesencephalic (dots), commissural vestibular neurons (circles) and double labeled neurons (asterisks) is indicated in transverse sections through the vestibular nuclei and in horizontal diagrams.

described, related to the use of double label tracer techniques (see Huisman et al, 1983 for a survey). False double labeling can occur by leakage of the tracer out of the neuron followed by successive uptake by glia and other neurons. This problem was especially described for NY (Bentivoglio et al, 1980a). Choosing optimal survival times and using short processing times can prevent leakage out of neurons (Bentivoglio et al, 1980a). Introductory experiments to determine optimal periods for NY (not illustrated) confirmed studies of Kuypers et al. (1980)

I. Distribution of single labeled neurons

Neurons projecting to the oculomotor region

The injections in the mesencephalon were large because they were intended to label the entire oculomotor nucleus. Consequently most of them spilled over the midline and all involved areas around the oculomotor nucleus. Thus, the experiments do not permit conclusions on the laterality of the projection of neurons in each vestibular complex. For details concerning the trajectories of the vestibulo-oculomotor projection and the termination in different populations of motoneurons innervating specific eye muscles the reader is referred to Thunnissen (1990).

The fluorescent tracer injections into the oculomotor region of the mesencephalon confirm previous findings in similar studies with HRP as a retrograde tracer (Graybiel and Hartwieg, 1974; Graybiel, 1977; Yamamoto et al, 1978; Epema et al, 1983; Thunnissen, 1990). The neurons that participate in the ascending projection were present bilaterally in all vestibular nuclei, except for the LV.

Qualitatively, the labeling is very similar in all cases, despite differences in size and extent of the injection sites.

In the present study group Y contained labeled neurons, which in the rostral part of this cell group were separated in a dorsal and a ventral cluster by the fibers of the floccular peduncle (Chapter 2). This observation is in accordance with Graybiel and Hartwieg (1974) and Highstein and McCrea (1988) in the cat, and Yamamoto et al. (1978) in the rabbit, who found labeled neurons in a dorsal and ventral subdivision of group Y following HRP injections into the oculomotor nucleus. Gacek (1977) in the cat, designated a cluster of neurons dorsal to group Y as the infracerebellar nucleus (Inf). There is disagreement in the literature with respect to borders of these two nuclei (see for a discussion Mehler and Rubertone, 1985). The confusion started with Gacek's (1977) subdivision of the cellgroups that cap the restiform body during its initial course upon entering the cerebellum. In his figure 11, Gacek (1977) labels group Y of Brodal and Pompeiano (1957) as Inf and their group l as Y. In the present study group l is considered as the caudolateral part of the SV.

The vestibulo-oculomotor projection is the second link in the three neuronal vestibulo-ocular reflex (VOR) arc (Lorente de Nó, 1933; Szentagothai, 1943). Certain aspects of the VOR are influenced through the cerebellum. The flocculus is implicated in the adaptive modification of the VOR in two different hypotheses (Ito, 1984; Lisberger, 1988 a, b). The effect of floccular stimulation

on oculomotor neurons has been demonstrated in the rabbit (Fukuda, 1972). Since the vestibular nuclei form the only target of the efferent floccular Purkinje cell axons (Langer et al, 1985 a; Haines, 1977; Chapter 7), it is of interest to compare the localization of the vestibulo-oculomotor neurons with the distribution of these floccular efferents. Dense flocculus projections reach group Y, the central SV, the dorsal and central MVpc, the rostral MVc, and the rostral DV. Pre-oculomotor neurons are present in the same areas. However, there is in addition a substantial number of pre-oculomotor neurons present in the ventral MVpc, the MVmc, and in the PH. Thus, these latter neurons cannot be influenced directly through the flocculus. Interestingly, the MVmc and the ventral MVpc receive a dense input from the fastigial nucleus, which is involved in the execution of saccadic eye movements (Ikeda et al., 1989). These vestibular nuclei are involved in horizontal and vertical eye movements (Highstein and McCrea, 1988). Neurons related to ipsilateral eye position, which generate bursts of spikes during saccades, have been identified in ventral MVpc and ventral MVmc (McCrea et al., 1987). Therefore, an alternative pathway for the control of saccades, which passes through the vestibular nuclei, is available, apart from the one consisting of fibers ascending through the brachium conjunctivum and relaying in the brainstem as proposed by Noda et al. (1989).

Neurons projecting to the spinal cord

The experiments with HRP and fluorescent tracers in the present study demonstrated spinal projections with an origin in the LV, the MVmc, the MVc, the DV, and the IN. Labeling is present in neurons of different size. In the LV and MVmc giant and large neurons were labeled. The other nuclei, and again the MVmc, contained small and medium-sized labeled neurons. Throughout the MVmc neurons of almost any size were labeled, which is in agreement with data obtained in the rat (Leong et al, 1984). The origin of the two components of the vestibulospinal projection, the lateral (LVST) and medial (MVST) vestibulospinal tracts, has been described with retrograde tracers in the cat (Kuypers and Maisky, 1975; Peterson and Coulter, 1977).

In the literature there is agreement on the exclusive origin of the LVST in the LV (Pompeiano and Brodal, 1957). The LV in Brodal's terminology (1974) includes the LV and MVmc (Epema et al., 1988), which are treated separately in the present study. The large calibre fibers of the LVST descend ipsilaterally in ventral and lateral part of the anterior funiculus (Busch, 1961; Kuypers and Maisky, 1975) and could be traced into the lumbosacral cord. Termination was found in laminae VII and VIII of Rexed (for a review see Brodal, 1981). Electrophysiological data support the origin of LVST from LV (Akaike, 1983). Fibers of large and giant neurons in both LV and MVmc seem to contribute to the LVST.

Based on lesion material, the origin of the MVST was located in the MV. Busch (1961) distinguished two descending vestibular pathways in the flm. The uncrossed MVST consists of thin fibers with an origin in the rostral MV. The crossed MVST is characterized by a large proportion of coarse fibers, which originate from neurons in the MV and DV with a location caudal to the ones that contribute to the uncrossed MVST. The crossed MVST constitutes the largest component of the flm apart from the reticulospinal tract. It is located medially

in the flm and could not be mistaken for the LVST. With degeneration methods, fibers of the MVST could be traced bilaterally in the flm, but not further than mid-thoracic levels of the spinal cord (Busch, 1961; Nyberg-Hansen, 1964). This restricted projection was questioned in electrophysiological studies, which identified many MVST neurons projecting to the lumbar cord (Ito et al, 1969; Wilson et al, 1967). More recent HRP studies clearly demonstrated that both the MV and the DV contributed to MVST (Peterson et al, 1977; Tohyama et al, 1979), and that neurons in these nuclei could be labeled by HRP injections into the lumbar cord. A later HRP study in the rat (Leong et al, 1984) and the present data in the rabbit show a similar distribution of MVST neurons following injections into the lumbar spinal cord. In addition these studies in rat and rabbit describe a projection from SV to cervical levels of the spinal cord.

Pompeiano and Brodal (1957) proposed a clear somatotopy in the LV using retrograde cell degeneration studies. Projections to lumbar levels of the cord emerged from the caudodorsal LV, projections to cervical levels from the rostroventral LV (Pompeiano and Brodal, 1957). In an electrophysiological study in the cat and the rabbit, Akaike (1983) found a less distinct somatotopy in the LV, because neurons projecting to cervical levels were also present dorsally in the LV. In agreement with the situation in the rat (Leong et al., 1984) the present study demonstrated labeling throughout the LV after lumbar HRP injections. Although some degree of somatotopy may exist, it could not be demonstrated unequivocally with either physiological or anatomical techniques.

Electrophysiological studies demonstrated that many vestibulospinal neurons send axon collaterals to cervical and lumbar levels of the spinal cord (Abzug et al., 1974; Rapoport et al., 1977). This intraspinal branching was confirmed in an anatomical double labeling study (Huisman et al., 1984). A high degree of collateralization was found for ipsilaterally projecting neurons in the LV and MV, and for contralateral projections arising in the DV. The extensive branching of vestibulospinal axons may explain why the recent studies with sensitive tracer techniques were unable to find a similar somatotopy in the LV as advocated by the retrograde degeneration studies (Brodal, 1981).

Neurons projecting to the cerebellum and to the contralateral vestibular nuclei

The distribution of vestibulocerebellar neurons with a projection to either the caudal vermis or the flocculus is similar to the results obtained in previous studies (Epema et al., 1990; Chapter 5). Labeled neurons were found bilaterally in all vestibular nuclei, except in the LV, and in the PH. Differences in the cerebellar projection of group x are clearly illustrated by cases with injections in the flocculus and the caudal vermis. Only the latter cases show labeling in group x (Gould, 1980; Rubertone and Haines, 1981; Epema et al, 1990).

Commissural neurons are present in the PH (McCrea, 1988) and throughout the vestibular complex, except in the LV and group x. Their distribution is in agreement with reports (Mehler and Rubertone, 1985; Epema et al., 1988; Chapter 4). There is much overlap between distribution of commissural and vestibulocerebellar neurons.

This study is the first that demonstrates collateralization of vestibular nucleus axons with retrograde labeling following simultaneous injection of different tracers in different efferent targets of the vestibular nuclei. It confirms and expands data obtained in experiments with intra-axonal labeling of physiological identified neurons (McCrea, 1988). Double labeled neurons were found in two sets of experiments.

Mesencephalon and cerebellum: absence of collateralization

All experiments with a combination of injections in the cerebellum and the mesencephalon exclusively contained single labeled neurons. This is in agreement with morphological studies of electrophysiologically identified vestibulocerebellar neurons (Mitsacos et al., 1983a, 1983b; Highstein et al., 1987). In a fluorescent tracer study of neurons in the PH collateralization has been described for the projection to the oculomotor nuclei and the flocculus (Yingcharoen and Rinvik, 1982), but no data were provided on labeling in the vestibular nuclei by these authors. An explanation for this difference with the present results could be either the involvement of the paraflocculus in their injection sites, or diffusion of tracer into group Y, which is very close to the flocculus. The latter possibility could have resulted in labeling PH neurons via their extensive ipsi- and contralateral connections with the vestibular nuclei (Epema et al., 1988). Double labeling in the PH was indeed found in the present study after combined injections of the vestibular nuclei and oculomotor region.

II Distribution of double labeled neurons.

Mesencephalon, spinal cord, and contralateral vestibular nuclei

The combination of injections into spinal cord and mesencephalon showed a cluster of double labeled neurons in the LV, MVmc, MVc and DV. Labeling in the MVc and DV is chiefly concentrated rostrally around the border between these nuclei. Double labeled neurons were almost exclusively found contralateral to the injection site in the mesencephalon. The largest proportion of double labeled neurons was in the rostral art of the cluster.

Simultaneous injections into the contralateral vestibular nuclei and the mesencephalon resulted in double labeled neurons with a roughly similar distribution. In addition, these experiments displayed double labeled cells in and around the PH. The largest proportion of double labeled cells was at caudal levels of the MVc (up to 45% of the total number of mesencephalon projecting neurons).

Several studies combining electrophysiological characterization of neurons with intracellular or intra-axonal labeling demonstrated branching secondary vestibular neurons (Isu and Yokota, 1983; Graf and Ezure, 1986; McCrea et al., 1986). Graf et al. (1983) in the rabbit, labeled neurons in the rostral MV relaying posterior canal information with intra-axonal injection of HRP. They could trace axon collaterals to the contralateral vestibular nuclei, the PH, and the abducens nucleus. Through the contralateral flm, ascending collaterals reached the oculomotor nucleus (OMN) and interstitial nucleus of Cajal, while a crossed descending branch could be traced till below the obex. In a subsequent study of

vertical canal related neurons, Graf and Ezure (1986) identified and labeled individual neurons in the MV and DV. These neurons showed a similar pattern of termination. Axons travelled in the contralateral flm. The descending branch headed for the spinal cord, while the ascending part of the axon provided terminal branches in the contralateral MV, DV, SV, INC and trochlear nucleus, and bilaterally in the OMN. Highstein et al. (1987) identified 21 secondary vestibular neurons of which 11 could be activated from the rostral flm, 8 from the spinal cord, and 2 from both the spinal cord and the oculomotor area. McCrea et al. (1987) stated that the bifurcating secondary vestibular neurons differ from normal VOR relay neurons. Their rostral branch is much thinner than the caudal branch, which was previously described by Busch (1961) in a degeneration study.

Thus some secondary vestibular neurons in the MV give off axonal branches to the contralateral OMN and the spinal cord. Others in the MV, DV, MVmc project to the contralateral vestibular nuclei and the contralateral OMN (McCrea et al., 1987).

Thus the vestibular nuclei contain several populations of neurons which are for the cerebellar and OMN projections completely separated. The projection of OMN neurons to the contralateral VN and the OMN clearly is collateralized.

TABLE 6.1

exp	tracer	level	vol(ul)	surv (h)
C 571	33%HRP	C3-5	6	48
C1829	33%HRP	L3-5	1	48
C2047	33%HRP	Lumb	0.3	26
C2094	5%WGA-HRP	Lumb	0.3	24

LEGEND TO TABLE 6.1
Listing of experiments, type of tracer, level of injection site in the spinal cord, injected volume ul (vol) and survival time in hours (surv).

TABLE 6.2

injections in:

exp	MES	SPC	CV	FL	VN
C 1263	DY 8	FB 14			
C 1272	FB 8	DY 5			
C 730	FB 14		NY 1.2		
C 788	NY 1.7		FB 12		
C 1318	FB 18		DY 9		
C 1328	FB 11		DY 11		
C 737	FB 8			NY 1.4	
C 808	FB 14			NY 0.8	
C 859	FB 14				NY 0.9
C 1236	FB 10				DY 5
C 1310	FB 12				DY 8
C 1317	FB 15				DY 7

LEGEND TO TABLE 6.2

Listing of experiments (exp), type of tracer, and survival time (days). FB: fast blue; NY: nuclear yellow; DY: diamidino yellow; MES: mesencephalon; SPC: spinal cord; CV: caudal vermis; FL: flocculus; VN: vestibular nuclei.

7 CEREBELLOVESTIBULAR PROJECTIONS[6]

INTRODUCTION

The vestibular nuclei (VN) receive two types of cerebellar input. 1 Corticovestibular projections which originate from Purkinje cells of the vermis of the anterior lobe and the simple lobule (lobule VI), the lobules VIII - X of the caudal vermis and the flocculus. 2 Nucleovestibular projections arise from the medial cerebellar nucleus (fastigial nucleus: FN).

In 1899 Klimoff (1899) described a direct uncrossed projection from different parts of the vermis in the rabbit to the vestibular complex. These early observations on corticovestibular connections (Ferrier and Turner, 1894; Klimoff, 1899 and Probst, 1902) were later elaborated in the cat (Hohman, 1929). Fibers were traced from an intermediate zone, located at the lateral border of the vermis of the anterior lobe, to the ipsilateral lateral vestibular nucleus (LV). A medially located zone of the vermis projected to the FN. The hemisphere projected to the interposed and lateral cerebellar nuclei. Later degeneration studies (Jansen and Brodal, 1940, 41; Walberg and Jansen, 1961) confirmed these findings.

Voogd (1964, 1967 and 1969) suggested a more detailed longitudinal pattern in the corticonuclear and vestibular projection. He studied the distribution of fibers of different calibers in the cerebellar white matter of the cat and ferret. Areas of thicker Purkinje cell fibers were separated by thin fiber areas, raphes. A thick and thin fiber area formed a compartment. Thus the white matter was composed of several compartments with a longitudinal parasagittal orientation. It was postulated that each compartment carries the fibers of a narrow, longitudinal strip of Purkinje cells on their way to a particular cerebellar nucleus. The compartments and the corresponding zones were labeled A to D. A total of seven zones were identified. Thus, Purkinje cells of the anterior lobe located in the B zone, projected to the LV. Later studies using axonal degeneration methods (rabbit: van Rossum, 1969; primates: Haines, 1976; Haines et al, 1982), anterograde transport of WGA-HRP (rat: Bernard, 1987) and retrograde transport of HRP (cat: Corvaja and Pompeiano, 1979, Voogd and Bigaré, 1980; rabbit: Balaban, 1984) confirmed the B-zone projection to LV. The medially located A zone was found to project to the FN (rabbit: van Rossum, 1969, cat: Voogd and Bigaré, 1980) and the medial vestibular nucleus (MV) (cat: Voogd and Bigaré, 1980, Voogd, 1989). Ekerot and Larson (1982) added one more zone, the x-zone, using electrophysiological criteria. The x zone occupied a wedge-shaped area between the A- and B-zones in dorsal parts of the anterior lobe (Ekerot and Larsen, 1982). Trott and Armstrong (1987a, b), in an autoradiography study, traced the termination of Purkinje cells of the X-zone to the junction area of the fastigial and posterior interpositus nucleus. An X compartment and a corresponding zone were delimited in retrograde tracer and histochemical studies in cat and monkey (Marani, 1986; Hess and Voogd, 1986; Voogd, 1989).

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[6] Part of this chapter is based on the paper Reciprocal connections between the caudal vermis and the vestibular nuclei in the rabbit AH Epema, JM Guldemond and J Voogd Neurosci Lett (1985) 57: 273-278

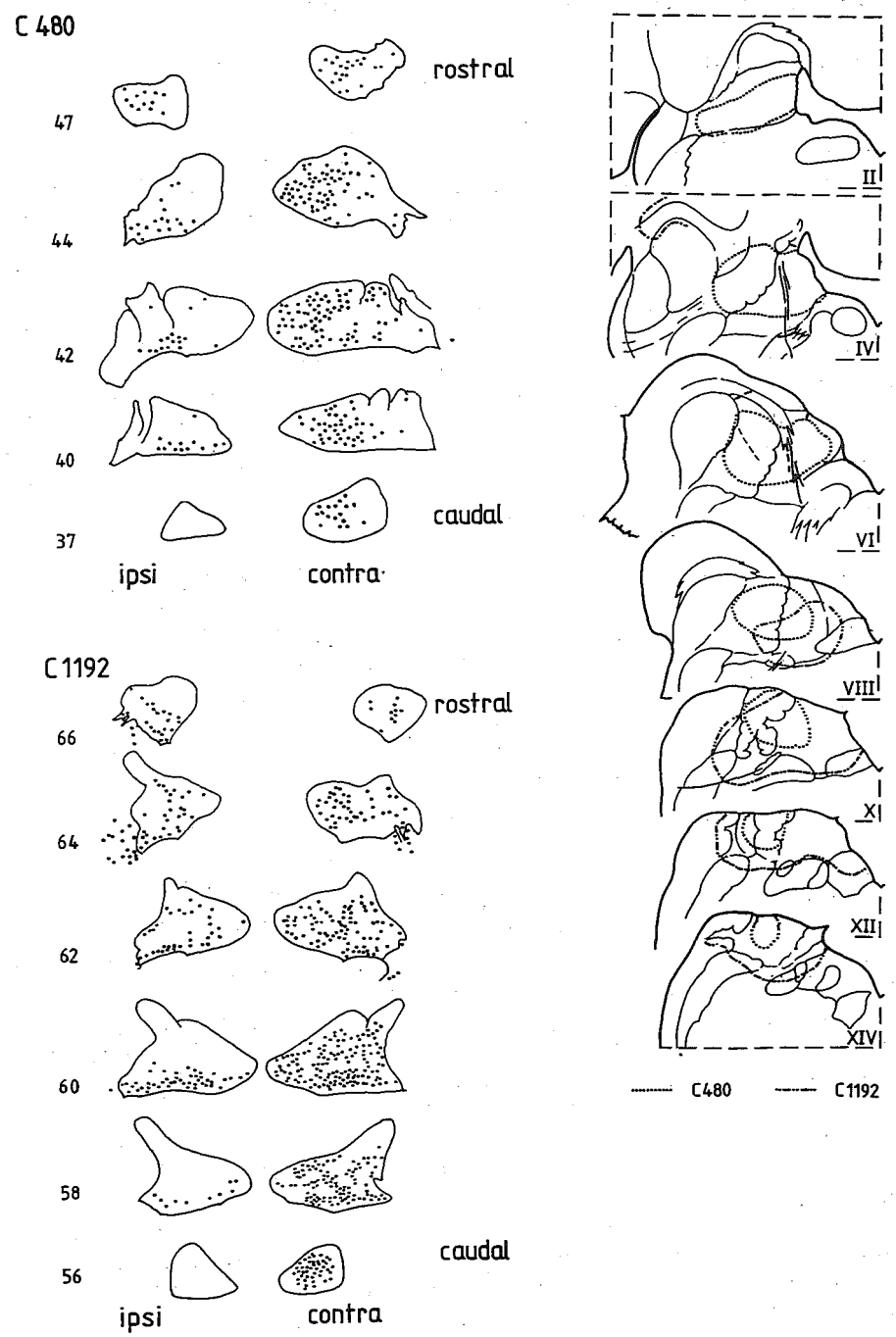

Fig 7.1 Experiments C 480 and 1192: Transverse sections of FN illustrating labeled neurons bilaterally. Right column shows diagrams illustrating the injection sites in the vestibular nuclei.

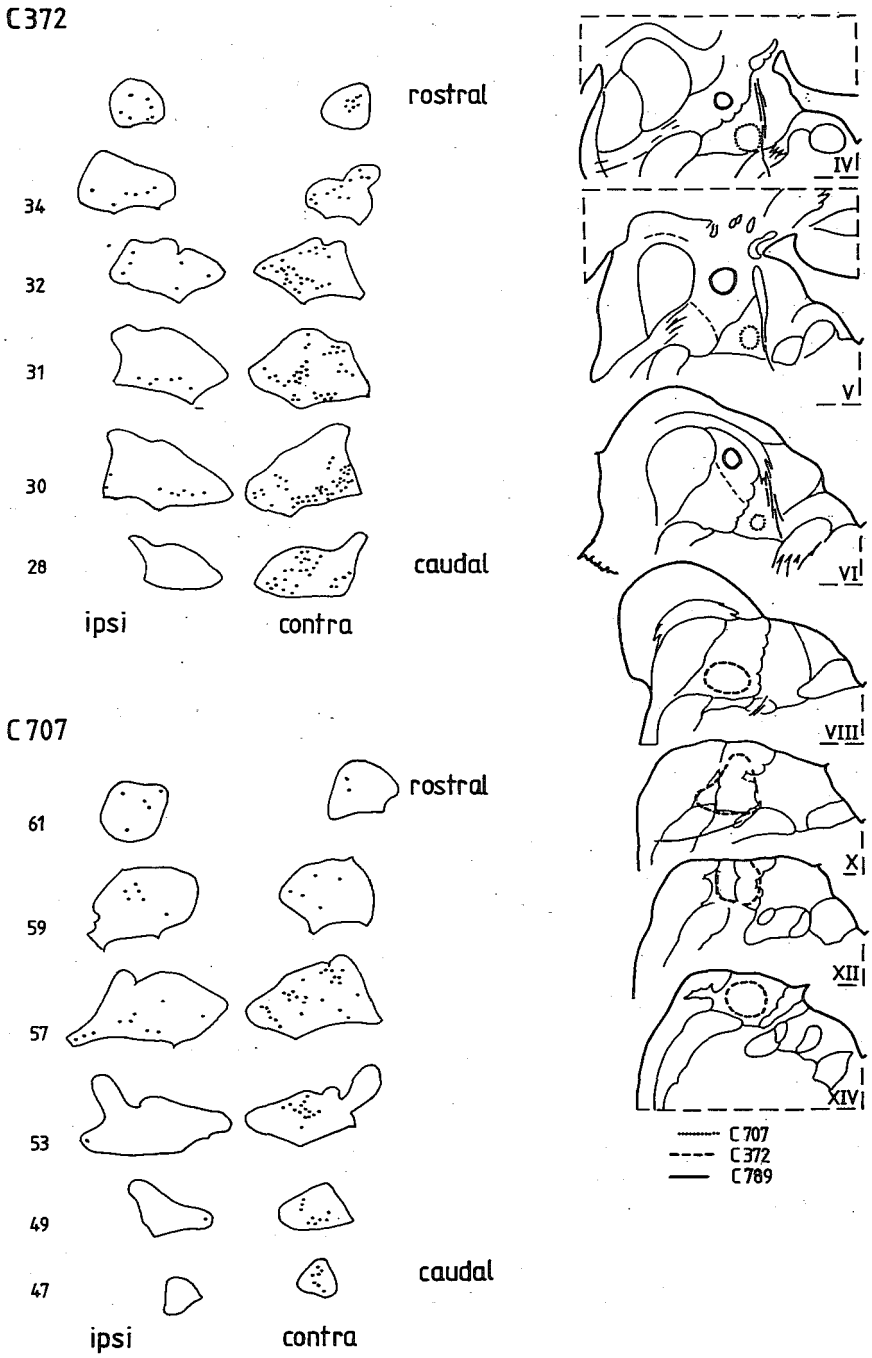

Fig 7.2 Experiments C707, C372, C789. Transverse section through the FN illustrating the extent of labeled neurons bilaterally. The right column shows the injection sites in sections through the VN.

Both cerebellar mossy and climbing afferents, are arranged in longitudinally oriented patterns. Climbing fibers from the contralateral inferior olive use the same compartments as Purkinje cell axons (see for review: Voogd, 1989; Arends and Voogd, 1989). They synapse in zonal patterns on Purkinje cell dendrites in the molecular layer. The organization of corticonuclear and olivonuclear projections seems to follow the same longitudinal zonal principle. Some mossy fiber projections show a parasagittal distribution (van Rossum, 1969; see Thunnissen, 1990 thesis for review). However, as a group, they are not so easy to fit in the longitudinal zonal model (Gerrits et al, 1984). Longitudinal patterns in the cerebellum emerge during development (Lakke et al, 1985; Marani et al, 1986; Marani, 1986).

A similar zonal arrangement of the Purkinje cells projecting to the VN was proposed for the lobule IX (the uvula) and X (the nodule) of the caudal vermis (van Rossum, 1969; Epema, 1983). There is disagreement in the literature on the extent and connections of these zones.

Anterograde degeneration studies (cat: Dow, 1936, Angaut and Brodal, 1967, Voogd, 1964; primate: Haines, 1977; rabbit: van Rossum, 1969) traced the corticovestibular fibers to the superior vestibular nucleus (SV), the medial vestibular nucleus (MV) and the descending vestibular nucleus (DV). No connections seem to exist between lobules IX and X, and the large cells of Deiters' lateral vestibular nucleus (LV). In a recent study in the rabbit Epema et al (1985) demonstrated a localization of Purkinje cells projecting to the VN in three zones on both sides of the uvula. For the nodule zonation was less clear or even absent. Slightly different zonal arrangements were described by Matsushita and Wang (1986) and Shojaku et al (1987) for the cat.

Bernard (1987) studied the distribution of antegradely labeled Purkinje cell axons in the VN using WGA-HRP injections in the cerebellum of the rat. According to this study the Purkinje cells projecting to the VN were arranged in overlapping zones. However, several studies of the olivocerebellar projection to the uvula and nodule in the rabbit demonstrated a clear zonal arrangement of this projection (Katayama and Nisimaru, 1988; Sato and Barmack, 1985).

Older lesion studies in cat and monkey demonstrated flocculovestibular fibers to the entire vestibular complex (Dow, 1936; Jansen and Brodal, 1940; Voogd, 1964). According to Voogd (1964), Angaut and Brodal (1967) in the cat, and Haines (1977) and Langer et al (1985 b) in primates the flocculus projects to all vestibular subnuclei, except for the LV. A zonal origin of the projections of Purkinje cells of the flocculus to different VN was demonstrated with retrograde transport of HRP rabbit (Yamamoto and Shimoyama, 1977) and the cat (Voogd and Bigaré, 1980; Sato et al., 1982a, 1982b). These studies suggested a pattern of three or four zones. The bands showed an alternating projection to the SV, the MV, the SV and the y-group, and the dentate nucleus.

Crossed and uncrossed pathways taking their origin from the FN were first described in the last century (Ferrier and Turner, 1894; Thomas, 1897; Russell, 1897; Klimoff, 1899). Later degeneration studies (Thomas et al, 1956, Carpenter et al., 1959, Walberg et al, 1962; Voogd, 1964) established termination in LV,

DV and MV. Autoradiographic tracer studies confirmed these findings. However, termination in ipsilateral LV proved to be due to transection of passing corticovestibular fibers (Batton et al, 1977; Andrezik, 1984). Similar observations were made in a study of human degeneration material (Gerrits, 1990).

This study describes different sets of experiments, performed in the Dutch belted rabbit, with the aim to determine the origin and the termination of cortico-nuclear and nucleo-cortical projections within the VN of this species. Autoradiography of 3H-leucine injections was used to determine the projections of the caudal vermis, the flocculus and the FN. Retrograde transport of HRP and WGA-HRP injected into the VN was used to reveal the zonal arrangement of Purkinje cells in the caudal vermis and the anterior lobe. Unilateral HRP injections of the VN are illustrated to detail the fastigio-vestibular projection.

RESULTS

Fastigiovestibular projection

HRP injections into the vestibular nuclei.

Some of the experiments with unilateral injections of HRP or WGA-HRP into the VN, were used to determine the origin of the nucleovestibular projection. Large injections covered the caudal (C1192) and rostral (C480) parts of the vestibular complex (Fig 7.1). Overlap of the injection sites occurred in parts of DV and MV (see fig 7.1). In the other depicted cases the injection sites were restricted to DV (C372) and MVmc (C707))Fig 7.2), the major targets of the fastigiovestibular projection. All experiments resulted in remarkable similar distributions of labeled neurons. Ipsilateral labeling was found clustered in ventral portions, with scattered neurons throughout rostral parts of FN. The majority of the labeling was found on the contralateral side. It was present throughout the entire FN. The dorsolateral protuberance was virtually free from labeled neurons. Some labeled neurons were found in a ventro-lateral position, between the fibers in the transition area of the FN, the posterior interposed nucleus and the VN. Very few labeled cells were observed in FN after an injection into LV (see for injection site Fig 7.2 C789).

Tritiated leucine injections into the central cerebellar nuclei

Three injections of 3H-leucine into the central cerebellar nuclei covered different parts of the FN. A dorsal approach was used and the injection needle passed through parts of the anterior lobe. The isotope diffused to a varying degree into the cerebellar cortex, the white matter and the other central cerebellar nuclei. All injection sites were unilateral.

In experiment C1240 the isotope labeled ventromedial parts of the rostral half of FN and extended ventrally into lobule I. A large injection was present in case C809 which covered most of FN and adjacent interpositus posterior nucleus. The tracer reached parts of the cortex of the ipsilateral lobules I, V and, IXd and X of the caudal vermis. The injection in C786 covered most of the rostrocaudal extent of the FN. The injection site involved the cortex of lobule V. Labeled

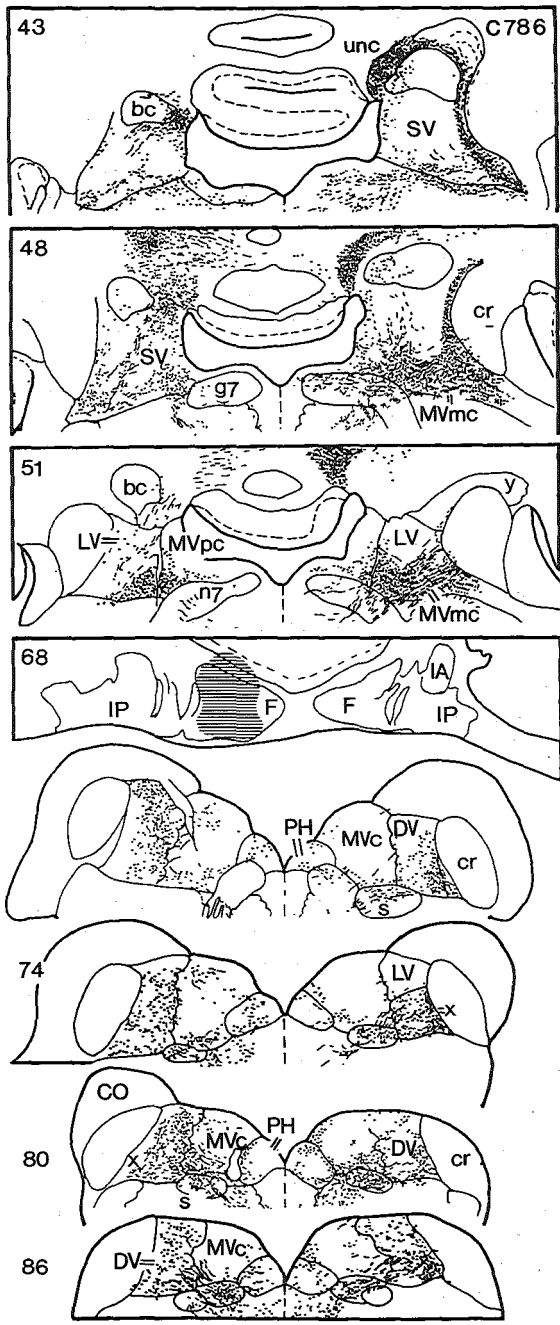

Fig 7.3 Experiment C786; Transverse sections through brainstem and cerebellum illustrating fiber course and termination resulting from 3H-leucine injection into the fastigial nucleus. Extent of injection site is shown in section 68.

fibers followed the same trajectory in the three experiments and their distribution over the VN was very similar. The fibers entered the VN via the ipsilateral juxtarestiform body and the contralateral uncinate tract (unc).

The following description is based on experiment C786 (Fig 7.3). Fibers of the uncinate tract (unc) cross rostral to the level of the FN (not illustrated). They reach a position dorsomedial to the brachium conjunctivum, from where the labeled fibers arched over the brachium to enter the VN from dorsolaterally. At these rostral levels some labeling was observed in ventral and medial SV. The descending fibers of unc occupied a lateral position in LV and DV. At the level of entry of the vestibular nerve, part of the labeled fibers turned ventromedially and terminated in MVmc and rostral DV. Heavy axosomatic termination was present around medium and large MVmc neurons. Some labeling was found in MVpc, mainly in ventral parts. Many fibers entered the reticular formation from MVmc. Heavy labeling of passing fibers obscured a possible termination in INVIII. Labeling was observed throughout DV. Fibers from the lateral descending bundle took a medial course at all levels of DV and entered MVc and the nucleus of the solitary tract. Termination was present in peripheral parts of MVc and PH. Fibers running through the x group terminated in this nucleus.

Labeled fibers of the direct fastigiobulbar tract entered the VN ipsilaterally via medial parts of LV. The majority of the termination was found in MVmc and DV, in the same areas as on the contralateral side. Few terminals were observed in peripheral SV, in ventral and lateral MVpc, and in peripheral MVc and PH. Labeling was never observed in the ipsilateral group x, the group y, NVpar and the contralateral LV.

The experiments differed with respect to the density and the distribution of the labeling on the ipsilateral side. Experiment C1240, with diffusion of the injection site into lobule I, contained few labeled fibers in LV. Label over the VN was more dense in C809 than in the other experiments. Moreover ipsilateral SV, MVpc and MVc contained label throughout their extent.

Corticovestibular projections

Projections from the flocculus to the vestibular nuclei.

Three rabbits received injections of the labeled amino acid in the flocculus. Injections sites covered parts of the flocculus and adjacent ventral paraflocculus. (see Fig. 7.4). Diffusion of the isotope to the central cerebellar nuclei or the group y was not observed. The label in case C677 covered parts of folium f1, f2 and f3. The injection site of C669 was present in folium f2 and f3, with some diffusion to folium f4 and p (Yamamoto and Shimoyama, 1977). Experiment C659 contained a small injection in folium f2 and f3 (not illustrated). All injections resulted in roughly similar distribution of the labeling in SV, MVpc, MVc and the group y.

The trajectory of the Purkinje cell axons of the flocculus to the VN is very characteristic. Labeled fibers collected at the base of the flocculus lateral to the restiform body. More caudally they arch over the restiform body in the floccular peduncle. This bundle is located ventral to the dentate nucleus and contains the fusiform cells belonging to group y. At the level of SV and rostral LV the

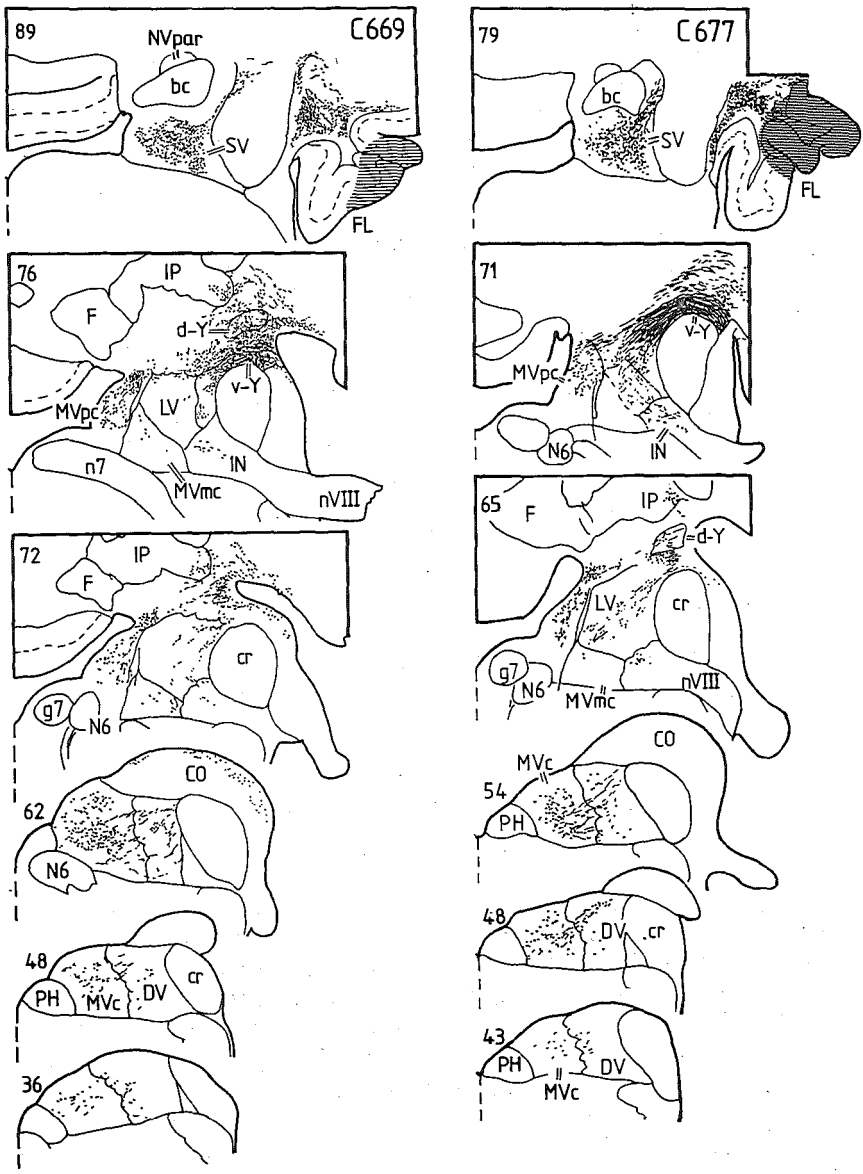

Fig 7.4 Distribution of labeled fibers and termination (dots) in transverse sections through the vestibular nuclei following injection of tritiated leucine in the flocculus (exps C677, C669).

floccular peduncle enters the VN. Some fibers pass more dorsally through the cerebellar nuclei and join the VN via the juxtarestiform body (Fig. 7.4 section 72 and 76). The most rostral fibers of the floccular peduncle entered SV at its dorsolateral edge. They fanned out to terminate in central SV. Some labeling was present in the periphery of this nucleus. Labeled fibers terminated in and passed around dorsal group y. Heavy labeling consisting of passing fibers but also of dense termination was present over the entire ventral group y, located immediately dorsal to the restiform body. The labeling reached the ventrolateral corner of group y where it is continuous with the cochlear nucleus. Moreover, in experiment C669 clear and in C677 faint label was found in the cochlear nucleus, mainly in its dorsal part (Fig. 7.4 section 62). The caudal part of the floccular peduncle divided in a dorsal and a ventral branch. The dorsal branch passed dorsal to LV and entered MVpc at its dorsal border, in the lateral angle of the fourth ventricle. It corresponds to Loewy's angular (1916) bundle. Termination occurred chiefly in dorsal and medial parts of MVpc. Some fibers ran through MVmc, but no sign of termination could be seen. The ventral branch followed the restiform body, to reach the IN and to descend through dorsolateral LV and DV. At more caudal levels the labeled fibers fanned out to terminate in rostral MVc. At mid levels of MVc, which was the most caudal level of termination, labeled fibers occupied the dorsal and central parts of the nucleus. Some fine labeling was found in rostral DV throughout its extent.

Outside the VN some labeling was clearly present in rostrolateral parts of the posterior interposed nucleus (Fig. 7.4 sections 72, 76 and 65). Termination was never found in LV, PH, group x, nVI and the most caudal aspects of MVc and DV.

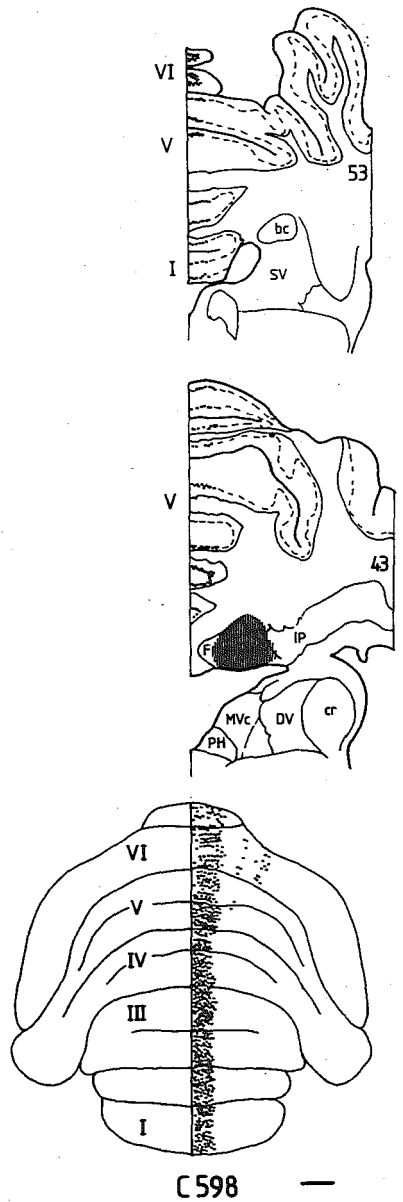

Fig 7.5 Experiment C598; two transverse sections and horizontal reconstruction of the vermis illustrating labeled Purkinje cells in A-zone, following injection of HRP into FN. Injection site in section 43.

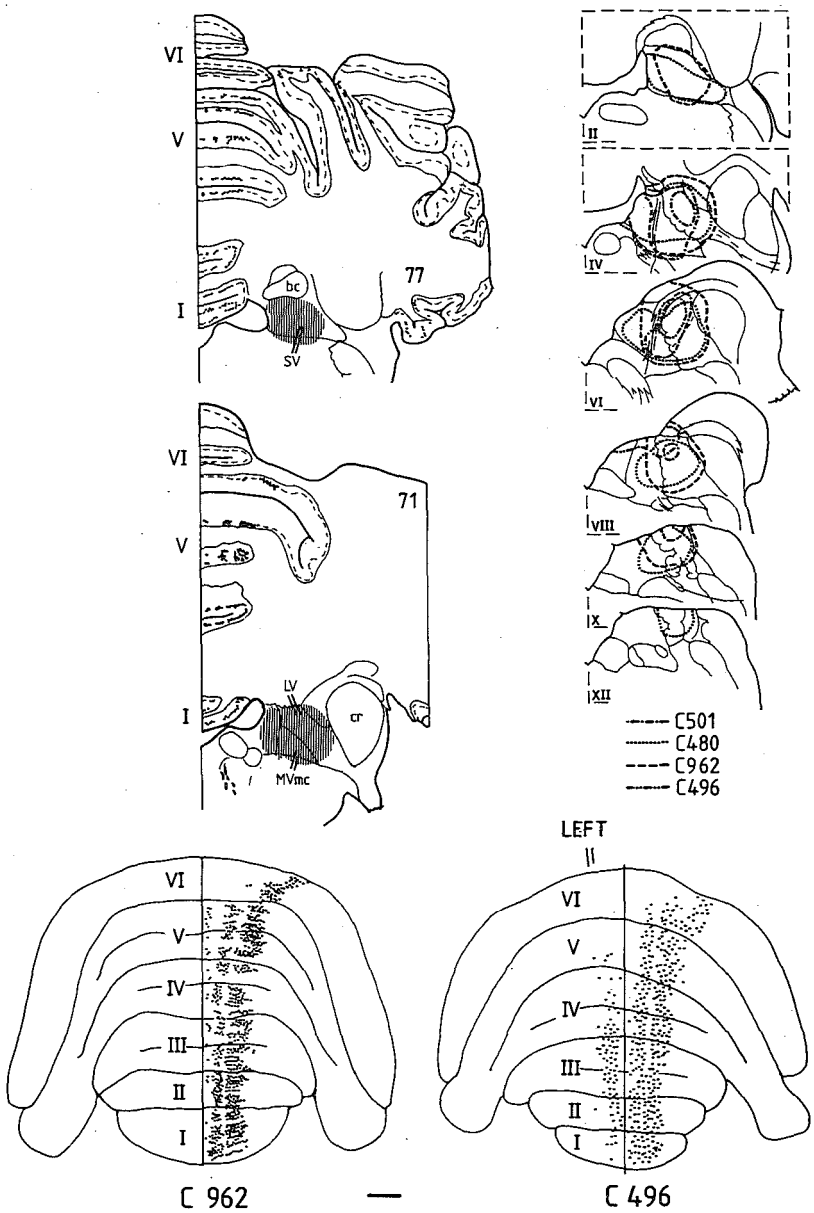

Fig 7.6 Experiments C962, C496; Transverse sections (top left case C962) and horizontal reconstructions of the vermis illustrating labeled Purkinje cells in the A- and B zones, following large injections into the VN. Injection sites are drawn in transverse diagrams through different levels of the VN (top right).

Projections from the anterior lobe to the fastigial and the vestibular nuclei

In ten experiments injections of HRP or WGA-HRP were placed in different parts of the VN or the FN (see Table 7.1). All experiments resulted in labeling of Purkinje cells in one or more longitudinally oriented zones in the vermis of the anterior lobe. Most of them were continuous throughout the lobules I to V, some crossed the primary fissure and extended into lobule VI (simple lobule).

The axons of the Purkinje cells were clearly labeled in some of the experiments. The labeling in these cases could be traced from the labeled Purkinje cells to the injection site. The distribution and the number of labeled fiber compartments was similar to that of the overlying labeled Purkinje cell strips. These fibers always occupied a more lateral position than the corresponding Purkinje cell zones. This correspondence was most distinct in dorsal aspects of the vermis. From the white matter they passed along and through the cerebellar nuclei in the juxtarestiform body to enter the LV nucleus. Labeled fibers from medial zones ran through lateral FN, and from the lateral zones just lateral to FN.

An injection restricted to the FN (C598, Fig.7.5) labeled a single strip of Purkinje cells extending from the midline to 0.5-0.9 mm. laterally in the lobules I through V. Caudal to the primary fissure it extends into the posterior lobe. It corresponds to the A zone of Voogd (1964). Large injections into LV and MVmc, and various parts of the VN always resulted in labeling of 3 longitudinal oriented strips of Purkinje cells (Fig. 7.6). A narrow medial strip with a width of only 2-3 Purkinje cells and a somewhat wider, 0.4 mm., strip located at the lateral border of the A zone occupied the same area as the FN zone. Still more laterally a third strip, corresponding to Voogd's (1964) B zone is labeled. Its width varied between 0.8 and 1 mm, and it extended into lobule VI. Few labeled neurons were present in the hemisphere. Between the A zone and the B zone a wedge shaped area remained free of labeling. This area was most distinct in dorsal parts of the anterior lobe, but also clearly present as a narrow slit in the in lobules II and III (Fig.7.6). This label-free zone is found in a position similar to that of the x zone in the cat and monkey (Trott and Armstrong, 1987 a and b; Voogd, 1982 and 1989). The labeling in the A and the B zones seemed to fuse in lobule I since no clear pattern was observed in this lobule. Labeling, restricted to the lateral A zone, was found in cases with injections in MVmc (C 707, C296 Fig 7.7). The B zone in the vermis was selectively labeled in case C654 with an injection into caudal LV diffusing into dorsal DV (Fig 7.8). The small iontophoretic HRP injection of case C789 resulted in labeling in the B zone and some labeled Purkinje cells in the medial strips. This could be due to interruption of fibers in LV on their way to MVmc (Fig 7.8).

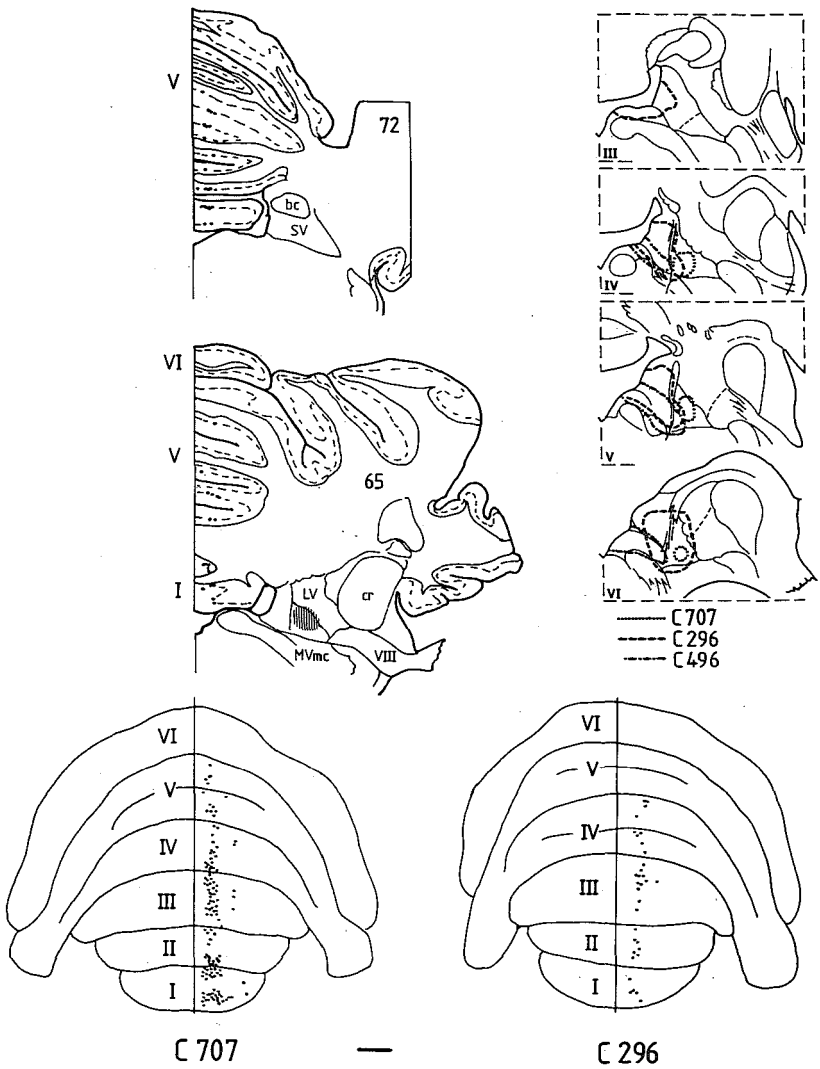

Fig 7.7 Experiments C707, C296; Transverse sections (top left case C707) and horizontal reconstructions of the vermis (bottom) illustrating labeled Purkinje cells in the A zone, following injections of HRP into MVmc. Injection sites in diagrams of the VN (top right)

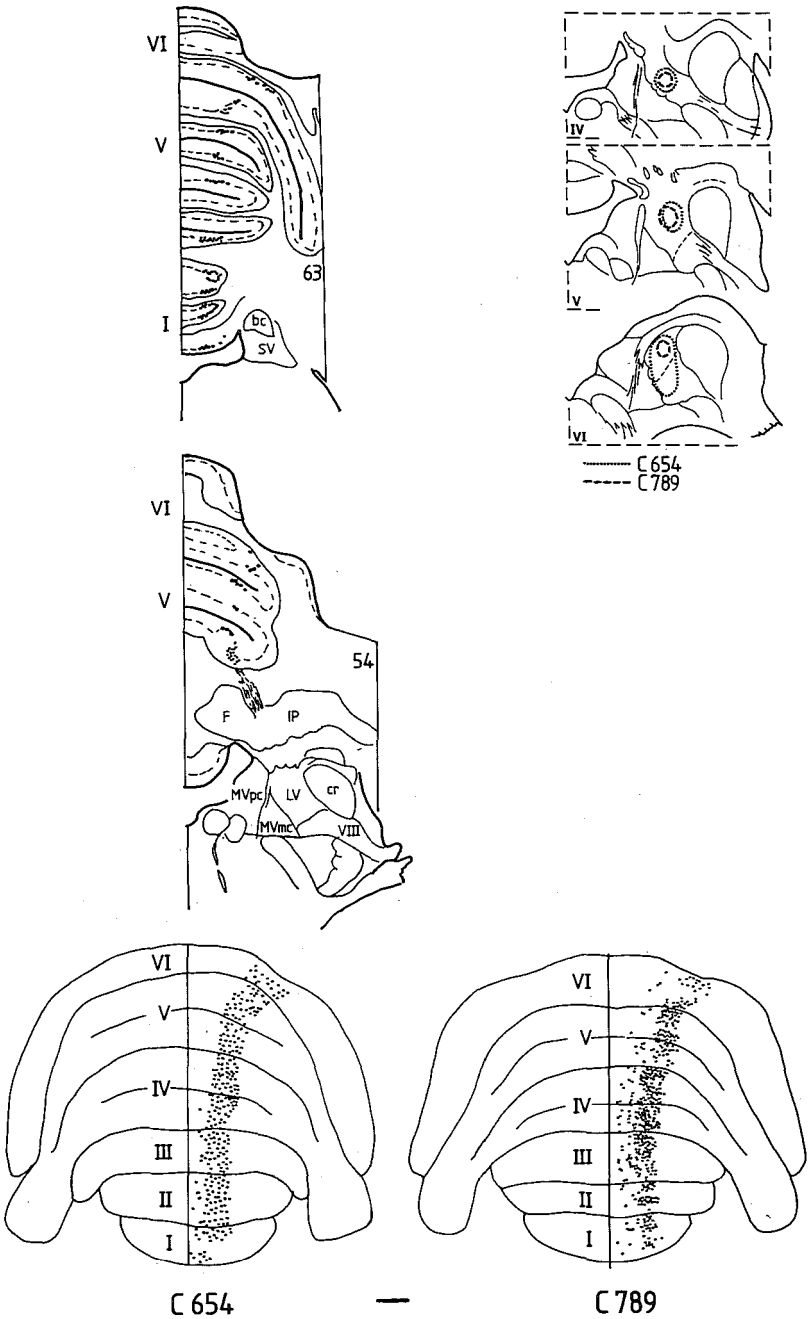

Fig 7.8 Experiments C654, C789; Transverse sections (top left case C654I) and horizontal diagrams illustrating labeled Purkinje cells in the B zone, following injections of HRP into LV. Transverse diagram of the VN illustrating the extent of the injection sites.

Projections from the uvula and the nodule to the vestibular nuclei

HRP injections into the vestibular nuclei

The corticovestibular projection was studied with injections of WGA-HRP and HRP in the different vestibular nuclei (Figs. 7, 9 and 7,10-12; Table.7.2). Unilateral experiments always resulted in labeled Purkinje cells on the ipsilateral side. A medial zone of lobules IX-a, IX-b and part of IX-c was labeled following an injection into the FN (C 598, Fig. 7.10; for injection site see Fig. 7.5). The rest of uvula and nodule was necrotic and could not be studied. Large injections in the caudal VN including MVc and DV (C 1192) resulted in the labeling of three strips of Purkinje cells in lobule IX (Fig 7.10). The three zones were most distinct towards the top of the lobules. In the depth of IX a-c the lateral two of the zones seemed to fuse. Diffusely scattered labeled Purkinje cells were present in the nodule and adjacent ventral uvula. The most lateral part of lobule IX remained free of labeling in all experiments. A similar distribution with less discrete labeling in the two lateral bands of lobule IX was found after an injection of dorsal DV extending into LV (C 654I, Fig. 7.10).

After injections restricted to the MVc (C 468, C654 II, Fig. 7.11) the labeling was restricted to two discrete strips of Purkinje cells, one laterally and the other medially in the nodule and ventral uvula (IXd). The area of the midline was never labeled. Towards the rostroventral border of the nodule the zones fused into a single cluster.

Injections into central SV (C 1201, Fig. 7.12) produced labeling of Purkinje cells of the entire nodule and ventral uvula. Lobules IX a-c contained few scattered cells. An injection into lateral SV (C 1102, Fig. 7.12) mainly labeled a medial cluster along the posterolateral fissure in lobules X and IX d, with few neurons in the rest of the nodule and uvula. In case C524 (Fig. 7.12) the injection into SV, MVpc and MVmc was made from ventrally and thus did not interrupt fibers of passage in caudal parts of the vestibular complex. Labeling of Purkinje cells was found in a intermediate strip in lobule IX and scattered in a more lateral position in lobule IX. A medial strip of Purkinje cells in lobule IX remained unlabeled. The nodule contained scattered labeled Purkinje cells.

Tritiated leucine injections into the cortex of the uvula and the nodule

In nine rabbits uni and bilateral injections of tritiated leucine were placed into the cortex of the nodule and the uvula (Figs. 7.13 and 7.14). The injection site of case C1056 (not illustrated) was located in the midline of lobule X, but diffused slightly into rostral parts of the FN. Few labeled fibers were found in the uncinate tract of both sides. Diffusion of the injected tracer into central cerebellar or VN was not observed in the other cases.

All injections covered the cortex of the caudal vermis to a varying extent. Unilateral injections resulted in strictly ipsilateral termination in the VN and the FN. Similar observations were made in the HRP material of the corticovestibular projection.

Labeled fibers from an injection located in the centrolateral part of lobule IX (C 646, left side; Fig. 7. 13B) took up a position in the folial white matter corresponding to the medio-lateral extent of the injection site and kept this

position during their central course. Fibers collected ventrolateral to the FN and passed through the cerebellar nuclei in the juxtarestiform body to enter the LV. A number of axons terminated in the posterior interposed nucleus and group y. Within LV most fibers changed their course and descended in fascicles between Deiters' neurons. They continued as fiber bundles in the dorsomedial DV. Termination in the MVc was restricted to its ventrolateral corner. Some fibers ascended along the mediodorsal edge of SV where they also seemed to terminate.

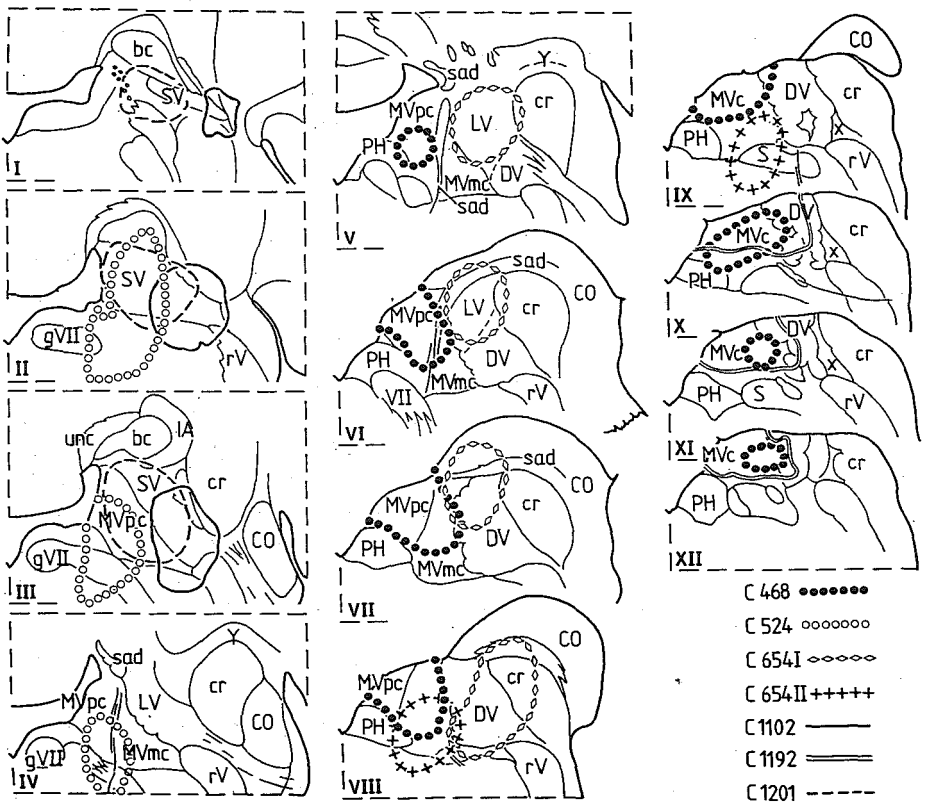

Fig 7.9 Diagram of transverse sections through the VN illustrating the extent of injection sites of experiments with labeled Purkinje cells in the caudal vermis.

Injections of the medial one third of lobule IX in the cases C570 (right side Fig 7.14 B) and K54 (Figs. 7.14 A) resulted in the labeling of axons in the medial half of the lobular white matter. These fibers assembled ventral to the FN where they terminated along its ventral periphery and at the junction of the FN with the posterior interposed nucleus. The lateral part of the posterior interposed nucleus remained free from labeled fibers. Some fibers could be traced to group y. The trajectory in the juxtarestiform body and the termination of the fibers in the VN

was the same as in the previous case. When the injection is located in the lateral one third of lobule IX (K53, Fig. 7. 13 A) the labeled axons in the lateral extremity of the folial white matter could be traced to the lateral half of the posterior interposed nucleus were they terminated. Only few labeled fibers were present in DV in this case,

Injections limited to the nodule were absent from our material. Injections of the centrolateral part of lobule X in C 646 (right side Fig 7.13 B) and C 692 (Fig. 7.13 C) also involved the lateral and the intermediate cortex of lobule IX in the bottom of the posterolateral fissure. The bundle of labeled fibers in the white matter of the nodule diverged laterally where they leave the lobule and merge with the fibers from lobule IX ventral to the posterior interposed nucleus and lateral to the FN. The course of the labeled fibers through the cerebellar nuclei was similar to the previous cases. Terminations were found in the interposed nucleus and group y, but were absent from the FN. Rostrally directed fibers entered SV to terminate in its periphery. Labeling in the fiber bundles and the neuropil of DV was similar to the cases with injections restricted to lobule IX. In addition labeling was found in dorsal and central parts of MVpc, which continues in central MVc. Labeling was absent from the LV, the MVmc and the PH. In C 570 (left side, Fig. 7.14 B) the injection involved the apex of lobule X together with lobule IXd. The distribution of the labeling was the same as in the previous two cases, but was somewhat more extensive in the MVc.

DISCUSSION

Fastigiovestibular projection

The present study is the first to reveal the fastigiovestibular projections with autoradiographic and retrograde transport methods in the rabbit. All HRP injections into target areas of this projection resulted in similar patterns of labeled neurons in the FN of both sides. On the ipsilateral side labeling was clustered ventrally in FN with scattered neurons throughout the nucleus. Most labeled cells were found throughout the FN contralaterally. These results differ from older studies which used axonal degeneration methods (Thomas et al, 1956; Carpenter et al, 1959; Walberg et al., 1962; Voogd, 1964), which described a crossed projection from the caudal half and uncrossed projection from rostral parts of the nucleus. Our findings are in accordance with the more recent axoplasmatic transport studies of Batton et al (1977) in the monkey and Andrezik et al (1984) in the dog. Following unilateral tritiated leucine injections heavy labeling occurred in MVmc, DV, and the group x bilaterally. The presence in some of the older studies of labeling in LV almost certainly was due to interruption of passing Purkinje cell fibers. In addition to Batton's (1977) and in keeping with Andrezik's study we found termination in peripheral parts of the superior vestibular nucleus. Ipsilaterally this could be due to involvement of the cortex in the injection site, because several studies (van Rossum, 1969; Angaut and Brodal, 1967; this thesis) demonstrated Purkinje cell projections from the caudal vermis to peripheral SV and MVc. However, Purkinje cell projections are

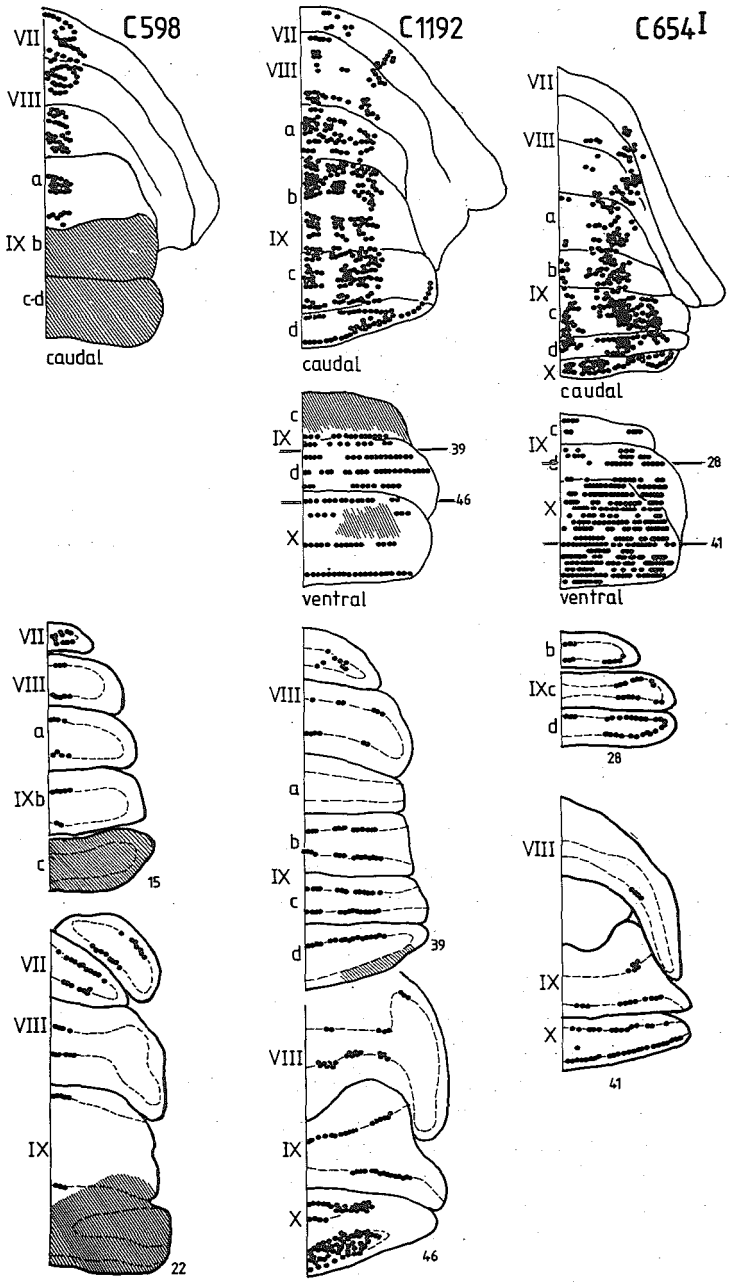

Fig 7.10 Experiments C598, C 1192, C654; Transverse sections (bottom) and horizontal reconstructions (top) of labeled Purkinje cells in the uvula and nodule following injections into FN (C598), into MVc and DV (C1192), and into DV and adjacent caudal LV (C654I)(Fig 7.9). See text for explanation of labeled Purkinje cell zones in these experiments.

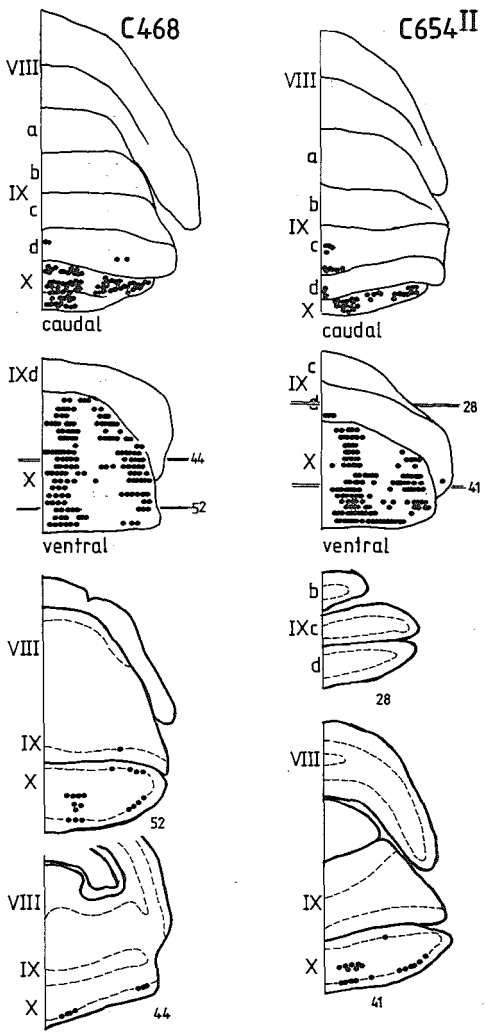

Fig 7.11 Experiments C468, C654 II; transverse sections (bottom) and horizontal recunstructions of the caudal vermis illustrating the distribution of labeled Purkinje cells in two zones in lobule X and IXd following injections of HRP into MVc. Injection sites in Fig 7.9.

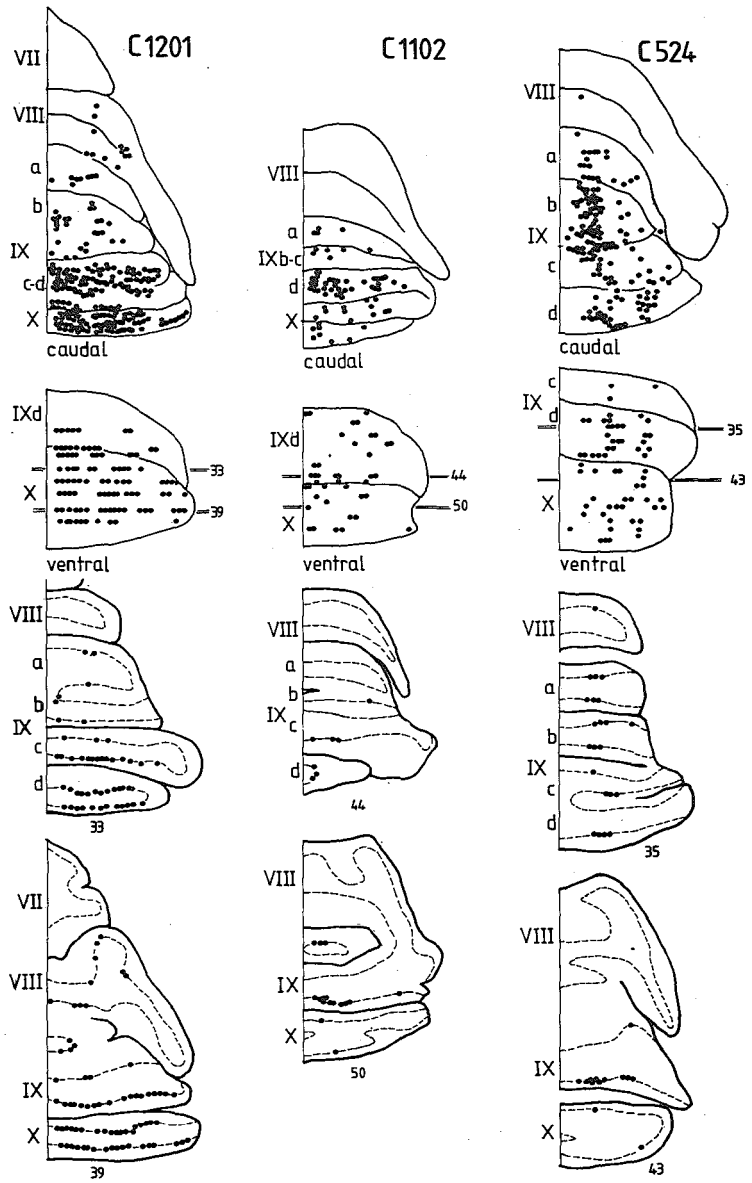

Fig 7.12 Experiments C1201, C1102, C524; transverse sections (bottom) and horizontal reconstruction of the caudal vermis illustrating labeled Purkinje cells following injections of HRP into different parts of SV (Fig 7.9).

always ipsilateral. Thus the contralateral label in SV and in MVc is due to the injection of fastigial neurons. Some termination was found over the PH, which was not reported in previous tracer studies.

Corticovestibular projection

Flocculus

Anterograde transport of tritiated leucine to trace the flocculovestibular projection was used by Langer et al. (1985 b) in the monkey and in the present study in the rabbit. Anterogradely transported aminoacids exclusively label Purkinje cell axons when injected into the cortex. In contrast to WGA-HRP, which was used to study this projection in the rat (Bernard, 1987), tritiated leucine does not cause retrograde labeling of axons and neurons and thus facilitates the interpretation of the results. Secondary vestibulocerebellar mossy fibers to the flocculus have been demonstrated to originate from the same areas of the VN which receive a projection from the flocculus (Epema et al, 1990; Haines, 1977; Langer et al, 1985 b). Some of the labeled structures in studies of corticovestibular projections using WGA-HRP as a tracer, therefore, could have represented retrogradely labeled axons or even dendrites. This methodological problem was also encountered in studies of the projections of the caudal vermis (Bernard, 1987; Dietrichs et al, 1983; Buisseret-Delmas, 1988). The original studies of the flocculovestibular projection employed axonal degeneration methods (Dow, 1936; Voogd, 1964; Angaut and Brodal, 1967; Haines, 1977).

The present study shows dense projections to the y group, central SV, parts of MVpc and MVc in keeping with other students (Voogd, 1964; Haines, 1977; Angaut and Brodal, 1967; Langer; 1985). Bernard (1987) who injected WGA-HRP into the flocculus of the rat did not observe labeling in SV. Yamamoto (1978) in a retrograde tracer study found labeled Purkinje cells in the flocculus of the rabbit following injections into the prepositus hypoglossal nucleus. In our cases and in the study of Langer et al. (1985b) labeling never reached the PH, but was observed close to this nucleus in MVc. Uptake of the tracer from the periphery of the HRP injections in Yamamoto's (1979) experiments could explain the difference. Neurons giving rise to secondary vestibulocerebellar mossy fibers to the flocculus occupy a larger area in the VN than the flocculocerebellar projection. They extend into the caudal MVc and DV, and in PH (Epema et al, 1990; Rubertone and Haines, 1981; Sato et al, 1983; Langer, et al 1985 a).

The findings in this study do not permit conclusions on the proposed zonal organization of the floccular Purkinje cell projections (Yamamoto and Shimoyama, 1977; Sato et al, 1983 and b, Balaban, 1984).

Anterior lobe vermis

The results of this study demonstrated a zonal organization of Purkinje cell projections from the anterior lobe vermis to the vestibular complex in the rabbit. The general pattern of projections of the medial A zone to the FN and the MVmc, and of the lateral B zone to LV confirmed earlier reports on other species is supported in the literature (see Voogd, 1989 for a review). In the

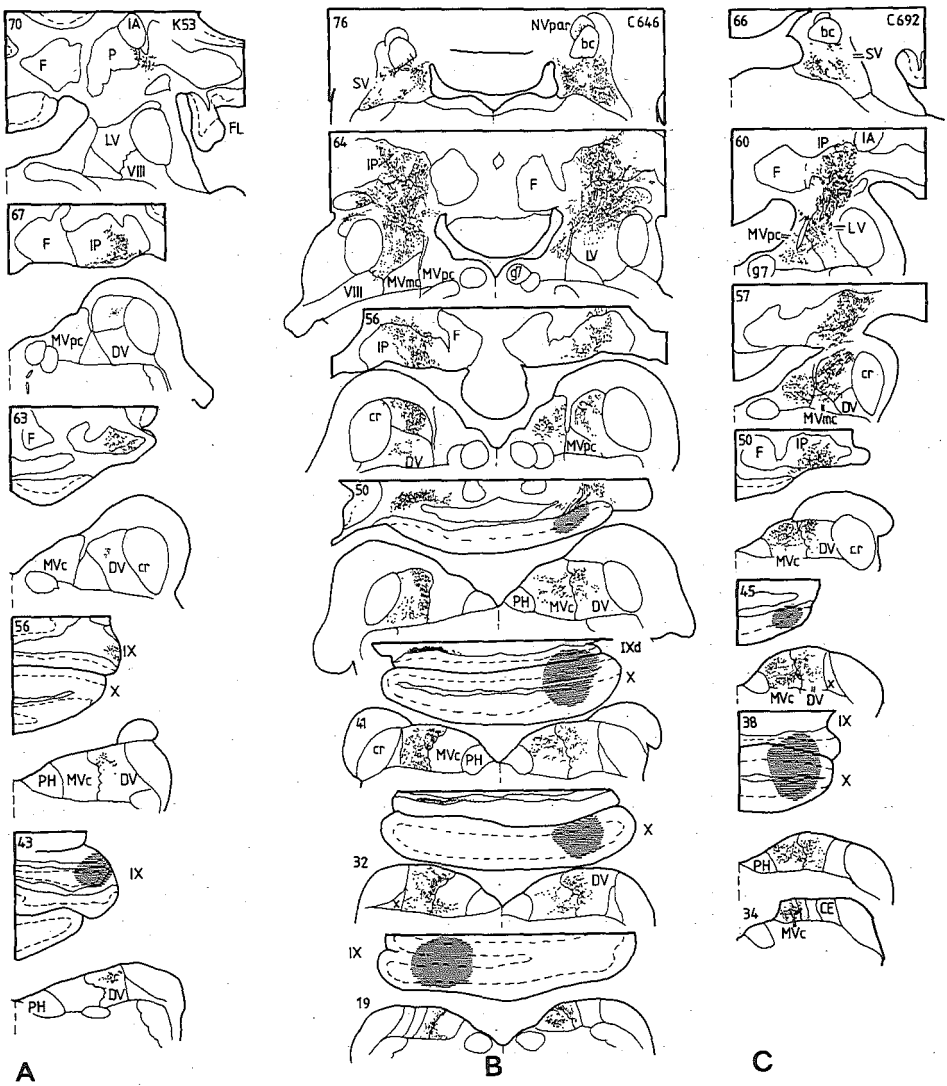

Fig 7.13 Experiments K53 (A), C646 (B), C692 (C); transverse sections of the VN illustrating the fiber course and termination of labeled Purkinje cell axons, following injections of tritiated leucin into different parts of the caudal vermis (K 53, section 43; C646 section 19-50; C 692 sections 38 and 45).

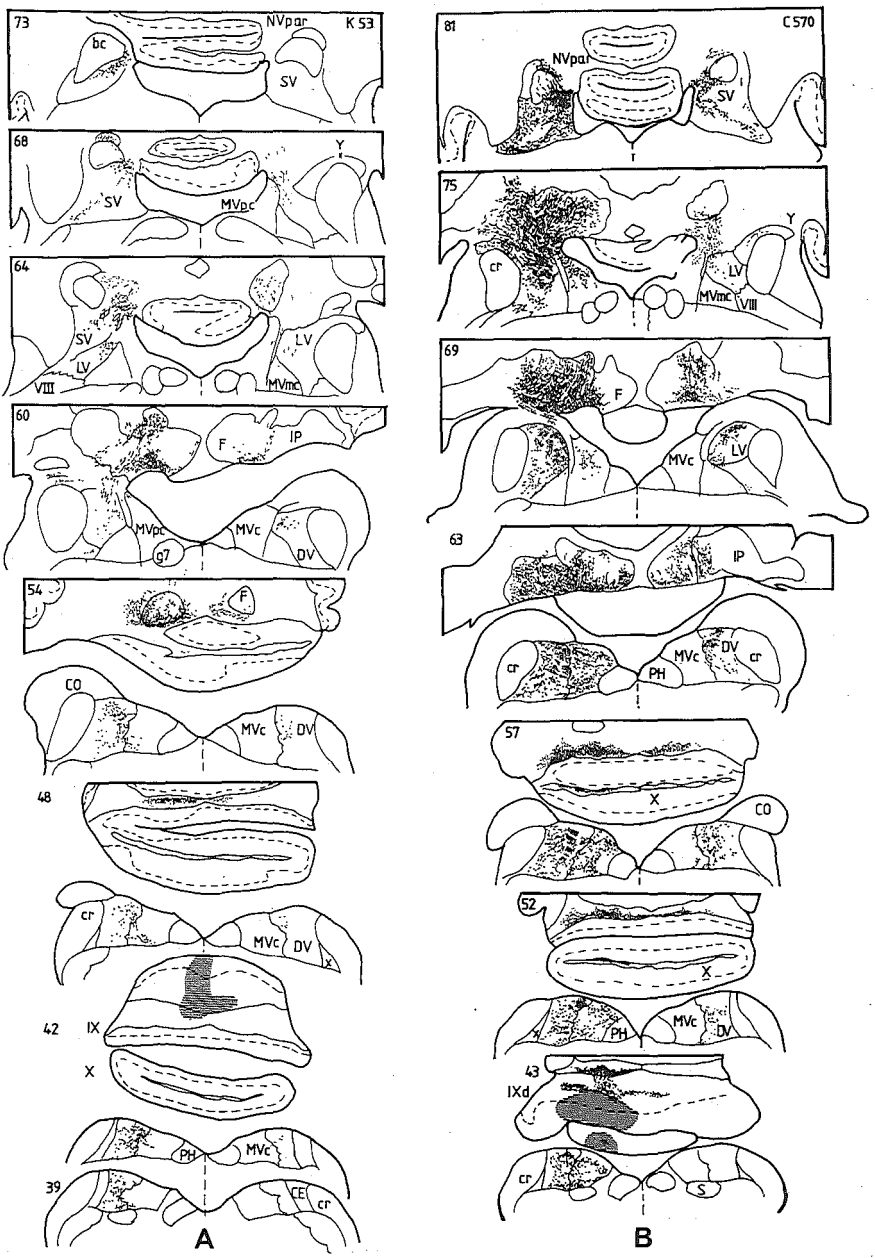

Fig 7.14 Experiments K54 (A), C570 (B); transverse sections of the VN illustrating fiber course and termination of labeled Purkinje cell axons after injections of tritiated leucine into parts of the caudal vermis (injection site: K54 section 42; C570 section 43).

rabbit a narrow, third zone was found to be located medial to the A2 zone of the former studies (Voogd and Bigaré, 1980, Voogd, 1989; Balaban, 1984).

A wedge-shaped zone between the A and the B zone always remained free of labeled corticovestibular Purkinje cells. This zone probably represents the X zone (Trott and Armstrong, 1987a and b; Voogd, 1989). This zone projects to the junction of the fastigial and posterior interposed nucleus (Trott and Armstrong, 1987b). Our material suggest that this zone is not only present in lobules V and IV, but that it extends ventrally into the lobules III and II as a zone with a width of one or two Purkinje cells.

Caudal vermis

Projections from Purkinje cells of the caudal vermis and the flocculus overlap to some degree but mostly terminate in complementary regions of the VN. This was demonstrated most clearly for the SV where the central part of the nucleus received fibers from the flocculus whereas fibers from the nodule terminate in the periphery, with fibers from the uvula terminating along its dorsomedial and ventrolateral edge. Similar observations were made in the MVc, where fibers from the flocculus which terminate in rostral and central portions are surrounded by a projections from the nodule, with a slight contribution from the uvula to its ventrolateral corner. The labeling in the MVpc was also complementary, with projections from the flocculus concentrated in dorsal parts and from the nodule in its more ventral parts. The remaining area of MVmc received a dense input from the FN. These results are is in keeping with and extend former studies in the cat (Voogd, 1964; Angaut and Brodal, 1967) in Galago (Haines, 1977) and rabbit (van Rossum, 1969).

With respect to the zonal organization of the Purkinje cells in the caudal vermis which project to the VN the situation is rather complicated. Discrete Purkinje cell strips were labeled in lobule X after injections of the MVc. Purkinje cells in the remaining cortex of the nodule and in lobule IX could be labeled to a varying extent from injections into DV and SV.

Large, caudal injections of MVc and DV and injections of DV alone, which presumably also label the MVc efferents which pass through this nucleus, produced a clear zonal pattern in lobule IX, except for its lateral one third. Injections restricted to the MVc labeled only a few cells in the ventral uvula. The projection of the uvula to DV, therefore, takes its origin from zonally organized Purkinje cells in its medial two thirds. Medially located Purkinje cells also project to the FN. An intermediate strip of Purkinje cells also projects to the junction of FN with the posterior interposed nucleus, to the MVpc and/or the SV. Purkinje cells in the lateral one third of lobule IX exclusively project to the posterior interposed nucleus.

Purkinje cells which were labeled from the SV are present in the same regions of lobule IX and X which project to DV and MVc. A medial cluster of Purkinje cells located in the depth of the posterolateral fissure was consistently labeled from SV.

The presence in the uvula of strips of Purkinje cells which could be labeled from injections of caudal DV and MVc is in accordance with the observations of Matsushita and Wang (1986)in the cat. In general our findings confirmed the

description of the Purkinje cells in the caudal vermis with projections to the VN of Shojaku et al. (1987) in the cat. The main difference concerns the presence of a zonation in the distribution of the Purkinje cells in the uvula in our experiments in the rabbit. Overlapping distributions of Purkinje cells with projections to the different vestibular nuclei also were observed by Bernard (1987) and Tabuchi et al (1989) in the rat.

Purkinje cells strips in the anterior and the posterior lobe had a common feature. Zones on both edges of the vermis in lobule I and in the nodule fused and formed a cluster. Moreover, the zones at caudal and rostral ends of the cerebellar cortex become smaller on their way to the primary fissure. This could point to a system of zones which are infringed from both ends and from the center of the cerebellum. A similar pattern of fusing zones was observed in enzyme histochemical studies (Marani, 1986).

Several explanations are possible for the zones in similar areas which projected to different nuclei. One zone projects to different nuclei or one zone contains smaller strips each projecting to a specific nucleus.

The experiments with retrograde labeling of Purkinje cells after single injections of HRP in the VN reported in this thesis are not well suited to decide whether Purkinje cells project to more than one vestibular nucleus. Collateralization in the projection of the Purkinje cells to VN and their zonal organization should be studied with double labeling techniques to resolve this question.

The differential projections of the flocculus, the caudal vermis and the FN to the VN are important in the interpretation of experiments on the influence of the cerebellum on eye movements and vestibular mediated postural adjustments. The vestibulo-oculomotor projection is the second link in the three neuronal vestibulo-ocular reflex (VOR) arc (Lorente de Nó, 1933; Szentágothai, 1943). The VOR can be influenced through the cerebellum. The flocculus was implicated in the adaptive modification of the VOR in two different hypotheses (Ito, 1984; Lisberger, 1988). The effect of floccular stimulation on oculomotor neurons has been demonstrated in the rabbit (Fukuda, 1972; Ito et al, 1982; Nagao et al, 1985). The caudal vermis assists in the adaptation of the VOR but is not essential for this process (Mason and Baker, 1989). However, the caudal vermis has a major role in the mechanism of 'velocity storage' of the VOR (Waespe et al, 1985). The FN is involved in saccades (Yamado and Noda, 1987).

The localization of the vestibulo-oculomotor neurons (see Chapter 6) can be compared with the distribution of these cerebellar cortical efferents and with the projection of the FN. Dense projections from the flocculus and the caudal vermis reach group Y, central and dorsal and MVpc, the rostral MVc, and the rostral DV. Pre-oculomotor neurons are present in the same areas. However, a substantial number of pre-oculomotor neurons is present in the ventral MVpc, the MVmc, and in the PH. These neurons which are involved in horizontal and vertical eye movements (Highstein and McCrea, 1988) cannot be influenced directly from the flocculus or the caudal vermis. Interestingly, the MVmc and the ventral MVpc receive a dense input from the FN, which is involved in the execution of saccadic eye movements (Noda et al., 1988). Neurons related to ipsilateral eye position, which generate bursts of spikes during saccades, have

been identified in ventral MVpc and ventral MVmc (McCrea et al., 1987). Therefore, an alternative pathway for the control of saccades, which passes through the VN, is available, apart from the one consisting of fibers ascending through the brachium conjunctivum and relaying in the brainstem as proposed by Ikeda et al. (1989).

Table 7.1

exp	tracer	vol ul	surv days	FN	LV	MVmc
C598	33%HRP	0.3	2	+		
C480	33%HRP	1	2		+	+
C501	20%HRP	0.4	2		+	+
C496right	33%HRP	0.3	2		+	+
C962	5%WGA-HRP	0.3	1		+	+
C654*	33%HRP	0.18	2		+	
C789	HRP#		2		+	
C707	HRP#		2			+
C296	33%HRP	0.5	2			+
C496left	33%HRP	0.2	2			+

*: injected from ventrally
#: iontophoresis of 5% HRP in 0.01M Trisbuffer (Graybiel and Devor, 1974)

LEGEND TO TABLE 7.1.
Listing of the experiments, with type of tracer, injected volume and survival time. The plus sign indicates which vestibular subnuclei are included in the different injection sites.

Table 7.2

exp	tracer	vol	SV	MVpc	LV	MVc	DV
C1102	33%HRP	0.15	+				
C1201	5%WGA-HRP	0.15	+				
C524*	33%HRP	0.25	+	+			
C654I*	33%HRP	0.18			+		+
C1181	5%WGA-HRP	0.25				+	+
C1192	5%WGA-HRP	0.25				+	+
C654II*	33%HRP	0.15				+	
C468	33%HRP	0.12				+	
C468	33%HRP	0.1				+	
C372	33%HRP	0.15					+
C371	33%HRP	0.2					+

*: injected from ventrally
#: iontophoresis of 5% HRP in 0.01M Trisbuffer (Graybiel and Devor, 1974)

LEGEND TO TABLE 7.2.
Listing of the experiments, with type of tracer, injected volume and survival time. The plus sign indicates which vestibular subnuclei are included in the different injection sites.

8 SUMMARY AND CONCLUSIONS

This thesis describes the afferent, efferent and intrinsic connections of the vestibular nuclei in the Dutch belted rabbit. Different anatomical tracing techniques were used to study these projections. A description of the vestibular complex was added, since recent data for the rabbit are scarce (Chapter 2).

A comparison between cytoarchitecture and staining for acetylcholinesterase and cytochromoxidase supported the subdivision of the central magnocellular area of the vestibular complex into a dorsal region comprising the lateral vestibular nucleus of Deiters and the ventrally located magnocellular portion of the medial vestibular nucleus. Additional evidence supporting this distinction came from a detailed analysis of the primary vestibular input in the rabbit (Chapter 3).

The central projections of the vestibular nerve were investigated with anterograde axonal transport of wheatgerm agglutinin conjugated horseradish peroxidase (WGA-HRP) and tritiated leucine following injection in the vestibular ganglion. Labeled fibers and terminal ramifications were observed throughout the vestibular complex, including the magnocellular part of the medial vestibular nucleus, but they were absent from lateral vestibular nucleus. The absence of a projection of the vestibular nerve to lateral vestibular nucleus is in accordance with the findings in other mammals (Voogd, 1964, Korte, 1979, Carleton and Carpenter, 1984).

Termination in the cortex was restricted to the vermis. Small numbers of mossy fiber terminals were present bilaterally, close to the midline in lobules I and II, and in the depth of the main fissures separating the lobules II - VI. In the posterior vermis labeled mossy fiber terminals were found in lobule X and the ventral aspect of lobule IXd. Here the entire ipsilateral hemivermis contained many terminals, while contralaterally fewer mossy fiber terminals were present in the medial one-third of these lobules. Labeled mossy fibers and terminals were absent from the flocculus and adjacent ventral paraflocculus.

Intrinsic connections and the commissural projection of the vestibular nuclei were investigated with retrograde transport of horseradish peroxidase and WGA-HRP (Chapter 4). It was found that the superior, medial and descending vestibular nuclei within each vestibular complex, were reciprocally interconnected. A rostrocaudally oriented column of medium-sized and large neurons, consisting of the central superior vestibular nucleus and the magnocellular part of the medial vestibular nucleus received an input from the surrounding neurons but did not reciprocate this projection. Efferents from group y terminated in the superior, medial and descending vestibular nucleus. The infracerebellar nucleus as well as the interstitial nucleus of the VIIIth nerve supplied fibers to the medial and the descending vestibular nucleus.

The neurons that participated in the commissural projection were distributed throughout the vestibular complex with the exception of the lateral vestibular nucleus and group x. The greatest number of cells was found in the medial vestibular nucleus. The HRP labeled cells showed a tendency to cluster into rostrocaudally oriented groups. Each nucleus projected to more than one

contralateral nucleus. The contralateral projection of group y was more extensive than that of the bordering infracerebellar nucleus.

It was concluded that quantitative differences in connectivity were present between a core region of the vestibular complex and its peripheral parts. This core region included the central part of superior vestibular nucleus, the lateral vestibular nucleus, the magnocellular part of the medial vestibular nucleus and extended into the rostral descending vestibular nucleus. It received a predominantly intrinsic input from the surrounding vestibular neurons and was only minimally involved in the commissural projection, in contrast to the peripherally located neurons.

The distribution of vestibular neurons projecting to the flocculus and the lobules IX and X (uvula and nodule) of the caudal vermis was investigated with retrograde axonal transport of horseradish peroxidase and the fluorescent tracers Fast Blue, Nuclear Yellow and Diamidino Yellow. The presence of branching neurons which projected both to the flocculus, and to nodule and uvula, was studied with injections of two different fluorescent tracers in these two regions of the cerebellum in the same animal. The distribution of vestibular neurons projecting to either flocculus or caudal vermis was rather similar and bilaterally symmetrical. The projection from the magnocellular part of the medial vestibular nucleus was very sparse and projections from the lateral vestibular nucleus were absent. The majority of labeled neurons was found in the medial, superior, and descending vestibular nuclei, in this order. Double-labeled neurons were distributed in a similar way as the single-labeled ones. Labeled neurons projected to nodulus and uvula, to the flocculus, and to both parts of the cerebellum simultaneously in a ratio of 12 : 4 : 1. Five different populations of vestibulocerebellar neurons could be distinguished on the basis of their projection to the (1) ipsilateral flocculus, (2) contralateral flocculus, (3) ipsilateral flocculus and nodulus/uvula, (4) contralateral flocculus and nodulus/uvula, and (5) nodulus/uvula.

The distribution and collateralization of vestibular neurons to the oculomotor nucleus and to the spinal cord was studied using the fluorescent double labeling technique (chapter 6). Projections to the spinal cord took their origin from the superior vestibular nucleus, the lateral vestibular nucleus, the magnocellular, parvocellular and caudal parts of the medial vestibular nucleus, and the descending vestibular nucleus. The projection from the superior vestibular nucleus was restricted to the cervical spinal cord. Vestibulospinal axons from the other vestibular nuclei reached the lumbar region. A topical arrangement in the projection of the lateral vestibular nucleus to the spinal cord could not be established. Oculomotor projecting neurons were found in the entire vestibular complex except for the lateral vestibular nucleus. Neurons with branching axons to the spinal cord and the oculomotor nuclei in the mesencephalon occurred in the magnocellular and caudal parts of the medial vestibular nucleus and the descending vestibular nucleus. These branching neurons were almost exclusively found contralateral to the injection site in the mesencephalon. Similar distributions of double labeled neurons were found after combinations of injections of different tracers in the mesencephalon and

the contralateral vestibular nuclei. In addition neurons in and around PH had branching axons to the contralateral vestibular nuclei and the mesencephalon. Moreover the fluorescent double labeling technique clearly revealed that vestibular neurons with projections to either the cerebellum or the mesencephalon belonged to different populations.

The cortical and nuclear projections of the cerebellum to the vestibular nuclei were analyzed in Chapter 7, using anterograde axonal transport of tritiated leucine and retrograde transport of HRP and WGA-HRP. Projections to the lateral vestibular nucleus and to the magnocellular part of the medial vestibular nucleus originated from discrete strips of Purkinje cells in the anterior vermis. In the magnocellular part of the medial vestibular nucleus these projections overlapped with the bilateral projections from the fastigial nucleus. The flocculus and the lobules IX and X of the caudal vermis projected to complementary regions in the superior vestibular nucleus, the parvocellular and caudal parts of the medial vestibular nucleus and the descending vestibular nucleus. Overlap with the terminations of the anterior vermis and the fastigial nucleus was minimal. Purkinje cells in the lobules IX and X gave rise to fibers terminating in the descending and superior vestibular nuclei; projections to the caudal part of the medial vestibular nucleus mainly came from the nodule. The question of the zonal organization of the Purkinje cells with projections to the vestibular nuclei and of the existence of Purkinje cells with projections to different nuclei was discussed. The differential projections of the cortex of the flocculus and the caudal vermis and of the fastigial nucleus were compared to the distribution of vestibulo-oculomotor relay cells in the vestibular nuclei.

CONCLUSIONS

1 In the rabbit the subdivision of the large cell areas in the vestibular nuclei, into lateral vestibular and medial vestibular nuclei, is supported by the absence of a projection of the vestibular nerve to the lateral vestibular nucleus and by differences in acetylcholinesterase staining.
2 The vestibular nerve in the rabbit projects to the entire vestibular complex except for the lateral vestibular nucleus Termination in the cerebellum is restricted to lobules IXd and X, and small paramedian areas in the lobule I-VI.
3 Reciprocal intrinsic connections are established by the peripheral part of the superior vestibular nucleus and by the medial and the descending vestibular nucleus. A core region including the central part of the superior vestibular nucleus and the magnocellular portion of the medial vestibular nucleus receive projections from the other vestibular subnuclei with the exception of the lateral vestibular nucleus, but do not reciprocate these projections
4 A diffusely organized commissural projection interconnects the vestibular complex of both sides, except for the lateral vestibular nuclei.
5 Experiments with the fluorescent double labeling technique revealed branching of 5-10% of the secondary vestibular mossy fiber projections to the flocculus and the caudal vermis. The single-labeled and branching

neurons are scattered throughout the vestibular complex except for the lateral vestibular nucleus.

6 Branching of axons to the contralateral vestibular nuclei and the spinal cord is present for neurons in a central region of the vestibular nuclei, consisting of the magnocellular portion of the medial vestibular nucleus, and bordering areas of the medial vestibular and the descending vestibular nucleus. Neurons with branching axons terminating in the oculomotor nuclei and the contralateral vestibular complex occupy a somewhat larger area including caudal parts of the medial and descending vestibular nucleus and the prepositus hypoglossal nucleus.

7 Fastigiovestibular projections take their origin from ipsilateral clusters in the ventral fastigial nucleus and from the entire contralateral fastigial nucleus. These crossed and uncrossed projections terminate in the magnocellular portion of the medial vestibular nucleus and the descending vestibular nucleus, with a limited projection to the superior vestibular, the medial vestibular and the prepositus hypoglossal nucleus.

8 Corticovestibular projections from the anterior lobe are organized in a zonal pattern. The medial A zone projects to the fastigial nucleus and the magnocellular portion of the medial vestibular nucleus. The lateral B zone to the lateral vestibular nucleus. The zonal organization of the Purkinje cells of the caudal vermis which project to the vestibular is difficult to interpret.

9 Corticovestibular and nucleovestibular projections of the cerebellum occupy complementary projections in the vestibular nuclei of the rabbit.

10 Vestibulo-oculomotor neurons are located both within the target area of the corticovestibular projection of the flocculus and the caudal vermis and the nucleovestibular projection of the fastigial nucleus.

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9 Samenvatting en conclusies

Dit proefschrift omvat een onderzoek naar de afferente, efferente en intrinsieke verbindingen van de vestibulaire kernen van het konijn (Hollander). Verschillende anatomische tracing technieken werden gebruikt om deze verbindingen zichtbaar te maken. Een beschrijving van de vestibulaire kernen werd toegevoegd aangezien recente gegevens over het konijn ontbreken (Hoofdstuk 2).

Een vergelijking tussen cytoarchitectuur en de resultaten van histochemische kleuringen voor acetylcholinesterase en cytochroomoxidase ondersteunde een verdeling van het centrale, grootcellige gebied van de vestibulaire kernen in een dorsaal gelegen laterale vestibulaire kern van Deiters en een ventraal gelegen grootcellig gedeelte van de mediale vestibulaire kern. Aanwijzingen voor deze onderverdeling werden bovendien gevonden bij een gedetailleerde analyse van de primaire wortel input uit de nervus vestibularis van het konijn (Hoofdstuk 3).

De centrale projecties van de nervus vestibularis werden onderzocht met behulp van antegraad axonaal transport van in het vestibulaire ganglion gespoten wheatgerm agglutinine gekoppeld aan mierikswortel peroxidase (WGA-HRP) en getritieerd leucine. Gelabelde vezels en eindigingen werden in het gehele vestibulaire kerncomplex, inclusief de grootcellige mediale vestibulaire kern aangetroffen, maar zij ontbraken in de laterale vestibulaire kern. De afwezigheid van een projectie van de nervus vestibularis naar de laterale vestibulaire kern is in overeenstemming met de bevindingen bij andere zoogdieren (Voogd 1964, Korte 1979, Carleton en Carpenter 1984).

De eindiging van de nervus vestibularis in de schors van het cerebellum was beperkt tot de vermis. Een klein aantal mosvezel eindigingen bevond zich in de lobuli I and II beiderzijds van de mediaanlijn en in de diepte van de voornaamste fissuren tussen de lobuli II-VI. In de vermis van de lobus posterior bevonden zich gelabelde mosvezeleindigingen in lobulus X en het ventrale aspect van lobulus IXd. Het eindigingsgebied besloeg hier de gehele hemivermis aan de ipsilaterale zijde terwijl zich contralateraal een kleiner aantal mosvezeleindigingen in het mediale een-derde van de vermis bevonden. Gelabelde mosvezels ontbraken in de flocculus en de aangrenzende ventrale paraflocculus.

Intrinsieke en commissurale verbindingen van de vestibulaire kernen werden onderzocht met behulp van retrograad transport van mierikswortel peroxidase en WGA-HRP (Hoofdstuk 4). Binnen het vestibulaire kerncomplex waren de nucleus vestibularis superior, medialis en descendens reciprook met elkaar verbonden. Een rostrocaudale kolom van middelgrote en grote neuronen, die het centrale deel van de nucleus vestibularis superior en de grootcellige nucleus medialis omvatten, ontving verbindingen afkomstig uit omgevende neuronen maar gaf geen oorsprong aan een reciproke verbinding. Efferenten van de groep y eindigden in de nucleus vestibularis superior, medialis en descendens. De infracerebellaire kern evenals de nucleus interstitialis van de nervus vestibularis projecteren op de nucleus vestibularis medialis en descendens.

Neuronen die oorsprong geven aan commissurale verbindingen kwamen in alle

vestibulaire kernen voor met uitzondering van de laterale vestibulaire kern en de groep x. Het grootste aantal cellen bevond zich in de mediale vestibulaire kern. De HRP gelabelde cellen neigden tot clustering in rostrocaudale groepen. Iedere kern was met meer dan één contralaterale kern verbonden. De projectie van groep y was uitgebreider dan die van de aangrenzende infracerebellaire kern.

De conclusie werd getrokken dat een centraal gelegen gebied en de periferie van het vestibulaire kerncomplex in hun verbindingen verschillen. Het centrale gebied omvatte het centrale deel van de nucleus vestibularis superior, de laterale vestibulaire kern en de grootcellige nucleus medialis en liep uit in het rostrale deel van de nucleus descendens. Het ontving voornamelijk intrinsieke verbindingen van omgevende vestibulaire neuronen en nam, in tegenstelling tot deze perifere neuronen, nauwelijks deel aan de commissurale verbindingen.

De verdeling over de vestibulaire kernen van neuronen met een projectie naar de flocculus en de nodulus en de uvula van de caudale vermis (Larsell's lobuli X en IX) werd onderzocht met retrograad transport van mierikswortel peroxidase en de fluorescente tracers Fast Blue, Nuclear Yellow en Diamidino Yellow. De aanwezigheid van neuronen met een vertakkend axon dat zowel in de flocculus, als in nodulus en uvula eindigt, werd aangetoond door middel van injecties van twee verschillende fluorescerende tracers in deze twee gebieden van het cerebellum bij hetzelfde proefdier. De verdeling over de vestibulaire kernen van neuronen die of naar de flocculus of naar nodulus en uvula projecteren, was identiek en bilateraal symmetrisch. De projectie van het magnocellulaire deel van de mediale vestibulaire kern was gering, de laterale vestibulaire kern nam geen deel aan deze verbinding. Het grootste deel van de gelabelde neuronen bevond zich, in afnemende volgorde, in de nucleus vestibularis medialis, superior en descendens. De verdeling over de vestibulaire kernen van dubbel- en enkel gelabelde neuronen was gelijk. De aantallen neuronen met projecties op nodulus en uvula, op de flocculus, en op beide gebieden verhielden zich als 12:4:1. Vijf verschillende populaties vestibulocerebellaire neuronen werden onderscheiden op basis van hun efferente verbindingen naar (1) de ipsilaterale flocculus, (2) de contralaterale flocculus, (3) de ipsilaterale flocculus en nodulus/uvula, (4) de contralaterale flocculus en nodulus/uvula, en (5) nodulus/uvula.

De verdeling en de collateralisatie van vestibulaire neuronen met verbindingen naar de oogspierkernen en het ruggemerg werd onderzocht met behulp van dubbel labeling van fluorescerende tracers (Hoofdstuk 6). Verbindingen naar het ruggemerg ontsprongen aan de nucleus vestibularis superior, lateralis en descendens en aan de groot- en kleincellige en caudale delen van de mediale vestibulaire kern. De verbindingen van de nucleus vestibularis superior beperkten zich tot het cervicale ruggemerg. Vestibulospinale neuronen uit andere subnuclei bereikten het lumbale merg. Een duidelijke topische rangschikking binnen de projectie van de laterale vestibulaire kern op het ruggemerg was niet aanwezig. Neuronen met projecties op de oogspierkernen bevonden zich in het gehele vestibulaire kencomplex behalve in de laterale vestibulaire kern. Neuronen met axonen die vertakken naar het ruggemerg en de oogspierkernen kwamen voor in het groot-en kleincellige deel van de nucleus

vestibularis medialis en in de nucleus descendens. Deze neuronen met vertakkende axonen bevonden zich vrijwel uitsluitend contralateraal ten opzichte van de injectieplaats in het mesencephalon. Een identieke localisatie werd gevonden voor neuronen met een dubbele projectie op het mesencephalon en de contralaterale vestibulaire kernen. Dergelijke neuronen bevonden zich ook in en rond de nucleus prepositus hypoglossi. Uit experimenten met de dubbel-labeling techniek bleek duidelijk dat de vestibulaire neuronen die of op het cerebellum, of op het mesencephalon projecteren gescheiden celpopulaties vormen.

De verbindingen van de schors en de centrale kernen van het cerebellum met de vestibulaire kernen werden geanalyseerd in hoofdstuk 7, met behulp van antegraad axonaal transport van getritieerd leucine en retrograad transport van HRP en WGA-HRP. Verbindingen naar de laterale vestibulaire kernen en het magnocellulaire deel van de mediale vestibulaire kern ontsprongen aan afzonderlijke stroken Purkinje cellen in de vermis van de lobus anterior. In het magnocellulaire deel van de mediale vestibulaire kern overlapte de eindiging van deze vezels met de bilaterale verbindingen uit de nucleus fastigii. De flocculus en de lobuli IX en X van de caudale vermis projecteerden naar complementaire gebieden in de nucleus vestibularis superior, de parvocellulaire en caudale delen van de nucleus vestibularis medialis en de nucleus vestibularis descendens. Overlap van deze projecties met de verbindingen uit de vermis van de lobus anterior en de nucleus fastigii was slechts minimaal. Purkinje cellen in de lobuli IX en X gaven oorsprong aan vezels die eindigden in de nucleus descendens en de nucleus vestibularis superior. De verbindingen naar het caudale deel van de mediale vestibulaire kern ontsprongen voornamelijk aan de nodulus. De zonale organisatie van Purkinje cellen met projecties naar de vestibulaire kernen en het bestaan van Purkinje cellen met verbindingen die eindigen in de verschillende vestibulaire kernen werden besproken. De projecties van de schors en de nucleus fastigii op de vestibulaire kernen werden vergeleken met de verdeling van de vestibulo-oculomotorische relaiscellen.

Conclusies

1. De onderverdeling van het centraal gelegen grootcellige gebied in de vestibulaire kernen van het konijn, in de laterale vestibulaire en de mediale vestibulaire kern wordt ondersteund door afwezigheid van eindigingen uit de nervus vestibularis in de laterale vestibulaire kern en het bestaan van kleurings verschillen met betrekking tot acetylcholinesterase.
2. De nervus vestibularis van het konijn eindigt in het gehele vestibulaire kerncomplex, met uitzondering van de laterale vestibulaire kern. Eindigingen in het cerebellum beperken zich tot lobuli IXd en X, en kleine paramediane gebieden in de lobuli I en IV.
3. Het perifere deel van de nucleus vestibularis superior, de nucleus vestibularis medialis en de nucleus vestibularis descendens onderhouden reciproke intrinsieke verbindingen. Een centraal gebied bestaande uit het centrale deel van de nucleus vestibularis superior en het magnocellulaire deel van de

mediale vestibulaire kern ontvangt verbindingen uit andere vestibulaire kernen behalve uit de laterale vestibulaire kern.

4. Een diffuus georganiseerd commissuraal systeem verbindt de vestibulaire kernen van beide zijden, met uitzondering van de laterale vestibulaire kernen.
5. Uit dubbel-labeling experimenten met behulp van fluorescerende tracers bleek dat 5-10% van de secundaire vestibulaire mosvezels zich vertakken naar de flocculus en de caudale vermis. Enkel-gelabelde neuronen en neuronen met vertakkende axonen bevinden zich overal in de vestibulaire kernen met uitzondering van de LV.
6. Neuronen met axonen die vertakken naar het ruggemerg en de contralaterale vestibulaire kernen liggen centraal in de vestibulaire kernen in een gebied dat bestaat uit het magnocellulaire deel van de mediale vestibulaire kern en aangrenzende gebieden van de nucleus vestibularis medialis en descendens. Neuronen met projecties naar de oogspierkernen en de contralaterale vestibulaire kernen bevinden zich in een wat groter gebied dat ook caudalere delen van de nucleus vestibularis medialis en descendens en de nucleus prepositus hypoglossi omvat.
7. Fastigiovestibulaire verbindingen ontspringen aan cel-clusters in het ventrale deel van de gelijkzijdige nucleus fastigii en aan de gehele contralaterale kern. Deze gekruiste en ongekruiste verbindingen eindigen beide op het magnocellulaire deel van de mediale vestibulaire kern en de nucleus vestibularis descendens. De projecties naar de nucleus vestibularis superior, de rest van de nucleus vestibularis medialis en de nucleus prepositus hypoglossi zijn zeer beperkt.
8. De Purkinje cellen waaraan de corticovestibulaire verbindingen van de lobus anterior ontspringen zijn gerangschikt in een aantal longitudinale zones. De mediale A zone projecteert op de nucleus fastigii en het magnocellulaire deel van de mediale vestibulaire kern. De laterale B zone projecteert op de nucleus vestibularis lateralis. De zonale organisatie van Purkinje cellen in de caudale vermis met projecties naar de vestibulaire kernen is moeilijk the interpreteren.
9. Vestibulaire neuronen met verbindingen naar de oogspierkernen bevinden zich zowel binnen de gebieden die projecties ontvangen uit de flocculus en de caudale vermis als binnen het projectiegebied van de nucleus fastigii.

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11 ABBREVIATIONS

ANS	ansiforme lobe
ANT	anterior lobe
bc	brachium conjunctivum
CE	external cuneate nucleus
cr	restiform body
CO	cochlear nuclei
DV	descending vestibular nucleus
DX	dorsal vagal nucleus
f	fastigial nucleus
f.pl	posterolateral fissure
f.prima	primary fissure
FL(O)	flocculus
flm	medial longitudinal fascicle
gV	vestibular ganglion
gVII	facial genu
IA	anterior interpositus nucleus
Inf	infracerebellar nucleus (= dorsal group y)
IN(VIII)	interstitial nucleus of the eighth nerve
LV	lateral vestibular nucleus
ME	medial extension of the PFLV
MVc	caudal medial vestibular nucleus
MVmc	magnocellular medial vestibular nucleus
MVpc	parvocellular medial vestibular nucleus
NVpar	parabrachial vestibular nucleus
nVII	facial nerve
N 6(VI)	abducens nucleus
PFL(v,d)	paraflocculus (ventral, dorsal)
PH	prepositus hypoglossal nucleus
PMD	paramedian
rV	descending root of the trigeminal nerve
S	solitary tract and nucleus
sad	dorsal acoustic striae
Sl	simple lobule
SV	superior vestibular nucleus
unc	uncinate tract
U-NL	uvulo-nodular lobule
tu	uncinate tract
X	group x
Y	group y (d-Y: dorsal, and v-Y: ventral parts)
VI	abducens nucleus

12 CURRICULUM VITAE

De auteur van dit proefschrift werd in 1955 te Amsterdam geboren. In 1972 werd het examen HBS-B behaald aan de Rijks Hogere Burgerschool te Woerden. De studie geneeskunde werd aangevangen in 1973. Tijdens de studie vervulde hij student assistentschappen bij respectievelijk de afdeling Anatomie en Embryologie, en de afdeling Pathologie. Na het behalen van het doctoraalexamen in 1979 volgde een aanstelling bij de afdeling Anatomie en Embryologie van de Rijks Universiteit te Leiden. Tijdens deze periode was hij ondermeer docent anatomie en fysiologie bij de opleiding operatie-assistenten in het opleidingscentrum van het Academisch Ziekenhuis te Leiden. De geneeskunde studie werd hervat in 1984 en voltooid met het behalen van het artsexamen in 1986. Na een korte aanstelling bij de afdeling Anatomie van de Erasmus Universiteit Rotterdam is de schrijver van dit proefschrift sinds 1 juli 1986 als arts-assistent in opleiding op de afdeling Anesthesiologie van het Academisch Ziekenhuis te Leiden (opleider: Prof.Dr. Joh. Spierdijk). Hij hoopt de opleiding per 1 januari 1991 te voltooien. Daarnaast is hij sinds 1985 bestuurslid van de stichting RIAGG Zuid-Holland Noord (regionale instelling voor ambulante geestelijke gezondheidszorg), en maakt hij deel uit van de centrale opleidings commissie van het Academisch Ziekenhuis te Leiden.