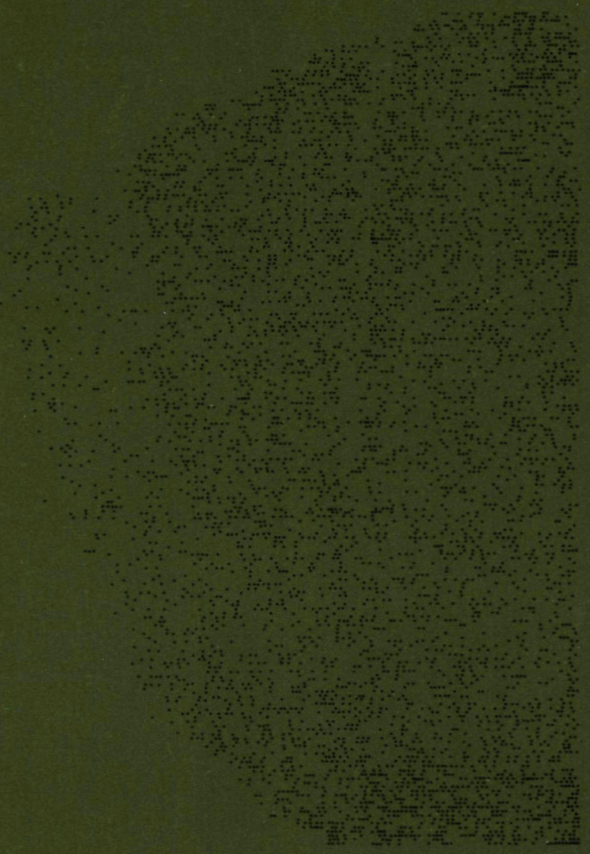


Cerebellar connections in some reptiles

G.C.Bangma

CEREBELLAR CONNECTIONS IN SOME REPTILES

G.C. BANGMA

PROMOTOR : PROF.DR. R. NIEUWENHUYS

CO-REFERENT : DR. H.J. TEN DONKELAAR

CEREBELLAR CONNECTIONS IN SOME REPTILES

proefschrift

ter verkrijging van de graad van doctor
in de geneeskunde
aan de katholieke universiteit te nijmegen
op gezag van de rector magnificus
prof.dr. j.h.g.i. giesbers
volgens besluit van het college van dekanen
in het openbaar te verdedigen
op donderdag 8 december 1983
des namiddags te 4 uur

door

GESINA CHRISTINA BANGMA
geboren te Baarn

Nijmegen, 1983

Voor mijn ouders,
voor Peke en Joost.

Dit proefschrift is gebaseerd op de volgende publikaties :

Bangma, G.C., and ten Donkelaar, H.J. (1982). Afferent connections of the cerebellum in various types of reptiles. J. comp. Neurol. 207, 255-273.

Bangma, G.C., ten Donkelaar, H.J. and Pellegrino, A. (1983). Cerebellar corticonuclear projections in the red-eared turtle *Pseudemys scripta elegans*. J. comp. Neurol. 215, 258-274.

ten Donkelaar, H.J., and Bangma, G.C. (1984). The Cerebellum. In 'Biology of the Reptilia', Vol. 17: Neurology C (C. Gans and R.G. Northcutt, eds.). Academic Press, London, in press.

De volgende publikaties beschrijven resultaten welke eveneens uit dit onderzoek werden verkregen :

Bangma, G.C. and ten Donkelaar, H.J. (1983). Some afferent and efferent connections of the vestibular nuclear complex in the turtle *Pseudemys scripta elegans*. J. comp. Neurol. 220, in press.

ten Donkelaar, H.J., and Bangma, G.C. (1983). A crossed rubrobulbar projection in the snake *Python regius*. Brain Res., in press.

ten Donkelaar, H.J., Bangma, G.C., and de Boer-van Huizen, R. (1983). Reticulospinal and vestibulospinal projections in the snake *Python regius*. Anat. Embryol., in press.

Dit onderzoek werd gesteund door de Stichting voor Medisch Wetenschappelijk Onderzoek FUNGO, die wordt gesubsidieerd door de Nederlandse Organisatie voor Zuiver-Wetenschappelijk Onderzoek (Z.W.O.).

De voltooiing van dit proefschrift biedt mij de gelegenheid diegenen te bedanken die aan de uitvoering van het onderzoek en aan het tot stand komen van dit proefschrift hebben meegewerkt. In het bijzonder denk ik daarbij aan:
Hendrik-Jan Janssen, Gerrie Grutters en de heer P. Spaan van het Centraal Dierenlaboratorium voor de verzorging van de proefdieren, hun technische bijstand tijdens de operaties en de vele perfusies die zij voor mij uitvoerden;
de heer M. van Megen voor het verzorgen van de waterschildpadden;
de medewerkers van de afdeling Medische Fotografie, in het bijzonder de heren F. de Graaf en C. de Bruin;
Chris van Huijzen voor het ontwerpen van de omslag van dit proefschrift en zijn adviezen met betrekking tot een groot aantal figuren;
Joop Russon, Joep de Bekker en Ed Noyons voor het vervaardigen van de vele figuren;
Roelie de Boer-van Huizen, Jos Dederen, Annelies Pellegrino en René van Rheden voor hun analytische en fotografische hulp. Jos dank ik met name voor zijn hulp bij de autoradiografische experimenten en de zorgvuldige bewerking van dit materiaal. Roelie ben ik in het bijzonder dankbaar voor de wijze waarop zij mij in de geheimen van het praktisch werk op het lab heeft ingewijd en haar grote inzet bij de uitvoering en bewerking van de experimenten;
Wanda de Haan en Riet Fliervoet voor het typen van de publikaties. Daarnaast dank ik Wanda voor de uitnemende wijze waarop zij de definitieve versie van dit proefschrift heeft verzorgd, als ook voor haar open oog en luisterend oor gedurende deze drie jaar.

De afdeling Anatomie en Embryologie dank ik voor de gastvrijheid die zij mij gedurende de afgelopen drie jaar heeft verleend. De 'bewoners' van de tweede verdieping en in het bijzonder de leden van de werkgroep Experimentele en Vergelijkende Neuro-anatomie dank ik voor de goede samenwerking en de gezellige momenten die deze jaren kenmerkten.

Dit proefschrift vormt in zekere zin de afsluiting van mijn biologie studie. Mijn ouders wil ik bij deze gelegenheid bedanken voor de mogelijkheid die zij mij hebben geboden deze studie te volgen.

CONTENTS

I. INTRODUCTION

The cerebellum varies greatly in both size and form throughout vertebrates (Larsell, '67: Nieuwenhuys, '67). Based on extensive comparative studies Larsell ('67) considered the cerebellum to consist of two fundamental divisions present in all vertebrate classes, i.e. a corpus cerebelli and a floccular part (the lobus vestibulolateralis or lobus auricularis).

Comparative studies have demonstrated a striking similarity between the primitive and more advanced cerebellar cortices (Hillman, '69; Llinás and Hillman, '69; Llinás, '81) including a molecular layer, a Purkyně cell layer, and a granular layer. The cerebellum is connected with the central nervous system by two sets of input systems, the mossy and climbing fibers, and one output system, formed by the axons of the Purkyně cells.

The first adequate experimental studies to reveal the function of the cerebellum were carried out by Rolando (1809) whose studies on a wide variety of animals, including reptiles, showed that by removing portions of the cerebellum ipsilateral motor activity was impaired. Later on Flourens (1824) and Luciani (1891) formulated the more general idea that the cerebellum functions to coordinate movements rather than to generate them.

Reptiles are particularly interesting objects for neurobiological research since their great variation in form and locomotion has remarkable repercussions in the organization of the central nervous system (see e.g. ten Donkelaar, '76 a, b; Kusuma *et al.*, '79; ten Donkelaar, '82; ten Donkelaar and Bangma, '84). The cerebellum also differs greatly among the several reptilian orders (Larsell, '67; Nieuwenhuys, '67). Larsell ('67) divided the reptilian corpus cerebelli into a median part, the pars interposita, flanked on each side by the pars lateralis. The floccular part or lobus auricularis is located lateral to the pars lateralis. Larsell noted that the pars interposita in snakes and limbless lizards is relatively prominent, whereas the pars lateralis is reduced. In turtles, solely dependent on their extremities for locomotion, the pars interposita is reduced whereas the pars lateralis is well developed. Based on these observations Larsell suggested that the pars lateralis is concerned with movements of the limbs, and the pars interposita with movements of the axial musculature.

The aim of the present study is to analyse the connectivity of the reptilian cerebellum to reveal the ways by which the cerebellum influences motor activity. With regard to their mode of progression roughly three groups of reptiles can be distinguished: (1) turtles, solely dependent for locomotion on their extremities; (2) lizards and crocodiles, using both trunk musculature and extremities; (3) snakes and limbless lizards, moving only by way of trunk musculature. For the present inquiry of each of these three groups one representative was chosen, viz., the turtle *Pseudemys scripta elegans*, the lizard *Varanus exanthematicus*, and the snake *Python regius*.

It was felt necessary to include some notes on the morphology of the cerebellum of the three reptilian species studied (Chapter III). The following aspects will be dealt with: (1) gross anatomy; (2) the topographical relationships of the cerebellum; (3) aspects of the histology of the cerebellar cortex; (4) the organization of the Purkyně cell layer including a topological analysis of this layer in *Pseudemys scripta elegans* and *Varanus exanthematicus*.

The experimental part of this study comprises: (1) the origin of cerebellar afferents, (2) the organization of corticonuclear projections, i.e. efferent connections arising in the cerebellar Purkyně cell layer, (3) the efferent connections of the cerebellar nuclei to the brainstem and spinal cord. The fiber connections of the reptilian cerebellum have been studied both with the classical anterograde degeneration techniques (Nauta and Gygax, '54, Fink and Heimer, '67) as well as with the more modern tracer techniques, making use of axonal transport phenomena.

The origin of cerebellar afferents (Chapter IV) has been studied with several retrograde tracers horseradish peroxidase (HRP), wheat-germ agglutinin conjugated HRP (WGA-HRP), and 'Fast Blue' (FB). In addition in the lizard *Varanus exanthematicus* the olivocerebellar projection has been studied anterogradely by injecting WGA-HPP and the autoradiographic tracer ^{3}H-leucine in the vicinity of the inferior olive. Finally, the distribution of monoaminergic afferent fibers in the cerebellum of the lizard *Varanus exanthematicus* is described in this chapter.

As regards the cerebellar efferents, the corticonuclear projections, studied retrogradely with HRP, are described in Chapter V. The projections of the cerebellar nuclei, i.e the nucleus cerebellaris medialis and lateralis, described in Chapter VI, were studied with various techniques. Anterograde degeneration techniques showed an overview of these projections. HRP injections at various levels of the brainstem and the spinal cord demonstrated the existence of ascending and descending projections of the cerebellar nuclei. Axonal branching of the efferent fibers of the cerebellar nuclei was analysed with the multiple retrograde fluorescent tracer technique (Kuypers *et al.*, '80). Injection of the anterogradely transported tracer ^{3}H-leucine into the cerebellar nuclei showed the efferent ascending and descending projections of the cerebellar nuclei more in particular. The latter findings were confirmed by making use of anterograde transport of HRP resulting from the implantation of HRP slow-release gels into the cerebellar nuclei.

In the general discussion (Chapter VII) a comparison between the organization and connectivity of the cerebellum in reptiles and other terrestrial vertebrates is made. Moreover, an attempt is made to discuss cerebellar function in reptiles as inferred from ablation studies, stimulation experiments, and the available experimental anatomical data

II. MATERIALS AND TECHNIQUES

For the description of the topographical relationships of the cerebellum series, sectioned in the three conventional planes have been used of each reptile studied, stained either with cresylechtviolet, Haggqvist's ('36) modification of the Alzheimer-Mann methylblue-eosin stain, or according to Kluver and Barrera ('53).

For the experimental part of this study altogether 39 turtles (36 *Pseudemys scripta elegans* and 3 *Testudo hermanni*), varying in weight from 350 to 1000 gm, with a carapace length of 10 to 18 cm, 44 lizards (*Varanus exarthematicus*), varying in weight from 450 to 1000 gm, with a total length of 50 to 70 cm and a snout-vent length of 25 to 40 cm, and 26 snakes (*Python regius*), varying in weight from 700 to 950 gm, with a total length of 90 to 115 cm were used.

All experiments were carried out under surgical anesthesia. The animals were intubated and received endotracheal anesthesia for which a mixture of 0 2-0.5 l oxygen, 50-250 ml nitrous oxide with 0 25-0 50 volumen % halothane was used Operations were performed under aseptic conditions with the aid of a Zeiss binocular operation microscope. Following a midline skin incision and separation of the dorsal neck musculature, an opening was drilled in the skull at the intended level and the dura was incised. In those cases in which the spinal cord was involved, following separation of the dorsal musculature laminectomy was performed and the dura was incised The experiments carried out can be divided into the following groups

1. Anterograde degeneration experiments

In five turtles (*Pseudemys scripta elegans*) and four lizards (*Varanus exanthematicus*) a lesion of the cerebellar peduncle was made with a von Graefe cataract knife.

2 Application of the enzyme horseradish peroxidase (HRP)

After exposure of the cerebellum, the brainstem or the spinal cord, HRP (Boehringer) was applied either by injection or in a slow-release gel. HRP injections were made with a glass micropipette attached to a Hamilton syringe placed in a micromanipulator under control of the operation microscope. The HRP was dissolved in distilled water in a 30% solution. HRP slow-release gels were implanted into the brain with a fine-tipped forceps under control of the operation microscope. The slow-release gels, containing 20% HRP and 0.5% dimethylsulfoxide (DMSO), were prepared according to Griffin *et al.* ('79), slightly modified after Wolters *et al.* ('82a) HRP injections, in portions of 0 1 µl, were made into the cerebellum, or at various levels of the brainstem or the spinal cord of 16 turtles, 11 lizards, and 9 snakes. HRP slow-release gels, with a midline of 0 16 - 0.35 mm and various lengths, were implanted in 11 turtles, 12 lizards and 17 snakes.

In addition, in three turtles (*Pseudemys scripta elegans*) 1-3 unilateral injections were made into the cerebellum of 0.1 µl 5% WGA-HRP in physiological saline (Sigma). In three lizards 1-3 injections of 0 1 µl WGA-HPP were made into the caudal part of the brainstem at the level of the obex

3 Multiple labeling experiments application of the fluorescent tracers 'Fast Blue'

(FB) and 'Nuclear Yellow' (NY). The recently introduced multiple fluorescent tracer technique (Kuypers *et al.*, '80) was used to investigate the existence of axonal branching of cerebellar efferent fibers. FB (Dr. Illing K.G., Gross Umstadt) was injected with a glass micropipette attached to a Hamilton syringe placed in a micromanipulator under control of the operation microscope. FB was dissolved in distilled water in a 3% solution and 1-3 injections of 0.1 µl were made. Since the retrograde transport of the fluorescent tracer NY (Hoechst) by the nerve fibers requires less time than the retrograde transport of FB, the implantation of NY slow-release gels occurred in a second operation, several days later. The gels (1-3) were implanted with a fine-tipped forceps under control of the operation microscope at various levels of the cervical spinal cord. The NY slow-release gels, containing 8% NY and 0.5% DMSO were prepared in a similar way as the HRP gels. In three turtles and four lizards this multiple labeling technique was applied.

In addition in one turtle and one lizard the fluorescent tracer FB was used to determine the origin of cerebellar afferent connections by 2-3 unilateral injections of 0.1 µl 3% FB into the cerebellum following the operation technique described above.

4. Injection of L-(4.5-^{3}H) leucine into the cerebellar peduncle

The anterograde transport of the autoradiographic tracer L-(4.5-^{3}H) leucine (Radiochemical Centre, Amersham) was used to analyse the efferent connections of the cerebellar nuclei. Following incision of the dura one injection of 0.5 µl (15 µCi/µl) L-(4.5-^{3}H) leucine (specific activity 53 Ci/m mole) was made into the cerebellar peduncle with a glass micropipette attached to a Hamilton syringe placed in a micromanipulator, under control of the operation microscope. The injection was made over a period of 10 minutes. Upon completion of the injection the micropipette was left in place for an additional period of 10 minutes. Six lizards received an unilateral injection of ^{3}H-leucine into the cerebellar peduncle. In addition one lizard received an unilateral injection of ^{3}H-leucine into the caudal brainstem at the level of the obex.

Following surgery the animals were kept at an environmental temperature ranging from 24^o to 27^o C (the snakes 27^o to 30^o C) and sacrificed after postoperative survival times of 7 to 25 days for animals in which a lesion was performed; of 4 to 10 days for animals in which HRP or WGA-HRP was injected or implanted in a slow-release gel. The optimal survival time for animals which received both FB injections and NY slow-release gels appeared to be 7 to 10 days for FB and 3 days for NY. Consequently these animals were always sacrificed 3 days after the implantation of the NY slow-release gels. The lizards which received a ^{3}H-leucine injection were sacrificed after a postoperative survival time of 14 days.

All animals were perfused transcardially under deep Nembutal anesthesia. The reptiles used in lesion experiments were perfused with physiological saline followed by 4% formaldehyde. After their removal the brain and selected segments of the spinal cord were further fixed in 4% formaldehyde for periods varying from 2 to 10 weeks. The material obtained was, in order to study the anterograde fiber degeneration, embedded in albumin, sectioned transversely on a freezing microtome at 25 µm thickness and subsequently stained with the Nauta-Gygax ('54) and Fink-Heimer ('67) techniques.

The brains and selected segments of the spinal cord of the reptiles in which HRP or WGA-HRP was either injected or implanted in a slow-release gel, were processed as follows. The animals were perfused with physiological saline, followed by a mixture of 1% formaldehyde and 1 25% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). After their removal the brain and spinal cord were further fixed in the above-mentioned solution for 1 to 3 hours. Thoroughly fixed the material was stored for at least 3 hours in 0.1 M phosphate buffer (pH 7.4) containing 30% sucrose at room temperature. The brain and the spinal cord segments were embedded in a 30% sucrose-phosphate buffer solution containing 15% gelatin. These blocks were stored overnight at room temperature in a 4% formaldehyde solution, and subsequently rinsed in a 30% sucrose-phosphate buffer solution (pH 7.4) for 15 to 30 minutes. The embedded material was frozen in dry ice and cut into transverse sections of 40 µm on a freezing microtome. The sections were stained according to a slightly modified Mesulam ('78) technique, using tetramethylbenzidine (TMB), described by ten Donkelaar *et al.* ('80) and counterstained with neutral red. The incubation procedure using 3.'3-diaminobenzidine tetrahydrochloride (DAB), described by Graham and Karnovsky ('66), or the incubation procedure using DAB described by Adams ('81), was used for a part of the sections, which were counterstained, respectively, with cresylecht violet or neutral red.

Following injection of FB and implantation of NY slow-release gels the animals were perfused according to the procedure described by Kuypers *et al.* ('80). To optimize the results obtained in reptiles this procedure was slightly modified. The animals were perfused with, in this order, physiological saline, a solution of 4% formaldehyde in 0.1 M phosphate buffer (pH 7.4), and the final solution of 4% formaldehyde in 0.1 M phosphate buffer (pH 7.4) containing 10% sucrose. Later on, the latter step in this perfusion, which was intended to make immediate cutting possible, was left out. Instead the brains were further fixed after the perfusion in 0.1 M phosphate buffer (pH 7.4) containing 4% formaldehyde and 10% sucrose for 1 to 2 hours. After perfusion and fixation the brains were frozen in dry ice and cut into sections of 40 µm or 30 µm on a freezing microtome. Immediately after cutting the sections were mounted on slides from a 10% sucrose-phosphate buffer solution and air-dried. The sections were coverslipped with paraffin oil, later on with Depex and stored in the dark at 4^{o} C. The material was viewed with a Zeiss fluorescence microscope, equipped with an UV excitation filter (combination 487702) providing excitation light at a wavelength of 365 nm.

The lizards which received an injection of L-(4.5-^{3}H) leucine were perfused with physiological saline, followed by 4% formaldehyde. After their removal the brain and selected segments of the spinal cord were further fixed in 4% formaldehyde for a period of 2 weeks. The material was embedded in paraplast and sectioned. The 7 µm sections were mounted on slides prepared with chrome-alum-gelatin solution, deparaffinated, rinsed in distilled water, dipped with Ilford G5-emulsion, and exposed at 4^{o} C for 12 weeks. Subsequently the slides were developed in Brussels amidol at 15^{o} C during 10 minutes, rinsed in distilled water, fixed with 30% sodium thiosulphate, rinsed in water, and counterstained with haematoxylin-eosin.

5. Immunohistochemical procedure

The cerebellum of two lizards, *Varanus exanthematicus*, was studied to determine the distribution

of serotonin, metenkephalin, leu-enkephalin, and substance P. For the immunohistochemical procedure used reference is made to Wolters *et al.* ('83 a, b). The material, kindly made available by Drs. J.G. Wolters, was viewed with a Zeiss fluorescence microscope equipped with an excitation filter (combination 487709) providing excitation light at a wavelength of 455 - 490 nm. Kodak Ektachrome EPD 200 film was used for photomicrography.

III. NOTES ON THE MORPHOLOGY OF THE REPTILIAN CEREBELLUM

A. GROSS ANATOMY

The cerebellum differs greatly, both in form and size, among the various reptilian species (Larsell, '67; Nieuwenhuys, '67; ten Donkelaar and Bangma, '84).

In the turtle *Pseudemys scripta elegans* the cerebellum forms a caudally directed arch which roofs the fourth ventricle like a helmet (Larsell, '32) (Figs. 1, 4-7). On the ventricular side of the cerebellum a median sulcus, i.e. the sulcus medianus dorsalis (Cruce and Nieuwenhuys, '74) is present, separating the two halves of the corpus cerebelli. In the rostral and middle part of the cerebellum a bilateral groove is present separating the pars interposita and the pars lateralis as distinguished by Larsell ('32) (Figs. 6, 7). As in most turtles, the flocculus is ill-defined in *Pseudemys scripta elegans*. Larsell ('32) regarded the small rostrolateral part of the cerebellum which is separated from the corpus cerebelli by a shallow ventricular groove, the sulcus flocculi internus dorsalis, as the flocculus (Fig. 6). According to Mugnaini *et al.* ('74) the flocculus continues caudally as a small marginal rim of the cerebellum. No external grooves such as the fissura posterolateralis can be distinguished on the surface of the corpus cerebelli.

In the lizard *Varanus exanthematicus* the cerebellum is tilted forward, i.e. everted, resulting in a dorsal position of part of the granular layer (Figs. 2, 8-11). A sulcus medianus dorsalis is present, but no clear grooves separating the pars interposita and the pars lateralis can be distinguished (Figs. 8-11). In *Varanus exanthematicus* the flocculus is well-developed and present as a clear lateral lobe of the cerebellum, the so-called lobus auricularis (Figs. 8, 9). The external groove between the flocculus and the corpus cerebelli can be regarded as the fissura posterolateralis (Larsell, '67; Nieuwenhuys, '67).

The most simple cerebellum of the reptilian species studied is found in the snakes *Python reticulatus* and *Python regius*. In these species the cerebellum consists of a dorsocaudally directed plate, partly covering the fourth ventricle (Figs. 3, 12-15). Except the median sulcus medianus dorsalis no ventricular grooves are present. No definite flocculus can be distinguished.

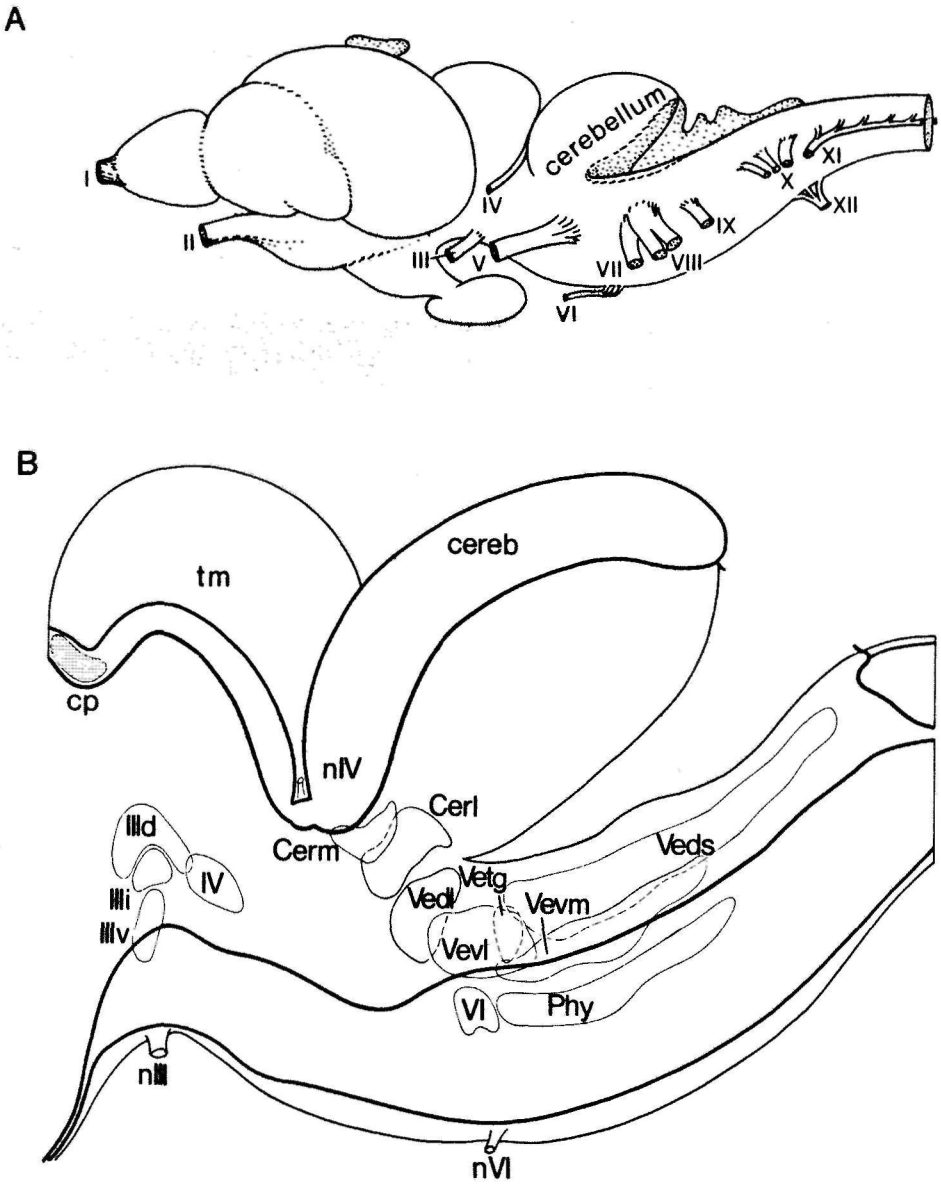


Fig. 1 *A, Lateral view of the brain of Pseudemys scripta elegans, ×3.5. I-XII, the cranial nerves. B, Topographical reconstruction of some cell masses in the brainstem of Pseudemys scripta elegans as projected upon a sagittal plane. For abbreviations cf. pages 28-29.*

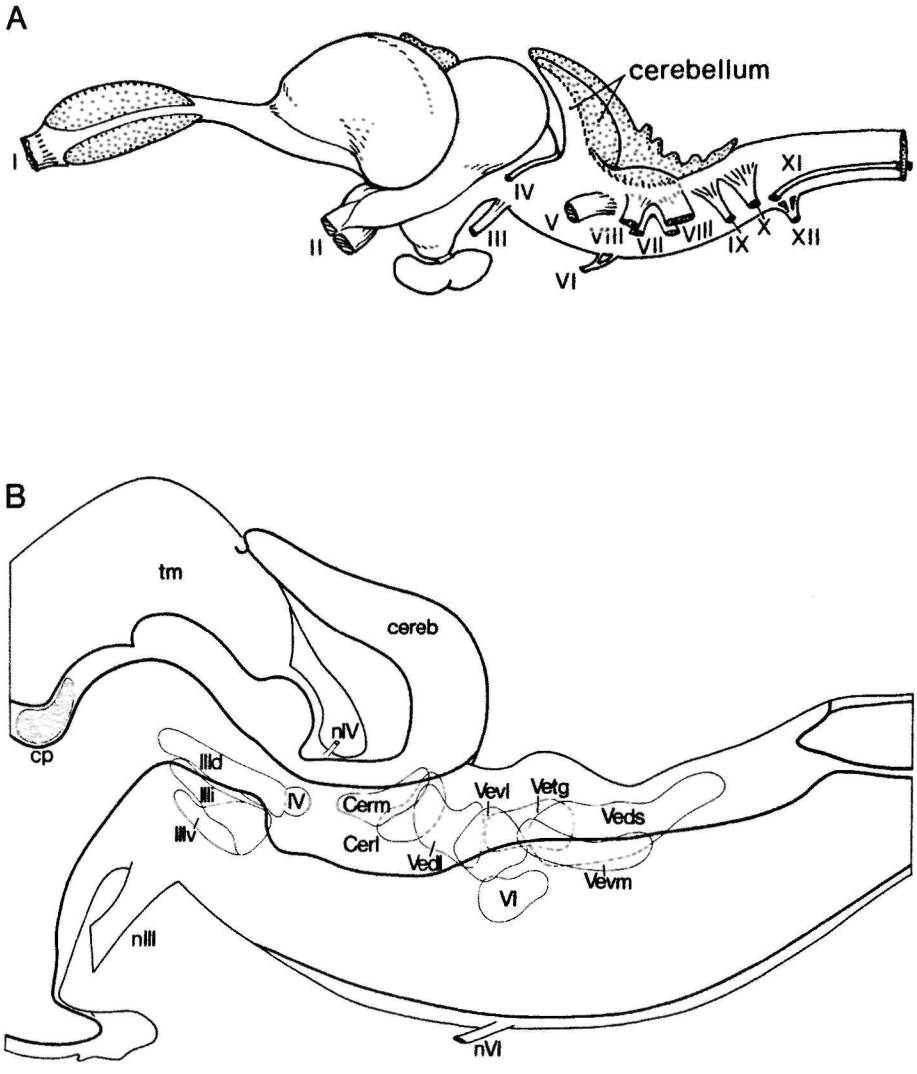


Fig. 2 *A, Lateral view of the brain of Varanus exanthematicus, ×2.7. I-XII, the cranial nerves. B, Topographical reconstruction of some cell masses in the brainstem of Varanus exanthematicus as projected upon a sagittal plane. For abbreviations cf. pages 28-29.*

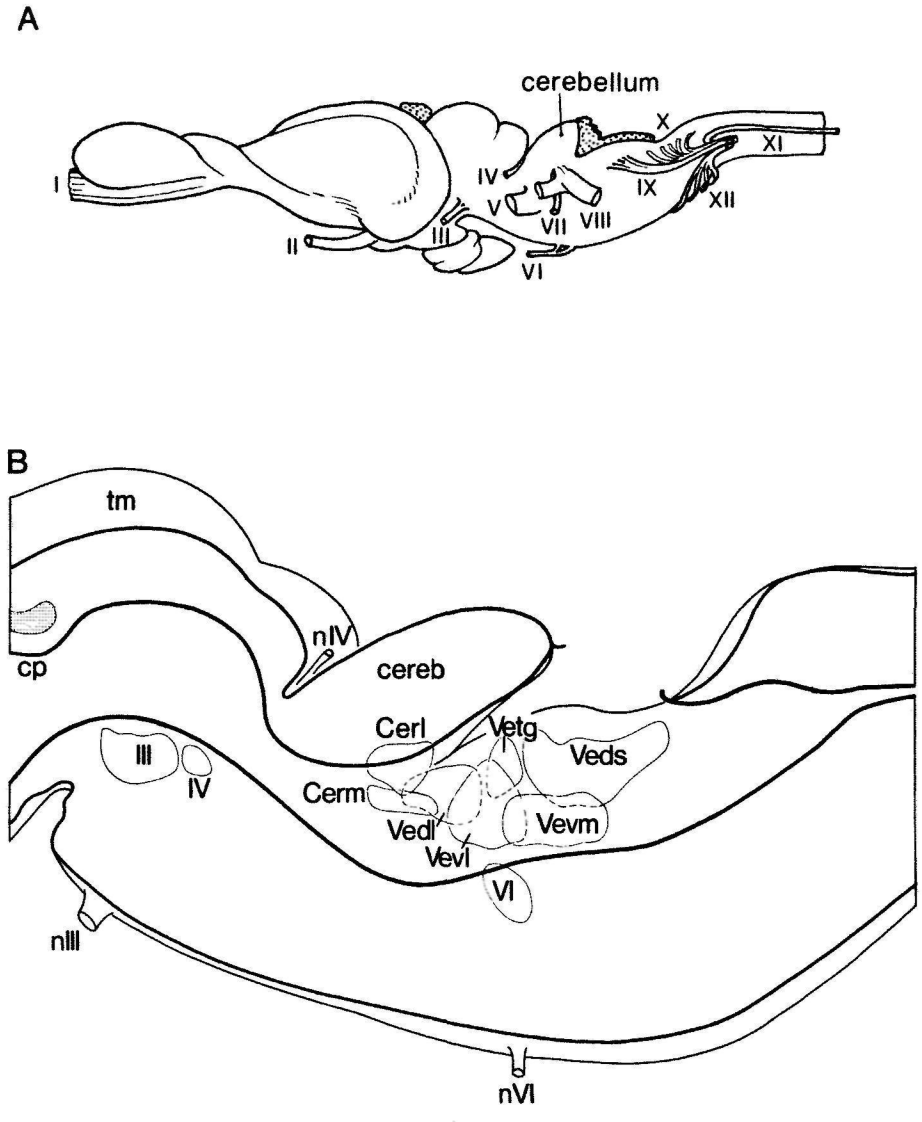


Fig. 3 *A, Lateral view of the brain of Python regius, ×3.5. I-XII, the cranial nerves. B, Topographical reconstruction of some cell masses in the brainstem of Python reticulatus as projected upon a sagittal plane. For abbreviations cf. pages 28-29.*

B. TOPOGRAPHICAL RELATIONSHIPS

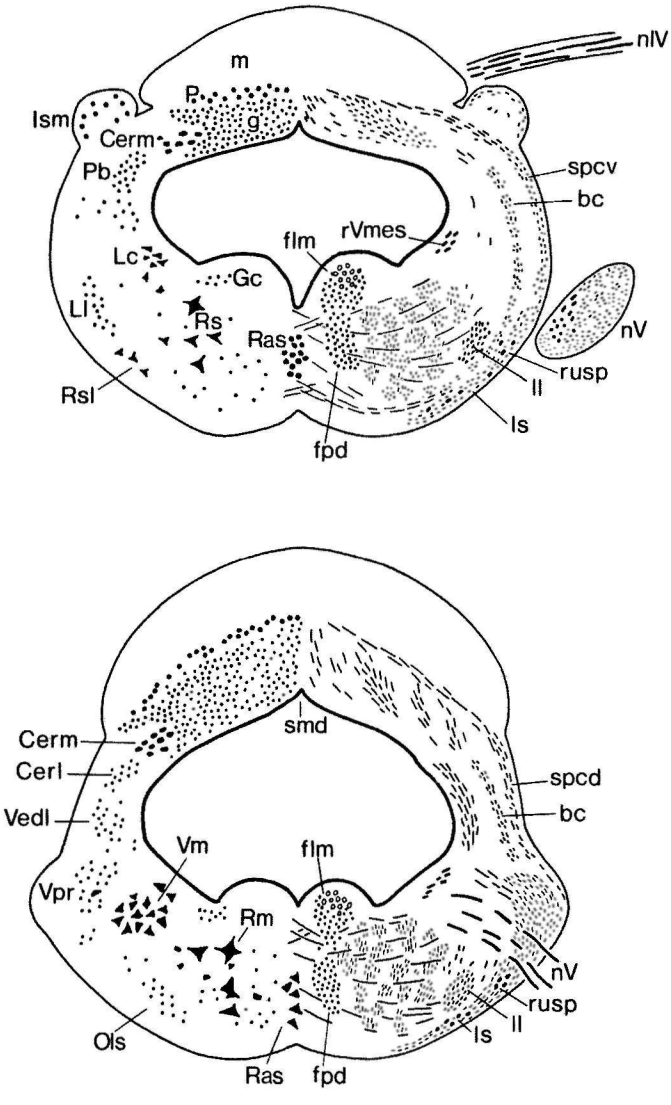
The topographical relationships of the cerebellum are illustrated in some transverse sections of the brainstem of the turtle *Pseudemys scripta elegans* (Figs. 4-7), the lizard *Varanus exanthematicus* (Figs. 8-11), and the snake *Python reticulatus* (Figs. 12-15). Each figure shows the cell bodies at the left and the fiber systems at the right. As in previous analyses of the brainstem (ten Donkelaar and Nieuwenhuys, '79) and spinal cord (Kusuma *et al.*, '79) the general terms small, medium-sized, and coarse are applied to the fibers of the various systems in these figures. These terms are not strictly defined, but the small fibers generally range from 0 to 3 μm, the medium-sized from 3 to 6 μm, and the coarse fibers from 6 to 12 μm in diameter.

As first pointed out by Stieda (1875) the reptilian cerebellar cortex is composed of the three typical layers, i.e. the molecular, Purkyně cell, and granular layer, that characterize the cerebellum in vertebrates (Figs 4-7; 8-10, 12-15, 16). Some histological aspects of the cerebellar cortex in the three reptilian species studied, as well as the organization of the Purkyně cell layer will be dealt with in the next two sections of this chapter

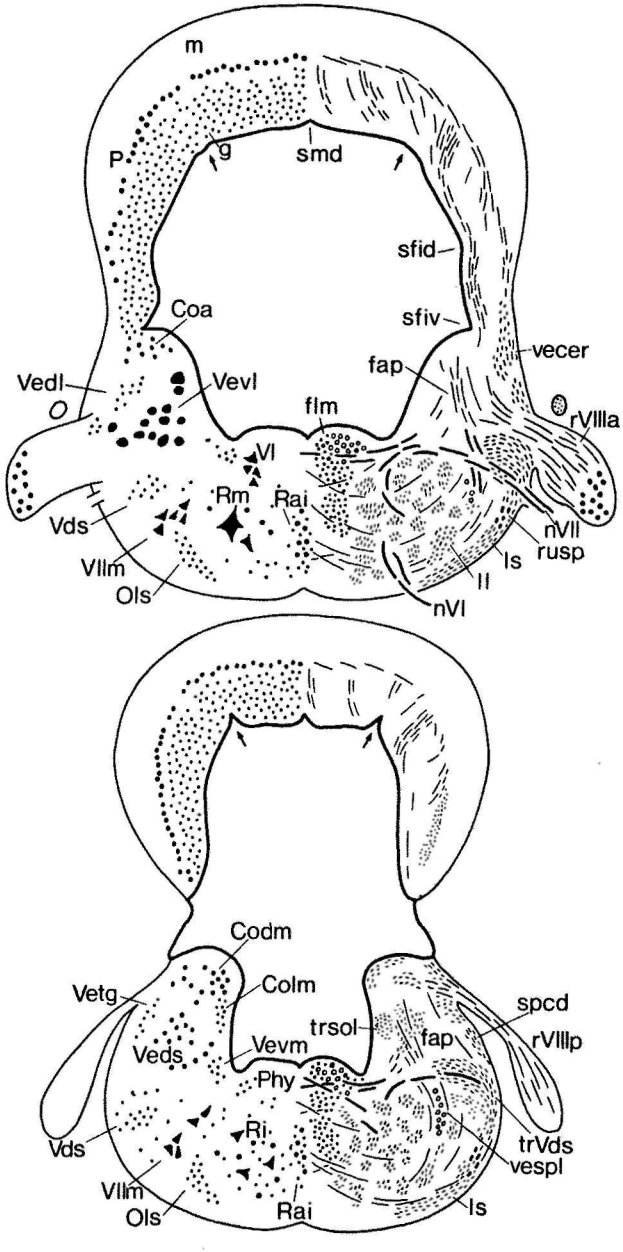
The cerebellar and vestibular nuclei in reptiles form a more or less continuous complex located in the dorsal part of the rhombencephalon (Figs. 1B, 2B, 3B). Two cerebellar nuclei, viz., the *nucleus cerebellaris medialis* and the *nucleus cerebellaris lateralis*, can be distinguished. The nucleus cerebellaris medialis (Figs. 4, 5; 8, 9; 13), characterized by relatively large oval neurons intermingled with smaller elements, is located in the basal part of the corpus cerebelli, dorsal to the fourth ventricle. Its most rostral neurons are dispersed among the fibers of the cerebellar commissure whereas caudolaterally the medial cerebellar nucleus is directly adjacent to the lateral cerebellar nucleus. This latter nucleus (Figs. 5, 9, 13) consists chiefly of small and medium-sized cells. The boundaries between the two cerebellar nuclei are indistinct in all three species, but especially in the snake. Rostrally the cerebellar nuclei border on the nuclei of the isthmic region, viz., the magno- and parvocellular isthmic nuclei and the so-called parabrachial nucleus (ten Donkelaar and de Boer-van Huizen, '81 b, see Figs. 4, 8, 12). Surrounded by neurons of the latter nucleus a distinct brachium conjunctivum can be distinguished in *Pseudemys scripta elegans* and *Varanus exanthematicus*, but not in *Python reticulatus*, in Kluver-Barrera and Haggqvist-stained material (Figs. 4, 8; 12).

Caudally the cerebellar nuclei border on the vestibular nuclear complex. The reptilian vestibular nuclear complex is usually divided into at least five nuclei (Weston, '36), viz , the dorsolateral, ventrolateral, tangential, ventromedial, and descending vestibular nuclei. In turtles, in addition a superior vestibular nucleus is distinguished (Weston, '36, Cruce and Nieuwenhuys, '74, Miller and Kasahara, '79). In the present study no distinction is made between the dorsolateral and superior vestibular nuclei since the boundaries between these nuclei are very ill-defined. The superior vestibular nucleus is regarded as a rostral elongation of the medial part of the *nucleus vestibularis dorsolateralis*. This nucleus (Figs. 5, 6, 9, 10, 13) which has also been called superior vestibular nucleus by several authors

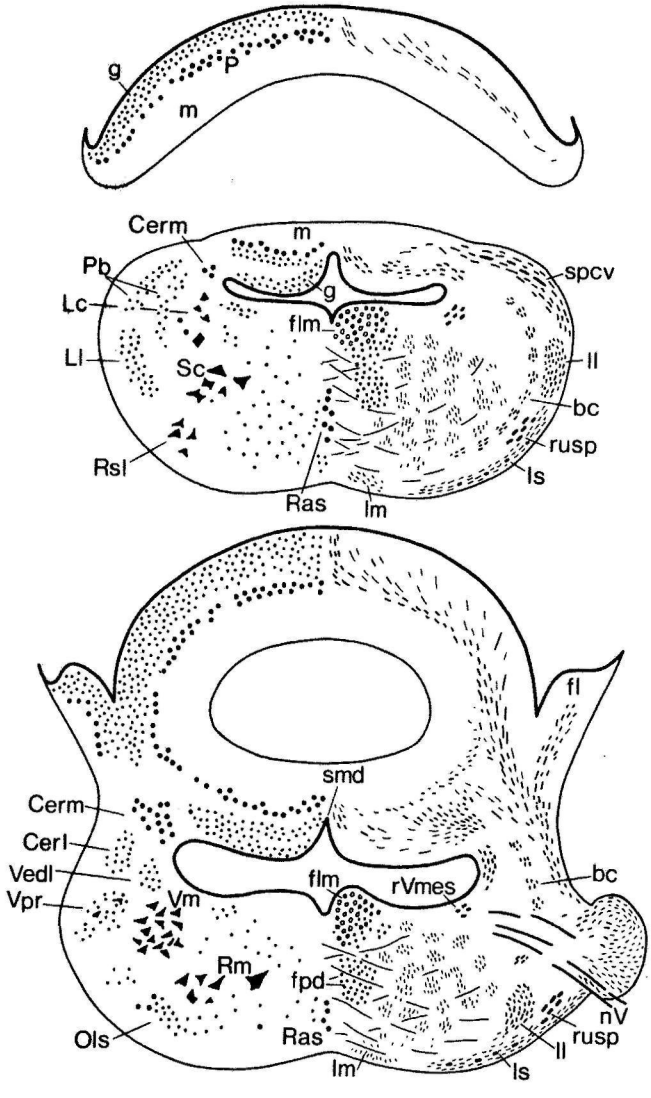
(Beccari, '11; Larsell, '26; Papez, '29; Stefanelli, '44 a), borders dorsorostrally on the lateral cerebellar nucleus, ventromedially on the ventrolateral vestibular nucleus. Particularly its border with the lateral cerebellar nucleus is ill-defined. The *nucleus vestibularis ventrolateralis* (Figs. 6; 11; 14) consists of large neurons among which smaller elements are scattered. It is generally considered to be homologous to the mammalian nucleus of Deiters (ten Donkelaar, '76 b; '82). The *nucleus vestibularis tangentialis* (Figs. 7; 11; 14) consists of small cells dispersed between the entering fibers of the vestibular root. This nucleus, located lateral to the ventrolateral vestibular nucleus, is rather well-developed in *Varanus exanthematicus* and *Python reticulatus*, but only sparsely in *Pseudemys scripta elegans*. Both Weston ('36) and Stefanelli ('44 a, b) have suggested that the degree of development of the tangential nucleus is correlated with the relative development of the trunk musculature. The absence of trunk musculature in turtles is, according to Weston ('36), correlated with the great reduction of the tangential nucleus in the order Testudines, which is in keeping with the sparse development of the tangential nucleus in *Pseudemys scripta elegans*. The *nucleus vestibularis ventromedialis* (Figs. 7; 11; 14, 15) is located at the angle of the fourth ventricle, ventromedial to the ventrolateral vestibular nucleus, and consists of medium-sized cells. It extends along the caudal one-half of the vestibular nuclear complex (Figs. 1B; 2B; 3B). The *nucleus vestibularis descendens* (Figs. 7; 15), consisting of small and medium-sized cells borders rostrally on the ventrolateral vestibular nucleus and extends along the brainstem to the level of the nucleus funiculi dorsalis (Figs. 1B; 2B; 3B).



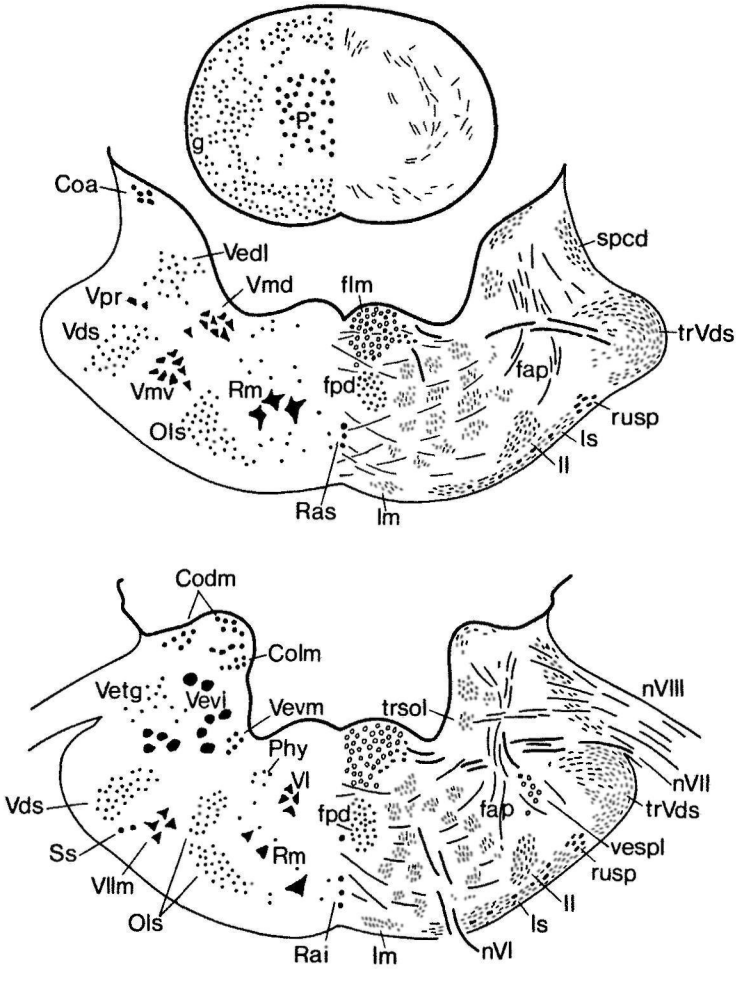
Figs. 4, 5 Transverse sections through the rostral part of the rhombencephalon in the turtle Pseudemys scripta elegans. At the left the cell picture, based on a Nissl-stained series; at the right the fiber systems based on Häggqvist and Klüver-Barrera preparations. For abbreviations cf. pages 28-29.



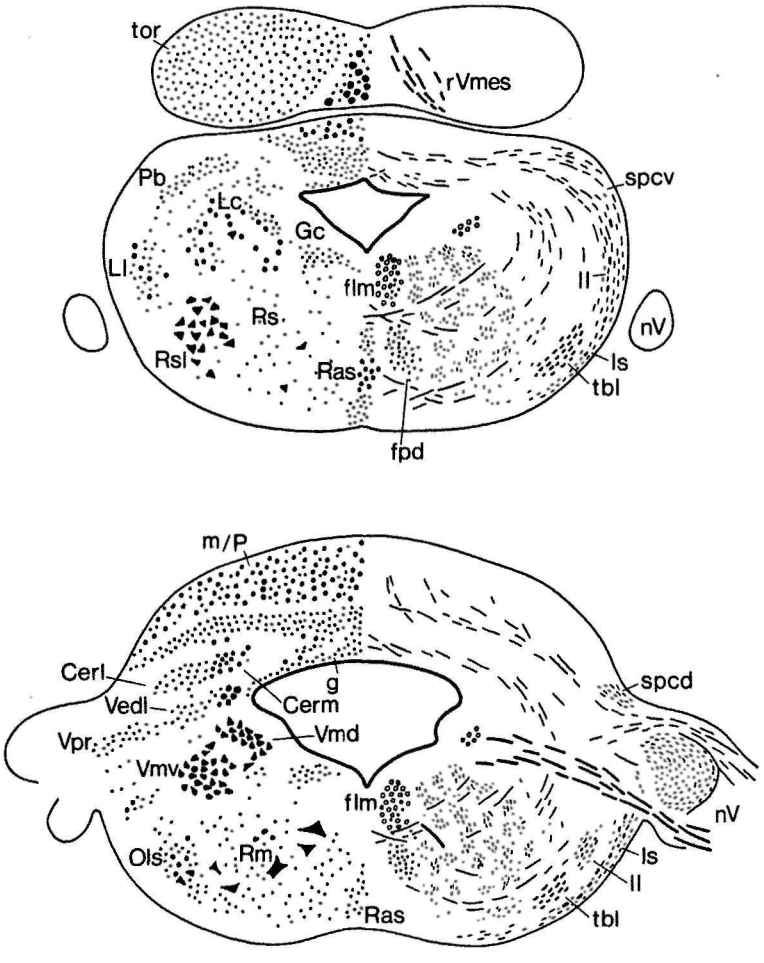
Figs. 6, 7 Transverse sections through the middle part of the rhombencephalon in the turtle Pseudemys scripta elegans. For abbreviations cf. pages 28-29.



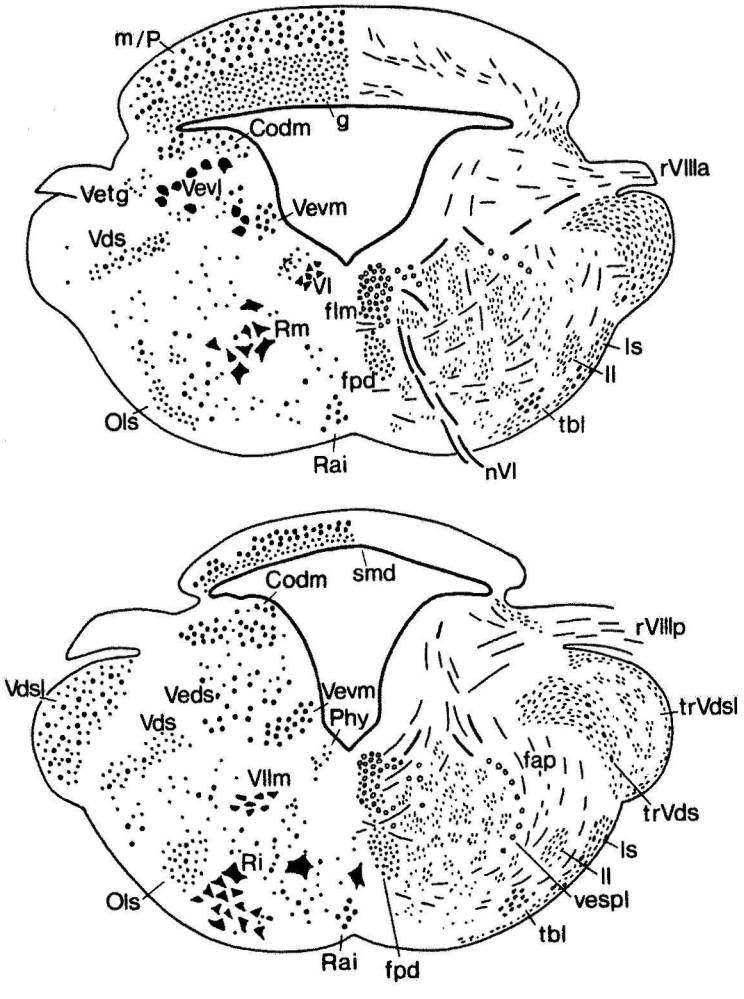
Figs. 8, 9 Transverse sections through the rostral part of the rhombencephalon in the lizard Varanus exanthematicus. For abbreviations cf. pages 28-29.



Figs. 10, 11 Transverse sections through the middle part of the rhombencephalon in the lizard Varanus exanthematicus. For abbreviations cf. pages 28-29.



Figs. 12, 13 Transverse sections through the rostral part of the rhombencephalon in the snake Python reticulatus. For abbreviations cf. pages 28-29.



Figs. 14, 15 Transverse sections through the middle part of the rhombencephalon in the snake Python reticulatus. For abbreviations cf. pages 28-29.

Abbreviations :

Bor	,	nucleus of the basal optic root
bc	,	brachium conjunctivum
Cerl	,	nucleus cerebellaris lateralis
Cerm	,	nucleus cerebellaris medialis
Co	,	cochlear nuclear complex
Coa	,	nucleus cochlearis angularis
Codm	,	nucleus cochlearis dorsalis magnocellularis
Colm	,	nucleus cochlearis laminaris
cereb	,	cerebellum
cho	,	chiasma opticum
cp	,	commissura posterior
Dm	,	nucleus dorsalis myelencephali
dbc	,	decussation of the brachium conjunctivum
EW	,	nucleus of Edinger-Westphal
Fun	,	nucleus funiculi dorsalis
fap	,	fibrae arcuatae profundae
fl	,	flocculus
flm	,	fasciculus longitudinalis medialis
fpd	,	fasciculus predorsalis
fr	,	fasciculus retroflexus
fu	,	fasciculus uncinatus
Gc	,	griseum centrale
g	,	granular layer
Iflm	,	nucleus interstitialis of the flm
Ism	,	nucleus isthmi, pars magnocellularis
Lc	,	locus coeruleus
Ll	,	nucleus lemnisci lateralis
ll	,	lemniscus lateralis
lm	,	lemniscus medialis
ls	,	lemniscus spinalis
m	,	molecular layer
m/P	,	molecular/Purkyně cell layer
Nflm	,	nucleus of the flm
nIII	,	nervus oculomotorius
nIV	,	nervus trochlearis
nV	,	nervus trigeminus
nVI	,	nervus abducens
nVII	,	nervus facialis
nVIII	,	nervus octavus
nX	,	nervus vagus
nXII	,	nervus hypoglossus
Oli	,	oliva inferior
Ols	,	oliva superior
P	,	Purkyně cell layer
Pb	,	nucleus parabrachialis
Ph	,	nucleus periventricularis hypothalami
Phy	,	perihypoglossal nuclear complex
Rai	,	nucleus raphes inferior
Ras	,	nucleus raphes superior
Rf	,	reticular formation
Ri	,	nucleus reticularis inferior
Rm	,	nucleus reticularis medius
Rs	,	nucleus reticularis superior
Rsl	,	nucleus reticularis superior, pars lateralis
Rub	,	nucleus ruber
rusp	,	tractus rubrospinalis
rVmes	,	radix mesencephalicus nervi trigemini
rVIIIa	,	radix anterior nervi octavi
rVIIIp	,	radix posterior nervi octavi

Sc , subcoeruleus area
Sol , nucleus tractus solitarii
Ss , nucleus salivatorius superior
sfid , sulcus flocculi internus dorsalis
sfiv , sulcus flocculi internus ventralis
smd , *sulcus medianus dorsalis*
sol , tractus solitarius
spcd , tractus spinocerebellaris dorsalis
spcv , tractus spinocerebellaris ventralis
Torc , torus semicircularis, nucleus centralis
Torl , torus semicircularis, nucleus laminaris
tbl , tractus tectobulbaris lateralis
tm , tectum mesencephali
tor , torus semicircularis
tr opt , tractus opticus
tr sol , tractus solitarius
trVds , tractus descendens nervi trigemini
trVdsl , tractus descendens lateralis nervi trigemini
Ve , vestibular nuclear complex
Vedl , nucleus vestibularis dorsolateralis
Veds , nucleus vestibularis descendens
Vetg , nucleus vestibularis tangentialis
Vevl , nucleus vestibularis ventrolateralis
Vevm , nucleus vestibularis ventromedialis
vecer , fibrae vestibulocerebellares
vespl , tractus vestibulospinalis lateralis
vespm , tractus vestibulospinalis medialis
III , nucleus nervi oculomotorii
IIId , nucleus nervi oculomotorii, pars dorsalis
IIIi , nucleus nervi oculomotorii, pars intermedia
IIIv , nucleus nervi oculomotorii, pars ventralis
IV , nucleus nervi trochlearis
Vds , nucleus descendens nervi trigemini
Vdsl , nucleus descendens lateralis nervi trigemini
Vm , nucleus motorius nervi trigemini
Vmd , nucleus motorius nervi trigemini, pars dorsalis
Vmv , nucleus motorius nervi trigemini, pars ventralis
Vpr , nucleus princeps nervi trigemini
VI , nucleus nervi abducentis
VII , nucleus nervi facialis
VIIm , nucleus motorius nervi facialis
Xmd , nucleus motorius dorsalis nervi vagi
XII , nucleus nervi hypoglossi

C. HISTOLOGY

The histological structure of the cerebellum of *Pseudemys scripta elegans*, *Varanus exanthematicus* and *Python reticulatus* is shown in a diagrammatic representation of a transverse Nissl-stained section of the cerebellum of each of these reptiles (Fig. 16 A, B, C respectively) As stated before the reptilian cerebellar cortex consists of the three typical layers of this part of the brain, the molecular, Purkyně cell, and granular layer. Several cell types, characteristic for these layers, can be distinguished. the stellate cells, located in the molecular layer, the Purkyně cells, located as a separate layer between the molecular and granular layer in *Pseudemys scripta elegans* and *Varanus exarthematicus*, but with a widespread distribution throughout the molecular layer in *Python reticulatus* (and *Python regius*), the small granule cells which form the granular layer and, scattered throughout this layer, the larger Golgi cells. In all three species studied occasionally so-called 'displaced' Purkyně cells are found scattered in the granular layer.

The abovementioned neuronal elements of the cerebellar cortex, as well as the afferent climbing and mossy fibers all have been demonstrated in reptiles with light microscopic (Golgi) techniques (P Ramón, 1896, Ramón y Cajal, '11, Larsell, '26, '32, Ochoterena, '32, Hillman, '69; Llinás and Hillman, '69) and electronmicroscopy (Hillman, '69, Llinás and Hillman, '69, Mugnaini *et al.*, '74). Together, the afferent mossy and climbing fibers, the granule cells, and the Purkyně cells with their efferent axons form the so-called basic cerebellar circuitry as distinguished in all vertebrates (Llinás and Hillman, '69, Llinás and Nicholson, '69, Llinás, '81)

Climbing fibers, already described by Ochoterena ('32) in the lizard *Phrynosoma*, form direct contacts with the dendrites of Purkyně cells. The climbing fibers consist of an arborizing axon which follows the main Purkyně cell branches as a single fiber Contacts are made with the dendritic spines of the Purkyně cells (*Caiman sclerops*, Hillman, '69, Llinás and Hillman, '69). Since in mammals the climbing fibers have been demonstrated to arise in the inferior olive the presence of such fibers in the reptilian cerebellar cortex suggests that also in reptiles an inferior olive is present (see Chapter IV)

Mossy fibers, arising in many subcerebellar centers (see Chapter IV) form an indirect input to the Purkyně cells, being relayed through the granule cells.

The granule cells, in *Pseudemys scripta elegans* with a diameter of 6-9 μm (Mugnaini *et al.*, '74), send one axon into the molecular layer. Here these axons divide in a T-shaped manner forming the parallel fibers which run perpendicularly through the dendritic trees of the Purkyně cells. In reptiles, as in other vertebrates, the climbing and mossy fibers and the parallel fibers have been demonstrated to make excitatory contacts on the dendritic trees of the Purkyně cells (Kitai *et al.*, '69, Kennedy *et al.*, '70, Llinás and Nicholson, '69, '71).

Ramón y Cajal ('11) was the first to demonstrate the *Purkyně cell* dendritic tree in the lizard *Lacerta stirpium*. The Purkyně cells in *Lacerta stirpium* (Ramón y Cajal, '11) and the turtle *Cistudo carolina* (Larsell, '32) were described having a long primary dendrite which after short branching arborizes into spiny branchlets. These observations were confirmed and extended in the alligator *Caiman sclerops* (Hillman, '69, Llinás and Hillman, '69) The flask-

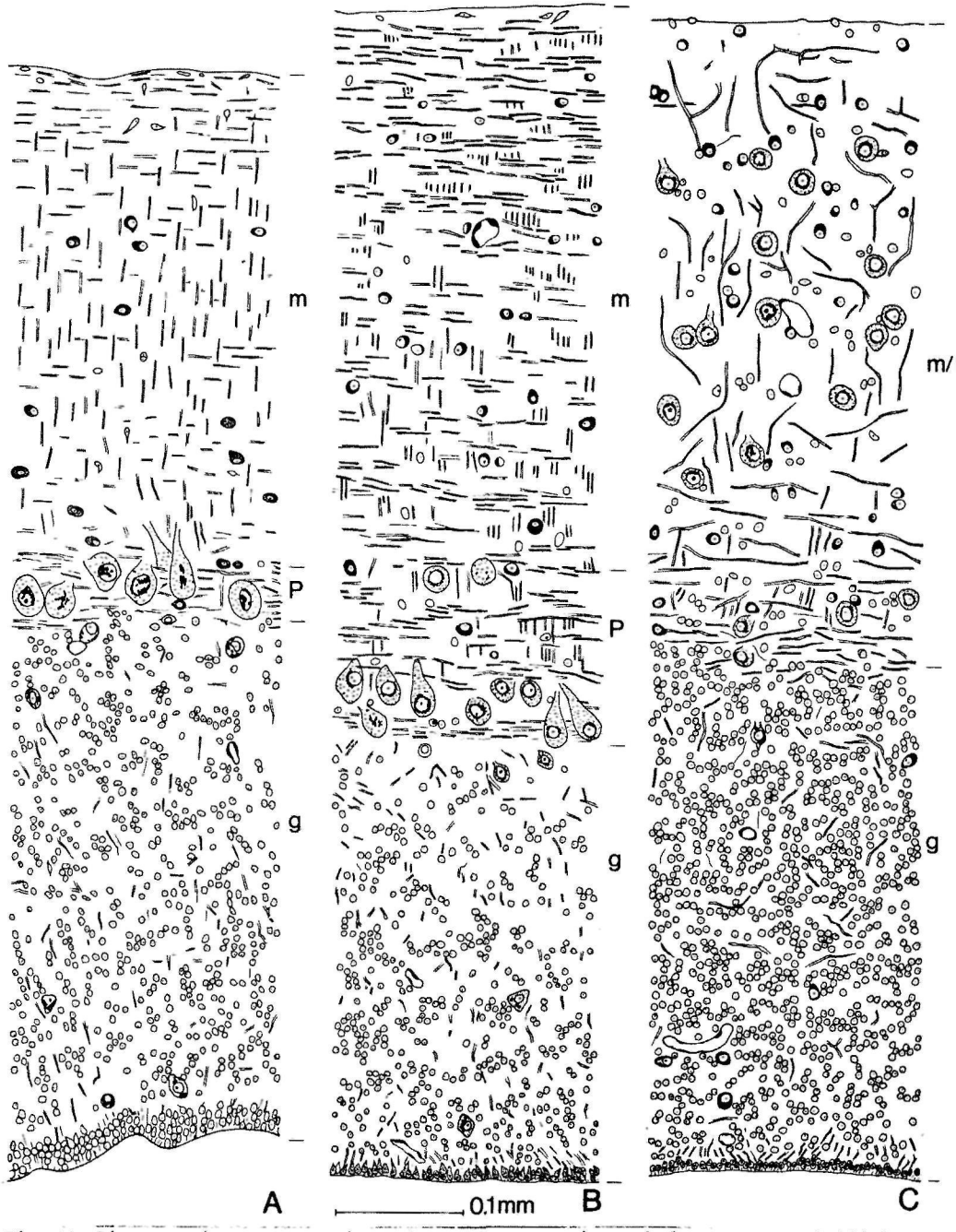


Fig. 16 Diagrammatic representations of transverse sections of the corpus cerebelli in: A, Pseudemys scripta elegans; B, Varanus exanthematicus; C, Python reticulatus. At the bottom the ventricular side is shown. g, granular layer; m, molecular layer; m/P, molecular-Purkyně cell layer; P, Purkyně cell layer.

shaped Purkyně cells of reptiles (Fig. 16) with a diameter of 25-35 µm have a flattened dendritic tree oriented sagittally in the cerebellar cortex, the lateral spread being slight. The organization of the Purkyně cell layer, i.e. the arrangement of the Purkyně cells, and the possible implication of this organization for the efferent cerebellar connections in the reptilian species studied are dealt with in section D of this chapter and in Chapter V.

Superimposed upon the basic cerebellar circuitry the inhibitory influence of interneurons is found (Llinás, '81). In reptiles several types of interneurons can be distinguished: (1) *Stellate cells* (10-15 µm) present in the molecular layer (Fig. 16). Ramón y Cajal ('11) and Larsell ('32) first demonstrated these cells and their various dendritic and axonal patterns, (2) A variety of the stellate cells was distinguished in *Chamaeleo vulgaris* (P. Ramón, 1896) and the lizard *Phrynosoma* (Ochoterena, '32), having dendrites extending towards the cerebellar surface - between the dendritic trees of the Purkyně cells - and axons terminating on the cell bodies of Purkyně cells. These neurons were indicated as primitive *basket cells* (Larsell, '67), (3) *Golgi cells* (18-25 µm) (Fig. 16), scattered in the granular layer, were described in various reptilian species including *Pseudemys scripta elegans* (Mugnaini *et al.*, '74).

Besides the inhibitory influence of these various interneurons a second inhibitory system is represented by catecholaminergic afferents (Llinás, '81). A catecholaminergic input arising in the locus coeruleus has been demonstrated in birds (Mugnaini and Dahl, '75) and mammals (e.g. Hokfelt and Fuxe, '69, Hoffer *et al.*, '71; Olson and Fuxe, '71). Serotonergic afferent fibers to the cerebellum have also been demonstrated (e.g. Chan-Palay, '77). In reptiles a monoaminergic innervation of the cerebellum has been shown (Yamamoto *et al.*, '77, Parent, '79). This innervation is further discussed in Chapter IV.

In short, the reptilian cerebellar cortex comprises all elements of the so-called basic cerebellar circuitry, i.e. the mossy and climbing fibers, granule cells, and Purkyně cells, each present in a characteristic cortical layer, as well as elements which form a superimposed inhibitory system upon this basic cerebellar circuit, i.e. stellate and Golgi cells, and in addition an inhibitory catecholaminergic input.

D. ORGANIZATION OF THE PURKYNE CELL LAYER

As already noted, the Purkyně cell layer forms the only output system of the cerebellum. In 'higher' vertebrates as birds and mammals a longitudinal pattern of organization of the cerebellum has been demonstrated with various anatomical techniques. Myeloarchitectonic studies of Häggqvist-stained sections of the cerebellum of birds (*Gallus domesticus*; Feirabend, '76; '83) and mammals (ferret; cat; Voogd, '64, '67, '69) revealed a number of rostrocaudally oriented compartments of Purkyně cell axons in the lobular white matter. In the cat each compartment appeared to be associated with a specific target site of its Purkyně cell axons. With silver impregnation techniques and retrograde tracer studies a longitudinal pattern of the corticonuclear projections has now been established in birds (*Gallus domesticus*; Wold, '81) and various mammals (see for review, Voogd and Bigaré, '80; Haines *et al.*, '82). Häggqvist-stained sections of the cerebellum of the turtle *Pseudemys scripta elegans* and the lizard *Varanus exanthematicus* did not reveal a subdivision of the white matter into series of compartments by repeating local differences in axon diameter as in birds and mammals. For this reason a different approach was chosen to find out whether a zonal pattern of organization exists in the reptilian cerebellar output zone, i.e. a topological analysis of the Purkyně cell layer. Topological analysis of the Purkyně cell layer of the turtle *Testudo hermanni* (Gerrits and Voogd, '73) showed that in each cerebellar half the Purkyně cell layer can be divided into a medial and a lateral zone.

In most reptiles the Purkyně cell layer consists of several rows of Purkyně cells located between the molecular and granular layer (Larsell, '67). However, in several chelonian species such as e.g. *Chelonia mydas* (Larsell, '67) and *Pseudemys scripta elegans* (Figs. 4-7, 16A) only a single row of Purkyně cells is found throughout most of the cerebellum. In *Pseudemys scripta elegans* only in the most rostral part of the cerebellum several rows of Purkyně cells are found. At certain places, apparently corresponding with the ventricular grooves, no Purkyně cells are present (Figs. 6, 7). In the lizard *Varanus exanthematicus* the Purkyně cell layer in the everted caudal part as well as the middle part of the cerebellum is composed of several rows of cells (Figs. 8-10, 16B). In the most rostral part, i.e. the ventral attachment site, only a single row of Purkyně cells is found (Fig. 8). In the snakes *Python regius* and *Python reticulatus* (Figs. 12-15, 16C) the Purkyně cells are scattered throughout the molecular layer. In the most caudal part of the cerebellum of these snakes separate groups of Purkyně cells seem to be present, especially laterally (Fig. 15). At certain places of the remaining part of the corpus cerebelli (e.g. Figs. 13, 14), a less dense packing of Purkyně cells is present, suggesting a subdivision of the corpus cerebelli into at least two longitudinally oriented parts. However, due to the, apparently random, widespread distribution of the Purkyně cells in the molecular layer, it appeared to be impossible to study this subdivision by way of a topological analysis of the cerebellar Purkyně cell layer. For this reason a topological analysis of the cerebellar Purkyně cell layer was carried out only in the turtle *Pseudemys scripta elegans* and the lizard *Varanus exanthematicus*.

The topological analysis of the cerebellar Purkyně cell layer of the turtle *Pseudemys scripta elegans*, for which a 10 µm transversely sectioned Nissl series was used, involved the following sequence of steps (Fig. 17), based on a procedure developed by Nieuwenhuys ('74):

-1. With the aid of a Zeiss drawing apparatus the outlines of every second section were drawn at a magnification of 60 times (Fig. 17.1). In order to avoid plotting of one Purkyně cell into more than one drawing, only those Purkyně cells were drawn in which a nucleolus was clearly visible. In addition the midline, indicated by the sulcus medianus dorsalis (Figs 4-7), and the borderlines between the molecular and granular layer were indicated.
-2. The Purkyně cells were projected perpendicularly to the borderline between the molecular and granular layer (Fig. 17.2). The midline and the lateral borders were also indicated on this borderline
-3. The curved borderline of each section was transformed into a straight line on which the positions of the Purkyně cells, the midline, and the lateral borders were indicated (Fig. 17.3).
-4. The analysis was completed by transferring the straight lines, representing the drawn sections, into a chart in the right rostrocaudal sequence and at the right distance, using the midline as point of reference (Fig. 17.4). The procedure followed for the topological analysis of the lizard *Varanus exanthematicus*, for which also a 10 µm Nissl-stained series was used, included basically the same steps. However, the following adaptations had to be made, due to the eversion of the cerebellum in this animal. In normally cut transverse series of the brain of *Varanus exanthematicus* (Figs. 8-11) a large number of Purkyně cells is present in sections through the curved part of the cerebellum (Fig 10). To avoid this crowding of Purkyně cells, which makes a topological analysis as described for *Pseudemys scripta elegans* impossible, the cerebellum of the lizard was rotated twice during the sectioning. The dorsal, completely everted part of the cerebellum of *Varanus exanthematicus*, which is comparable to the caudal (uneverted) part of the cerebellum of *Pseudemys scripta elegans*, was cut transversely as in *Pseudemys scripta elegans*. At the level of the curvature of the cerebellum, i.e. the middle part, the cerebellum was rotated twice as indicated in figure 19C (arrows) In this way strongly oblique, or tangential cutting of the Purkyně cell layer could be avoided. However, due to these rotations it was impossible to make a topological analysis of the most ventral, i.e. the rostral part of the cerebellum of *Varanus exanthematicus* (Fig. 19C).

The result of the topological analysis of *Pseudemys scripta elegans* is illustrated in figure 18. The part of the Purkyně cell layer which was analysed is shown in figure 18C in a sagittal plane of part of the brainstem. Of the total length of the Purkyně cell layer, measuring 4.3 mm, 4.0 mm was analysed. The most rostral and caudal parts of the cerebellum were excluded from the analysis since in these parts some crowding of Purkyně cells occurs due to the slight bending of the cerebellum (Fig. 18B). The topological analysis revealed a distinct pattern in the Purkyně cell layer (Fig. 18A). The two halves of the cerebellum are clearly separated by an almost Purkyně cell free strip along the whole length of the cerebellum. In each half of the cerebellum, three longitudinally oriented zones of Purkyně cells, viz., a *medial*, an *intermediate*, and a *lateral* zone can be distinguished. The medial zone is separated from the intermediate zone by an almost Purkyně cell free strip, most clearly visible in the rostral and middle parts of the cerebellum. In the rostral part of the cerebellum, the lateral zone is separated from the intermediate zone by a distinct cell free area. In the middle part, the lateral zone is characterized by a less dense pattern of Purkyně cells as compared to the other zones. This difference diminishes in the most caudal

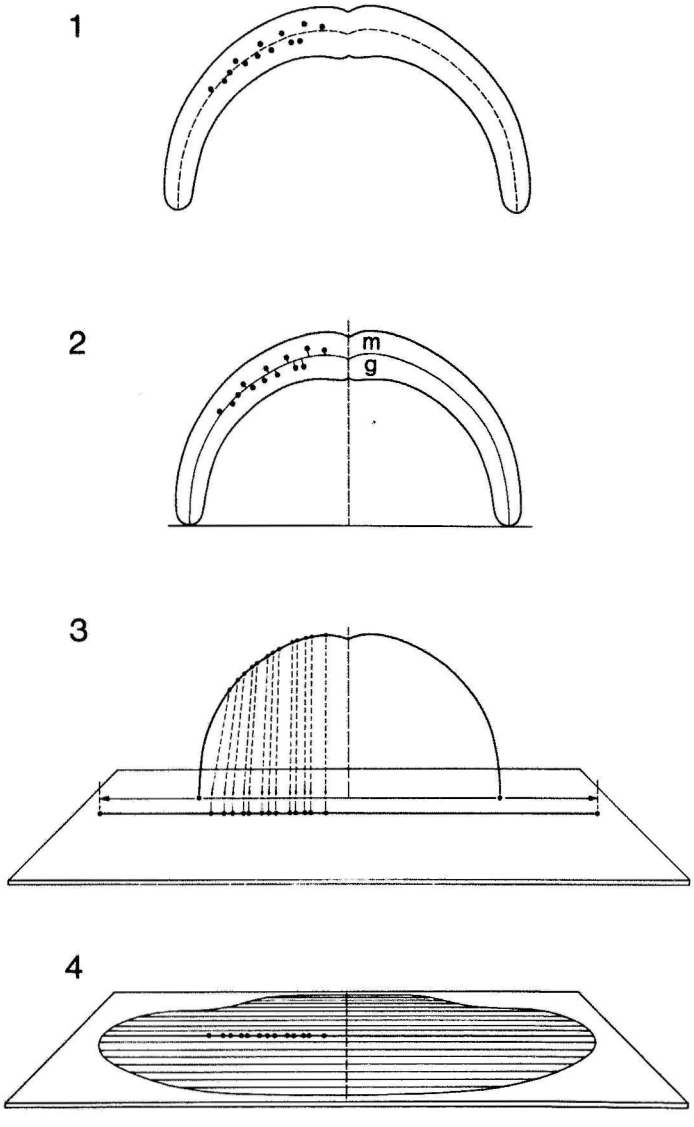


Fig. 17 Schematic diagram of the subsequent steps involved in the preparation of a topological analysis of the cerebellum. The numbers of the steps correspond to those employed in the text. g, granular layer; m, molecular layer.

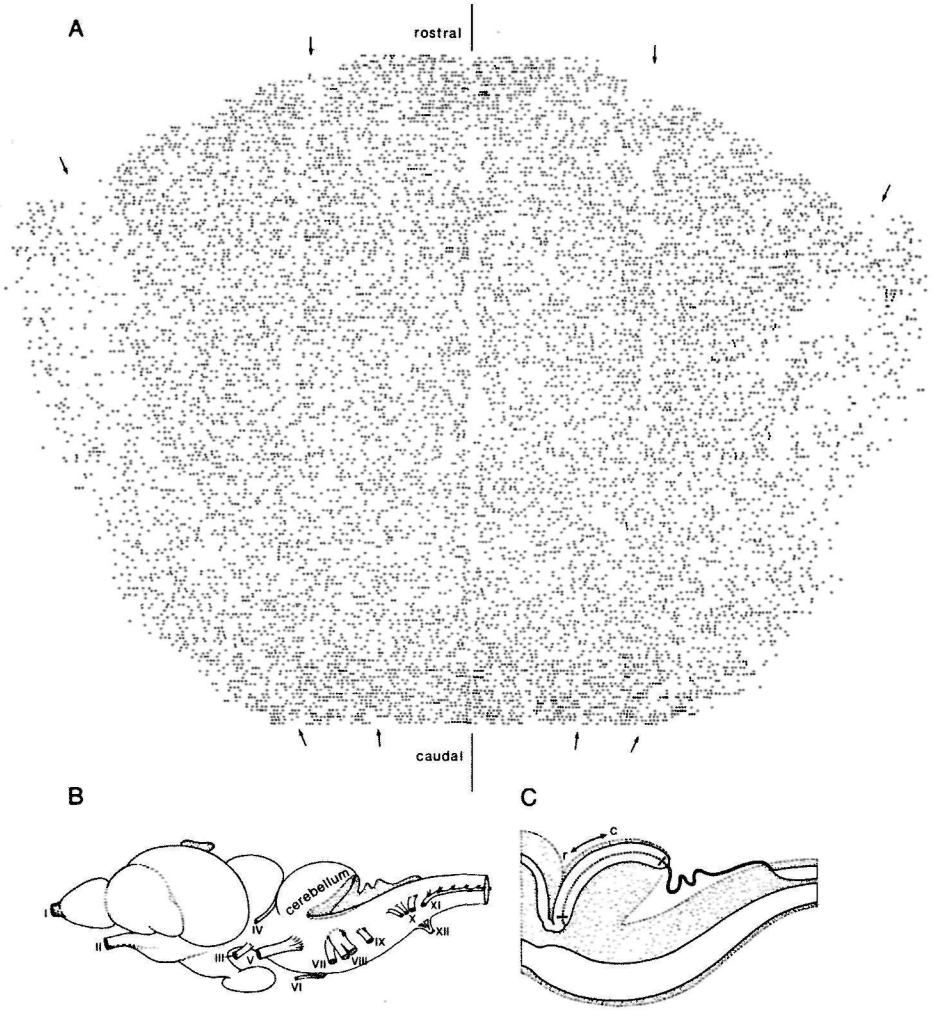


Fig. 18 A, Topological chart showing the distribution of the Purkynĕ cells in both halves of the cerebellum of Pseudemys scripta elegans. The vertical lines indicate the midline, the arrows the borderlines between the medial, intermediate, and lateral Purkynĕ cell zones (×20). B, Lateral view of the brain of Pseudemys scripta elegans (×1.8). C, Sagittal plane of part of the brainstem showing the analysed part (dotted line) of the Purkynĕ cell layer. c, caudal; r, rostral.

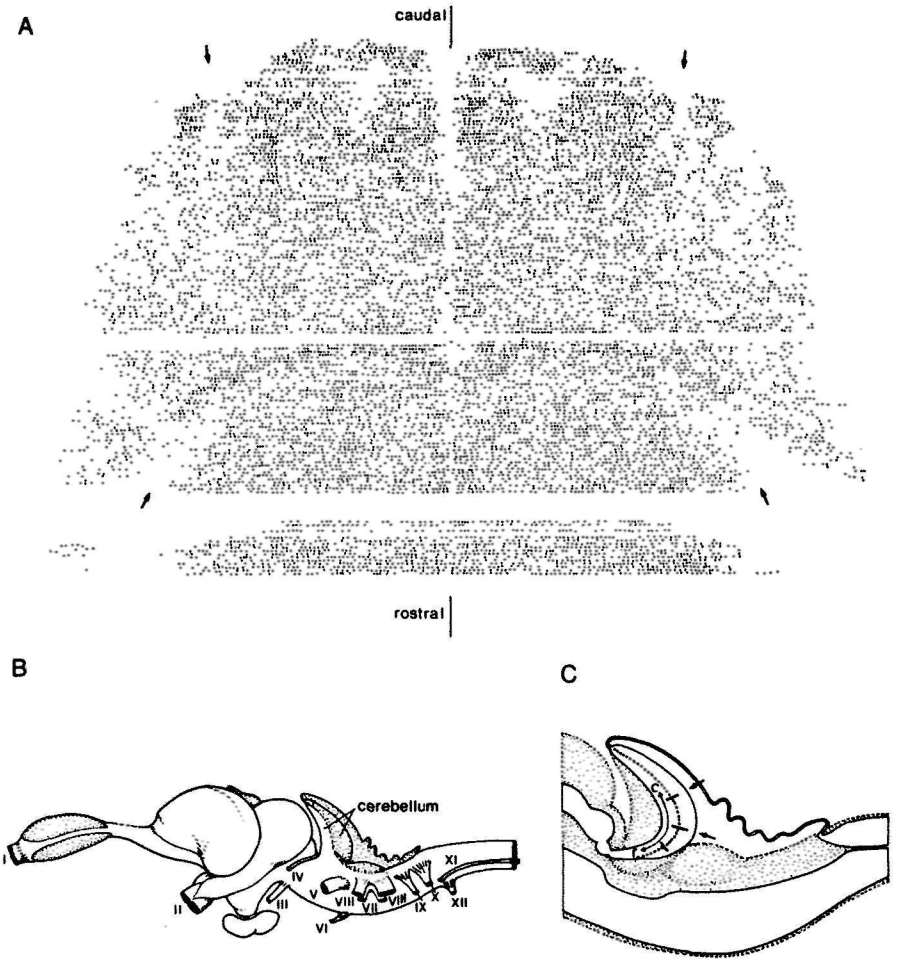


Fig. 19 *A, Topological chart showing the distribution of the Purkyně cells in both halves of the cerebellum of Varanus exanthematicus. The vertical lines indicate the midline, the arrows the borderline between the corpus cerebelli and the floccular zone (×20). B, Lateral view of the brain of Varanus exanthematicus (×1.6). C, Sagittal plane of part of the brainstem showing the analysed part (dotted line): the distribution of Purkyně cells has been studied in three parts. c, caudal; r, rostral.*

part of the cerebellum. These zones correspond in part to the three different components of the cerebellum as distinguished by Larsell ('26, '32, '67). The medial zones of the two cerebellar halves together correspond to his pars interposita. Larsell's pars lateralis corresponds to the intermediate zone described in this chapter. The flocculus as distinguished by Larsell corresponds to the rostral part of the lateral zone. The remaining part of the lateral zone of Purkyně cells corresponds to the caudal elongation of the flocculus, i.e. the so-called marginal rim (Mugnaini *et al.*, '74; Brand and Mugnaini, '80).

The result of the topological analysis in *Varanus exanthematicus* is shown in figure 19. The part of the Purkyně cell layer which was analysed included approximately two-thirds of the total Purkyně cell surface and is shown in figure 19C. In the Purkyně cell chart (Fig. 19A) the two horizontal interruptions correspond to the sites of the two rotations (Fig. 19C, arrows). In *Varanus exanthematicus* a less clear subdivision of the Purkyně cell layer is present than in *Pseudemys scripta elegans*, although at certain places a similar longitudinal subdivision seems to exist. However, a lateral, floccular zone (indicated by the arrows) can be clearly distinguished.

Based on these topological analyses it is concluded that in the reptiles studied a longitudinal pattern of Purkyně cells can be distinguished, which is most clearly present in the turtle *Pseudemys scripta elegans*.

IV. AFFERENT CONNECTIONS OF THE CEREBELLUM

INTRODUCTION

In the preceding chapter the presence of mossy and climbing fibers, and monoaminergic afferent fibers in the cerebellar cortex of reptiles has been described. So far our knowledge on the origin of these cerebellar afferents has been rather limited. Based on the study of Weigert and pyridine-silver material of various reptiles, several afferent fiber systems were described, including:

a) tectocerebellar projections (Ariëns Kappers, '21; Huber and Crosby, '26, '33; Larsell, '26, '32; Shanklin, '30; Hindenach, '31; Weston, '36);

b) trigeminocerebellar projections (Ariëns Kappers, '21; Huber and Crosby, '26, '33; Larsell, '26, '32; Shanklin, '30; Hindenach, '31; Weston, '36);

c) primary and secondary vestibulocerebellar fibers (Edinger, '08; Beccari, '11; Ingvar, '18; Ariëns Kappers, '21; Huber and Crosby, '26; Larsell, '26, '32; Weston, '36; Kawakami, '54);

d) olivocerebellar fibers (van Hoevell, '16; Leblanc, '23; Larsell, '26, '32; Shanklin, '30) as part of the spinocerebellar system. The presence of an inferior olive in reptiles has been disputed by other authors (e.g. Kooy, '17; Weston, '36);

e) spinocerebellar fibers (Edinger, '08; de Lange, '17; Ariëns Kappers, '21; Huber and Crosby, '26; Larsell, '26, '32; Shanklin, '30; Hindenach, '31; Weston, '36; Kawakami, '54). Separate dorsal and ventral spinocerebellar tracts, both ascending by way of the lateral funiculus were distinguished. Moreover, Banchi ('03) noted a nucleus of Clarke located in the dorsal horn of the turtle *Emys europaea* with Golgi-impregnation techniques.

With anterograde degeneration techniques and the recently developed axonal tracing techniques (^{3}H-leucine, HRP) the aforementioned observations were partly confirmed and extended, e.g. the primary and secondary vestibulocerebellar projections (Leake, '74; ten Donkelaar, '76 b), spinocerebellar connections (Ebbesson, '67, '69; Jacobs, '68; Pedersen, '73). Also a striocerebellar pathway was reported in the lizard *Tupinambis nigropunctatus* (Hoogland, '77; Voneida and Sligar, '79). Reiner and Karten ('78) demonstrated cerebellar afferents from the nucleus of the basal optic root in the turtle *Chrysemys picta picta*, whereas Schwarz and Schwarz ('80) demonstrated cerebellar afferents in several turtle species from other brainstem nuclei, including the vestibular nuclear complex and the parvocellular isthmic complex.

Within the frame of the present study the origin of the brainstem and spinal cerebellar afferents has been investigated retrogradely with the modern tracing techniques (HRP, WGA-HRP, FB) in the turtles *Pseudemys scripta elegans* and *Testudo hermanni*, the lizard *Varanus exanthematicus*, and the snake *Python regius*. Making use of the anterograde transport of the tracer WGA-HRP the olivocerebellar pathway and the termination pattern of climbing fibers in the cerebellar molecular layer of *Varanus exanthematicus* were demonstrated. In addition the distribution of monoaminergic fibers in the cerebellum of the lizard *Varanus exanthematicus* is shown.

The observations obtained in 12 HRP experiments on these two turtle species were roughly comparable, although differences existed related to site and size of the injections. One experiment on *Pseudemys scripta elegans*, case 6151 (Figs. 20, 21, 23), and one experiment on *Testudo hermanni*, case 6121 (Fig. 22), will be described. The results obtained after FB injection into the cerebellum of the turtle *Pseudemys scripta elegans* were comparable with the illustrated case 6151. Generally more neurons appeared to be labeled after FB injection than after HRP injections

In case 6151 (Figs. 20, 21, 23) three HRP injections were made into the rostrolateral part of the cerebellum. The injections were restricted to the ipsilateral part of the cerebellum (Fig. 20H, I). Spread of HRP occurred in the rostral direction to the cerebellar commissure, in the caudal direction into the lateral cerebellar nucleus. In case 6121 (Fig. 22) one HRP injection was made, slightly more rostral, at the level of the isthmic nucleus (Fig. 22E). In experiments with large HRP injections extending into the cerebellar peduncle labeled neurons were observed as far rostrally as the diencephalon and as far caudally as the lumbar segments of the spinal cord. In experiments with smaller injections restricted to the corpus cerebelli the number of labeled neurons was much reduced. The distribution of the labeled neurons will be described separately for each division of the central nervous system

No labeled neurons were present in the *telencephalon* in any of the experiments. In some cases labeled neurons were observed in the *diencephalon*: in case 6151 a few labeled cells were present bilaterally in the regio preoptica (Fig. 20A), in the dorsal hypothalamic area (Fig. 20B, C), and in the nucleus periventricularis hypothalami (Fig. 20C). In three experiments a few labeled neurons were observed in the ventrolateral part of the ipsilateral nucleus geniculatus pretectalis, a cell mass which is known to receive a direct retinal projection (Knapp and Kang, '68). The latter results are in keeping with the findings of Reiner and Karten ('78) in the eastern painted turtle *Chrysemys picta picta*.

In the *mesencephalon* labeled neurons were observed in the periventricular gray, in the nucleus opticus tegmenti or nucleus of the basal optic root (Reiner and Karten, '78), scattered throughout the tegmentum mesencephali, in the nucleus interstitialis of the fasciculus longitudinalis medialis (flm), in the so-called nucleus of the fasciculus longitudinalis medialis (Tuge, '32), in the nucleus ruber, in the nucleus laminaris of the torus semicircularis and in the stratum griseum periventriculare of the tectum mesencephali. In almost all cases labeled neurons were present in the ipsilateral nucleus of the basal optic root (Figs 20D, E, 22B). Retrogradely labeled fibers could be traced running from the commissura cerebelli in the velum medullare anterior through the ipsilateral mediobasal part of the tegmentum mesencephali to their site of origin, i.e. the nucleus of the basal optic root. Adjacent to the nucleus of the basal optic root labeled cells were observed scattered throughout the ipsilateral tegmentum mesencephali (Fig. 20D, E). In a few cases some labeled cells were also observed contralaterally in the tegmentum (Fig. 22A, B). Some of these cells were situated directly mediolateral to the nucleus of the basal optic root (Figs. 20E, 22B). This area has been termed 'A' by Reiner and Karten in a recent study of the bisynaptic retinocerebellar pathway in the turtle *Chrysemys*

picta picta ('78) Other labeled cells belonged to the interstitial nucleus of the flm (Figs. 20E, 22B). Some cells of the ipsilateral nucleus of the flm (Tuge, '32) were also labeled (Fig. 20D) In both experiments illustrated the contralateral nucleus ruber contained some labeled cells probably due to spread of HRP to the deep cerebellar nuclei (Figs 20E, F, 22C). In this respect it should be noted that in the lizard *Tupinambis nigropunctatus* the nucleus ruber was demonstrated to project to the contralateral nucleus cerebellaris lateralis (ten Donkelaar, '76b). Similar observations were made in the lizard *Varanus exanthematicus* (ten Donkelaar and Dederen, unpublished autoradiographic data). In three experiments the laminar nucleus of the torus semicircularis contained a few labeled cells (Fig. 20E, F). In case 6121 some labeled neurons were found in the ipsilateral stratum griseum periventriculare of the tectum mesencephali (Fig. 22C). This is most probably due to spread of HRP to the isthmic nucleus, which is known to receive tectal projections (Foster and Hall, '75, ten Donkelaar, '76b, Ulinski, '77).

Most of the cerebellar afferents were found to arise in nuclei of the *rhombencephalon*. In its rostral part two cell groups contained ipsilaterally labeled neurons· the locus coeruleus (Fig. 20G) and a cell group (Fig. 22D) probably homologous to the mammalian nucleus parabrachialis (ten Donkelaar and de Boer-van Huizen, '81b). Caudal to the isthmic level labeled neurons were present in the deep cerebellar nuclei, the vestibular nuclear complex including the vestibular ganglion, in cell groups presumably comparable to respectively the perihypoglossal nuclei and the inferior olive of mammals, in two somatosensory nuclei (the descending nucleus of the trigeminal nerve and the dorsal funicular nucleus), in the nucleus of the solitary tract, and throughout the reticular formation.

In the vestibular nuclear complex retrogradely labeled neurons were observed in three cell masses, i e. the dorsolateral, ventromedial, and descending vestibular nuclei (Figs. 21A-F, 22G-L). The most distinct vestibular projection to the cerebellum was found to arise in the nucleus vestibularis descendens (Fig. 31B). Even in experiments with a more restricted extension of the HRP injection labeled neurons were present throughout this nucleus. Retrogradely labeled neurons were also present in the vestibular ganglion whereas labeled primary vestibulocerebellar fibers could be traced along the vestibular nerve.

In the rhombencephalon two other distinct cell groups, most probably corresponding to the perihypoglossal nuclei and the inferior olive of mammals, were found to project to the cerebellum. A conspicuous group of bilaterally labeled neurons was observed in a cell mass directly adjacent to the flm between the levels of the abducens and hypoglossal nuclei (Figs. 21E, F, 22J,K, 31C). In snakes and lizards this cell mass was termed the nucleus parvocellularis medialis by Ebbesson ('67, '69). According to this author this nucleus can not be distinguished in Nissl series of *Pseudemys scripta elegans*. In its location and spinal afferents (Ebbesson, '67, '69) the nucleus parvocellularis medialis resembles the perihypoglossal nuclei in mammals. The present finding that the cell mass in question projects extensively to the cerebellum is also in keeping with experimental data in mammals (Brodal, '52; Kotchabhakdi *et al.*, '78; Batini *et al* , '78). Therefore, in the present study this important site of origin of cerebellar afferents will be termed the perihypoglossal nuclear complex.

In almost all experiments a conspicuously labeled cell mass was present contralateral

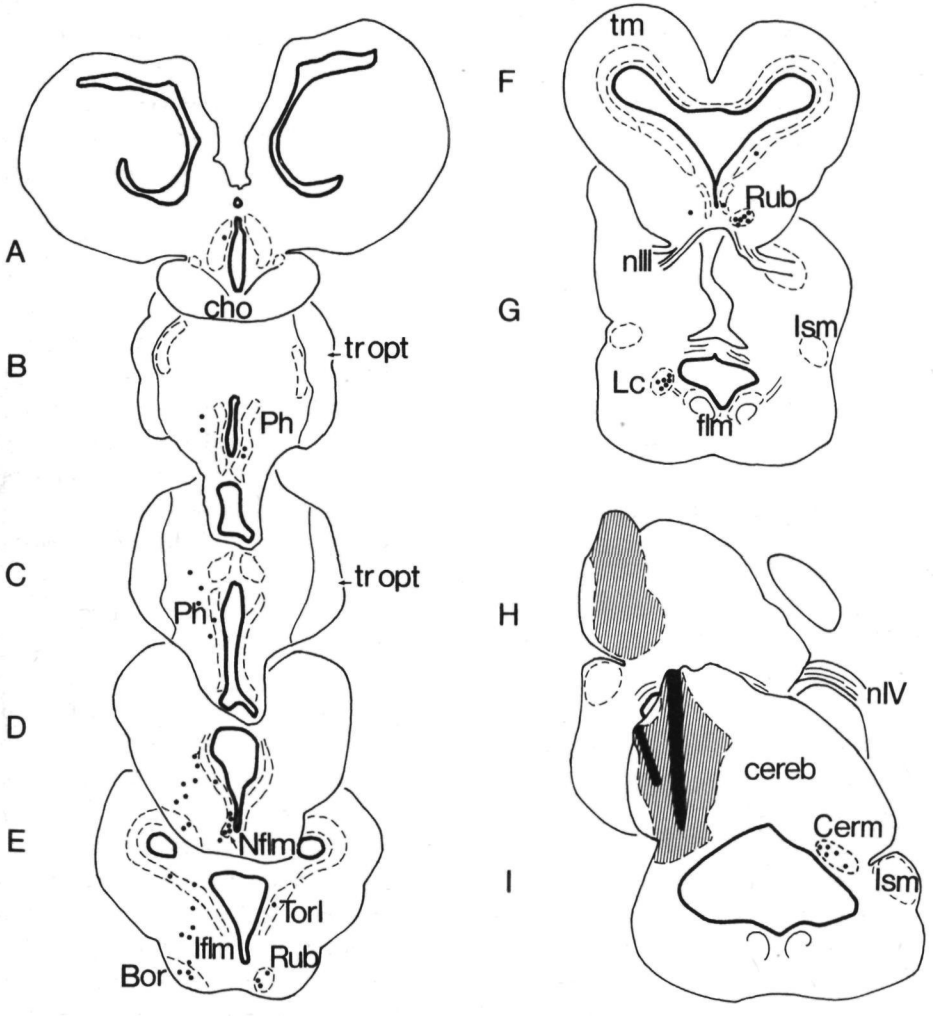


Fig. 20 The distribution of labeled neurons in the diencephalon and rostral part of the brainstem after HRP injections into the cerebellum of the turtle Pseudemys scripta elegans. At each level the labeled neurons found in one section are plotted. For abbreviations cf. pages 28-29.

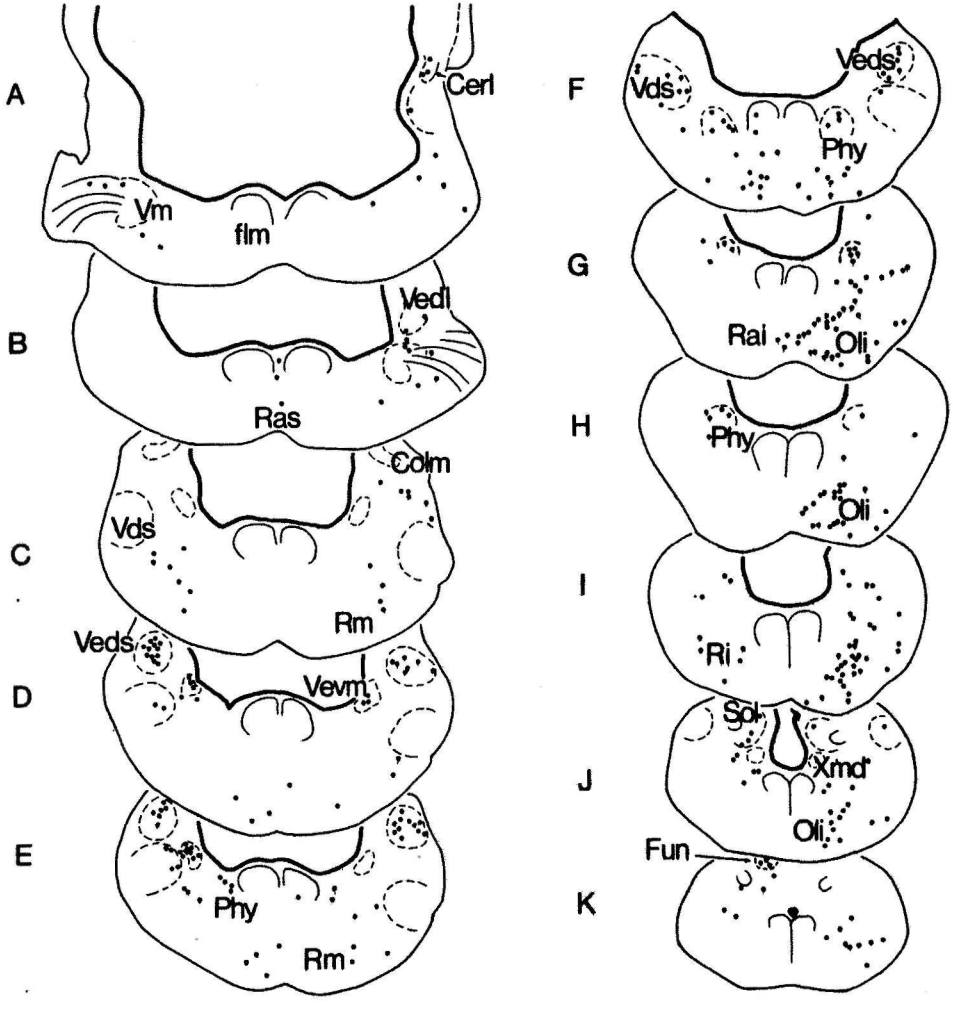


Fig. 21 The distribution of labeled neurons in the middle and caudal part of the brainstem after HRP injections into the cerebellum of the turtle Pseudemys scripta elegans. At each level the labeled neurons found in one section are plotted. For abbreviations cf. pages 28-29.

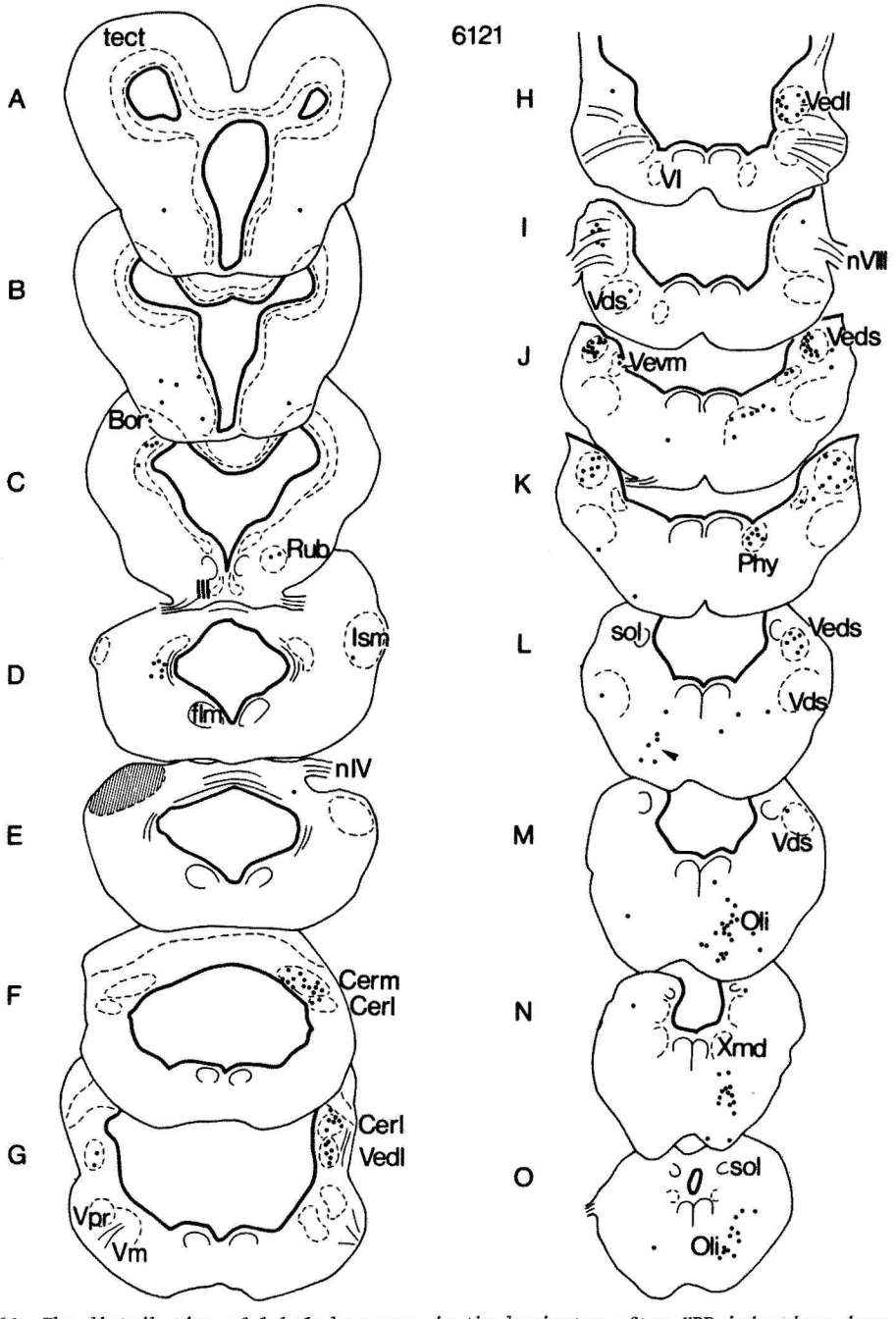


Fig. 22 The distribution of labeled neurons in the brainstem after HRP injections into the cerebellum of the turtle Testudo hermanni. At each level the labeled neurons found in one section are plotted. For abbreviations cf. pages 28-29.

to the injection site in the mediobasal part of the brainstem, adjacent to the nucleus raphes inferior, between the level of the caudal part of the descending vestibular nucleus and the nucleus of the hypoglossal nerve (Figs. 22G-J; 22M-O; 32A, B). Also, some labeled fibers could be traced coursing in the ipsilateral spinal lemniscus and crossing the midline to their apparent site of origin. This location and the constant contralateral labeling after both small and large cerebellar injections point to the conclusion that this cell mass most probably represents the reptilian homologue of the inferior olive of other vertebrates.

In mammals the pontine nuclei, the inferior olive and the tegmental, lateral, and paramedian reticular nuclei are referred to as precerebellar nuclei because all of these centers give off most of their efferent fibers to the cerebellum (Brodal, '81). As regards these precerebellar nuclei, in the turtles studied no evidence has been found for the existence of pontine nuclei, and tegmental and paramedian reticular nuclei. In case 6121 a few labeled neurons observed ipsilaterally at the border of the caudal brainstem (Fig. 22L, arrow) might represent the lateral reticular nucleus or nucleus funiculi lateralis. In other experiments, however, a distinct nucleus funiculi lateralis could not be distinguished.

In experiments with a large extension of the injection site, e.g. case 6151, labeled neurons were located bilaterally in the descending nucleus of the trigeminal nerve throughout its course along the brainstem (Figs. 21B-F, 22I-M). A second somatosensory nucleus, the dorsal funicular nucleus, contained a few labeled neurons only in case 6151 (Fig. 21K).

No evidence was found for the existence of an afferent projection to the cerebellum of the nucleus cuneatus externus as distinguished by Ebbesson ('67, '69) in several reptilian species.

Other cells of origin of cerebellar afferents in the rhombencephalon were found in the nucleus of the solitary tract (Figs. 21J; 22N) and throughout the reticular formation. The most rostrally labeled neurons were observed in the nucleus raphes superior (Fig. 21B) and the nucleus reticularis medius (Fig. 21 C-F). Caudally the nucleus raphes inferior (Fig. 21G) and the nucleus reticularis inferior (Fig. 21I) contained labeled neurons. In cases with a limited injection site, e.g. case 6121, almost no cerebellar afferents from the raphe nuclei were observed.

In the present study both primary and secondary projections of the *spinal cord* to the cerebellum were found. Primary projections were observed only after injections of the sensitive tracer WGA-HRP into the cerebellum of *Pseudemys scripta elegans* (ten Donkelaar and Bangma, '84). Labeled spinal ganglion cells were found in the ipsilateral dorsal root ganglia of a large number of spinal segments throughout the length of the cord (Fig. 31D). Also contralaterally labeled ganglion cells were found. These observations are in keeping with the results of Künzle ('82) in the turtle *Pseudemys scripta elegans*. After injections of ^{35}S-methionine into the cervical and lumbar dorsal root ganglia, labeled terminals were observed in the cerebellar granular layer, indicating a primary projection of the dorsal root ganglia to the cerebellum.

Secondary projections of the spinal cord to the cerebellum appeared to arise also throughout almost the whole spinal cord (Fig. 23). In case 6151 the injection was directed to the cerebellar commissure in which at least part of the spinocerebellar fibers decussate (Weston, '36; Ebbesson, '67. '69, Jacobs, '68; Pedersen, '73; ten Donkelaar and Nieuwenhuys, '79 ; Fig. 27). In the cervical intumescence the labeled neurons turned out to be more or less

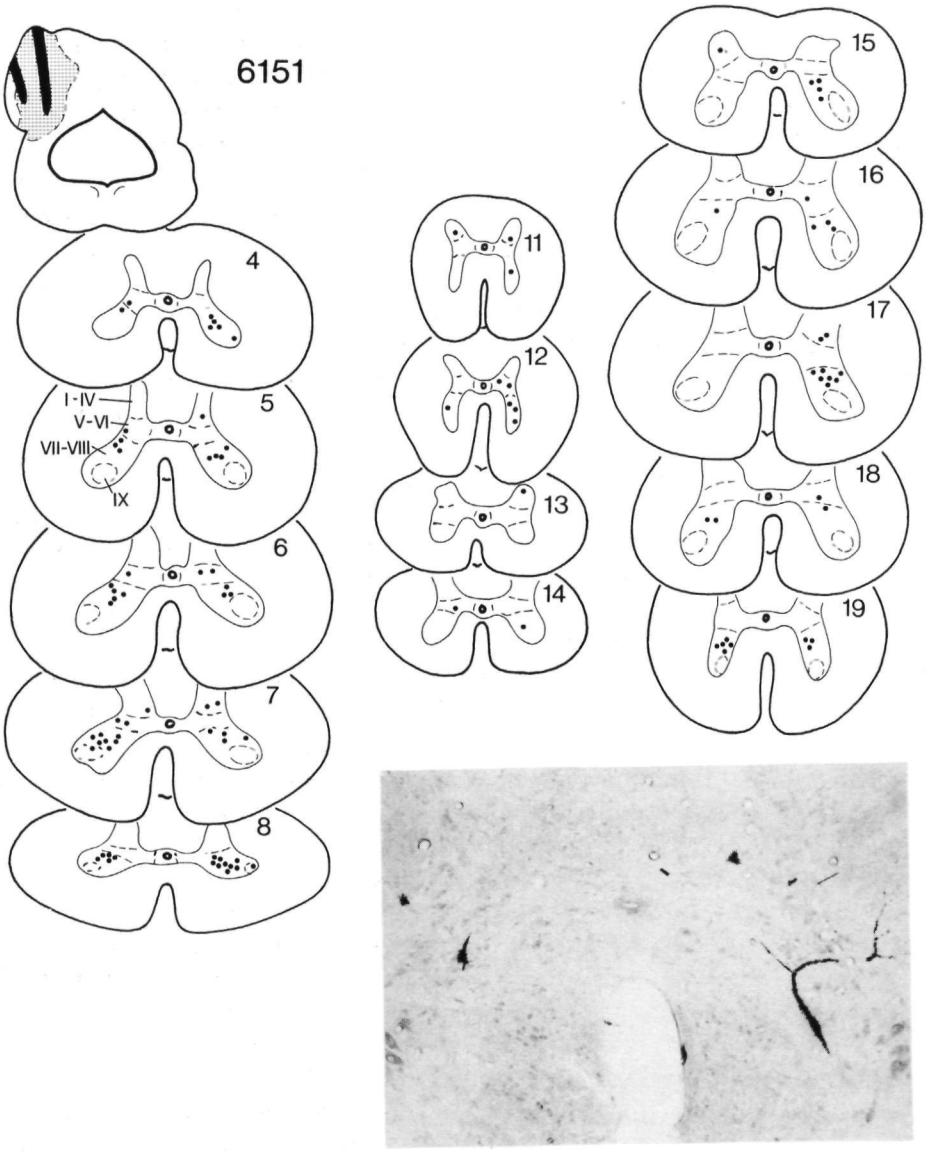


Fig. 23 The distribution of labeled neurons in cervical (4-8), thoracic (11-14), and lumbar (15-19) segments of the spinal cord after HRP injections into the cerebellum of the turtle Pseudemys scripta elegans. Each level represents the composite of the plots of ten consecutive sections. Insert: retrogradely labeled neurons in the 16th segment of the spinal cord, TMB incubation, ×70.

evenly distributed between the ipsilateral and contralateral side of the spinal cord. In area I-IV (subdivision of the reptilian spinal gray after Kusuma *et al.*, '79) only a few labeled neurons occurred. Area V-VI contained more labeled neurons but the greatest number was present in area VII-VIII. The area of the motoneurons (IX) also contained a few labeled cells. In thoracic segments the total number of labeled cells was rather limited. In the lumbar intumescence area I-IV and area V-VI contained only few labeled cells. Most of the labeled cells at lumbar levels were present in area VII-VIII, especially on the contralateral side of the spinal cord. In other experiments labeled cells were also observed in the spinal cord but, probably due to the limited extent of the injection, in a strongly reduced number as compared with the illustrated case.

CEREBELLAR AFFERENTS IN THE LIZARD VARANUS EXANTHEMATICUS

The description of the results obtained after injection of HRP and FB into the cerebellum of the lizard *Varanus exanthematicus* will be limited to a comparison with the results obtained in the turtles *Pseudemys scripta elegans* and *Testudo hermanni*. In figure 24, case 6145, representing the series of HRP injections into the cerebellum of five lizards is shown. Figure 25 shows the results obtained from a FB injection into the cerebellum of a lizard (case 6219). In case 6145 two HRP injections were made into the lateral part of the cerebellum (Fig. 24C). Some spread of the enzyme occurred to the region of the deep cerebellar nuclei, the cerebellar peduncle and the most rostral part of the vestibular nuclear complex (Fig. 24D). As regards the extent of the HRP injections, this case is comparable to the experiment on the turtle *Pseudemys scripta elegans* shown in figures 20 and 21. In case 6219 one injection of FB was placed in the lobus auricularis or flocculus (Larsell, '67) of the cerebellum (Fig. 25D). The tip of the injection needle also reached a very small part of the dorsal brainstem nearby the cochlear nuclei (Fig. 25E).

As in the turtles studied, the number of labeled neurons observed in the *prosencephalon* was limited. In the telencephalon no labeled neurons were present. In the diencephalon only a few labeled neurons were observed in the dorsal hypothalamus in regions comparable to those found in turtles (not illustrated). After FB injections no labeled neurons were observed in the diencephalon.

In the *mesencephalon* labeled neurons were present in the ipsilateral and contralateral nucleus of the basal optic root (Figs. 24A, B; 25A, B; 31A), the ipsilateral and contralateral interstitial nucleus of the flm (Figs. 24A, B; 25A), the nucleus of the flm (Figs. 24A, B; 25A, B), and the tegmentum mesencephali. Some labeled cells were also present in the contralateral nucleus ruber (Figs. 24B; 25B).

In the *rhombencephalon*, as in the turtles studied, labeled neurons were present in the contralateral deep cerebellar nuclei (Figs. 24D; 25C, D), the vestibular nuclear complex, the perihypoglossal nuclear complex, the inferior olive, the descending nucleus of the trigeminal nerve (Figs. 24G-J; 25 F-L), the nucleus of the solitary tract and the reticular formation.

In the vestibular nuclear complex labeled cells were present in the nucleus vestibularis dorsolateralis (Figs. 24E; 25D, E), the ipsilateral and contralateral nucleus

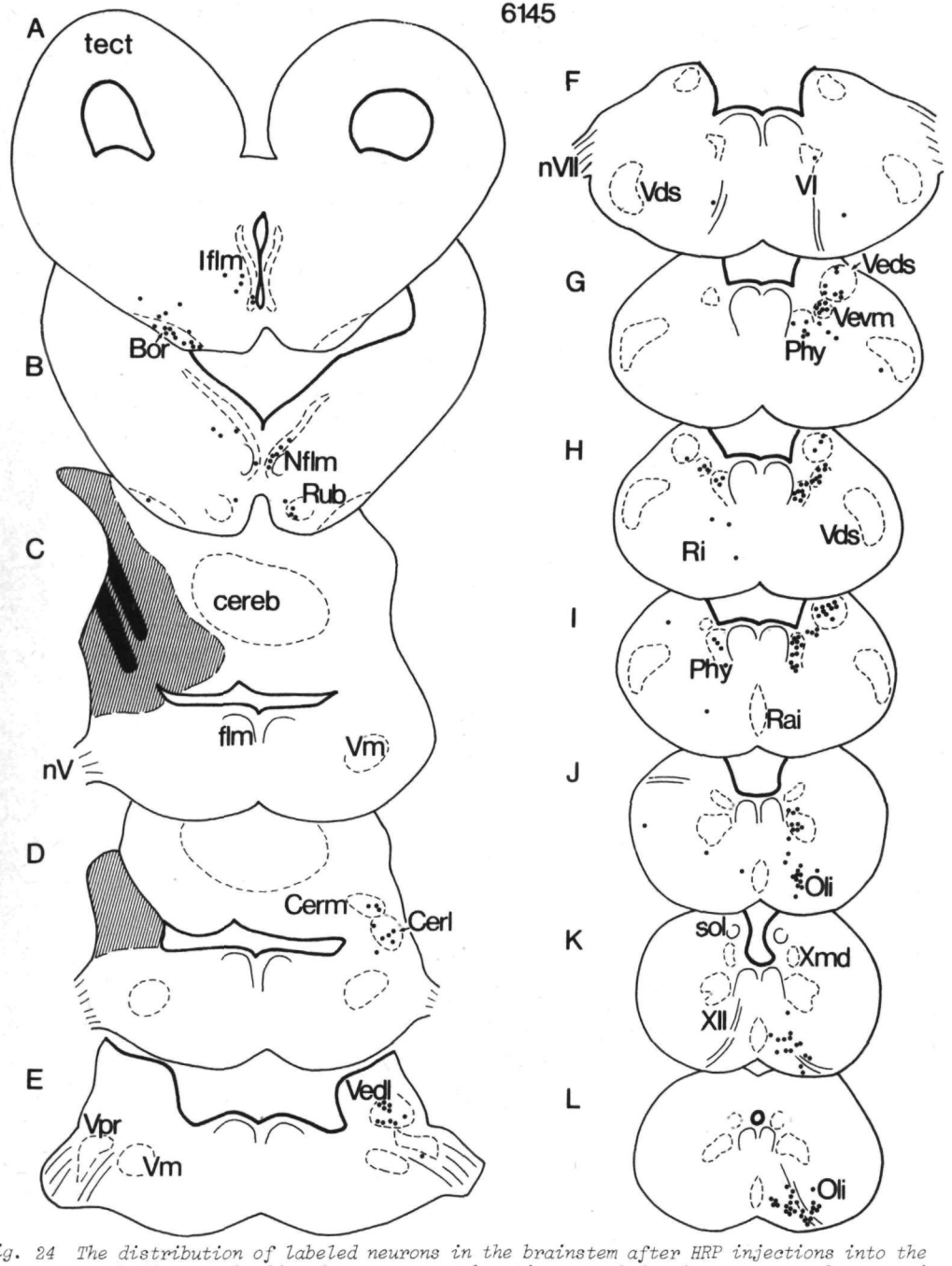


Fig. 24 The distribution of labeled neurons in the brainstem after HRP injections into the cerebellum of the lizard Varanus exanthematicus. Each level represents the composite of the plots of two sections. For abbreviations cf. pages 28-29.

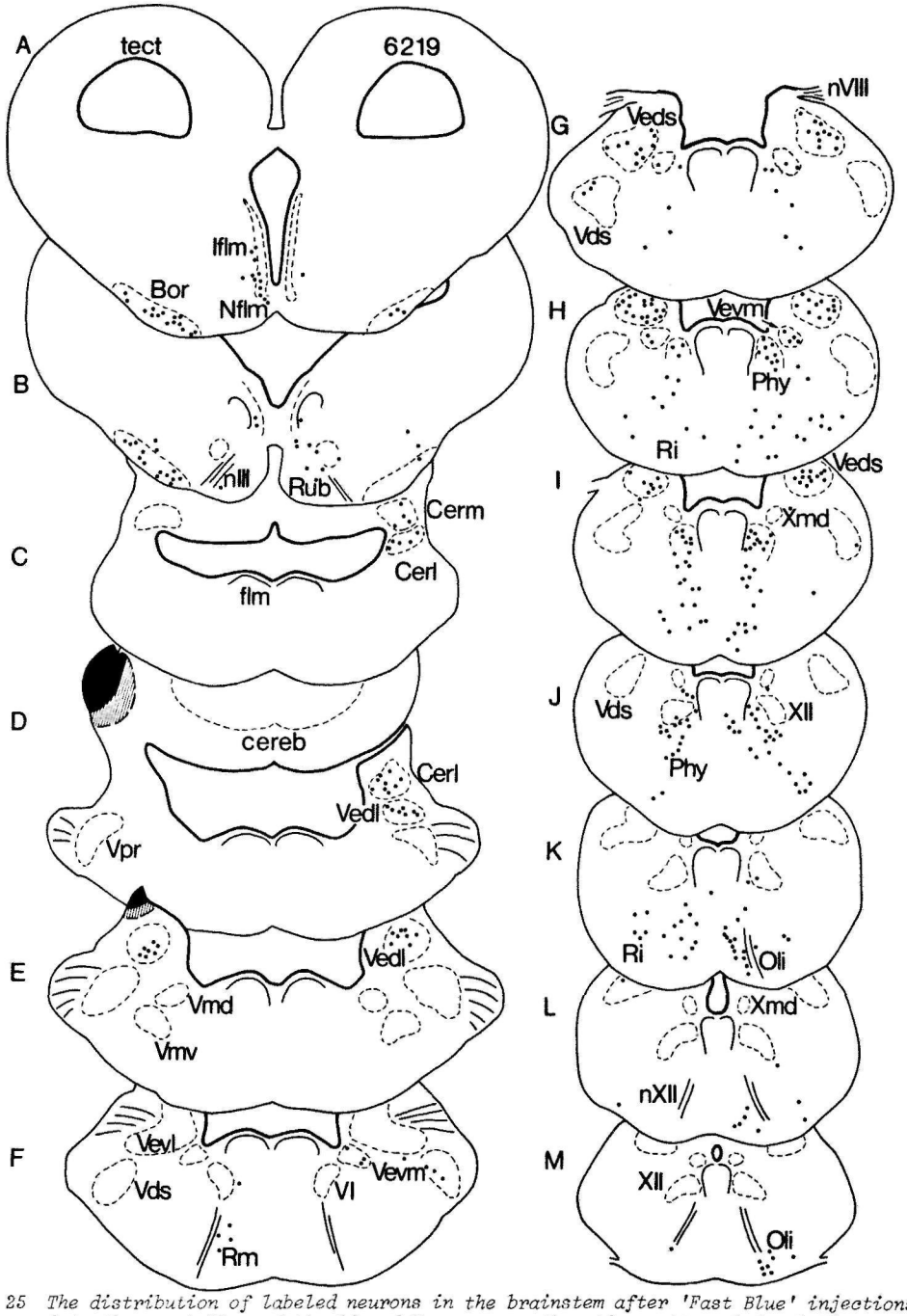


Fig. 25 The distribution of labeled neurons in the brainstem after 'Fast Blue' injections into the cerebellum of the lizard Varanus exanthematicus. At each level the neurons found in one section are plotted. For abbreviations cf. pages 28-29.

vestibularis ventromedialis (Figs. 24G-I, 25 F-H), and bilaterally in the nucleus vestibularis descendens (Figs 24G-I, 25G-I).

An extensive bilateral projection to the cerebellum was observed to arise in the so-called perihypoglossal nuclear complex (Figs. 24G-J, 25H-J), previously termed nucleus parvocellularis medialis by Ebbesson ('67).

In the caudal brainstem a distinct group of labeled neurons contralateral to the injection site was observed (Figs. 24J-L; 25K-M, 32C). In case 6219 relatively less fluorescent neurons appeared to be present in this region, but in all experiments a cell mass contralateral to the injection site was present. In Nissl series of *Varanus exanthematicus* no separate cell mass is discernable in the reticular formation at this level of the brainstem. It seems likely that this labeled cell mass represents the inferior olive of the lizard *Varanus exanthematicus*.

Injection of the anterogradely transported tracer ^{3}H-leucine into the caudal part of the inferior reticular formation resulted in labeled terminals located in the molecular layer of the cerebellum of *Varanus exanthematicus* (Wolters, Dederen, and ten Donkelaar, unpublished observations). In three cases the tracer WGA-HRP was injected into the vicinity of the inferior olive. As shown in figure 26 anterogradely labeled fibers were found in the molecular layer of the cerebellum. These fibers, at some places clearly showing an arborizing axon (Fig. 26C), appeared to be present at certain places of the cerebellar cortex (Fig 26A). Topological reconstruction of the observed labeled fibers, basically following the procedure described in Chapter IIID, revealed a distinct longitudinally oriented pattern of labeled fibers (Fig. 26B). These observations not only confirm the existence of an olivocerebellar climbing fiber projection in *Varanus exanthematicus* but also suggest that this projection is organized in longitudinally oriented zones

No evidence was found for the existence of other precerebellar nuclei such as the pontine nuclei and the tegmental and lateral reticular nuclei. In case 6219 (Fig. 25I, J) a large number of fluorescent neurons was observed bilaterally in the reticular formation, ventrally to the perihypoglossal nuclear complex and laterally to the fasciculus predorsalis. In mammals this part of the reticular formation is designated as the paramedian reticular nucleus and known to have a bilateral projection to the cerebellum (see, e g., Brown-Gould,'80, Brodal, '81) These results might indicate the existence of a precerebellar paramedian reticular nucleus in the lizard *Varanus exanthematicus*

As regards the somatosensory projection to the cerebellum, labeled neurons were observed, although in a restricted number, bilaterally in the descending nucleus of the trigeminal nerve (Fig 25F-L). In this series of experiments, however, no labeled neurons were observed in the dorsal funicular nucleus, nor in the so-called nucleus cuneatus externus as distinguished in the lizard *Tupinambis nigropunctatus* by Ebbesson ('67). In the nucleus of the solitary tract only a few labeled cells were observed in this series of experiments (not illustrated).

As regards the projection of the *spinal cord* to the cerebellum of the lizards studied the following results were obtained. Compared with the case of *Pseudemys scripta elegans* shown in figure 23 less labeled neurons appeared to be present in the spinal cord of the lizard *Varanus exanthematicus* after injection of HRP into the cerebellum itself. Nevertheless, labeled

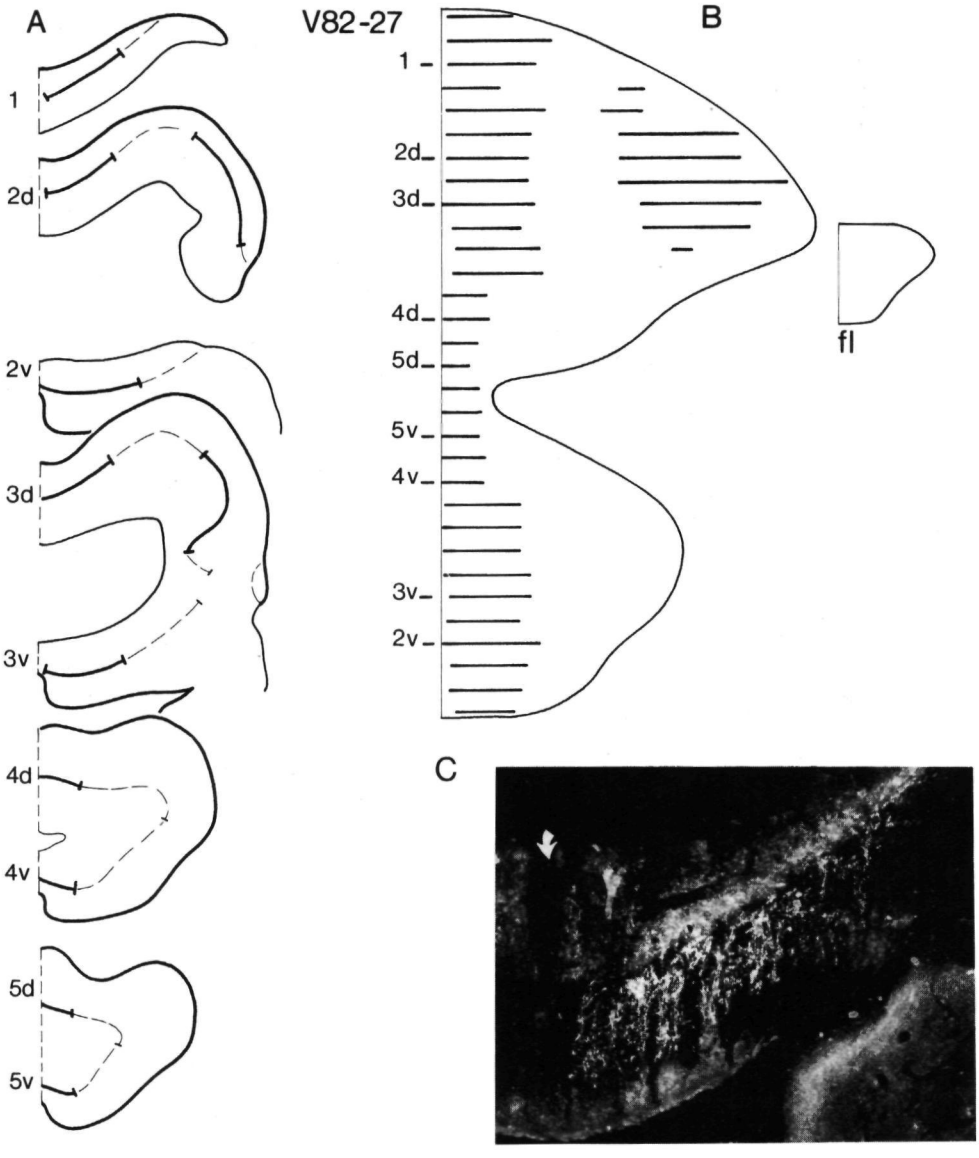


Fig. 26 The distribution of anterogradely labeled fibers and terminal structures after WGA-HRP injections into the caudal brainstem, including the inferior olive, of the lizard Varanus exanthematicus. A, Transverse sections of the cerebellum, the bold lines indicate the area of termination of olivocerebellar fibers; B, A chart in which the termination fields of olivocerebellar fibers are indicated. c, caudal; r, rostral; fl, flocculus; C, Dark-field photomicrograph, showing the labeled fibers and terminal structures in the molecular layer, the arrow indicates the midline (section 1 of A), TMB incubation, ×80.

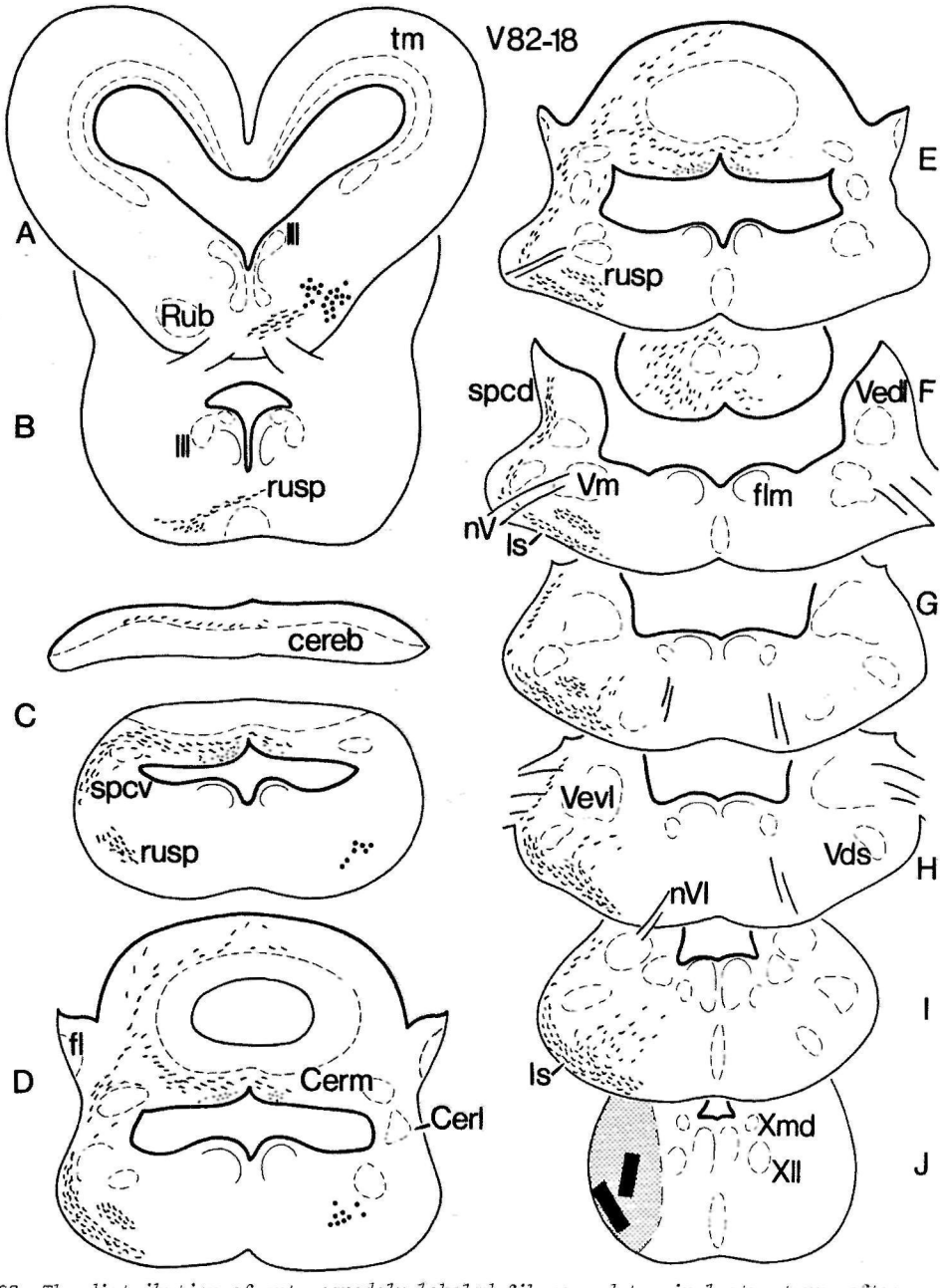


Fig. 27 The distribution of anterogradely labeled fibers and terminal structures after implantation of HRP slow-release gels into the spinal lemniscus of the lizard Varanus exanthematicus. Broken lines indicate anterogradely labeled fibers (and the retrogradely labeled rubrospinal tract), small dots indicate labeled terminal structures, large dots retrogradely labeled neurons. For abbreviations cf. pages 28-29.

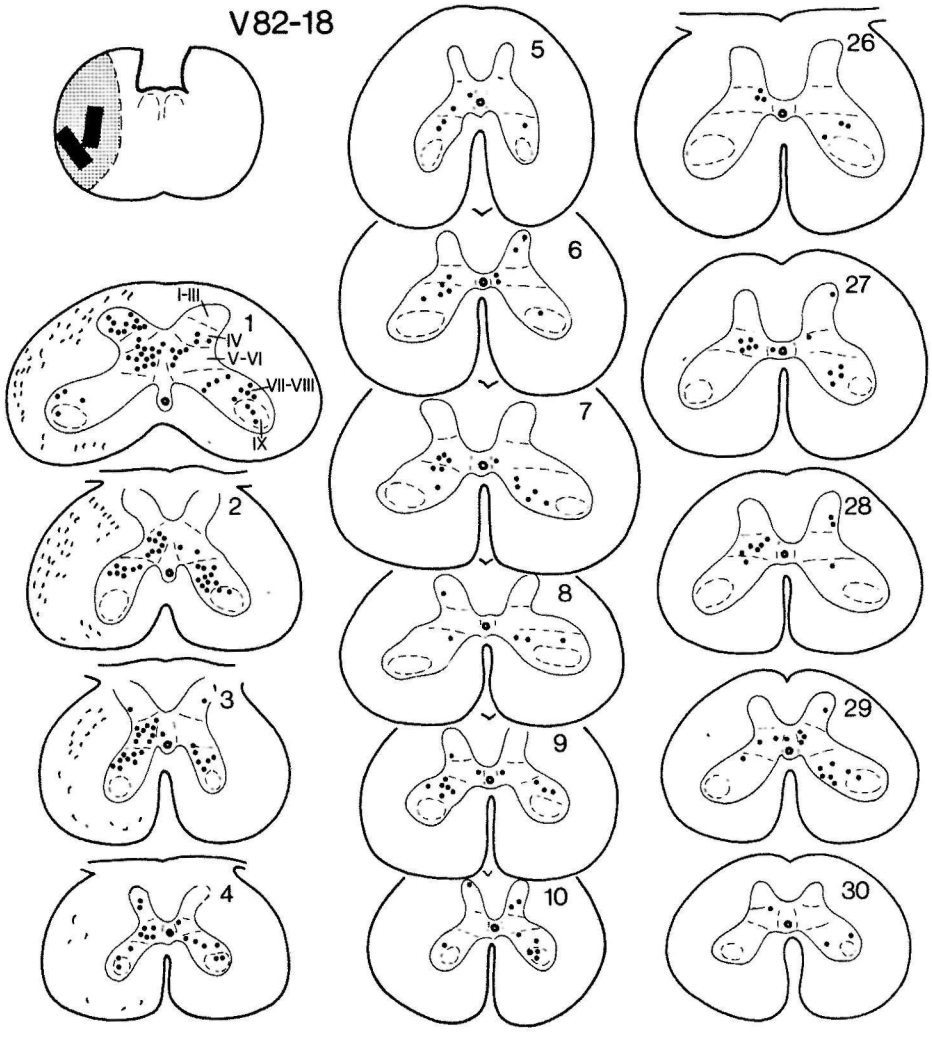


Fig. 28 The distribution of labeled neurons (mainly spinocerebellar tract cells) in cervical (1-4; 5-10) and lumbar (26-30) segments of the spinal cord after implantation of HRP slow-release gels into the spinal lemniscus of the lizard Varanus exanthematicus. Each level represents the composite of the plots of ten consecutive sections.

neurons were observed throughout the spinal cord in cervical, thoracic, and lumbar segments As in the turtle *Pseudemys scripta elegans* most of these neurons were located in area VII-VIII of the spinal gray. In the cervical intumescence the labeled neurons were more or less evenly distributed between the ipsilateral and contralateral side of the spinal cord. The total number of labeled neurons observed in the thoracic and lumbar segments in these cases was too limited to discern a particular distribution between the ipsilateral and contralateral side After implantation of two HRP slow-release gels into the spinal lemniscus in the caudal brainstem of *Varanus exanthematicus* labeled neurons were found in the spinal cord in a comparable distribution as found in the turtle *Pseudemys scripta elegans* (case V 82-18, Figs. 27, 28) It should be noted that in this particular experiment labeled cells in the spinal cord could be partly due to damage of spinoreticular and spinothalamic fibers However, as shown in figure 27, the dorsal and ventral spinocerebellar tracts, both arising from the spinal lemniscus, are extensively labeled anterogradely Therefore it seems likely that a large part of the labeled cells in the spinal cord are cells of origin of spinocerebellar fibers. As in *Pseudemys scripta elegans* spinocerebellar tract neurons were present mainly in area VII-VIII of the spinal gray. In addition, however, distinct groups of labeled cells were found, ipsilaterally, in area V-VI (segments 1-4, 26-29). In mammals spinal cord cells giving rise to uncrossed cerebellar projections were found in comparable areas (Matsushita and Hosoya, '79, Matsushita *et al.*, '79; Grant *et al.*, '82). The course of the dorsal and ventral spinocerebellar tracts in the brainstem as well as their entrance into the cerebellar peduncle could be clearly followed in this experiment (Fig. 27). As demonstrated in degeneration experiments the dorsal tract enters the cerebellum at a more caudal level (Fig. 27D, E) compared with the ventral spinocerebellar tract (Fig. 27C). In the cerebellar granular layer the spinocerebellar fibers could be traced, mainly ipsilaterally, over about two-thirds of the total length of the cerebellum. In the most distal part no spinocerebellar fibers were observed. Comparable data were found in a recent study in *Pseudemys scripta elegans* (Künzle and Woodson, '82) in which HRP was used as an anterograde tracer.

CEREBELLAR AFFERENTS IN THE SNAKE PYTHON REGIUS

Finally, the results obtained after implantation of an HRP slow-release gel into the cerebellum of the snake *Python regius* will be discussed.

In case 6217 (Fig. 29), the HRP gel was implanted into the caudal part of the cerebellum and the underlying dorsal part of the brainstem (Fig. 29E, F). The caudal part of the cerebellum as well as the nucleus vestibularis dorsolateralis, the nucleus vestibularis ventrolateralis, and their immediate surroundings were damaged by the implantation of the gel. Because of extensive reaction of the enzyme both after the TMB and DAB incubation procedures, it was not possible to discern the HRP labeled cells individually at the ipsilateral side of the brainstem adjacent to the implantation site.

In the *prosencephalon* no labeled neurons were observed while in the *mesencephalon* only the interstitial nucleus of the fim and the adjacent tegmentum mesencephali contained some labeled neurons (Fig. 29A, B). Unlike the results obtained in the other reptilian species

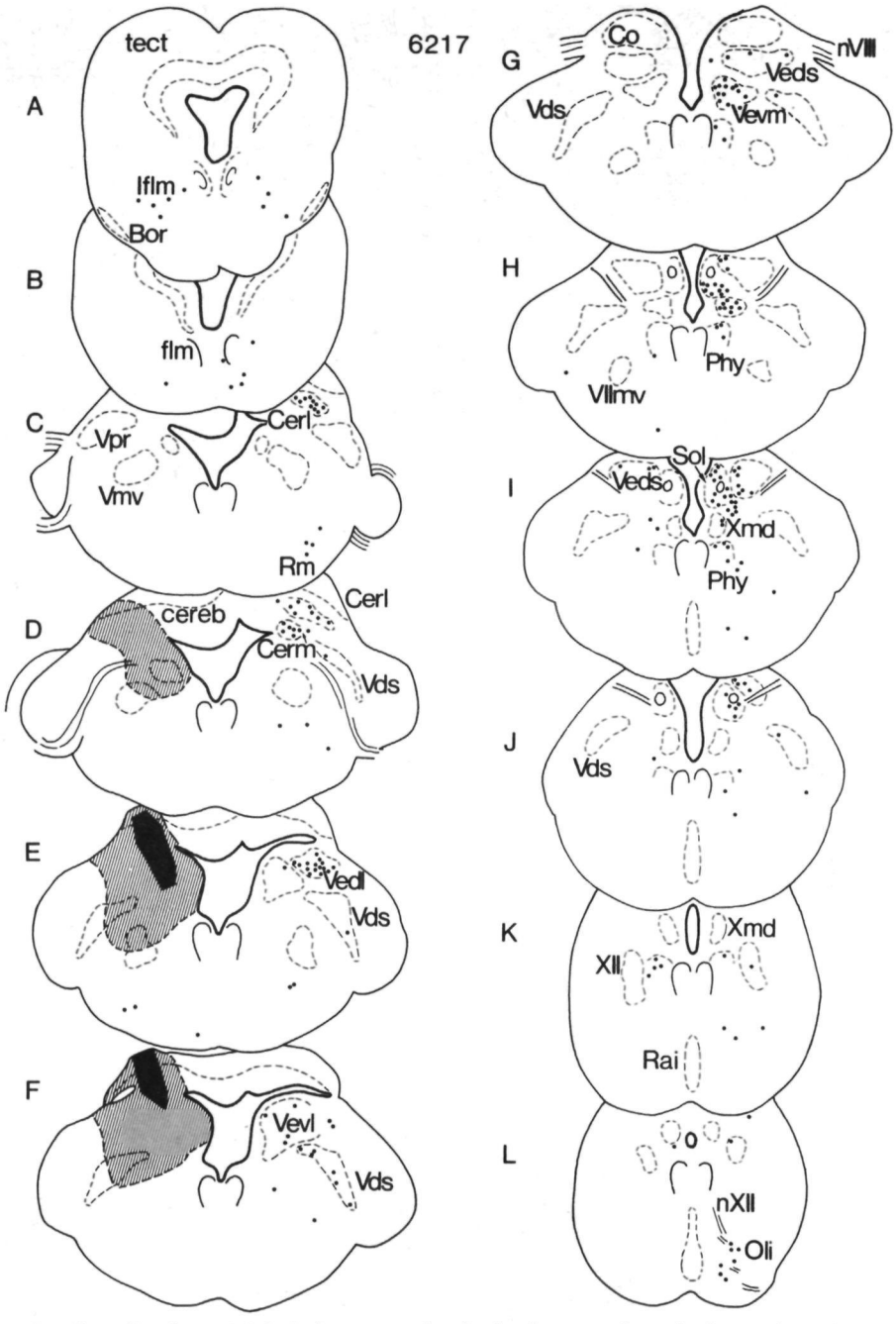


Fig. 29 The distribution of labeled neurons in the brainstem after implantation of an HRP slow-release gel into the cerebellum of the snake Python regius. At each level the labeled neurons found in one section are plotted. For abbreviations cf. pages 28-29.

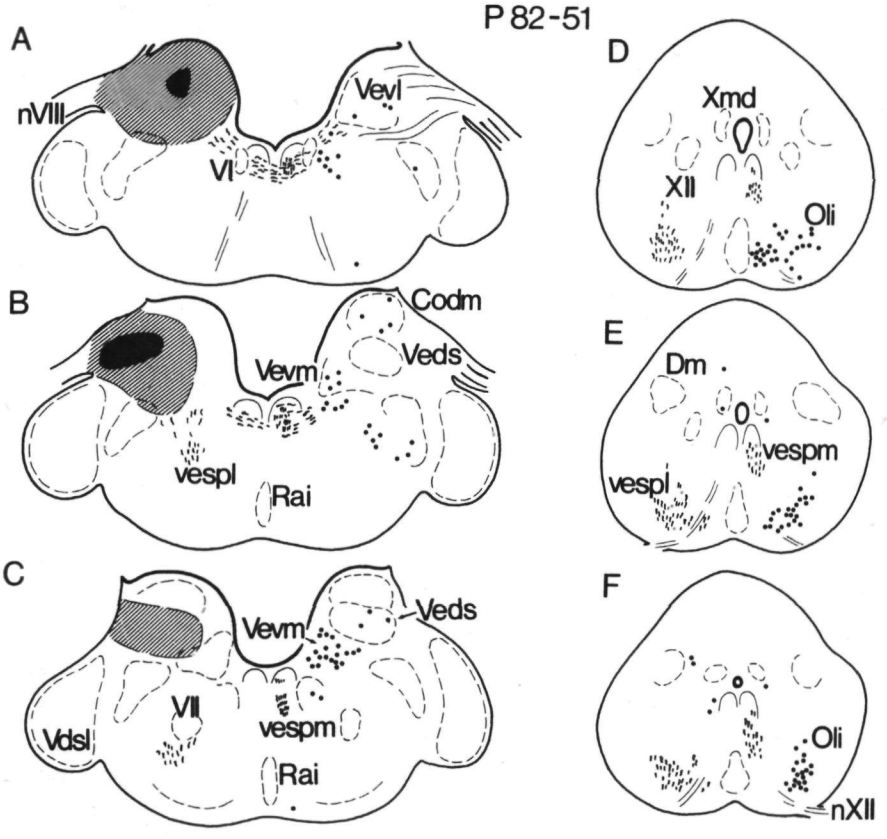


Fig. 30 The distribution of labeled neurons in the inferior olive after implantation of an HRP slow-release gel into the vestibular nuclear complex of the snake Python regius, also interrupting the olivocerebellar pathway. At each level the labeled neurons found in one section are plotted. For abbreviations cf. pages 28 - 29.

studied no labeled neurons were observed so far in the nucleus of the basal optic root (Fig. 29A).

In the *rhombencephalon* the greatest number of labeled neurons was observed. The contralateral nucleus cerebellaris medialis and nucleus cerebellaris lateralis contained labeled neurons (Fig. 29C, D). In the vestibular nuclear complex labeled neurons were observed in the nucleus vestibularis dorsolateralis (Fig. 29E), the nucleus vestibularis ventrolateralis (Fig. 29E, F), the nucleus vestibularis ventromedialis (Fig. 29G, H) and the nucleus vestibularis descendens (Fig. 29G-J). Part of these cells are probably labeled due to the presence of commissural connections between the vestibular nuclei.

Like in the turtles *Pseudemys scripta elegans* and *Testudo hermanni* and in the lizard *Varanus exanthematicus* labeled neurons were observed bilaterally in the cell mass adjacent to the flm between the nucleus of the nervus abducens and the nucleus of the hypoglossal nerve (Fig. 29G-K). Previously this cell mass was termed the nucleus parvocellularis medialis in the boa *Constrictor constrictor* by Ebbesson ('69) but, for reasons mentioned before, in the present study this area was called the perihypoglossal nuclear complex.

In the caudal part of the brainstem of the snake *Python regius* a small contralaterally labeled cell mass adjacent to the nucleus raphes inferior at the level of the hypoglossal nerve was observed (Fig. 29L). Since this labeled cell mass resembles in its location the contralaterally labeled cell mass in the caudal brainstem of the turtles *Pseudemys scripta elegans* and *Testudo hermanni* and of the lizard *Varanus exanthematicus* this nucleus has been termed oliva inferior.

As regards somatosensory afferent connections to the cerebellum in the snake, labeled neurons were observed throughout the whole length of the descending nucleus of the trigeminal nerve. No labeled neurons were observed in the nucleus funiculi dorsalis.

Compared with the data obtained in the other reptilian species studied a great number of labeled neurons was observed in the nucleus of the solitary tract (Fig. 29H-J). In the reticular formation labeled neurons were observed scattered throughout the medial and inferior cell masses (Fig. 29C-K).

In the present series of experiments no cells of origin of spinocerebellar fibers were observed in the spinal cord of *Python regius*.

In figure 30 part of an experiment (case P82-51) is shown in which an HRP slow-release gel was implanted into the vestibular nuclear complex at the level of the eight nerve (Fig. 30A, B). Spread of HRP occurred to the middle part of the vestibular nuclear complex including mainly the ventrolateral, ventromedial, and descending vestibular nuclei. The implantation site of the HRP slow-release gel renders it probable that in addition to connections with the vestibular nuclear complex, e.g. corticovestibular projections (Chapter V), also some afferent pathways to the cerebellum were interrupted. In particular the olivocerebellar pathway appeared to be damaged, resulting in extensive labeling of the inferior olive in the contralateral caudal brainstem (Figs. 30D-F, 32D).

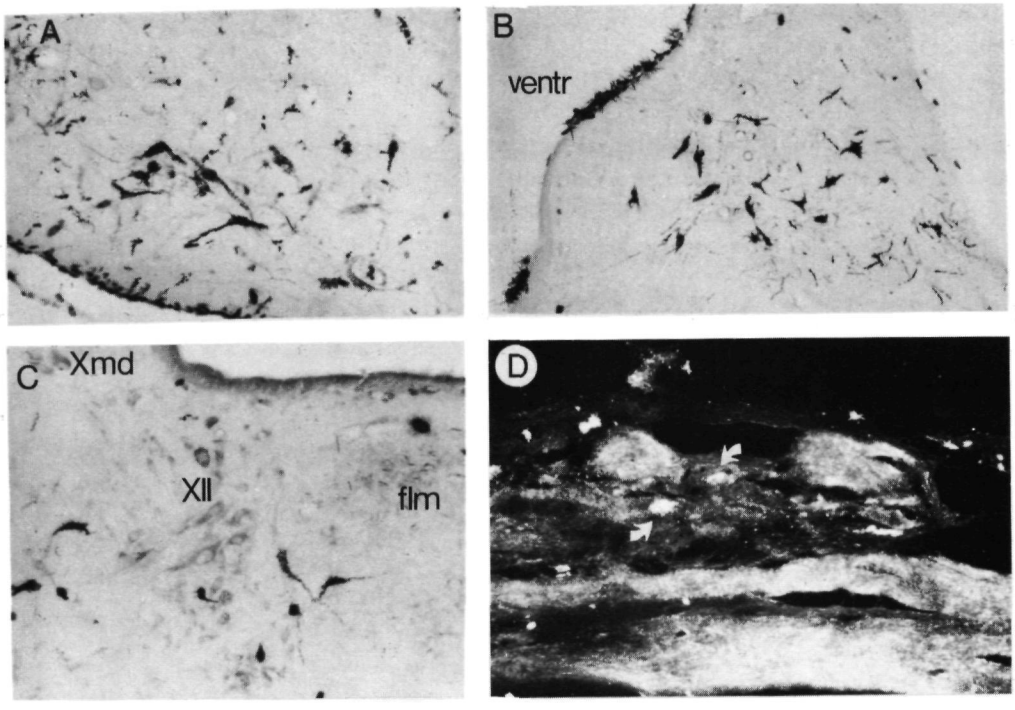


Fig. 31 Retrogradely labeled neurons after HRP injections (A-C, TMB incubation) or WGA-HRP injections (D, TMB incubation) into the cerebellum of the lizard Varanus exanthematicus (A) and the turtle Pseudemys scripta elegans (B-D) in the following cell masses: A, The nucleus of the basal optic root, ×105; B, The nucleus vestibularis descendens, ×80; C, The perihypoglossal nuclear complex, ×115; D, Spinal ganglion cells (arrows), ×80.

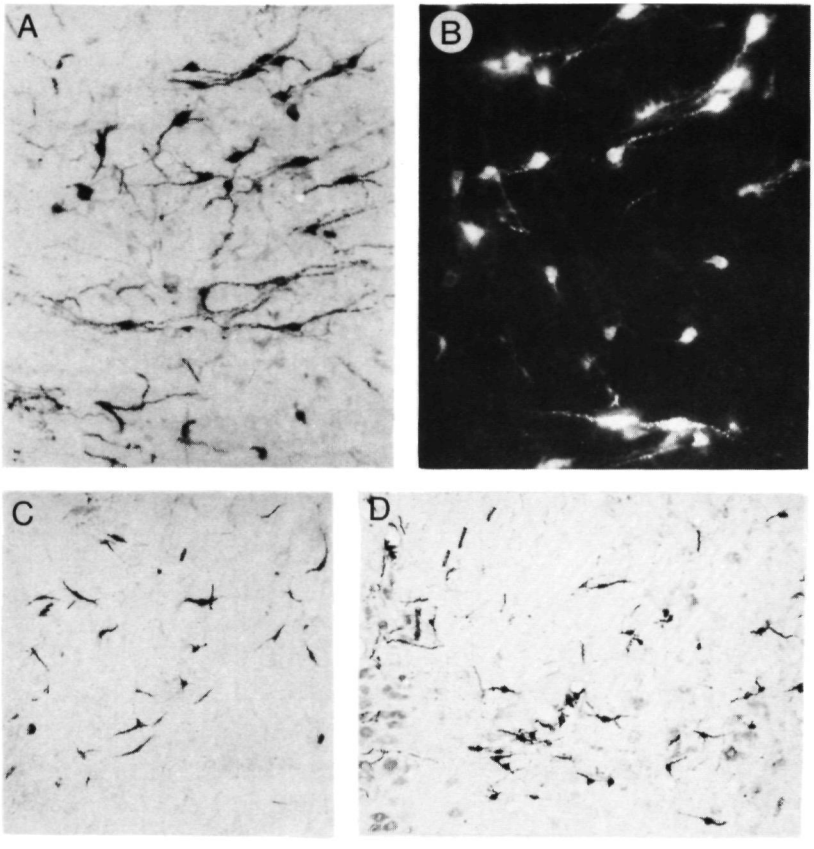


Fig. 32 Retrogradely labeled neurons in the inferior olive after application of HRP (A, C, D, TMB incubation) or FB (B) to the cerebellum of the turtle Pseudemys scripta elegans (A, ×112; B, ×150), and the lizard Varanus exanthematicus (C, ×80), and the snake Python regius (D, case P82-51, Fig. 30, ×80).

The immunohistochemical localization of some monoaminergic as well as peptidergic substances in the brainstem and spinal cord was studied in the lizard *Varanus exanthematicus* (Wolters *et al.*, '82b, '83 a, b).

With the indirect immunofluorescence technique (Steinbusch *et al.*, '78, '82) the presence of serotonin in *Varanus exanthematicus* was demonstrated (Wolters *et al.*, '82b, '83a). In the cerebellum of *Varanus exanthematicus* a distinct afferent serotonin-containing fiber system appeared to be present (Fig. 33). Most serotonin-containing fibers and terminals were found in the rostral and middle parts of the cerebellar granular layer, whereas the number of serotonergic fibers decreased in the caudal part of the cerebellum. Serotonin-containing neurons appeared to be located in several cell masses of the brainstem, including the nucleus raphes inferior (Wolters *et al.*, '83a). In HRP experiments a restricted projection of the nucleus raphes inferior appeared to be present in reptiles In the turtle *Chrysemys picta* serotonergic neurons were found scattered within the raphe region of the entire rhombencephalon, but mainly in the nucleus raphes inferior (Parent, '73, '79).

As noted before a cerebellar projection of the locus coeruleus was found in HRP studies in turtles (Schwarz and Schwarz, '80; the present study, Fig. 20G). In mammals the locus coeruleus is known to give rise to a catecholaminergic (i.e. noradrenergic) projection to the cerebellum. In several turtle species the locus coeruleus was demonstrated to contain catecholamines (Parent and Poirier, '71; Parent, '73, '79; Yamamoto *et al.*, '77). In *Varanus exanthematicus* the presence of catecholamines was determined by an indirect technique, viz., the demonstration of tyrosine hydroxylase (TH), present in catecholamine-containing neurons and fibers (Wolters *et al.*, '82b, '83a). However, practically no TH-immunoreactivity was found in the cerebellum of *Varanus exanthematicus*.

Apart from a monoaminergic afferent cerebellar projection, peptidergic projections to the cerebellum are found in reptiles. With immunohistochemical techniques (Wolters *et al.*, '83b) mossy fibers and terminals containing substance P-like immunoreactivity were observed in the cerebellum of the lizard *Varanus exanthematicus*, especially in the rostrolateral part. Korte *et al.* ('80) demonstrated similar fibers and terminals in the cerebellum of the turtle *Chrysemys scripta elegans* entering the cerebellum from the tegmentum at the level of the isthmic nuclei in a distinct bundle of axons. In the lizard *Varanus exanthematicus* substance P-containing neurons were observed in the nucleus periventricularis hypothalami, the adjacent lateral hypothalamic area, the nucleus of the flm, and some in the nucleus raphes superior (Wolters *et al.*, '83b). The nucleus of the flm could be a source of origin of substance P-containing afferents of the cerebellum, since this nucleus was labeled distinctly in HRP and FB studies both in *Pseudemys scripta elegans* and *Varanus exanthematicus* (Figs. 20D, 24A, B, 25A, B). Other possible sites of origin might be e.g. the descending trigeminal nucleus, the raphe nuclei, and the perihypoglossal nuclear complex, which nuclei all send axons to the cerebellum. Immunohistochemical studies in the rat (Cuello and Kanazawa, '78, Ljungdahl *et al.*, '78) showed substance P-positive cells in these areas.

A very sparse distribution of leu- and metenkephalin-containing terminals was found

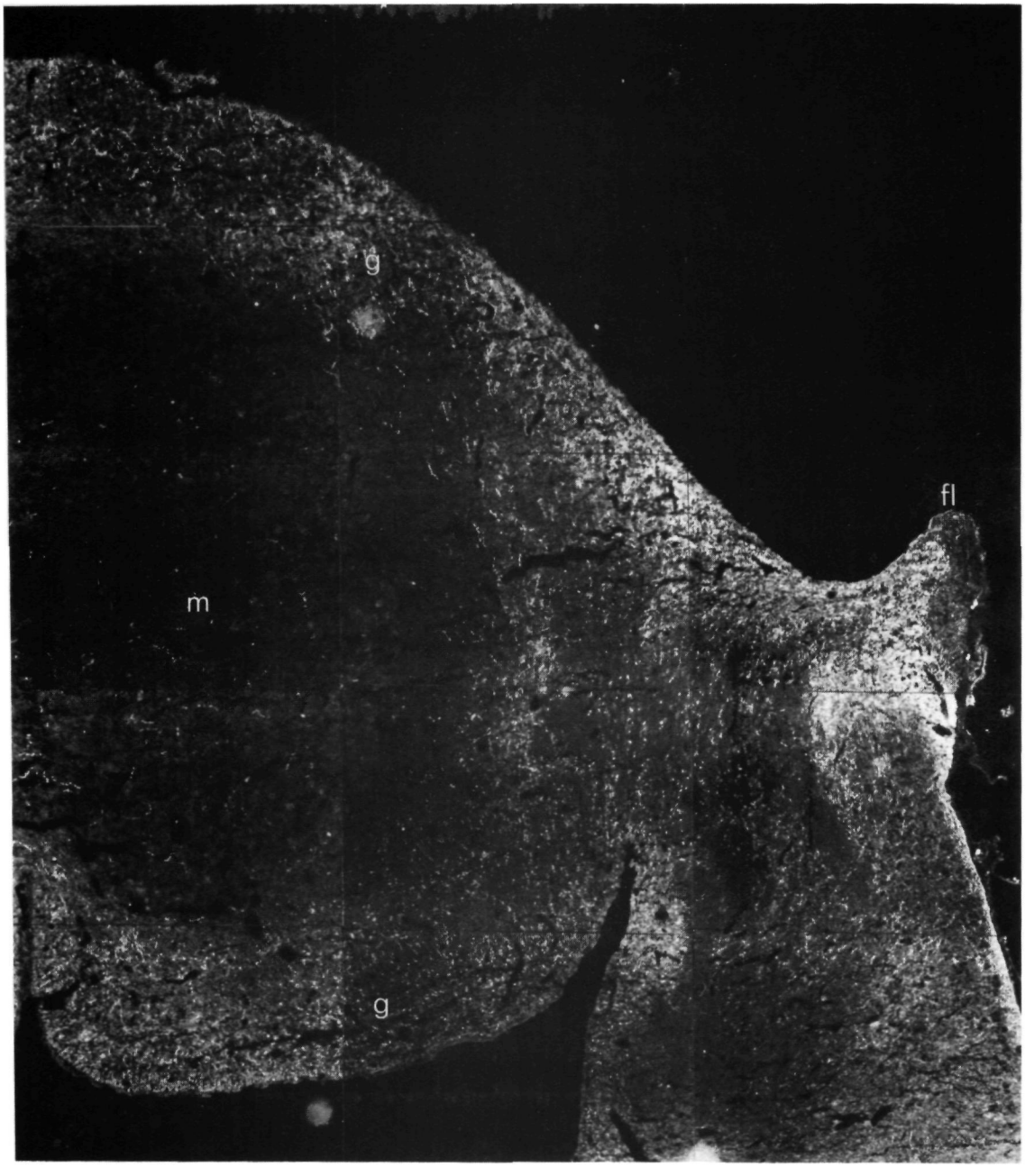


Fig. 33 The serotonergic innervation of the cerebellum in Varanus exanthematicus. fl, flocculus; g, granular layer; m, molecular layer, ×56.

DISCUSSION

In this part of the present study the cells of origin of afferent projections to the cerebellum have been demonstrated with retrograde tracer techniques in the turtles *Pseudemys scripta elegans* and *Testudo hermanni*, the lizard *Varanus exanthematicus*, and the snake *Lytorhynchus diadema*. The results obtained with the HRP technique and with the retrograde fluorescent tracer 'Fast Blue' were largely comparable, although with FB in many instances more labeled neurons were observed than with HRP. Furthermore, it should be noted that particularly after HRP injections into or directly above the cerebellar peduncle the widest distribution of labeled cells was observed. A disadvantage in these cases, however, is the spread of the tracers to the deep cerebellar nuclei, to the locus coeruleus and to the parabrachial region. Therefore, it seems likely that the presence of labeled cells in the red nucleus is due to spread of HRP to the lateral cerebellar nucleus, since anterograde degeneration (ten Donkelaar, '76b) and anterograde tracer studies (ten Donkelaar and Dederen, unpublished observations) in lizards revealed a projection of the red nucleus to the lateral cerebellar nucleus.

The results obtained in the various reptilian species studied will now be discussed for each division of the central nervous system separately.

1. Cerebellar afferents from the prosencephalon

Cerebellar afferents from the prosencephalon in the reptiles studied seem to be rather limited. In the telencephalon no labeled neurons were observed in any of the experiments. A striocerebellar pathway to the ipsilateral nucleus cerebellaris lateralis as reported by Hoogland ('77) and Voneida and Sligar ('79) in the lizard *Tupinambis nigropunctatus* could not be confirmed although in several cases spread of the HRP to the nucleus cerebellaris lateralis occurred. Implantations of HRP slow-release gels into the cerebellar peduncle of both *Pseudemys scripta elegans* and *Varanus exanthematicus*, including the cerebellar nuclei (Chapter V), never resulted in retrogradely labeled neurons in the striatum. However, in one case in *Varanus exanthematicus* the HRP slow-release gel not only reached both cerebellar nuclei but also the parabrachial isthmic region (see Chapter V, Fig. 46). In this particular case a large number of labeled neurons was observed in the ipsilateral ventral striatum. These results might indicate a striatal projection to the parabrachial isthmic region, in keeping with previous data of Reiner ('79) in the turtle *Chrysemys picta*. In the diencephalon in the turtle *Pseudemys scripta elegans* a few labeled neurons were observed in the preoptic region, the nucleus periventricularis hypothalami and in the dorsal hypothalamic area, in the latter areas also in the lizard *Varanus exanthematicus* a few labeled neurons were observed. As noted before, these labeled neurons could well be due to spread of HRP to the locus coeruleus.

Both in the turtle *Pseudemys scripta elegans* and the lizard *Varanus exanthematicus* in some cases labeled neurons were observed in the ipsilateral nucleus geniculatus pretectalis, in keeping with results obtained in the turtles *Chrysemys picta picta* by Reiner and Karten ('78) and *Pseudemys scripta elegans* (Kunzle, '83). Comparable projections to the cerebellum have been

demonstrated in the pigeon (Clarke, '77; Brecha *et al.*, '80) and the duck (Arends and Blok, '83) arising in the nucleus lentiformis mesencephali.

The ways by which the reptilian telencephalon might influence the cerebellum are still unclear. Possible relay stations are present in the rostral mesencephalon, as aforementioned in the parabrachial region, and in the caudal brainstem. In *Caiman crocodilus* two nuclei in the vicinity of the posterior commissure, i.e. the nucleus circularis and the interstitial nucleus of the posterior commissure, were found to project to the cerebellum (Brauth *et al.*, '78). These authors noted the similarity of the nucleus circularis to the avian nucleus spiriformis medialis (see Karten and Finger, '76), since both project to the cerebellum. In teleosts (see Karten and Finger, '76; Ito *et al.*, '82) also an indirect striocerebellar pathway was found, with a relay in the nucleus paracommissuralis. In *Varanus exanthematicus* HRP injections into the caudal brainstem (including the inferior olive) showed retrogradely labeled neurons in the striatum (ten Donkelaar and de Boer-van Huizen, '81a), which might indicate a projection from the telencephalon to the inferior olive.

2. Cerebellar afferents from the mesencephalon

In the mesencephalon the most prominent afferent projection to the cerebellum was found to arise, both in the turtles and in the lizard, in the nucleus of the basal optic root, or nucleus opticus tegmenti. These findings are in keeping with results of Reiner and Karten ('78) in *Chrysemys picta picta*, Schwarz and Schwarz ('80), and Künzle ('83; *Pseudemys scripta elegans*). Although this projection could not be demonstrated so far in *Python regius* it seems likely that such a bisynaptic retinal input to the cerebellum is common to most reptilian species.

In the reptiles studied labeled neurons were also scattered throughout the tegmentum mesencephali, including the interstitial nucleus of the flm and the nucleus of the flm as distinguished by Tuge ('32). The interstitial nucleus of the flm is known to project to the spinal cord by way of the flm (Robinson, '69; ten Donkelaar, '76a, b; ten Donkelaar *et al.*, '80; Cruce and Newman, '81; Wolters *et al.*, '82a) and the present study suggests another major projection of this magnocellular nucleus, i.e. to the cerebellum. In the opossum (Henkel and Martin, '77) and the cat (Pompeiano and Walberg, '57) the interstitial nucleus of the flm is known to project to the ipsilateral ventromedial vestibular nucleus. HRP injections and HRP slow-release gel implantations into the vestibular nuclear complex of *Pseudemys scripta elegans* revealed a distinct projection of the interstitial nucleus of the flm to the ipsilateral vestibular nuclear complex, especially the ventromedial vestibular nucleus (Bangma and ten Donkelaar, '83). Similar results were obtained in *Varanus exanthematicus* (ten Donkelaar and de Boer-van Huizen, in preparation). The observed projection of the so-called nucleus of the flm to the cerebellum was confirmed by recent results of Künzle ('83) in *Pseudemys scripta elegans*. This nucleus might be a source of origin of substance P-containing afferents of the cerebellum (Wolters *et al.*, '83b; this Chapter).

A tectocerebellar pathway, as suggested by Weston ('36) based on the study of normal material in various reptiles, could not be confirmed in the present study.

3. Cerebellar afferents from the rhombencephalon

The greatest number of cerebellar afferent sources was observed in the rhombencephalon, including the vestibular nuclear complex, the oliva inferior and various other structures, i.e. the perihypoglossal nuclear complex, somatosensory nuclei, the nucleus of the solitary tract, the locus coeruleus, the parabrachial nucleus and throughout the reticular formation.

(a) Vestibular afferents. Both primary and secondary vestibulocerebellar projections were demonstrated. In the turtle *Pseudemys scripta elegans* vestibular ganglion cells were found to project to the ipsilateral half of the cerebellum, in keeping with data of Leake ('74) who reported terminal degeneration in the granular layer of the cerebellum of *Caiman crocodilus* after lesion of the intra-otic and intracranial vestibular ganglion cells. Extensive secondary vestibulocerebellar projections were demonstrated to originate bilaterally in the dorsolateral, ventromedial, and descending vestibular nuclei in the turtles *Pseudemys scripta elegans* and *Testudo hermanni* and in the lizard *Varanus exanthematicus*. Although part of the contralaterally labeled neurons in the dorsolateral, ventromedial, and descending vestibular nuclei of the snake *Python regius* could be due to commissural connections between the vestibular nuclei, it seems likely that also in the snake *Python regius* a projection of the vestibular nuclear complex exists comparable to the projection observed in the other reptiles studied. The results of the present series of experiments concerning secondary vestibulocerebellar projections are in agreement with the findings of Schwarz and Schwarz ('80) in various turtle species and Künzle ('83) in *Pseudemys scripta elegans*.

(b) Precerebellar nuclei. The term precerebellar nuclei refers, as mentioned before, to nuclei that send most of their efferent fibers to the cerebellum (Brodal, '81). In mammals these include the pontine nuclei (absent in reptiles), the tegmental reticular nucleus, the lateral reticular nucleus or nucleus funiculi lateralis, the paramedian reticular nucleus and the inferior olive. In the present study only the reptilian homologue of the inferior olive of mammals can be designated as a precerebellar nucleus.

Although as previously mentioned in older studies of the reptilian brainstem the presence of an inferior olive and an olivocerebellar tract was claimed, definite proof for such a projection was obtained only quite recently in HRP studies. After almost all HRP injections into the cerebellum carried out in the present study as well as by Künzle ('83) a conspicuously labeled cell mass, contralateral to the injection site was present in the mediobasal part of the brainstem, adjacent to the nucleus raphes inferior (Figs. 21G-J; 22M-O; 24J-L; 25K-M; 29L; 30D-F; 32). Also some labeled fibers could be traced coursing in the ipsilateral spinal lemniscus and crossing the midline to their apparent site of origin. This location and the constant contralateral labeling both after small (mainly molecular layer) and large cerebellar HRP injections point to the conclusion that this mass most probably represents the reptilian homologue of the inferior olive of other vertebrates. This cell mass was also labeled following injection of radioactive D-aspartate into the cerebellar cortex of *Pseudemys scripta elegans* (Künzle and Wiklund, '82). In rat, similar D-aspartate injections have recently been shown to selectively label the climbing fibers and their cells of origin within the inferior olive, while mossy fibers or monoaminergic cerebellar afferents remained unlabeled (Wiklund *et al.*, '82).

The following experimental data provide additional proof for the presence of an inferior olive in the caudal brainstem of reptiles:

- rather massive terminations of spinal afferents in the caudal brainstem of reptiles (Ebbesson, '67, '69, Jacobs, '68, Ebbesson and Goodman, '81), indicating spino-olivary projections (see also ten Donkelaar and Bangma, '84);
- ipsilateral descending projections from the red nucleus to the caudal brainstem in *Varanus exanthematicus* (see ten Donkelaar and de Boer-van Huizen, '81a), indicating a rubro-olivary connection as present in mammals,
- ipsilateral connections of the nucleus of the basal optic root to the caudal brainstem in *Varanus exarthematicus* (see ten Donkelaar and de Boer-van Huizen, '81a), comparable to projections of the nucleus of the basal optic root to the inferior olive in the pigeon (Clarke, '77, Brecha *et al.*, '80);
- anterograde tracer (^{3}H-leucine) studies in *Varanus exanthematicus* revealed contralaterally terminating fibers in the molecular layer of the cerebellar cortex after injections into the caudal brainstem (Wolters, Dederen and ten Donkelaar, unpublished observations),
- after injections of WGA-HRP into the vicinity of the inferior olive in *Varanus exanthematicus* a distinct pattern of termination in the contralateral molecular layer of the cerebellar cortex was observed, arranged as longitudinally oriented zones (Fig. 26)

The aforementioned observations all together indicate the existence of an inferior olive in the caudal brainstem of reptiles. Schwarz and Schwarz ('80), however, suggested that a cell mass in the rostral rhombencephalon (their parvocellular isthmic complex) represents a source of climbing fibers in reptiles, possibly corresponding to the inferior olive of mammals. This suggestion seems to be quite conflicting with the phylogenetically constant appearance of the inferior olive in the caudal brainstem as described in all vertebrate classes except amphibians and reptiles (Kooy, '17, Ariens Kappers *et al.*, '36). Recent experiments in amphibians also revealed the presence of an inferior olive at this position in the caudal brainstem (Cochran and Hackett, '77, '80; Grover and Grusser-Cornehls, '80). Schwarz and Schwarz ('80) parvocellular isthmic complex might correspond to the parabrachial nucleus as distinguished by ten Donkelaar and de Boer-van Huizen ('81b), which contained retrogradely labeled cells in several cases of HRP injections into the cerebellum (Fig. 22D). As noted before Reiner ('79) demonstrated projections of the paleostriatum augmentatum to the parabrachial isthmic region, which might indicate a relay function of this region to the cerebellum for telencephalic information (ten Donkelaar and Bangma, '84).

In the present study no conclusive evidence was found for the existence of two other precerebellar nuclei, i e. the lateral funicular nucleus and the paramedian reticular nucleus, although the results of two cases (6121 and 6219 respectively) might suggest the presence of primordia of these nuclei. A lateral funicular nucleus has been distinguished in Nissl-stained sections (see e.g. ten Donkelaar and Nieuwenhuys, '79). Kunzle ('83) mentioned, as in case 6121, the occurrence of some labeled neurons in the region of the lateral funicular nucleus after HRP injections into the cerebellum of *Pseudemys scripta elegans*.

(c) Other brainstem nuclei. Other cerebellar projections appeared to originate in all reptiles studied in a number of brainstem nuclei, i.e. the perihypoglossal nuclear complex,

reticular formation, somatosensory nuclei and the nucleus of the solitary tract

An extensive projection of the perihypoglossal nuclear complex was demonstrated (see also Künzle, '83). Previously, at least a part of this cell mass was termed nucleus parvocellularis medialis in snakes and lizards by Ebbesson ('67; '69). In the cat three perihypoglossal nuclei can be distinguished, all projecting to the cerebellar vermis (Brodal, '52, Torvik and Brodal, '54; Batini *et al.*, '78; Kotchabhakdi *et al.*, '78). Based on their afferent and efferent connections these nuclei seem to be involved in the control of eye movements and possibly head movements in relation to the cerebellum and the vestibular system (Kotchabhakdi *et al.*, '78).

Cells of origin of reticulocerebellar fibers were found scattered throughout the medial and inferior reticular nuclei. Also the superior and inferior raphe nuclei contained labeled neurons in some experiments but, compared with the rather extensive projection of the raphe nuclei as demonstrated in the cat (Taber Pierce *et al.*, '77) this connection seems to be rather limited in reptiles. At least part of the raphecerebellar projection in reptiles is serotonergic (Parent and Poirier, '71; Parent, '73, '79; Wolters *et al.*, '83a)

Somatosensory projections to the cerebellum were demonstrated to arise in the descending nucleus of the trigeminal nerve in all four reptilian species studied. On the basis of normal material a trigeminocerebellar connection has already been suggested in several reptiles by Weston ('36), Larsell ('67) and Nieuwenhuys ('67). However, a connection of the nucleus princeps nervi trigemini and the nucleus mesencephalicus nervi trigemini with the cerebellum could not be confirmed in the present study. Kunzle ('83) reported a small projection of the principal trigeminal nucleus to the cerebellum in HRP studies. In mammals cerebellar projections of these latter nuclei and of the nucleus descendens nervi trigemini were demonstrated (Saigal *et al.*, '80; Brown-Gould, '80). In addition in *Pseudemys scripta elegans* a small projection of the dorsal funicular nucleus to the cerebellum was observed, in keeping with such projections in mammals (see e.g. Brown-Gould, '80).

Projections of the nucleus of the solitary tract to the cerebellum were demonstrated in all reptilian species studied, but in the lizard this projection was rather limited. In mammals a projection of the solitary tract has been demonstrated in the cat (Batini *et al* , '78) and in sheep (Saigal *et al.*, '80).

In the turtle *Pseudemys scripta elegans* labeled neurons were observed in the locus coeruleus and in the parabrachial nucleus. Both the locus coeruleus and the parabrachial nucleus were demonstrated to project to the cerebellum in mammals (Batini *et al* , '78, Saigal *et al.*, '80).

No evidence was found in the present study for projections of motor nuclei of cranial nerves as recently shown in mammals (Kotchabhakdi and Walberg, '77, Saigal *et al.*, '80).

4. Cerebellar afferents from the spinal cord

In the classical studies on spinocerebellar projections in reptiles two spinocerebellar tracts, i.e. the dorsal and ventral spinocerebellar tract, were distinguished. Both tracts ascend to the brainstem by way of mainly the lateral funiculus of the spinal cord, in which they cannot be delimited as distinct bundles (Kusuma *et al.*, '79). At caudal levels of the

brainstem both tracts are included in the spinal lemniscus (ten Donkelaar and Nieuwenhuys, '79). At more rostral rhombencephalic levels two separate spinocerebellar tracts become visible, apparently arising from the spinal lemniscus (Fig. 27). The ventral tract extends more rostrally than the dorsal one, and partly decussates in the anterior medullary velum. Degeneration studies by Jacobs ('68) in *Lacerta viridis* confirmed the subdivision into a dorsal and ventral spinocerebellar tract, both arising from the lateral funiculus. According to Jacobs ('68) the dorsal spinocerebellar tract is composed of fibers situated dorsally in the periphery of the lateral funiculus of the spinal cord and entering the cerebellar peduncle directly rostral to the trigeminal nerve root. The ventral spinocerebellar tract is formed by fibers in the intermediate part of the lateral funiculus, which enter the cerebellar peduncle more rostrally. However, Ebbesson ('67, '69, see also Pedersen, '73; Ebbesson and Goodman, '81) holds that the dorsal spinocerebellar tract consists only of spinocerebellar fibers which are located in the dorsal funiculus of the spinal cord, whereas the ventral spinocerebellar tract is considered to consist of all spinocerebellar fibers passing by way of the lateral funiculus.

These discrepancies were probably solved by some degeneration and HRP studies (ten Donkelaar and Bangma, '84). From these experiments it was concluded that the dorsal spinocerebellar tract is composed of fibers located in the dorsal funiculus and of fibers located in the dorsal part of the lateral funiculus. The remaining spinocerebellar fibers in the lateral funiculus form the ventral spinocerebellar tract (ten Donkelaar and Bangma, '84). Both tracts enter the cerebellar peduncle separately, as shown in figure 27.

In the present study both primary and secondary spinocerebellar projections were found. The primary projections, arising in the dorsal root ganglia, were observed only after injections of the tracer WGA-HRP into the cerebellum. After HRP injections into the cerebellum only some labeled fibers could be traced passing through the dorsal funiculus of the spinal cord as far as the thoracic level. Injection of ^{35}S-methionine into cervical and lumbar root ganglia also showed primary projections to the cerebellum of *Pseudemys scripta elegans* (Kunzle, '82). It should also be noted that in *Pseudemys scripta elegans* after application of HRP to peripheral nerves innervating hindlimb muscles labeled fibers could be traced ipsilaterally to the cerebellar granular layer (Ruigrok, personal communication).

As regards the localization of the cells of origin of secondary spinocerebellar fibers it should be emphasized that they are mainly found in area VII-VIII of the spinal gray (subdivision after Kusuma *et al.*, '79). No evidence for a column of Clarke in thoracic segments could be noted, in keeping with previous data by ten Donkelaar and de Boer-van Huizen ('78b) in the lizard *Lacerta galloti*. In rostral cervical segments no definite evidence was found for a reptilian homologue of the central cervical nucleus, which in mammals has recently been shown to project extensively to the cerebellum (Matsushita and Ikeda, '75, Snyder *et al.*, '78; Matsushita and Hosoya, '79; Matsushita *et al.*, '79). It should be noted, however, that distinct groups of labeled cells were present ipsilaterally in area V-VI of the segments 1-4 in *Varanus exanthematicus*. The presence of spinocerebellar tract neurons in area VII-VIII suggests a resemblance with the rostral (Oscarsson, '73, Petras and Cummings, '77, Matsushita *et al.*, '79) and ventral spinocerebellar tracts (Hubbard and Oscarsson, '62, Matsushita *et al.*, '79) in mammals which arise, respectively, ipsilaterally and contralaterally mainly in lamina VII of

the spinal gray (Rexed, '54). The ventral spinocerebellar tract is related only to the hindlimbs and posterior trunk, whereas the rostral spinocerebellar tract represents the forelimb equivalent of the ventral spinocerebellar tract (Oscarsson and Uddenberg, '64).

In short, although the data presented here on the origin of spinocerebellar projections in reptiles are still somewhat fragmentary, it can be concluded that distinct spinocerebellar projections in reptiles exist.

5. Summary

Summarizing the results of the present study concerning the origin of cerebellar afferents in reptiles, it can be concluded that the following main afferent pathways, schematically summarized in figure 34, were demonstrated:

- projections of the nucleus of the basal optic root, relaying retinal input to the cerebellar cortex;
- a rubrocerebellar pathway, directed to the lateral cerebellar nucleus (except in *Python regius*);
- primary and secondary vestibulocerebellar projections;
- projections of the perihypoglossal nuclear complex, probably related to oculomotor and vestibulomotor control;
- a crossed olivocerebellar projection, originating in the inferior olive and terminating as climbing fibers into the molecular layer;
- somatosensory projections, originating in the nucleus descendens nervi trigemini and the nucleus funiculi dorsalis;
- primary and secondary spinocerebellar projections, organized into dorsal and ventral spinocerebellar tracts.

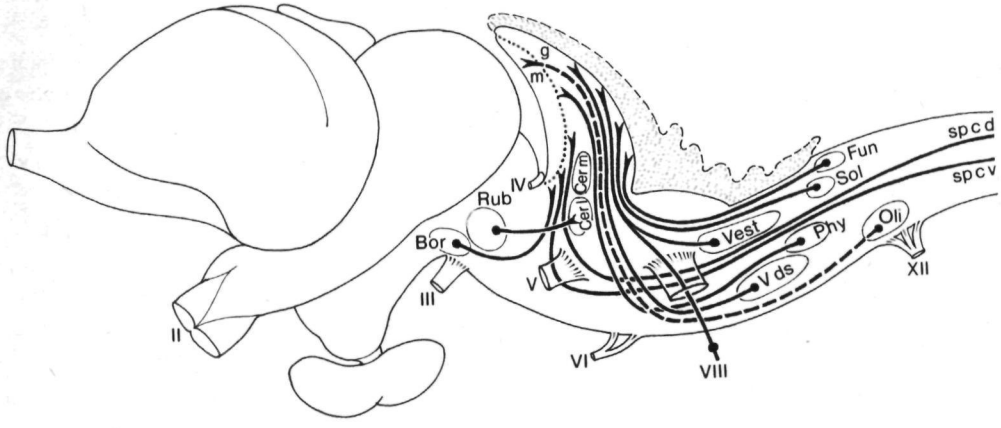


Fig. 34 Diagram summarizing the reptilian cerebellar afferent connections illustrated in Varanus exanthematicus. Bor, nucleus of the basal optic root; Cerl, Cerm, lateral and medial cerebellar nuclei; Fun, nucleus funiculi dorsalis; g, granular layer; m, molecular layer; Oli, oliva inferior; Phy, perihypoglossal nuclear complex; Rub, nucleus ruber; Sol, nucleus tractus solitarii; spcd, spcv, dorsal and ventral spinocerebellar tracts; Vest, vestibular nuclear complex; II, nervus opticus; III, nervus oculomotorius; IV, nervus trochlearis; V, nervus trigeminus; Vds, nucleus descendens nervi trigemini; VI, nervus abducens; VIII, nervus octavus; XII, nervus hypoglossus.

V. EFFERENT CONNECTIONS OF THE CEREBELLAR CORTEX

INTRODUCTION

The cerebellar corticonuclear projections have been the subject of investigations since 1897, in which year Klimoff presented the results of experiments in the rabbit. Using the Marchi technique, Klimoff (1897, 1899) showed cerebellar corticonuclear projections to the deep cerebellar nuclei and the vestibular nuclear complex. Klimoff's work was extended by Hohman ('29) and particularly by Jansen and Brodal ('40, '42) in several mammalian species, also with the Marchi technique. Jansen and Brodal described a topographical pattern in the corticonuclear projections, consisting of three longitudinally oriented zones, i.e., a medial (vermal) zone, projecting to the fastigial nucleus and vestibular nuclei, an intermediate zone with efferents to the interposed nuclei, and a lateral zone projecting to the dentate nucleus. Behavioral studies of Chambers and Sprague ('55 a, b) provided physiological support for the subdivision of the cerebellum into three longitudinal zones. A different pattern of motor deficits resulted from lesions placed in each of these anatomically defined zones. Although Chambers and Sprague's concept on functional localization in the cerebellum has been modified by more recent investigations (for recent reviews see e.g. Brooks and Thach, '81, Gilman *et al.*, '81), in broad general terms it can be stated that the medial zone is concerned with posture and mainly influences trunk and proximal limb musculature, whereas the intermediate and lateral zones are involved in finely coordinated movements of the extremities, particularly of the distal parts.

Myeloarchitectonic studies by Voogd ('64, '67, '69) revealed that more than three longitudinally directed zones are present in the cerebellar cortex. Typically six to seven zones of Purkyně cell axons can be distinguished in the lobular white matter. Each zone has been associated with a specific target for the Purkyně cells passing their axons via that particular compartment. The corticonuclear zonal pattern appears to be also equivalent to the projection of climbing fibers from the inferior olive (Brodal, '40, '80; Oscarsson, '69, '80, Groenewegen and Voogd, '77; Groenewegen *et al.*, '79). With silver impregnation techniques and retrograde tracer (horseradish peroxidase) studies a longitudinal pattern in the cerebellar corticonuclear projections has now been established in various mammals, including opossum (Haines *et al.*, '76), rat (Haines and Koletar, '79), rabbit (van Rossum, '69; Yamamoto, '78), cat (Courville *et al.*, '73; Courville and Diakiw, '76; Dietrichs and Walberg, '79, '80; Voogd and Bigaré, '80, Sato *et al.*, '82), and primates (*Galago senegalensis*. see e.g. Haines, '76; *Macaca fascata* Balaban *et al.*, '81, for further references see Haines *et al.*, '82), and even in birds (*Gallus domesticus*· Feirabend *et al.*, '76; Wold, '81, Feirabend, '83). In 'lower' vertebrates as e.g. reptiles, no pertinent experimental data on corticonuclear projections are available.

As noted before Larsell ('26, '32, '67) divided the corpus cerebelli into longitudinally oriented zones, viz., a median pars interposita, and a lateral zone on each side. Larsell noted that the pars interposita of snakes and limbless lizards is relatively prominent, whereas the pars lateralis is reduced. In turtles, solely dependent on their extremities for locomotion, the pars interposita is reduced, whereas the pars lateralis is well developed. Based on these observations Larsell suggested that the pars lateralis is

concerned with movements of the limbs and the pars interposita with movements of the axial musculature. In the South American alligator *Caiman sclerops* stimulation studies (Goodman and Simpson, '60, Goodman, '64, '69) and lesion experiments (Senn and Goodman, '69) suggest a zonal organization of the cerebellar cortex. Three types of postural patterns appeared to be related to two longitudinally oriented zones (vermal and paravermal) present throughout the cerebellum, and a floccular zone, only present in the lobus posterior. Further evidence for a longitudinal subdivision of the reptilian cerebellar cortex was presented by Gerrits and Voogd ('73) in a topological analysis of the Purkyně cell layer of the cerebellum of the turtle *Testudo hermanni*, in which at least two longitudinally oriented zones of Purkyně cells were observed. Within the frame of the present investigation, the organization of the cerebellar corticonuclear connections has been studied by two different approaches (1) a topological analysis of the cerebellar Purkyně cell layer in the turtle *Pseudemys scripta elegans* and the lizard *Varanus exanthematicus*, described in Chapter IIID, (2) experimentally, by applying HRP to the cerebellar and vestibular nuclei in *Pseudemys scripta elegans*, *Varanus exanthematicus*, and *Python regius*. The results of the experimental part are described in the present chapter

CORTICONUCLEAR PROJECTIONS IN THE TURTLE PSEUDEMYS SCRIPTA ELEGANS

HRP injections and HRP slow-release gel implantations were made into the cerebellar and vestibular nuclei of 15 red-eared turtles. Of these experiments 5 cases, representative for the whole series, are shown in the figures 35-39. It should be noted that in all experiments of the present study the projections of the cerebellar cortex to the cerebellar nuclei and the vestibular nuclear complex appeared to be strictly ipsilateral. To visualize the pattern of labeled Purkyně cells in the cerebellum of *Pseudemys scripta elegans*, the procedure was followed as described for the topological analysis of the whole Purkyně cell layer in *Pseudemys scripta elegans* (Chapter IIID), using 40 µm TMB-incubated transverse sections of the cerebellum.

Corticovestibular projections

In case 6245 (Fig. 35) a gel was implanted into the ventral part of the nucleus vestibularis ventrolateralis (Fig 35A). Topological analysis of the HRP labeled Purkyně cells revealed a distribution of these cells as shown in figure 35B The lines indicated in the chart represent the boundaries between the medial, intermediate, and lateral zones as distinguished by topological analysis of both labeled and unlabeled Purkyně cells of this animal. These boundaries appeared to be comparable to those found in the topological analysis of the Nissl series of the cerebellum as described in Chapter IIID (Fig 18A). Labeled Purkyně cells were found in all three zones Most of them were present in the caudolateral part of the cerebellum, especially in the intermediate and lateral zones, whereas less labeled cells were present in the medial zone In this latter zone labeled Purkyně cells were more concentrated in the lateral part. The number of labeled Purkyně cells in the medial zone decreased distinctly in the rostral direction, leaving a large area of Purkyně cells in the rostral and middle part of the medial zone unlabeled In the intermediate zone only relatively few labeled Purkyně cells were found in its rostral part, whereas HRP containing neurons were found

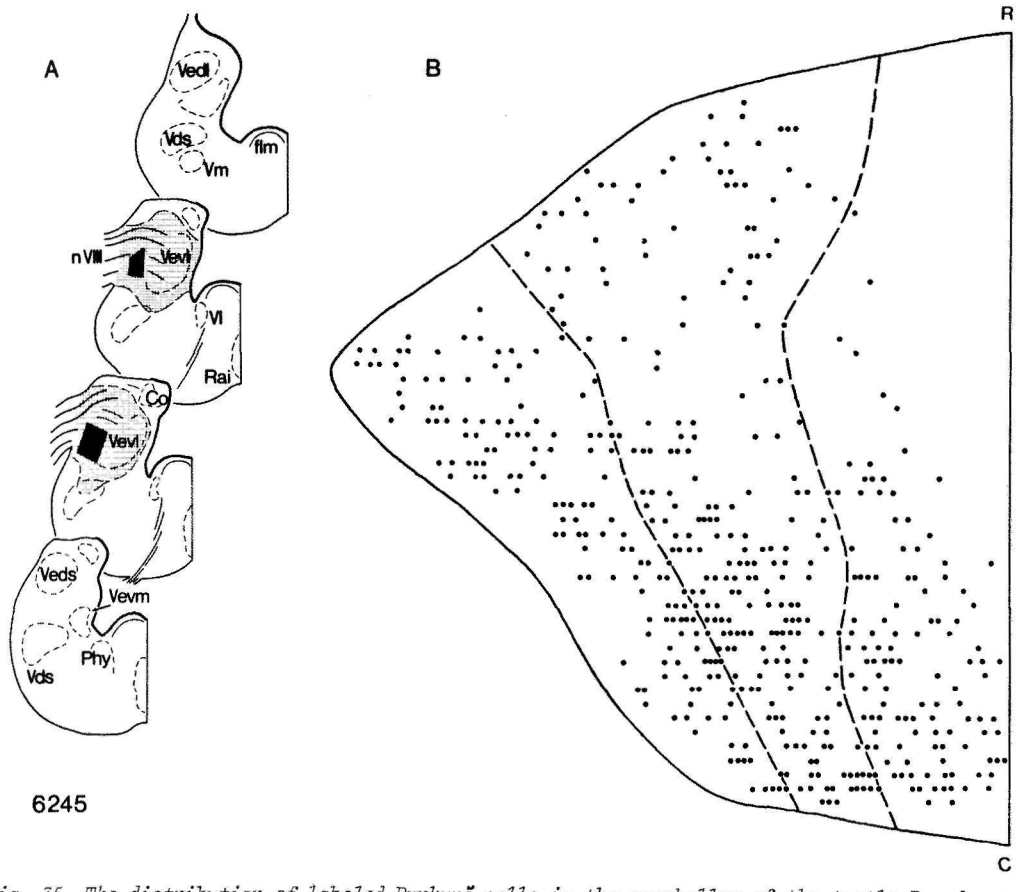


Fig. 35 The distribution of labeled Purkyně cells in the cerebellum of the turtle Pseudemys scripta elegans (Fig. 35 B), following HRP slow-release gel implantation into the ventrolateral vestibular nucleus (Fig. 35 A). C, caudal; R, rostral. For further abbreviations cf. pages 28-29.

throughout the lateral zone.

It should be noted that in this experiment probably corticovestibular fibers to the ventromedial and descending vestibular nuclei were also damaged, since in three cases, in which small HRP injections were made into the descending and ventromedial vestibular nucleus, labeled Purkyně cells were found in both the lateral and intermediate zones (not illustrated). In one experiment (case 6212, not illustrated) two HRP injections were made into the brainstem medial to the sulcus limitans at the level of the ventromedial vestibular nucleus and the rostral part of the perihypoglossal nuclear complex. In this case labeled Purkyně cells were present in a distinct rostrocaudal strip in the intermediate zone. In addition, labeled Purkyně cells were present, although in a reduced number, in the lateral zone. In all the above-mentioned cases only a few labeled Purkyně cells were present scattered in the medial zone.

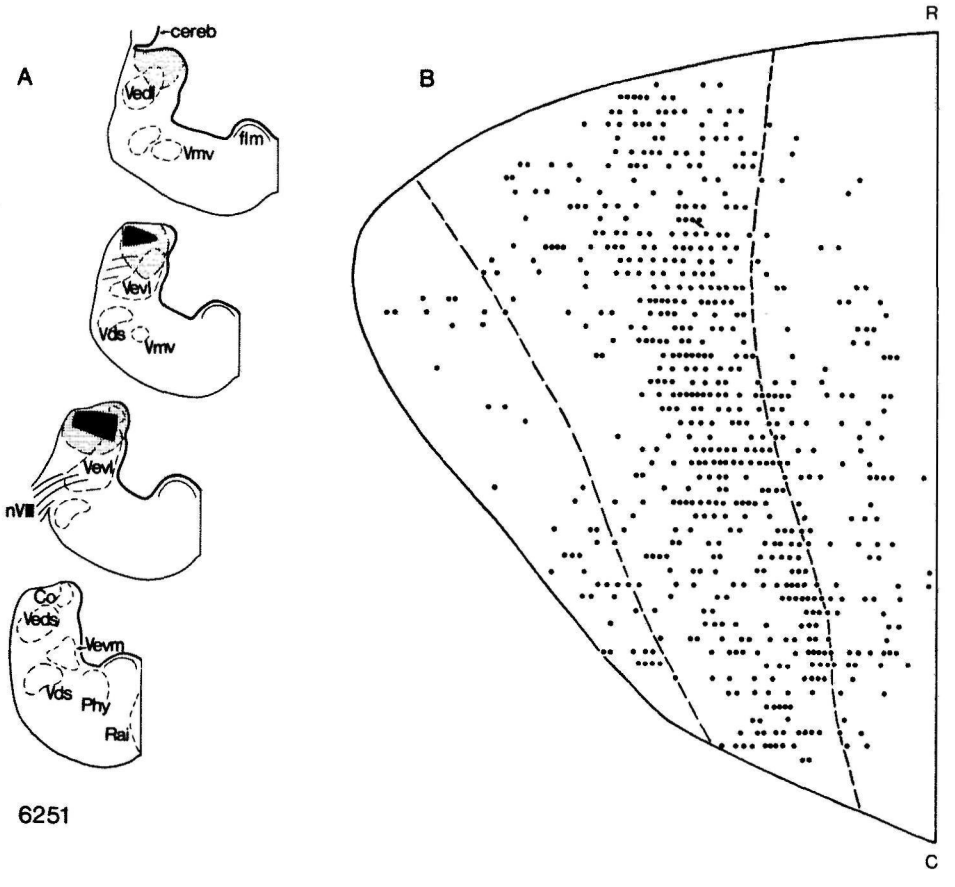


Fig. 36 The distribution of labeled Purkyně cells in the cerebellum of the turtle Pseudemys scripta elegans (Fig. 36B), following HRP slow-release gel implantation into the ventrolateral and dorsolateral vestibular nuclei (Fig. 36A). C, caudal; R, rostral. For further abbreviations cf. pages 28-29.

In figure 36 (case 6251) an experiment is shown in which an HRP slow-release gel was implanted into the caudal part of the dorsolateral vestibular nucleus and the dorsal part of the ventrolateral vestibular nucleus (Figs. 36A, 40B). A photomicrograph of a representative section of this experiment is shown in figure 40A. As in case 6245 a topological analysis of both the labeled and unlabeled Purkyně cells was made, which revealed the boundaries between the medial, intermediate, and lateral zones. Most of the labeled neurons appeared to be present in a distinct rostrocaudally oriented strip in the intermediate zone. The lateral zone contained a few labeled neurons in its rostral, floccular part, as well as at more caudal levels. In the medial zone a distinct rostrocaudally oriented strip of labeled Purkyně cells

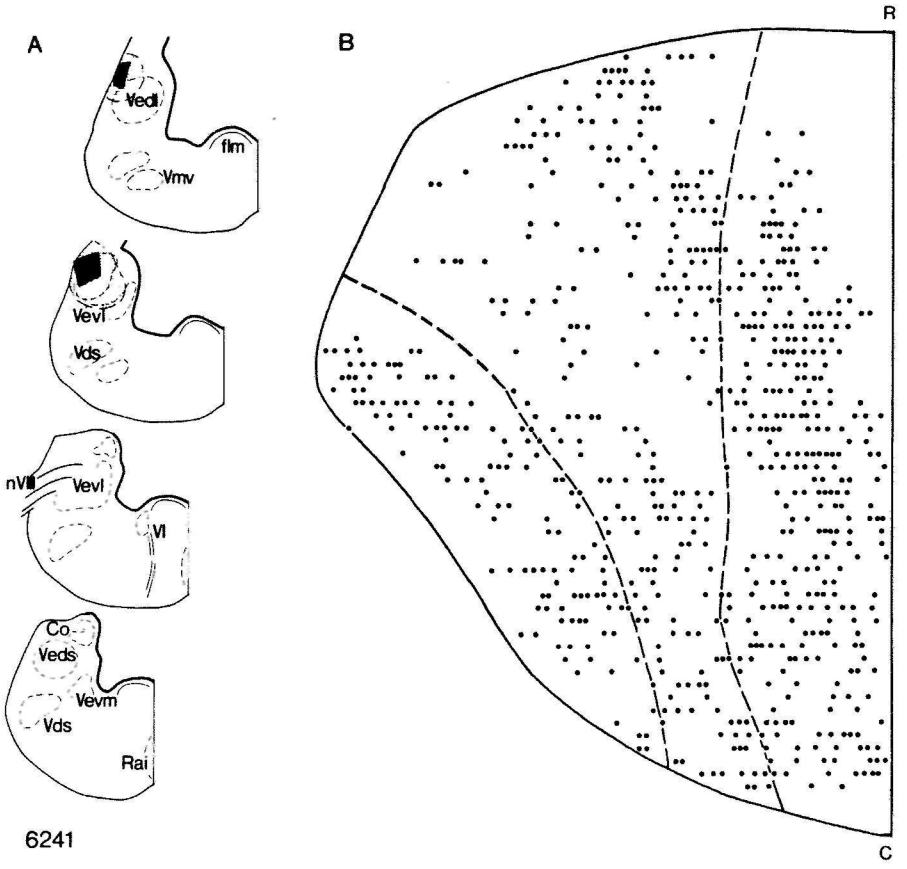


Fig. 37 The distribution of labeled Purkyně cells in the cerebellum of the turtle Pseudemys scripta elegans (Fig. 37B), following HRP slow-release gel implantation into the rostral part of the dorsolateral vestibular nucleus (Fig. 37A). C, caudal; R, rostral. For further abbreviations cf. pages 28-29.

bordering on the intermediate zone was present. As in case 6245 (Fig. 35B), the remaining part of the medial zone contained only a few labeled Purkyně cells.

In case 6241 (Fig. 37) an HRP slow-release gel was implanted into the dorsolateral vestibular nucleus (Figs. 37A, 41B), at a more rostral level as compared to case 6251 (Fig. 36A). A photomicrograph of a part of a representative section of this experiment is shown in figure 41A. In contrast to case 6251, in case 6241 some spread of HRP occurred to the cerebellar nuclei, due to the more rostral position of the gel. As in the previous cases topological analysis of both the labeled and unlabeled neurons revealed the boundaries between the three cerebellar zones. Labeled neurons were present in all three zones (Fig. 37B), but the

distribution of the labeled neurons was quite different as compared to the previous cases The most distinct rostrocaudal strip of labeled neurons appeared to be present in the lateral part of the medial zone, continuing in the medial part of the intermediate zone. As in the previous cases a strip of Purkyně cells remained unlabeled in the medial part of the medial zone, although reduced in width along its whole rostrocaudal length. The density of the labeled neurons in the medial part of the intermediate zone was reduced in respect to case 6251 (Fig 36B). As in the other vestibular experiments illustrated, only a few labeled Purkyně cells were found in the rostrolateral part of the intermediate zone. The gel also interrupted corticovestibular fibers, as judged by the presence of labeled terminal structures in the vestibular nuclei, mainly to the ventrolateral vestibular nucleus In the lateral zone a considerable number of labeled neurons was present in case 6241, both in its rostral floccular part and in the more caudal parts of this zone.

In short, the HRP slow-release gel implantations and HRP injections into the vestibular nuclear complex show that most of the labeled Purkyně cells appeared to be present in the lateral and intermediate Purkyně cell zones, and in the lateral part of the medial zone. In the medial part of the medial zone and the rostrolateral part of the intermediate zone only few labeled neurons were found.

Corticonuclear projections to the cerebellar nuclei

In figure 38 one of three comparable cases is shown in which an HRP slow-release gel was implanted from the lateral side of the brain into the cerebellar peduncle at the level of the lateral and medial cerebellar nuclei, aimed to reach the lateral cerebellar nucleus (case 6301, Fig. 38A). The spread of the enzyme from the gel appeared to be mainly restricted to the lateral cerebellar nucleus. However, the gel penetrated the cerebellar peduncle, thereby interrupting corticovestibular fibers. Topological analysis of the labeled Purkyně cells revealed the distribution pattern shown in figure 38B. In all three mentioned cases, two distinct strips of labeled Purkyně cells were present. In the lateral part of the medial zone, and in the intermediate zone a strip of labeled Purkyně cells was present, comparable to the strip of neurons in case 6251 (Fig. 36B), in which an HRP slow-release gel was implanted into the dorsolateral vestibular nucleus. In case 6301 this strip was reduced in its rostrocaudal length as compared to case 6251. The second distinct strip of labeled Purkyně cells was located in the rostrolateral part of the intermediate zone, toward the boundary with the floccular part of the lateral zone. Such a distinct strip of labeled neurons was present only in these experiments in which the gel was located in the lateral cerebellar nucleus In addition, more labeled Purkyně cells were present in the medial part of the medial zone as compared to the experiments described before, probably due to some spread of HRP to the medial cerebellar nucleus. Almost no labeled Purkyně cells were found in the lateral zone.

In figure 39, one of three experiments is shown (case 6231), aimed to place an HRP slow-release gel into the medial cerebellar nucleus. Therefore the gel was implanted at a more rostral position in the cerebellar peduncle as compared to case 6301 (Fig 38) In case 6231 (Fig. 39A, 41D) the HRP gel appeared to be placed into the medial cerebellar nucleus, as well

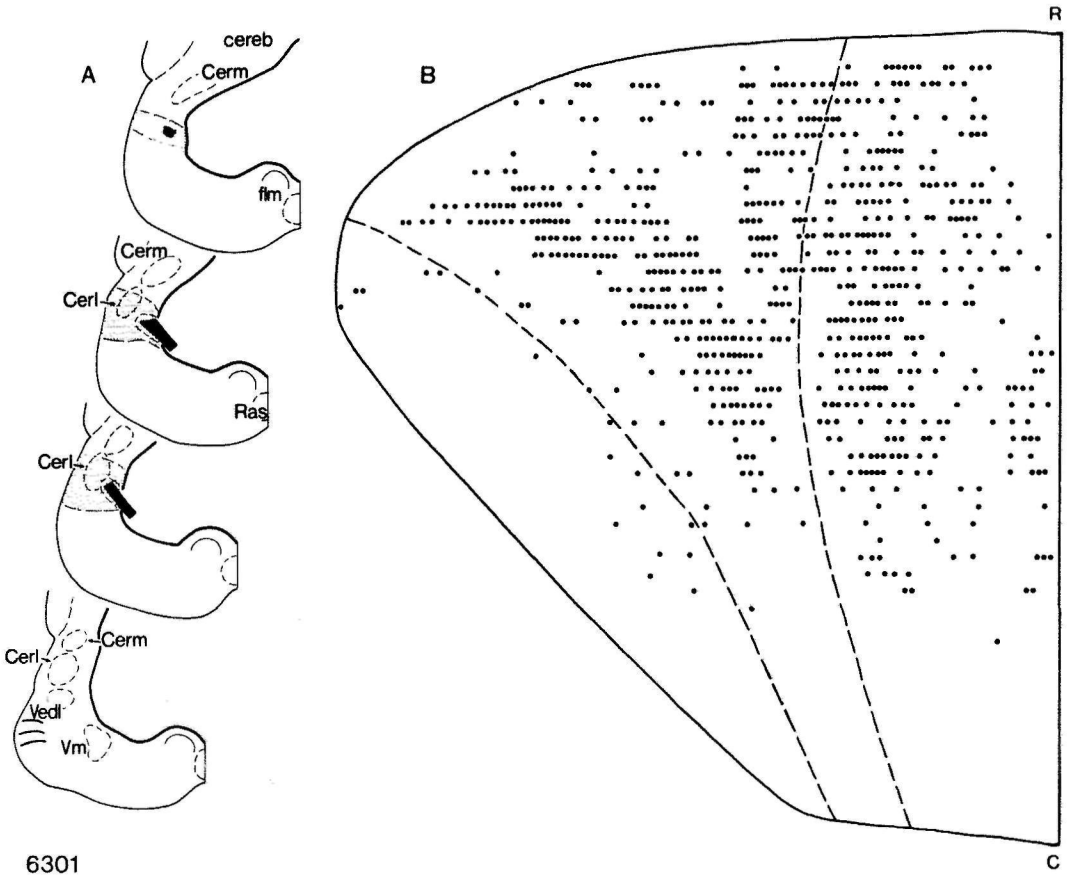


Fig. 38 The distribution of labeled Purkyně cells in the cerebellum of the turtle Pseudemys scripta elegans (Fig. 38 B), following HRP slow-release gel implantation into the cerebellar peduncle at the level of the lateral and medial cerebellar nuclei (Fig. 38A). C, caudal; R, rostral. For further abbreviations cf. pages 28-29.

as the most rostral part of the lateral cerebellar nucleus. The distribution pattern of the labeled Purkyně cells is shown in figure 39B. A photomicrograph of a part of a representative section of this experiment is shown in figure 41C. In this experiment almost all Purkyně cells of the medial zone appeared to be labeled, whereas in the intermediate zone a large part of the Purkyně cells was also labeled (Fig. 39B). In the lateral zone considerably fewer labeled neurons were present. These data indicate that in case 6231 a great number of the corticonuclear fibers projecting to the medial and lateral cerebellar nuclei, as well as to the vestibular nuclear complex, has taken up the enzyme HRP, either by terminals or by damage to corticovestibular fibers passing through the area of the HRP gel implantation.

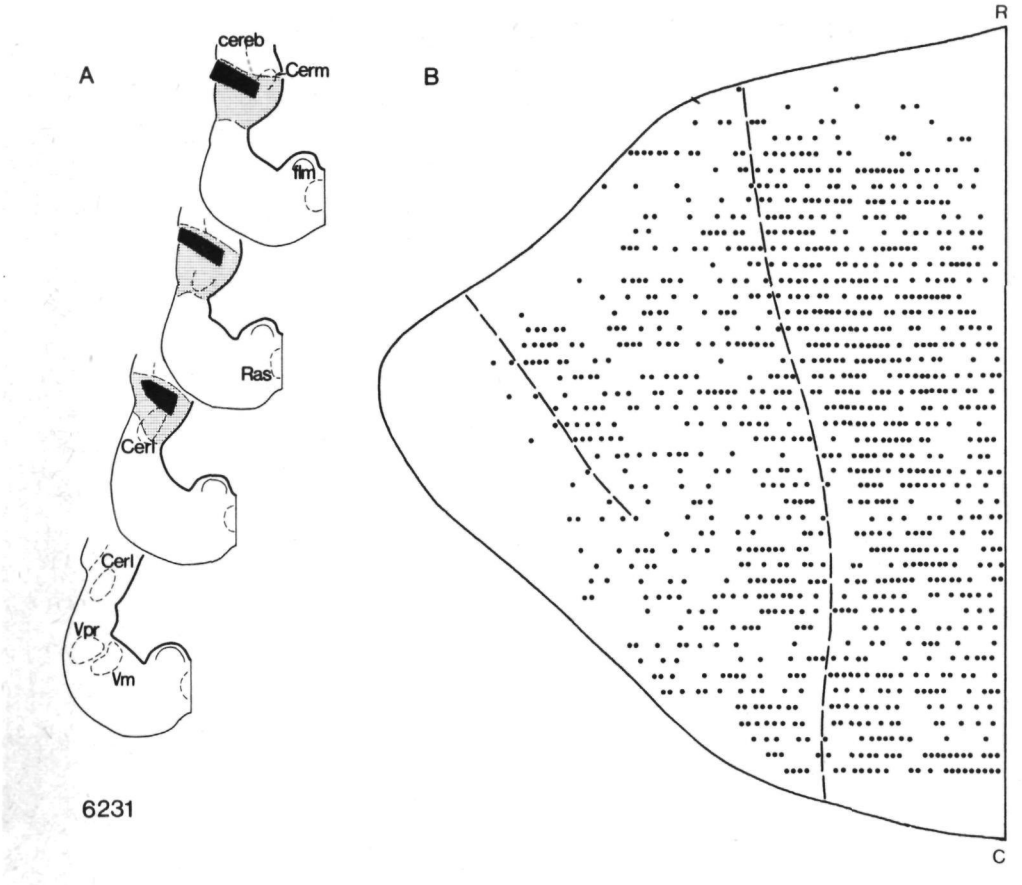


Fig. 39 The distribution of labeled Purkyně cells in the cerebellum of the turtle Pseudemys scripta elegans (Fig. 39B), following HRP slow-release gel implantation into the cerebellar peduncle at the level of the lateral and medial cerebellar nuclei (Fig. 39A). C, caudal; R, rostral. For further abbreviations cf. pages 28-29.

In summary, HRP slow-release gel implantations aimed at the cerebellar nuclei revealed two areas of labeled Purkyně cells not found after the vestibular cases. After HRP gel implantations mainly restricted to the lateral cerebellar nucleus a distinct strip of labeled Purkyně cells was present in the rostrolateral part of the intermediate zone (case 6301, Fig. 38), whereas after gel implantations including the medial cerebellar nucleus the major change in the labeling pattern was the labeling of almost all Purkyně cells in the medial part of the medial zone (case 6231, Fig. 39).

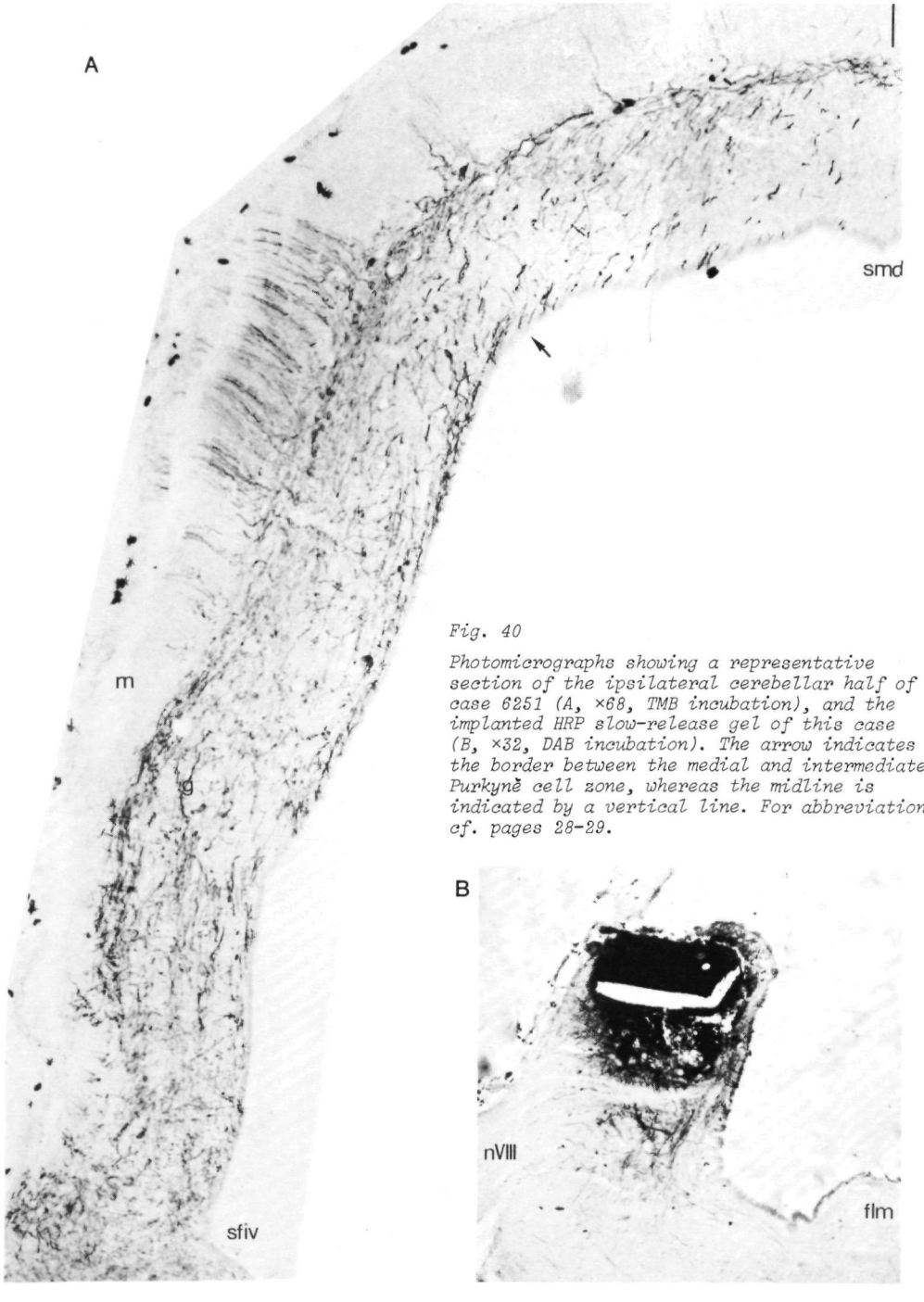


Fig. 40

Photomicrographs showing a representative section of the ipsilateral cerebellar half of case 6251 (A, ×68, TMB incubation), and the implanted HRP slow-release gel of this case (B, ×32, DAB incubation). The arrow indicates the border between the medial and intermediate Purkyně cell zone, whereas the midline is indicated by a vertical line. For abbreviations cf. pages 28-29.

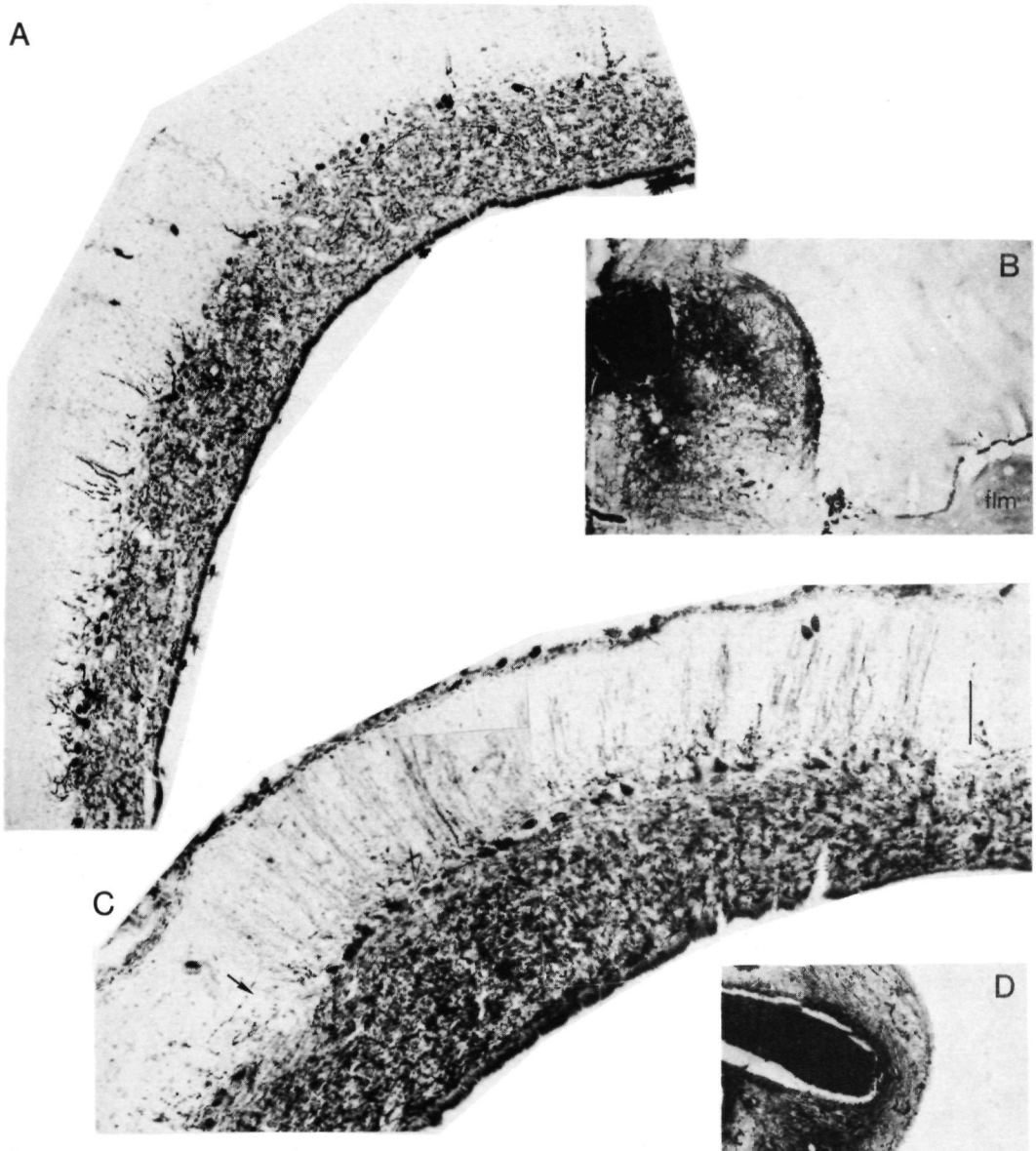


Fig. 41 Photomicrographs showing: A, A part of a representative section of the cerebellum of case 6241 (×84, TMB incubation); B, The site of implantation of the HRP slow-release gel of case 6241 (×36, DAB incubation); C, A part of a representative section of the cerebellum of case 6231 (×133, TMB incubation); D, The site of implantation of the HRP slow-release gel of case 6231 (×36, DAB incubation). In C the arrow indicates the border between the medial and intermediate Purkynĕ cell zone, whereas the midline is indicated by a vertical line.
For abbreviations cf. pages 28-29.

HRP injections and HRP slow-release gel implantations were made into the cerebellar and vestibular nuclei of 13 lizards. Of these experiments 4 cases, representative for the whole series, are shown in the figures 43-46. As in *Pseudemys scripta elegans*, the projections of the cerebellar cortex to the cerebellar and vestibular nuclei appeared to be strictly ipsilateral in *Varanus exanthematicus*.

To visualize the pattern of labeled Purkyně cells in *Varanus exanthematicus* the procedure of the topological analysis of the Purkyně cell layer in the lizard as described in Chapter IIID had to be modified. Instead of turning the cerebellum during the sectioning, the whole brain was sectioned in the normal transverse plane (Fig. 42). The cerebellar outlines, the borderline between the molecular and granular layer, and the labeled Purkyně cells of every second, 40 µm TMB-incubated section were drawn. Subsequently the curved borderlines with the plotted neurons were transferred to straight lines, as described in Chapter IIID. This latter step did not render any difficulties for the morphologically rostral (i.e.'ventral') and caudal (i.e. 'dorsal') parts of the cerebellar Purkyně cell layer, nor for those sections of the middle part where the ventral and dorsal parts remained separate (see Fig. 8). However, in a number of transverse sections through the middle part of the cerebellar cortex, the ventral and dorsal parts of the Purkyně cell layer fuse, due to the eversion of the cerebellar plate (see Figs. 9, 42). In those sections the Purkyně cell layer, i.e. the borderline between the molecular and granular layer, was divided equally into a ventral and a dorsal part. Also these curved borderlines were transferred to straight lines. All the straight lines were entered into a chart of the Purkyně cell layer in the correct rostrocaudal (i.e. ventrodorsal) sequence, as described in Chapter IIID. By this procedure the cerebellar Purkyně cell layer of *Varanus exanthematicus* was flattened, resulting in a chart composed of a rostral or ventral, and a caudal or dorsal part. In all figures of the illustrated experiments in addition to this chart some transverse sections of the cerebellum are shown, as is there position in the chart.

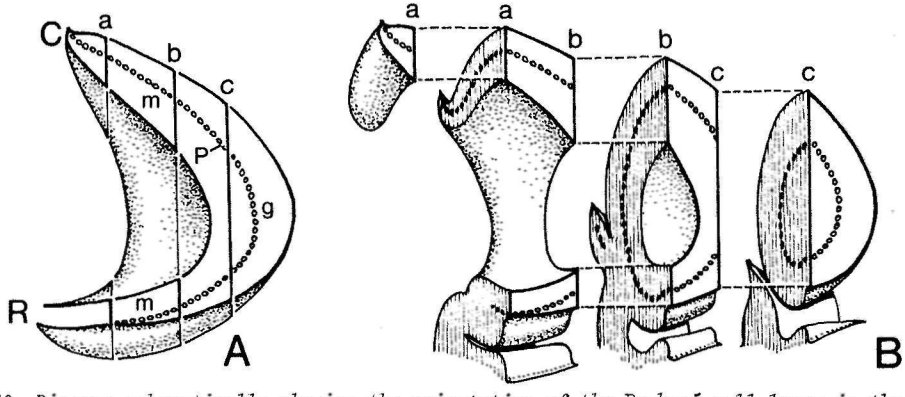


Fig. 42 Diagram schematically showing the orientation of the Purkyně cell layer in the everted cerebellum of the lizard Varanus exanthematicus in (A) a sagittal plane and (B) a number of transverse sections (a, b, c) through the cerebellum. In section (a) a separate morphologically rostral (i.e. 'ventral') and caudal (i.e. 'dorsal') part of the Purkyně cell layer are present. In the sections (b) and (c) the ventral and dorsal parts of the Purkyně cell layer are fused, due to the eversion of the cerebellar plate. C, caudal; g, granular layer; m, molecular layer; P, Purkyně cell layer; R, rostral.

Corticovestibular projections

In figure 43 case V82-26 is shown, in which an HRP slow-release gel was implanted into the middle part of the vestibular nuclear complex. The site of implantation included the ventrolateral and part of the descending vestibular nucleus; spread of the enzyme occurred to the ventromedial vestibular nucleus (Fig. 43C). Topological analysis of the HRP labeled Purkyně cells revealed a distribution of these cells as shown in figure 43B. Labeled neurons were present scattered throughout the Purkyně cell layer, in both the ventral and dorsal parts. In the flocculus and part of the adjacent lateral area of the dorsal layer a distinct group of labeled cells was observed (see also Fig. 43A, sections 2d-4d). Two other cases, in which HRP injections were made into the middle and caudal part of the vestibular nuclear complex, including a small part of the ventrolateral vestibular nucleus as well as the ventromedial and descending vestibular nuclei, also resulted in a distinct labeling of the flocculus. In one of these cases the remaining part of the Purkyně cell layer contained hardly any labeled neurons, whereas in the other experiment the Purkyně cell layer contained labeled neurons in a pattern as shown in figure 43.

In figure 44 case V82-43 is shown, in which an HRP slow-release gel was implanted into the middle part of the vestibular nuclear complex at a slightly more rostral level compared to the previous illustrated case. In this experiment the implantation site included the ventrolateral and part of the ventromedial and dorsolateral vestibular nuclei (Fig. 44C). Topological analysis of the HRP labeled Purkyně cells revealed a distribution of these cells as shown in figure 44B. From the midline lateralwards the following areas of Purkyně cells could be distinguished. First, a medial longitudinal strip is present in which labeled Purkyně cells occurred in a rather diffuse pattern. The number of labeled neurons increased in lateral direction towards a second, distinctly labeled area. The latter area appeared to be heavily labeled especially in the rostral and middle part of the cerebellum (see also Fig. 44A, sections 2-5). In caudal direction the distinct labeling of this area decreased (Fig. 44A, section 1). Lateral to this broad zone of labeled Purkyně cells only few HRP-positive neurons were found. In the flocculus relatively few labeled neurons were present in this experiment.

In summary, these experiments indicate that the corticovestibular projections in *Varanus exanthematicus* arise mainly in two separate longitudinally oriented zones of the Purkyně cell layer. One zone is formed by the flocculus and probably part of the directly adjacent area of the Purkyně cell layer, as illustrated by the experiment shown in figure 43. This zone was labeled especially after application of HRP to the caudal and middle parts of the vestibular nuclear complex, i.e. the descending, ventromedial, and part of the ventrolateral vestibular nuclei. The second zone is formed by a longitudinal zone located in the intermediate part of the Purkyně cell layer (Fig. 44). The main target of this cortical zone appeared to be the dorsal and middle part of the vestibular nuclear complex, i.e. the dorsolateral and ventrolateral vestibular nuclei. After application of HRP to the vestibular nuclear complex two regions in the Purkyně cell layer contained only few labeled neurons: (1) a rostrocaudal zone in the medial part of the cerebellar cortex adjacent to the midline; (2) an area in the caudal part of the cerebellum located between the broad 'vestibular' zone and the most caudal part of the flocculus.

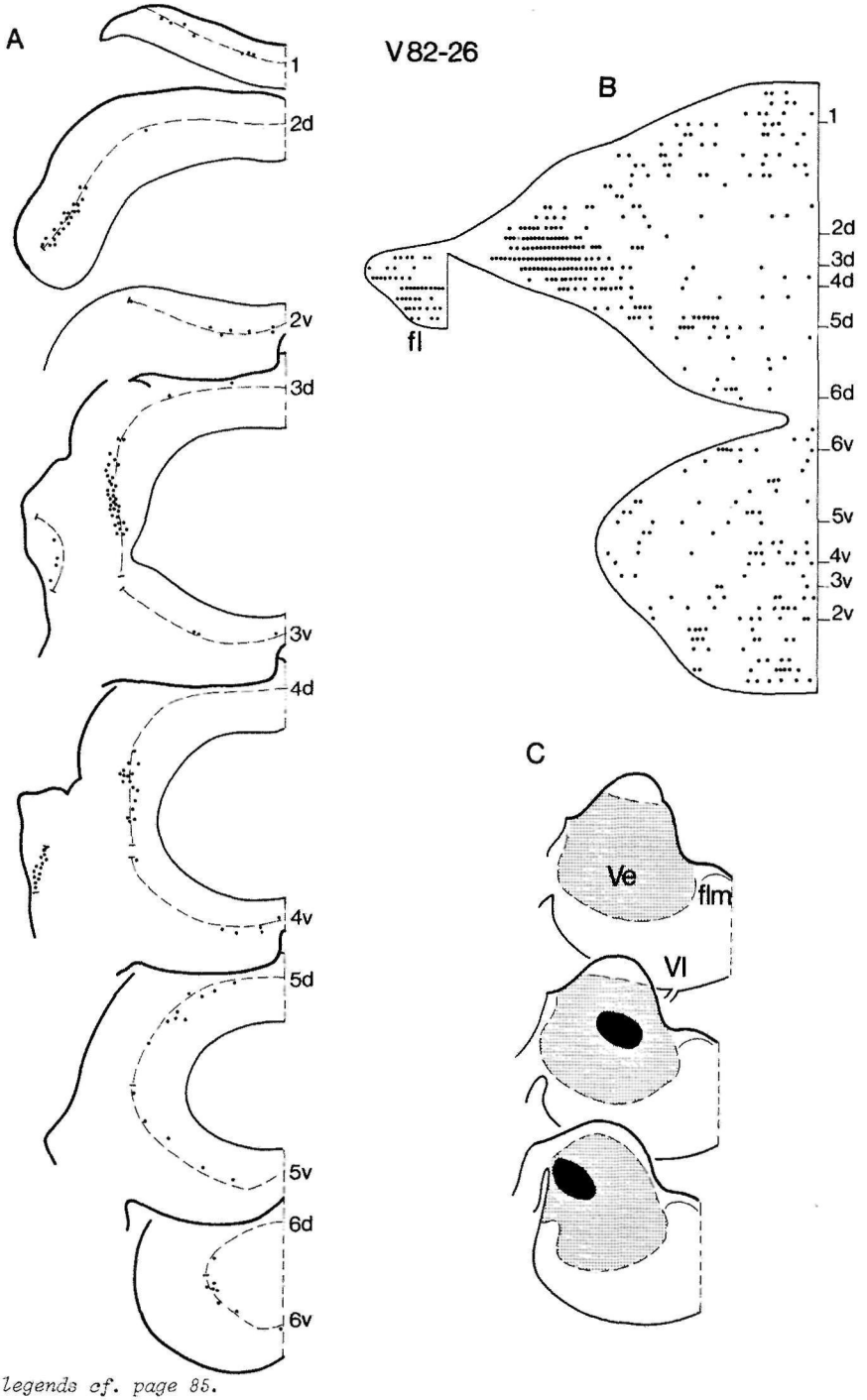


Fig. 43 For legends cf. page 85.

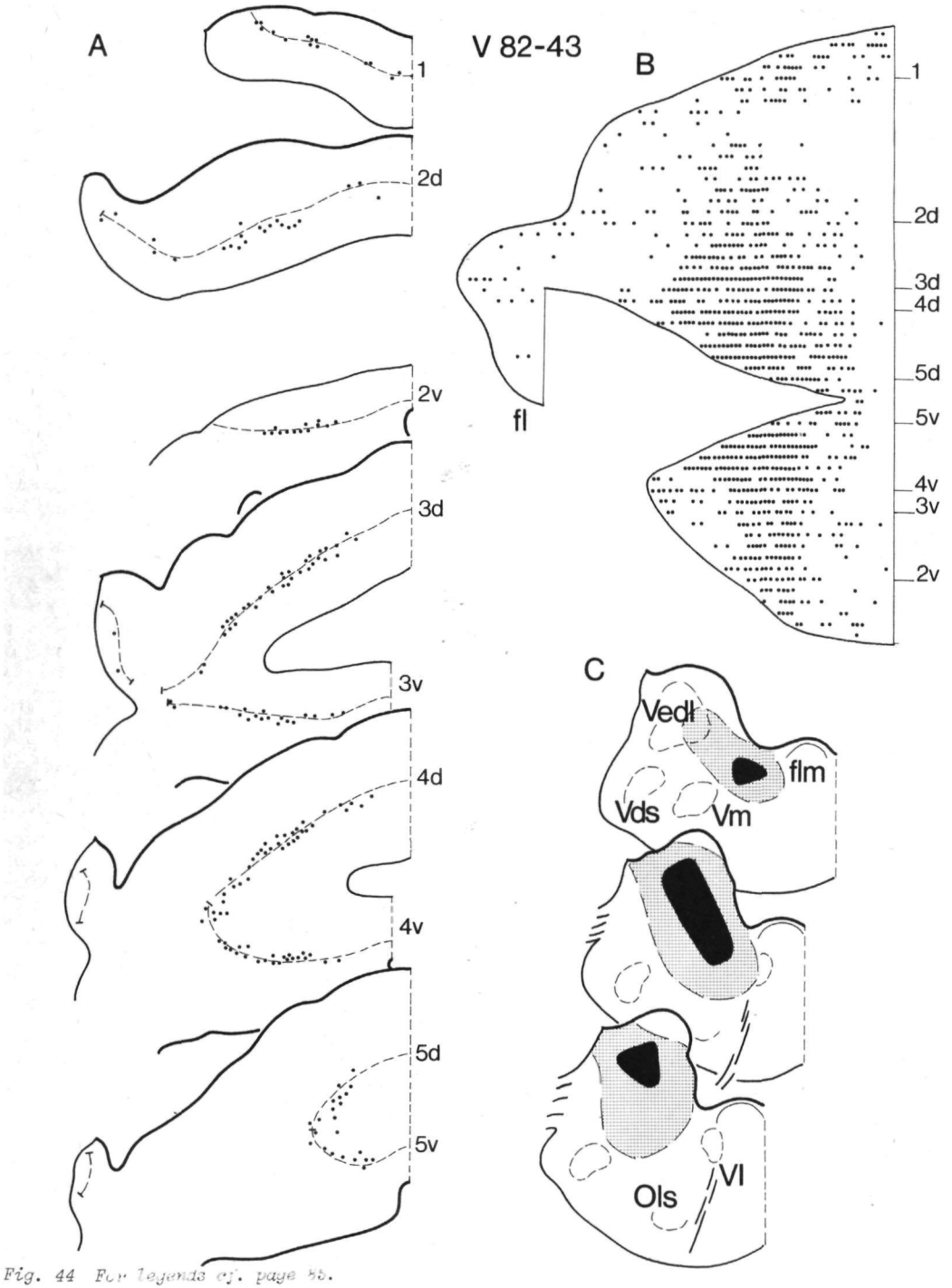


Fig. 44 For legends cf. page 85.

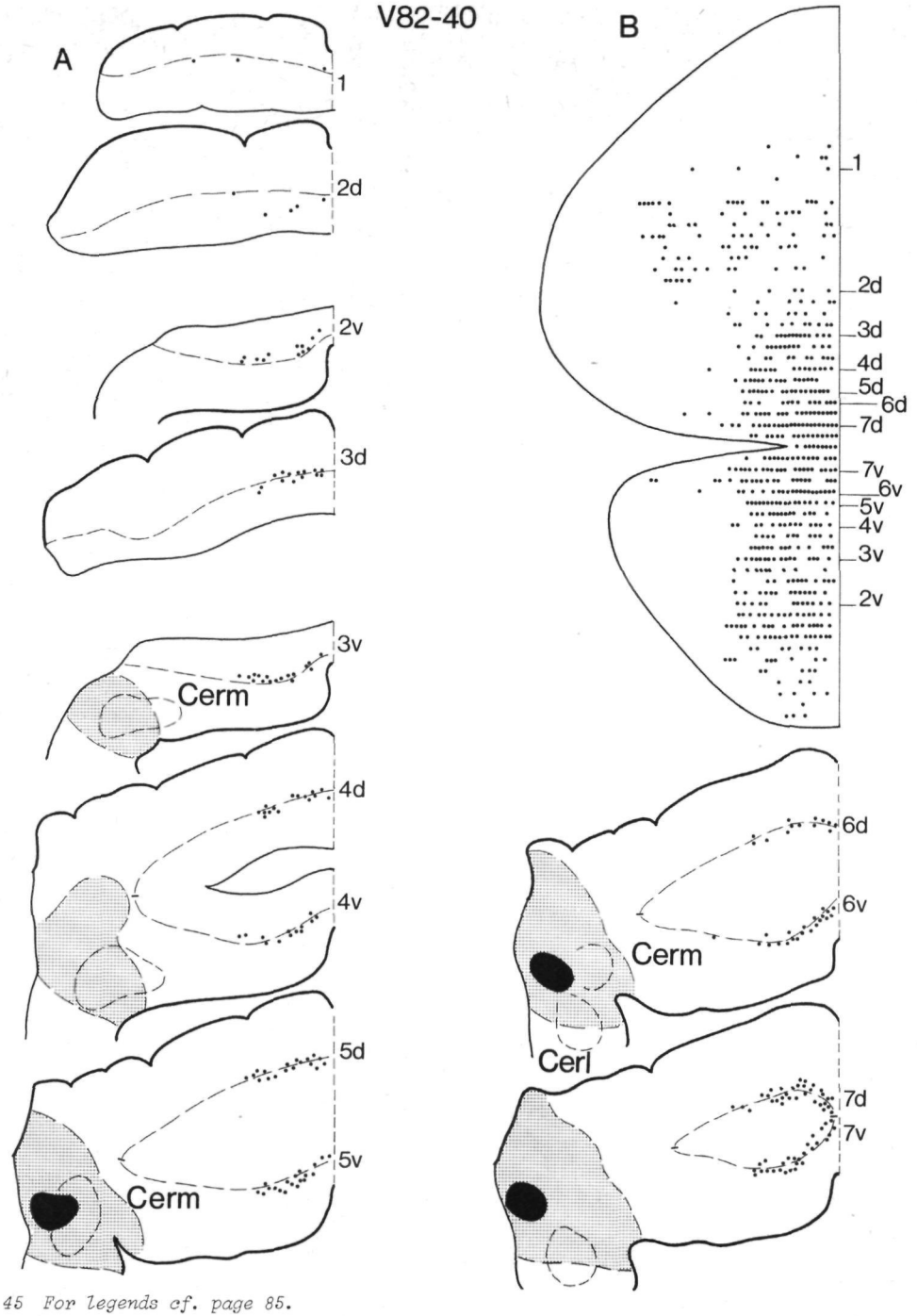


Fig. 45 For legends cf. page 85.

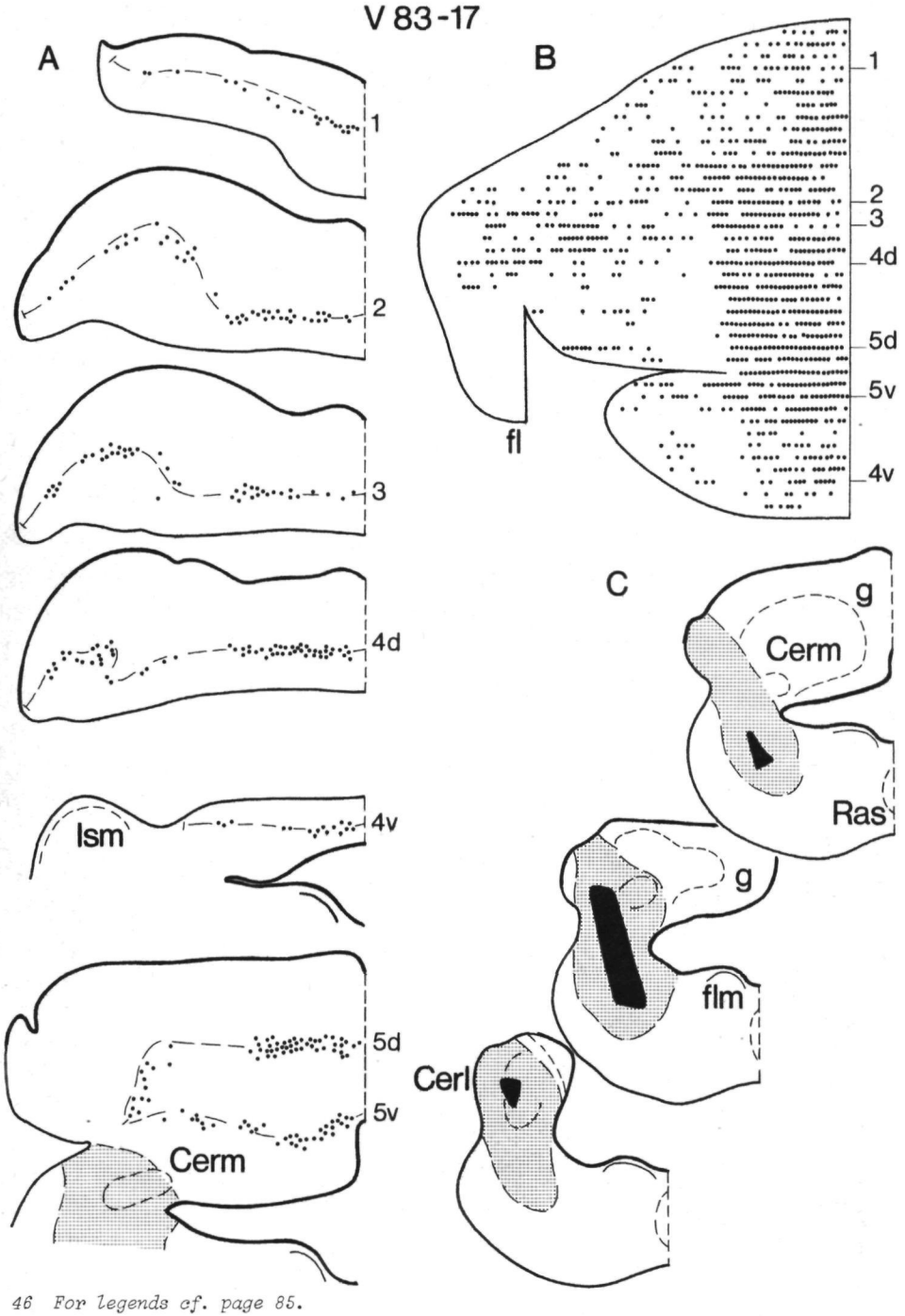


Fig. 46 For legends cf. page 85.

Legends figure 43 - 46:

Fig. 43 The distribution of labeled Purkynĕ cells in the cerebellum of the lizard Varanus exanthematicus following HRP slow-release gel implantation into the caudal part of the vestibular nuclear complex. A, Some transverse sections of the cerebellum with retrogradely labeled Purkynĕ cells; B, A chart of the cerebellum in which the distribution of the labeled neurons is indicated; C, The site of implantation of the HRP slow-release gel. C, caudal; R, rostral. For further abbreviations cf. pages 28-29.

Fig. 44 The distribution of labeled Purkynĕ cells in the cerebellum of the lizard Varanus exanthematicus following HRP slow-release gel implantation into the middle part of the vestibular nuclear complex. A, Some transverse sections of the cerebellum with retrogradely labeled Purkynĕ cells; B, A chart of the cerebellum in which the distribution of the labeled neurons is indicated; C, The site of implantation of the HRP slow-release gel. C, caudal; R, rostral. For further abbreviations cf. pages 28-29.

Fig. 45 The distribution of labeled Purkynĕ cells in the cerebellum of the lizard Varanus exanthematicus following HRP slow-release gel implantation into the medial cerebellar nucleus. A, Some transverse sections of the cerebellum with retrogradely labeled Purkynĕ cells; B, A chart of the cerebellum in which the distribution of the labeled neurons is indicated; C, The site of implantation of the HRP slow-release gel. C, caudal; R, rostral. For further abbreviations cf. pages 28-29.

Fig. 46 The distribution of labeled Purkynĕ cells in the cerebellum of the lizard Varanus exanthematicus following HRP slow-release gel implantation into the lateral and medial cerebellar nuclei. A, Some transverse sections of the cerebellum with retrogradely labeled Purkynĕ cells; B, A chart of the cerebellum in which the distribution of the labeled neurons is indicated; C, The site of implantation of the HRP slow-release gel. C, caudal; R, rostral. For further abbreviations cf. pages 28-29.

Corticonuclear projections to the cerebellar nuclei

In figure 45 and 46 two cases are shown in which an HRP slow-release gel was implanted into the cerebellar peduncle, aimed to reach the cerebellar nuclei. In case V82-40 (Fig. 45) the gel appeared to be located mainly in the medial cerebellar nucleus, with some spread of enzyme to the lateral cerebellar nucleus. The gel was implanted into the medial cerebellar nucleus through the flocculus, which made it impossible to score any label in this part of the cerebellar cortex (Fig. 45C). Topological analysis of the labeled Purkyně cells revealed a distribution of these neurons as shown in figure 45B. The greatest number of labeled neurons was found in a distinct, longitudinally oriented zone adjacent to the cerebellar midline. In addition some labeled neurons were found more laterally in the caudal part of the cerebellar cortex. In three other cases, in which the HRP gel was located in the medial cerebellar nucleus also labeled neurons were found in this medial zone of the cerebellum.

In case V83-17 (Fig. 46) the HRP slow-release gel was located both in the medial and in the lateral cerebellar nucleus. In this case the cerebellum was slightly compressed, which is clearly visible in the topological reconstruction (Fig. 46B), and the cerebellar nuclei were cut in a different angle compared with the other cases (Fig. 46C). Topological reconstruction of the labeled Purkyně cells revealed a distribution pattern shown in figure 46B. Labeled Purkyně cells were found particularly in two areas: (1) a rostrocaudal zone adjacent to the midline, (2) an area in the caudal part of the cerebellum. Between these two areas labeled neurons were present in a reduced number. In the flocculus labeled neurons were observed only in a restricted part.

In summary, these experiments indicate that in the lizard *Varanus exanthematicus* the corticonuclear projections to the cerebellar nuclei arise in two separate areas of the Purkyně cell layer. As illustrated in figures 45 and 46 one zone is located in the medial part of the cerebellar cortex, directly adjacent to the midline. In this zone labeling occurred particularly after HRP slow-release gel implantations involving the medial cerebellar nucleus. In the caudal part of the cerebellar cortex a second area of labeled neurons was observed, but only after HRP slow-release gel implantations which included the lateral cerebellar nucleus.

CORTICONUCLEAR PROJECTIONS IN THE SNAKE PYTHON REGIUS

HRP slow-release gel implantations were made into the brainstem of 10 snakes, *Python regius*, aimed to reach the cerebellar or vestibular nuclei. Of these experiments three cases are shown in figures 48-50. Since topological reconstruction of the Purkyně cells in *Python regius* was not possible the experimental data are shown in a number of rostrocaudally arranged transverse sections through the cerebellum. Like in *Pseudemys scripta elegans* and *Varanus exanthematicus*, the corticonuclear projections in *Python regius* appeared to be strictly ipsilateral.

In figure 48 an experiment is shown (case 6274), in which an HRP slow-release gel was implanted into the middle part of the vestibular nuclear complex. The site of implantation included the ventrolateral, ventromedial, and part of the descending vestibular nuclei (Fig. 48B). Spread of the enzyme remained restricted to the vestibular nuclear complex. As shown in

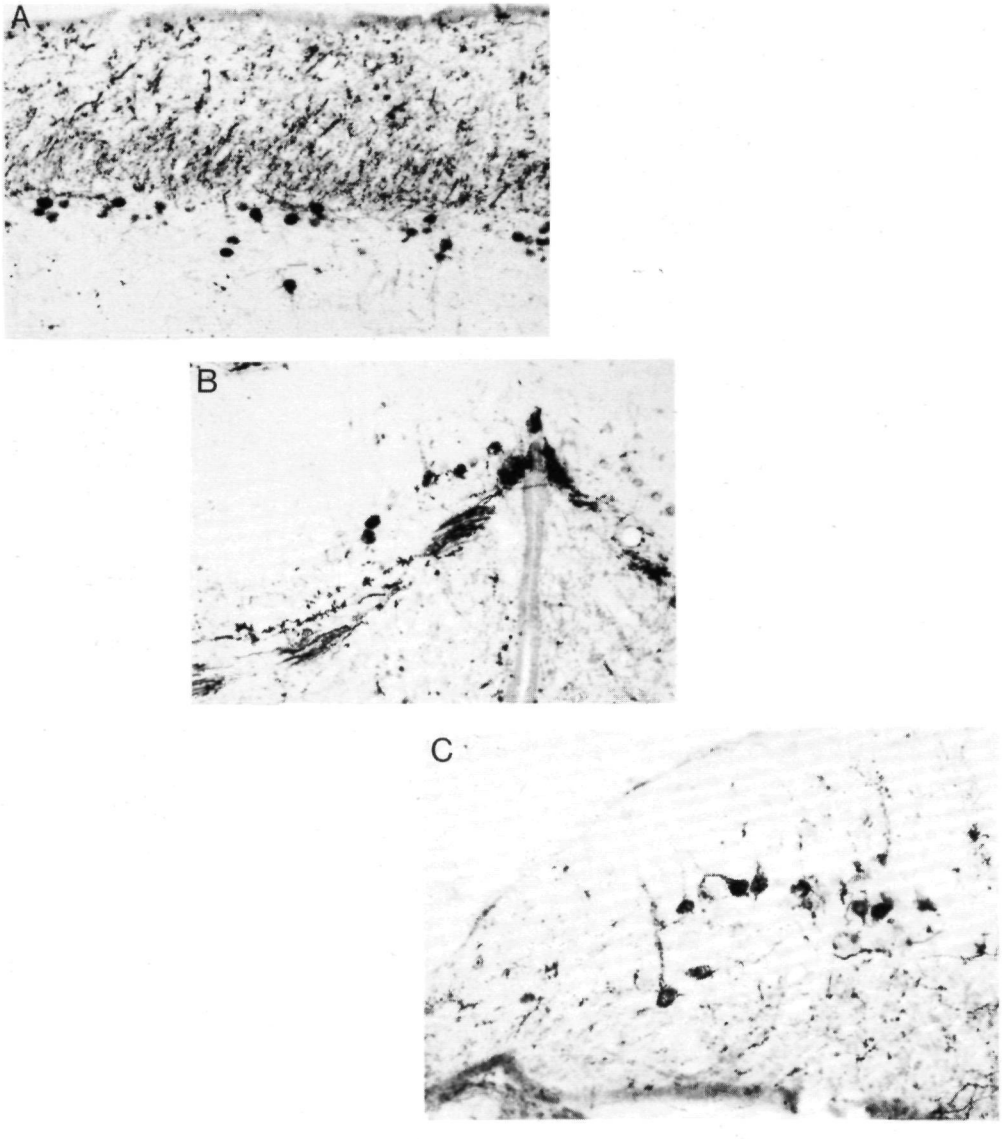


Fig. 47 Photomicrographs showing HRP labeled Purkyně cells in the cerebellum of the lizard Varanus exanthematicus (A, B) and the snake Python regius (C). A, A group of labeled neurons in the intermediate zone of the dorsal cerebellar cortex after HRP slow-release gel implantation into the vestibular nuclear complex. TMB incubation, ×115; B, A group of labeled neurons in the medial zone of the ventral cerebellar cortex after HRP slow-release gel implantation into the medial cerebellar nucleus. TMB incubation, ×115; C, A group of labeled neurons in the lateral part of the cerebellum after HRP slow-release gel implantation into the vestibular nuclear complex. TMB incubation, ×80.

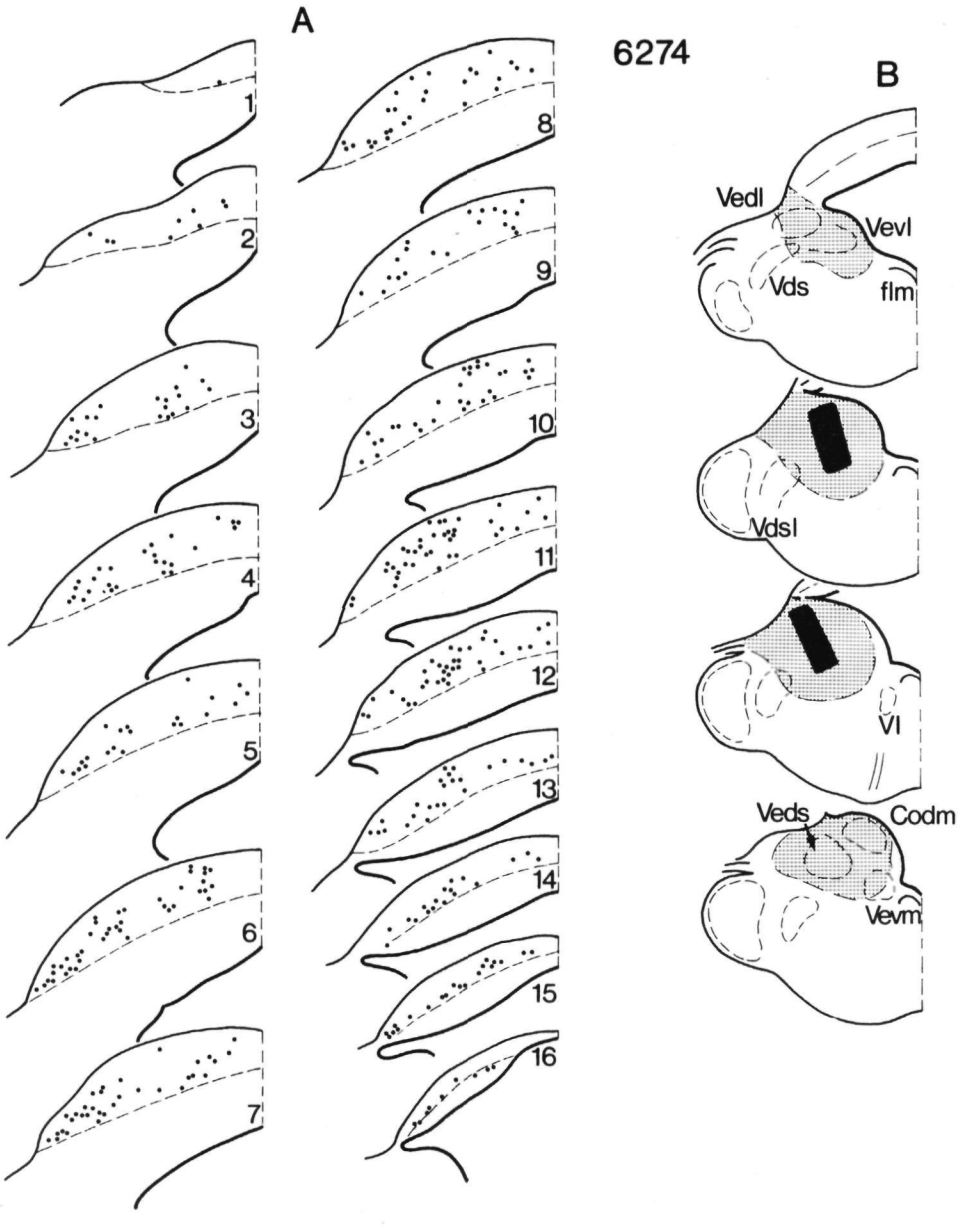


Fig. 48 The distribution of labeled Purkynĕ cells in the cerebellum after implantation of an HRP slow-release gel into the vestibular nuclear complex of the snake Python regius. A, Series of transverse sections of the cerebellum with the labeled Purkynĕ cells; B, Site of implantation of the HRP slow-release gel. For abbreviations cf. pages 28-29.

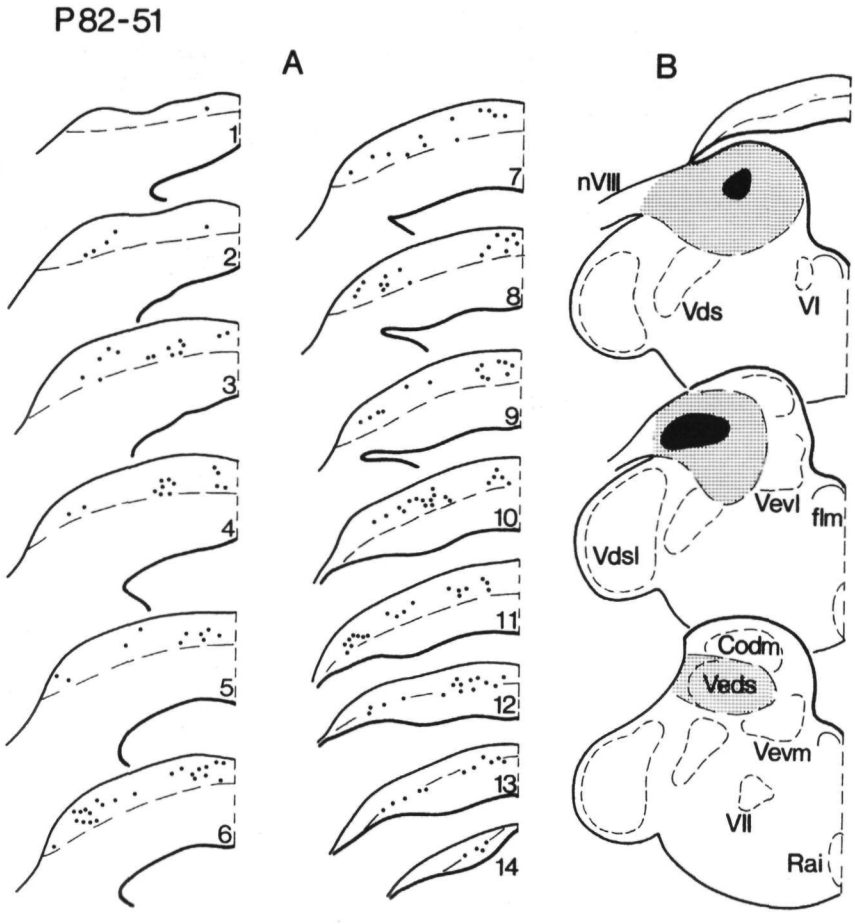


Fig. 49 The distribution of labeled Purkyně cells in the cerebellum after implantation of an HRP slow-release gel into the vestibular nuclear complex of the snake Python regius. A, Series of transverse sections of the cerebellum with the labeled Purkyně cells; B, Site of implantation of the HRP slow-release gel. For abbreviations cf. pages 28-29.

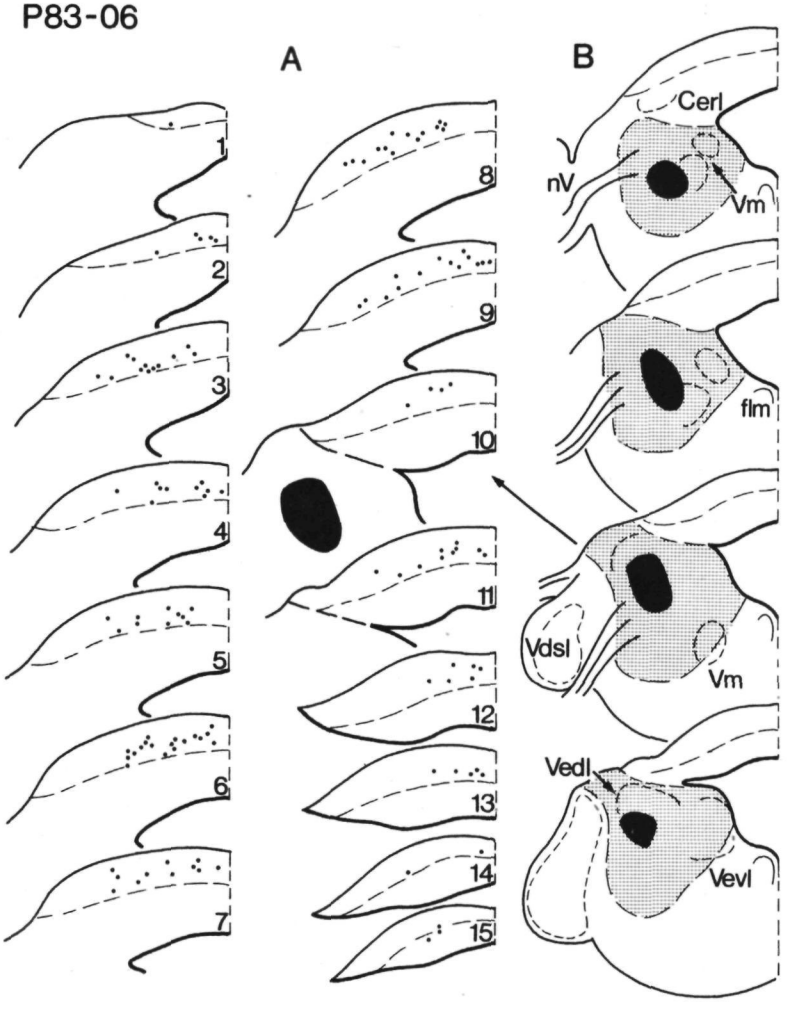


Fig. 50 The distribution of labeled Purkyně cells in the cerebellum after implantation of an HRP slow-release gel into the vestibular nuclear complex of the snake Python regius. A, Series of transverse sections of the cerebellum with the labeled Purkyně cells; B, Site of implantation of the HRP slow-release gel. For abbreviations cf. pages 28–29.

the sections through the cerebellum a certain pattern of labeling could be distinguished (Fig. 48A). Along almost the whole rostrocaudal length of the cerebellum a distinct lateral strip of labeled neurons was present, increasing in width in more caudal sections (sections 3-14). This zone of labeled neurons appeared to be bordered by an area in which almost no labeled neurons were present, especially in the rostral part of the cerebellum (sections 2-9). More medially again a zone of labeled Purkyně cells could be observed (sections 2-11), whereas the most medial part of the cerebellar cortex contained few labeled cells (e.g. sections 3, 6, 9, 10).

In case P82-51 (Fig. 49), in which experiment the HRP slow-release gel was located more caudally, a comparable pattern was observed, although less distinctly. Also in this case the most lateral part of a number of sections throughout the cerebellum contained a distinct group of labeled neurons (e.g. sections 6, 8, 10, 11), bordered by an area containing almost no labeled neurons. In the intermediate part of the cerebellum again a zone of labeled neurons was present (e.g. sections 5, 6, 11-13).

In case P83-06 (Fig. 50), the HRP slow-release gel was located mainly in the rostral part of the vestibular nuclear complex. In this case no labeled neurons were observed in the lateral part of the cerebellum. The greatest number was found in the intermediate part, leaving unlabeled an area directly adjacent to the midline in a number of sections (e.g. sections 3, 5, 8, 10). After HRP gel implantations which included the area of the cerebellar nuclei (case 6217) this midline zone was also labeled.

DISCUSSION

The organization of the cerebellar corticonuclear projections in the turtle *Pseudemys scripta elegans*, the lizard *Varanus exanthematicus*, and the snake *Python regius* was studied with the retrograde tracer HRP. The results of the experiments will be discussed separately for the three reptilian species studied. However, prior to a discussion of these results some comments should be given on the technique by which HRP was applied. Because of their position in and dorsomedial to the cerebellar peduncle in all three reptilian species studied (Chapter IIIB) the cerebellar nuclei could not be reached without considerable damage to the cerebellar cortex and possible spread of HRP within the cortex cerebelli. Therefore a lateral approach was preferred for delivering HRP to the cerebellar nuclei. In this way, however, the cerebellar nuclei were difficult to reach with a Hamilton syringe. Previous excellent results with the HRP slow-release gel technique in studies on descending pathways to the spinal cord (Griffin *et al.*, '79; Watkins *et al.*, '80; Wolters *et al.*, '82a) led to the use of gels in the present study. In the above-mentioned studies it was shown that the spread of HRP from slow-release gels is rather limited. It should be noted, however, that damage to passing fibers, e.g. corticovestibular fibers (e.g. cases 6231, V82-40) could not be avoided. But, the same disadvantage holds true for the use of HRP injections, and moreover, in that way considerable spread of the enzyme can also occur. The results of the experiments in which an HRP slow-release gel was implanted into the cerebellar peduncle (aimed to reach the cerebellar nuclei) indicated that the spread of this enzyme from such gels was rather limited.

Thus, since damage to passing corticovestibular fibers could not be avoided when

applying HRP gels into the cerebellar peduncle, first the experimental data on corticovestibular projections will be discussed, followed by a discussion of the experiments aimed at delivering HRP to the cerebellar nuclei. By a subtractive approach, however, the pattern of the corticonuclear projections to the cerebellar nuclei can be revealed.

Pseudemys scripta elegans

Figure 51 schematically summarizes the results of the present study concerning the organization of the corticonuclear projection in the turtle *Pseudemys scripta elegans*. From the topological analysis of the Purkyně cell layer (Chapter IIID), it was concluded that in both halves of the cerebellar cortex in *Pseudemys scripta elegans* three longitudinally oriented zones of Purkyně cells can be distinguished, i.e. a medial, an intermediate, and a lateral zone. In figure 51 these zones are indicated by the rostrocaudally running broken lines

From the implantations of HRP gels into the vestibular nuclear complex it can be concluded that corticovestibular fibers arise in the lateral (Larsell's flocculus, and Mugnaini's marginal rim), intermediate, and in the medial zone (particularly its lateral part) Several cases of small HRP injections into the *descending* and *ventromedial vestibular nuclei* resulted in labeled neurons in both the lateral and intermediate zones. Corticovestibular fibers to the *ventrolateral vestibular nucleus*, i.e. the presumable homologue of the mammalian nucleus of Deiters (see e.g. ten Donkelaar, '82) arise mainly in the intermediate zone (cases 6245, Fig. 35, 6251, Fig. 36). The labeled Purkyně cells in the lateral zone in these cases, particularly case 6245 (Fig. 35) might be due to damage to corticovestibular fibers passing to the descending and/or ventromedial vestibular nuclei. As noted before, after HRP deposits restricted to these nuclei, HRP positive Purkyně cells were observed mainly in the intermediate and lateral zones. In case 6251 (Fig. 36) the HRP implantation was not restricted to the ventrolateral vestibular nucleus, but also involved the *dorsolateral vestibular nucleus*. In case 6241 (Fig. 37) a somewhat more medially located zone of labeled Purkyně cells was observed as well as a distinctly labeled flocculus. In this experiment the HRP gel was largely confined to the dorsolateral vestibular nucleus, however, most likely also damaged some laterally situated corticovestibular fibers passing to the caudal part of the vestibular nuclear complex.

In all cases in which a gel was implanted into the vestibular nuclear complex relatively few labeled Purkyně cells were found in the medial part of the medial zone, and in the rostrolateral part of the intermediate zone. Only when HRP was delivered to the *lateral cerebellar nucleus* a strip of labeled Purkyně cells was observed in this part of the intermediate zone (case 6301, Fig. 38). In this particular case an increased number of labeled neurons was observed in the medial part of the medial zone, most likely due to the involvement of the *medial cerebellar nucleus*. In experiments like case 6231 (Fig. 39) in which the HRP slow-release gel reached the medial cerebellar nucleus practically all Purkyně cells in the medial zone were labeled. These observations suggest that the Purkyně cells which project to the medial cerebellar nucleus are located in the medial part of the medial zone.

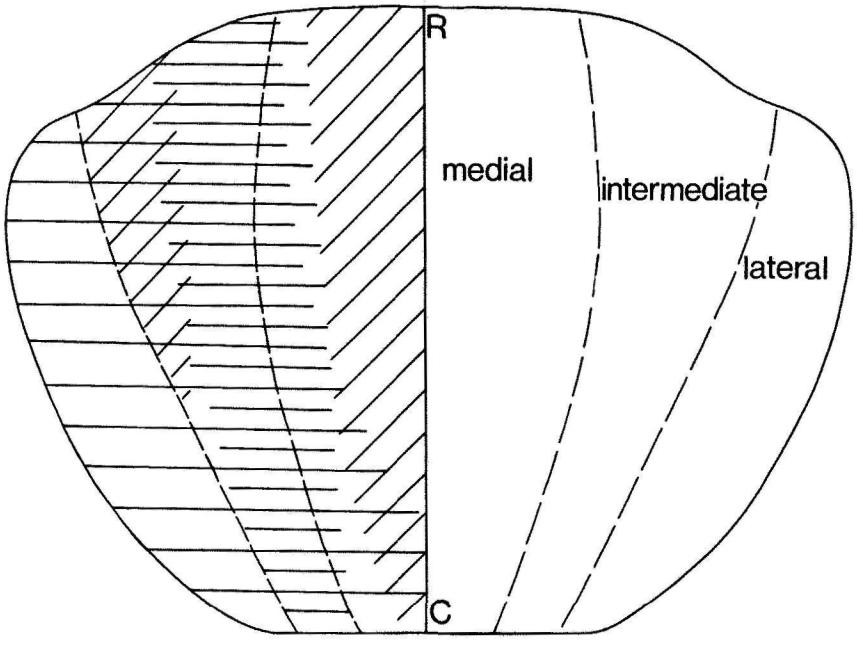


Fig. 51 Diagram, summarizing the corticonuclear projections to the cerebellar nuclei and the vestibular nuclear complex in the turtle Pseudemys scripta elegans (left). Oblique lines correspond to the Purkyně cell areas which project to the medial and lateral cerebellar nuclei, respectively. Horizontal lines correspond to the areas which project to the vestibular nuclear complex. On the right the three longitudinal zones of Purkyně cells as revealed by the topological analysis of the cerebellum of Pseudemys scripta elegans are shown. C, caudal; R, rostral.

In summary, our results strongly suggest that in the cerebellar cortex of the turtle studied four longitudinally oriented zones of Purkyně cells can be distinguished, each with a different target. From the midline lateralwards the following efferent zones are present (Fig. 51): (1) the medial part of the medial zone projecting to the medial cerebellar nucleus; (2) the lateral part of the medial zone and the main part of the intermediate zone projecting to the vestibular nuclear complex, particularly the dorsolateral and ventrolateral vestibular nuclei; (3) the rostrolateral part of the intermediate zone projecting to the lateral cerebellar nucleus; (4) the lateral zone projecting to the vestibular nuclear complex, presumably mainly to the descending and ventromedial vestibular nuclei. The lateral zone, which includes both Larsell's flocculus and its caudal extension, i.e. the marginal rim (Mugnaini *et al.*, '74; Brand and Mugnaini, '80), probably constitutes the floccular part of the cerebellum.

Varanus exanthematicus

In *Varanus exanthematicus* the topological analysis of the Purkyně cell layer revealed a less distinct distribution pattern of the Purkyně cells than in *Pseudemys scripta elegans* (Chapter IIID, Fig. 19). In the lizard only a lateral zone, including the flocculus, could be clearly distinguished. The remaining area of Purkyně cells presumably includes the medial and intermediate zones as distinguished in *Pseudemys scripta elegans*.

The results of the experimental part of this study on the corticonuclear projections in *Varanus exanthematicus* are schematically summarized in figure 52. From the application of HRP to various levels of the vestibular nuclear complex it can be concluded that the corticovestibular projections in the lizard arise in two different parts of the Purkyně cell layer. After HRP injections into the caudal part of the vestibular nuclear complex, including mainly the *descending* and *ventromedial vestibular nuclei*, labeled neurons were found especially in the caudolateral part of the cerebellar cortex, i.e. in the flocculus and part of the adjacent Purkyně cell layer. A similar result was found after HRP slow-release gel implantation into the middle part of the vestibular nuclear complex including the ventromedial, descending and part of the ventrolateral vestibular nuclei (Fig. 43). More rostrally located implantations including the *dorsolateral* and *ventrolateral vestibular nuclei* (e.g. case V82-43, Fig. 44) resulted in labeling of another part of the cerebellar cortex. In these cases a distinct rostrocaudally oriented strip of labeled neurons was found in the intermediate part of the Purkyně cell layer.

In all vestibular cases a small number of labeled neurons was found in a rostrocaudally oriented zone of the Purkyně cell layer (e.g. case V82-43, Fig. 44). In such experiments in the caudal part of the cerebellar cortex a second area with only few labeled neurons was observed. This latter area appeared to be labeled in case V83-17 (Fig. 46), in which experiment the HRP slow-release gel clearly involved the *lateral cerebellar nucleus*. The longitudinally oriented medial zone was labeled distinctly in all cases in which the HRP slow-release gels reached the *medial cerebellar nucleus* (cases V82-40, V83-17, Figs. 45, 46).

In summary, the present results strongly suggest that the corticonuclear projections in the lizard *Varanus exanthematicus* are organized into longitudinally oriented zones. As in the turtle *Pseudemys scripta elegans* (Fig. 51) four different zones of Purkyně cells can be distinguished, each with a different target: (1) a medial zone, projecting to the medial cerebellar nucleus; (2) an intermediate zone, projecting to the vestibular nuclear complex, especially the dorsolateral and ventrolateral vestibular nuclei; (3) a caudolaterally located area of the cerebellar cortex projecting presumably to the lateral cerebellar nucleus; (4) the flocculus and part of the adjacent lateral Purkyně cell layer projecting to the middle and caudal parts of the vestibular nuclear complex, i.e. the descending and ventromedial vestibular nuclei. Together, the latter two zones, i.e. (3) and (4), might be comparable with the lateral zone of Purkyně cells as distinguished in the topological analysis of the cerebellar cortex of *Varanus exanthematicus* (Chapter IIID, Fig. 19).

Comparing the results obtained in *Pseudemys scripta elegans* (Fig. 51) and *Varanus exanthematicus* (Fig. 52) a striking resemblance seems to be present in the organization of the

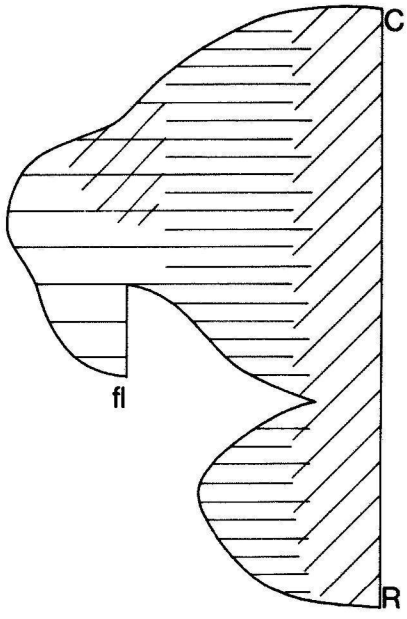


Fig. 52 Diagram, summarizing the corticonuclear projection to the cerebellar nuclei and the vestibular nuclear complex in the lizard Varanus exanthematicus. Oblique lines correspond to the Purkyně cell areas which project to the medial and lateral cerebellar nuclei, respectively. Horizontal lines correspond to the areas which project to the vestibular nuclear complex. C, caudal; R, rostral.

corticonuclear projections in these reptiles. The most obvious difference in the pattern of the corticonuclear projections is the location of the area of Purkyně cells in which the projections to the lateral cerebellar nucleus arise. In *Pseudemys scripta elegans* this area is probably located in the rostrolateral part of the cerebellar cortex, whereas in *Varanus exanthematicus* it is found in the caudolateral part of the cerebellar cortex.

Python regius

Due to the rather difficult access of the cerebellum and the underlying cerebellar nuclei in the snake, in the present study it has not been possible to apply HRP selectively to the cerebellar nuclei. Therefore, a subtractive approach is used to derive some conclusions on the organization of the corticonuclear projections in *Python regius*. The results of the HRP slow-release gel implantations into the vestibular nuclear complex of *Python regius* indicate that the corticonuclear projections in this animal are directed mainly to the vestibular

nuclei These corticovestibular projections appeared to arise in a broad zone including most of the cerebellar cortex except for a rather narrow paramedian strip of Purkyně cells along the whole length of the cerebellum. This broad vestibular zone might be subdivided into two separate subzones (1) a lateral zone, which was particularly labeled after HRP application to the *ventrolateral*, *ventromedial*, and *descending vestibular nuclei* (Figs 48, 49), almost no labeled Purkyně cells were found in this zone in case P83-06 (Fig 50), in which the HRP deposit remained restricted to the dorsolateral and ventrolateral vestibular nuclei; (2) a mediolateral part, labeled especially after HRP slow-release gel implantation into the *dorsolateral* and *ventrolateral* vestibular nuclei (Fig 50)

In all vestibular cases of the present study a small medial zone of Purkyně cells, adjacent to the midline, remained practically unlabeled, especially in the rostral and middle part of the cerebellum. In this particular zone labeled Purkyně cells were found after HRP implants including the cerebellar nuclei. In case 6274 (Fig. 48) a second, rostrally located zone containing only few labeled neurons could be distinguished, less evident in the other cases shown Although these data are somewhat scanty, the observed unlabeled zones might represent the areas of origin of corticonuclear projections to the *medial* and *lateral cerebellar nuclei*, respectively (see also Chapter VI, Fig. 68)

The present data in *Python regius* indicate that, as in the other reptiles studied, various rostrocaudally arranged zones of Purkyně cells can be distinguished, each projecting to a different target

The above-mentioned findings strongly suggest that the corticonuclear projections in the three reptilian species studied are longitudinally organized Further data on the organization of corticonuclear projections in reptiles are scanty, except for the South American alligator *Caiman sclerops* The cerebellum of *Caiman sclerops* was divided by Larsell ('32, '67), as in other reptilian species, into a median pars interposita, flanked by a pars lateralis on each side, and a flocculus. Stimulation and ablation experiments in *Caiman sclerops* (Goodman and Simpson, '60, Goodman, '64, '69) demonstrated three definable postural patterns, viz., a vermal, a paravermal, and a floccular pattern (Chapter VII, Fig. 69). The vermal zone pattern was evoked in the medial two-thirds of the middle lobe and the medial one-half of the posterior lobe. Goodman's vermal zone is probably comparable to Larsell's pars interposita and the medial zone of the present study. The paravermal zone pattern in *Caiman sclerops* was evoked in the lateral half of the corpus cerebelli, which corresponds to the pars lateralis of Larsell and presumably also to the intermediate zone. A floccular zone pattern was obtained from stimulation of the flocculus Lesion experiments (Senn and Goodman, '69) in *Caiman sclerops* indicated that corticonuclear projections to the cerebellar nuclei originate mainly in the vermal zone, but also in the paravermal zone. Corticovestibular projections appeared to be derived from Purkyně cells located in the paravermal zone, especially of the posterior lobe (no lesions were made in the flocculus). The above-mentioned data indicate that in various reptilian species representative for the major reptilian groups a longitudinal organization of corticonuclear projections exists, which may be basic for terrestrial vertebrates (see Chapter VII).

VI. EFFERENT CONNECTIONS OF THE CEREBELLAR NUCLEI

INTRODUCTION

The cerebellar cortex projects to the cerebellar nuclei and the vestibular nuclear complex (Chapter V). In the present chapter the efferent connections of the medial and lateral cerebellar nuclei will be discussed.

Projections of the cerebellar nuclei were already described in the classical studies on the reptilian cerebellum. Weston ('36) regarded the cerebellar efferent fibers, except those to the vestibular nuclei and the tectum mesencephali, as forming the cerebellotegmental system. This system was subdivided into the rostrally directed tractus cerebellomotorius et -tegmentalis mesencephali, and the caudally directed tractus cerebellomotorius et -tegmentalis bulbaris. In both tracts Weston distinguished a motor component, directed to the motor nuclei of the cranial nerves, and a tegmental component, directed to other brainstem nuclei. The tractus cerebellomotorius et -tegmentalis mesencephali is considered to arise largely in the lateral cerebellar nucleus, but also in the medial cerebellar nucleus and the Purkyně cell layer of the cerebellar cortex. This tract includes cerebellar fibers to the red nucleus, described as the brachium conjunctivum by other students of the reptilian central nervous system (Ariens Kappers, '21; Larsell, '26, '32; Papez, '29; Shanklin, '30, Frederikse, '31; Hindenach, '31). No cerebellothalamic connections have been observed in these classical studies. The tractus cerebellomotorius et -tegmentalis bulbaris, the tractus cerebellovestibularis, and the tractus cerebellospinalis form the descending fiber system of the cerebellum. According to Weston these tracts are composed of efferent fibers, mainly arising in the medial cerebellar nucleus, but also in the lateral cerebellar nucleus and the cerebellar cortex. The crossed vestibular and spinal components of this fiber system, presumably originating mainly in the medial cerebellar nucleus, are considered to represent the reptilian homologue of the fasciculus uncinatus or hook bundle, as found in mammals (Russell, 1895). In a number of experimental studies, not directly aimed at clarifying the connections of the cerebellar nuclei, various targets of these nuclei have been demonstrated in several reptilian species (Lohman and van Woerden-Verkley, '78; ten Donkelaar and de Boer-van Huizen, '78a, '81b, Brauth and Kitt, '80; ten Donkelaar *et al.*, '80, Wolters *et al.*, '82a, Woodson and Kunzle, '82).

In the present study the connections of the cerebellar nuclei have been studied with various experimental anatomical techniques: (1) Anterograde degeneration studies, which present an outline of the efferent connections of the cerebellar nuclei. In five turtles, *Pseudemys scripta elegans*, and four lizards, *Varanus exanthematicus*, surgical lesions of the cerebellar peduncle were carried out, (2) Anterograde tracer studies, i.e. ^{3}H-leucine injections into the cerebellar nuclei of six lizards, and HRP slow-release gel implants into the cerebellar nuclei of six turtles, seven lizards, and two snakes, *Python regius*, revealed the efferent connections of the cerebellar nuclei more in detail; (3) Retrograde tracer studies, with HRP as well as fluorescent tracers, were used to demonstrate the precise localization of the cells of origin of ascending and descending connections of the cerebellar nuclei. Furthermore, the existence

of axonal branching of the efferents of the cerebellar nuclei was studied with the recently introduced multiple fluorescent tracer technique (Kuypers *et al.*, '80) in three turtles and four lizards.

EFFERENT CONNECTIONS OF THE CEREBELLAR NUCLEI IN THE TURTLE PSEUDEMYS SCRIPTA ELEGANS

Figure 53 shows one of five comparable cases in which a large lesion interrupted the cerebellar peduncle in the turtle *Pseudemys scripta elegans* (Fig. 53F, arrow). Both corticonuclear (including corticovestibular) connections and fibers arising in the cerebellar nuclei were damaged. Since, as demonstrated in the preceding chapter, projections from the cerebellar cortex are strictly ipsilateral and directed only to the cerebellar and vestibular nuclei, degenerating fibers present in the ipsilateral vestibular nuclear complex are at least partly due to damage to corticovestibular fibers. However, two bundles of degenerating fibers were observed, one ascending and one descending, both arising in the cerebellar nuclei.

The ascending pathway, i.e. the brachium conjunctivum, could be traced as far rostrally as the mesodiencephalic junction (Fig. 53A). Degenerating fibers were found bending ventrally, coursing along the lateral wall of the brainstem (Fig. 53D, E), decussating in the most caudal part of the midbrain (Fig. 53D) and ascending in the ventromedial part of the contralateral midbrain tegmentum. In Fink-Heimer sections terminal degeneration was found in the red nucleus and the interstitial nucleus of the flm (Fig. 53B, C). The descending pathway, i.e the fasciculus uncinatus, appeared to decussate partly in the cerebellar commissure and partly in the base of the corpus cerebelli (Fig. 53F). Degenerating fibers were observed passing dorsal to and through the medial cerebellar nucleus, thereby hooking around the cerebellar nuclei (Fig. 53F, G). These fibers could be traced to various levels of the vestibular nuclear complex (Fig. 53 G-L). Throughout the vestibular nuclei terminal degeneration was observed. Degenerating fibers could not be traced beyond the obex region

After HRP slow-release gel implants into the cerebellar peduncle, including the cerebellar nuclei (Chapter V), labeled fibers of the brachium conjunctivum and the fasciculus uncinatus could be traced anterogradely in a comparable way as found in the degeneration studies. So, with both anterograde degeneration and anterograde tracer techniques two main pathways were demonstrated, most likely arising in the cerebellar nuclei The origin of these pathways has been studied with retrograde tracers (HRP, and the fluorescent tracers FB and NY). HRP slow-release gel implantations into various levels of the vestibular nuclear complex demonstrated the origin of the cerebellovestibular projections (e.g. cases 6241 and 6245, Fig. 54). In such experiments retrogradely labeled neurons were observed in the medial cerebellar nucleus, mainly contralaterally (Fig. 54). In addition, labeled neurons were present bilaterally in the lateral cerebellar nucleus (Fig 54).

The use of the multiple fluorescent tracer technique in the turtle *Pseudemys scripta elegans* clearly showed the origin of the efferent pathways of the cerebellar nuclei, as well as the existence of axonal branching in neurons of these nuclei (case S83-15, Fig. 55). Injection of Fast Blue (FB) into the mesencephalon at the level of the nucleus ruber and the oculomotor nucleus resulted in a considerable number of FB-positive neurons in the contralateral

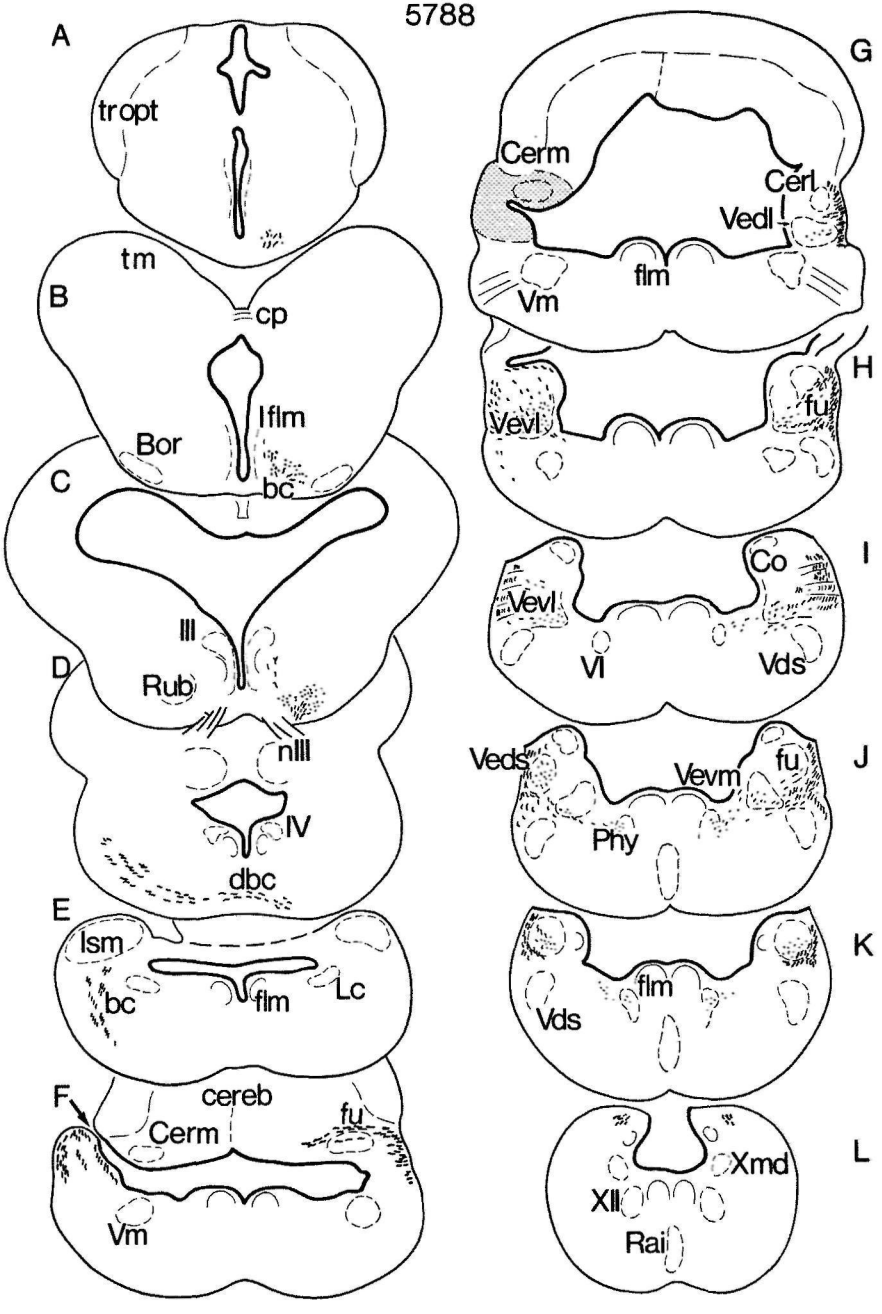


Fig. 53 Diagrammatic representation of the course and distribution of degenerating fibers within the brainstem following a surgical lesion of the cerebellar peduncle in the turtle Pseudemys scripta elegans; broken lines indicate degenerating fibers, dots evidence of terminal degeneration. For abbreviations cf. pages 28-29.

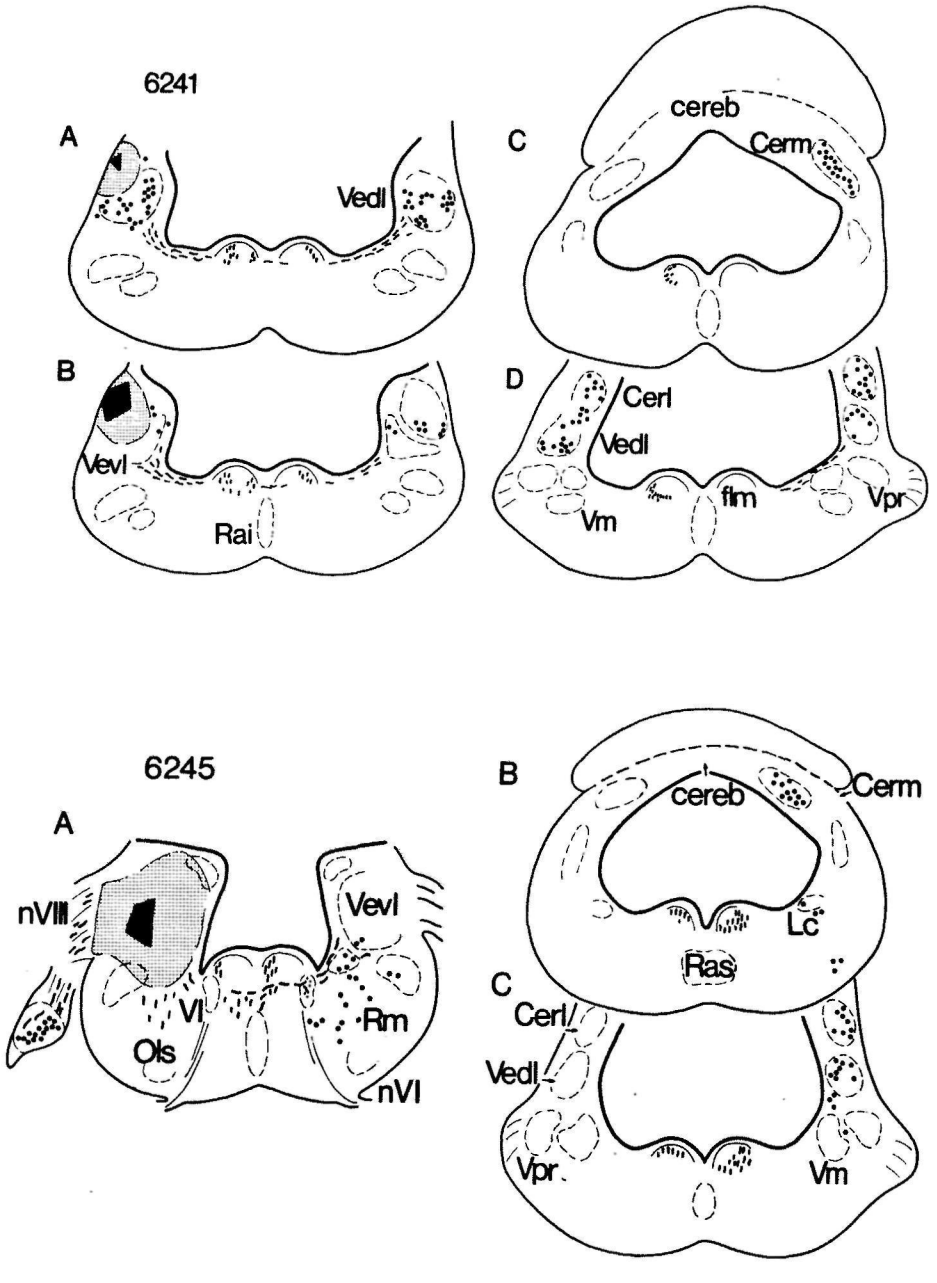


Fig. 54 The distribution of labeled neurons in the cerebellar nuclei following implantation of an HRP slow-release gel into the vestibular nuclear complex at two different levels in the turtle Pseudemys scripta elegans. For abbreviations cf. pages 28-29.

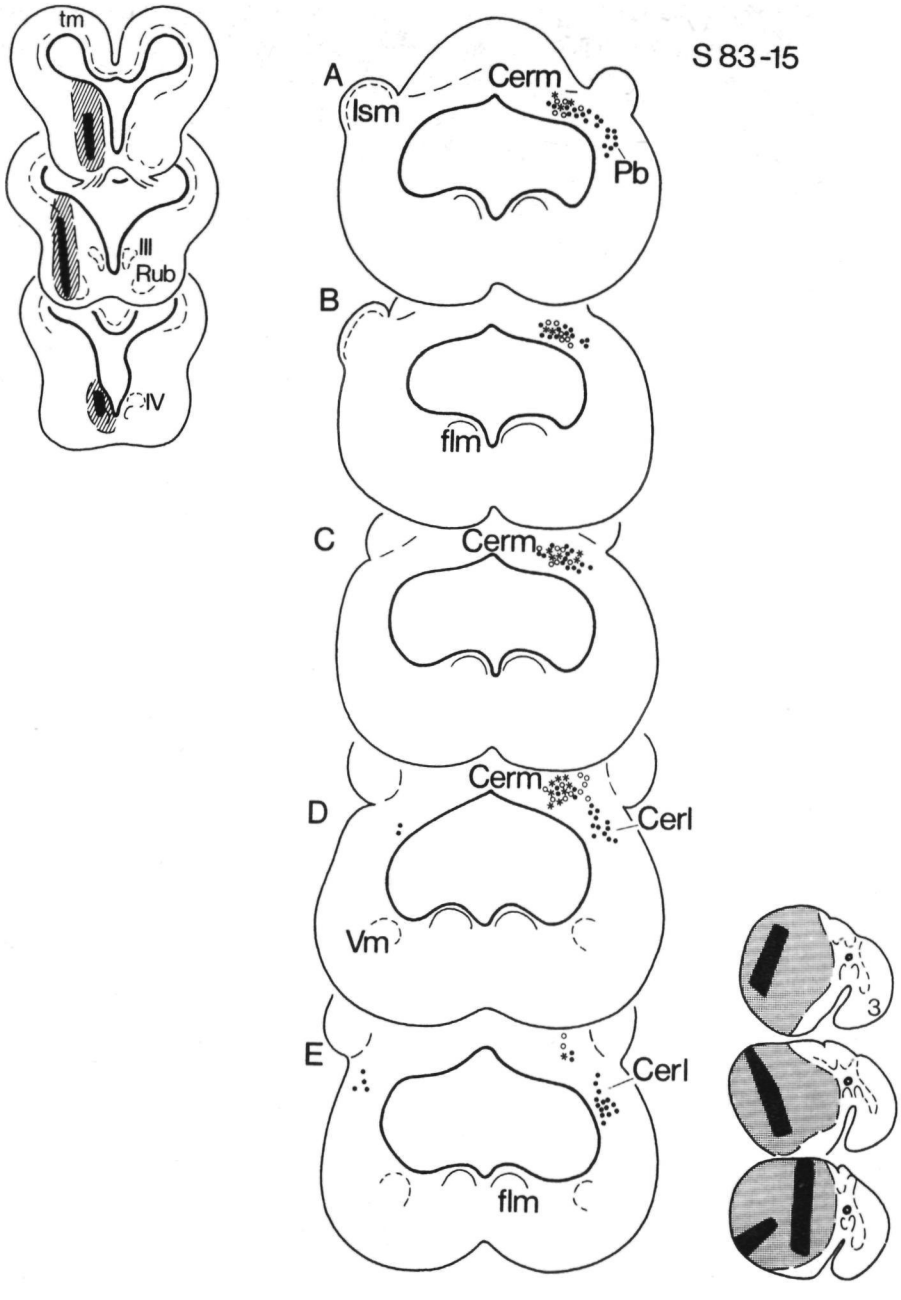


Fig. 55 The distribution of labeled neurons in the cerebellar nuclei following injection of 'Fast Blue' into the mesencephalon and implantation of 'Nuclear Yellow' slow-release gels into the spinal cord of the turtle Pseudemys scripta elegans. Filled dots indicate FB labeled neurons, open dots indicate NY labeled neurons, asterisks indicate double labeled neurons. For abbreviations cf. pages 28-29.

lateral and medial cerebellar nuclei (Fig. 55 A-E), indicating a projection of both nuclei to the mesencephalon. Implantation of a number of 'Nuclear Yellow' (NY) slow-release gels into the third spinal segment resulted in distinct labeling of the contralateral medial cerebellar nucleus (Fig. 55 A-E), showing the presence of a cerebellospinal projection arising in this nucleus. In the experiment shown in figure 55 a large part of the labeled neurons in the medial cerebellar nucleus contained both FB (in the cytoplasm) and NY (in the nucleus), i e. was double labeled. These data indicate that a number of neurons of the medial cerebellar nucleus projects both to the mesencephalon and the rostral spinal cord.

Thus, the efferent projections of the cerebellar nuclei in the turtle *Pseudemys scripta elegans* appeared to be organized mainly in two contralateral pathways. an ascending pathway, the brachium conjunctivum, arising partly in the medial but predominantly in the lateral cerebellar nucleus, and a descending pathway, the fasciculus uncinatus, arising mainly in the medial cerebellar nucleus.

EFFERENT CONNECTIONS OF THE CEREBELLAR NUCLEI IN THE LIZARD VARANUS EXANTHEMATICUS

Degeneration studies, as illustrated in figure 56 (case 6087), showed in outline the projections of the cerebellar nuclei in the lizard *Varanus exanthematicus*. In case 6087 a large lesion interrupted the cerebellar peduncle. Both corticonuclear fibers and efferent fibers of the cerebellar nuclei were damaged. As in *Pseudemys scripta elegans* two contralateral bundles of degenerating fibers were found, one ascending and one descending, both arising in the cerebellar nuclei. The ascending brachium conjunctivum bends ventrally along the lateral wall of the brainstem (Fig 56 H-J), decussates in the most caudal part of the midbrain (Fig. 56F, G), further ascends in the ventromedial part of the midbrain tegmentum, terminates extensively in the red nucleus (Fig. 56D, E) and continues rostrally at least as far as the mesodiencephalic junction. The descending fasciculus uncinatus decussates in the cerebellar commissure and the base of the corpus cerebelli (Fig. 56H, I), passes dorsal to and through the medial cerebellar nucleus, hooking around the cerebellar nuclei and the brachium conjunctivum, and reaches the vestibular nuclear complex along its whole length (Fig. 56 K-N). No evidence for terminal degeneration was observed in the vestibular nuclei.

With anterograde tracing techniques (^{3}H-leucine and HRP) these two main efferent pathways of the cerebellar nuclei have been further studied. Figure 57 shows an experiment (case V29) in which an injection of ^{3}H-leucine was made into the cerebellar peduncle. In figure 58 an experiment is illustrated (case V82-37), in which an HRP slow-release gel was implanted into the caudal part of the lateral cerebellar nucleus and the dorsolateral vestibular nucleus. In both cases the ascending brachium conjunctivum was extensively labeled and could be traced anterogradely at least as far as the mesodiencephalic junction Evidence for terminal structures was found in the interstitial nucleus of the flm (Figs. 57A, 58A) and the red nucleus (Figs. 57B, C, 58B, 59A). Also the fasciculus uncinatus was extensively labeled in these cases. It was observed decussating in the basal part of the corpus cerebelli (Figs. 57F, 58E), hooking around the medial cerebellar nucleus (Figs. 57F, G, 58E, 59C), and reaching the vestibular nuclear complex from the lateral side of the brainstem (Figs 57 H-L; 58 F-J). Especially in case V82-37 HRP labeled terminal structures were observed throughout

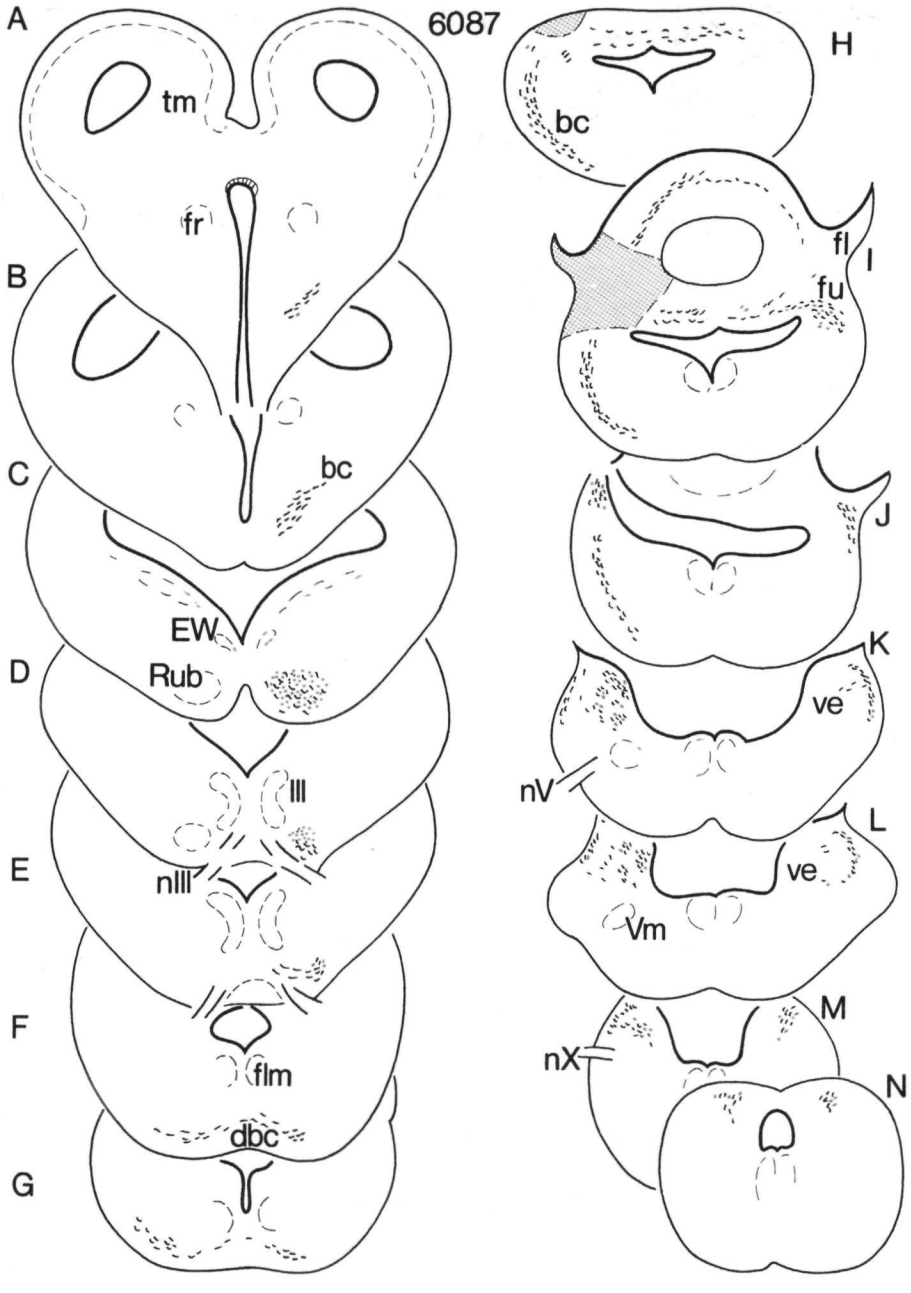


Fig. 56 Diagrammatic representation of the course and distribution of degenerating fibers within the brainstem after a surgical lesion of the cerebellar peduncle in the lizard Varanus exanthematicus; broken lines indicate degenerating fibers, dots evidence of terminal degeneration. For abbreviations cf. pages 28-29.

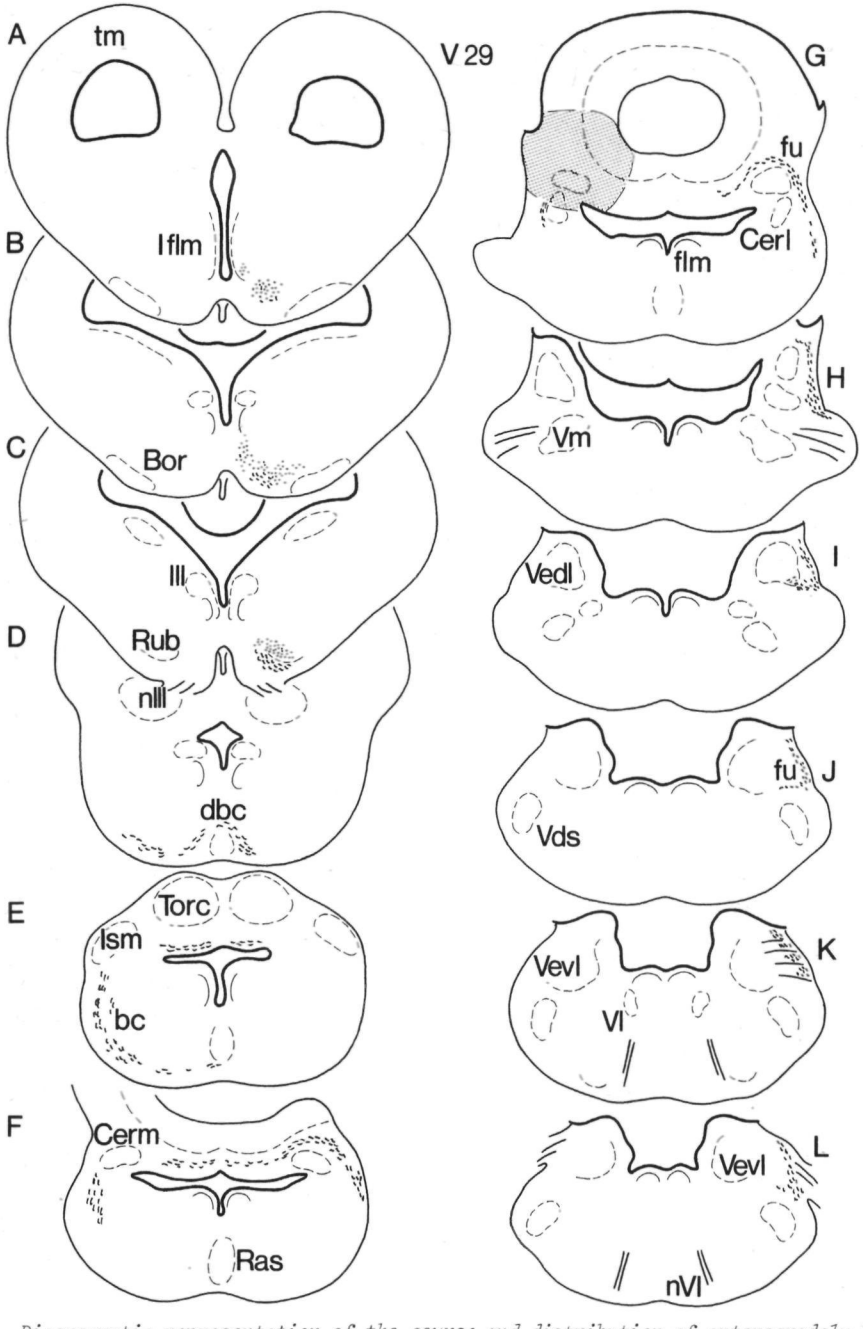


Fig. 57 Diagrammatic representation of the course and distribution of anterogradely labeled fibers in the brainstem after injection of ^{3}H-leucine into the cerebellar nuclei of the lizard Varanus exanthematicus; broken lines indicate labeled fibers, dots evidence of labeled terminal structures. For abbreviations cf. pages 28-29.

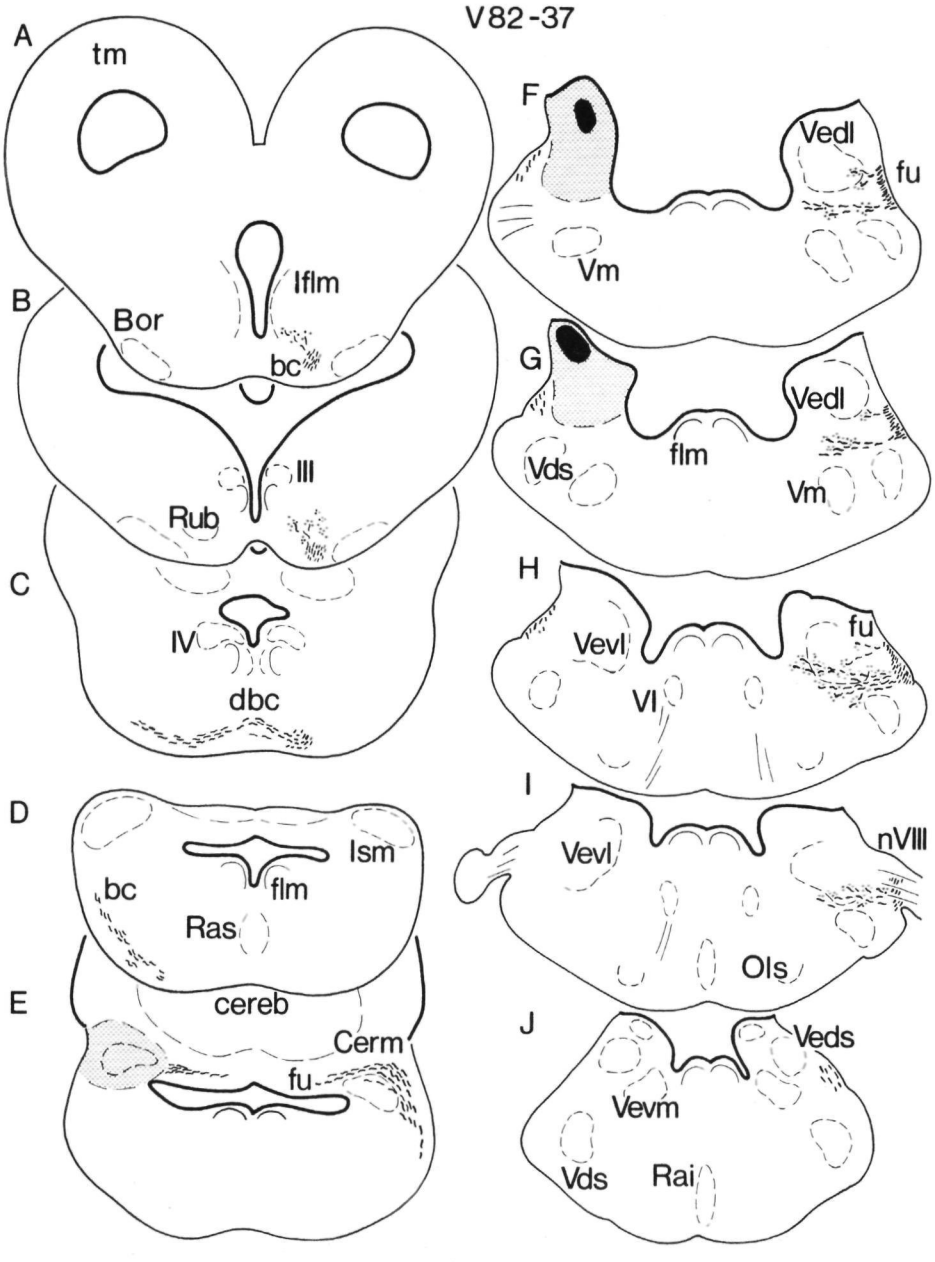


Fig. 58 Diagrammatic representation of the course and distribution of anterogradely labeled fibers in the brainstem after implantation of an HRP slow-release gel into the dorsolateral vestibular nucleus and the caudal part of the lateral cerebellar nucleus in the lizard Varanus exanthematicus; broken lines indicate labeled fibers, dots evidence of labeled terminal structures. For abbreviations cf. pages 28-29.

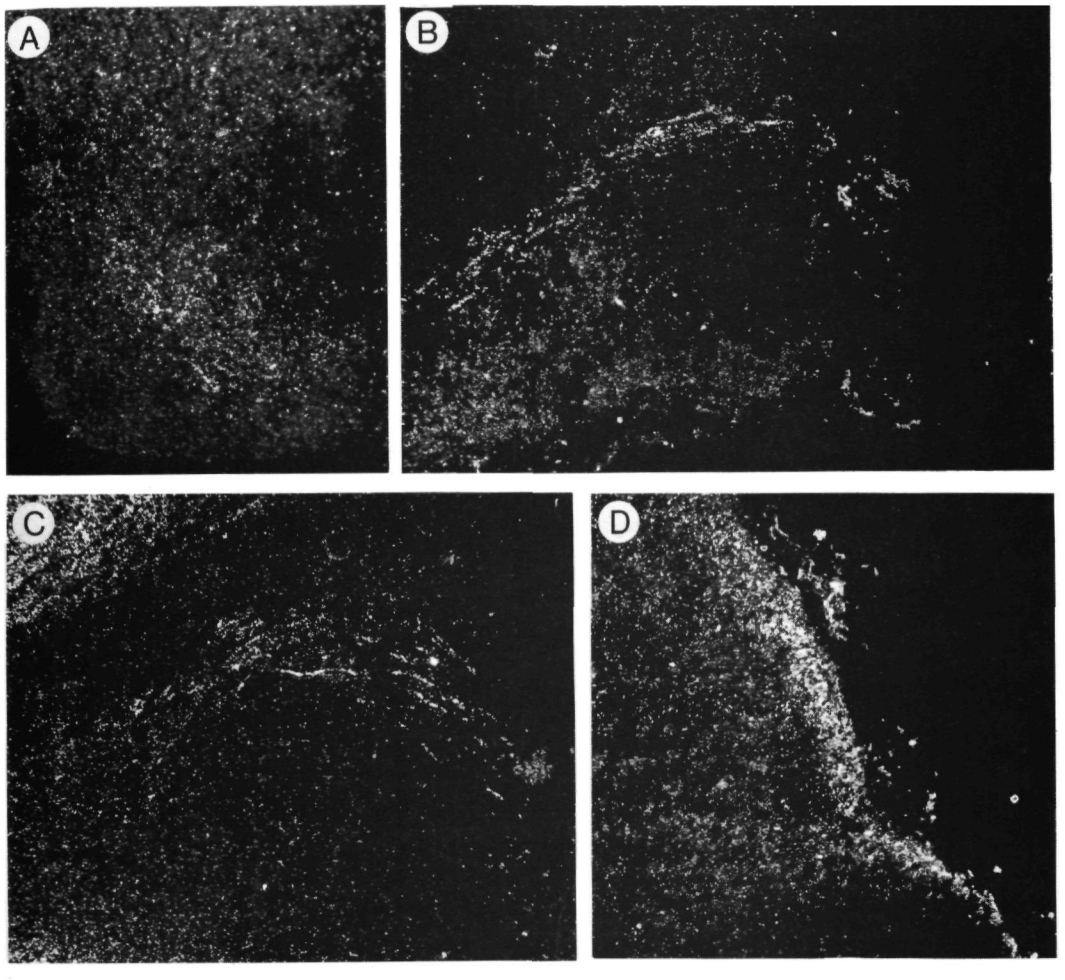


Fig. 59 Darkfield photomicrographs of autoradiographic labeling in the brainstem after injection of ^{3}H-leucine into the cerebellar nuclei of the lizard Varanus exanthematicus (case V29; see also Fig. 57). A, Labeled terminal structures in the nucleus ruber, ×50; B, Labeled decussating fibers of the brachium conjunctivum, ×80; C, Labeled fibers of the fasciculus uncinatus hooking around the contralateral medial cerebellar nucleus, ×80; D, Labeled fibers of the fasciculus uncinatus lateral to the vestibular nuclear complex, ×80. For abbreviations cf. pages 28-29.

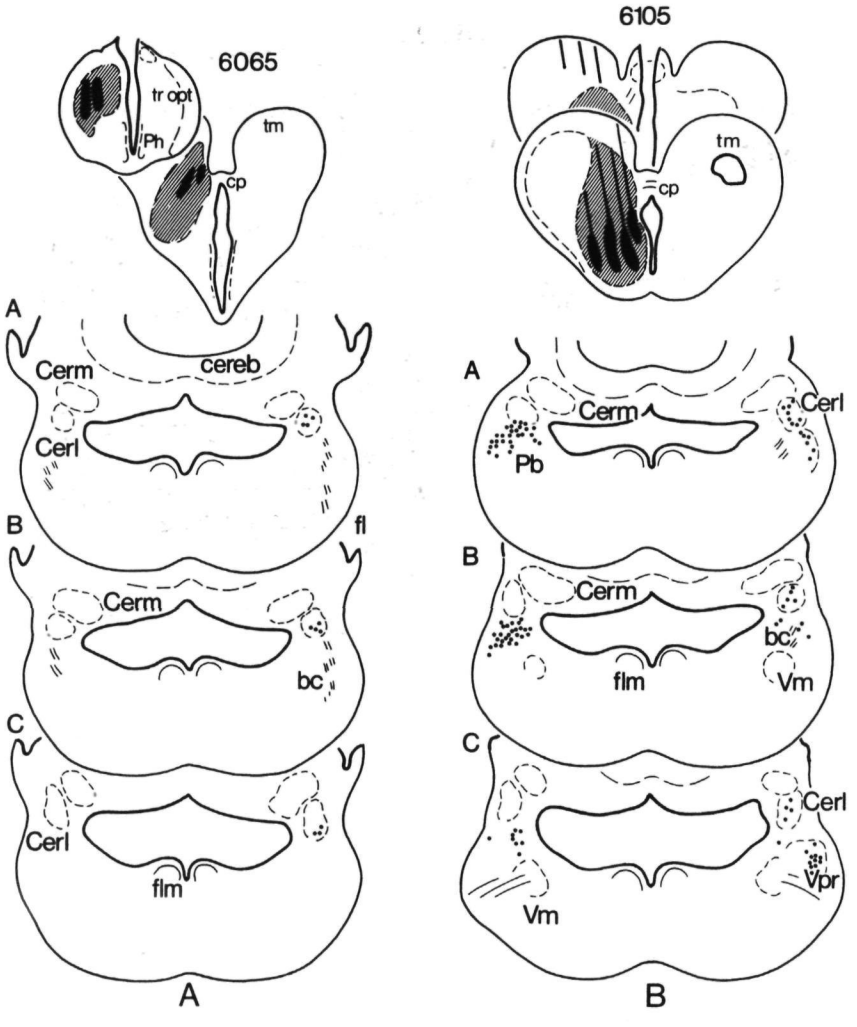


Fig. 60 The distribution of labeled neurons in the cerebellar nuclei following injection of HRP into: A, the diencephalon (case 6065); B, the rostral mesencephalon (case 6105) of the lizard Varanus exanthematicus (after ten Donkelaar and de Boer-van Huizen, '81b). The indicated brachium conjunctivum contained no labeled fibers. For abbreviations cf. pages 28-29.

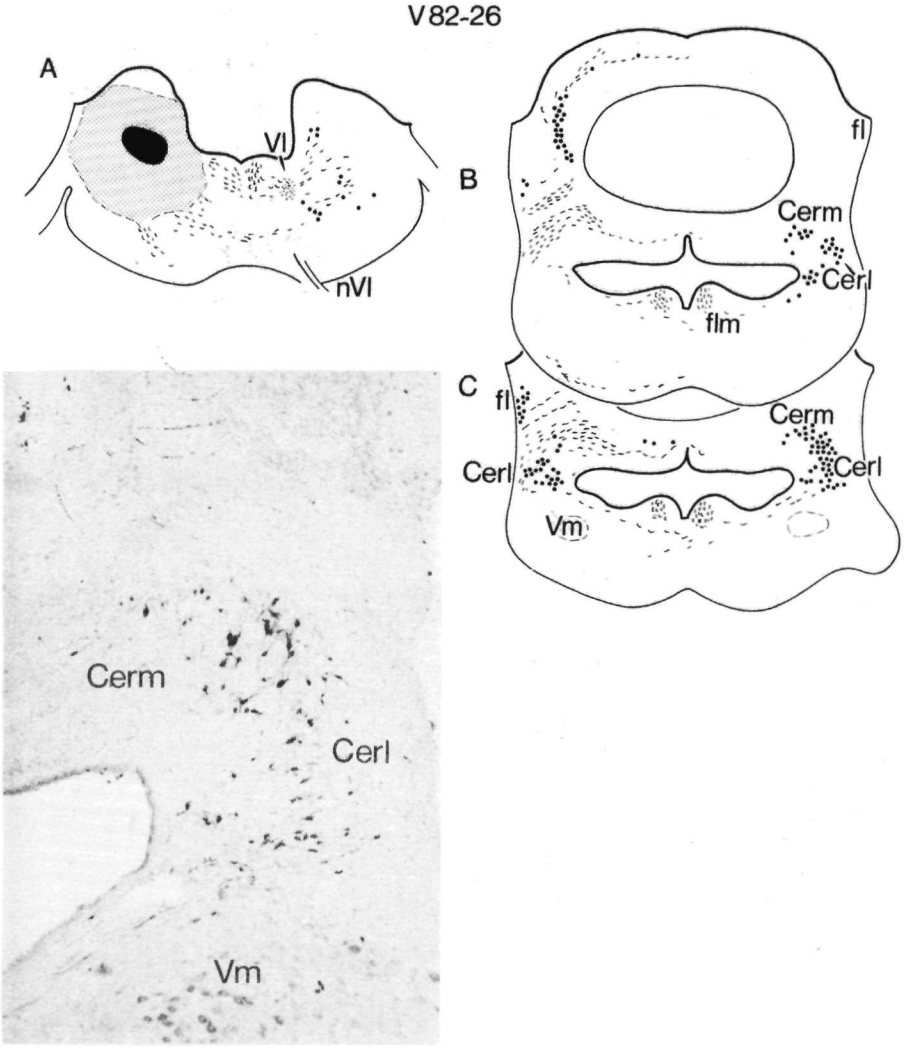


Fig. 61 The distribution of labeled neurons in the cerebellar nuclei following implantation of an HRP slow-release gel into the ventrolateral vestibular nucleus of the lizard Varanus exanthematicus. Insert: retrogradely labeled neurons in the medial and lateral cerebellar nuclei, TMB incubation, ×61. For abbreviations cf. pages 28-29.

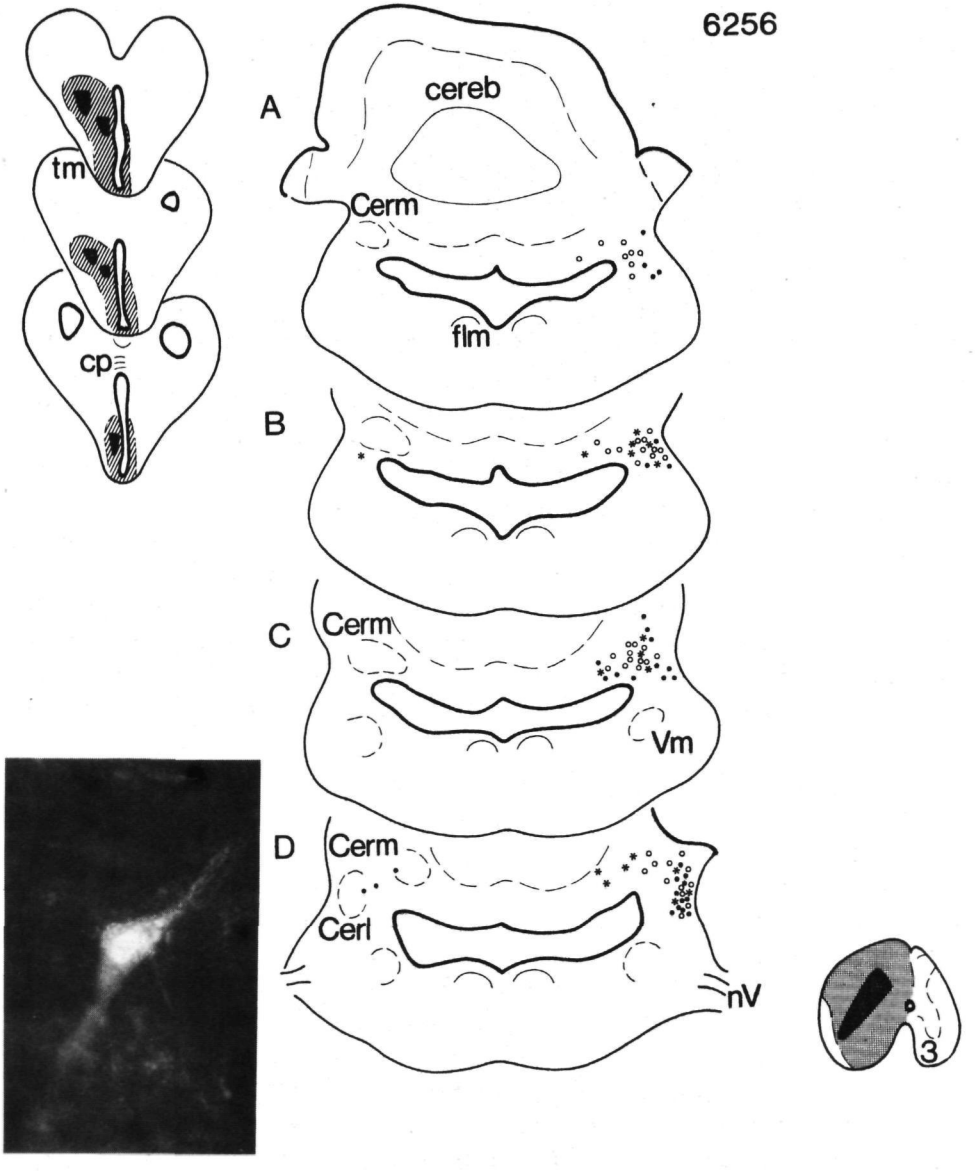


Fig. 62 The distribution of labeled neurons in the cerebellar nuclei following injection of 'Fast Blue' into the rostral mesencephalon and implantation of 'Nuclear Yellow' slow-release gels into the spinal cord of the lizard Varanus exanthematicus. Filled dots indicate FB labeled neurons, open dots indicate NY labeled neurons, asterisks indicate double labeled neurons. Insert: double labeled neuron in the contralateral medial cerebellar nucleus, × 350.

the ventral part of the vestibular nuclear complex (Fig. 58 F-I) and in adjacent parts of the reticular formation (Fig. 58H, I). Ipsilaterally almost no labeled descending fibers arising in the cerebellar nuclei could be traced anterogradely in these studies.

The cells of origin of the ascending and descending projections of the cerebellar nuclei in the lizard *Varanus exanthematicus* were demonstrated both with HRP and fluorescent tracers HRP injections into the diencephalon (Fig. 60A), and at the level of the mesodiencephalic junction (Fig. 60B) showed retrogradely labeled neurons in the lateral cerebellar nucleus contralateral to the injection side. HRP slow-release gel implants into various levels of the vestibular nuclear complex (e.g. case V82-26, Fig. 61) resulted in labeled neurons bilaterally in both cerebellar nuclei, but especially in the contralateral medial cerebellar nucleus (Fig 61B, C).

In figure 62 one of four comparable cases is shown (case 6256) in which FB injections into the thalamus and rostral mesencephalon were combined with NY slow-release gel implants into the rostral segments of the spinal cord. In such experiments the pattern of cell labeling in the medial and lateral cerebellar nuclei confirmed the results of HRP experiments. The greatest number of FB positive neurons was found in the contralateral lateral cerebellar nucleus, although also ipsilaterally labeled neurons were present in this nucleus (Fig. 62C, D). However, also in the contralateral medial cerebellar nucleus a considerable number of FB positive neurons was present. These data indicate that in *Varanus exanthematicus*, as in *Pseudemys scripta elegans*, both cerebellar nuclei have ascending projections to the mesencephalon, the main part arising in the lateral cerebellar nucleus. Regarding the spinal projections, the pattern of labeling appeared to be the reverse. Most NY labeled neurons were found in the contralateral medial cerebellar nucleus (Fig. 62 A-D), indicating a cerebellospinal projection arising mainly in the medial cerebellar nucleus. Double labeled neurons were observed in both cerebellar nuclei, but mainly in the medial cerebellar nucleus (Fig. 62 A-D).

EFFERENT CONNECTIONS OF THE CEREBELLAR NUCLEI IN THE SNAKE PYTHON REGIUS

To establish the efferent connections of the cerebellar nuclei in the snake *Python regius* a number of HRP injections or HRP slow-release gel implants was made into various levels of the brainstem and spinal cord, known to receive cerebellar afferents in other reptiles. Some of these experiments are shown in the figures 63 and 64. In those experiments of the present series in which HRP slow-release gels included the cerebellar nuclei no ascending labeled fibers were observed.

In the first experiment shown in figure 63 (case 6202) an HRP injection was made into the mesencephalic tegmentum at the level of the interpeduncular nucleus. Labeled neurons were present in the lateral cerebellar nucleus, especially contralaterally. In case P82-01 (Fig. 63) an HRP slow-release gel was implanted into the ventrolateral vestibular nucleus. Retrogradely labeled neurons appeared to be present in both cerebellar nuclei, strictly contralaterally. As shown in the sections A-C, in rostrocaudal direction labeled neurons gradually shift mediolaterally In section D labeled neurons were also present in the rostral part of the dorsolateral vestibular nucleus In the third experiment illustrated in figure 63 (case 6274),

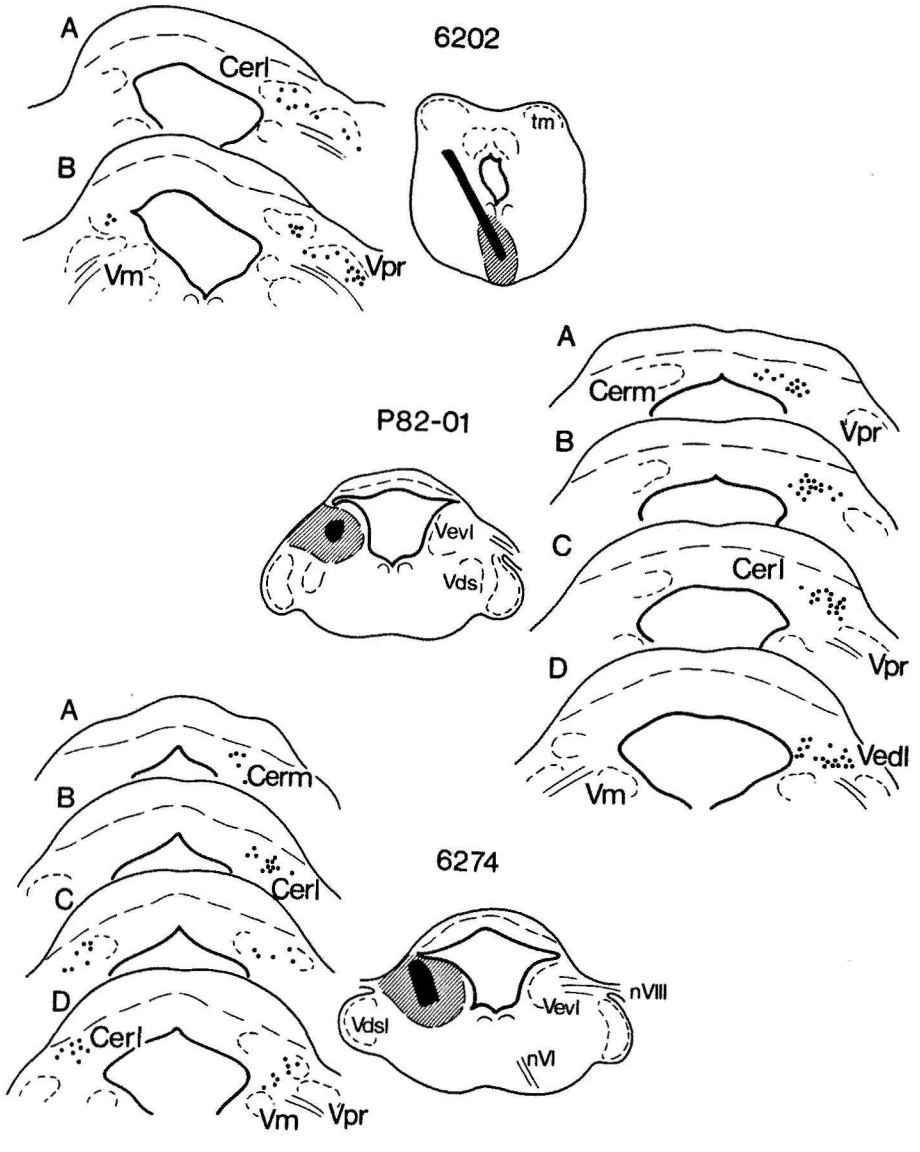


Fig. 63 The distribution of labeled neurons in the cerebellar nuclei following application of HRP to various levels of the brainstem in the snake Python regius. For abbreviations cf. pages 28-29.

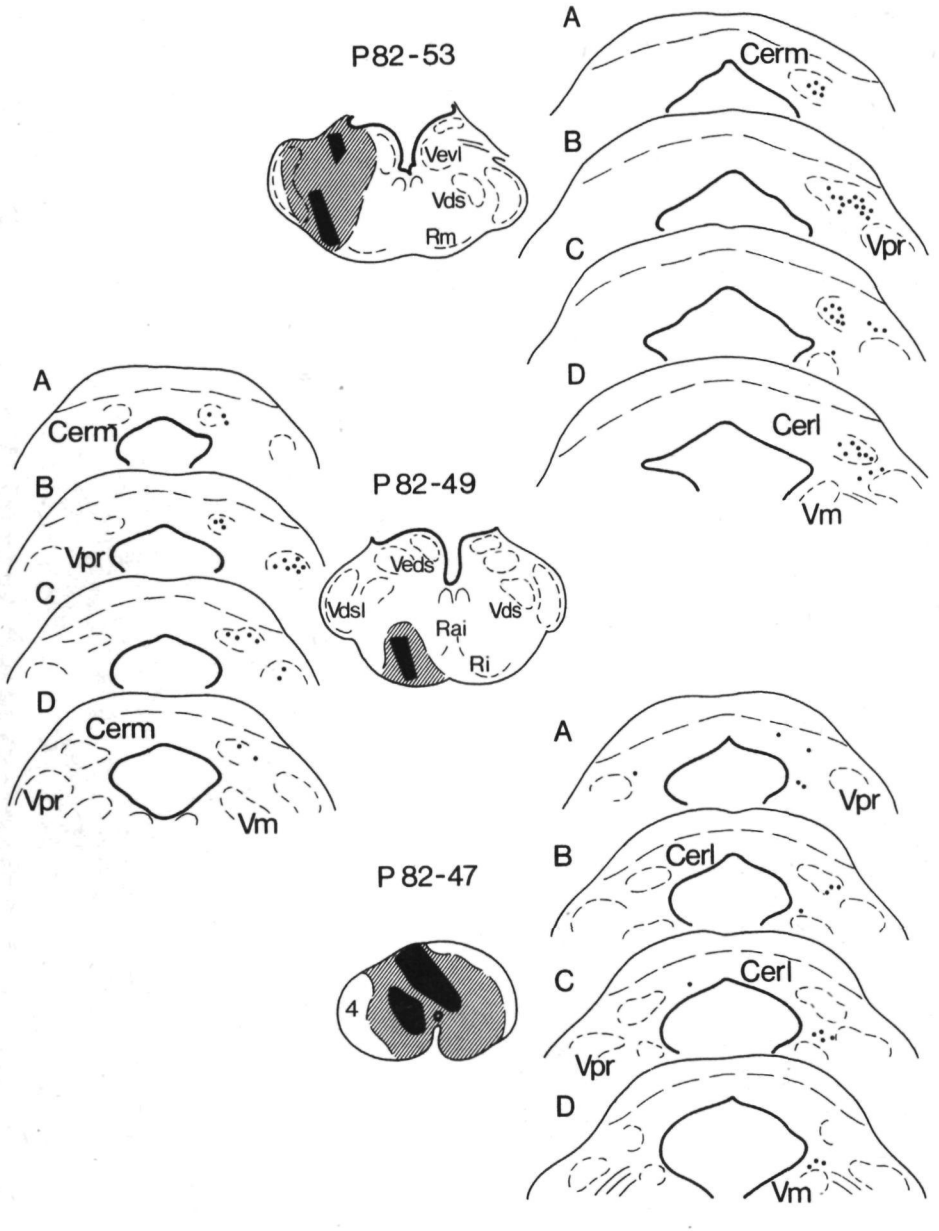


Fig. 64 The distribution of labeled neurons in the cerebellar nuclei following application of HRP to various levels of the brainstem and the spinal cord in the snake Python regius. For abbreviations cf. pages 28-29.

an HRP slow-release gel was implanted into the vestibular nuclear complex, slightly more caudally than in case P82-01. The contralateral medial cerebellar nucleus contained only few labeled neurons (section A), but the lateral cerebellar nucleus was distinctly labeled in this case, both ipsilaterally and contralaterally (section B-D). In case P82-53 (Fig. 64) two HRP slow-release gels were implanted one into the vestibular nuclear complex, and one into the lateral part of the rhombencephalic reticular formation. Both the contralateral medial and lateral cerebellar nuclei were distinctly labeled. As in case P82-01 (Fig 63) a gradual shift of labeled neurons in mediolateral direction was observed. In the second experiment illustrated in figure 64 (case P82-49), in which an HRP slow-release gel was implanted into the caudal part of the rhombencephalic reticular formation, particularly the contralateral medial cerebellar nucleus was labeled. Case P82-47 of figure 64 is representative for eight experiments in which HRP was either injected or implanted as a slow-release gel into the rostral part of the spinal cord In all such cases only few labeled neurons were found in the cerebellar nuclei which were distinctly labeled in the aforementioned experiments. Instead, after application of HRP to the spinal cord, a small distinctly labeled contralateral cell group was observed, located directly ventral to the cerebellar nuclei and lateral to the fourth ventricle In Nissl-stained material this small cell mass appeared to consist of rather large multipolar neurons Apparently this cell mass also belongs to the cerebellar nuclei, and presumably forms part of the medial cerebellar nucleus.

In summary, the results of the HRP experiments in *Python regius* indicate the presence of only a rather sparse contralateral projection of the lateral cerebellar nucleus to the tegmentum mesencephali (case 6202). The medial cerebellar nucleus projects predominantly contralaterally to the vestibular nuclear complex (cases P82-01, 6274, P82-53), whereas this complex also receives a distinct bilateral projection from the lateral cerebellar nucleus (cases P82-01, 6274, P82-53). The medial cerebellar nucleus projects contralaterally to the reticular formation (case P82-49) Cerebellospinal projections seem to arise only for a minor part in the medial and lateral cerebellar nuclei. Instead, a distinct contralateral spinal projection was observed from a separate cell mass adjacent to the cerebellar nuclei (case P82-47).

DISCUSSION

The data obtained in the present investigation on the efferent connections of the cerebellar nuclei of some reptiles are schematically summarized in figure 65. Figure 65A shows the projections found in *Varanus exanthematicus*, but also applies to *Pseudemys scripta elegans*, figure 65B summarizes the projections observed in *Python regius*

The *lateral cerebellar nucleus* in the turtle *Pseudemys scripta elegans* and the lizard *Varanus exanthematicus* (Fig. 65A) gives rise to a well-developed ascending projection, i.e. the brachium conjunctivum. With anterograde degeneration and tracing techniques this projection could be followed at least as far rostrally as the mesodiencephalic junction, profusely terminating on the red nucleus A smaller contralateral projection was found to the interstitial nucleus of the flm HRP studies in the lizard *Varanus exanthematicus* also indicated projections of the lateral cerebellar nucleus to the contralateral diencephalon and prosencephalon (ten

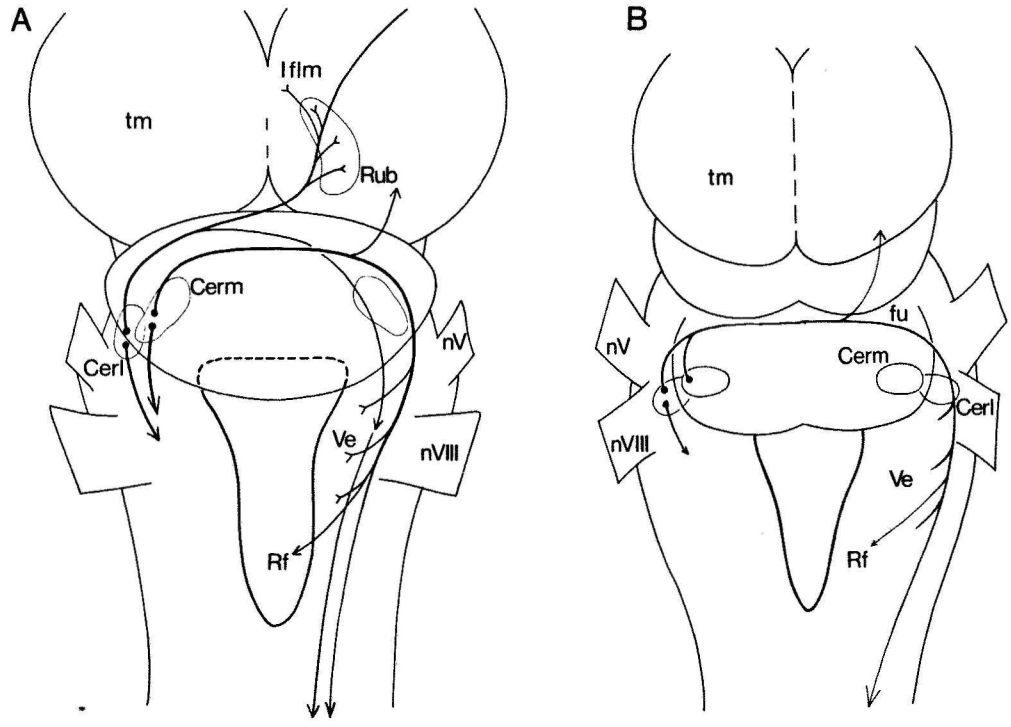


Fig. 65 Diagrams, summarizing the efferent projections of the cerebellar nuclei in Varanus exanthematicus (A), also applying to Pseudemys scripta elegans, and Python regius (B). For abbreviations cf. pages 28-29.

Donkelaar and de Boer-van Huizen, '81b; the present investigation), in keeping with experimental data of Lohman and van Woerden-Verkley ('78) in the lizard *Tupinambis nigropunctatus*. In *Caiman crocodilus* the lateral cerebellar nucleus was found to project to the contralateral thalamus, i.e. the ventral lateral and ventral medial thalamic area. Neurons of the ventral lateral area project to an area of the rostral telencephalon external to the ventrolateral area (Brauth and Kitt, '80), which suggests the existence of a bisynaptic cerebellotelencephalic projection in *Caiman crocodilus*. In *Python regius* (Fig. 65B) only a rather small ascending projection of the lateral cerebellar nucleus was found. With the tracer HRP a small projection of this nucleus to the contralateral mesencephalic tegmentum was observed. In Häggqvist and Klüver-Barrera stained material of *Python reticulatus* a brachium conjunctivum could not be distinguished, in contrast with the other reptiles studied (see Chapter III, figs. 4-15, ten Donkelaar and Nieuwenhuys, '79).

In all three reptilian species studied a well-developed descending contralateral

projection of the lateral cerebellar nucleus was observed (Fig. 65A, B). This projection appeared to be directed mainly to the vestibular nuclear complex, but is probably also directed to the reticular formation (ten Donkelaar and de Boer-van Huizen, unpublished observations in *Varanus exanthematicus*, the present investigation). In keeping with previous HRP data (ten Donkelaar and de Boer-van Huizen, '78a, ten Donkelaar *et al.*, '80) in the present study a contralateral projection of the lateral cerebellar nucleus to the spinal cord was observed in the lizard *Varanus exanthematicus* with the retrogradely transported tracer NY. After NY implants no spinal projection of the lateral cerebellar nucleus was observed in *Pseudemys scripta elegans* (Fig 55), although HRP data (ten Donkelaar *et al* , '80) suggest a projection of this nucleus to the contralateral spinal cord in this reptile. The descending contralateral projection of the lateral cerebellar nucleus presumably passes via the fasciculus uncinatus, since in the present study no separate descending limb of the brachium conjunctivum was observed. In addition, the lateral cerebellar nucleus was found to project ipsilaterally to the vestibular nuclear complex in all reptiles studied.

In both the turtle *Pseudemys scripta elegans* and the lizard *Varanus exanthematicus* an ascending projection of the *medial cerebellar nucleus* to the contralateral mesencephalon was demonstrated, especially after FB injections into the mesencephalon Previously, only sparse ascending projections of this nucleus have been described in the lizard *Varanus exanthematicus* (ten Donkelaar and de Boer-van Huizen, '81b) Since in the present study no separate ascending branch of the fasciculus uncinatus could be distinguished, the efferent fibers of the medial cerebellar nucleus to the mesencephalon might join the brachium conjunctivum. In *Python regius* no evidence was found for an ascending projection of the medial cerebellar nucleus.

In all three reptilian species studied, the main part of the efferent fibers arising in the medial cerebellar nucleus appeared to be organized in a distinct descending fiber tract, decussating in the cerebellar commissure and the basal part of the corpus cerebelli, i.e. the fasciculus uncinatus (Fig 65) This fiber tract, which could be traced anterogradely in degeneration studies and with HRP and ^{3}H-leucine to the caudal brainstem, appeared to be directed mainly to the vestibular nuclear complex, whereas a minor projection to the reticular formation was observed

A distinct contralateral spinal pathway was observed arising in the medial cerebellar nucleus in *Pseudemys scripta elegans*, *Varanus exanthematicus* and *Python regius*, in keeping with previous data (ten Donkelaar and de Boer-van Huizen, '78a; ten Donkelaar *et al.*, '80, ten Donkelaar, '82, Wolters *et al* , '82a, Woodson and Kunzle, '82). This projection, mainly aimed at the rostral cervical segments, reaches as far as the lumbar intumescence in *Varanus exanthematicus* (ten Donkelaar, '82)

In addition, in *Pseudemys scripta elegans* and *Varanus exanthematicus*, an ipsilateral projection of the medial cerebellar nucleus to the vestibular nuclear complex was observed

The results of the multiple fluorescent tracer studies in *Pseudemys scripta elegans* and *Varanus exanthematicus* indicate that in these reptilian species at least part of the neurons of the cerebellar nuclei, especially the medial cerebellar nucleus, have both ascending and descending axon collaterals to the mesencephalon and the rostral part of the spinal cord, respectively.

In summary, the efferent projections of the cerebellar nuclei in the turtle *Pseudemys scripta elegans*, the lizard *Varanus exanthematicus*, and the snake *Python regius* appeared to be organized mainly in two, more or less developed, contralateral pathways. An ascending pathway, the brachium conjunctivum, particularly evident in the quadrupedal reptiles studied, arising in the lateral and partly in the medial cerebellar nucleus, was demonstrated, as well as a descending pathway, the fasciculus uncinatus, arising mainly in the medial cerebellar nucleus.

VII. GENERAL DISCUSSION

In this study the afferent and efferent connections of the cerebellum have been investigated with various experimental anatomical techniques in the turtle *Pseudemys scripta elegans*, the lizard *Varanus exanthematicus*, and the snake *Python regius*. The results of these experimental studies, presented in the chapters IV - VI, will now be discussed in a comparative perspective. Furthermore, an attempt will be made to discuss some aspects of cerebellar function in reptiles, as inferred from ablation studies, stimulation experiments, and the presently available experimental anatomical data on the connectivity of the reptilian cerebellum.

COMPARATIVE ASPECTS OF CEREBELLAR AFFERENTS IN TERRESTRIAL VERTEBRATES

The origin of cerebellar afferents in the turtles *Pseudemys scripta elegans* and *Testudo hermanni*, the lizard *Varanus exanthematicus* and the snake *Python regius* has been demonstrated with the aid of the retrograde axonal transport of the enzyme HRP and the fluorescent tracer 'Fast Blue'. Following injection of these tracers into the cerebellum of the reptiles studied, a number of afferent projections appeared to exist, present in the four reptilian species studied (see Chapter IV), and schematically summarized in figure 66:

- projections of the nucleus of the basal optic root, relaying retinal input to the cerebellar cortex;
- a rubrocerebellar pathway, directed to the lateral cerebellar nucleus (except in *Python regius*);
- primary and secondary vestibulocerebellar projections;
- projections of the perihypoglossal nuclear complex, probably related to oculomotor and vestibulomotor control;
- a crossed olivocerebellar projection, originating in the inferior olive and terminating as climbing fibers in the molecular layer;
- somatosensory projections, originating in the nucleus descendens nervi trigemini and the nucleus fasciculi dorsalis;
- primary and secondary spinocerebellar projections, organized as dorsal and ventral spinocerebellar tracts.

In the lizard *Varanus exanthematicus* the crossed olivocerebellar projection was confirmed by means of anterograde axonal transport of both ^{3}H-leucine and WGA-HRP. In such experiments the olivocerebellar projection appeared to be characterized by a longitudinally oriented pattern of labeled terminal structures in the molecular layer.

Comparing the organization of cerebellar afferent projections of reptiles as demonstrated in the present study with the corresponding fiber systems in amphibians, birds, and mammals a basic pattern of cerebellar afferents appears to exist common to these groups of vertebrates including retinal, vestibular, precerebellar, somatosensory, and spinal projections.

Bisynaptic retinal afferents to the cerebellum by way of the nucleus of the basal optic root have been demonstrated experimentally in reptiles, birds (Clarke, '77; Brecha *et al.*, '80; Arends and Blok, '83), and mammals (Winfield *et al.*, '78). In birds the basal optic root

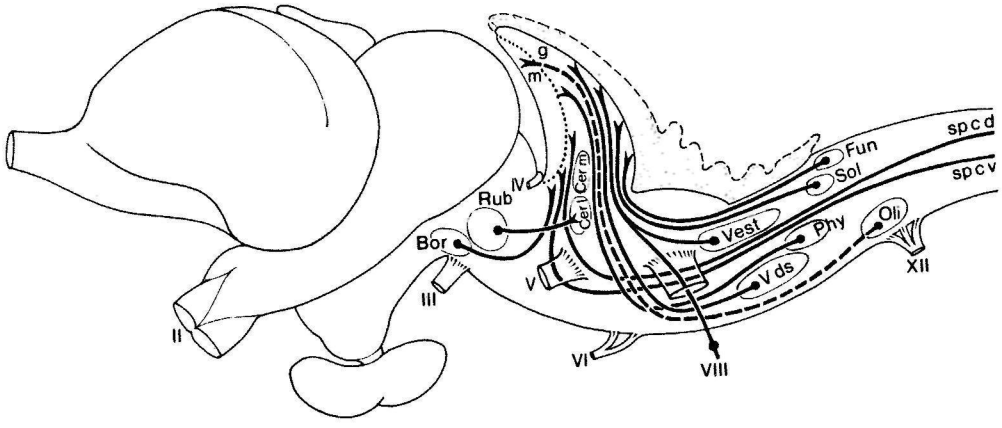


Fig. 66 Diagram summarizing the reptilian cerebellar afferent connections illustrated in Varanus exanthematicus. Bor, nucleus of the basal optic root; Cerl, Cerm, lateral and medial cerebellar nuclei; Fun, nucleus funiculi dorsalis; g, granular layer; m, molecular layer; Oli, oliva inferior; Phy, perihypoglossal nuclear complex; Rub, nucleus ruber; Sol, nucleus tractus solitarii; spcd, spcv, dorsal and ventral spinocerebellar tracts; Vest, vestibular nuclear complex; II, nervus opticus; III, nervus oculomotorius; IV, nervus trochlearis; V, nervus trigeminus; Vds, nucleus descendens nervi trigemini; VI, nervus abducens; VIII, nervus octavus; XII, nervus hypoglossus.

arises from displaced ganglion cells in the inner nuclear layer of the retina (Reiner *et al.*, '79). In the turtle (Reiner, '81) and the chinchilla (Kimm *et al.*, '79) the fibers of this root originate in large ganglion cells in the ganglionic layer of the retina. In amphibians this retinocerebellar connection by way of the basal optic root has not yet been demonstrated anatomically although destruction of the nucleus of the basal optic root (Lazar, '73) evoked physiological effects, i.e. optokinetic nystagmus, comparable to those reported for reptiles and birds (Fite *et al.*, '79). Moreover, visual responses in the cerebellum of the frog have been reported (Ansorge and Grüsser-Cornehls, '78).

Primary vestibulocerebellar connections have been demonstrated in amphibians (Llinás *et al.*, '67; Fuller, '74), reptiles (the present study), birds (Brecha *et al.*, '80), and mammals (e.g. Kotchabhakdi and Walberg, '78a; Korte and Mugnaini, '79). Likewise secondary vestibulocerebellar fibers from the vestibular nuclear complex have been demonstrated in amphibians (Fuller, '74), reptiles (Schwarz and Schwarz, '80; Künzle, '83; the present study), birds (Brecha *et al.*, '80; Arends and Blok, '83), and mammals. In mammals, the secondary vestibulocerebellar fibers arise mainly in the ventromedial and descending vestibular nuclei as well as in several subgroups of the vestibular nuclear complex (see e.g. Brown-Gould, '80 for review).

Considerable differences appear to exist in the projections of the so-called precerebellar nuclei in amphibians, reptiles, birds, and mammals. In birds and mammals cerebellar afferent projections of the pontine nuclei mediating mainly telencephalic efferent

impulses to the cerebellum have been demonstrated and extensively studied (Clarke, '77; Brecha *et al.*, '80, Arends and Blok, '83; Brodal, '81 for review). Until now in reptiles and amphibians a primordium of pontine nuclei relaying input from the telencephalon to the cerebellum has not been demonstrated. The same holds true for two other precerebellar nuclei known in mammals, the nucleus funiculi lateralis and the nucleus reticularis paramedianus, which are at best only present as primordia in the reptilian brainstem. The only precerebellar nucleus common to amphibians, reptiles, birds, and mammals seems to be the inferior olivary nucleus. Recently an olivocerebellar projection was demonstrated in the frog (Cochran and Hackett, '77, '80; Grover and Grusser-Cornehls, '80). In birds (Feirabend *et al.*, '76; Freedman *et al.*, '77; Feirabend, '83), reptiles (the present study), and mammals (e.g. Brodal, '40, '80; Oscarsson, '69, '80, Groenewegen and Voogd, '77, Groenewegen *et al.*, '79) efferents of the inferior olive terminate as climbing fibers in a longitudinal pattern in the cerebellum.

Somatosensory afferent fibers to the cerebellum originating in the trigeminal nuclear complex were demonstrated in mammals (see e.g. Brown-Gould, '80), birds (Arends and Blok, '83) and reptiles (Kunzle, '83, the present study), but in amphibians a cerebellar projection of the trigeminal nuclear complex has not been demonstrated experimentally.

Relatively few data have been reported on the origin of spinocerebellar fibers in nonmammalian vertebrates. In the frog primary spinal afferents ascending by way of the dorsal funiculus to the cerebellum have been demonstrated by Joseph and Whitlock ('68) and recently confirmed with the cobalt tracer technique by Antal *et al.* ('80) and Szêkely *et al.* ('80). In addition cells of origin of spinocerebellar fibers have been found in the dorsal and ventral horns of the frog spinal cord (Grover and Grüsser-Cornehls, '80). In the pigeon Brecha *et al.* ('80) observed some neurons projecting to the cerebellum located in high cervical segments of the spinal cord, but no further data exist on the origin of spinocerebellar connections in birds. In mammals the spinocerebellar pathways have been studied in great detail. Five main spinocerebellar tracts are known, the dorsal , ventral, cuneocerebellar, rostral, and cervical spinocerebellar tracts (see e.g. Brodal, '81 for review). The reptilian spinocerebellar projection as demonstrated in the present study resembles the mammalian rostral and ventral spinocerebellar projections as regards its origin. In reptiles direct spinocerebellar fibers arise mainly in area VII-VIII of the spinal gray (subdivision after Kusuma *et al.*, '79). In mammals the rostral and ventral spinocerebellar tracts originate mainly in lamina VII of the spinal gray. As mentioned before no reptilian homologues of a column of Clarke, a nucleus cuneatus externus or a central cervical nucleus, which in mammals give rise to the dorsal, cuneocerebellar, and cervical spinocerebellar tracts, respectively, were found. Although our observations on the spinal afferents to the cerebellum are still rather incomplete it can be tentatively concluded that these connections in lower vertebrates are more simply organized than the spinocerebellar pathways in mammals.

Surveying the present knowledge on cerebellar afferents throughout terrestrial vertebrates it should be emphasized that in 'lower' vertebrates as reptiles and presumably also amphibians the cerebellum is dominated by vestibular afferents and connections from the nucleus of the basal optic root and perihypoglossal nuclear complex, all related to the control of eye, head, and neck movements. In 'higher' vertebrates such as birds and mammals

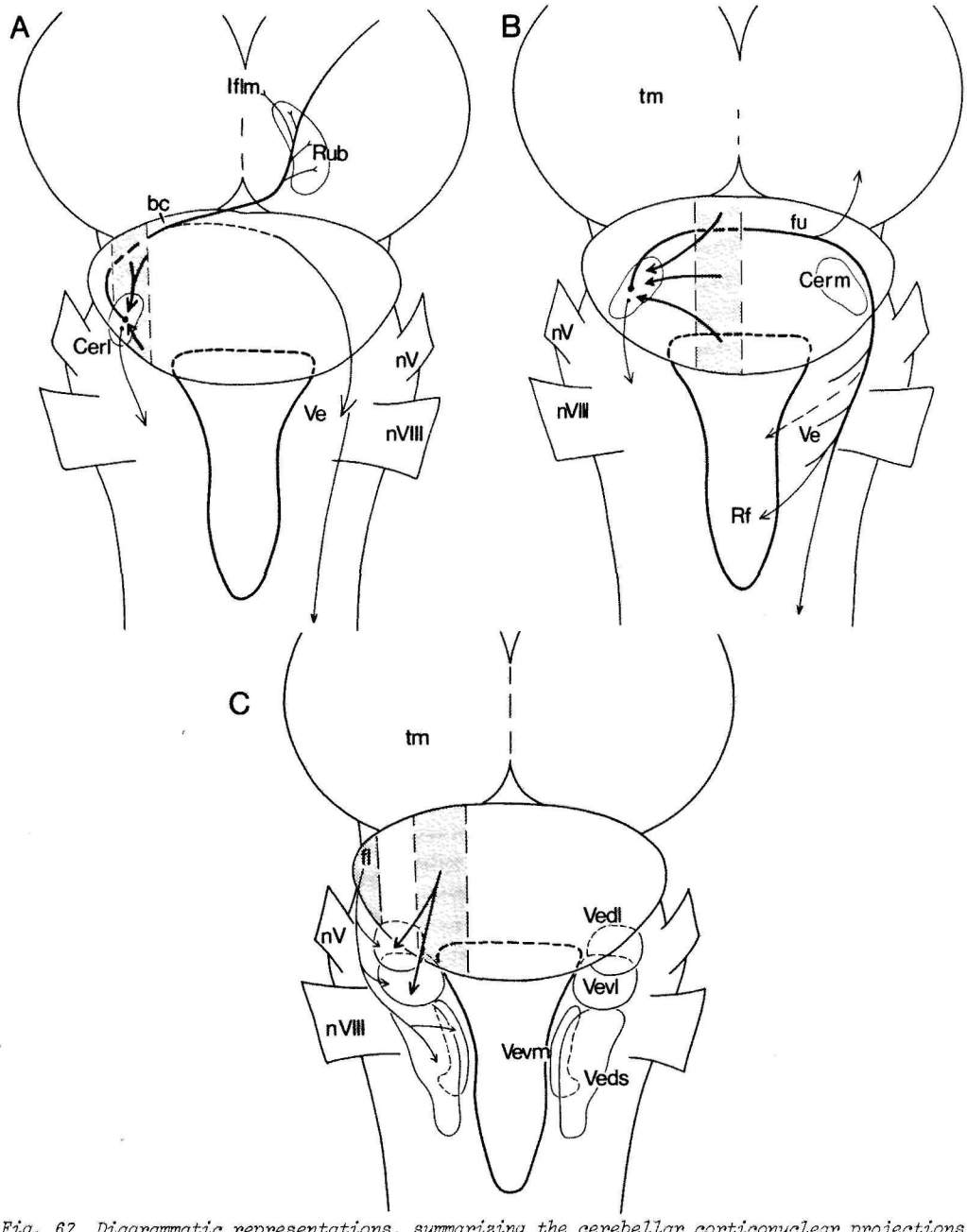


Fig. 67 Diagrammatic representations, summarizing the cerebellar corticonuclear projections to and the efferent connections of the lateral (A) and medial (B) cerebellar nuclei, and the cerebellar corticovestibular projections (C) illustrated in Varanus exanthematicus, also applying to Pseudemys scripta elegans. Shaded areas represent the zones of Purkynĕ cells projecting to the cerebellar nuclei and the vestibular nuclear complex, respectively. For abbreviations cf. pages 28-29.

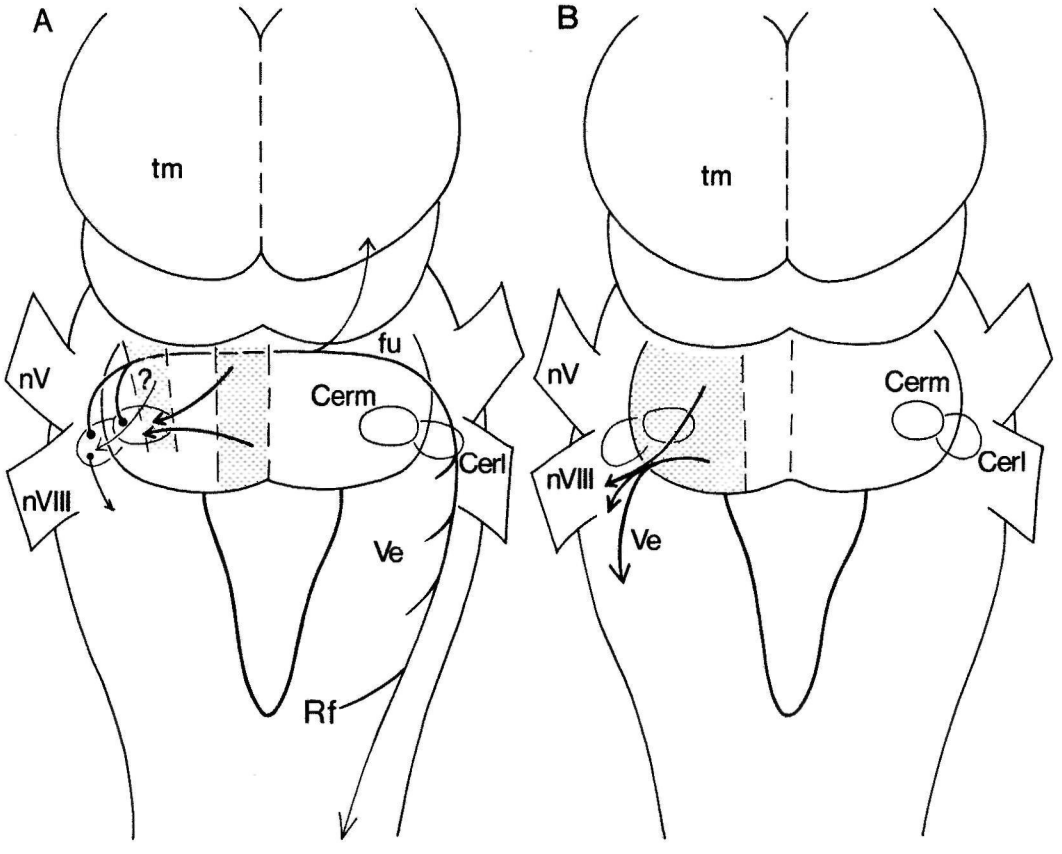


Fig. 68 Diagrammatic representations, summarizing the cerebellar corticonuclear projections to and the efferent connections of the lateral and medial cerebellar nuclei (A), and the cerebellar corticovestibular projections (B) in Python regius. Shaded areas represent the zones of Purkyně cells projecting to the cerebellar nuclei and the vestibular nuclear complex, respectively. For abbreviations cf. pages 28-29.

phylogenetically 'new' input, i.e. the afferents from the telencephalon, becomes superimposed upon this 'ancient' input to the cerebellum.

COMPARATIVE ASPECTS OF CEREBELLAR EFFERENT CONNECTIONS IN TERRESTRIAL VERTEBRATES

The efferent connections of the reptilian cerebellar cortex and cerebellar nuclei, as demonstrated in the present investigation (Chapters V, VI), are schematically summarized in figures 67 and 68. Figure 67 represents the efferent connections of the cerebellum in the lizard *Varanus exanthematicus*, but also applies to the turtle *Pseudemys scripta elegans*, figure 68 shows the cerebellar efferents observed in the snake *Python regius*.

Corticonuclear projections

In the turtle *Pseudemys scripta elegans*, the lizard *Varanus exanthematicus*, and the snake *Python regius* projections of the cerebellar cortex to the cerebellar nuclei and the vestibular nuclear complex appeared to be organized in a number of longitudinally oriented zones of Purkyně cells, each projecting to a different target (Figs. 67, 68; Chapter V). In *Pseudemys scripta elegans* and *Varanus exanthematicus* (Fig. 67) from the midline lateralwards the following zones could be distinguished: (1) a medial zone, projecting to the medial cerebellar nucleus (Fig. 67B); (2) an intermediate zone, projecting to the vestibular nuclear complex, especially the dorsolateral and ventrolateral vestibular nuclei (Fig. 67C); (3) a caudolaterally (in *Varanus exanthematicus*) or a rostrolaterally (in *Pseudemys scripta elegans*) located area of the intermediate part of the cerebellum projecting presumably to the lateral cerebellar nucleus (Fig. 67A; see also Figs. 51, 52); (4) a lateral zone, including the flocculus, projecting mainly to the middle and caudal parts of the vestibular nuclear complex, i.e. the descending and ventromedial vestibular nuclei (Fig. 67C). In *Python regius* (Fig. 68), although less distinctly, a comparable pattern of efferent corticonuclear projections seems to be present, including: (1) a small paramedian zone, projecting to the medial cerebellar nucleus (Fig. 68A); (2) an area of Purkyně cells located in the lateral half of the cerebellar cortex, probably projecting to the lateral cerebellar nucleus (Fig. 68A); (3) a broad zone, including the most lateral, floccular, part of the cerebellum with projections to the vestibular nuclear complex (Fig. 68B).

The organization of cerebellar corticonuclear projections has been studied extensively in a great number of vertebrate species. Goodman ('64) presented comparative data (Fig. 69) on cerebellar stimulation experiments in the bullfrog *Rana catesbeiana* (Goodman, '58), *Caiman sclerops* (Goodman and Simpson, '60), the duck *Anas domesticus* (Goodman *et al.*, '63b), and the rat (Goodman and Simpson, '61; see also Goodman *et al.*, '63a). In the bullfrog only a central vermal and a superficial floccular zone pattern were found, in *Caiman sclerops* vermal, paravermal, and floccular patterns, but in the duck and particularly in the rat an additional lateral zone pattern was observed. Considering the aforementioned anatomical data on corticonuclear projections the vermal zone as distinguished by Goodman comprises both the projection zones to the medial cerebellar nucleus and the (ventro)lateral vestibular nucleus. The paravermal zone has projections to the lateral cerebellar nucleus in reptiles, the

intermediate nucleus (and dorsal part of the lateral nucleus) in birds (see Wold, '81), and the interposed nuclei in mammals. The lateral zone, only found in birds and mammals, projects to the dentate (or lateral nucleus) and its avian homologue, i.e. the ventrolateral part of the lateral nucleus (see Larsell, '67; Wold, '81).

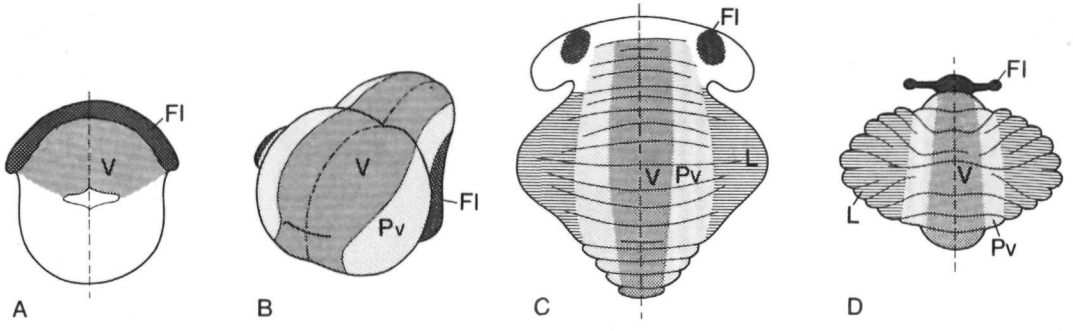


Fig. 69 Schematic representations showing the basic subdivisions of the cerebellum in terrestrial vertebrates in: A, The bullfrog Rana catesbeiana (after Goodman, '58); B, Caiman sclerops (after Goodman and Simpson, '60); C, The duck Anas domesticus (after Goodman et al., '63b); D, The rat (after Goodman and Simpson, '61). Fl, flocculus zone; L, lateral zone; Pv, paravermal zone; V, vermal zone.

In birds a longitudinal organization of the efferent connections of the cerebellar cortex has been shown in both myeloarchitectonic studies (Feirabend *et al.*, '76; Feirabend, '83) and degeneration as well as HRP experiments on cerebellovestibular projections (Wold, '81) in the white leghorn *Gallus domesticus*. On each side of the midline the cerebellar white matter could be divided into at least six longitudinal zones corresponding to six compartments of afferent and efferent connections of the cerebellar cortex. The main part of the corticovestibular fibers in the domestic hen appeared to be derived from Purkyně cells located in a longitudinal zone at a certain distance of the cerebellar midline, comparable to zone 3 of Feirabend *et al.* ('76). A smaller part of the labeled Purkyně cells was located in more lateral zones. In addition, corticovestibular projections were found to originate in the flocculus. These data indicate that the most medial part of the cerebellum of birds, which did not contain retrogradely labeled Purkyně cells after HRP injections into the vestibular nuclei, projects to a cerebellar nucleus.

In mammals (see e.g. Voogd, '64, '67; Voogd and Bigaré, '80) at least seven longitudinal zones can be distinguished. Of these, the most medial zone (zone A, i.e. the medial part of the vermis) projects to the nucleus fastigii, the lateral part of the vermis (zone B) projects to the lateral vestibular nucleus, the intermediate part (C zones) to the interposed nuclei, and the lateral part of the cerebellar hemisphere (D zones) to the dentate nucleus. Recent HRP experiments in the cat (Bigaré, '80; Voogd and Bigaré, '80) have shown a wider distribution in the cerebellar cortex of the cells of origin of corticovestibular fibers than previously known. The A zone also projects to the superior, medial, lateral, and descending vestibular nuclei,

whereas the flocculus also has extensive projections to these vestibular nuclei (see also Balaban *et al.*, '81, Sato *et al.*, '82).

In broad general terms a parallel can be drawn between the present experimental data in the reptiles studied, especially the turtle *Pseudemys scripta elegans* and the lizard *Varanus exanthematicus*, and the abovementioned findings in mammals. The medial zone and a large part of the intermediate zone of the present study show clear similarities with the A and B zones in mammals, whereas the C zones of mammals, projecting to the interposed nuclei, seem to be represented in the rostrolateral part (in *Pseudemys scripta elegans*) and the caudolateral part (in *Varanus exanthematicus*) of the cerebellar cortex, respectively. The lateral zone of the present study, which only projects to the vestibular nuclear complex, represents the reptilian homologue of the mammalian flocculus. The flocculus, with its close connection with the vestibulo-ocular reflex path, is found throughout vertebrates.

Efferent connections of the cerebellar nuclei

In all three reptilian species studied the efferent connections of the cerebellar nuclei appeared to be organized mainly in two contralateral pathways (Figs. 67, 68), i.e. the brachium conjunctivum and the fasciculus uncinatus (Chapter VI).

The *lateral cerebellar nucleus* in the turtle *Pseudemys scripta elegans* and the lizard *Varanus exanthematicus*, receiving afferents from a lateral strip of Purkyně cells, gives rise to a well-developed ascending, contralateral projection, the brachium conjunctivum (Fig. 67A). This fiber tract is directed in particular to the red nucleus. In addition, HRP studies in *Varanus exanthematicus* indicate projections to the contralateral diencephalon, in keeping with data in *Caiman crocodilus* (Brauth and Kitt, '80), and telencephalon (Lohman and van Woerden-Verkley, '78; ten Donkelaar and de Boer-van Huizen, '81b). In the snake *Python regius* (Fig. 68A) only a sparse contralaterally ascending projection of the lateral cerebellar nucleus to the mesencephalic tegmentum was observed. A well-developed descending projection of the lateral cerebellar nucleus, probably passing by way of the fasciculus uncinatus, appeared to be present in all species studied (Figs. 67A, 68A), directed mainly to the vestibular nuclear complex, but also to the reticular formation. Also a spinal projection of the lateral cerebellar nucleus was found, comparable to the spinal projection of the nucleus interpositus in mammals (e g. Fukushima *et al.*, '77, Matsushita and Hosoya, '78).

In amphibians, only a single cerebellar nucleus is present, which according to Larsell ('23) gives rise to a brachium conjunctivum However, until now no experimental data on the ascending projections of the amphibian cerebellar nucleus are available. In birds, a brachium conjunctivum, arising in the lateral cerebellar nucleus, has been described by several authors (e.g. Shimazono, '12; Zecha, '68, Verhaart, '74) In the pigeon *Columba livia* a distinct projection of the lateral cerebellar nucleus to the contralateral red nucleus was demonstrated with anterograde degeneration techniques (Karten, '64; Karten and Revzin, '66). Whereas in the present study only sparse projections of the reptilian lateral cerebellar nucleus beyond the mesodiencephalic junction were observed, in birds and mammals the ascending projections of the lateral cerebellar nucleus, and the interposed and dentate nuclei, respectively, proceed further rostrally. In the pigeon *Columba livia* cerebellothalamic projections have been

demonstrated (Karten, '64, Karten and Revzin, '66). In mammals the brachium conjunctivum proceeds, after reaching the red nucleus, further rostrally to the thalamus. In the thalamus fibers are mainly distributed to the ventrolateral and intralaminar nuclei (e.g. Martin *et al.*, '74, Rinvik and Grofová, '74; Chan-Palay, '77; Faull and Carman, '78, Stanton, '80, Sugimoto *et al.*, '81, Bentivoglio, '82, see for a review also Gilman *et al.*, '81).

In mammals a distinct contralateral descending limb of the brachium conjunctivum is present, which is apparently absent as such in reptiles. In mammals the descending limb of the brachium conjunctivum courses, immediately rostral to the decussation of the brachium conjunctivum, caudally and is distributed predominantly to the pontine and medullary reticular formation (e.g. Voogd, '64, Achenbach and Goodman, '68, Martin *et al.*, '74, Chan-Palay, '77; Faull, '78; Watt and Mihailoff, '83). An ipsilaterally descending limb of the brachium conjunctivum (e g. Faull, '78, Watt and Mihailoff, '83) proceeds ventrally from the brachium conjunctivum prior to its decussation and terminates mainly in the lateral parvocellular reticular formation of the lower brainstem In the present study no evidence was found for comparable descending components of the brachium conjunctivum in reptiles.

The efferent connections of the medial cerebellar nucleus in reptiles (Figs 67B, 68A) show striking resemblances with the projections of the mammalian medial (or fastigial) cerebellar nucleus. The reptilian medial cerebellar nucleus receives afferents from a medial zone of Purkyně cells and gives rise to an extensive descending contralateral projection, the fasciculus uncinatus (Figs, 67B, 68A) This fiber tract is directed mainly to the vestibular nuclear complex and the spinal cord, whereas only relatively sparse projections to the reticular formation were observed In *Pseuderys scripta elegans* and *Varanus exanthematicus* ascending projections of the medial cerebellar nucleus to the contralateral mesencephalon were observed, reaching at least the level of the red nucleus (Fig. 67B).

The fasciculus uncinatus or hook bundle, arising in the medial cerebellar nucleus is probably common throughout terrestrial vertebrates. Stern and Rubinson ('71) made cerebellar lesions in *Rana pipiens* and noted decussating fibers within the cerebellum passing to the contralateral vestibular nuclear complex. These findings suggest a projection of the amphibian nucleus cerebelli to the contralateral vestibular region In the domestic hen, *Gallus domesticus*, Wold ('81) showed bilateral projections of both the medial and intermediate cerebellar nucleus to the vestibular nuclei. In mammals the tractus (or fasciculus) uncinatus arises in the nucleus fastigii. The crossed efferents of this fiber tract hook around the brachium conjunctivum, giving of an ascending and a descending limb (e.g. Thomas *et al.*, '56, Cohen *et al.*, '58, Angaut and Bowsher, '70, Batton *et al.*, '77 see for review Gilman *et al.*, '81). The ascending branch courses dorsal to the brachium conjunctivum. In the midbrain, it does not distribute fibers to the red nucleus but some of its fibers terminate in the mesencephalic reticular formation and the deep layers of the superior colliculus (e.g Angaut and Bowsher, '70, Kievit and Kuypers, '72, Batton *et al.*, '77). The tractus uncinatus proceeds further rostrally to the thalamus and its fibers are distributed predominantly to the ventromedial and ventrolateral nuclei (e.g Martin *et al.*, '74, Haroian *et al.*, '81; Sugimoto *et al.*, '81). In reptiles a separate ascending fiber tract arising in the medial cerebellar nucleus could not be distinguished (the present study) although distinct ascending projections of the medial

cerebellar nucleus were demonstrated both in *Pseudemys scripta elegans* and *Varanus exanthematicus* (Chapter VI). Therefore it is concluded that the ascending efferent fibers of the reptilian medial cerebellar nucleus probably pass by way of the brachium conjunctivum. In this respect it is noteworthy that in the cat some ascending efferents of the medial cerebellar nucleus were reported to follow the ascending limb of the brachium conjunctivum and, after crossing in the decussation of the brachium conjunctivum, join the ascending limb of the tractus uncinatus (Voogd, '64; Kievit and Kuypers, '72).

The descending branch of the mammalian tractus uncinatus terminates in the contralateral vestibular nuclei and the medial pontine and medullary reticular formation (see e.g. Thomas *et al.*, '56; Cohen *et al.*, '58; Walberg *et al.*, '62a, b; Martin *et al.*, '74; Batton *et al.*, '77; Watt and Mihailoff, '83). A part of the crossed descending efferents of the fastigial nucleus reach the spinal cord (e.g. Fukushima *et al.*, '77; Matsushita and Hosoya, '78; Bharos *et al.*, '81; Bentivoglio, '82). In addition, the fastigial nucleus has uncrossed efferents which are distributed primarily to the ipsilateral vestibular nuclei (Thomas *et al.*, '56; Walberg *et al.*, '62a; Batton *et al.*, '77).

The data presented in this study concerning the existence of neurons in the lateral and predominantly in the medial cerebellar nucleus with projections to both the rostral mesencephalon and the upper part of the spinal cord are in keeping with recent results in rat (Bentivoglio, '82; Bentivoglio and Kuypers, '82) and cat (Bharos *et al.*, '81). In these mammals many neurons of the cerebellar nuclei were demonstrated to distribute divergent axon collaterals, not only to thalamus and spinal cord, but also to thalamus and tectum, and thalamus and medulla oblongata, respectively.

FUNCTIONAL ASPECTS OF CEREBELLAR CONNECTIVITY IN REPTILES

The first adequate experiments to reveal the function of the cerebellum in reptiles were carried out by Rolando (1809) who demonstrated in several animals, including reptiles, that by removing parts of or the whole cerebellum ipsilateral motor activity was impaired. Cerebellar ablations were also performed by Fano (1884) in the turtle *Emys europaea*, Steiner (1886) in the lizard *Lacerta viridis*, and Leblanc ('23) in the lizards *Uromastix acanthinurus* and *Varanus griseus*, and the chamaeleon *Chamaeleo vulgaris*. Leblanc observed difficulties in gait and posture, described as ataxia, lack of coordination, posture problems, and tremor of the limbs. The extent of the lesions was not verified histologically. Hacker ('31) made cerebellar ablations in two lizard species, i.e. the limbless lizard *Ophisaurus apus* and the quadrupedal *Lacerta viridis*. In *Ophisaurus*, disturbances in locomotion, particularly a lack of coordination in the serpentine movement, was observed. After two weeks, however, the normal pattern of movement returned. Also hypotonia of the muscles was observed. In *Lacerta viridis*, the following symptoms were observed: hypotonia alternated with hypertonia, the head could not be kept up, ataxia, lack of coordination, tremor of the limbs. Furthermore, dysmetria was found: the fore- and hindlimbs stepped on each other during locomotion, and when catching mealworms the movement was too fast and the animal reached beside or above the 'target'.

More recently, cerebellar ablations (Goodman, '69) as well as stimulation experiments

(Goodman and Simpson, '60; Goodman, '64, '69) were performed in the South American alligator *Caiman sclerops* (Fig. 69). Unilateral cerebellar ablations involved either the functional vermal or paravermal zone. Lesions in the vermal zone, i.e. the medial two-thirds of the corpus cerebelli, resulted in postural effects, and a general hypoflexia. Lesions in the paravermal zone, i.e. the lateral one-third of the cerebellum, resulted in postural effects with the reverse pattern observed to lesions in the vermal zone, but caused minimal or no detectible effects on the animal's reflexes. Dysmetria was observed after both kinds of lesions (Goodman, '69), but was more severe in animals with vermal-zone lesions.

Stimulation of the cerebellar cortex in the unanesthetized and unrestrained South American alligator *Caiman sclerops* (Goodman and Simpson, '60; Goodman, '64, '69) elicited three definable postural patterns:

- Stimulation of the medial two-thirds of the middle lobe and the medial half of the posterior lobe (*vermal zone pattern*) resulted in: ipsilateral forelimb flexion, adduction and protraction, ipsilateral hindlimb flexion, adduction and retraction, contralateral forelimb extension, abduction and retraction, contralateral hindlimb extension, abduction and protraction; the head turned ipsilaterally, and the body and tail became concave toward the ipsilateral side;
- stimulation of the flocculus resulted in a *floccular zone pattern* that is the mirror-image to that of the vermal zone pattern with the exception that the head was rotated with the contralateral occiput down instead of the head turning laterally;
- stimulation of the lateral half of the anterior lobe, the lateral one-third of the middle lobe and the lateral half of the posterior lobe resulted in a third definable postural pattern. This *paravermal zone pattern* consisted of body and limb movements similar to those observed for the floccular zone pattern with the exception that the head was turned contralaterally.

An additional type of postural adjustment concerned with the axial musculature was obtained: rotation of the head, body and tail about the longitudinal axis was superimposed upon all three postural patterns: stimulation of the vermal zone was associated with clockwise axial rotation, of the paravermal zone with counterclockwise rotation, of the floccular zone with a 'corkscrew' axial posture. These rotation movements along the longitudinal body axis caused by cerebellar stimulation may be related to movements peculiar to feeding and agressive behavior of the alligator. After a relatively large object has been seized and held firmly by the jaws, the alligator rotates his entire body and head in an attempt to tear away a small piece (Goodman, '69).

Altogether, the observations after cerebellar ablations and stimulation experiments in various reptiles indicate that the reptilian cerebellum plays a role in the regulation of muscle tone and the coordination of movements, permitting a rather precise control of both posture and locomotion. Major signs of cerebellar dysfunction in reptiles are, as in higher vertebrates (see e.g. Dow and Moruzzi, '58; Gilman *et al.*, '81): (1) loss of coordination, expressed in ataxia, gait disturbances, and postural effects; (2) dysmetria; (3) compensation: since Flourens (1824) it has been known that an animal can recover from motor disturbances produced by surgical lesions of the cerebellum. Negative findings after cerebellar ablations as reported by some authors (Fano, 1844; Steiner, 1886; Leblanc, '23) might be due to the ability of undamaged parts of the cerebellum to compensate for the functions destroyed by the

- incomplete - lesions

For a better understanding of the influence of the cerebellum on motor activity in reptiles not only cerebellar dysfunction and connectivity have to be known, but also the connections of the main cerebellar targets in the brainstem involved in motor control, viz., the vestibular nuclear complex and the nucleus ruber.

The function of the cerebellum in the control of spinal motor mechanisms is further established by the elegant studies of Orlovsky ('70a, b; '72a, b; Shik and Orlovsky, '76). In cat rubrospinal, vestibulospinal and reticulospinal neurons are known to be phasically active during so-called controlled locomotion (Orlovsky, '70a; '72a, b). In these experiments the forelimbs were fixed and only the hindlimbs were stepping on a treadmill band. Most vestibulospinal neurons are maximally active at the beginning of the stance phase, most rubrospinal and reticulospinal neurons in the swing phase. An acute cerebellectomy almost exclusively abolishes the phasic activity in these different types of neurons (Orlovsky, '70b; '72a, b). As shown by ablation studies, locomotion can still be carried out after cerebellectomy, but the movements become less coordinated. Cerebellar activity during locomotion contributes to the coordination by somehow matching the different types of inputs, such as those related to limb activity (spinocerebellar input), and the equilibrium information (vestibular input) to produce an output appropriate in a given situation (Grillner, '81; see also Wetzel and Stuart, '76, '77).

As demonstrated in Chapters V and VI, the main output of the reptilian cerebellum is directed to the vestibular nuclear complex, both by way of the direct, ipsilateral corticovestibular projections (Chapter V), as well as by way of the medial and lateral cerebellar nuclei (Chapter VI). The latter projection appears to be predominantly contralateral. Studies in the turtle *Pseudemys scripta elegans*, the lizard *Varanus exanthematicus*, and the snake *Python regius* established the importance of vestibular control for eye movements and spinal motor mechanisms (ten Donkelaar, '82). In the turtle *Pseudemys scripta elegans* (Bangma and ten Donkelaar, '83), the lizard *Varanus exanthematicus* (ten Donkelaar, '76b; ten Donkelaar and de Boer-van Huizen, '83) and the snake *Python regius* (Bangma and ten Donkelaar, unpublished observations) distinct vestibulo-oculomotor projections were established: the dorsolateral vestibular nucleus appeared to project ipsilaterally to the nuclei of the extrinsic eye muscles (III, IV, VI), the ventromedial vestibular nucleus mainly contralaterally. Both projections were demonstrated to pass by way of the fasciculus longitudinalis medialis (flm) (Fig 70). Vestibulospinal projections in reptiles (Robinson, '69; ten Donkelaar, '76a, b; '82; ten Donkelaar *et al.*, '80, '83; Cruce and Newman, '81, Woodson and Künzle, '82) are organized in two pathways: the ipsilaterally descending tractus vestibulospinalis lateralis, arising in the magnocellular part of the ventrolateral vestibular nucleus, and the contralaterally descending tractus vestibulospinalis medialis, arising in the ventromedial and descending vestibular nucleus. Both in the turtle *Pseudemys scripta elegans* (Bangma and ten Donkelaar, '83), the lizard *Varanus exanthematicus* (ten Donkelaar and de Boer-van Huizen, '83) and in the snake *Python regius* (ten Donkelaar *et al.*, '83) direct vestibular projections on neck motoneurons, i.e. vestibulocollic projections, were demonstrated anterogradely with HRP. The latter projections pass particularly by way of the medial vestibulospinal tract. Direct

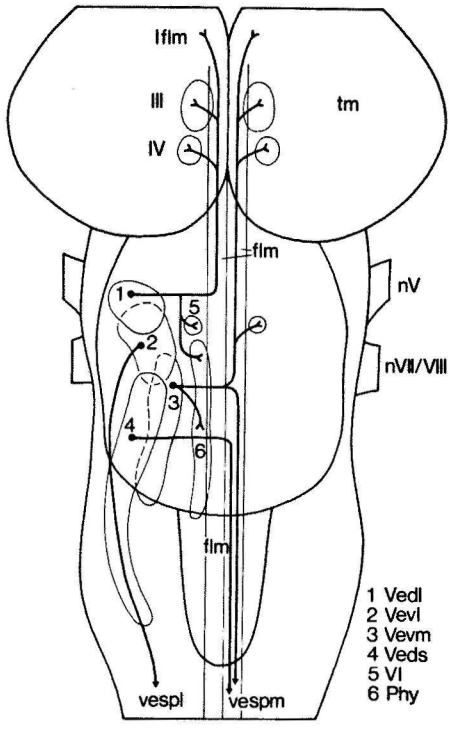


Fig. 70 Diagrammatic representation, summarizing the efferent connections of various parts of the vestibular nuclear complex in Pseudemys scripta elegans. For abbreviations cf. pages 28-29.

projections of the medial vestibulospinal tract on neck motoneurons are also found in the pigeon (Eden and Correia, '82) and mammals (see e.g. Wilson and Melvill Jones, '79; Boyle and Pompeiano, '81). Ipsilateral projections of the ventrolateral vestibular nucleus were found throughout the spinal cord to the ventromedial part of area VII-VIII in various reptiles (e.g. Robinson, '69; ten Donkelaar, '76b; ten Donkelaar *et al.*, '80; ten Donkelaar, '82), and in the lizard *Tupinambis nigropunctatus* also to the medial column of motoneurons innervating axial musculature (ten Donkelaar, '76b). The main vestibular connections have been summarized in figure 70 for *Pseudemys scripta elegans* (Bangma and ten Donkelaar, '83). By way of its connections with the vestibular nuclear complex the cerebellar influence on the vestibular control of both eye movements and spinal motor mechanisms can be summarized in the following way: direct corticovestibular connections are organized in two separate zones:
(1) The intermediate zone of the cerebellar cortex with projections predominantly to the dorsolateral and ventrolateral vestibular nuclei. Through these connections the cerebellum

exerts influence on. (a) the extrinsic eye muscles by way of the ipsilaterally ascending vestibulo-oculomotor pathway arising in the dorsolateral vestibular nucleus, (b) the spinal cord, by way of the lateral vestibulospinal tract, arising in the ventrolateral vestibular nucleus.

(2) The lateral zone of the cerebellar cortex, including the flocculus, with projections to all vestibular nuclei but in particular to the ventromedial and descending vestibular nuclei. These connections enable the cerebellum to influence: (a) the extrinsic eye muscles by way of the contralaterally ascending vestibulo-oculomotor pathway, arising in the ventromedial vestibular nucleus, (b) the spinal cord, via the medial vestibulospinal tract.

Indirectly, the cerebellum exerts influence on the vestibular nuclear complex by way of the crossed projection arising mainly in the medial cerebellar nucleus, the fasciculus uncinatus. In addition, ipsilateral projections of the lateral and medial cerebellar nuclei are directed to the vestibular nuclei.

In the turtle *Pseudemys scripta elegans* and the lizard *Varanus exanthematicus* a distinct efferent projection of the cerebellum is mediated by the lateral cerebellar nucleus, the brachium conjunctivum, and directed to the red nucleus. In *Python regius* a comparable fiber tract could not be demonstrated, although a small projection of the lateral cerebellar nucleus to the contralateral mesencephalon was observed (Chapter VI). In quadrupedal reptiles the nucleus ruber gives rise to a well-developed rubrospinal tract (Robinson, '69, ten Donkelaar, '76a, b, ten Donkelaar *et al.*, '80, Cruce and Newman, '81; Woodson and Kunzle, '82). However, this tract appears to be absent in limbless reptilian species as *Pythor reticulatus* (ten Donkelaar, '76a, b) and *Python regius* (ten Donkelaar, '82, ten Donkelaar and Bangma, '83). In the lizard *Tupinambis nigropunctatu* the rubrospinal tract was demonstrated to descend contralaterally along the lateral wall of the brainstem, immediately after its decussation in the mesencephalon, and to distribute fibers to the lateral cerebellar nucleus, several nuclei of the trigeminal nerve, and the motor nucleus of the facial nerve. HRP studies in the lizard *Varanus exanthematicus* also suggested an ipsilateral rubro-olivary projection as found in mammals (ten Donkelaar and de Boer-van Huizen, '81a). In the spinal cord the rubrospinal tract, at this level located in the most dorsolateral part of the lateral funiculus, terminates in the lateral parts of area V - VI, especially at the level of the cervical and lumbar intumescences (ten Donkelaar, '76b, '82; ten Donkelaar and Bangma, '84) A comparable rubrospinal tract has not been demonstrated in the limbless reptiles studied. Recently, HRP implants in *Python regius* involving the descending trigeminal nucleus and the motor nucleus of the facial nerve resulted in distinct labeling of the contralateral red nucleus, suggesting a crossed rubrobulbar projection in this snake (ten Donkelaar and Bangma, '83). In lizards (ten Donkelaar, '76b, '82) and several mammalian species (e g. Courville, '66, Edwards, '72, Martin and Dom, '70, Miller and Strominger, '73, Panneton and Martin, '79, '83) also a crossed rubrobulbar projection has been described.

In the turtle *Pseudemys scripti elegans* and the lizard *Varanus exanthematicus* a seperate, laterally located area of the cerebellar cortex projects to the lateral cerebellar nucleus (Chapter V) By way of the brachium conjunctivum this nucleus exerts influence on the nucleus ruber In *Python regius* the sparse ascending projection of the lateral cerebellar

nucleus (Chapter VI) is probably also directed to the nucleus ruber. In the quadrupedal reptiles studied the cerebellum influences by way of the descending connections of the red nucleus (i.e. rubrospinal and rubrobulbar projections, respectively) both the spinal motor mechanisms, viz., movements of the limbs, as well as motor functions localized in the brainstem, e g. feeding mechanisms. In the limbless snake the influence of the cerebellum by way of the lateral cerebellar nucleus and the rubrobulbar projection seems to be related to a refined control of feeding mechanisms. In reptiles the facial motor nucleus is mainly involved in the control of jaw muscles as the musculus depressor mandibulae and several constrictors which play an important role in the process of mastication (Haas, '73; Gorniak *et al.*, '82; Smith, '82).

In the present study also a projection from the medial cerebellar nucleus to the brainstem reticular formation was noted (Figs. 67B, 68B), however, much less extensive than the reticular projections from the medial (or fastigial) nucleus found in mammals (see e.g. Thomas *et al.*, '56, Cohen *et al.*, '58, Walberg *et al.*, '62a, b, Martin *et al.*, '74; Batton *et al.*, '77) The rather simple locomotor patterns in reptiles (see e g. Grillner, '75, '81) presumably require a less extensive reticular control than the more advanced movements in mammals.

In summary, the influence of the cerebellum on spinal motor mechanisms is exerted through vestibulospinal, rubrospinal, and reticulospinal pathways, but also by way of direct cerebellospinal connections mediated by the medial cerebellar nucleus. The latter contralateral projection is presumably mainly involved in control of neck motoneurons. In cat (see e.g. Wilson *et al.*, '78) direct fastigiospinal connections to neck and shoulder movements have been demonstrated.

In reptiles, as in mammals (Kuypers, '64, '81; Lawrence and Kuypers, '68a, b) a classification of the descending pathways to the spinal cord has been made regarding their origin, course, and site of termination into a medial and a lateral system (ten Donkelaar, '76a, b, '82). The medial system terminates in the mediodorsal parts of the ventral horn and the adjacent parts of the intermediate zone. This system (interstitiospinal, reticulospinal and vestibulospinal pathways) is functionally related to postural activities and progression and constitutes a basic system by which the brain exerts control over movements. The lateral system of brainstem pathways, i e the rubrospinal tract terminates in lateral and dorsal parts of the intermediate zone The latter system, at least in regard to the extremities, superimposes upon the general motor control by the medial system, the capacity for the independent use of the extremities, particularly of the hand. The classification of descending pathways into medial and lateral systems renders it likely that in snakes and limbless lizards the absence of a rubrospinal tract is correlated to the absence of limbs (see ten Donkelaar, 1982).

So, it appears that in reptiles the medial strip of Purkyně cells via the medial cerebellar nucleus, but presumably also the direct corticovestibular projections (arising in the intermediate strip of Purkyně cells) exert their influence on spinal motor mechanisms via the *medial* system of brainstem pathways, the lateral strip of Purkyně cells projecting to the lateral cerebellar nucleus via the *lateral* system of brainstem pathways, at least in quadrupedal reptiles. Such an organization within the cerebellum is in keeping with the concept of

localization in the mammalian cerebellum going back to Chambers and Sprague ('55a, b; see also Brooks and Thach, '81). Two longitudinally organized corticonuclear zones in the cat are also found in reptiles: (1) the medial, vermal cortex and the fastigial nucleus are concerned with postural tonus, equilibrium and locomotion of the entire body, (2) the intermediate, paravermal cortex and the interposed nuclei are involved in a more discrete control of the use of the ipsilateral limbs only, including the management of postural placing reflexes in these limbs (Chambers and Sprague, '55a, b). In mammals, in addition a lateral zone with projections via the dentate (or lateral) nucleus is present, involved in skilled and spatially organized movements of the ipsilateral limbs and face.

Comparing the experimental anatomical data on cerebellar connectivity presently available in amphibians, reptiles, birds, and mammals, it can be concluded that the connections of the reptilian cerebellum are organized in a pattern basic to these vertebrate classes. By way of these connections the reptilian cerebellum seems to be involved in the control of several aspects of motor activity, viz. movements of the eyes, feeding mechanisms, and in particular locomotion.

VIII. SUMMARY

In the present study the afferent and efferent connections of the cerebellum have been investigated in three reptilian species, each with a different mode of locomotion, viz. the turtle *Pseudemys scripta elejans*, the lizard *Varanus exanthematicus*, and the snake *Python regius*, in order to reveal the ways in which the reptilian cerebellum influences motor activity.

The origin of cerebellar afferents, the organization of the corticonuclear projections, i e. the efferent connections arising in the cerebellar Purkyně cell layer, and the efferent connections of the cerebellar nuclei to the brainstem and the spinal cord have been studied both with the classical anterograde degeneration techniques (Nauta-Gygax, Fink-Heimer), as well as with modern tracer techniques making use of axonal transport phenomena (HRP, WGA-HRP, fluorescent tracers, ^{3}H-leucine).

In the three reptilian species studied cerebellar afferents were demonstrated to arise mainly in the following cell masses the nucleus of the basal optic root, the nucleus ruber (projecting to the lateral cerebellar nucleus), the vestibular ganglion and the vestibular nuclear complex, the perihypoglossal nuclear complex, some somatosensory nuclei (the descending trigeminal nucleus and the dorsal funicular nucleus), dorsal root ganglia and throughout the spinal cord These subcerebellar centres are all known to project to the granular layer of the cerebellum by way of mossy fibers. In addition, a well-developed projection of climbing fibers arising in the inferior olive was demonstrated, in *Varanus exanthematicus* terminating in the molecular layer in a longitudinally arranged pattern.

Topological analysis of the cerebellar Purkyně cell layer in the turtle *Pseudemys scripta elegans* and the lizard *Varanus exanthematicus* showed a distinct longitudinally oriented zonal pattern of Purkyně cells. In *Pseudemys scripta elegans* in each cerebellar half a medial, an intermediate, and a lateral zone of Purkyně cells could be distinguished Also the efferent projections of the Purkyně cell layer appeared to be organized in a longitudinal pattern. After HRP slow-release gel implants into the cerebellar nuclei and at various levels of the vestibular nuclear complex of these two reptilian species, from the midline lateralwards four zones of Purkyně cells could be distinguished, each projecting to a different target. (1) a medial zone, projecting to the medial cerebellar nucleus, (2) an intermediate zone, projecting to the vestibular nuclear complex, especially the dorsolateral and ventrolateral vestibular nuclei, (3) an area with projections to the lateral cerebellar nucleus, (4) a lateral zone including the floccular part of the cerebellum with projections to the vestibular nuclear complex, especially the ventromedial and descending vestibular nuclei. Experimental data in *Python regius* indicate that also in this limbless species comparable zones are present, although only a rather small area with projections most likely to the lateral cerebellar nucleus was observed.

The efferent projections of the cerebellar nuclei in the three reptiles studied appeared to be organized mainly in two contralateral pathways In the quadrupedal reptiles *Pseudemys scripta elegans* and *Varanus exanthematicus* the lateral cerebellar nucleus gives rise to a well-developed ascending projection, the brachium conjunctivum With anterograde tracing techniques this projection could be traced as far rostrally as the mesodiencephalic junction,

profusely terminating on the nucleus ruber. HRP data in *Varanus exanthematicus* also indicated projections of the lateral cerebellar nucleus to the diencephalon and telencephalon. In the limbless snake *Python regius* only a sparse ascending projection arising in the lateral cerebellar nucleus was demonstrated. In all three reptiles studied the medial cerebellar nucleus gives rise to a well-developed descending projection, the fasciculus uncinatus This projection appeared to be directed predominantly to the vestibular nuclear complex, but also minor projections to the reticular formation were observed. Furthermore the medial cerebellar nucleus projects extensively to the contralateral spinal cord. Results of multiple fluorescent tracer studies in the turtle and the lizard indicate that in these reptiles at least part of the neurons of the cerebellar nuclei, especially the medial cerebellar nucleus, have both ascending and descending axon collaterals to the mesencephalon and the rostral spinal cord, respectively.

The influence of the reptilian cerebellum on spinal motor mechanisms is exerted through vestibulospinal, rubrospinal (except in the snake), and reticulospinal pathways, but also by way of direct cerebellospinal connections mediated by the medial cerebellar nucleus. The latter contralateral projection is presumably mainly involved in control of neck motoneurons. It appears that in reptiles the medial strip of Purkynĕ cells via the medial cerebellar nucleus, and presumably also the direct corticovestibular projections exert their influence on spinal motor mechanisms via the medial system of brainstem pathways (i.e. vestibulospinal and reticulospinal tracts). It is known that these pathways are functionally related to postural activities and progression. The lateral strip of Purkynĕ cells projecting to the lateral cerebellar nucleus on the other hand, influences via the lateral system of brainstem pathways, i.e. the rubrospinal tract, the capacity for the independent use of the extremities. In limbless species as the snake *Python regius* a lateral strip of Purkynĕ cells with connections via the lateral cerebellar nucleus to the red nucleus and the bulbar projection arising in the latter nucleus, seems to be related to a refined control of feeding mechanisms. Via its extensive connections with the vestibular nuclear complex the reptilian cerebellum is also involved in the control of eye movements.

Comparing the experimental anatomical data on cerebellar connections presently available in terrestrial vertebrates, i.e. amphibians, reptiles, birds, and mammals, it can be concluded that the connections of the reptilian cerebellum are organized in a pattern basic to these vertebrate classes. In mammals this basic pattern in the organization of cerebellar afferents, corticonuclear projections, as well as in pathways arising in the cerebellar nuclei, becomes superimposed by the development of an extensive corticopontocerebellar system and by the development of the lateral zone (the main part of the cerebellar hemisphere). The latter zone exerts its influence via the dentate nucleus over spinal motor mechanisms mainly via the corticospinal tract

SAMENVATTING

Dit proefschrift beschrijft een onderzoek naar de afferentie en efferentie van het cerebellum bij drie, zich op verschillende wijze voortbewegende reptielen, nl. de roodwangwaterschildpad, *Pseudemys scripta elegans*, de steppevaraan, *Varanus exanthematicus*, en de koningspython, *Python regius*. Het doel van dit onderzoek is na te gaan op welke wijze het cerebellum van invloed is op de motoriek van deze reptielen.

De oorsprongscentra van de cerebellaire afferenten, de organisatie van de projekties van de cerebellaire schors - d w z. de projekties van de Purkyně cellen -, en de projekties van de cerebellaire kernen naar de hersenstam en het ruggemerg werden onderzocht zowel met klassieke anterograde degeneratietechnieken (Nauta-Gygax, Fink-Heimer), als met moderne tracertechnieken welke gebaseerd zijn op het transport van stoffen door axonen (HRP, WGA-HRP, fluorescerende tracers, ^{3}H-leucine).

De afferenten van het cerebellum blijken voornamelijk uit de volgende celgebieden te ontspringen de nucleus van de basale optische wortel; de nucleus ruber, welke met name op de laterale cerebellaire kern projekteert, het ganglion vestibulare en het vestibulaire kerncomplex, het perihypoglossuscomplex, enkele somatosensibele kernen (de nucleus trigeminus descendens en de nucleus funiculi dorsalis), spinale ganglia en neuronen gelegen in bepaalde laminae van het ruggemerg. Bovengenoemde precerebellaire celgebieden eindigen als mosvezels in de granulaire laag van het cerebellum. Naast deze mosvezelprojekties werd een belangrijke klimvezelprojektie, afkomstig uit de onderste olijf, naar de moleculaire laag van het cerebellum gevonden Bij de steppevaraan vertoont deze klimvezelprojektie een longitudinaal gerangschikt eindigingspatroon

Een topologische analyse van de Purkyně cellaag van het cerebellum bij *Pseudemys scripta elegans* en *Varanus exanthematicus* bracht aan het licht dat dit deel van de cerebellaire schors is opgebouwd uit een aantal longitudinaal gerangschikte zones van Purkyně cellen. In de cerebellaire schors van de waterschildpad kunnen aan beide zijden drie zones worden onderscheiden een mediale, een intermediaire en een laterale zone De projekties van de Purkyně cellen vertonen eveneens een longitudinale organisatie. Deze projekties werden onderzocht met behulp van implantaties van HRP slow-release gels in de cerebellaire kernen en in het vestibulaire kerncomplex Bij de waterschildpad en de steppevaraan kunnen van de mediaanlijn naar lateraal vier zones Purkyně cellen worden onderscheiden (1) een mediale zone met projekties naar de mediale cerebellaire kern, (2) een daaropvolgende zone met projekties naar het vestibulaire kerncomplex (vnl de dorsolaterale en ventrolaterale vestibulaire kernen), (3) een strook Purkyně cellen met efferenten naar de laterale cerebellaire kern, (4) het meest laterale deel van de cerebellaire schors, inclusief de flocculus, dat naar het vestibulaire kerncomplex projekteert (vnl de ventromediale en descenderende vestibulaire kernen). Experimentele gegevens wijzen erop dat ook bij de slang *Python regius* vergelijkbare zones aanwezig zijn. In dit reptiel werd slechts een zeer smalle strook in het laterale deel van de cerebellaire schors gevonden welke waarschijnlijk op de laterale cerebellaire kern projekteert.

De efferentie van de cerebellaire kernen blijkt in hoofdzaak te bestaan uit twee, contralateraal projekterende baansystemen. De nucleus cerebellaris lateralis is, bij de waterschildpad en de varaan, de oorsprong van een belangrijke ascenderende projektie, het brachium conjunctivum. In anterograde degeneratie experimenten kon dit baansysteem worden vervolgd tot aan de overgang tussen mesencephalon en diencephalon. In het tegmentum mesencephali bleek de nucleus ruber een belangrijk eindigingsgebied van het brachium conjunctivum te zijn. Uit gegevens van HRP experimenten bij *Varanus exanthematicus* kan worden opgemaakt dat de laterale cerebellaire kern ook naar het diencephalon en telencephalon projekteert. In *Python regius* kon slechts een kleine ascenderende projektie van de laterale cerebellaire kern worden aangetoond. Uit de nucleus cerebellaris medialis ontspringt bij alle drie proefdieren een belangrijke descenderende projektie, de fasciculus uncinatus. Deze projektie is met name gericht op het vestibulaire kerncomplex, doch er bleek ook een kleine projektie naar de reticulaire formatie aanwezig te zijn. De mediale cerebellaire kern heeft een uitgebreide contralaterale projektie op het ruggemerg. Met behulp van de multiple fluorescerende tracertechniek werd aangetoond dat in beide cerebellaire kernen, en vooral in de mediale cerebellaire kern, neuronen aanwezig zijn met zowel ascenderende projekties naar het mesencephalon als descenderende projekties naar het rostrale deel van het ruggemerg.

Het cerebellum van reptielen beïnvloedt de motoriek zowel via vestibulospinale, rubrospinale (niet aanwezig in de slang) en reticulospinale verbindingen als via direkte verbindingen tussen het cerebellum en het ruggemerg, nl. de contralaterale projektie van de mediale cerebellaire kern naar het ruggemerg. Deze laatste projektie is waarschijnlijk met name betrokken bij de regulatie van motoneuronen die nekspieren innerveren. Bij reptielen blijken de mediaal gelegen zone van Purkyně cellen (via de mediale cerebellaire kern) en de direkte corticovestibulaire projekties van het cerebellum de motoriek te beïnvloeden via het zogenaamde 'mediale systeem' van descenderende baansystemen (d.w.z vestibulospinale en reticulospinale banen). Deze baansystemen zijn funktioneel betrokken bij houding en voortbeweging. De lateraal gelegen strook Purkyně cellen, die op de laterale cerebellaire kern projekteert, beïnvloedt daarentegen, via het 'laterale systeem' van descenderende baansystemen (nl. de rubrospinale baan), het vermogen tot het onafhankelijke gebruik van de extremiteiten. Bij reptielen zonder extremiteiten, zoals de slang, staat een kleine laterale strook van Purkyné cellen van het cerebellum via de laterale cerebellaire kern in verbinding met de nucleus ruber en de projektie van deze kern op de caudale hersenstam, de rubrobulbaire baan. Via deze verbinding lijkt dit deel van het cerebellum een rol te spelen bij de regulatie van muskulatuur betrokken bij de voedselopname. Tenslotte is het cerebellum via een uitgebreid systeem van verbindingen met het vestibulaire kerncomplex, ook betrokken bij de regulatie van oogbewegingen.

Vergelijking van de resultaten van dit onderzoek met experimenteel-anatomische gegevens betreffende amfibieen, reptielen, vogels en zoogdieren leert dat de verbindingen van het cerebellum bij reptielen geordend zijn in een voor landvertebraten basaal patroon. Dit basale patroon in de organisatie van cerebellaire afferenten, corticonucleaire projekties, en uit de cerebellaire kernen ontspringende baansystemen wordt bij zoogdieren overvleugeld door de ontwikkeling van een zeer uitgebreid corticopontocerebellair systeem. Hieraan gerelateerd is de vergaande ontwikkeling van de beide laterale delen van het cerebellum, de hemisferen.

Via de nucleus dentatus beïnvloedt dit laterale deel van het cerebellum de motoriek via het corticospinale baansysteem.

Achenbach, K.E. and Goodman, D.C. (1968). Cerebellar projections to pons, medulla and spinal cord in the albino rat. Brain Behav. Evol. 1, 43-57.

Adams, J.C. (1981) Heavy metal intensification of DAB-based HRP reaction product. J. Histochem. Cytochem. 29, 775.

Angaut, P. and Bowsher, D. (1970). Ascending projections of the medial cerebellar (fastigial) nucleus: an experimental study in the cat. Brain Res. 24, 49-68.

Ansorge, K. and Grusser-Cornehls, U. (1978). Visual and visual-vestibular responses in frog cerebellar and spinal cord neurons. Neurosci. Lett., Suppl. 1, S 350.

Antal, M , Tornai, I. and Székely, G. (1980). Longitudinal extent of dorsal root fibres in the spinal cord and brain stem of the frog. Neuroscience 5, 1311-1322.

Arends, J.J.A. and Blok, A. (1983). The efferent connections of the nuclei of the descending trigeminal tract in the mallard (*Anas platyrhynchos L.*). Submitted.

Ariens Kappers, C.U. (1921). 'Vergleichende Anatomie des Nervensystems'. E.G. Bohn, Haarlem.

Ariens Kappers, C.U., Huber, G.C. and Crosby, E.C. (1936). 'The Comparative Anatomy of the Nervous System of Vertebrates, Including Man'. Macmillan, New York.

Balaban, C.D., Ito, M. and Watanabe, E. (1981). Demonstration of zonal projections from the cerebellar flocculus to vestibular nuclei in monkeys (*Macaca fuscata*). Neurosci. Lett. 27, 101-105.

Banchi, A. (1903). La minuta struttura della midollo spinale dei Chelonii (*Emys europaea*). Arch. ital. Anat. Embriol. 2, 291-307.

Bangma, G.C. and ten Donkelaar, H.J. (1983). Some afferent and efferent connections of the vestibular nuclear complex in the turtle *Pseudemys scripta elegans*. J. comp. Neurol., in press.

Batini, C., Buisseret-Delmas, C., Corvisier, J., Hardy, O. and Jassik-Gerschenfeld, D. (1978). Brain stem nuclei giving fibers to lobulus VI-VII of the cerebellar vermis. Brain Res. 153, 241-261.

Batton, III, R.R., Jayaraman, A., Ruggiero, D. and Carpenter, M.B. (1977). Fastigial efferent projections in the monkey: an autoradiographic study. J. comp. Neurol. 174, 281-306.

Beccari, N. (1911). La costituzione, i nuclei terminali, e le vie di connessione del nervo acustico nella *Lacerta muralis*, Merr. Arch. ital. Anat. Embriol. 10, 646-698.

Bentivoglio, M. (1982). The organization of the direct cerebellospinal projections. In 'Progress in Brain Research', Vol. 57: 'Descending Pathways to the Spinal Cord' (H.G.J.M. Kuypers and G.F. Martin, eds.). Elsevier Biomedical Press, Amsterdam, pp. 279-291.

Bentivoglio, M., and Kuypers, H.G.J.M. (1982). Divergent axon collaterals from rat cerebellar nuclei to diencephalon, mesencephalon, medulla oblongata and cervical cord. Exp. Brain Res. 46, 339-356.

Bharos, T.B., Kuypers, H.G.J.M., Lemon, R.N. and Muir, R.B. (1981). Divergent collaterals from deep cerebellar neurons to thalamus and tectum, and to medulla oblongata and spinal cord: retrograde fluorescent and electrophysiological studies. Exp. Brain Res. 42, 399-410.

Bigaré, F. (1980). 'De Efferente Verbindingen van de Cerebellaire Schors van de Kat'. Thesis, University of Leiden.

Boyle, R. and Pompeiano, O. (1981). Relation between cell size and response characteristics of vestibulospinal neurons to labyrinth and neck inputs. J. Neurosci. 1, 1052-1066.

Brand, S. and Mugnaini, E. (1980). Pattern of distribution of acetylcholinesterase in the cerebellar cortex of the pond turtle, with emphasis on parallel fibers. Anat. Embryol. 158, 271-287.

Brauth, S.E. and Kitt, C.A. (1980). The paleostriatal system of *Caiman crocodilus*. J. comp. Neurol. 189, 437-465.

Brauth, S.E., Kitt, C.A. and Ferguson, J.L. (1978). The crocodilian midbrain tegmentum: a key to understanding the avian thalamus. Soc. Neurosci. Abstr. 4, 98.

Brecha, N., Karten, H.J. and Hunt, S.P. (1980). Projections of the nucleus of the basal optic root in the pigeon: an autoradiographic and horseradish peroxidase study. J. comp. Neurol. 189, 615-670.

Brodal, A. (1940) Experimentelle Untersuchungen uber die olivo-cerebellare Lokalisation. Z. Ges. Neurol. Psychiat. 169, 1-153.

Brodal, A. (1952). Experimental demonstration of cerebellar connections from the perihypoglossal nuclei (nucleus intercalatus, nucleus praepositus hypoglossi and nucleus of Roller) in the cat. J. Anat. 86, 110-129.

Brodal, A. (1980). Olivocerebellocortical projection in the cat as determined with the method of retrograde axonal transport of horseradish peroxidase. 2. Topographical pattern in relation to the longitudinal subdivision of the cerebellum. In 'The Inferior Olivary Nucleus: Anatomy and Physiology' (J. Courville, C. de Montigny, and Y. Lamarre, eds.). New York: Raven Press, pp. 185-205.

Brodal, A. (1981). 'Neurological Anatomy in Relation to Clinical Medicine'. 3rd ed. Oxford University Press, New York.

Brooks, V.B. and Thach, W.T. (1981). Cerebellar control of posture and movement. In 'Handbook of Physiology - The Nervous System' (J.M. Brookhart and V.B. Mountcastle, eds.). Sect. 1, Vol. II (Motor Control). Am. Physiol. Soc., Bethesda, pp. 877-946.

Brown-Gould, B. (1980). 'Organization of Afferents from the Brain Stem Nuclei, to the cerebellar Cortex in the Cat'. Adv. Anat. Embryol. Cell. Biol. Vol. 62. Springer Verlag, Berlin, Heidelberg.

Chambers, W.W. and Sprague, J.M. (1955 a). Functional localization in the cerebellum. I. Organization in longitudinal cortico-nuclear zones and their contribution to the control of posture, both extrapyramidal and pyramidal. J. comp. Neurol. 103, 105-129.

Chambers, W.W. and Sprague, J.M. (1955 b). Functional localization in the cerebellum. II. Somatotopic organization in cortex and nuclei. A.M.A. Arch. Neurol. Psychiat. 74, 653-680.

Chan-Palay, V. (1977). 'Cerebellar Dentate Nucleus: Organization, Cytology and Transmitters'. Springer-Verlag, Berlin-Heidelberg, New York.

Clarke, P.G.H. (1977). Some visual and other connections to the cerebellum of the pigeon. J. comp. Neurol. 174, 535-552.

Cochran, S.L. and Hackett, J.T. (1977). The climbing fiber afferent system of the frog. Brain Res. 121, 362-367.

Cochran, S.L. and Hackett, J.T. (1980). Phylogenetically consistent features of cerebellar climbing fibers present in the tadpole. Brain Res. 192, 543-549.

Cohen, D., Chambers, W.W. and Sprague, J.M. (1958). Experimental study of the efferent projections from the cerebellar nuclei to the brainstem of the cat. J. comp. Neurol. 109, 233-259.

Courville, J. (1966). Rubrobulbar fibers to the facial nucleus and the lateral reticular nucleus (nucleus of the lateral funiculus). An experimental study in the cat with silver impregnation methods. Brain Res. 1, 317-337.

Courville, J., Diakiw, N., and Brodal, A. (1973). Cerebellar corticonuclear projections in the cat. The paramedian lobule. An experimental study with silver methods. Brain Res. 50, 25-45.

Courville, J., and Diakiw, N. (1976). Cerebellar corticonuclear projection in cat. The vermis of the anterior and posterior lobes. Brain Res. 110, 1-20.

Cruce, W.L.R. and Newman (1981). Brain stem origins of spinal projections in the lizard *Tupinambis nigropunctatus*. J. comp. Neurol. 198, 185-207.

Cruce, W.L.R. and Nieuwenhuys, R. (1974). The cell masses in the brain stem of the turtle *Testudo hermanni*; a topographical and topological analysis. J. comp. Neurol. 156, 277-306.

Cuello, A.C. and Kanazawa, I. (1978). The distribution of substance P immunoreactive fibers in rat central nervous system. J. comp. Neurol. 178, 129-156.

de Lange, S.J. (1917). Das Hinterhirn, das Nachhirn und das Rückenmark der Reptilien. Folia Neuro-biol. (Lpz). 10, 385-422.

Dietrichs, E., and Walberg, F. (1979). The cerebellar corticonuclear and nucleocortical projections in the cat as studied with anterograde and retrograde transport of horseradish peroxidase. I. The paramedian lobule. Anat. Embryol. 158, 13-39.

Dietrichs, E., and Walberg, F. (1980). The cerebellar corticonuclear and nucleocortical projections in the cat as studied with anterograde and retrograde transport of horseradish peroxidase. II. Lobulus simplex, crus I and II. Anat. Embryol. 161, 83-103.

Dow, R.S. and Moruzzi, G. (1958). 'The Physiology and Pathology of the Cerebellum'. Univ. of Minnesota Press, Minneapolis.

Ebbesson, S.O.E. (1967). Ascending axon degeneration following hemisection of the spinal cord in the tegu lizard (*Tupinambis nigropunctatus*). Brain Res. 5, 178-206.

Ebbesson, S.O.E. (1969). Brain stem afferents from the spinal cord in a sample of reptilian and amphibian species. Ann. N. Y. Acad. Sci. 167, 80-101.

Ebbesson, S.O.E. and Goodman, D.C. (1981). Organization of ascending spinal projections in *Caiman crocodilus*. Cell Tissue Res. 215, 383-395.

Eden, A.R. and Correia, M.J. (1982). An autoradiographic and HRP study of vestibulocollic pathways in the pigeon. J. comp. Neurol. 211, 432-440.

Edinger, L. (1908). 'Vorlesungen über den Bau der nervosen Zentralorganen des Menschen und der Tiere'. Vogel Verl., Leipzig.
Edwards, S.B. (1972). The ascending and descending projections of the red nucleus in the cat: an experimental study using an autoradiographic tracing method. Brain Res. 48, 45-63.
Fano, G. (1884). 'Saggio sperimentale sul meccanismo dei movimenti volontari nella Testuggine palustre (*Emys europaea*)'. Pubbl. Ist. Stud. Super. Firenze, Firenze.
Faull, R.L.M. (1978) The cerebellofugal projections in the brachium conjunctivum of the rat. The ipsilateral and contralateral descending pathways. J. comp Neurol. 178, 519-536.
Faull, R.L.M. and Carman, J.B. (1978). The cerebellofugal projections in the brachium conjunctivum of the rat. I. The contralateral ascending pathway. J. comp. Neurol. 178, 495-518.
Feirabend, H.K.P. (1983). 'Anatomy and Development of Longitudinal Patterns in the Architecture of the Cerebellum of the White Leghorn *Gallus domesticus*'. Thesis, University of Leiden.
Feirabend, H.K.P., Vielvoye, G.J., Freedman, S.L. and Voogd, J (1976). Longitudinal organization of afferent and efferent connections of the cerebellar cortex of the white leghorn (*G. g. domesticus*). Exp. Brain Res., Suppl. 1, 72-78.
Fink, R.P. and Heimer, L. (1967). Two methods for selective impregnation of degenerating axons and their synaptic endings in the central nervous system. Brain Res. 4, 369-374.
Fite, K.V., Reiner, A. and Hunt, S.P. (1979). Optokinetic nystagmus and the accessory optic system of pigeon and turtle. Brain Behav Evol. 16, 192-202.
Flourens, P. (1824) 'Recherches expérimentales sur les propriétés et les fonctions du système nerveux dans les animaux vertébrés'. Crevot, Paris.
Foster, R.E. and Hall, W.C. (1975). The connections and laminar organization of the optic tectum in a reptile (*Iguana iguana*). J. comp Neurol. 163, 397-426.
Frederikse, A.(1931). 'The Lizards Brain'. Thesis, University of Amsterdam.
Freedman, S.L., Voogd, J. and Vielvoye, G.J. (1977). Experimental evidence for climbing fibers in the avian cerebellum. J. comp. Neurol. 175, 243-252.
Fukushima, K., Peterson, B.W., Uchino, Y., Coulter, J D. and Wilson, V.J. (1977). Direct fastigiospinal fibers in the cat. Brain Res. 126, 538-542.
Fuller, P.M. (1974). Projections of the vestibular nuclear complex in the bullfrog (*Rana catesbeiana*). Brain Behav. Evol. 10, 157-169.
Gerrits, N.M. and Voogd, J. (1973). The distribution of the Purkinje cells in the cerebellum of *Testudo hermanni* (turtle) Acta Morphol. Neerl.-Scand 11, 357-358
Gilman, S., Bloedel, J.R. and Lechtenberg, R. (1981). 'Disorders of the Cerebellum'. F.A. Davis Co., Philadelphia.
Goodman, D.C. (1958). Cerebellar stimulation in the unanesthethized bullfrog. J comp. Neurol. 110, 321-336.
Goodman, D.C. (1964). The evolution of cerebellar structure and function. Amer Zool. 4, 33-36.
Goodman, D C. (1969). Behavioral aspects of cerebellar stimulation and ablation in the frog and alligator and their relationship to cerebellar evolution. In 'Neurobiology of Cerebellar Evolution and Development' (R. Llinás, ed.). Am. Med. Ass.,Chicago, pp. 467-473
Goodman, D.C., Hallett, R.E. and Welch, R.B. (1963a). Patterns of localization in the cerebellar corticonuclear projections of the albino rat. J. comp. Neurol. 121, 51-67.
Goodman, D.C., Horel, J.A. and Freemon, F R. (1963b). Functional localization in the cerebellum of the bird and its bearing on the evolution of cerebellar function. J. comp. Neurol. 123, 45-54.
Goodman, D.C. and Simpson, J.T. (1960). Cerebellar stimulation in the unrestrained and unanesthetized alligator. J. comp. Neurol. 114, 127-136
Goodman, D.C. and Simpson, J.T. (1961) Functional localization in the cerebellum of the albino rat. Exp. Neurol. 3, 174-188.
Gorniak, G.C., Rosenberg, H.I. and Gans, C. (1982). Mastication in the tuatara, *Sphenodon punctatus* (Reptilia: Rhynchocephalia), structure and activity of the motor system. J. Morphol. 171, 321-353.
Graham, R.C. and Karnovsky, M.I. (1966) Glomerular permeability. Ultrastructural cytochemical studies using peroxidase as protein tracers J. exp Med. 124, 1123-1134.
Grant, G., Wiksten, B., Berkley, K.J and Aldskogius, H. (1982). The localization of cerebellar-projecting neurons within the lumbosacral spinal cord in the cat An anatomical study with HRP and retrograde chromatolysis J. comp. Neurol. 204, 336-348.
Griffin, G., Watkins, L.R. and Mayer, D J. (1979). HRP pellets and slow-release gels· two new techniques for greater localization and sensitivity. Brain Res. 168, 595-601
Grillner, S (1975). Locomotion in vertebrates central mechanisms and reflex interaction. Physiol. Rev. 55, 247-304.

Grillner, S. (1981). Control of locomotion in bipeds, tetrapods, and fish. In 'Handbook of Physiology - The Nervous System' (J M. Brookhart and V.B. Mountcastle, eds.). Sect. 1, Vol. II (Motor Control). Am. Physiol. Soc., Bethesda, pp. 1179-1236.
Groenewegen, H.J. and Voogd, J. (1977). The parasagittal zonation within the olivocerebellar projection. I. Climbing fiber distribution in the vermis of cat cerebellum. J. comp. Neurol. 174, 417-488.
Groenewegen, H.J., Voogd, J. and Freedman, S.L. (1979). The parasagittal zonation within the olivocerebellar projection. II. Climbing fiber distribution in the intermediate and hemispheric parts of cat cerebellum. J. comp. Neurol. 183, 551-602.
Grover, B.G. and Grusser-Cornehls, U. (1980). Some ascending and descending pathways in the frog revealed by horseradish peroxidase. Neurosci. Lett., Suppl. 5, S193.
Haas, G. (1973). Muscles of the jaws and associated structures in the Rhynchocephalia and Squamata. In 'Biology of the Reptilia', Vol. 4, Morphology D (C. Gans and T.S. Parsons, eds.) Academic Press, London, pp. 285-490.
Hacker, A. (1931) Zur Physiologie des Reptilienkleinhirnes. Z. vergl. Physiol. 15, 679-692.
Haggqvist, G. (1936). Analyse der Faserverteilung in einem Ruckenmarkquerschnitt (Th. 3). Z. mikr.-anat. Forsch. 39, 1-34.
Haines, D.E. (1976). Cerebellar corticonuclear and corticovestibular fibers of the anterior lobe vermis in a prosimian primate (*Galago senegalensis*). J. comp. Neurol. 170, 67-96.
Haines, D.E., Culberson, J.L. and Martin, G.F. (1976). Laterality and topography of cerebellar cortical efferents in the opossum (*Didelphis marsupialis virginiana*). Brain Res. 106, 152-158.
Haines, D.E. and Koletar, S.L. (1979). Topography of cerebellar corticonuclear fibers of the albino rat. Brain Behav. Evol. 16, 271-292.
Haines, D.E., Patrick, G.W. and Satrulee, P. (1982). Organization of cerebellar corticonuclear fiber systems. In 'The Cerebellum, New Vistas' (S.L. Palay and V. Chan-Palay, eds.). Exp. Brain Res., Suppl. 6, 320-371.
Haroian, A.J., Massopust, L.C. and Young, P.A. (1981). Cerebellothalamic projections in the rat· an autoradiographic and degeneration study. J. comp. Neurol. 197, 217-236.
Henkel, C.K. and Martin, G.F. (1977). The vestibular complex of the american opossum *Didelphis virginiana*. J. comp. Neurol. 172, 321-348
Hillman, D.E. (1969). Neuronal organization of the cerebellar cortex in amphibia and reptilia. In 'Neurobiology of Cerebellar Evolution and Development' (R. Llinás, ed.). Amer. Med. Ass., Chicago, pp. 279-325.
Hindenach, J.C.R. (1931). The cerebellum of *Sphenodon punctatum* J. Anat. (Lond.) 65, 283-318.
Hoffer, B.J., Siggins, G.R and Bloom, F.E. (1971). Studies on norepinephrine-containing afferents to Purkinje cells of rat cerebellum. II. Sensitivity of Purkinje cells to norepinephrine and related substances administered by microiontophoresis. Brain Res. 25, 523-534
Hohman, L.B. (1929). The efferent connections of the cerebellar cortex: Investigations based upon experimental extirpation in the cat. Res. Publ. Assoc. Res. Nerv. Ment. Dis. 6, 445-460.
Hökfelt, T. and Fuxe, K. (1969). Cerebellar monoamine nerve terminals, a new type of afferent fiber to the cortex cerebelli. Exp. Brain Res. 9, 63-72.
Hoogland, P.V.J.M. (1977). Efferent connections of the striatum in *Turinambis nigropunctatus*. J. Morph 152, 229-246.
Hubbard, J.I. and Oscarsson, O. (1962). Localization of the cell bodies of the ventral spinocerebellar tract in lumbar segments of the cat. J. comp. Neurol. 118, 199-204.
Huber, G.C. and Crosby, E.C. (1926). On thalamic and tectal nuclei and fiber paths in the brain of the American alligator. J. comp. Neurol. 40, 97-227.
Huber, G.C. and Crosby, E.C. (1933). The reptilian optic tectum. J. comp. Neurol. 57, 57-163.
Ingvar, S. (1918). Zur Phylo- und Ontogenese des Kleinhirns nebst ein Versuch zu einheitlicher Erklarung der zerebellaren Funktion und Lokalisation. Folia neuro-biol. 11, 205-495.
Ito, H., Muzakami, T. and Morita, Y. (1982) An indirect telencephalo-cerebellar pathway and its relay nucleus in teleosts. Brain Res. 249, 1-13.
Jacobs, V L. (1968). An experimental study of the course and termination of the spinocerebellar systems in a lizard (*Lacerta viridis*). Brain Res. 11, 154-176.
Jansen, J. and Brodal, A. (1940). Experimental studies on the intrinsic fibers of the cerebellum. II The corticonuclear projection. J. comp. Neurol. 73, 267-321.
Jansen, J. and Brodal, A. (1942). Experimental studies on the intrinsic fibers of the cerebellum. III The corticonuclear projection in the rabbit and the monkey. Skr. Norske Vidensk.-Akad., I.Mat.-nat. Kl., No. 3, pp. 1-50

Joseph, B.S. and Whitlock, D.G. (1968). Central projections of selected spinal dorsal roots in anuran amphibians. Anat Rec. 160, 279-288.
Karten, H.J. (1964). Projections of the cerebellar nuclei of the pigeon (*Columba livia*). Anat. Rec. 148, 297-298.
Karten, H.J. and Finger, T. (1976). A direct thalamo-cerebellar pathway in pigeon and catfish. Brain Res. 102, 335-338.
Karten, H.J. and Revzin, A.M. (1966). The afferent connections of the nucleus rotundus in the pigeon. Brain Res. 2, 368-377.
Kawakami, M. (1954). Contributions to the comparative anatomy of the cerebellar fiber connections in reptiles. Hiroshima J. Med. Sci. 2, 295-317.
Kennedy, D.T., Shimono, T. and Kitai, S.T. (1970). Parallel fiber and white matter activation of Purkinje cells in a reptilian cerebellum (*Iguana iguana*). Brain Res. 22, 381-385.
Kievit, J. and Kuypers, H.G.J.M. (1972). Fastigial cerebellar projections to the ventrolateral nucleus of the thalamus and the organization of the descending pathways. In 'Corticothalamic Projections and Sensorimotor Activities' (T. Frigyesi, E. Rinvik and M.D Yahr, eds.). Raven Press, New York, pp. 91-114.
Kimm, J., Winfield, J.A. and Hendrickson, A E. (1979). Visual-vestibular interactions and the role of flocculus in the vestibular ocular reflex. In 'Progress in Brain Research', Vol. 50: 'Reflex Control of Posture and Movement' (R. Granit and O. Pompeiano, eds.), Elsevier, Amsterdam, pp. 703-713.
Kitai, S.T., Shimono, T. and Kennedy, D.T. (1969). Inhibition in the cerebellar cortex of the lizard *Lacerta viridis*. In 'Neurobiology of Cerebellar Evolution and Development' (R. Llinás, ed.). Amer. Med. Ass., Chicago, pp. 481-489.
Klimoff, J. (1897). On the Conduction Paths of the Cerebellum. Experimental Anatomical Observations (in Russian). Dissertation, Imperial University of Kazan.
Klimoff, J. (1899). Ueber die Leitungsbahnen des Kleinhirns. Arch. Anat. Physiol., Anat. Abth. 11-27.
Klüver, H. and Barrera, E. (1953). A method for the combined staining of cells and fibers in the central nervous system. J. Neuropath. exp. Neurol. 12, 400-403.
Knapp, H. and Kang, D.S. (1968). The visual pathways of the snapping turtle (*Chelydra serpentina*). Brain Behav. Evol. 1, 19-42.
Kooy, F.H. (1917). The inferior olive in vertebrates. Folia neurobiol. 10, 205-259
Korte, G.E. and Mugnaini, E. (1979). The cerebellar projection of the vestibular nerve in the cat. J. comp. Neurol. 184, 265-278.
Korte, G.E., Reiner, A. and Karten, H.J. (1980). Substance P-like immunoreactivity in cerebellar mossy fibers and terminals in the red-eared turtle *Chrysemys scripta elegans*. Neuroscience 5, 903-914.
Kotchabhakdi, N., Hoddevik, G.H. and Walberg, F. (1978). Cerebellar afferent projections from the perihypoglossal nuclei: An experimental study with the method of retrograde axonal transport of HRP. Exp. Brain Res. 31, 13-29.
Kotchabhakdi, N. and Walberg, F (1977). Cerebellar afferents from neurons in motor nuclei of cranial nerves demonstrated by retrograde axonal transport of horseradish peroxidase. Brain Res. 137, 158-163.
Kotchabhakdi, N. and Walberg, F. (1978). Cerebellar afferent projections from the vestibular nuclei in the cat. Exp. Brain Res. 31, 591-604.
Künzle, H. (1982). Dorsal root projections to the cerebellum in turtle. Exp. Brain Res. 45, 464-466.
Künzle, H. (1983). Supraspinal cell populations projecting to the cerebellar cortex in the turtle, *Pseudemys scripta elegans*. Exp Brain Res. 49, 1-12.
Künzle, H. and Wiklund, L. (1982). Identification and distribution of neurons presumed to give rise to cerebellar climbing fibers in turtle. A retrograde axonal flow study using radioactive D-aspartate as a marker. Brain Res. 252, 146-150.
Kunzle, H. and Woodson, W. (1982). Mesodiencephalic and other target regions of ascending spinal projections in the turtle, *Pseudemys scripta elegans* J. comp. Neurol. 212, 349-364.
Kusuma, A., ten Donkelaar, H.J. and Nieuwenhuys, R. (1979). Intrinsic organization of the spinal cord. In 'Biology of the Reptilia', Vol. 10, Neurology B (C. Gans, R.G Northcutt and P.S. Ulinski, eds.). Academic Press, London, pp. 59-109.
Kuypers, H.G.J.M. (1964). The descending pathways to the spinal cord, their anatomy and function. In 'Progress in Brain Research', Vol. 11. 'Organization of the Spinal Cord' (J.C. Eccles and J.P. Schadé, eds.). Elsevier, Amsterdam, pp. 178-202.
Kuypers, H.G.J M. (1981) Anatomy of the descending pathways. In 'Handbook of Physiology - The Nervous System' (J.M. Brookhart and V.B. Mountcastle, eds.). Sect. 1, Vol II (Motor

Control). Am. Physiol. Soc., Bethesda, pp. 597-666.
Kuypers, H.J.G.M., Bentivoglio, M., Catsman-Berrevoets, C.E. and Bharos, A.T. (1980). Double retrograde neuronal labeling through divergent axons collaterals, using two fluorescent tracers with the same excitation wavelength which label different features of the cell. Exp. Brain Res. 40, 383-392.
Larsell, O. (1923). The cerebellum of the frog. J. comp. Neurol. 36, 89-112.
Larsell, O. (1926). The cerebellum of reptiles: lizards and snake. J. comp. Neurol. 41, 59-94.
Larsell, O. (1932). The cerebellum of reptiles: chelonians and alligator. J. comp. Neurol. 56, 299-345.
Larsell, O. (1967). 'The Comparative Anatomy and Histology of the Cerebellum from Myxinoids through Birds'. The University of Minnesota Press, Minneapolis.
Lawrence, D.G. and Kuypers, H.G.J.M. (1968 a). The functional organization of the motor system in the monkey. I. The effects of bilateral pyramidal lesions. Brain 91, 1-14.
Lawrence, D.G. and Kuypers, H.G.J.M. (1968 b). The functional organization of the motor system in the monkey. II. The effects of lesions of the descending brain-stem pathways. Brain 91, 15-36.
Lazar, G. (1973). Role of the accessory optic system in the optokinetic nystagmus of the frog. Brain Behav. Evol. 5, 443-460.
Leake, P.A. (1974). Central projections of the stato-acoustic nerve in *Caiman crocodilus*. Brain Behav. Evol. 10, 170-196.
Leblanc, E. (1923). L'acérébellation expérimentale chez les Lézards. C.r. Acad. Sci. (Paris), 176, 1182-1184.
Ljungdahl, A., Hokfelt, T. and Nilsson, G. (1978). Distribution of substance P-like immunoreactivity in the central nervous system of the rat. I. Cell bodies and nerve terminals. Neuroscience 3, 861-943.
Llinás, R.R. (1981). Electrophysiology of the cerebellar networks. In 'Handbook of Physiology - The Nervous System' (J.M Brookhart and V.B. Mountcastle, eds.) Sect. 1, Vol. II (Motor Control). Am. Physiol. Soc., Bethesda, pp. 831-876.
Llinás, R. and Hillman, D.E. (1969). Physiological and morphological organization of the cerebellar circuits in various vertebrates. In 'Neurobiology of Cerebellar Evolution and Development' (R Llinás, ed). Am. Med. Ass , Chicago, pp. 43-73.
Llinás, R. and Nicholson, C. (1969). Electrophysiological analysis of alligator cerebellar cortex: a study on dentritic spikes. In 'Neurobiology of Cerebellar Evolution and Development' (R. Llinás, ed.). Am. Med. Ass., Chicago, pp. 431-465.
Llinás, R. and Nicholson, C. (1971). Electrophysiological properties of dendrites and somata in alligator Purkinje cells. J Neurophysiol. 34, 532-551.
Llinás, R , Precht, W. and Kitai, S.T. (1967). Climbing fiber activation of Purkinje cells following primary vestibular afferent stimulation in the frog Brain Res. 6, 371-375.
Lohman, A.H.M. and van Woerden-Verkley, I. (1978). Ascending connections to the forebrain in the tegu lizard. J. comp. Neurol. 182, 555-594.
Luciani, L. (1891). 'Il Cervelletto. Nuovi studi de fisiologia normale e patologice'. Le Monnier, Firenze.
Martin, G.F. and Dom, R. (1970). Rubrobulbar projections of the opossum (*Didelphis virginiana*) J. comp. Neurol. 139, 199-214.
Martin, G.F., King, J.S. and Dom, R. (1974). The projections of the deep cerebellar nuclei of the opossum, *Didelphis marsupialis virginiana*. J. Hirnforsch 15, 545-573.
Matsushita, M. and Hosoya, Y. (1978). The location of spinal projection neurons in the cerebellar nuclei (cerebellospinal tract neurons) of the cat. A study with the horseradish peroxidase technique. Brain Res. 142, 237-248.
Matsushita, M. and Hosoya, Y. (1979). Cells of origin of the spinocerebellar tract in the rat, studied with the method of retrograde transport of horseradish peroxidase. Brain Res. 173, 185-200.
Matsushita, M., Hosoya, Y. and Ikeda, M. (1979). Anatomical organization of the spinocerebellar system in the cat, as studied by retrograde transport of horseradish peroxidase. J comp. Neurol. 184, 81-106.
Matsushita, M. and Ikeda, M. (1975). The central cervical nucleus as cell origin of a spinocerebellar tract arising from the cervical cord: a study in the cat using horseradish peroxidase. Brain Res. 100, 412-417.
Mesulam, M.M. (1978). Tetramethylbenzidine for horseradish peroxidase neurohistochemistry: a non-carcinogenic blue reaction product with superior sensitivity for visualizing neural afferents and efferents. J. Histochem. Cytochem. 26, 106-117.
Miller, M R. and Kasahara, M. (1979). The cochlear nuclei of some turtles. J. comp. Neurol. 185, 221-236.

Miller, R.A and Strominger, N.L. (1973) Efferent connections in the red nucleus in the brainstem and spinal cord of the rhesus monkey. J. comp. Neurol. 152, 327-346.
Mugnaini, E., Atluri, R.L. and Houk, J.C. (1974). Fine structure of granular layer in turtle cerebellum with emphasis on large glomeruli. J. Neurophysiol. 37, 1-29.
Mugnaini, E. and Dahl, A.L. (1975). Mode of distribution of aminergic fibers in the cerebellar cortex of the chicken. J. comp. Neurol. 162, 417-432.
Nauta, W.J.H. and Gygax, P.A. (1954). Silver impregnation of degenerating axons in the central nervous system a modified technique. Stain Technol. 29, 91-93.
Nieuwenhuys, R. (1967). Comparative anatomy of the cerebellum. In 'Progress in Brain Research', Vol. 25. 'The Cerebellum' (C.A. Fox and R.S. Snider, eds.). Elsevier, Amsterdam, pp. 1-93.
Nieuwenhuys, R. (1974). Topological analysis of the brain stem: a general introduction. J. comp. Neurol. 156, 255-276.
Ochoterena, I. (1932). Histologia del cerebelo del Tepayaxin (*Phrynosoma orbiculare*, Wieg). An. Inst Biol. (Méx) 3, 81-94.
Olson, L and Fuxe, U. (1971). On the projections from the locus coeruleus noradrenaline neurons. the cerebellar innervation. Brain Res. 43, 289-295.
Orlovsky, G N. (1970 a). Work of the reticulo-spinal neurones during locomotion. Biophysics 15, 761-771.
Orlovsky, G.N. (1970 b). Influence of the cerebellum on the reticulospinal neurones during locomotion. Biophysics 15, 928-936.
Orlovsky, G.N. (1972 a). Activity of vestibulospinal neurons during locomotion. Brain Res. 46, 85-98.
Orlovsky, G.N. (1972 b). Activity of rubrospinal neurons during locomotion. Brain Res 46, 99-112.
Oscarsson, O (1969). The sagittal organization of the cerebellar anterior lobe as revealed by the projection patterns of the climbing fiber system. In 'Neurobiology of Cerebellar Evolution and Development' (R. Llinás, ed.). Am. Med. Ass., Chicago, pp. 525-537.
Oscarsson, O. (1973). Functional organization of spinocerebellar pathways. In 'Handbook of Sensory Physiology', Vol. II, 'Somatosensory System' (A. Iggo, ed.). Springer-Verlag, Berlin, pp. 339-380.
Oscarsson, O. (1980). Functional organization of olivary projection to the cerebellar anterior lobe. In 'The Inferior Olivary Nucleus· Anatomy and Physiology' (J. Courville, C. de Montigny, and Y. Lamarre, eds.). Raven Press, New York, pp. 279-290.
Oscarsson, O. and Uddenberg, N. (1964). Identification of a spinocerebellar tract activated from forelimb afferents in the cat. Acta Physiol. Scand. 62, 125-136.
Panneton, W.M. and Martin, G.F. (1979). Midbrain projections to the trigeminal, facial and hypoglossal nuclei in the opossum. A study using axonal transport techniques. Brain Res. 168, 493-511.
Panneton, W.M. and Martin, G.F. (1983). Brainstem projections to the facial nucleus of the opossum. A study using axonal transport techniques. Brain Res. 267, 19-33.
Papez, J.W. (1929). 'Comparative Neurology'. Thomas Y. Crowell, New York.
Parent, A. (1973). Distribution of monoamine-containing nerve terminals in the brain of the painted turtle, *Chrysemys picta*. J. comp. Neurol. 148, 157-166.
Parent, A. (1979). Monoaminergic systems of the brain. In 'Biology of the Reptilia', Vol. 10: Neurology B (C. Gans, R.G. Northcutt and P.S. Ulinski, eds.). Academic Press, London, pp. 247-285.
Parent, A. and Poirier, L.J. (1971). Occurrence and distribution of monoamine containing neurons in the brain of the painted turtle, *Chrysemys picta*. J. Anat. 110, 81-90.
Pedersen, R. (1973). Ascending spinal projections in the three species of side-necked turtle: *Podocnemis unifilis*, *Pelusios subniger*, and *Pelomedusa subrufa*. Anat. Rec. 175, 409.
Petras, J.M. and Cummings, J F. (1977). The origin of spinocerebellar pathways. II. The nucleus centrobasalis of the cervical enlargement and the nucleus dorsalis of the thoracolumbar spinal cord. J. comp. Neurol. 173, 693-716.
Pompeiano, O. and Walberg, F. (1957) Descending connections to the vestibular nuclei. An experimental study in the cat. J. comp. Neurol. 108, 465-502.
Ramón, P. (1896). Las células estrelladas de la capa molecular del cerebelo de los reptiles. Rev. Trim. Micrográf. (Cit. S. Ramón y Cajal, 1909-1911).
Ramón y Cajal, S. (1911). 'Histologie du système nerveux de l'homme et des vertébrés'. Vol. II. A. Maloine, Paris.
Reiner, A. (1979). The paleostriatal complex in turtles. Soc. Neurosci. Abstr. 5, 146.
Reiner, A. (1981). A projection of displaced ganglion cells and giant ganglion cells to the accessory optic nuclei in the turtle. Brain Res 204, 403-409

Reiner, A. and Karten, H.J. (1978). A bisynaptic retinocerebellar pathway in the turtle. Brain Res. 150, 163-196.
Rexed, B. (1954). A cytoarchitectonic atlas of the spinal cord in the cat. J. comp. Neurol. 100, 297-379.
Rinvik, E. and Grofová, I. (1974). Cerebellar projections to the nuclei ventralis lateralis and ventralis anterior thalami. Experimental electron microscopical and light microscopical studies in the cat. Anat. Embryol. 146, 95-111.
Robinson, L.R. (1969). Bulbospinal fibres and their nuclei of origin in *Lacerta viridis* demonstrated by axonal degeneration and chromatolysis respectively. J. Anat. 105, 59-88.
Rolando, L. (1809). 'Saggio sopra la vera struttura del cervello dell'uomo e degli animali e sopra le funzioni del sistema nervosa'. Stampeia da S.S.R.M. Privilegiata, Sassari.
Russell, J.S.R. (1895). Degeneration consequent on experimental lesions of the cerebellum. Phil Trans. Roy. Soc. Lond. 186b, 633-660.
Saigal, R.P., Karamalidis, A.N., Voogd, J., Michaloudi, H. and Mangana, O. (1980). Cerebellar afferents from motor nuclei of cranial nerves, the nucleus of the solitary tract and nuclei coeruleus and parabrachialis in sheep, demonstrated with retrograde transport of horseradish peroxidase. Brain Res. 197, 200-206.
Sato, Y., Kawasaki, T. and Ikarashi, K. (1982). Zonal organization of the floccular Purkinje cells projecting to the vestibular nucleus in cats. Brain Res. 232, 1-15.
Schwarz, I.E. and Schwarz, D.W.F. (1980). Afferents to the cerebellar cortex of turtles studied by means of the horseradish peroxidase technique. Anat. Embryol. 160, 39-52.
Senn, D. and Goodman, D.C. (1969). Patterns of localization in the cerebellar corticofugal projections of the alligator (*Caiman sclerops*). In 'Neurobiology of Cerebellar Evolution and Development' (R. Llinás, ed.). Am. Med. Ass., Chicago, pp. 475-480.
Shanklin, W.M. (1930). The central nervous system of *Chameleon vulgaris*. Acta Zool. 11, 425-491.
Shik, M.L. and Orlovsky, G.N. (1976). Neurophysiology of locomotor automatism. Physiol. Rev. 56, 465-501.
Shimazono, J. (1912). Das Kleinhirn der Vogel. Arch. mikr. Anat. 80, 397-449.
Smith, K.K. (1982). An electromyographic study of the function of the jaw adducting muscles in *Varanus exanthematicus* (Varanidae). J. Morphol. 173, 137-158.
Snyder, R L , Faull, R.L M and Mehler, R W. (1978) A comparative study of neurons of origin of the spinocerebellar afferents in the rat, cat and squirrel monkey based on the retrograde transport of horseradish peroxidase. J. comp. Neurol. 181, 833-852.
Stanton, G.B. (1980). Topographical organization of ascending cerebellar projections from the dentate and interposed nuclei in *Macaca mulatta*: an anterograde degeneration study. J. comp. Neurol. 190, 699-731.
Stefanelli, A. (1944 a). I Centri Statici e della coordinazione motoria dei rettili. Commentat. pontif. Acad. Scient. 8, 147-293.
Stefanelli, A. (1944 b). La fisiologia dei centri statici alla luce delle ricerche di morfologia ecologia dei rettili. Arch. Fisiol. 44, 49-77.
Steinbusch, H.W.M., Verhofstad, A.A.J. and Joosten, H.W.J. (1978). Localization of serotonin in the central nervous system by immunohistochemistry: description of a specific and sensitive technique and some applications. Neuroscience 3, 811-819.
Steinbusch, H W.M., Verhofstad, A.A.J. and Joosten, H.W.J. (1982). Antibodies to serotonin for neuroimmunocytochemical studies. J. Histochem. Cytochem. 30, 765-759.
Steiner, J. (1886). 'Die Funktionen des Zentralnervensystems und ihre Phylogenese. IV. Abt. Reptilien'. Braunschweig.
Stern, T.A. and Robinson, K. (1971). Efferent projections of the cerebellar cortex of *rana pipiens*. Anat. Rec. 169, 438.
Stieda, L. (1875). Ueber den Bau des centralen Nervensystems der Amphibien und Reptilien. Z. wiss. Zool. 25, 1-74.
Sugimoto, T., Mizuno, N. and Itoh, K. (1981). An autoradiographic study on the terminal distribution of cerebellothalamic fibers in the cat. Brain Res. 215, 29-47.
Székely, G., Antal, M. and Gorcs, T. (1980). Direct dorsal root projection onto the cerebellum in the frog. Neurosci. Lett. 19, 161-165.
Taber Pierce, E., Hoddevik, G.H. and Walberg, F. (1977). The cerebellar projection from the raphe nuclei in the cat as studied with the method of retrograde transport of horseradish peroxidase. Anat. Embryol. 152, 73-87.
ten Donkelaar, H.J. (1976 a). Descending pathways from the brain stem to the spinal cord in some reptiles. I. Origin. J. comp Neurol. 167, 421-442.
ten Donkelaar, H.J. (1976 b) Descending pathways from the brain stem to the spinal cord in some reptiles. II. Course and site of termination. J. comp. Neurol. 167, 443-463.

ten Donkelaar, H.J. (1982). Organization of descending pathways to the spinal cord in amphibians and reptiles. In 'Progress in Brain Research', Vol. 57, 'Descending Pathways to the Spinal Cord' (H.G.J.M. Kuypers and G.F. Martin, eds.). Elsevier, Biomedical Press, Amsterdam, pp. 25-67.
ten Donkelaar, H.J. and Bangma, G.C. (1983). A crossed rubrobulbar projection in the snake *Python regius*. Brain Res., in press.
ten Donkelaar, H.J. and Bangma, G.C. (1984). The Cerebellum. In 'Biology of the Reptilia', Vol. 17: Neurology C (C. Gans and R.G. Northcutt, eds.), Academic Press, London, in press.
ten Donkelaar, H.J., Bangma, G.C. and de Boer-van Huizen, R. (1983). Reticulospinal and vestibulospinal projections in the snake *Python regius*. Anat. Embryol., in press.
ten Donkelaar, H.J. and de Boer-van Huizen, R. (1978 a). Cells of origin of pathways descending to the spinal cord in a lizard (*Lacerta gallotti*). Neurosci. Lett. 9, 123-128.
ten Donkelaar, H.J. and de Boer-van Huizen, R. (1978 b). Cells of origin of propriospinal and ascending supraspinal fibers in a lizard (*Lacerta gallotti*). Neurosci. Lett. 9, 285-290.
ten Donkelaar, H.J. and de Boer-van Huizen, R. (1981 a). Basal ganglia projections to the brain stem in the lizard *Varanus exanthematicus* as demonstrated by retrograde transport of horseradish peroxidase. Neuroscience 6, 1567-1590.
ten Donkelaar, H.J. and de Boer-van Huizen, R. (1981 b). Ascending projections of the brain stem reticular formation in a nonmammalian vertebrate (the lizard *Varanus exanthematicus*) with notes on the afferent connections of the forebrain. J. comp. Neurol. 200, 501-528.
ten Donkelaar, H.J. and de Boer-van Huizen, R. (1983). The medial longitudinal fasciculus in the lizard *Varanus exanthematicus*. Neurosci. Lett., Suppl. 14, S 370.
ten Donkelaar, H.J., Kusuma, A. and de Boer-van Huizen, R. (1980). Cells of origin of pathways descending to the spinal cord in some quadrupedal reptiles. J. comp. Neurol. 192, 827-851.
ten Donkelaar, H.J. and Nieuwenhuys, R. (1979). The brainstem. In 'Biology of the Reptilia', Vol. 10. Neurology B (C. Gans, R.G. Northcutt and P.S. Ulinski, eds.). Academic Press, London, pp. 133-200.
Thomas, D.M., Kaufman, R.P., Sprague, J.M. and Chambers, W.W. (1956). Experimental studies of the vermal cerebellar projections in the brain stem of the cat (fastigiobulbar tract). J. Anat. 90, 371-385.
Torvik, A. and Brodal, A. (1954). The cerebellar projection of the peri-hypoglossal nuclei (nuclei intercalatus, nucleus praepositus hypoglossi and nucleus of Roller) in the cat. J. Neuropath. Exp. Neurol. 13, 515-527.
Tuge, H. (1932). Somatic motor mechanisms in the midbrain and medulla oblongata of *Chrysemys elegans* (Wied). J. comp. Neurol. 55, 185-271.
Ulinski, P.S. (1977). Tectal efferents in the banded water snake, *Natrix sipedon*. J. comp. Neurol. 173, 251-274.
van Hoevell, J.J.L.D. (1916). De kernen der kleine hersenen. Proc. Acad. Sci. Amst. 24, 1485-1498.
van Rossum, J. (1969). 'Corticonuclear and corticovestibular projections of the cerebellum'. Thesis, University of Leiden. Van Gorcum, Assen.
Verhaart, W.J.C. (1974). Identification of fibre systems of the avian midbrain. J. Hirnforsch. 15, 379-386.
Voneida, T.J. and Sligar, C.M. (1979). Efferent projections of the dorsal ventricular ridge and the striatum in the tegu lizard, *Tupinambis nigropunctatus*. J. comp. Neurol. 186, 43-64.
Voogd, J. (1964). 'The Cerebellum of the Cat. Structure and Fiber Connections'. Thesis, University of Leiden. Van Gorcum, Assen.
Voogd, J. (1967). Comparative aspects of the structure and fibre connections of the mammalian cerebellum. In 'Progress in Brain Research', Vol. 25: 'The Cerebellum' (C.A. Fox and R.S. Snider, eds.). Elsevier, Amsterdam, pp. 94-134.
Voogd, J. (1969). The importance of fiber connections in the comparative anatomy of the mammalian cerebellum. In 'Neurobiology of Cerebellar Evolution and Development' (R. Llinás, ed.). Am. Med. Ass., Chicago, pp. 493-514.
Voogd, J. and Bigaré, F. (1980). Topographical distribution of olivary and corticonuclear fibers in the cerebellum. a review. In 'The Inferior Olivary Nucleus: Anatomy and Physiology' (J. Courville, C. de Montigny and Y. Lamarre, eds.). Raven Press, New York, pp. 207-234.
Walberg, F., Pompeiano, O., Brodal, A. and Jansen, J. (1962a). The fastigiovestibular projection in the cat. An experimental study with silver impregnation methods. J. comp. Neurol. 118, 49-75.
Walberg, F., Pompeiano, O., Westrum, L.E. and Hauglie-Hanssen, E. (1962b). Fastigioreticular fibers in the cat. An experimental study with silver methods. J. comp. Neurol. 119, 187-199.

Watkins, L.R., Griffin, G., Leichnetz, G R and Mayer, D.J. (1980). The somatotopic organization of the nucleus raphe magnus and surrounding structures as revealed by HRP slow-release gels. Brain Res 181, 1-15.
Watt, C B. and Mihailoff, G.A. (1983). The cerebellopontine system in the rat. I. Autoradiographic studies. J. comp. Neurol. 215, 312-330.
Weston, J.K. (1936). The reptilian vestibular and cerebellar gray with fiber connections. J. comp. Neurol. 65, 93-199.
Wetzel, M.C. and Stuart, D.G. (1976). Ensemble characteristics of cat locomotion and its neural control. Prog Neurobiol. 7, 1-98.
Wetzel, M.C. and Stuart, D.G. (1977). Activation and co-ordination of vertebrate locomotion. In 'Mechanics and Energetics of Animal Locomotion' (R. McN. Alexander and G. Goldspink, eds.). Chapman and Hall, London, pp. 115-152.
Wiklund, L., Toggenburger, G. and Cuénod, M. (1982). Aspartate: possible neurotransmitter in cerebellar climbing fibers. Science 216, 78-80.
Wilson, V.J. and Melvill Jones, G. (1979). 'Mammalian Vestibular Physiology'. Plenum Press, New York.
Wilson, V.J., Uchino, Y., Maunz, R.A., Susswein, A. and Fukushima, K. (1978). Properties and connections of cat fastigiospinal neurons. Exp. Brain Res. 32, 1-17.
Winfield, J.A., Hendrickson, A.E. and Kimm, J. (1978). Anatomical evidence that the medial terminal nucleus of the accessory optic tract in mammals provides a visual mossy fiber input to the flocculus. Brain Res. 151, 175-182.
Wold, J.E. (1981). The vestibular nuclei in the domestic hen (*Gallus domesticus*). VI. Afferents from the cerebellum. J. comp. Neurol. 201, 319-341.
Wolters, J G., de Boer-van Huizen, R and ten Donkelaar, H.J. (1982a). Funicular trajectories of descending brain stem pathways in a lizard. In 'Progress in Brain Research', Vol. 57: 'Descending Pathways to the Spinal Cord' (H.G.J.M. Kuypers and G.F. Martin, eds.). Elsevier, Biomedical Press, Amsterdam, pp. 69-78.
Wolters, J.G., ten Donkelaar, H.J. and Verhofstad, A A.J. (1983 a). Immunohistochemical localization of monoamines in the brain stem and spinal cord of the lizard *Varanus exanthematicus*. In preparation.
Wolters, J.G., ten Donkelaar, H.J. and Verhofstad, A A J (1983 b) Immunohistochemical localization of some peptides (substance P, leu- and met-enkephalin) in the brain stem and spinal cord of the lizard *Varanus exanthematicus*. In preparation.
Wolters, J.G., ten Donkelaar, H.J., Verhofstad, A.A.J., Steinbusch, H.W.M. and Joosten, H. (1982b). Immunohistochemical localization of tyrosine hydroxylase, serotonin, substance P, and leu- and met-enkephalin in the brain stem and spinal cord of the lizard *Varanus exanthematicus* Neurosci. Lett., Suppl. 10, S 525.
Woodson, W. and Kunzle, H. (1982). Distribution and structural characterization of neurons giving rise to descending spinal projections in the turtle *Pseudemys scripta elegans*. J. comp Neurol. 212, 336-348.
Yamamoto, K., Tohyama, M and Shimizu, N. (1977). Comparative anatomy of the topography of catecholamine containing neuron systems in the brain stem from birds to teleosts. J. Hirnforsch. 18, 229-240.
Yamamoto, M. (1978). Localization of rabbit's floccular Purkinje cells projecting to the cerebellar lateral nucleus and the nucleus prepositus hypoglossi investigated by means of the horseradish peroxidase retrograde axonal transport. Neurosci. Lett. 7, 197-202.
Zecha, A. (1968). The brachium conjunctivum in pigeons. Acta Morphol. Neerl.-Scand. 7, 97.

Gesina Christina Bangma werd geboren op 3 mei 1954 te Baarn. Na het behalen van het gymnasiumdiploma in 1972 studeerde zij biologie aan de Rijksuniversiteit van Utrecht. Het doctoraalexamen, hoofdrichting Embryologie, werd afgelegd in januari 1980. Van mei 1980 tot mei 1983 was zij als wetenschappelijk medewerkster in tijdelijke dienst werkzaam op het Laboratorium voor Anatomie en Embryologie (hoofd: Prof.Dr. H.J. Lammers) van de Faculteit der Geneeskunde aan de Katholieke Universiteit te Nijmegen.

STELLINGEN

I

De betrouwbaarheid van ontogenetisch onderzoek is mede afhankelijk van de nauwkeurigheid van de gehanteerde criteria ter bepaling van de opeenvolgende ontwikkelingsstadia.

A.A.M. Gribnau en L.G.M. Geysberts
Adv. Anat. Embryol. Cell Biol. 68, (1981).

II

De homologie van branchiomeren en metameren kan worden aangetoond m.b.v. experimenteel embryologisch onderzoek.

III

Het cerebellum coördineert bewegingspatronen en past deze aan de invloeden van de omgeving aan.

Yu. J. Arshavsky, J.M. Gelfand en G.N. Orlovsky
TINS (1983) 6, 417-422.

IV

De cerebellaire schors van landvertebraten is in longitudinale zones georganiseerd.

(Dit proefschrift)

V

Gezien de mogelijke toxische werking van pyridoxine (vitamine B_6) verdient het aanbeveling de dosering van dit als onschadelijk bekend staand vitamine te limiteren.

Schaumburg et al.
N. Engl. J. Med. (1983) 309, 445-448.

VI

De stelligheid waarmee de geneeskunde de genezingskansen t.a.v. een ziekte aanbiedt is mede oorzaak van het ontstaan van de euthanasieproblematiek.

VII

Financiële beheersing van de gezondheidszorg is onder meer afhankelijk van een juiste interpretatie van het begrip 'recht hebben op gezondheid'.

VII

In het anatomie-onderwijs aan medische studenten dient grotere aandacht te worden besteed aan de zogenaamde 'doorsneden-anatomie'.

IX

Het achterwege blijven van een gericht beschermingsbeleid van de overheid voor de otter ter voorkoming van een verdere achteruitgang van deze soort hier te lande, is in strijd met internationale verplichtingen zoals onder meer neergelegd in het Verdrag van Bern (Verdrag inzake het behoud van wilde dieren en planten en hun natuurlijke leefmilieu in Europa).

X

Er dient geen verband te worden gelegd tussen het in het kader van dit proefschrift gebruikte aantal proefdieren en de betrokkenheid van mijn echtgenoot bij het tot stand komen van de Wet bedreigde uitheemse dier- en plantesoorten.

G.C. Bangma

Nijmegen, 8 december 1983.