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A WELL DEFINED SPINOCEREBELLAR SYSTEM IN THE WEAKLY ELECTRIC TELEOST FISH *GNATHONEMUS PETERSII*

A tracing and immuno-histochemical study

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INTRODUCTION

In the last fifteen years a few publications (4, 6, 7, 14, 15) have appeared on the ascending pathways from the spinal cord in different species of teleost fish. The authors confirm the generally accepted fact that very little investigations has been made of the ascendings pathways in non mammalian vertebrates and even less in teleost fish. Indeed our present knowledge is based principally on data presented in the work of A. Kappers *et al.* (9) "Comparative Anatomy of the Nervous system of Vertebrates". The findings of Hayle (6, 7) (using Nauta's degeneration method) and of Oka *et al.* (15) (using cobaltic lysine axonal transport) bring a solid experimental support to the statement that ascending fibers arise from the dorsal and anterolateral funiculus and project to different cranial motor nuclei, to the medullary reticular formation, to the torus semicircularis and to several regions of the cerebellum. However, these results furnish little information about the existence of *long* ascending pathways since in both investigations the spinal sections or cobaltic-lysine applications were made at relative high spinal levels. Although the target of the ascending fibers could be determined, the described degenerated or labelled fibers appear to constitute a rather diffuse system and do not form well defined pathways. Furthermore, no experimental evidence is given concerning the cells of origin of the ascending fibers.

The mormyrid fish has a large cerebellum, and it has been established that this structure is a target for long ascending pathways originating in the spinal cord of higher vertebrates. We have looked for similar spinal pathways in *Gnathonemus petersii*, a mormyrid teleost, in which proprioceptive afferents (stretch receptors) (3) and corresponding sensory endings (17, 18) have been demonstrated. Two abstracts have been already published on this subject (11, 20).

METHODS

All together sixteen specimens of *Gnathonemus petersii* were used in this study: two for the Fink Heimer degeneration method, four for calbindin immunohistochemistry and ten

for HRP tracing. Prior to surgery, the animals were anaesthetized in each case with MS222 (tricaine methanesulfonate, Sandoz).

Fink Heimer degeneration.

To demonstrate long ascending spinal pathways by the secondary degeneration procedure, the vertebral column was exposed at the level of the anterior pole of the electric organ and the spinal cord, which is constituted in this fish of 47 segments, was transsected at the 34th segment. The wound was sealed with tissue glue and the fish were kept in their aquarium at 23°C for 21/28 days. After reaenesthesia with MS222 the fish were perfused transcardially with teleost Ringer solution (21); the perfusion was continued with 10% formaldehyde, in which the dissected brains and spinal cords were kept for one month. Both brains and spinal cords were then serially sectioned at 20 or 30 μm thick sections on a freezing microtome in the frontal plan. The sections were processed according to Fink-Heimer's (5) silver impregnation method and mounted without counterstaining.

Calbindin immunochistochemistry.

Anaesthetized with an overdose of MS222, the fish were perfused with teleost Ringer solution followed by 4% paraformaldehyde in 0.1M phosphate buffer at 4°C. The dissected brains and spinal cords were cut either with a vibratome or freezing microtome in 40 or 60 μm thick serial sections in the transverse or horizontal plane. The sections were placed first in 3% H_2O_2 in 0.05M Tris buffer, pH 7.6 for 1h in order to neutralize the action of endogenous peroxidase. Nonspecific binding was reduced by incubation of the sections in 20% normal goat serum in the same Tris buffer for 1h at room temperature. The sections were then incubated in anti CaBP28k antisera (dilution 1:2000) for 24-28h at 4°C with slow agitation this was followed by incubation in goat anti-rabbit IgG antiserum (Bionetics or Miles; 1:40) for 24h, and the sections were then transferred to rabbit-antiperoxidase complex (PAP, Miles) diluted 1:100 for 1h. The antiserum and the PAP were diluted in 0.05M Tris buffer, pH 7.6, containing 1% normal goat serum (NGS). The sections were washed several times in Tris buffer-NGS between each incubation step. The reaction product was visualized using a solution of 0.2 mg diaminobenzidine (DAB, Sigma) and 6mg of ammonium nickel sulfate dissolved in 1 ml 0.05M Tris buffer, pH 7.6, containing 1 μl H_2O_2. The immunostained sections were washed, dehydrated and mounted in DPX (BDH Chemicals).

Controls were performed: (1) by replacing the anti-CaBP28K antiserum with normal rabbit or goat serum; (2) by replacing the second antibody or the PAP by normal goat serum; (3) by using the anti-CaBP28K antiserum saturated with purified CaBP28K (500 micrograms CaBP/ml of undiluted antiserum).

Horseradish peroxidase tracing.

Two kinds of HRP applications were made: *a*) injection into the cerebellar caudal lobe, and, *b*) HRP pelet application to the highest spinal segmental roots.

a) Under anaesthesia the skull was opened over the otic bulla; the bulla was punctured and pushed the bottom of the cavity. The thin bone which covers the valvula was opened and by sucking away the valvula, the caudal lobed was exposed. A 30% HRP solution was injected by pressure by means of a micropipette held in a micromanipulator. The otic cavity was then packed with Gelfoam and the wound was sealed with tissue glue.

After a survival time of 4-5 days the fish was perfused transcardially perfused first with teleost Ringer solution and then with a fixative containing 2% glutaraldehyde and 2% formaldehyde in 0.1% phosphate buffer (pH7.4). The brains and spinal cords were dissected, removed and postfixed for a few hours in the same fixative. The brains were kept in a 30% sucrose buffer solution over night before serial sectioning at 40 μm on a freezing microtome in the frontal or horizontal planes. The sections were subsequently processed according to the modified Hanker Yates protocol (2), following mounted on gelatine coated slides, and counterstained with cresyl violet.

b) In the second case the procedure was similar. The cranial roots, runing together with the vagus nerve, were also approached through the otic cavity. These roots were sectioned and a pellet of HRP was placed on their central cut end.

Abbreviations

aLL : anterior lateral lobe
ax : axon
C : central canal
CaBp : calbindin immunoreactive fibers
CC : cerebellar corpus
CLg : caudal lobe pars granularis
DC : dorsal column
DG : dorsal gray
DH : dorsal horn
EG : eminentia granularis
El : electric lobe
FL_1 : lateral funicular one nucleus
FL_2 : lateral funicular two nucleus
FM : medial funicular nucleus
g : motoneurons of fundamental group
LC : lateral column
ll : lemniscus lateralis
ml : molecular layer
MFL : medial longitudinal fascicle
M : Mauthner axon
nEL : electrical nucleus
nlr : lateral reticular nucleus
pre : preeminentialis nucleus
PTA : paratrigeminal associated command nucleus
Sgl : spinal ganglion
Va : valvula
Vc : ventral column
Vd : descending trigeminal root
VH : ventral horn
vl : group of ventrolateral motoneurons
V : ventricle
Xn : vagal nerve
Xs : sensory vagal nucleus.

Figs. 1-6. – *Transverse sections of the spinal cord and the bulbe at different levels showing degenerated fibers and fiber tracts (Fig. 1, 2 and 3, Fink-Heimer) following transection of the spinal cord at the 3-4th segment and CaBP immunoreactivity of the same fiber tract at similar levels (Figs. 4, 5 and 6).*

Fig. 1. Spinal section at the 10th spinal segment, showing degenerated fiber tracts and fibers in the dorsal (DC) and lateral column (LC), respectively; *c*, central canal; VH, ventral horn. Arrows point to degenerated lateral column tract. ×100.

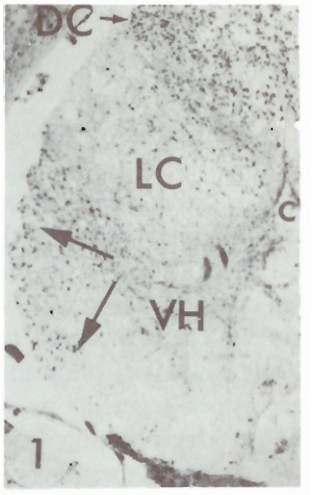
Fig. 2. Degenerated lateral column tract (arrow) at the level of the vagus nerve (Xn). *Xs*, sensory vagus lobe. ×65.

Fig. 3. Collateralisation (arrows) of the lateral column tract (double arrows) at the level of the lateral reticular nucleus (nrl). *Xn*, vagus nerve; *Xs*, sensory vagus lobe; *al*, olive. ×65.

Fig. 4. CaBP-IR lateral column tract (LC arrow) at the 8th spinal segment showing similar position as degenerated fibers in Fig. 1. Note the bundle-like appearance of CaBP-IR fibers. *DH*, dorsal horn; *DC*, dorsal column; *VH*, ventral horn; *c*, central canal. ×100.

Fig. 5. CaBP labelled lateral column tract (arrows) at level of the vagus nerve (Xn). *Xs*, sensory vagus lobe. ×65.

Fig. 6. Labelled collaterals of lateral column tract (arrows) terminating in the lateral reticular nucleus (nrl), *al*, olive ×125.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DC
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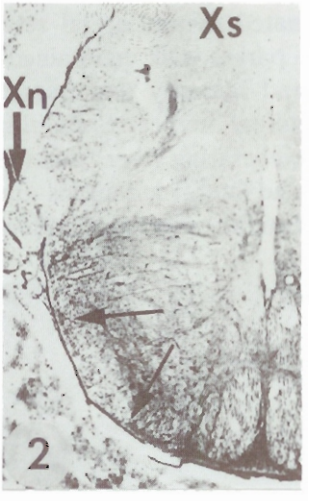
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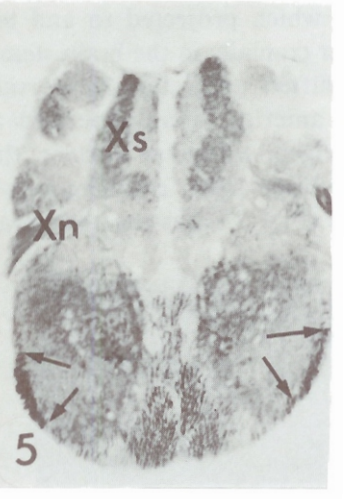
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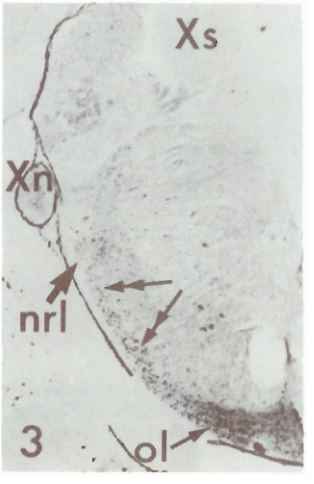
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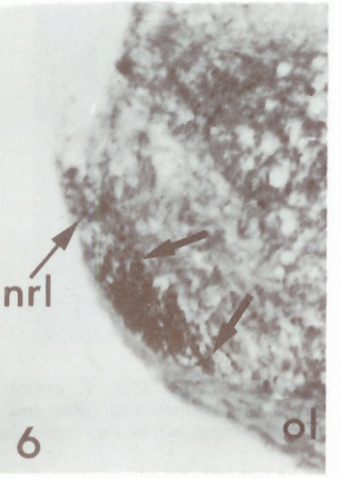
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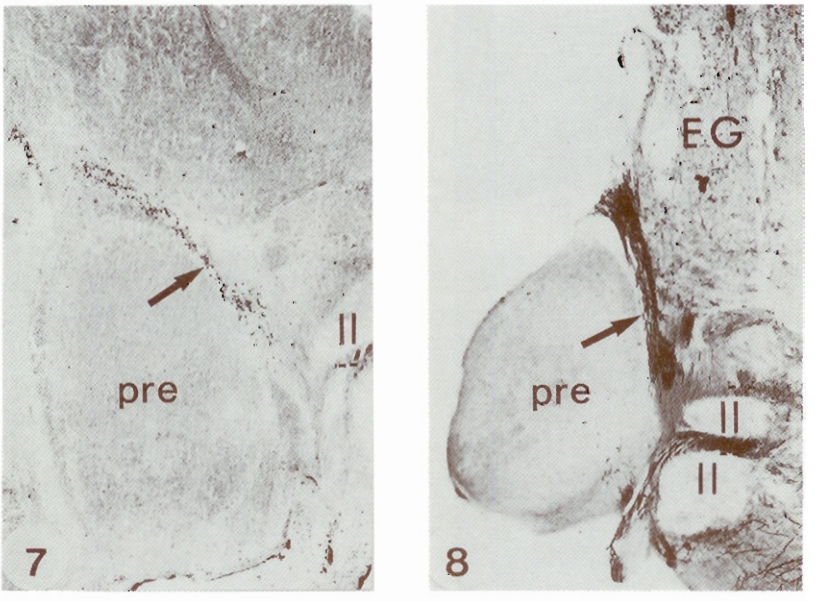
RESULTS

1. *Fink-Heimer degeneration.* — After total transsection of the spinal cord at the 34th segment a large number of degenerating fibers were seen in segments cranial to the section. These fibers were located in the dorsal and lateral columns (Fig. 1); in the former they constituted a well defined fascicle whereas in the latter they were rather scattered and displaced in the ventral part of the lateral column. In this investigation only *these lateral column fibers* will be considered. No degenerating fibers were observed in the dorsal column caudal to the section.

The degenerating fibers in each lateral column could be followed cranialwards throughout the whole spinal cord. In the most upper segments as well as in the lower medulla at the level of the vagal roots, the degenerating fibers assembled forming a narrow strip shaped tract at the ventrolateral periphery of the brain stem (Fig. 2). The tract coursed rostrally in the same position and gave off numerous collaterals which projected to and terminated in the lateral reticular nucleus (Fig. 3). Further cranially in the brain stem, the parent tract maintained its peripheral position but shifted progressivelly dorsally and laterally and passed the ventral border of the anterior lateral line and trigeminal nerves. Skimming the medial border of the trigeminal motor nucleus, the degenerating fiber tract then penetrated deeper into the brain stem, coursing between the lateral lemniscus and the nucleus preeminentialis (Fig. 7). Passing the paratrigeminal commande associated nucleus



Figs. 7-8. – *Passage of degenerated (Fig. 7, arrow) and CaBP-IR (Fig. 8, arrow) lateral column tract fibers between the nucleus preeminentialis (pre) and the eminantia granularis (EG) and lateral lemniscus (II); this part of the tract correspond to the ventrodorsal loop indicated in the schematic drawing Fig. 26*

Fig. 7, ×115; Fig. 8, ×100.

medially (Fig. 26F, PTA) the tract made a ventrodorsally oriented loop, coursed in the caudal direction and penetrated into the granular layer (CLG in Fig. 26 D, E) of the caudal cerebellar lobe. From here on the fibers ran caudalwards and shifted more and more medially and finally crossed in the midline with the contralateral degenerating fiber tract (Fig. 11). After crossing, the tract split into small bundles and single fibers which spread out all over the granular layer of the caudal lobe (Fig. 9 and 10). These fibers appear to produce only coarse terminal structures.

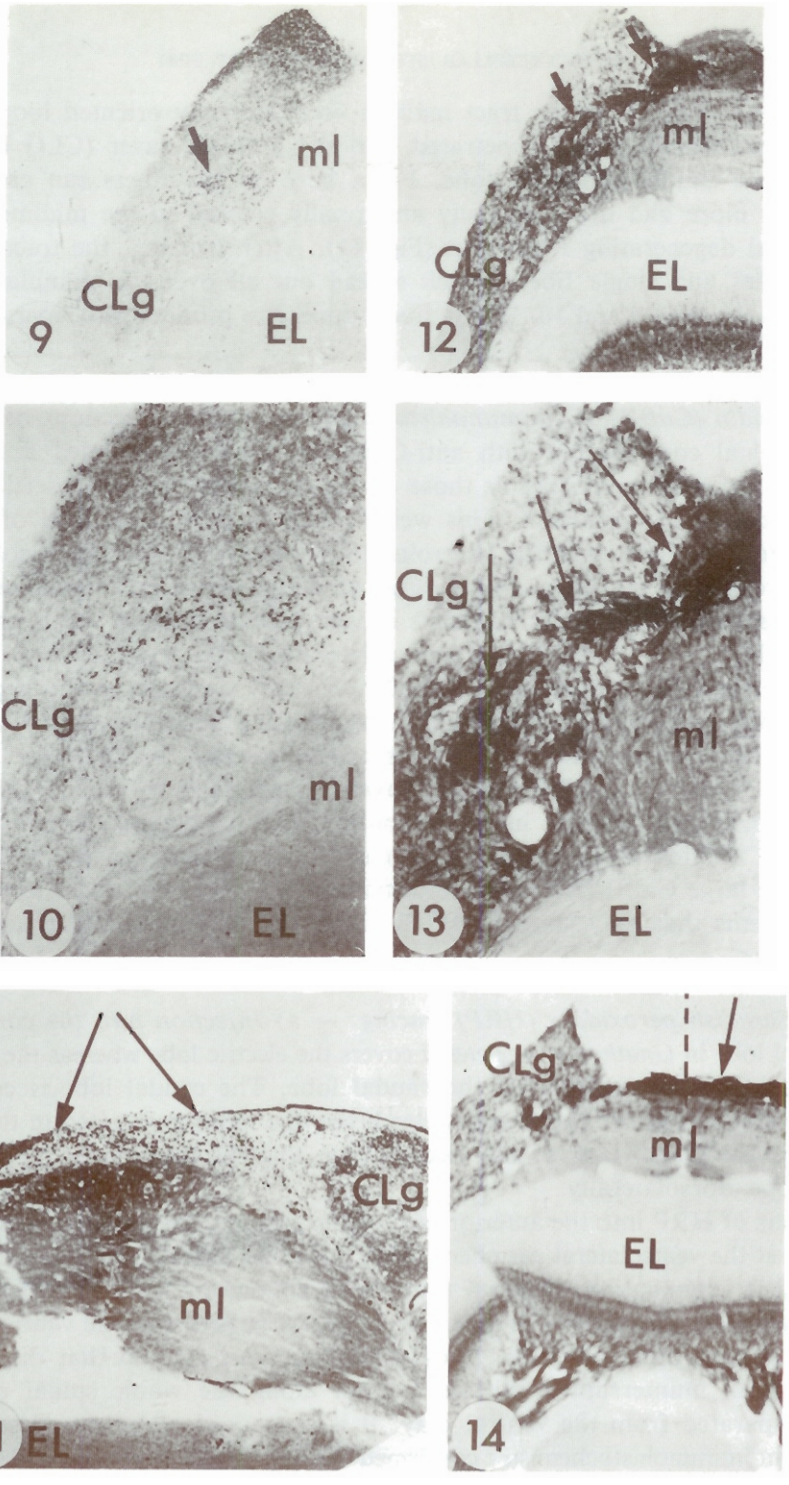
2. *Calbindin (CaBP28K) immunohistochemistry.* — Serial sections of the brain and the spinal cord treated with anti-CaBP28K antibody revealed a fiber tract having the same characteristics as those of the degenerating fiber fascicle consecutive to low spinal transsection. This well formed fascicle, composed of fibers of large diameter, is located at the ventrolateral periphery of the lateral column next to the extremity of the ventral horn (Fig. 4). Keeping the same position through all spinal segments, the bundle maintains its ventrolateral position within the medulla (Fig. 5) where it gives rise to many collaterals which terminate in the lateral reticular nucleus (Fig. 6); further cranially, it runs, similarly to the degenerating fibers, at the periphery of the brain stem, shifting progressively laterally until it reaches the paratrigeminal command associated nucleus. At this level the calbindin immunoreactive (CaBP-IR) tract makes a large loop in the dorsal direction (Fig. 8), turns caudalwards in the granular layer of the caudal lobe where it crosses the midline at more caudal levels (Fig. 14). The crossed bundles split off and the large CaBP-IR fibers are distributed all over the caudal lobe granular layer. It seems that only large CaBP-IR fibers are present in the granular layer.

3. *Horseradish peroxidase (HRP) tracing.* — a) *Injection into the caudal lobe.* The caudal lobe in *Gnathonemus petersii* covers the electric lobe whereas the eminentia granularis is located in front of the caudal lobe. The caudal lobe is constituted of two layers, a granular and a molecular layer: the latter is situated in the midline and covers the dorsal part of the electric lobe whereas the former surrounds the electric lobe dorsolaterally.

Injections of HRP into the anterior part of the granular layer retrogradely labelled large cells at the ventrolateral periphery along the whole spinal cord (Figs. 15 and 16).

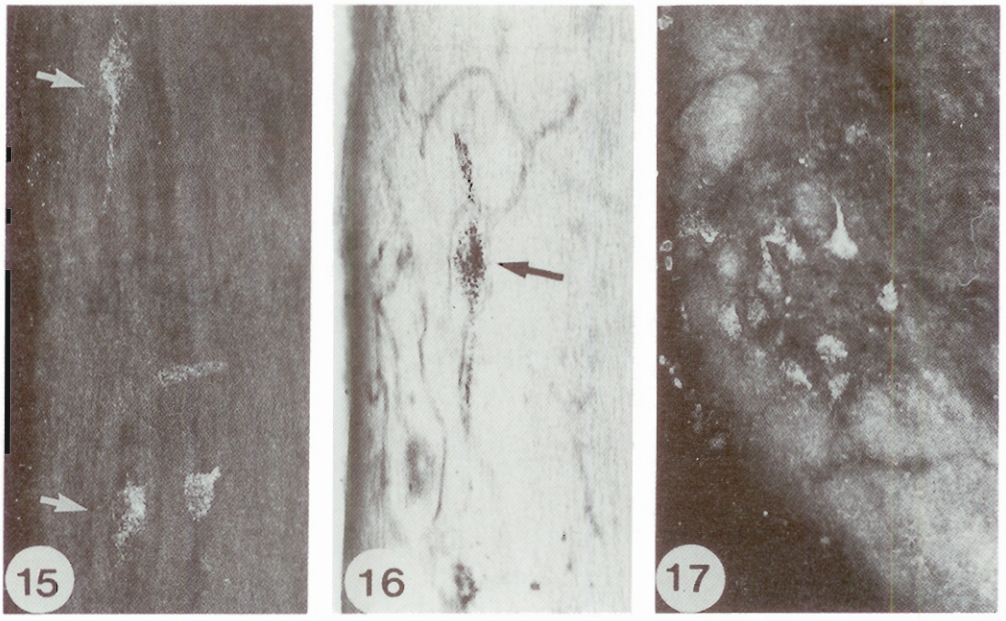
Systematic examination of serial transverse sections of the spinal cord stained with cresyl violet revealed the presence of a cellular structure at the ventral extremity of the ventral horn (Fig. 18). Longitudinal sections showed that this structure consists of an uninterrupted cellular column along the whole spinal cord (Fig. 19) and separated from the ventral gray. It is composed of large and small cells.

Calbindin immunohistochemistry confirmed the results obtained form HRP labelling and Nissl stain. In transverse sections large CaBP-IR cells were seen in the ventrolateral margin of the lateral column (Fig. 21); in longitudinal sections (Fig. 22) these cells appear to form a well circumscribed cellular column similar to



Figs. 9-14. – *Transverse sections of the brain showing terminal degeneration (Fig. 9 low, arrow, Fig. 10 high magnification) and CaBP-IR fibers (Fig. 12 low, arrow, Fig. 13 high magnification, arrows) in the caudal lobe granular layer (CLg) and the degenerated (Fig. 11, arrow) and CaBP-IR (Fig. 14, arrow) decussation of the lateral column tract at the dorsal surface of the caudal lobe molecular layer (ml).*

EL, electric lobe; *dashed line*, midline. Figs. 9, 12 and 14, ×40; Figs. 10, 11 and 13, ×115.



Figs. 15-16. – *Labelled large neurons (arrows) at the lateral periphery of the 10th spinal segment after HRP injection into the caudal lobe.*

Dark (Fig. 15) and light field (Fig. 16) microphotographs. ×450.

Fig. 17. – *Labelled neurons (arrows) in the lateral reticular nucleus after the same caudal lobe injection as indicated in Fig. 15.* ×300.

that seen in Nissl stained or HRP labelled material. In addition to giving their location, the immunohistochemically treated material revealed the morphological characteristics of these cells: they have a large diameter (6-7 μm), are spindle shaped and give rise to a cranially coursing axon and a 200-250 μm long caudally oriented dendrite (Fig. 20). Some of the cells present in addition several short dendrites which gives them a multipolar shape (Fig. 23). The immunotreated sections also showed that the CaBP-IR tract (described above) is located immediatelly dorsal to the CaBP-IR tract (described above) is located immediatelly dorsal to the CaBP-IR cellular column (Fig. 21) and that the axons of the CaBP-IR cells penetrate into (Fig. 20) and form this tract.

The same injections in the caudal lobe also labelled the cells of both lateral reticular nucleus retrogradely in the medulla. These cells which thus also project into the granular layer of the caudal lobe and which receive collaterals from the above described lateral column tract, represent a relay for spinal information towards the caudal lobe.

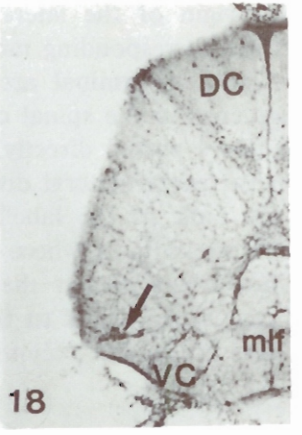
b) *HRP applications to segmental roots.* HRP pellets were applied to peripheral nerves of the most cranial levels of the spinal cord in order to see whether peripher-

Figs. 18-19. - *Cresyl violet stained transverse (Fig. 18) and longitudinal (Fig. 19) sections of the 15th and 25th segments showing the location of the cells of origine (arrow, Fig. 18, double arrows, Fig. 19) of the lateral column tract.*

Note the extreme lateral position of these cells (Fig. 18, arrow) at the lateral extremity of the ventral horn and the position of the cell column next to spinal cord surface (Fig. 19, double arrow). *DC, LC* and VC, dorsal, lateral and ventral column. *VH, DH*, ventral and dorsal horn. *mlf*, medial longitudinal fasciculus. ×60.

Figs. 20-23 – *CaBP-IR of lateral column cells shown in transverse (Fig. 21) and longitudinal sections at low (Fig. 22) and high (Fig. 20 and 23) magnification.*

Note the identical position and aspect of the cellular column in Nissl (Fig. 19) and CaBP-IR (Fig. 22) sections. In Fig. 20, typical spindle shaped cell with long caudally oriented dendrite (arrow) is shown. *ax*, axon. In Fig. 23 besides spindle shaped cell a multipolar cell (arrow) can be seen. Figs. 21 and 22, ×60; Figs. 20, ×120; Figs. 23, ×270.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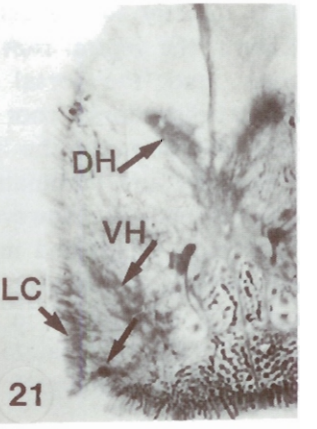
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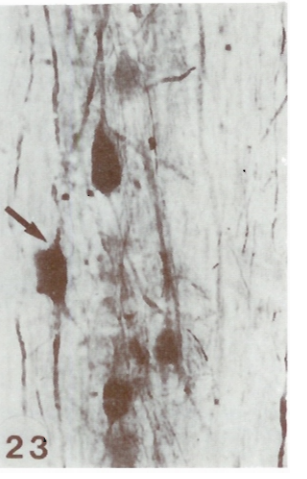
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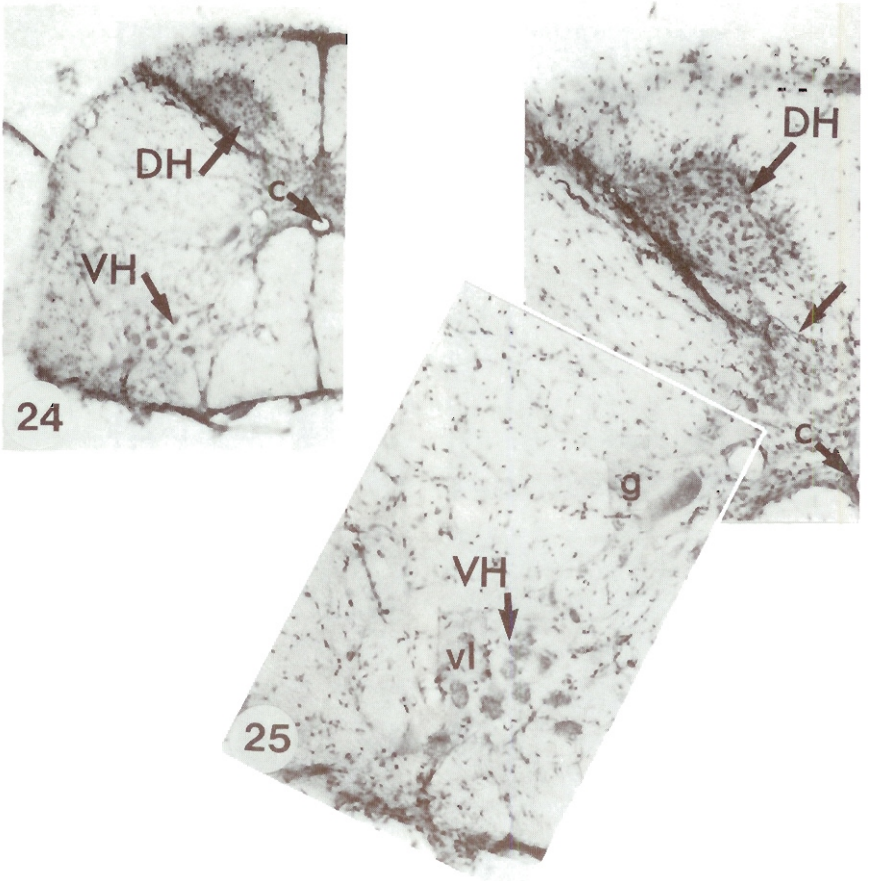
al afferents project directly to the cells of origin of the lateral column tract. After such application, the ventral root and the corresponding motoneurons were labelled retrogradely, and, the dorsal root and its terminal area were labelled anterogradely. The labelled dorsal root fibers entered the spinal cord at the level of the dorsal horn; here some of the large fibers passed directly into the dorsal column, coursed cranially and terminated in the posterolateral division of the second lateral funicular nucleus. However, the bulk of the labelled fibers, both small and large diameter, penetrated into the dorsal horn where they terminated in the dorsal and dorsolateral region. A few fibers ran at the ventral border of the dorsal horn (Fig. 24) to end in the central gray next to the central canal (Fig. 25). Some of them turned laterally into the lateral column to end there. The projection was strictly ipsilateral.



Figs. 24-25. – *Labelling of spinal afferents showing their terminal area limited to the dorsal half of the spinal cord. Same transverse section at low (Fig. 24) and high (Fig. 25) magnification.*

Note the presence of labelled terminal fibers in the dorsal horn (DH) and in the central gray next to the central canal (c), *g*, motoneuron of the fundamental group; *vl*, groupe of ventrolateral motoneurons. *VH*, ventral horn. Fig. 24, ×100; Fig. 25, ×200.

Labelled fibers were never observed in the ventral horn or in spinal region ventrally to the central canal (Fig. 25), showing that there was no direct peripheral fiber projection to the cells of origine of the lateral column tract.

DISCUSSION

The present experiments have clearly established the existence of a long ascending lateral column tract (Fig. 26), in the 47 segment long spinal cord of *Gnathonemus petersii*. Degeneration followed transection at the 34th segment (Fig. 27) indicates that this pathway rises beyond this spinal level and terminates in the caudal lobe of the cerebellum. The ascending pathway is ventrolaterally situated in the lateral column and runs ipsilaterally in the spinal cord and medulla but crosses the midline after entering the caudal lobe, before terminating in the granular layer. The calbindin immunoreactivity of the large axons which constitute the pathway confirms

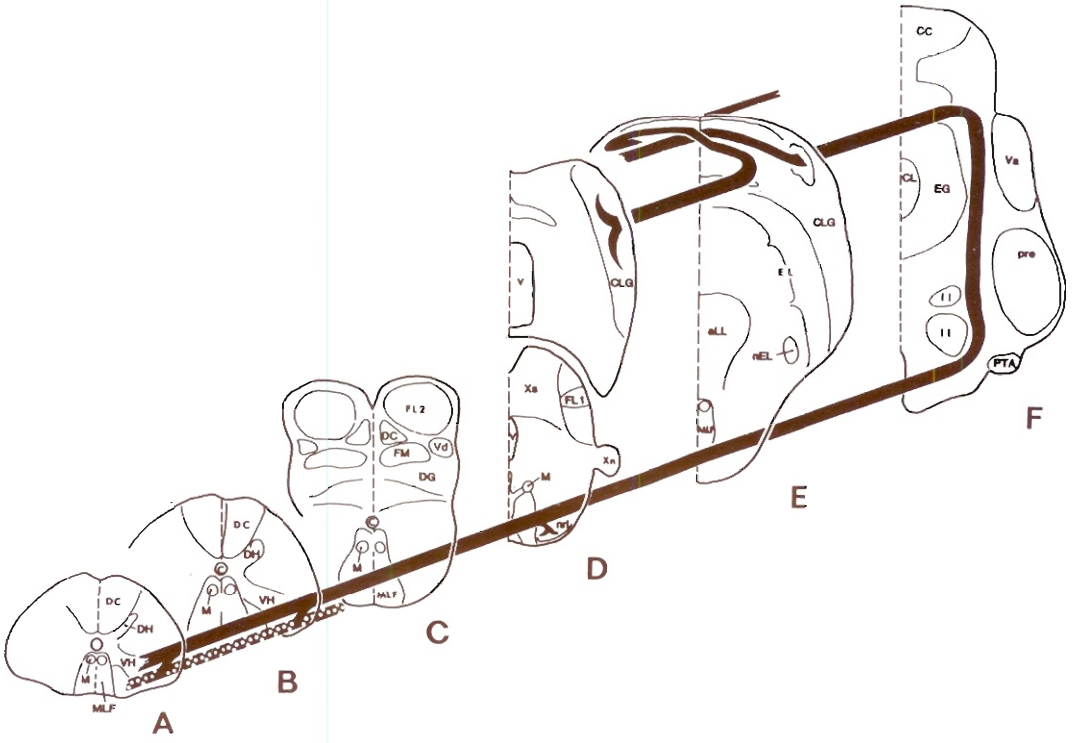


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Fig. 26. – *Schematic representation of the lateral column tract i.e. spinocerebellar pathway, in the weakly electric mormyrid,* Gnathonemus petersii.

Brain and spinal levels are indicated from caudal to rostral by capitals from A to F, A and B correspond to 25th and 7th spinal segments.

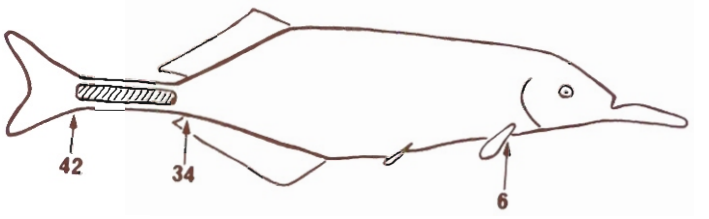

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Fig. 27. – *Schematic representation of the lateral column tract i.e. spinocerebellar pathway, in the weakly electric mormyrid,* Gnathonemus petersii.

Brain and spinal levels are indicated from caudal to rostral by capitals from A to F, A and B correspond to 25th and 7th spinal segments.

the results of the degeneration experiments exactly. Retrograde tracing showed that this pathway takes its origine in a cellular column which, located at the ventrolateral periphery of the spinal cord, constitutes a region gray matter which is separated from the ventral horn. As shown by Nissl stain an CaBP immunohistochemistry this cell column extends along the whole spinal cord confirming the fact that this long ascending pathway starts to arise at the very caudal levels of the spinal cord. It is the first time that such a *long ascending* pathway has been detected in a teleost fish.

The demonstrated *lateral column tract* has several characteristic which may allow it to be identified as homologous to the *ventral spinocerebellar pathway* of higher vertebrates (Fig. 26): it connects a large number of spinal segments *directly* with the cerebellum; it is located at the ventrolateral periphery of the spinal cord; it has an ipsilateral position in the spinal cord and medulla; it runs through more rostral levels than its entering point into the cerebellum; it decussates within the cerebellum and ends, finally, on the contralateral side. In contrast, it does not arises from dorsal horn cells but from a special spinal gray located between the lateral and ventral funiculi; it does not seem to possess a double midline crossing by sending fibers through the spinal ventral white commissure to the other side; it does not end in the corpus cerebelli but in a special cerebellar region and it does not receive *direct* input from the periphery.

The established spinocerebellar pathway in *G. petersii* has one particularity. In contrast to the ventral spinocerebellar system of other vertebrates which gives off on collaterals over its whole spinal and medullary course, the spinocerebellar tract in *G. petersii* produces a large number of collaterals at the medullary level, which project to the cells of the lateral reticular nucleus. It is intriguing that the lateral reticular nucleus projects to the same cerebellar area as the spinocerebellar tract itself.

Hayle (6, 7) and Oka *et al.* (15) also described ascending fibers in the spinal

cord in the rudd (*Scardinius erythropthalmus*) and in the himé salmon (*Onchorhynchus nerka*), respectively. Although the authors give no informations about the total number of the spinal segments in these experimental animals, the length of the fibers was not established since sections and tracers were apparently applied at high spinal levels (lowest level being in the experiments of the himé salmon between 10th and 15th segments). In both cases, the ascending spinal fibers constituted a rather diffuse system in the spinal cord which extended over almost the whole lateral and dorsal funiculi. However, according to Hayle's (7) drawings the degenerated fibers constitute a bundle (the so called spinal lemniscus according to Herrick, ref. 8) in the medulla at levels of the facial lobe and the trigeminal nerve. Both authors (7, 15) and also Murakami and Ito (14) claim that the spinocerebellar system branches from the spinal lemniscus or equivalent bundle (14). Oka *et al.* (15) add, more precisely, that the spinocerebellar fibers arches backwards while entering the eminentia granularis. Concerning the terminal region of the spinocerebellar tract, the results of Oka *et al.* (15) and those of Hayle (6, 7) differ: indeed, in the himé salmon the spinocerebellar fibers end in the eminentia granularis as well as in the valvula and corpus cerebelli whereas in the rudd no projection was seen either in the eminentia granularis or in the valvula, but was observed in the granular layer of the cerebellum. Our results are in agreement with this last statement since the spinocerebellar pathway in *G. petersii* terminates in the granular cerebellar layer although in a very restricted region, the caudal lobe of the cerebellum.

Hayle (6) did not confirm the cells of origin of the ascending spinal fibers. This author presumed that the degenerating spinal fibers could be derived from commissural cells described in elasmobranchs by von Lenhossek (10) and Retzius (16). These cells were found in the gray matter of the spinal cord in greatest abundance in the dorsal region of the ventral horn; their axons cross the midline and after bifurcating the rostrally projecting branch ascends in the lateral or anterolateral funiculus. Using retrograde axonal transport, Finger (4) also found some labelled cells in the catfish at mid-spinal levels (segment number not indicated) after HRP injections into different regions of the cerebellum. Most of these were located medially near the dorsal motor neuron pool, i.e. fundamental group of Beccari (1). No mention was given about the course and location of the ascending axons. None of the cells reported in elasmobranchs or in catfish can be compared with the cells of origin of the presently described lateral column tract since in *G. petersii* the cells of origin of the ascending fibers were located outside the ventral gray next to the border of the spinal cord.

A spinocerebellar path was described specifically in the mormyrid brain from two series of sagittal and transverse sections of *Mormyrops cashive* and *Mormyrops anguilloides* by Stendell (19). However this tract, according to the author, arises from the dorsal funicular nuclei in which the fibers of the dorsal funiculi terminate; coursing rostralwards and laterally from the IVth ventricle, it traverses the fiber plexus of the posterior then the anterior lateral line nerves and enters in the lower part of the eminentia granularis where it may give off some fibers. The symmetric

spinocerebellar tracts decussate in the corpus cerebelli where it probably terminates. The spinocerebellar tract described by Stendell (19) from normal histological material is quite different from the presently identified lateral column tract; it most probably corresponds to the bulbocerebellar tract described earlier by Libouban and Szabo (11). According to these authors, the latter originates, as also stated by Stendell (19), in the dorsal funicular nuclei but terminates in the caudal lobe and not in the corpus cerebelli.

In Stendell's (19) work the spinothalamic tract has the same ventrolateral position at the border of the medulla anda thus can be identified as a part of the presently described spinocerebellar tract; Stendell could not identify the origin of this spinothalamic tract and designation of the thalamus as its projection area is, according to our finding, inexact.

Turning to functional aspects, we may presume that the presently described spinocerebellar pathway carries proprioceptive and tactile information from all spinal levels as does its homologue in higher vertebrates. Proprioceptive afferent fibers have indeed been demonstrated in this fish by electrophysiological experiments (3). These fibers carry spontaneously active impulses which are modulated in a phasic-tonic fashion by stretch applied to the body. Furthermore, sensory nerve endings in the tendons and at the insertion points of tendons on the muscle have been described following light and electronmicroscopical observations (17, 18). The presence of a proprioceptive system in this teleost fish is not surprising since it possesses a highly developed orienting system based on its continuous electric emission. This system works by distortion of the emitted electric field and so the precision of sensing necessarily depends on the configuration of the body at each instant. These considerations are well supported by the fact that the spinocerebellar path projects to a cerebellar region which is in close connection with the electric lobe and which receives all electrosensory information. Thus the proprioceptive (or/and tactile) information can almost immediately operate on or modulate, the integration of electrosensory information.

Finally, two additional points should be taken in consideration: the first concerns the afferent input to the spinocerebellar pathway. Since the spinocerebellar cells do not receive direct synaptic contact from the periphery an integration of peripheral sensory impulses at the spinal level must be supposed. The morphology of the spinocerebellar cells bearing long (may be over the half of a segment), longitudinally oriented dendrites support this hypothesis.

The second point concerns the target area of the spinocerebellar tract. As retrograde labelling has shown, the caudal lobe of both sides receives, besides spinocerebellar afferents an input from the lateral reticular nucleus which represent in some way a feed forward back since the lateral reticular nucleus relais spinocerebellar impulses received by collaterals of the latter (Fig. 28). In addition, the caudal lobe receives proprioceptive or/and tactile impulses directly from the periphery *via* the dorsal funicular nuclei. It has been demonstrated that primary afferent fibers enter the spinal cord and penetrate directly into the dorsal column (see Results); these fibers constitute a long ascending pathway in the dorsal column

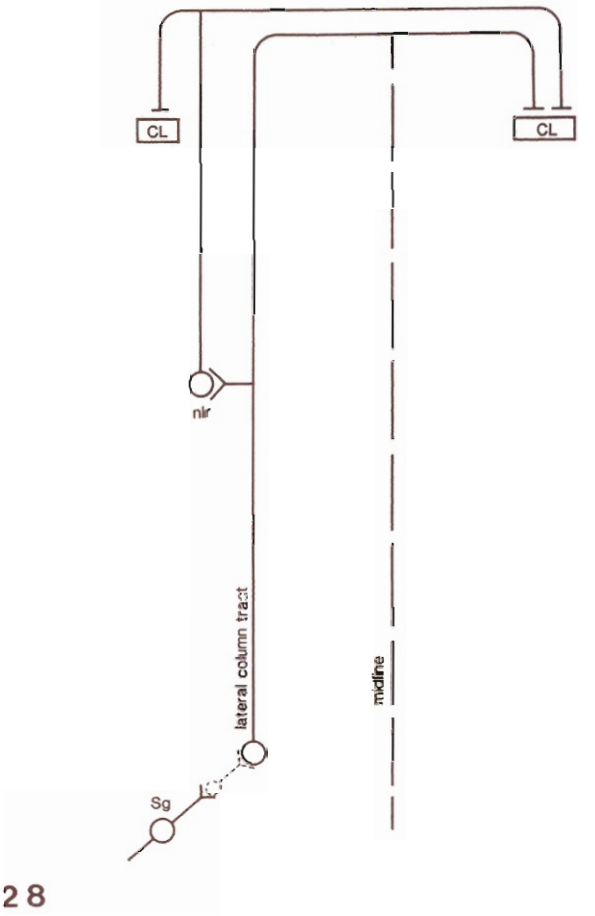

Fig. 28. – *Schematic representation of the spinocerebellar pathway and its connection in* Gnathonemus petersii *as demonstrated with degeneration and immunohistochemical technics.*

EG, eminentia granularis; *nrl*, lateral reticular nucleus; *Sg*, spinal ganglion.

and end in the second dorsal funicular nuclei (11, 20). Afferent fibers from the head region complete the system *via* the trigeminal nerves (12, 13).

Thus, the spinocerebellar pathway in *G. petersii* represents an important link in a spino-bulbo-cerebellar complex which may play an important role in processing of electrosensory information.

SUMMARY

Long ascending fiber systems were investigated in the spinal cord of a teleost fish, *Gnathonemus petersii*. Concomitant results of Fink-Heimer degeneration tracing as well as CaBP28K immunohistochemical labelling demonstrate the existence

of a well defined direct pathway from the very lowest spinal level to the caudal lobe of the cerebellum. HRP retrograde labelling shows that this pathway originates in a cellular column located in the most ventral part of the lateral column next to the lateral extremity of the ventral horn. From each spinal segment, the large axons of these cells gather and form a strip shaped tract at the periphery of the lateral column immediately dorsal to the cell column from which they originate. The spinal course of these fibers is ipsilateral; they give off a large number of collaterals to the lateral reticular nucleus. Bypassing the trigeminal motor nucleus, the lateral column tract courses dorsally to the paratrigeminal command associated nucleus between the lateral lemniscus and the nucleus preeminentialis and with a ventro-dorsally oriented large loop, turns in the caudal direction and penetrates into the cerebellar caudal lobe. Running caudally in the dorsal granular layer of the caudal lobe, it shifts more and more medially and crosses the midline whilst decussating with the contralateral tract on the dorsal margin of the molecular layer of the caudal lobe. Finally, the tract splits off and terminates throughtout the granular layer of the caudal lobe.

The main characteristics of this pathway are similar to those of the ventral spinocerebellar tract of higher vertebrates; it conveys information from all spinal levels directly to the contralateral cerebellum. However, it does not seem to receive direct synaptic input from the periphery, since projection of the dorsal root fibers appears to be limited to the dorsal ipsilateral half of the spinal cord. The appearance of such a pathway in a teleost fish is probably related to the existence of a well developed proprioceptive system in this species.

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