CHAPTER III

RESULTS

Results obtained from this study consists of two main parts, effect of electrical stimulation in specific areas of the FN on ABP and HR and efferent projection and their termination from the rostral , middle and caudal part of the FN.

Effect of Electrical Stimulation of Specific Areas of the FN on ABP and HR.

Electrical stimulation through glass micropipette were employed in the W.ant. rFN , rFN , mFN respectively.

1. Electrical Stimulation in W.ant.rFN

The electrical stimulation through glass micropipette were precisely placed in the white matter area between 200-400 μm anterior to the rostral pole. The FPR was first observed at the depth 6.5 mm from the dura matter. With 0.3 mA current stimulation , the SP and DP were first slightly decreased. Then increased rapidly to the highest response within 5 second. The response is maintained until the termination of stimulation. After that, the blood pressure gradually decrease to normal level within 3 seconds (Fig 6). Resting SP and DP are approximately 135±21.89 mmHg and 78. 33±21.79 mmHg. They decrease to 125±10 mmHg and 73.33±7.63 mmHg immediately after stimulation. The highest response are at 161±20.20 mmHg and 100 ±30.41 mmHg. Percentage change of SP , DP and MAP at the highest response are 20.35±6.74, 34.94±17.63 and 27.96±5.61 respectively (Table 3). These are

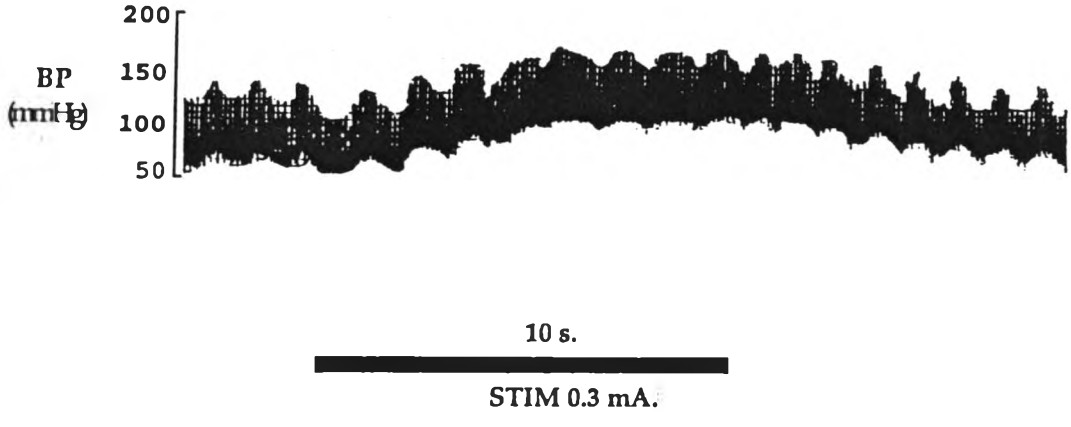

Fig 6 Tracing showing pattern ABP response after stimulation of white matter area anterior to rostral pole of FN

significant different to those of the resting stage. Likewise, heart rate increase in the same pattern as SP and DP. The percentage change at the highest response is 8.90 ±3.34 which significant different from that normal value (Table 4).

2. Electrical Stimulation in the rFN

Electrical stimulation through glass pipette were precisely placed in rostral part area of the FN between 100-400 μm posterior to the rostral pole. The stimulation current was set constant at 0.3 mA in every experiment. The FPR areas were always identified at depth approximately 6.5 mm. Percentage change of SP, DP and MAP at the highest response are 17.36±4.88, 24.30±9.38 and 20.97±6.79. These value are significant ($P<0.05$) (Table 3) higher than the normal value. Likewise, the stimulation induces an increase in HR. The percentage change is 8.74±2.21 which is significant higher ($P<0.05$) than the normal level (Table 4).

3. Electrical Stimulation in the mFN

Electrical stimulation through glass pipette in the middle portion of the FN , precisely in area between 400-500 μm posterior to the rostral pole. Percentage change of SP , DP and MAP at the highest response are 13.71±4.15, 16.66±3.60 and 16.47±1.88. These are significant higher ($P<0.05$) than resting value (Table 3). Likewise the stimulation also induce an increase in HR. The percentage change is 6.26±3.35 which is not significant higher ($P<0.05$) than the resting level (Table 4)

Table 3 The mean ± SD for percentage change in systolic pressure (SP), mean arterial pressure (MAP) and diastolic pressure (DP) during electrical stimulation of unilateral white matter area anterior to rostral pole of FN (W.ant.rFN) rostral of FN, medial of FN.

Area of FN stimulation	% SP	% DP	% MAP
control	0.00±0.00	0.00±0.00	0.00±0.00
W.ant.rFN (n = 3)	20.35±6.74*	34.94±17.63*	27.96±5.61*
rFN (n = 5)	17.36±4.88*	24.30±3.98*	20.97±6.79*
mFN (n = 3)	13.71±4.15*	16.66±3.6*	16.47±1.88*

n = number of tree shrews

* = $p < 0.05$ from resting control

Table 4 The mean ± SD for percentage change in heart rate (HR) during electrical stimulation of unilateral white matter area anterior to rostral pole of FN (W.ant.rFN), rostral of FN, medial of FN.

Area of FN stimulation	% HR
Control	0.00±0.00
W.ant.rFN	8.90±3.34*
(n = 3)	
rFN	8.74±2.21*
(n = 3)	
mFN	6.26±3.35
(n = 3)	

n = number of tree shrews

* = $p < 0.05$ from resting control

4. Electrical Stimulation in the cFN

Electrical stimulation through glass pipette in the caudal portion of the FN, precisely in the area between 700-900 μm posterior to the rostral pole. However, the stimulation failed to induce FPR and increase in HR.

Efferent Projections from the Rostral Part of the FN.

Long bundles of labelled fibers are observed curving in a dorsoventral direction from the BC to the spinal tract of trigeminal nerve (TS V) of both ipsilateral and contralateral side (Fig 7.1, Fig 8, Fig 9) at the level of the rostral inferior cerebellar peduncle (ICP). These bundles consist of short and medium-sized fibers with dark brown colour. They are observed to start at the medial end of the BC. Then, they move ventrolaterally, lying just dorsal to the peduncle, toward the medial boundary of the middle cerebellar peduncle and become the lateral boundary of the TS V; this tract end at the inferior border of the nerve.

At the caudal level of superior olivary nucleus (OS) the bundles were splitted into two separate bundles; the upper and lower. The upper bundles lie just dorsal to the brachium conjunctivum (BC) and run ventrolaterally along the medial border of the brachium pontis. The lower ones stretch along the lateral boundary of TS V extending to the lateral boundary of the OS (Fig 7.2, 7.3, Fig 10).

At the level of rostral part of the seventh cranial nerve (C VII), the upper bundles are observed lying close to the dorsal boundary of the BC from its medial

Fig 7 Atlas of the common three shrew brain from the rostral part of ICP to the caudal medulla (7.1-7.39) showing mapping of labelled fibers and terminals in different areas of the brainstem

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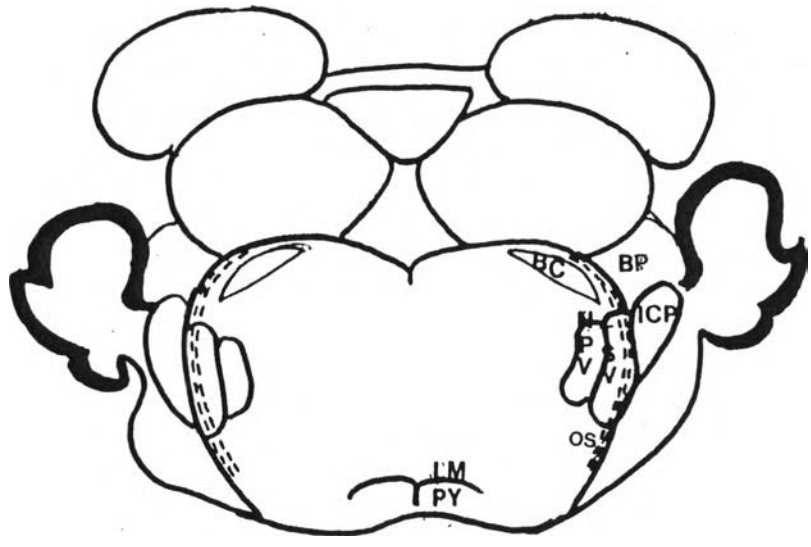

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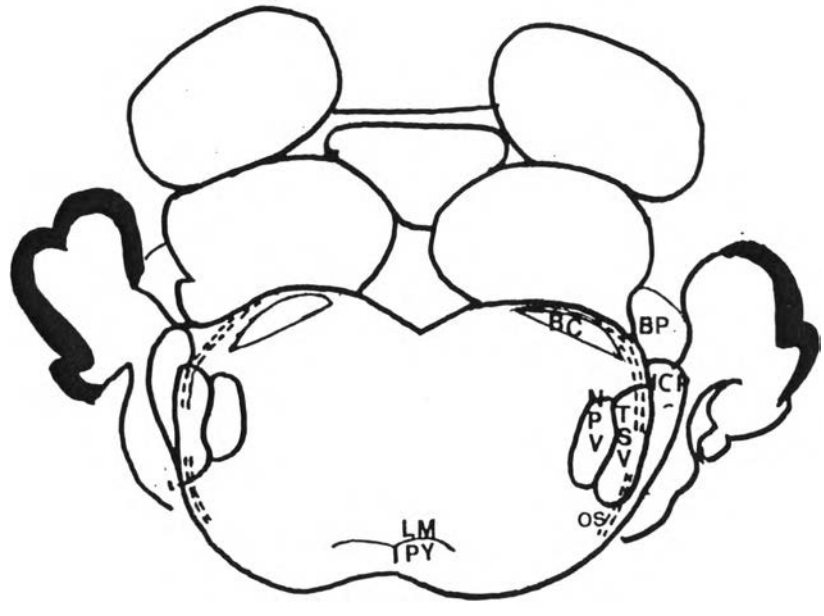

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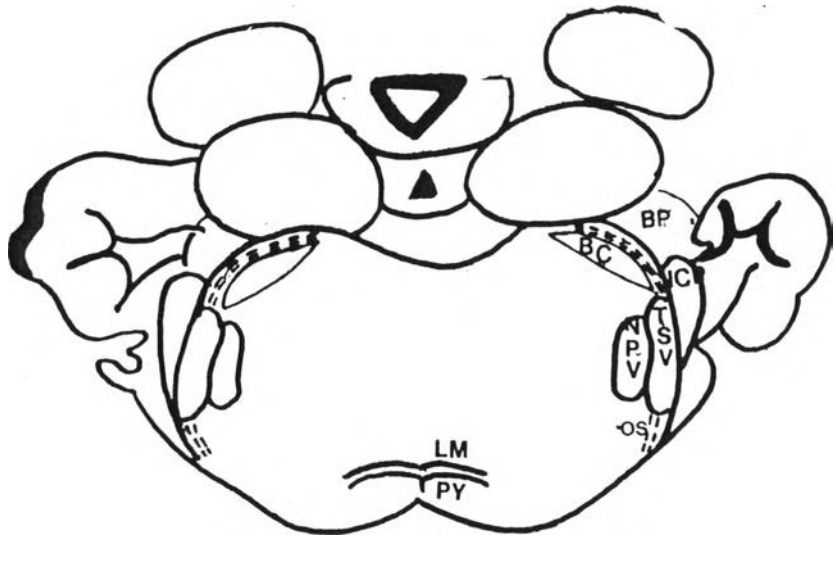

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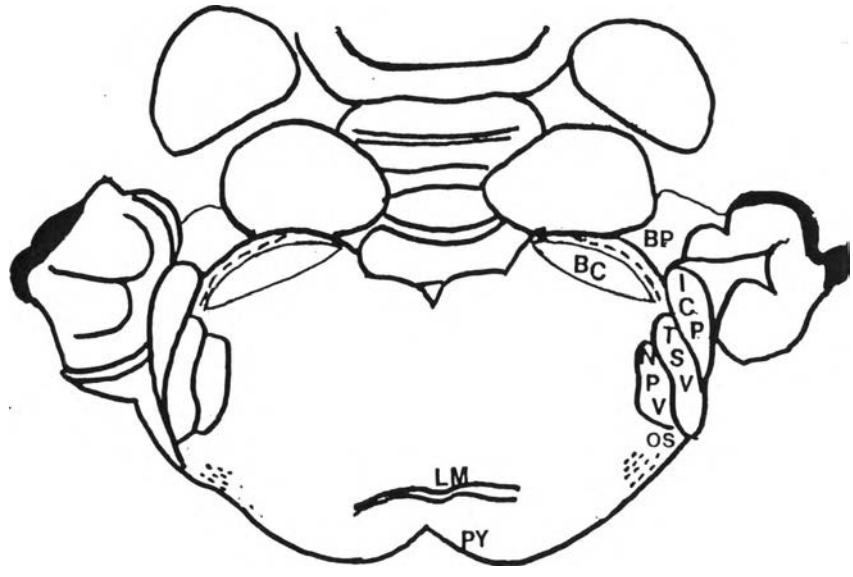
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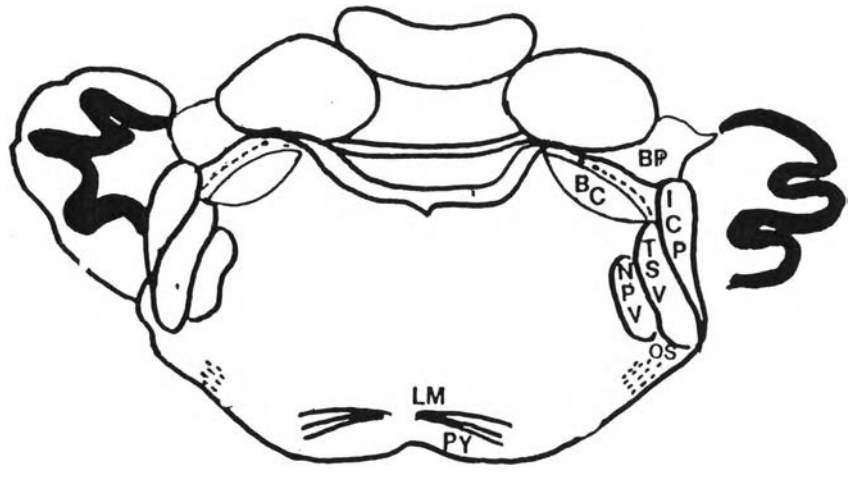
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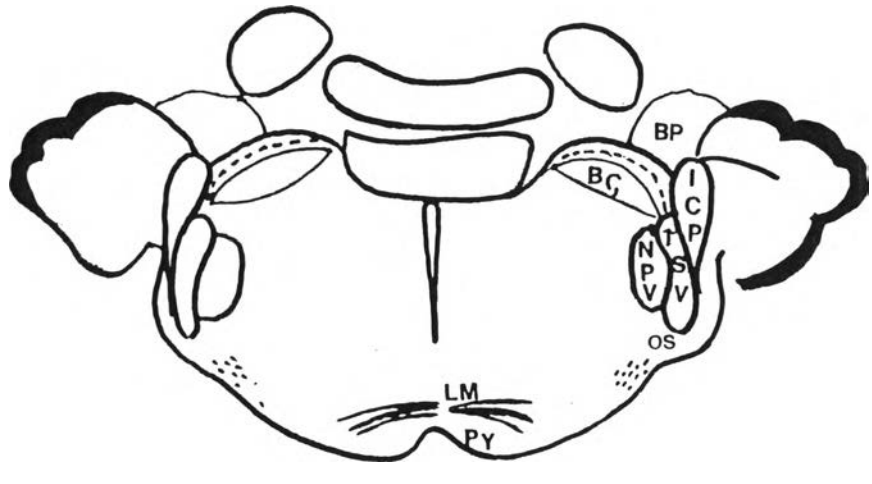
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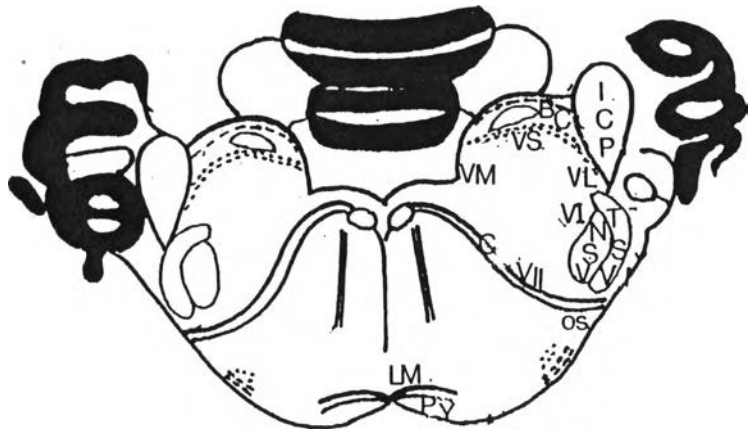
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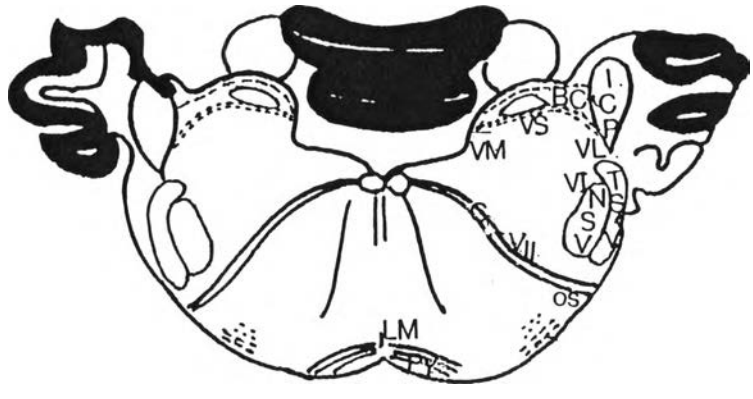
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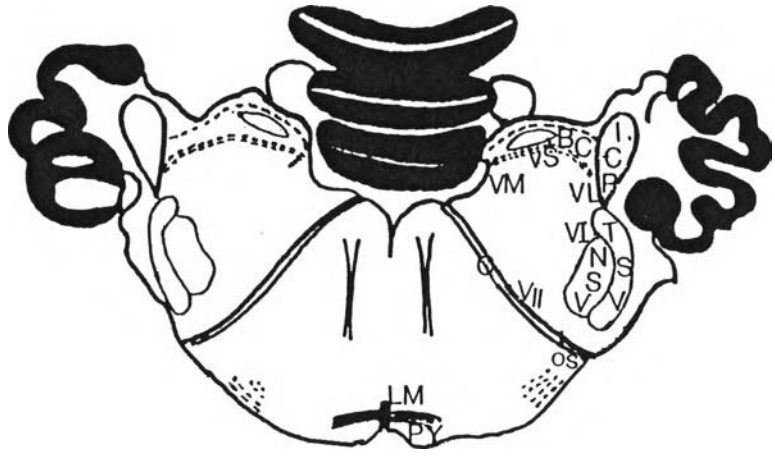
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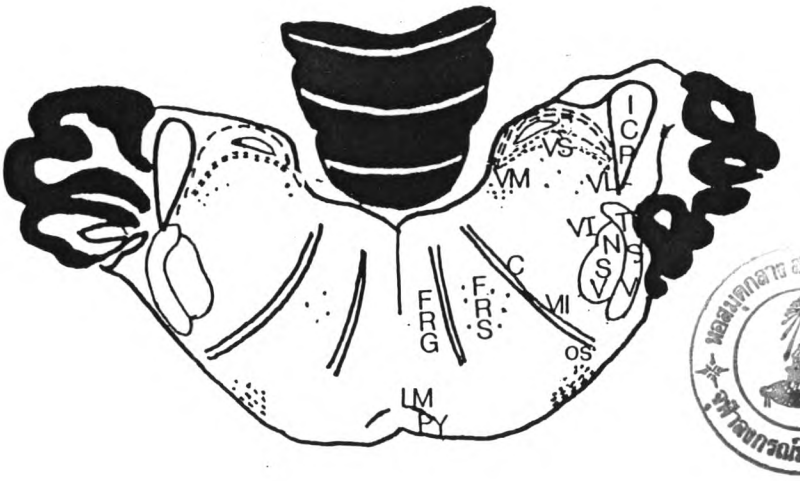

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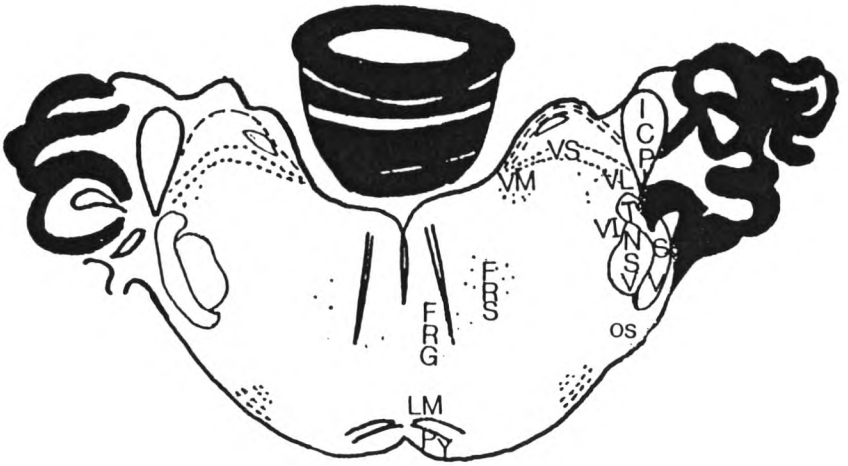

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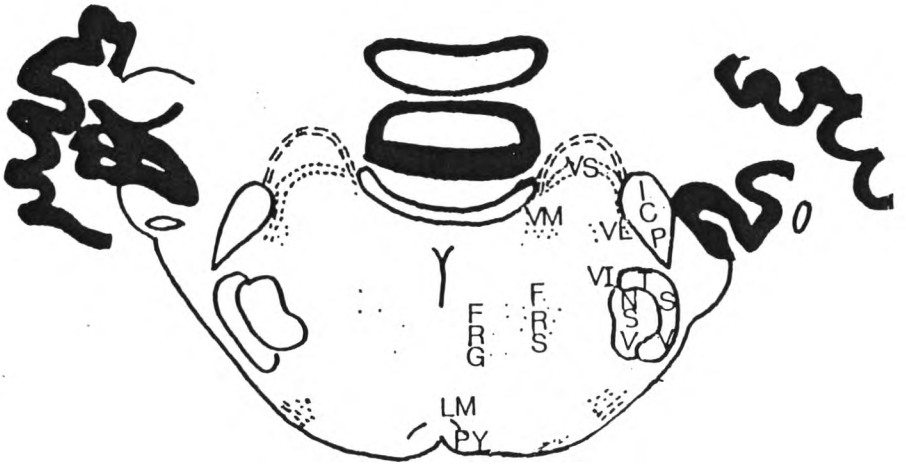

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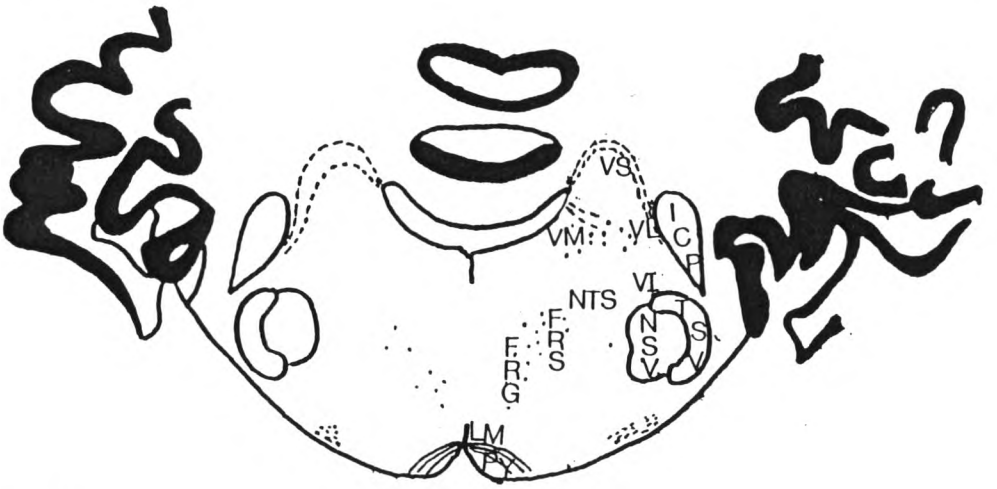
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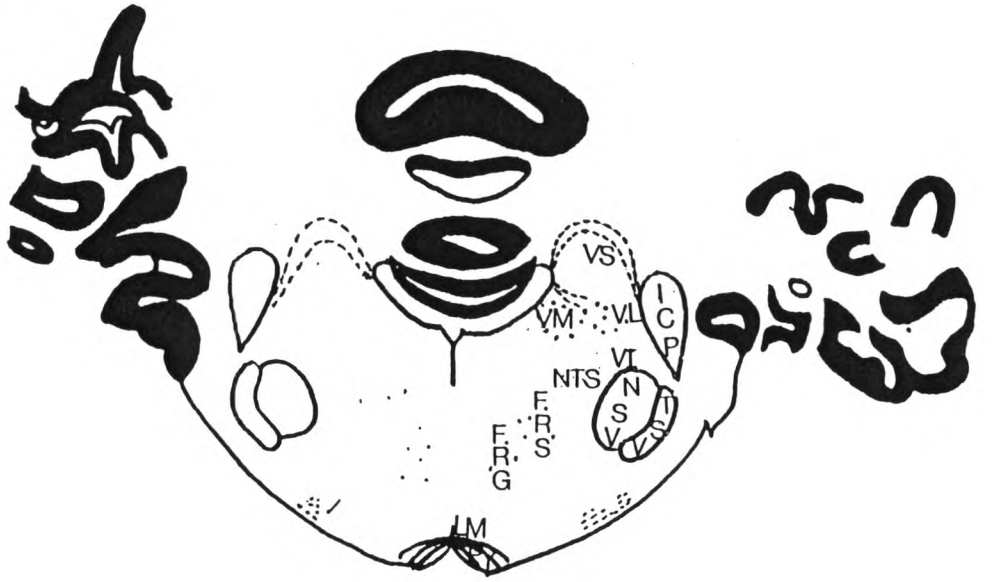
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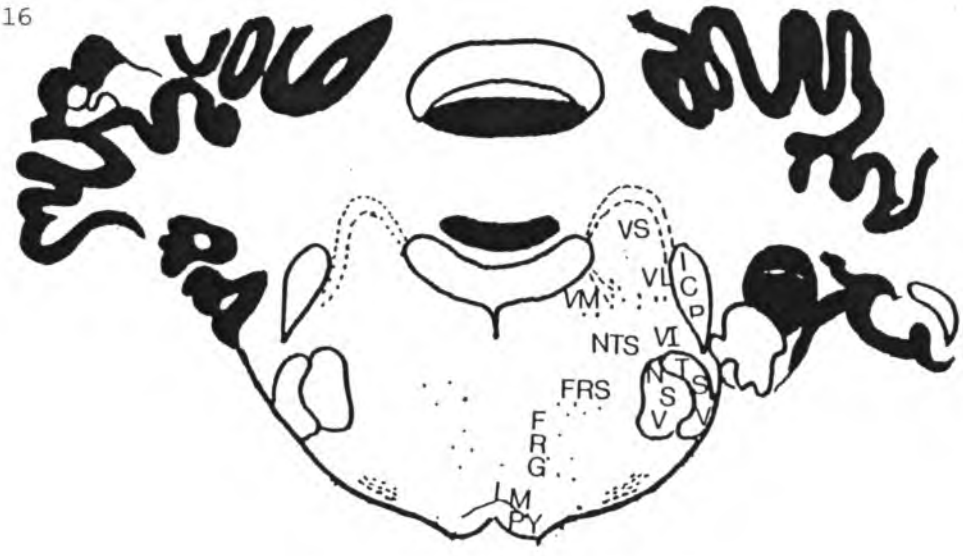
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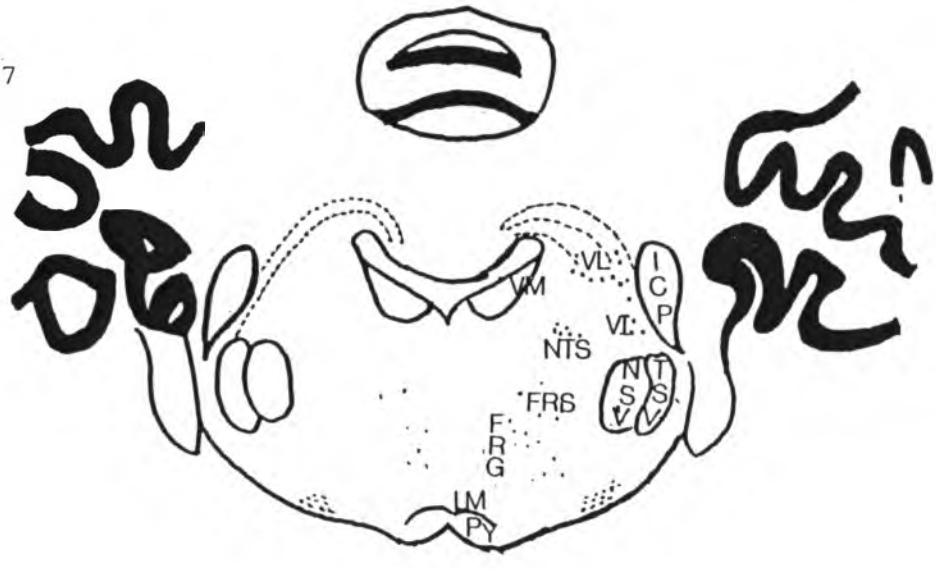
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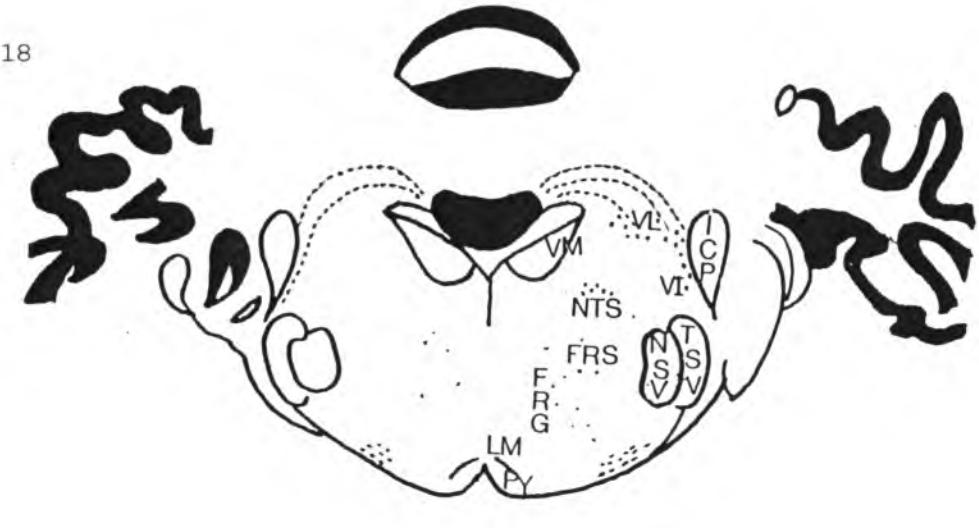
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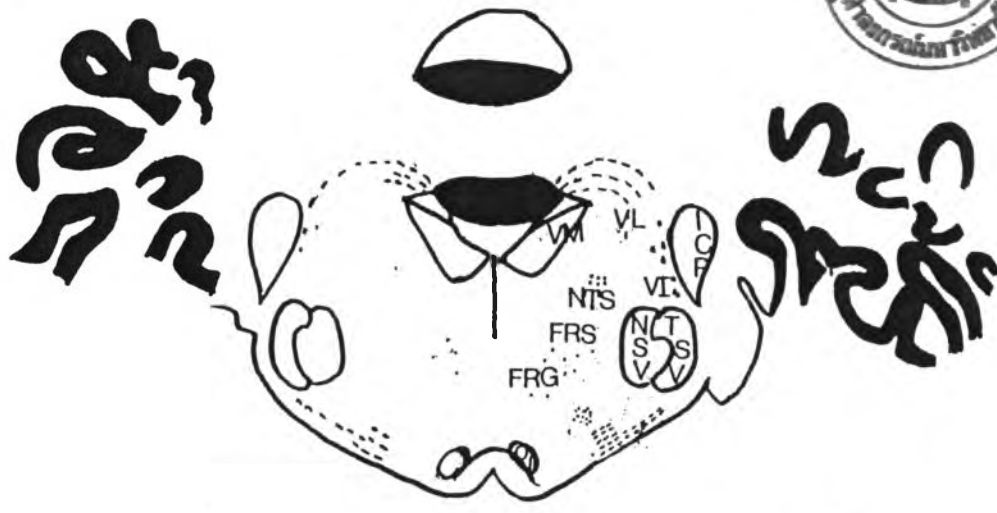
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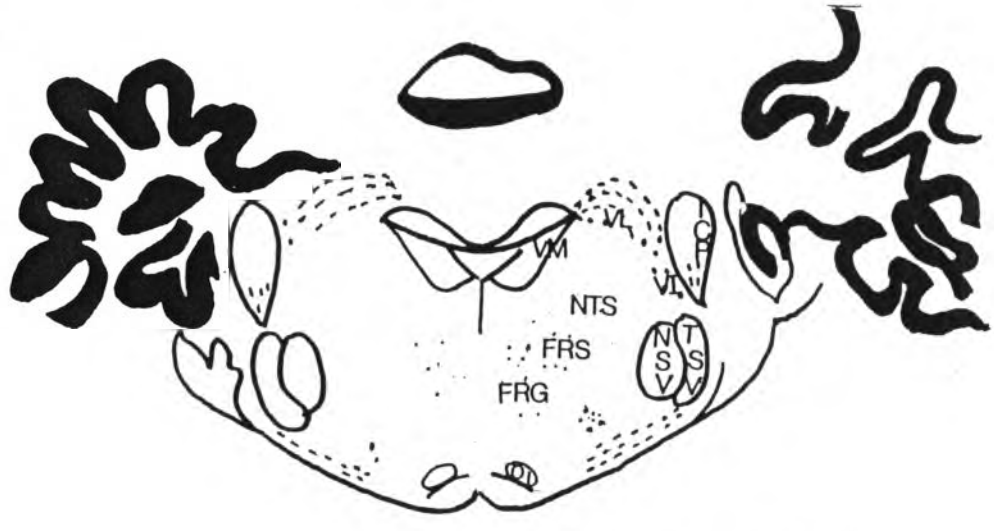
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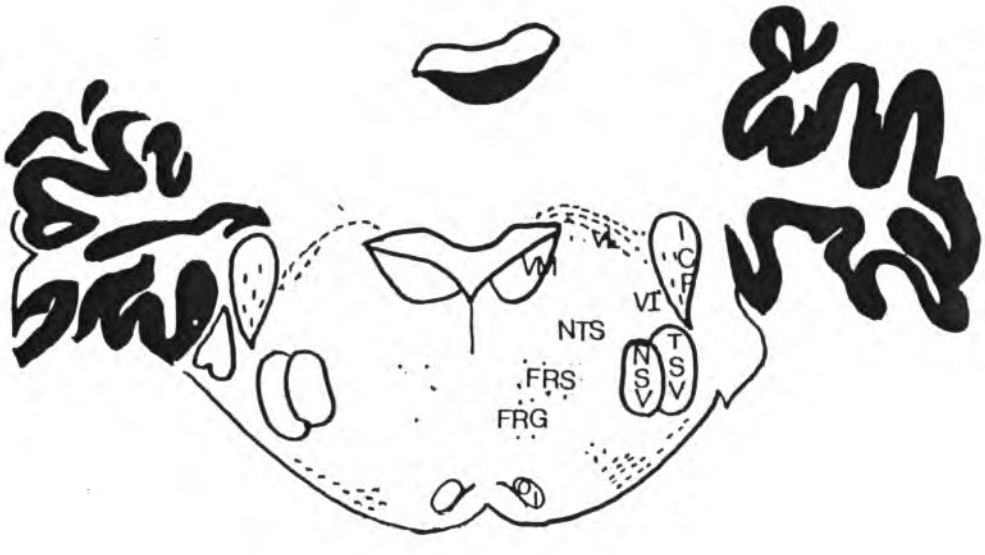
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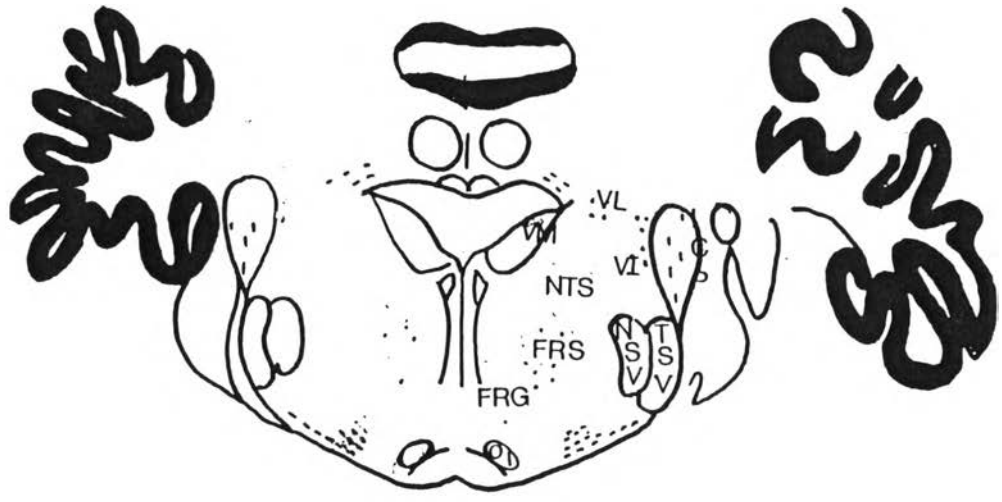
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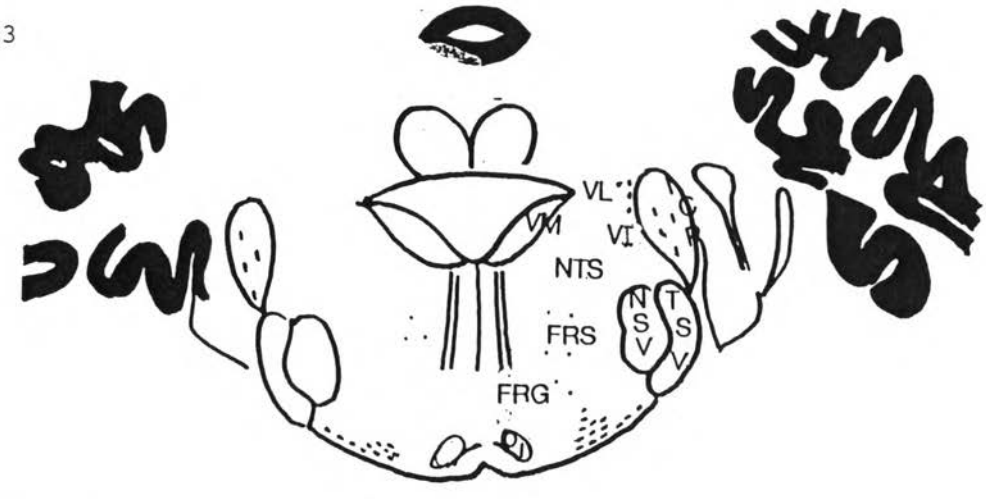
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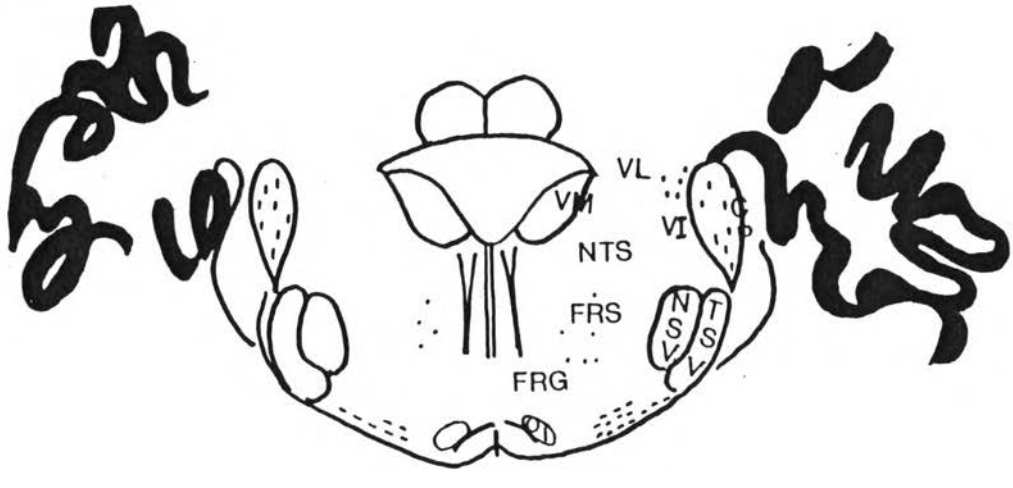
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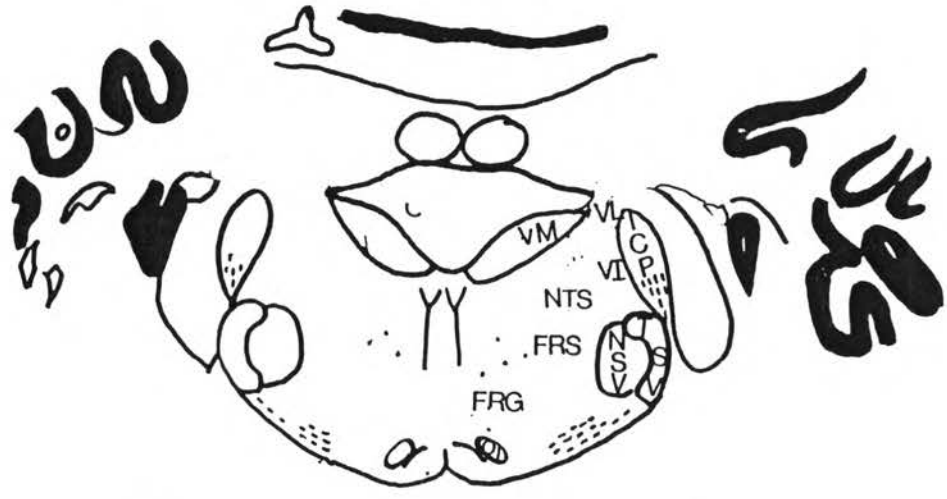
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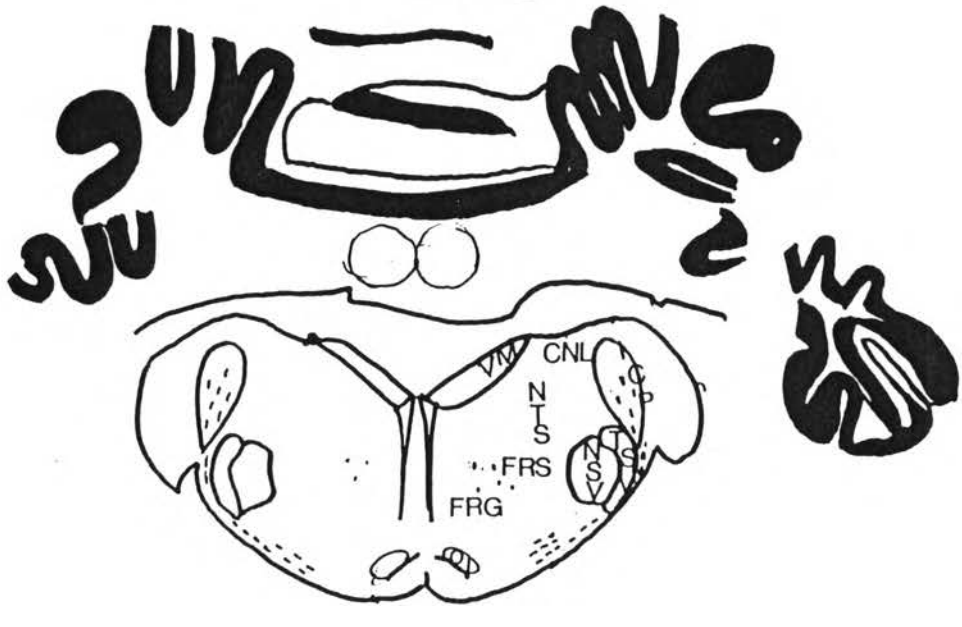
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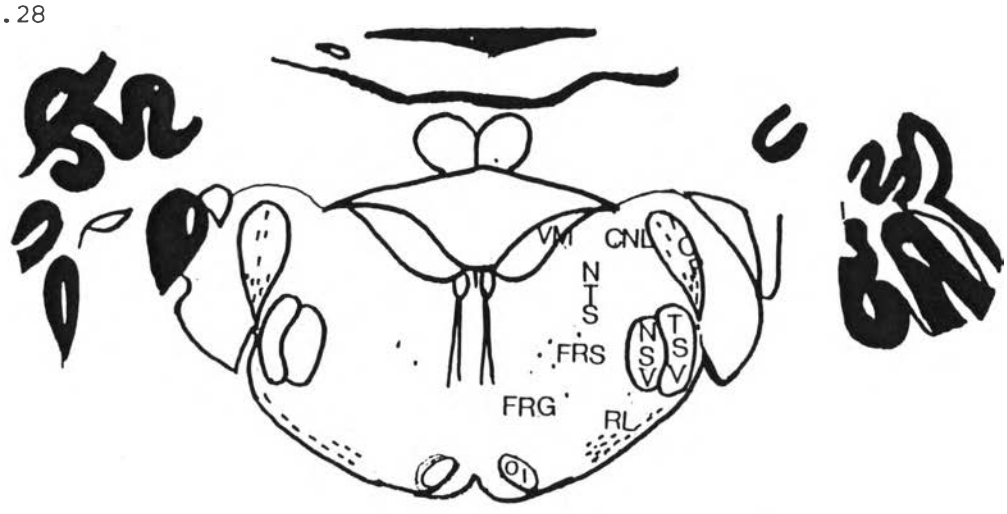
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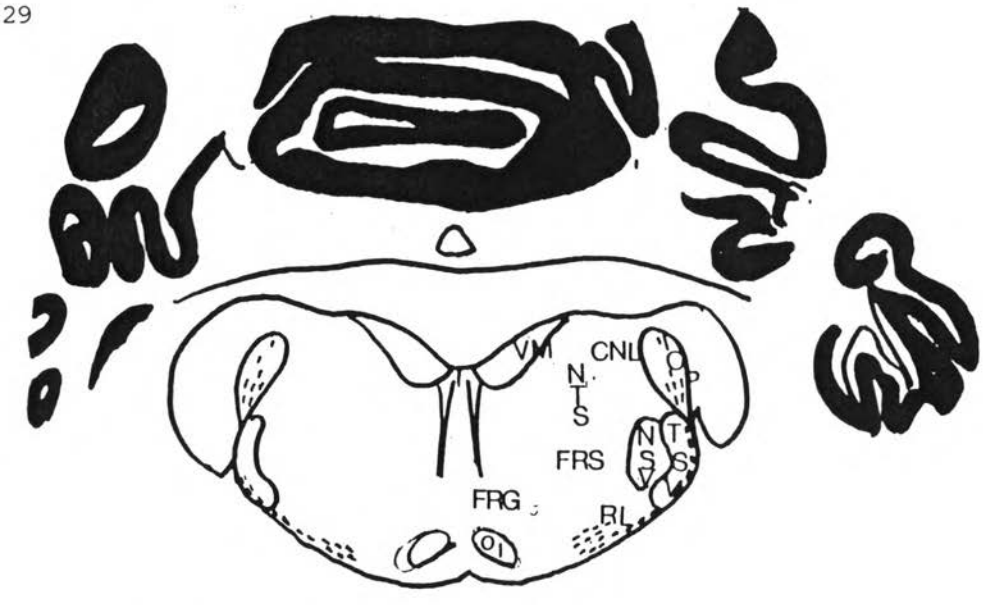
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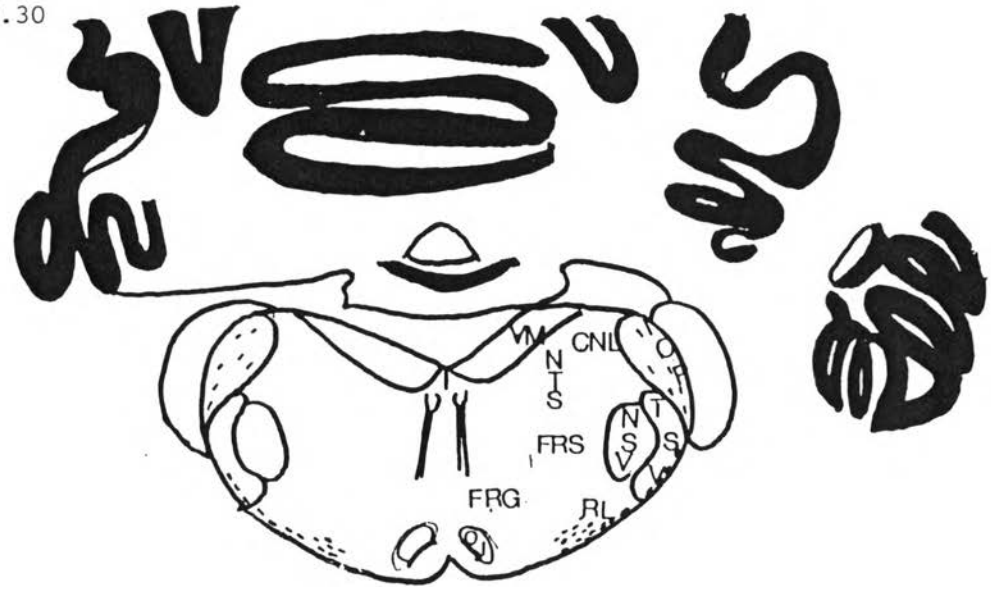
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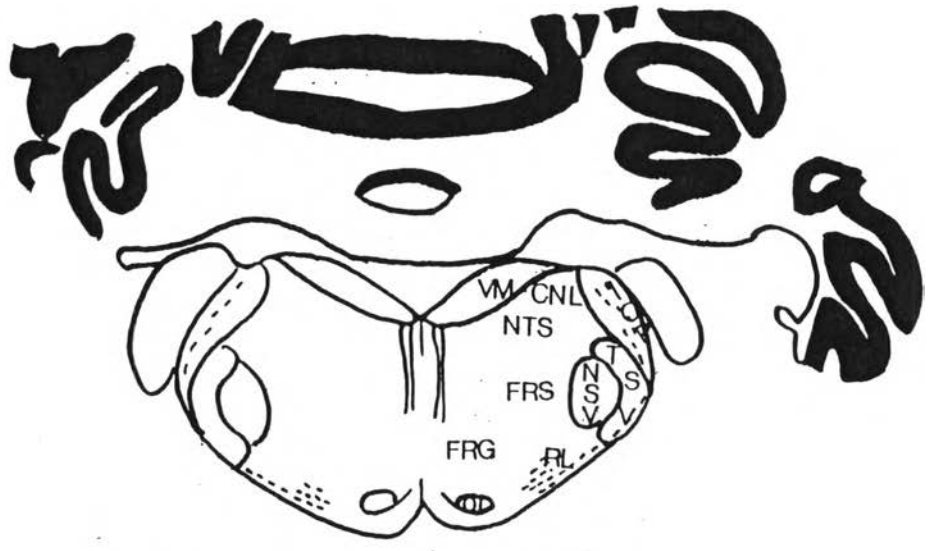

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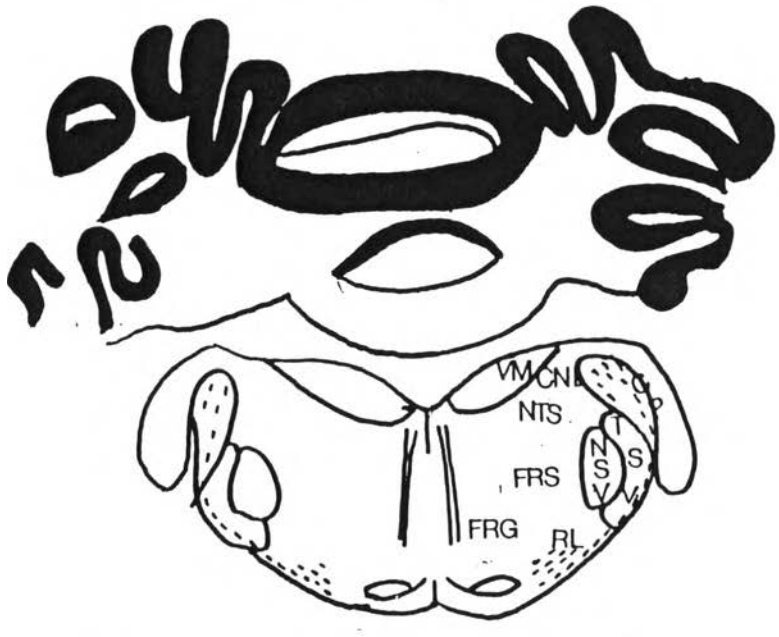

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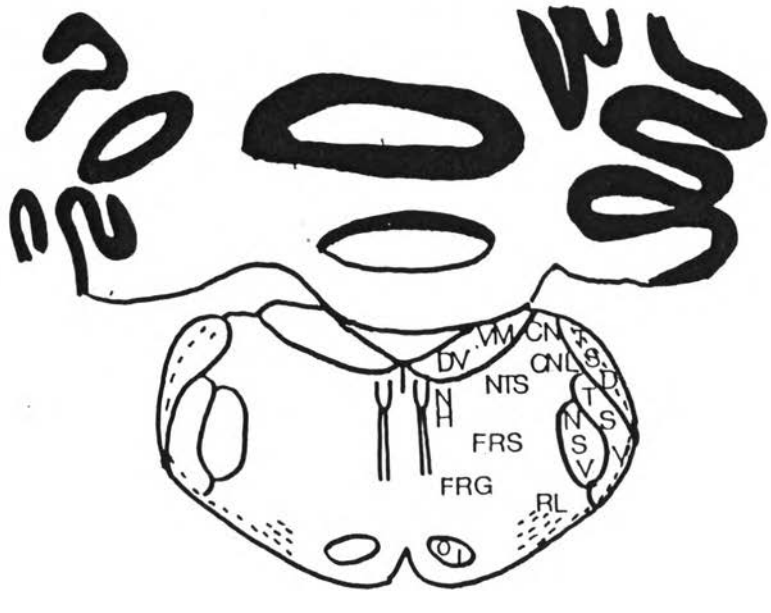

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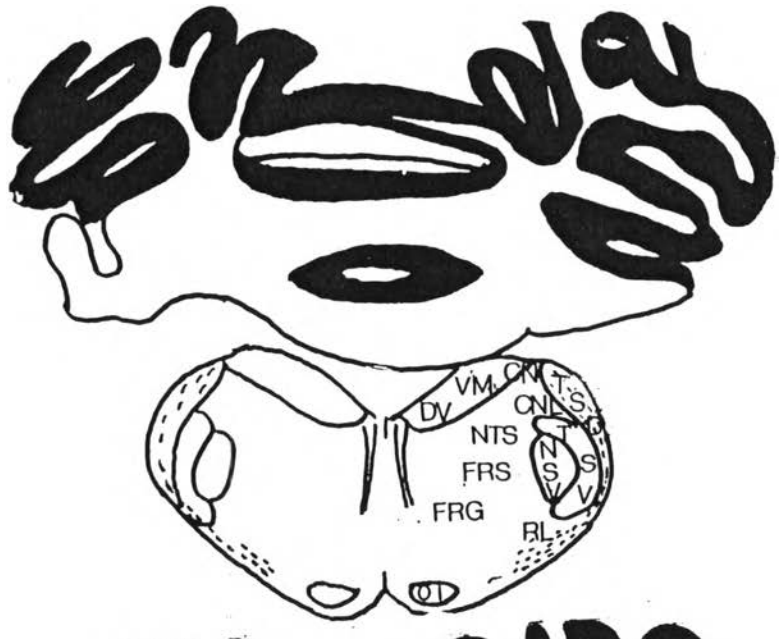
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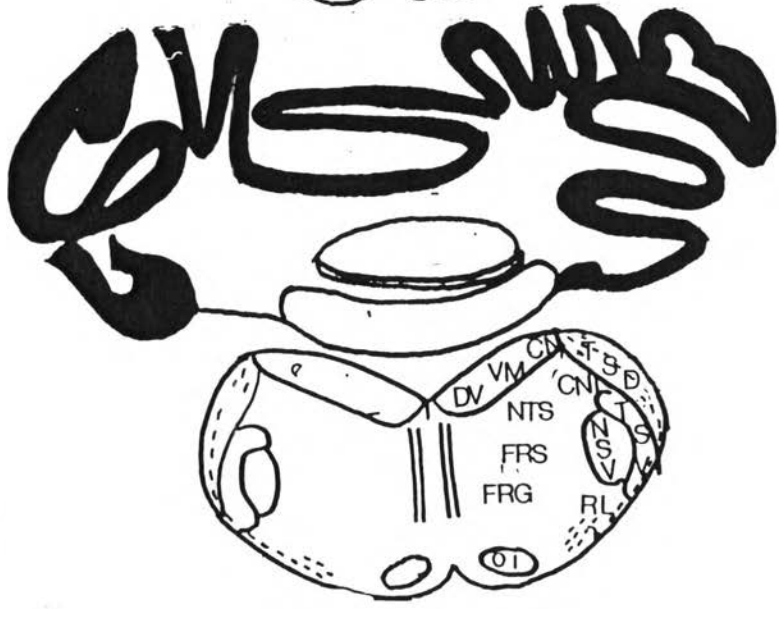
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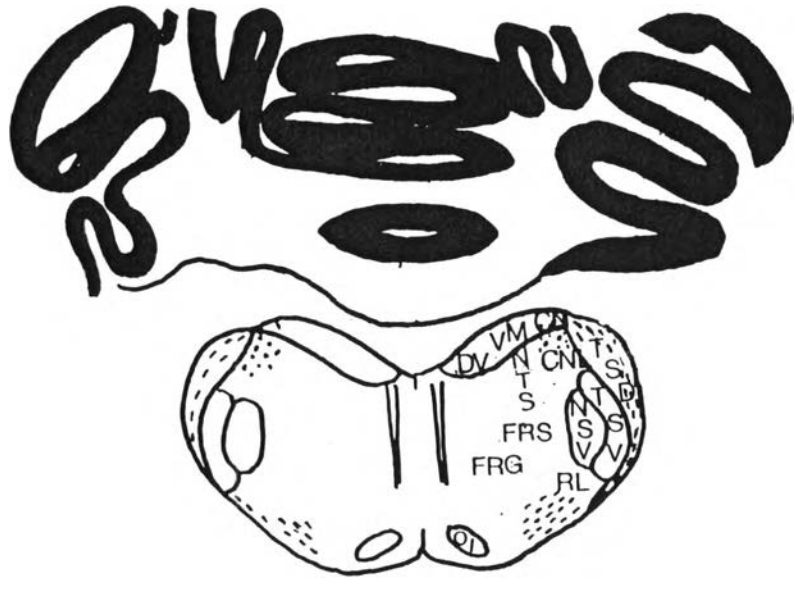
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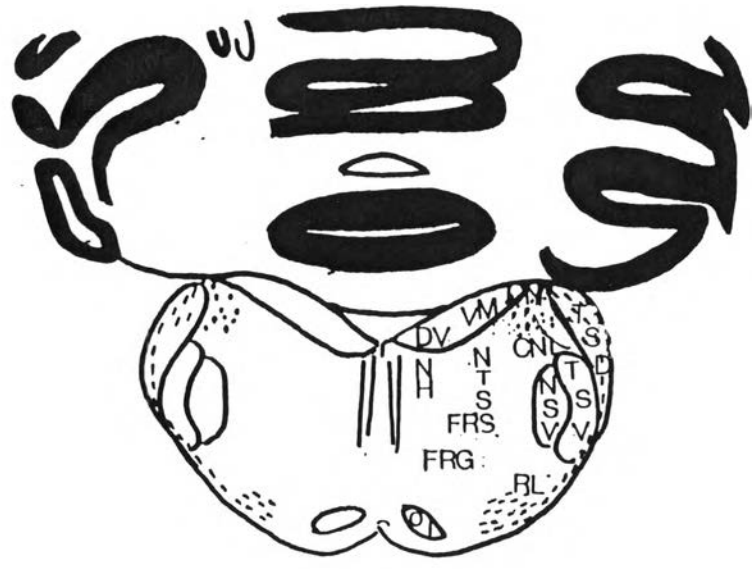
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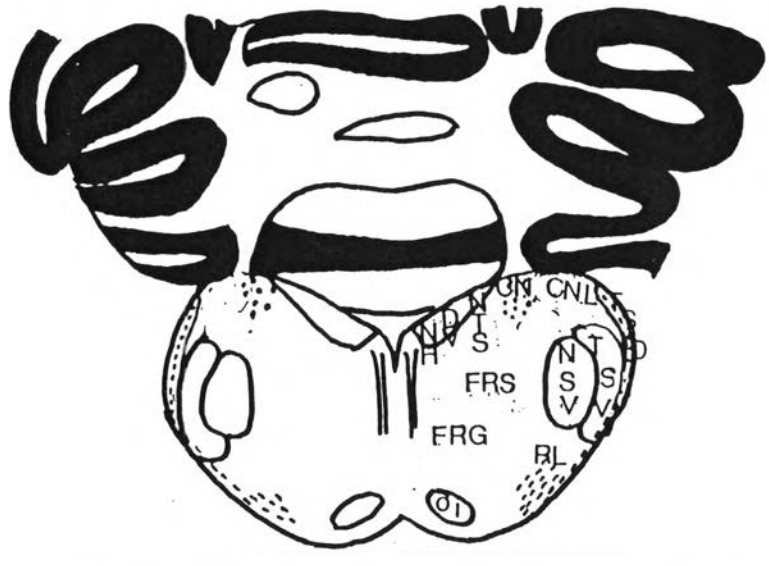
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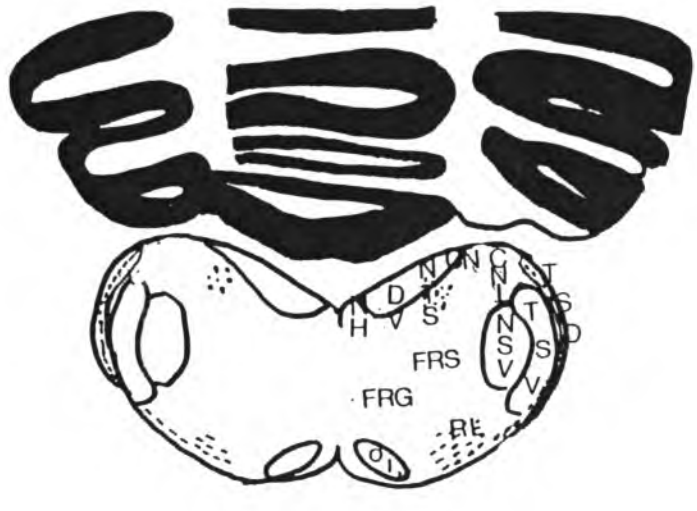
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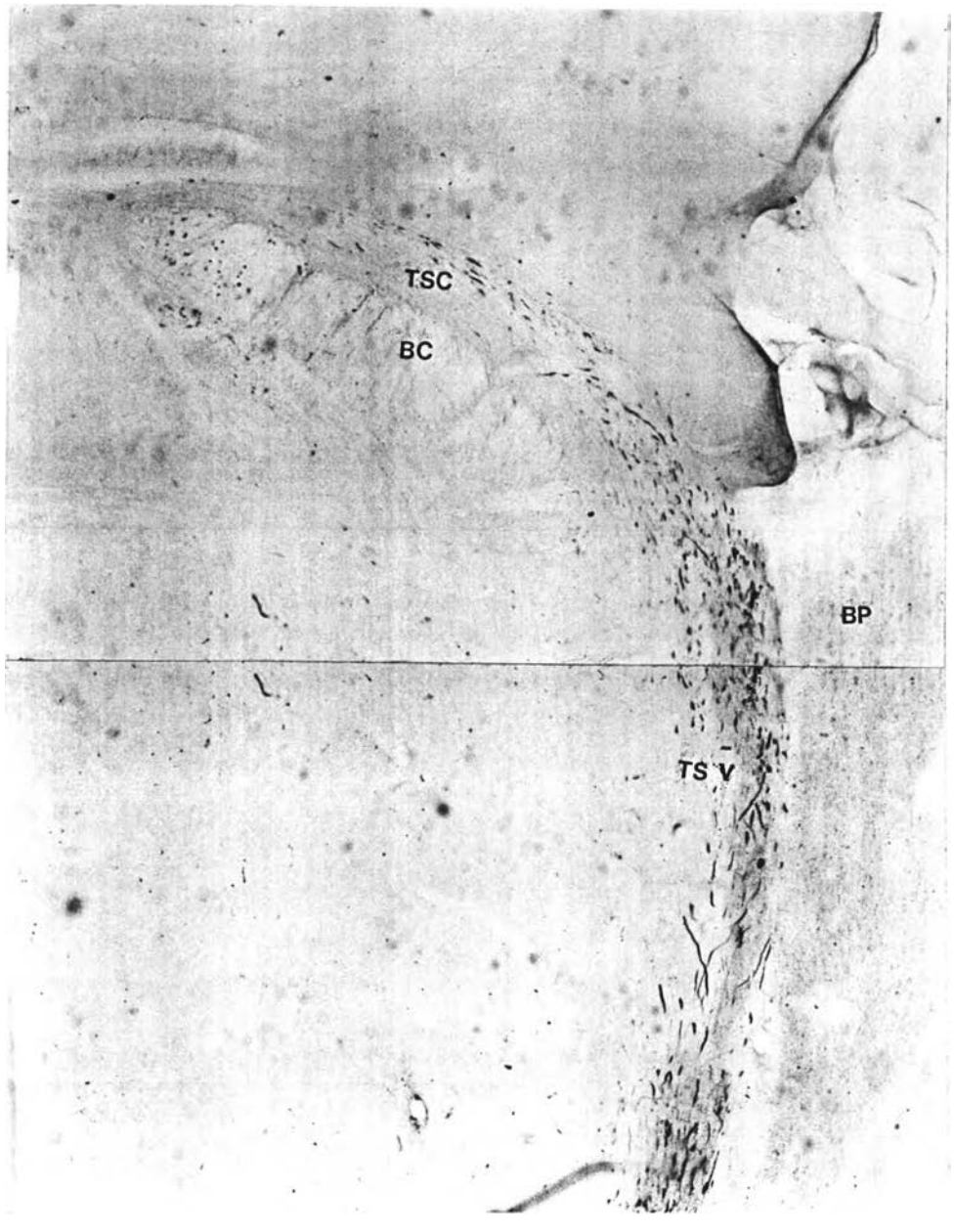

Fig 8 Photograph showing long bundles of labelled fibers curving in a dorsoventral direction from BC to TS V x100

BP = brachium pontis

BC = brachium conjunctivum

TS V = spinal tract of trigeminal nerve

TSC = ventral spinocerebellar tract

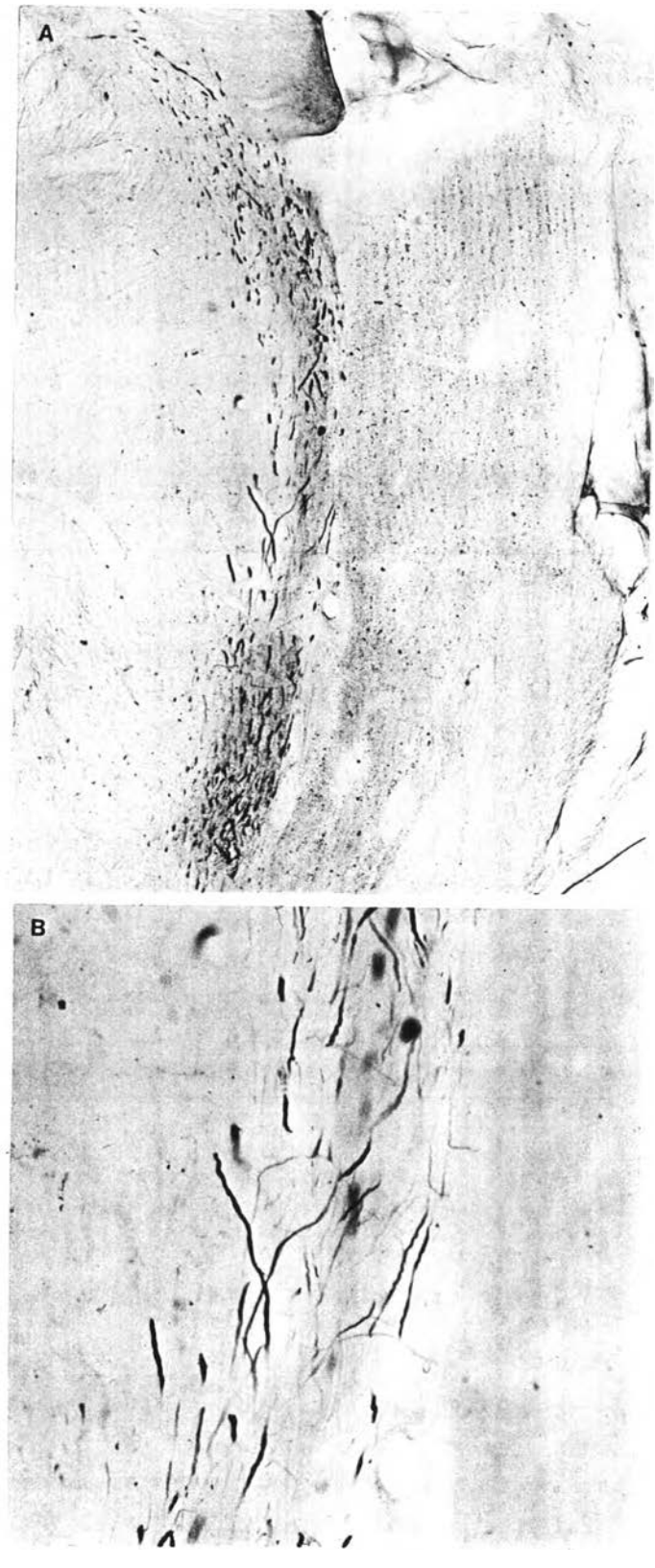

Fig 9 Photographs showing A) low magnification of long bundles of labelled fibers curving in a dorsoventral direction from BC to TS V x100 and B) high magnification x400
BC = brachium conjunctivum
TS V = spinal tract of trigeminal tract

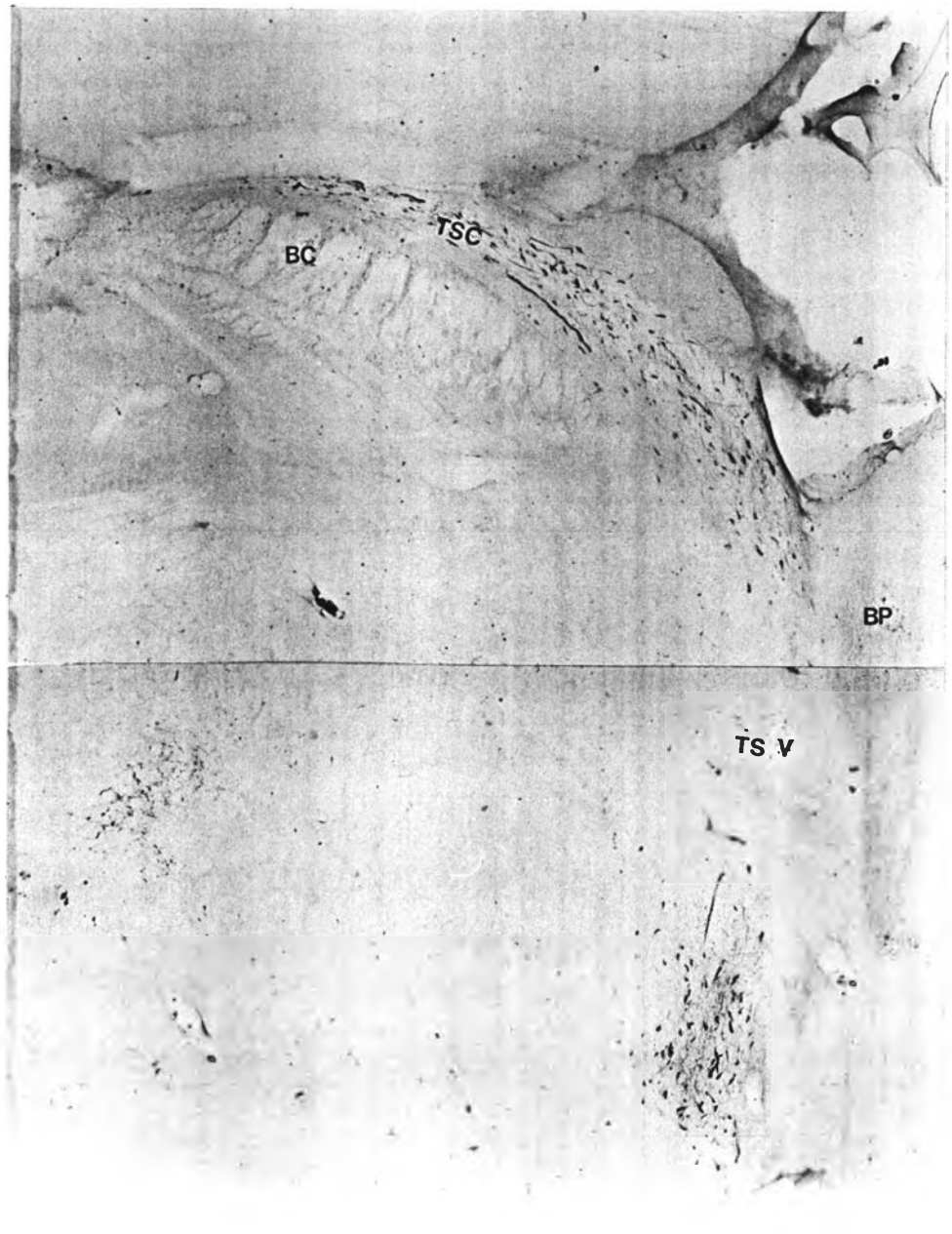

Fig 10 Photograph showing the bundles were splitted into two separate, the upper lie dorsal to the brachium conjunctivum and run ventrolaterally along the medial border of BP. The lower one stretch along the lateral boundary of TS V.

BC = Brachium conjunctivum, BP = brachium pontis

TSV = spinal tract of trigeminal nerve

TSC = ventral spinocerebellar tract

toward the lateral end and extending along the entire medial boundary of the rostral part of ICP (Fig 11). Two groups of short and small-sized labelled fibers, one on each side, are first observed inferior to the medial end of the ICP. Then, they gradually curve down, in a ventrolateral direction, into the area of lateral vestibular nucleus (VL) (Fig 7.7, 7.8, 7.9). Another two groups of labelled fibers, one on each side, are accumulated just inferior to lateral end of the OS. The ipsilateral group (Fig 12A) is clearly divided into two subdivisions. The lower subdivision consists of small fibers intermingled with fibers which form the base of the brain while the upper one consists of smaller fiber accumulated just dorsolateral to the lower one close to the C VII (Fig 7.7, 7.8 7.9, 7.10). However, the contralateral group, consists of fewer fibers (Fig 12B), especially the upper subdivision.

At the level of the caudal part of the C VII, number of fibers in the tract dorsal to the BC and medial to the ICP increased. However, number in the ipsilateral side is more than those of the contralateral one (Fig 7.10). These are two tracts of small-sized fibers, one on each side, stretch from the dorsal area of the VM along the inferior boundary of BC toward the upper VS then curved toward the lateral VL (Fig 7.10). Terminal varicosities are observed scattering in VM in a dorsoventral direction, few of them invade into the medial VL (Fig 7.10). Only few terminals are observed the dorsal area of contralateral VM and VL (Fig 7.12, 7.13). Likewise varicose terminals were seen scattering in the ipsilateral nucleus reticularis parvocellularis (FRS) area (Fig 7.10, Fig 13) very few was found in the contralateral side. The ventrolateral group of fibers are now located just underneath the lateral end of the facial nucleus presenting the same pattern as those of the rostral levels.

At the caudal level of BC, the long and curved fiber tracts are still observed continuously along the dorsal boundary of the BC and the lateral boundary of the of the TS V until the inferior border of the nerve. Numerous varicose terminals

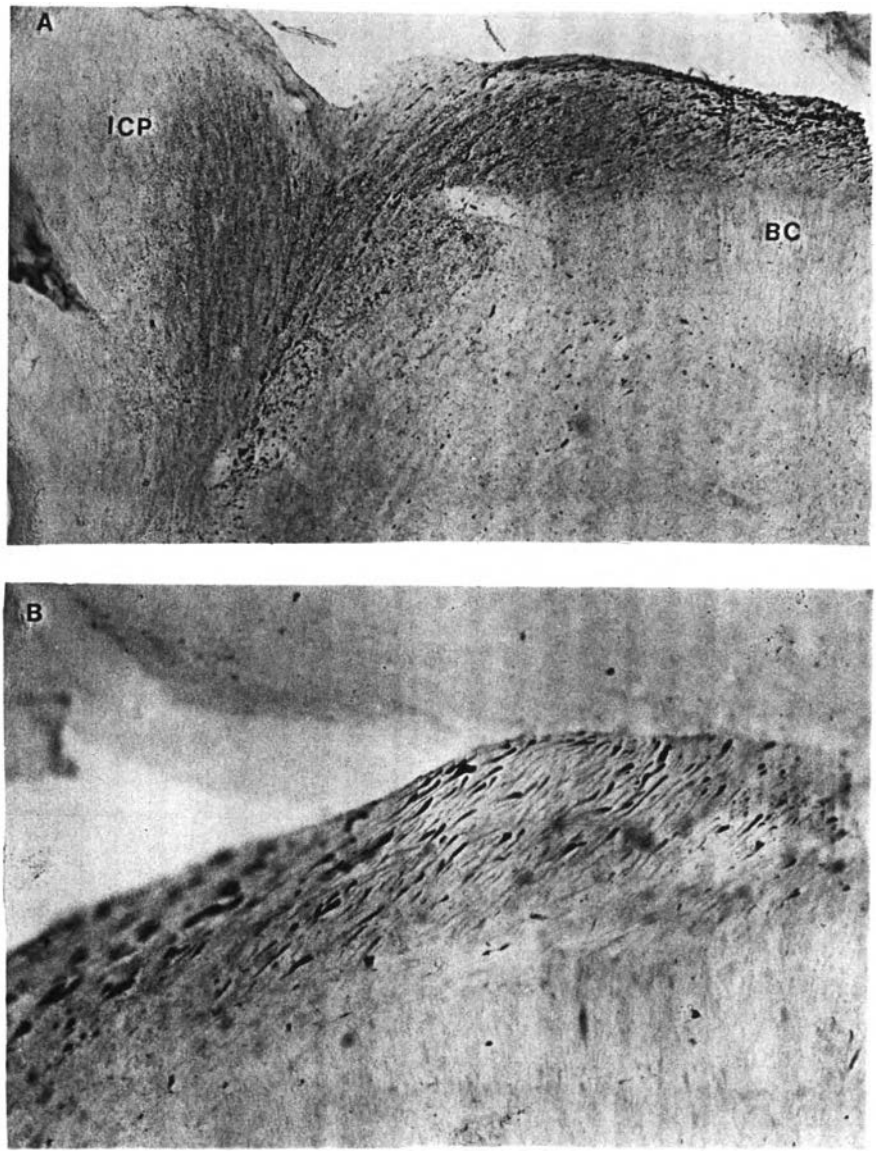

Fig 11 Photographs showing A) the bundles are observed lying close to the dorsal boundary of BC from its medial toward the lateral end and extending along the entire medial boundary of the rostral part of ICP x 40 B) high manification of the bundles from A x 100

BC = brachium conjunctivum

ICP = inferior cerebellar peduncle

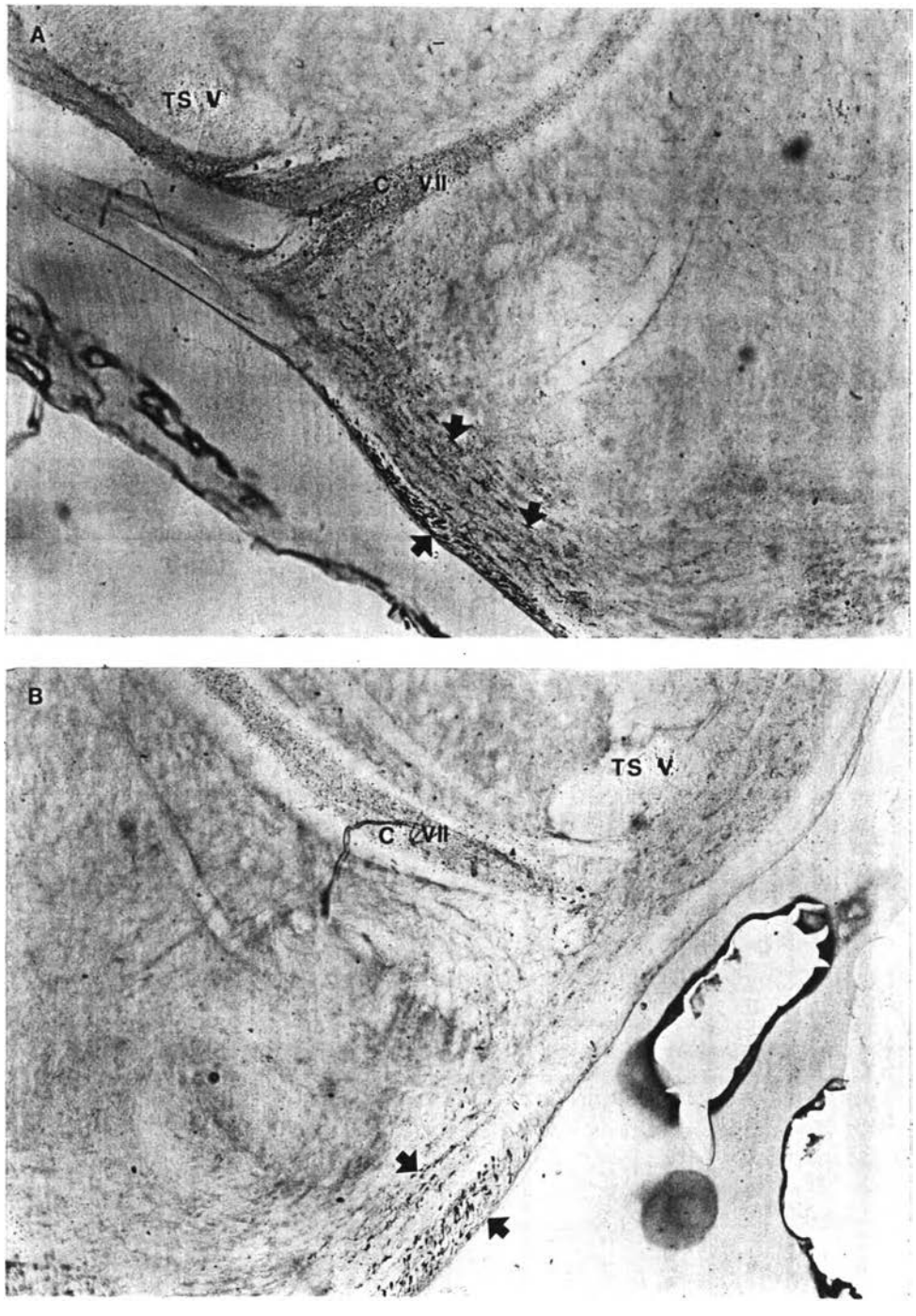

Fig 12 Photographs showing A) nerve fibers (arrow) in the ipsilateral fastigiospinal and B) in the contralateral side x40

C VII = the seventh cranial nerve

TS V = spinal tract of trigeminal nerve

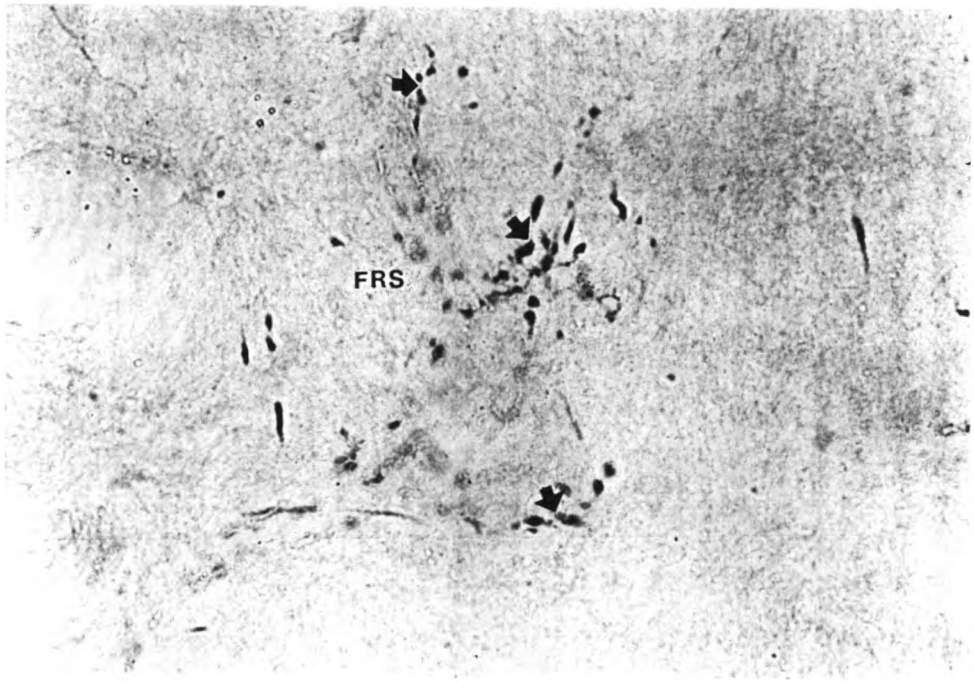

Fig 13 Photographs showing nerve terminals (arrow) in the FRS x400.

FRS = nucleus reticularis parvocellularis

are observed in the dorsal area of ipsilateral VM (Fig 14) extending ventromedially, while small tract stretches from the medial VM toward the dorsal area of VS. Numerous small labelled fibers were observed on the lateral area of ipsilateral VL (Fig 7.11), small number of terminals were seen intermingled with the tracts in the nucleus. Conversely, terminal could be observed in the contralateral VM. In VL, only few small fibers are seen with very few terminals (Fig 7.11, 7.12). More varicose terminals were seen scattering in the nucleus reticularis magnocellularis (FRG) and FRS (Fig 7.11, 7.12). Fiber tracts at the ventrolateral area of the base of the brain still occupied the same position.

At the level of caudal to the BC. Small fiber tracts are seen stretching laterally from the lateral boundary of the fourth ventricle toward the dorsal area of the ipsilateral VS (Fig 15). Then, they curved along the medial boundary of the VL. The second group of small fibers are in the dorsal area of the ipsilateral VM. Terminals could be observed at the end of these fibers. They extend into VL and ventral part of VM (Fig 7.13, 7.14, 7.15). Only aggregations of small fibers nerve found in VS and extending continuously into VL of contralateral side, these is no terminal in these two vestibular nuclei (Fig 7.13, 7.14, 7.15). Few terminals were found in the ipsilateral FRG and continuous with those in the FRS (Fig 8.13). In the contralateral side, most terminals are found in the FRG with very few in the FRS. Groups of small fibers at the ventrolateral position of the base of the brain move to more medial position. The ipsilateral groups are separated into two distinct groups, the medial and lateral groups. Those of contralateral side are single and long with larger fibers which are in the nerve tract at the base of the brain underneath those smaller ones (Fig 7.13, 7.14, 7.15).

At the level of rostral FN, labelled tracts are clearly seen running continuously from the FN toward the dorsal and lateral areas of VL, then VI of ipsilateral side (Fig

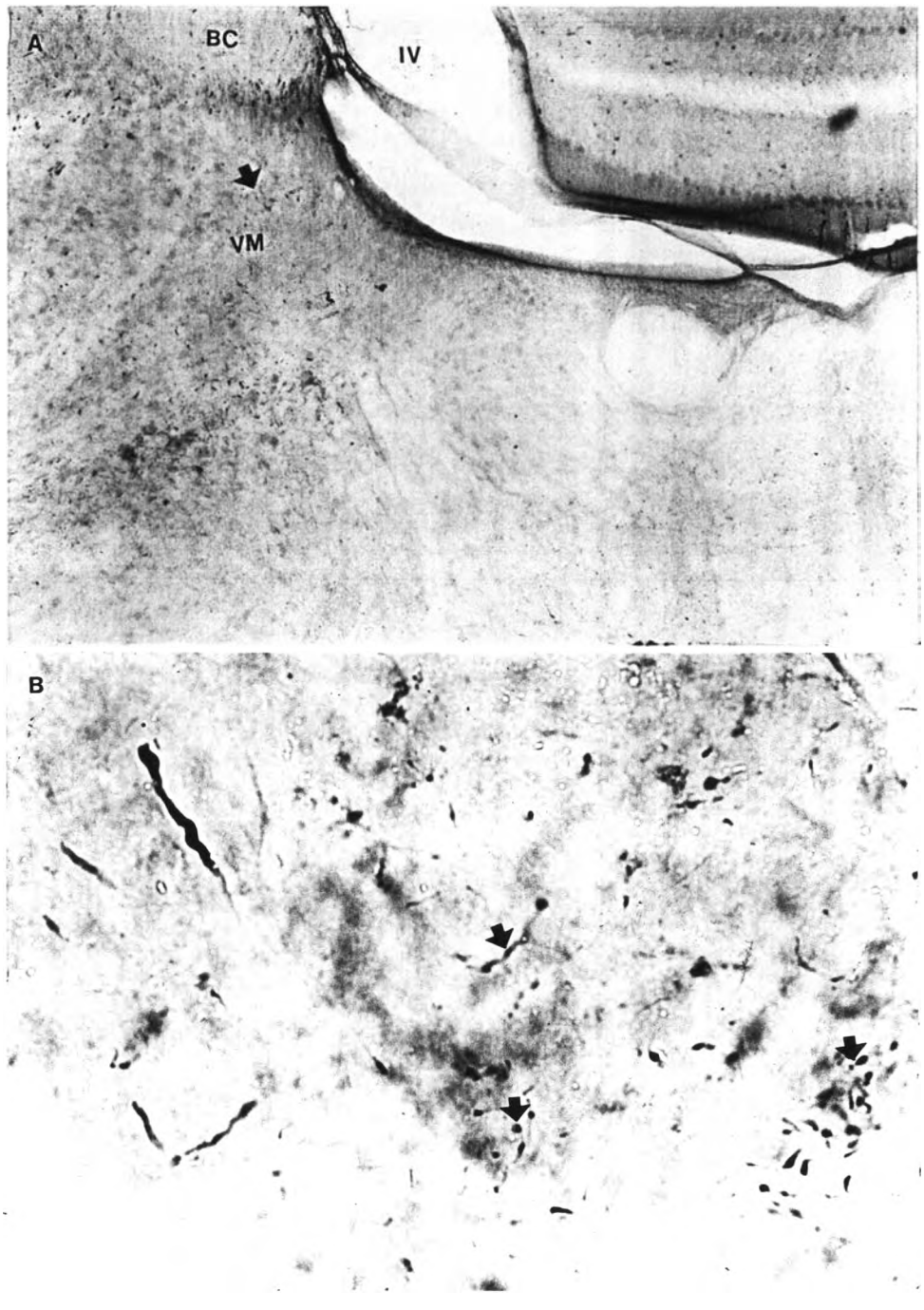

Fig 14 Photographs showing A) nerve terminals (arrow) in the VM x40 B) high magnification of nerve terminals in (A) arrow. x400.

IV = fourth ventricle

BC = brachium conjunctivum

VM = medial vestibular nucleus

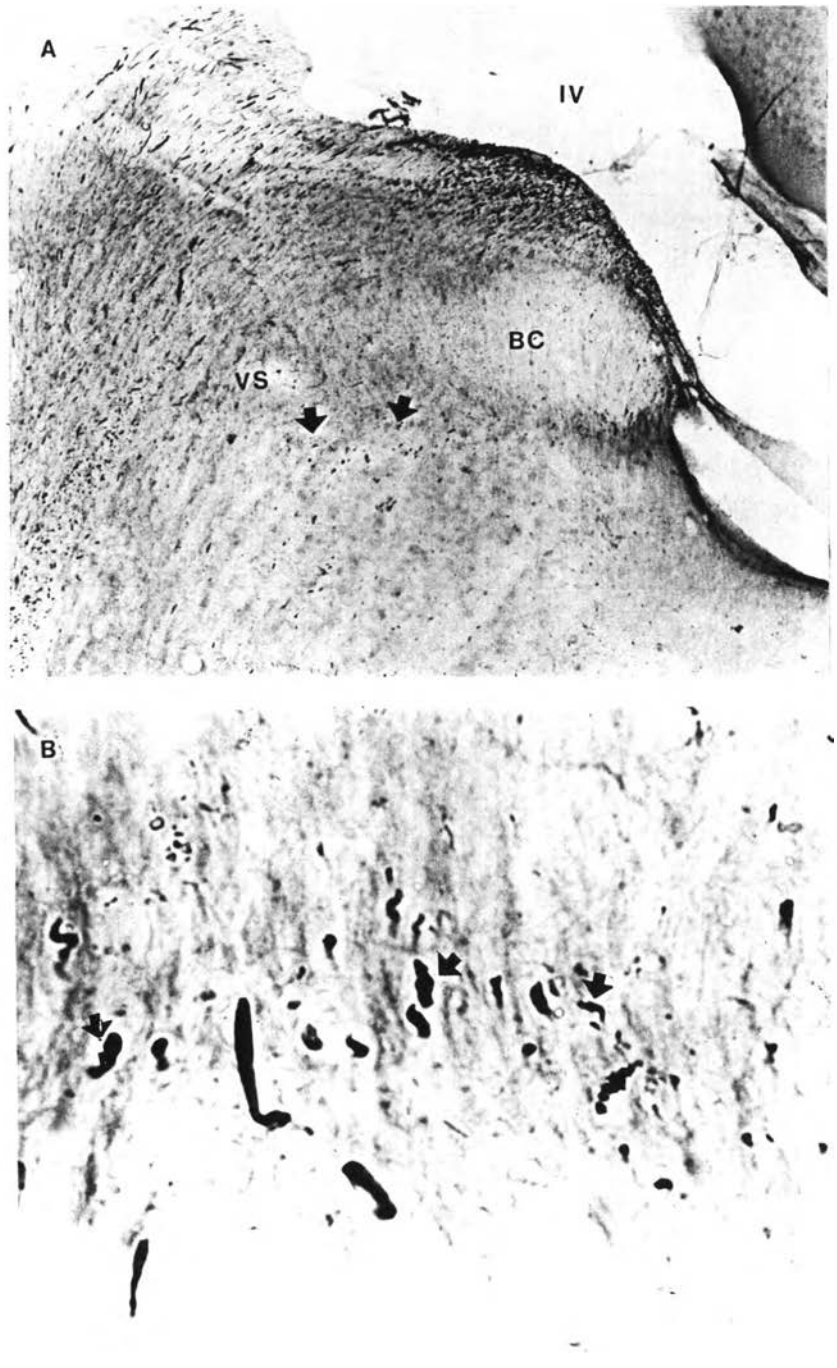

Fig 15 Photographs showing A) short nerve fibers (arrow) in the VS x40 B) high magnification of nerve fibers in (A) arrow x400.

VS = superior vestibular nucleus

BC = brachium conjunctivum

IV = fourth ventricle

7.17, 7.18). Terminals are intermingled with these fibers. They are numerous in VL (Fig 16) then continuous with fewer in the VL which lie along the medial border of the inferior part of ICP. Separate aggregation of terminals are located in the nucleus of tractus solitarius (NTS) as shown in Fig 7.16, 7.17, 7.18. Numerous terminals scatter in the FRG extending ventrolaterally toward dorsal area of the medial border of the ventrolateral group of fibers (Fig 7.16, 7.17, 7.18). Very few fibers were observed running from the contralateral FN. They scatter in the VL and VI (Fig 7.17, 7.18) ; in the lower part of VL and VI they lie along the medial boundary of ICP. Only few terminals are observed in this area. Terminals in FRG are fewer than those of the ipsilateral side. Likewise number of fibers in the ventrolateral group are clearly less than those of the centralateral side. At more caudal levels, fewer fibers are seen to project out of the both sides of FN. These fibers are short and smaller in size than those of the rostral levels. Ventrally they lie along the medial boundary of the ICP. Numerous terminals are intermingled with these fibers in the ipsilateral VL and VI (Fig 7.19, 7.20) ; only few are observed in the contralateral VL. Numerous terminals accumulate just dorsal to the medial border of the ventrolateral fiber group (Fig 7.19, 7.20, 7.21). Likewise terminals are seen scattering in FRG and FRS, those of contralateral size are fewer (Fig 7.19, 7.20, 7.21). Ventrolateral fibers group expands laterally on both sides toward the inferior border of the TS V. At 100-200 μm caudal to the level, fibers which project out of the FN are diappeared. Terminals are seen in the ipsilateral VL and VI along the medial border of ICP (Fig 17). However, those in the contralateral side are not observed. Accumulation of terminals are in the area just dorsal to the medial border of the ipsilateral ventrolateral fibers group; this area is designated as paragigantocellular reticular nucleus (PGl) (Fig 7.19, 7.20, 7.21, Fig 18). At this level fibers of the ventrolateral group spread more dorsolateral to lateral border of the inferior part of the TS V.

At the caudal level of FN, labelled fibers are observed scattering within the ICP of both sides. While terminals are accumulated along medial boundary of the

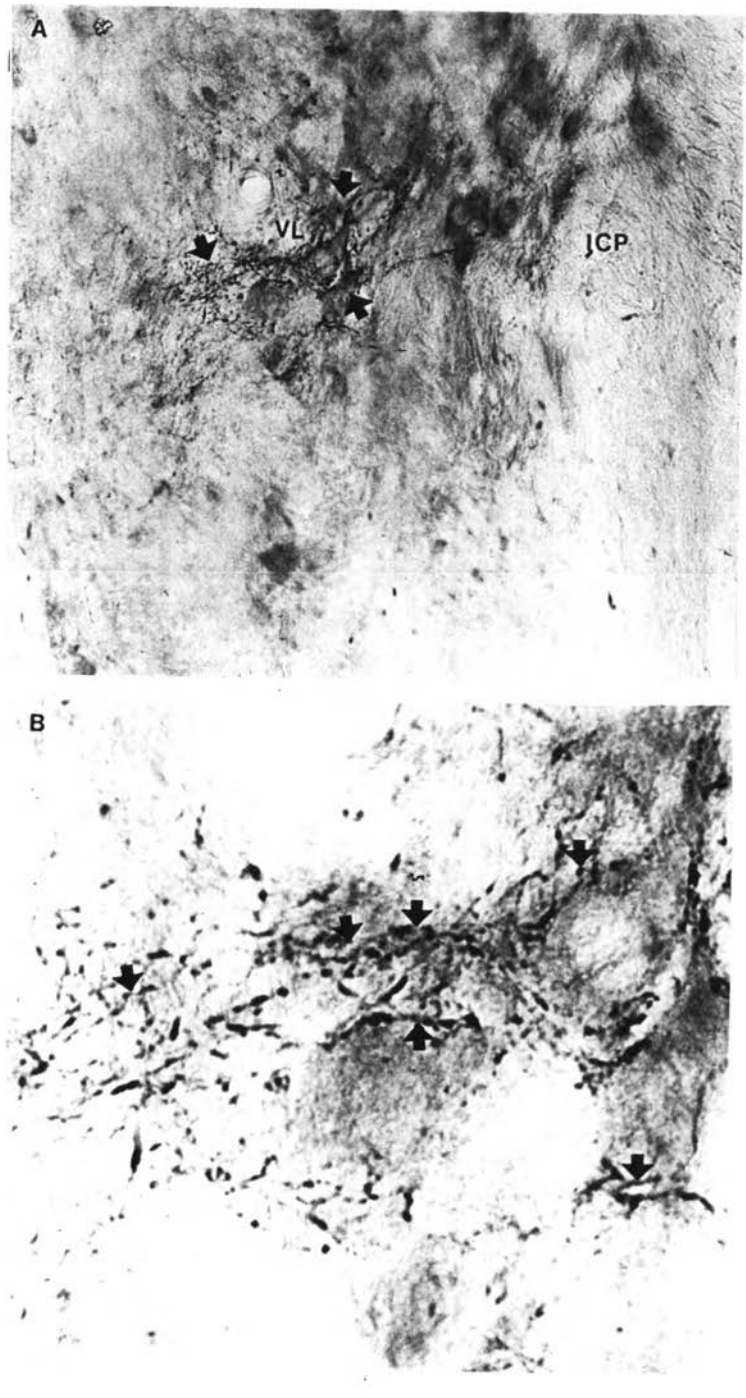

Fig 16 Photographs showing A) nerve terminals (arrow) in the VL x100 B) high magnification of nerve terminals in (A) arrow x400
VL = lateral vestibular nucleus
ICP = inferior cerebellar peduncle

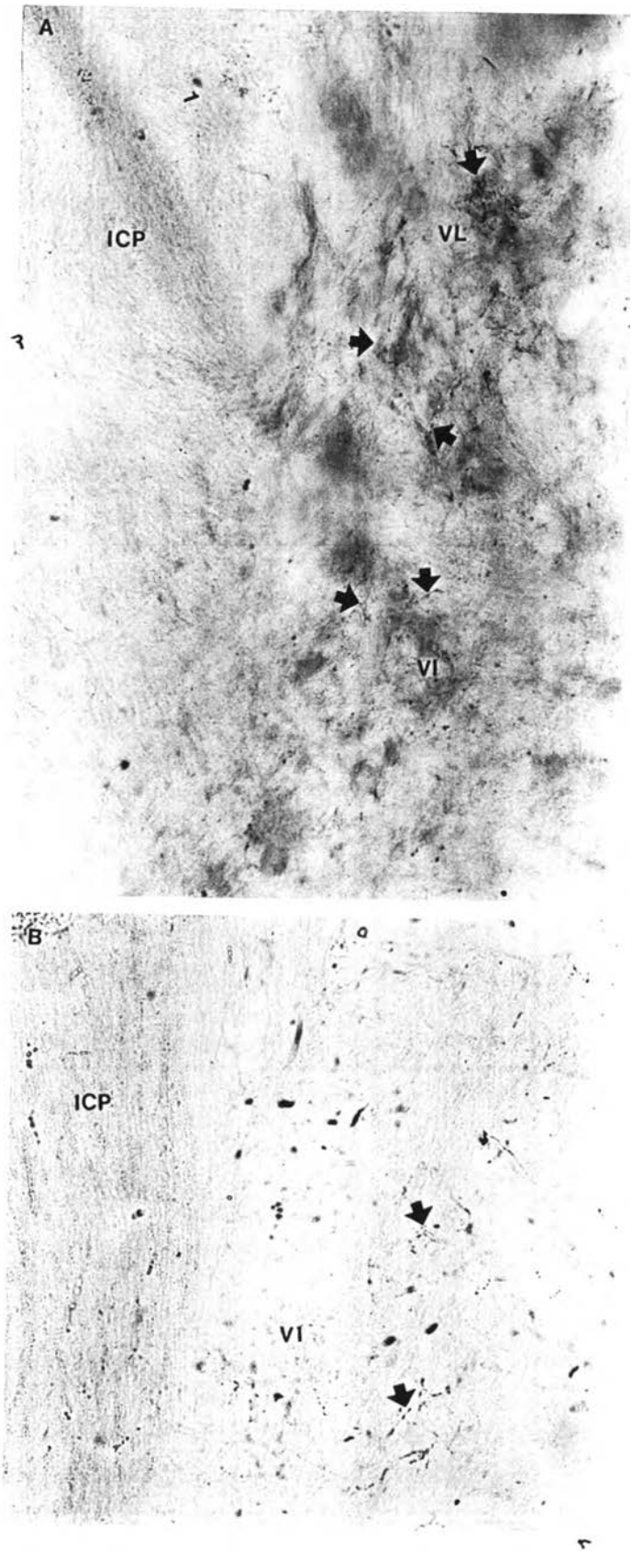

Fig 17 Photographs showing A) nerve terminals (arrow) in the VL, VI B) nerve terminals (arrow) in the VI x100

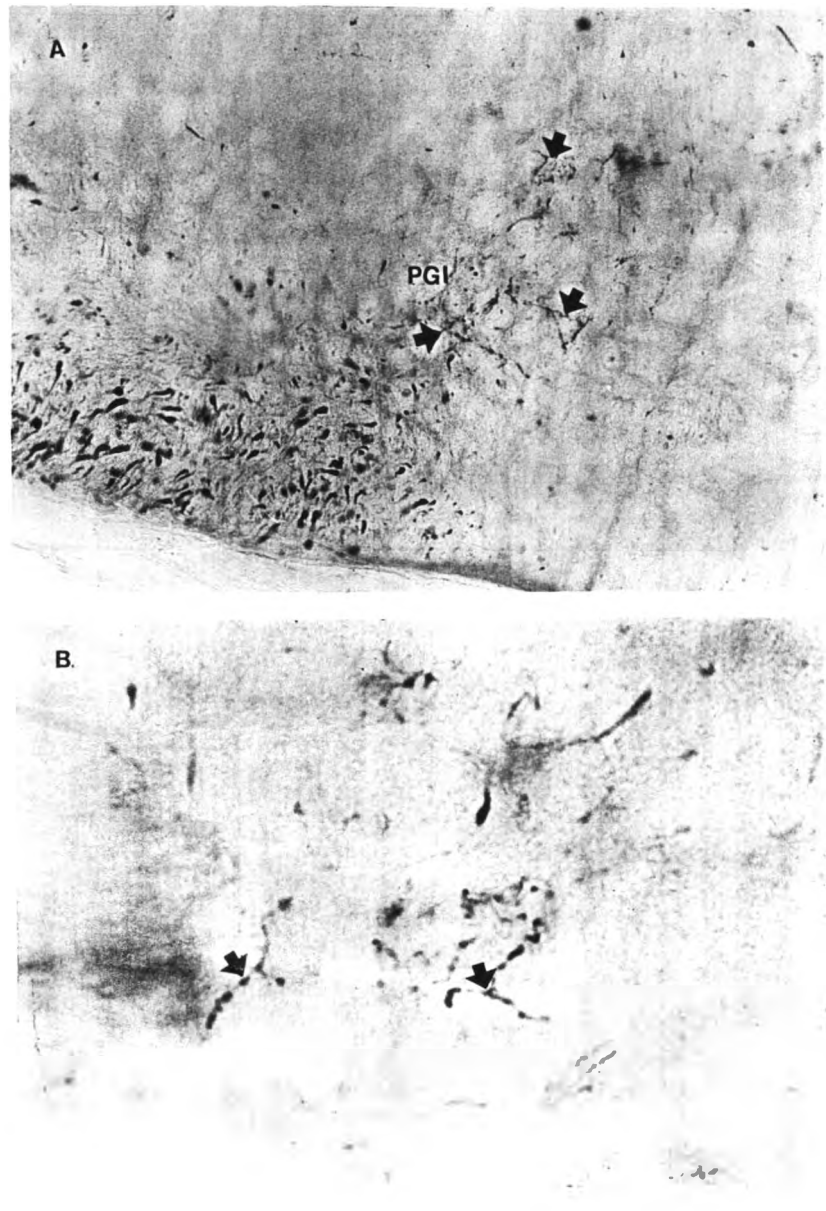

Fig 18 Photographs showing A) nerve terminals (arrow) in the PGI x100 B) high magnification of nerve terminals in (A) arrow x400

PGI = paragigantocellular reticular nucleus

upper part of the ipsilateral ICP (Fig 7.22, 7.23, 7.24). Labelled fibers are intermingled within the tract lateral to the TS V ; they are continuous with those in the ICP and the ventrolateral group (Fig 7.25, 7.26, 7.27). Terminals are scattered within the FRS ; number of those in the ipsilateral are more than that of the contralateral side (Fig 7.28). At levels caudal to the caudal pole of the FN, labelled fibers are clearly observed within the ICP (Fig 7.27, 7.28). They are continuous with those in the tract lateral to the TS V (Fig 7.28) and the ventrolateral groups of both side. No terminals are observed in the VL, VI, FRS and FRG.

At caudal levels of dorsal and ventral cochlear nucleus. Labelled fibers can be observed continuously from the ventrolateral groups, which lie next to lateral border of the inferior olivary nucleus (OI) to the ventral spinocerebellar tract (TSC), the dorsal spinocerebellar tract (TSD) and ICP (Fig 7.31, 7.32, Fig 19). At the level of dorsal vagal (DV) and hypoglossal (NH) nuclei, continuous labelled fiber tract are clearly observed in the TSD and TSC. Aggregations of terminals are found in both lateral cuneate nucleus (CNL) as shown in Fig 7.36, 7.37 ; numbers in the contralateral seem to be more than those of the ipsilateral side. Likewise accumulations of varicose terminals are also found in the cuneate nucleus (CN) as shown in Fig 7.38 ans Fig 20. Terminals in these two nuclei extend medially into the area of NTS (Fig 7.39). Numerous small labelled fibers are found to accumulate in the ipsilateral RL (Fig 21). They are just dorsal to the ventrolateral group in the TSC. Only few terminals are observed among the fibers in the nucleus. On the contralateral side, no fibers are observed in the RL. Fibers are found only in the TSC.

Efferent Projections from the Middle Part of the FN.

Patterns of distribution of fibers along the TSC, dorsal to BC, toward the

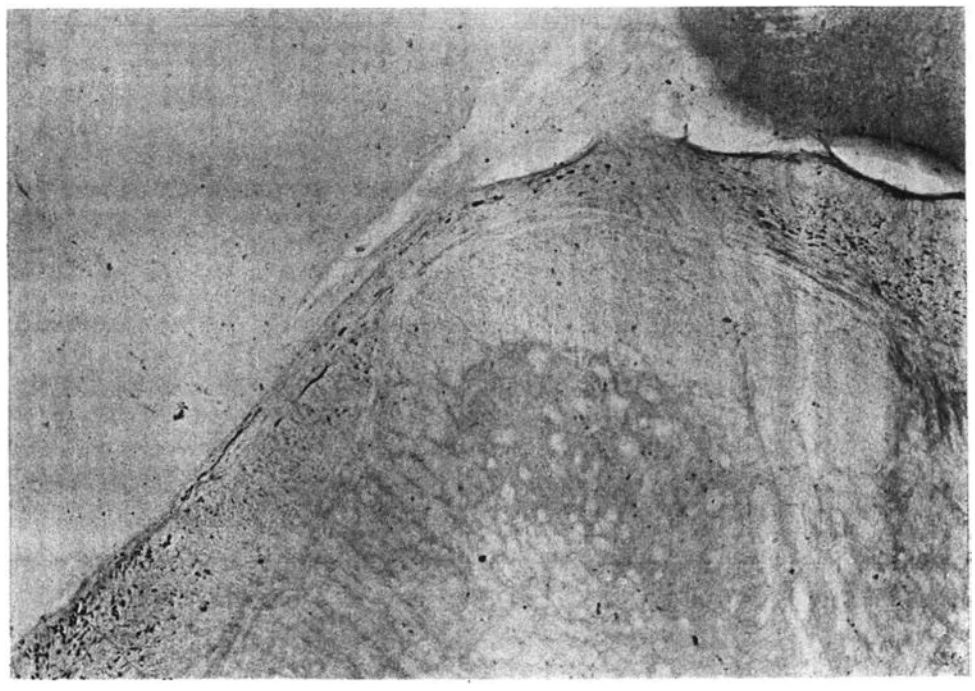

Fig 19 Photograph showing Labelled fibers can be observed continuously from the ventrolateral groupo which lie next to lateral border of the OI to TSC , TSD and ICP

OI = inferior olivary nucleus

TSC = ventral spinocerebellar tract

TSD = dorsal spinocerebellar tract

ICP = inferior cerebellar peduncle

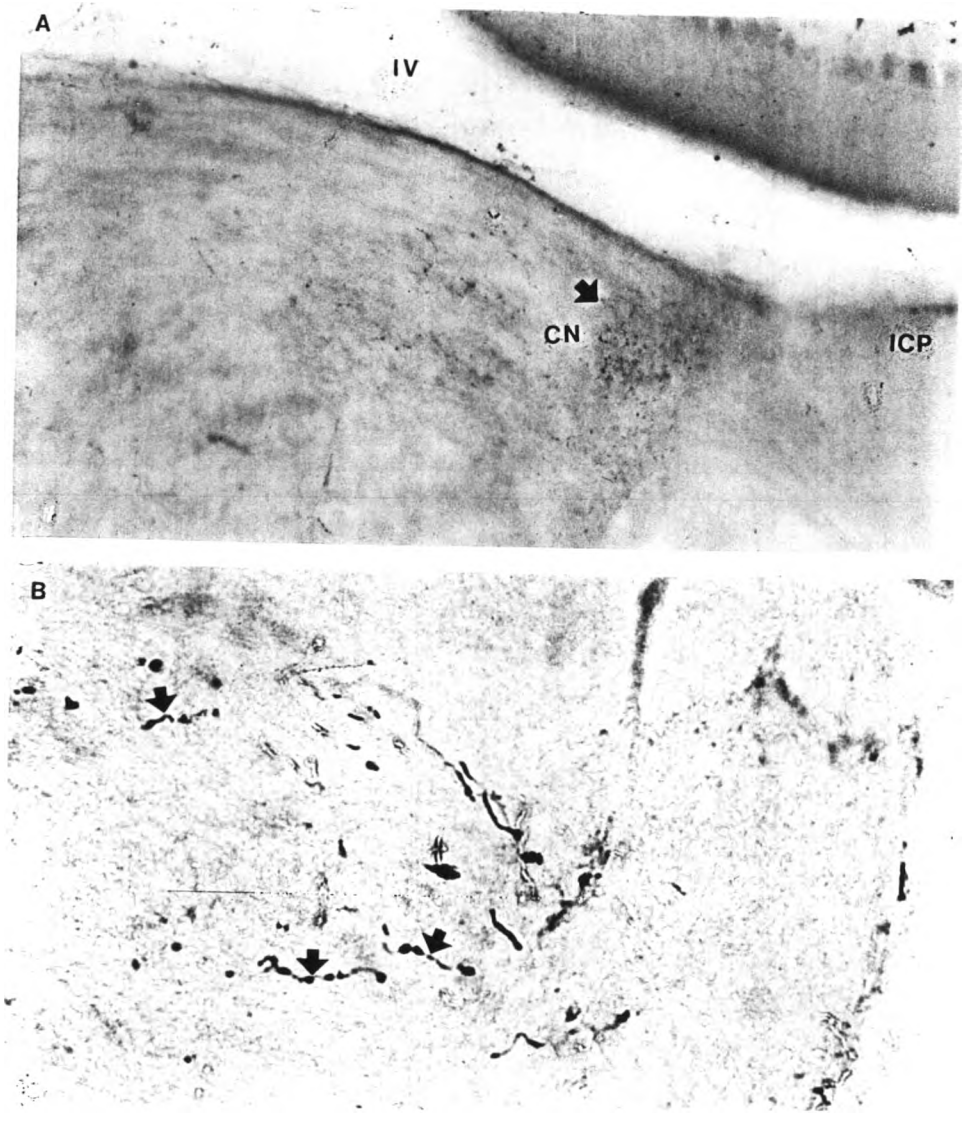

Fig 20 Photographs showing A) nerve terminals (arrow) in the CN x40 B) high magnification of nerve terminals in (A) arrow x400

CN = cuneate nucleus

IV = fourth ventricle

ICP = inferior cerebellar peduncle

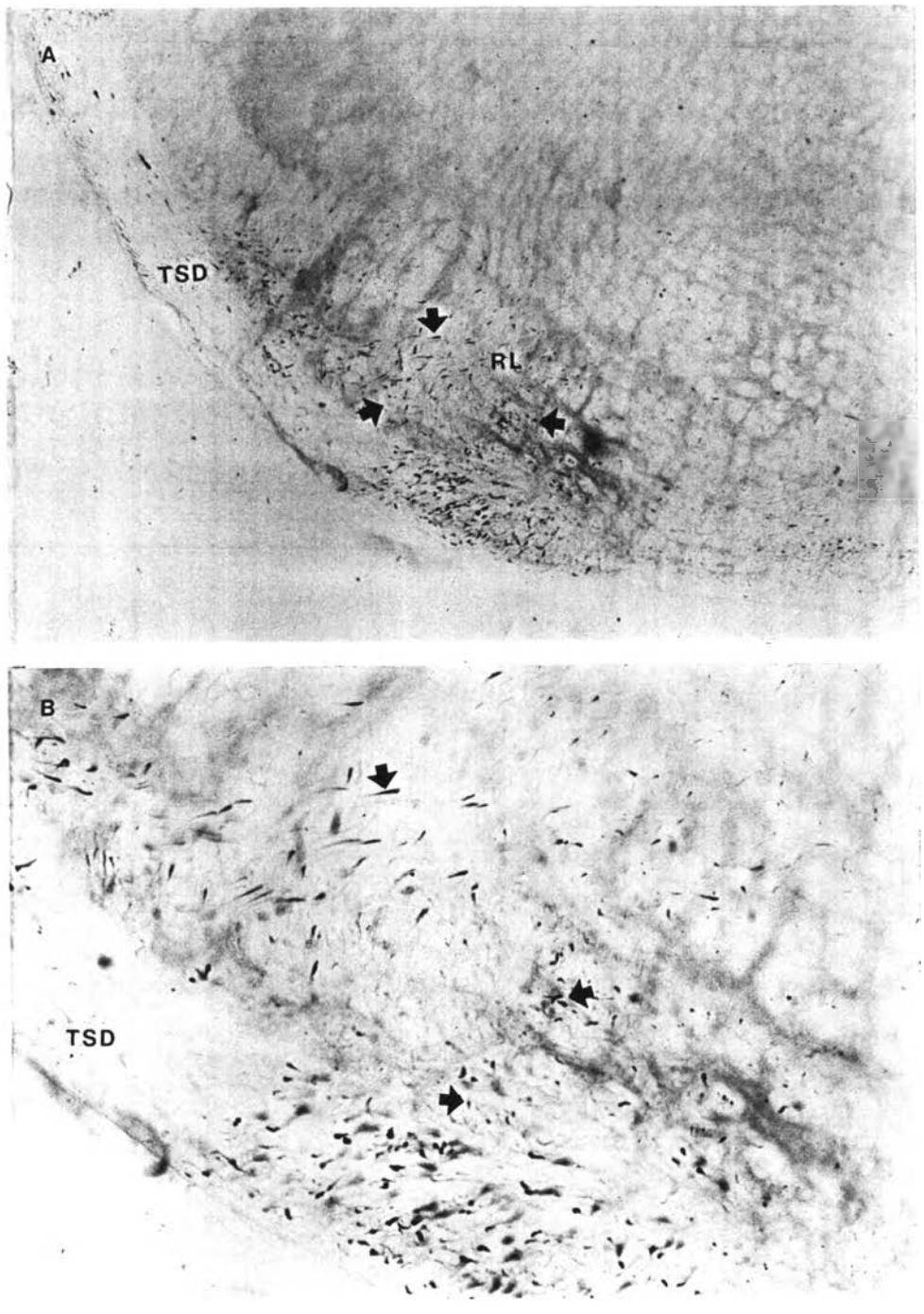

Fig 21 Photographs showing A) nerve fibers (arrows) in the RL x40 B) high magnification of nerve fibers in (A) arrow x100

RL = lateral reticular nucleus

TSD = dorsal spinocerebellar tract

medial boundary of the ICP and lateral boundary of TS V to the ventrolateral position of the base of the brain are similar to those shown in the rostral FN projections. However, numbers of fibers on each side are somewhat smaller. Likewise, numbers of fibers accumulate in the RL is also smaller than that of rostral FN projection.

Few fibers are observed at the ventral border of the medial end of the BC. They do not spread ventrally through the vestibular nuclei as found in the rostral FN projection.

Dense accumulation of fibers are found bilaterally in the area lateral to proximal part of the C VII close to nucleus abducen. They spread laterally to the lateral border of the VL. Small aggregation of the nerve terminals could be observed in the dorsal area of contralateral pontine reticular formation (FRP) as shown in Fig 22. No or very few terminals are found in the medullary FRS and FRG of the medulla. Small numbers of terminals can be observed bilaterally in the area of CNL (Fig 23). They are found to accumulate densely in the whole area of CN at more caudal levels. They are also extended into the NTS areas (Fig 24).

Efferent Projections from the Caudal Part of the FN.

At the level rostral to the C VII, fibers are observed lying along the dorsal border of BC. Small number of fibers are found in bilateral VS (Fig 25) ; they pass through the nucleus toward the VL with few varicose terminals. They lie in the dorsal boundary of the nucleus close to the ICP.

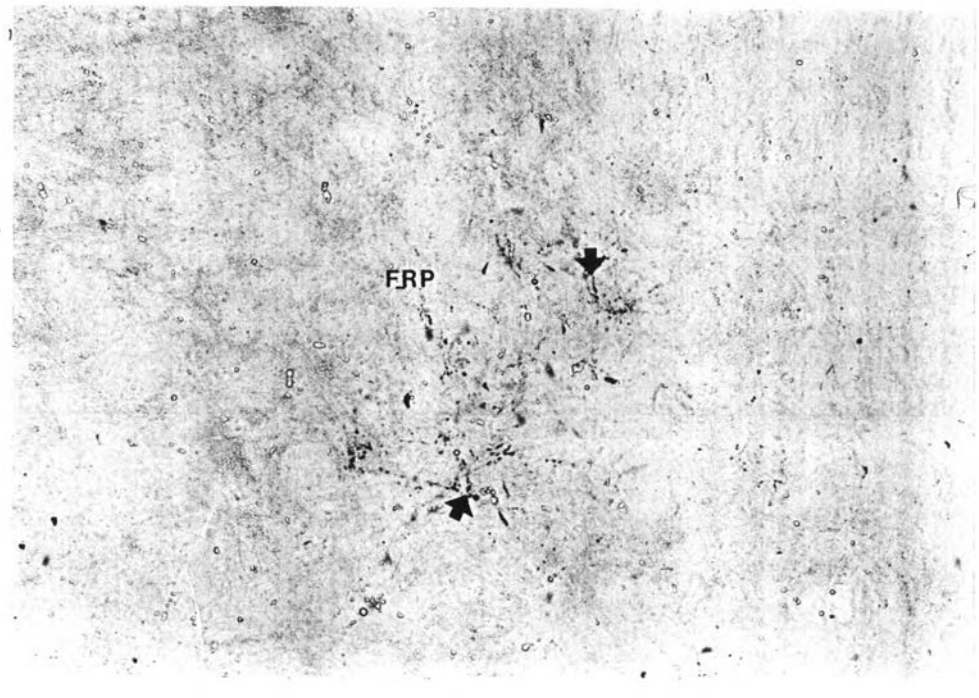

Fig 22 Photograph showing nerve terminals (arrow) in the FRP x100

FRP =pontine reticular formation

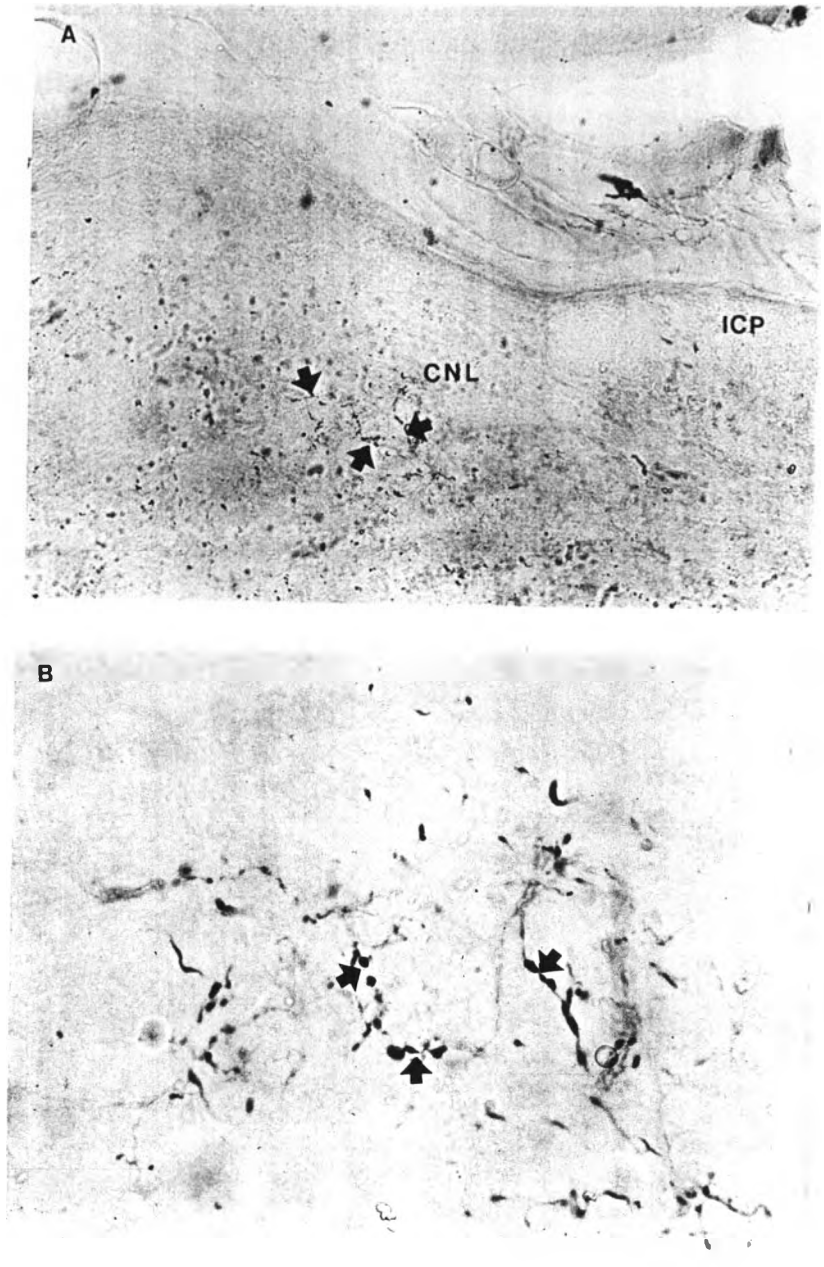

Fig 23 Photographs showing A) nerve terminals (arrow) in the CNL x40 B) high magnification of nerve terminals in (A) arrow x400

CNL = lateral cuneate nucleus

ICP = inferior cerebellar peduncle

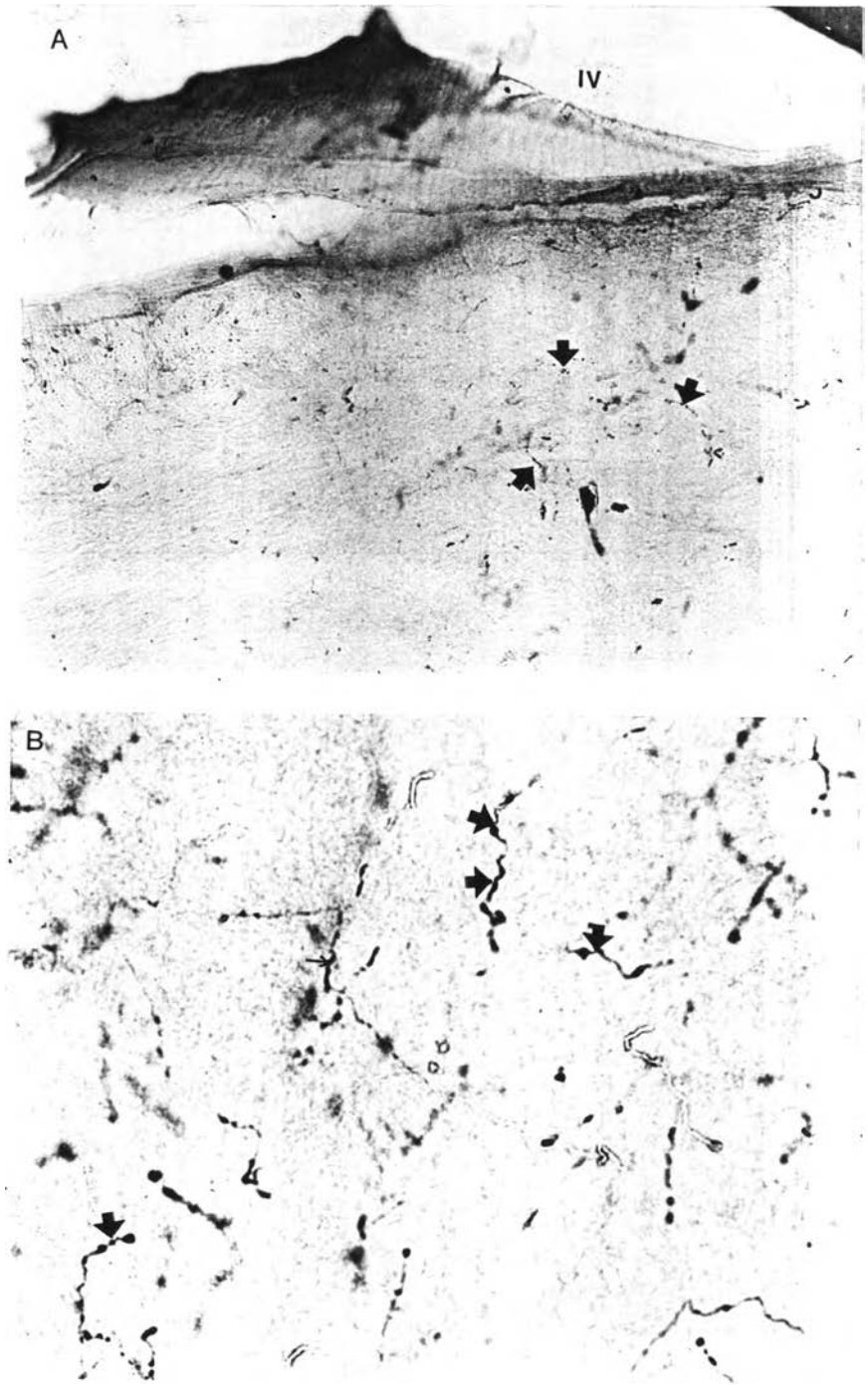

Fig 24 Photographs showing A) nerve terminals (arrow) in the NTS x100 B) high magnification of nerve terminals in (A) arrow x400

NTS = nucleus of tractus solitarius

IV = fourth ventricle

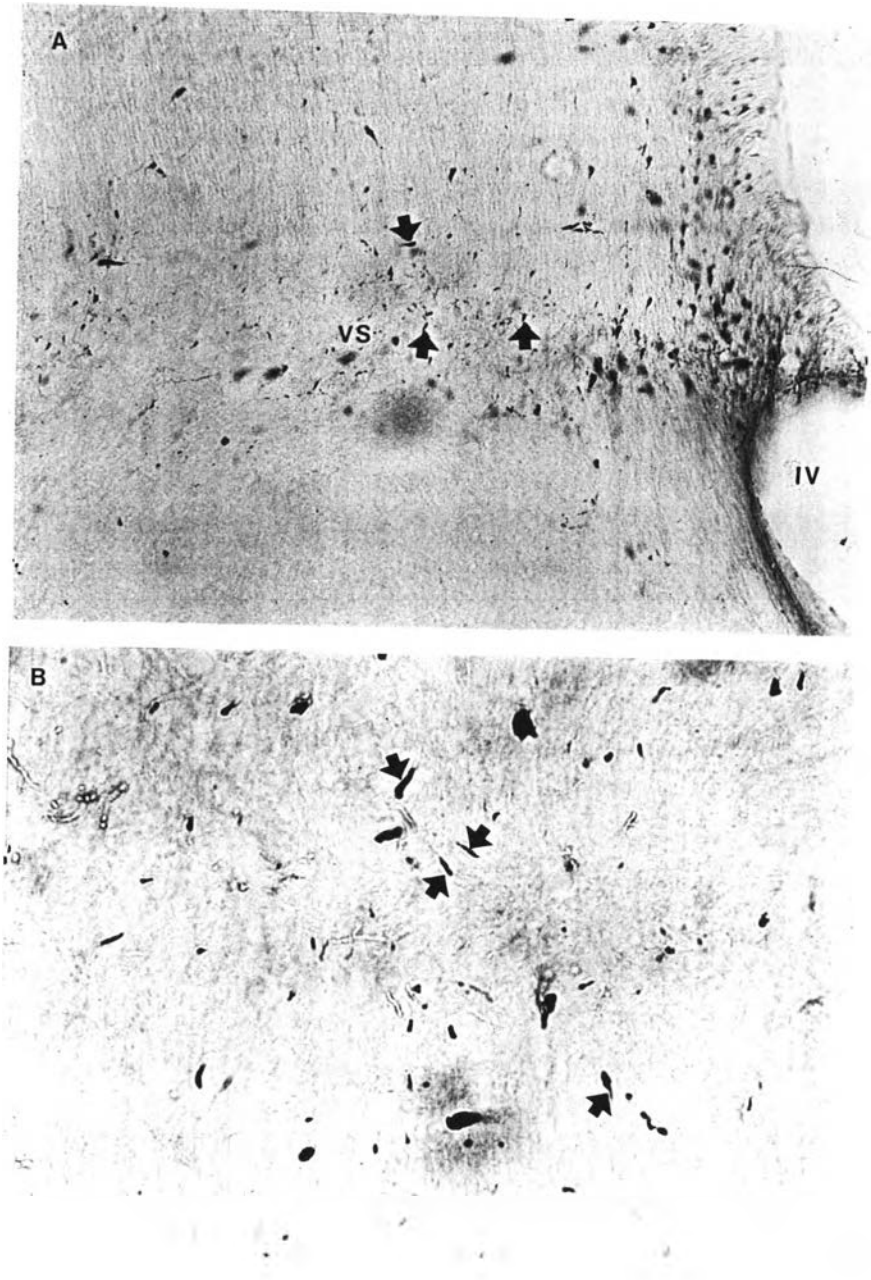

Fig 25 Photographs showing A) nerve fibers (arrow) in the VS x 100 B) high magnification of nerve fibers in (A) arrow x400

VS = superior vestibular nucleus

IV = fourth ventricle

At the rostral level of the C VII. Dense accumulation of terminals are located in the area ventral to bilateral VM (Fig 26). They spread laterally into the lateral area of the VL and VI and medially toward the proximal part of the C VII. This group of terminals increases at more caudal levels and spread more medially across the nerve into FRS. They gradually reduce and disappear at the level caudal to the C VII. At this level, terminals are still observed in VM (very few), VL and VI which lie along the medial boundary of ICP (Fig 27). Those in the VM spread ventrally into the FRS area (Fig 28). At caudal level of nucelus cochlearis dorsalis (NCD) and nucleus cochlearis ventralis (NCV), terminals in the contralateral VI spread medially into the FRS area.

At the levels caudal to the FN, terminals are found in the bilateral VM, CNL and NTS (Fig 29). At more caudal levels, terminals are mainly accumulated in the bilateral, CN, NTS. The NTS group diappears at more caudal levels. Summation of nerve terminals and fibers was shown in the Table 5 and 6.

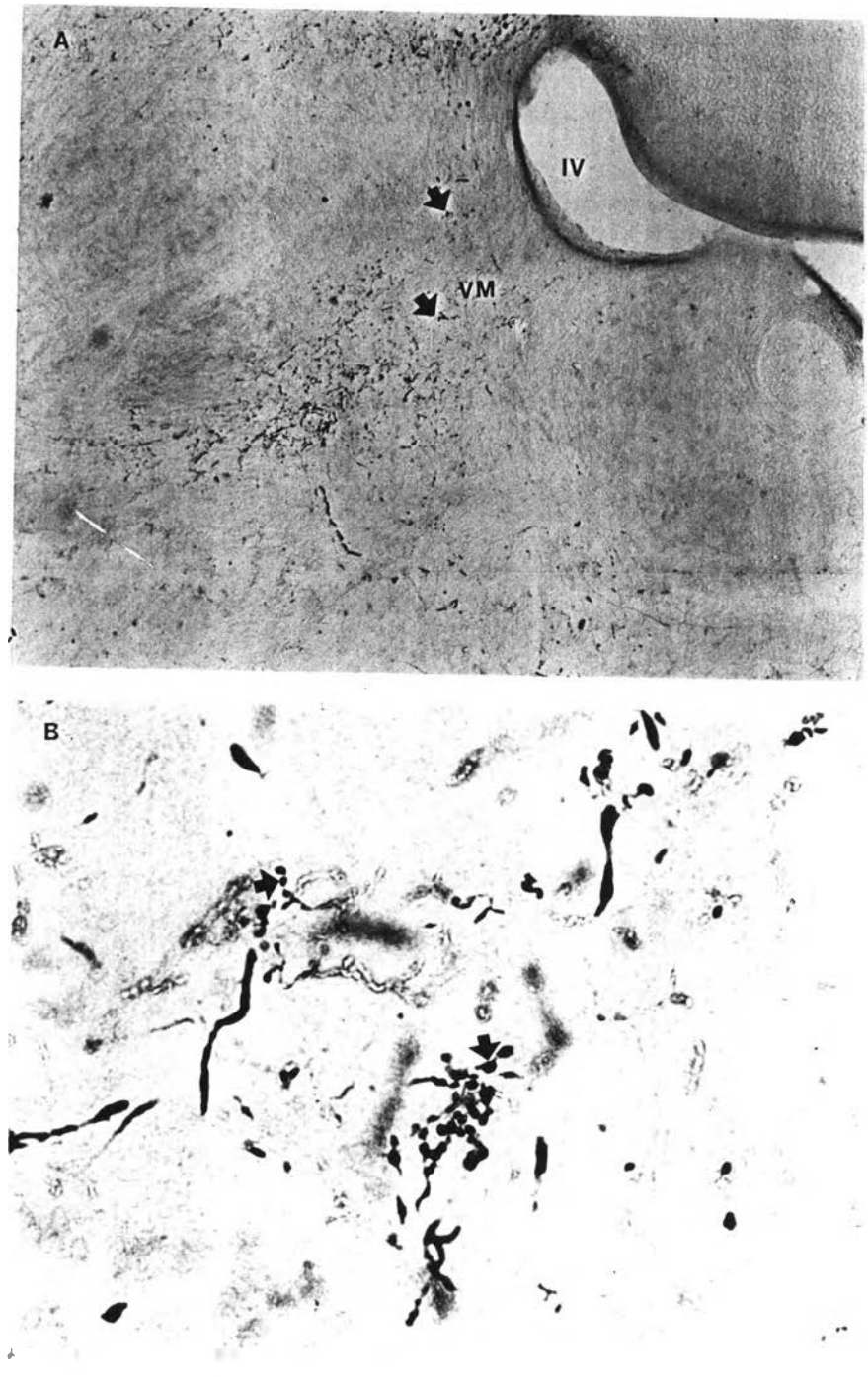

Fig 26 Photographs showing A) nerve terminals (arrow) in the VM x40 B) high magnification of nerve terminals in (A) arrow x400.

VM = medial vestibular nucleus

IV = fourth ventricle

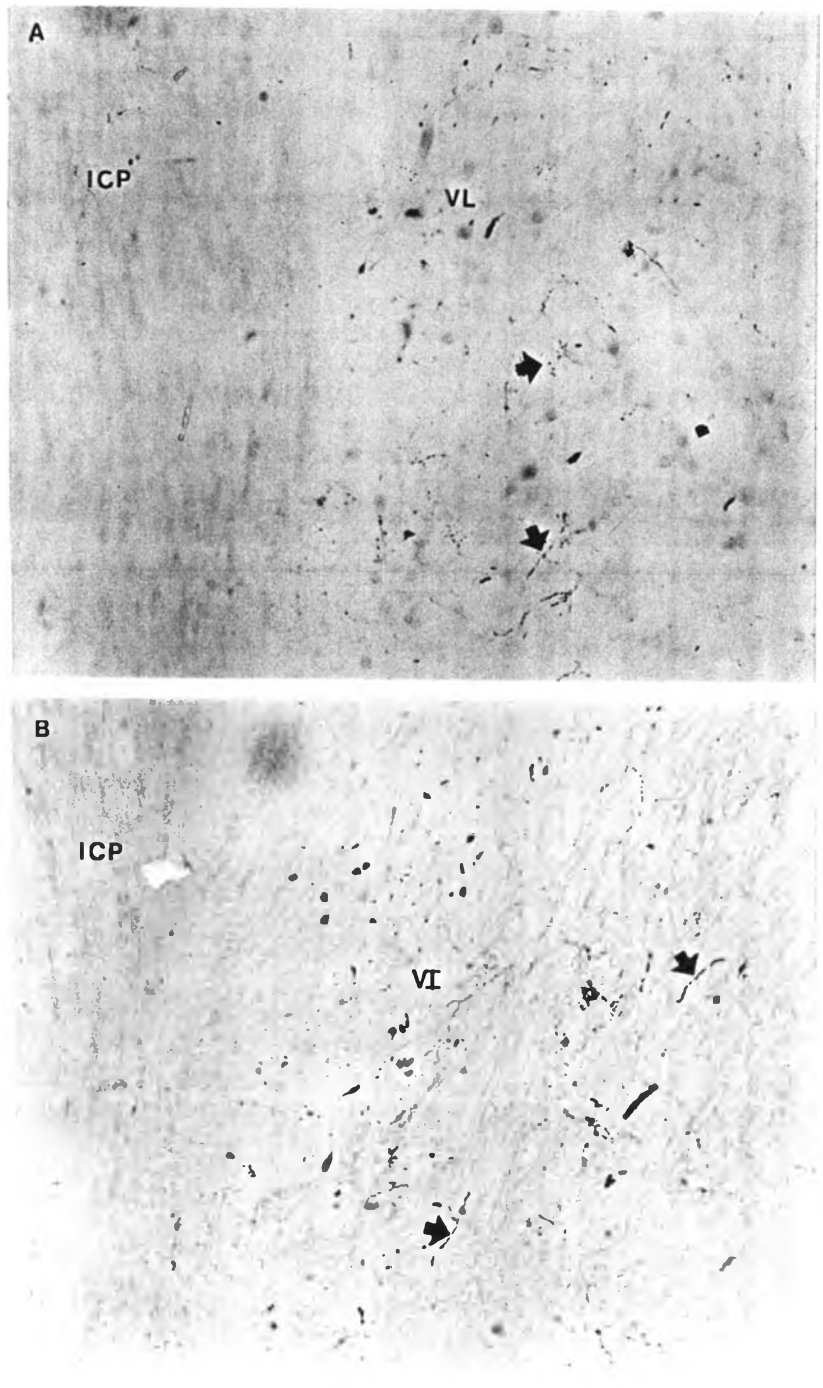

Fig 27 Photographs showing A) nerve terminals (arrow) in the VL x100 B) nerve terminals (arrow) in the VI x100

VL = lateral vestibular nucleus

VI = inferior vestibular nucleus

ICP = inferior cerebellar peduncle

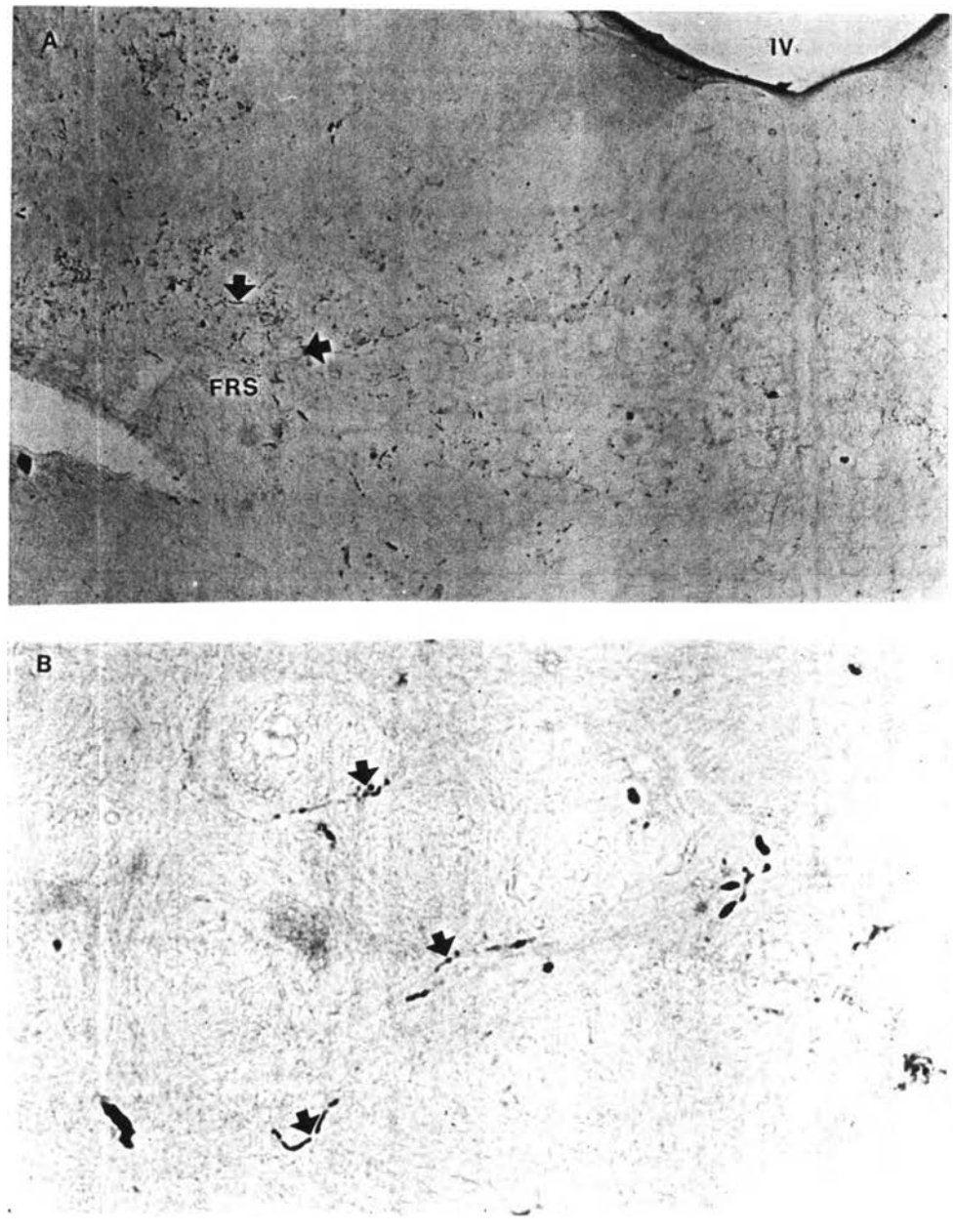

Fig 28 Photographs showing A) nerve terminals (arrow) in the FRS x40 B) nerve terminals (arrow) in the FRG x400.
FRS = nucleus reticularis parvocellularis
IV = fourth ventricle

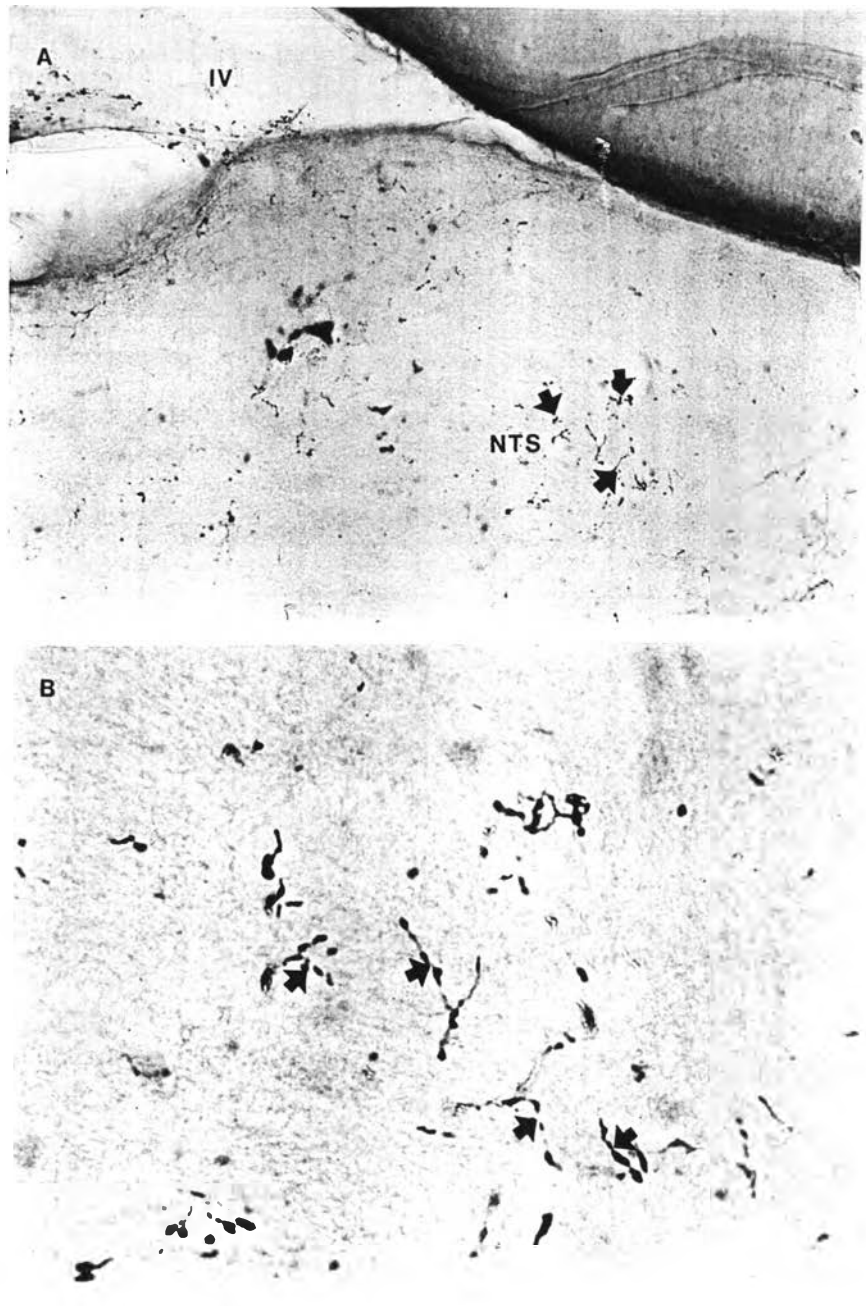

Fig 29 Photographs showing A) nerve terminals (arrow) in the NTS x40 B) high magnification of nerve terminals in (A) arrow x400

NTS = nucleus of tractus solitarius

IV = fourth ventricle

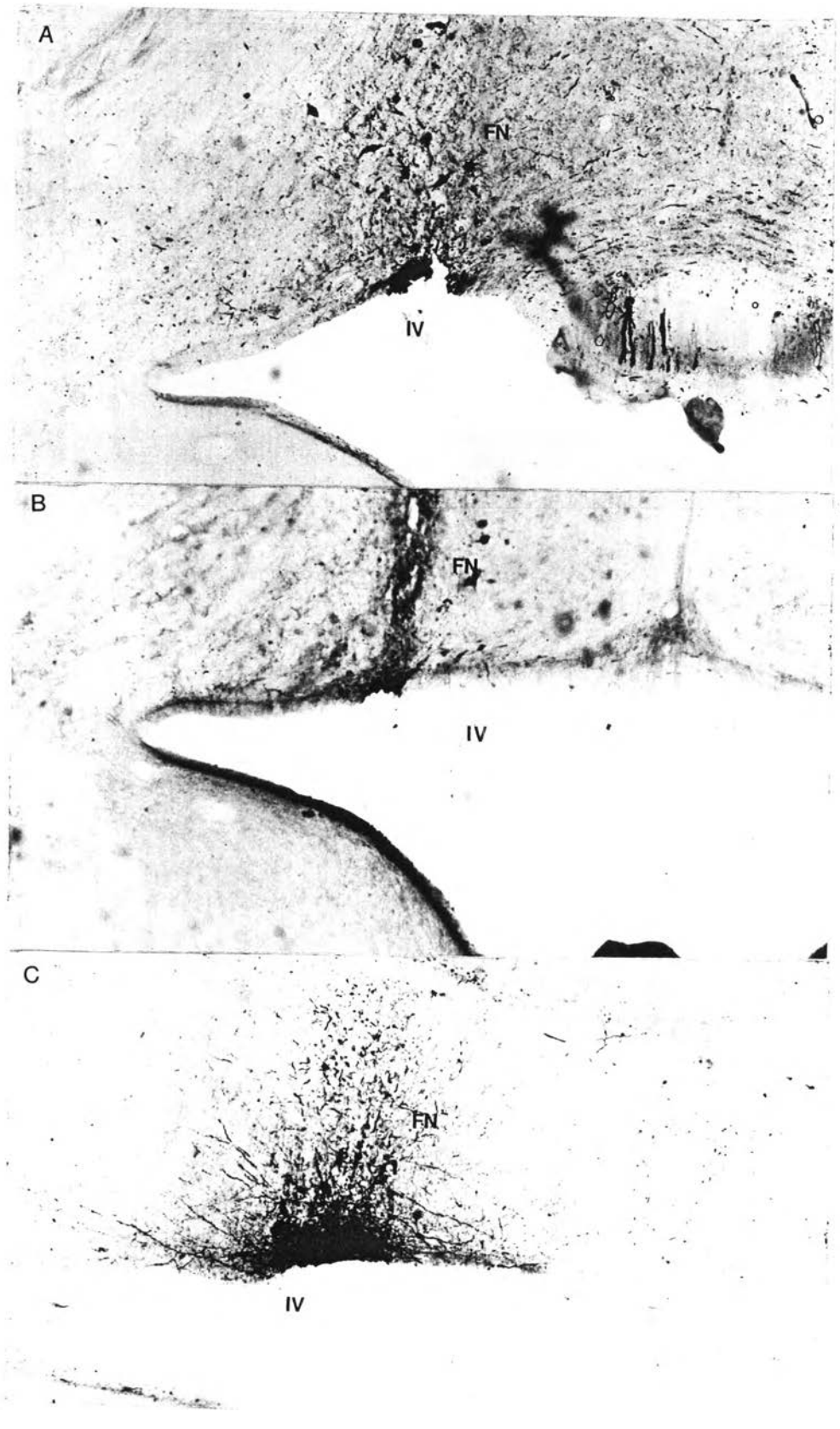

Fig 30 Photographs showing injection size of biocytin

A) rFN x100,B) mFN x100, C) cFN x100
rFN = rostral of fastigial nucleus, mFN = medial of fastigial nucleus, cFN = caudal of fastigial nucleus, VI = fourth ventricle

Table 5 Summation of nerve terminals in brainstem nuclei which was observed after biocytin injection at rostral, middle and caudal part of FN

Area	Rostral			Middle			Caudal		
	ipsilateral	contralateral	bilateral	ipsilateral	contralateral	bilateral	ipsilateral	contralateral	bilateral
1. vestibular									
VM	+++	++							++++
VL	+++	++							++++
VI	+++	++							++++
2. reticular									
FRP					++++				
FRS upper	++++	+++				+			+++
FRS lower	+++	++				+			++++
FRG upper	+++	++				+			++
FRG lower	++	+				+			+++
PGI	+++								
3. Other area									
CNL			+++			+++			+++
CN			+++			+++			+++
NTS			+++			+++			+++

\+ low density
++ medium density
+++ high density
++++ highest density

Table 6 Summation of nerve fibers in brainstem which was observed after biocytin injection at rostral, middle and caudal part of FN

Area	Rostral			Middle			Caudal		
	ipsilateral	contralateral	bilateral	ipsilateral	contralateral	bilateral	ipsilateral	contralateral	bilateral
1. dorsal BC	++++	+++		+++	++				
2. ventral BC	+++	++		++	+				
3.fastigio--spinal	++++	+++		++	+				
4. ICP	+++	++		++	+				
5. vestibular									
VS	+++	++							++
VL	+++	++		++	+				
6. Reticular									
RL	++++			+++					

\+ low density
++ medium density
+++ high density
++++ highest density