HS

AN EXPERIMENTAL STUDY OF THE OLIVO-CEREBELLAR CONNECTIONS IN THE CAT.

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A THESIS

Submitted to the Faculty of Medicine of the University of Ottawa in partial fulfillment of the Requirements for the Degree of Master of Science in the Department of Anatomy.

Ottawa, June 1970

ACKNOWLEDGEMENTS

This study was possible through the kind guidance and constant encouragement of Dr. Walter Hendelman, Assistant Professor, Department of Anatomy, Faculty of Medicine, University of Ottawa.

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CHAPTER I

INTRODUCTION

Investigators of the cerebellar cortex have observed that the afferent connections of the cerebellum terminate as two distinct fibre systems; the mossy and the climbing fibres. One or the other mode of termination has been assigned to each afferent system.

The climbing fibres were first discovered by Ramón y Cajal in 1888. In Cajal's word's:

> "Ces fibres que nous avons découvertes dans l'écorce cérébelleuse des oiseaux et des mammifères, doivent leur nom à un caratère singulier de leur terminaison. Elles portent, en effet, à leur extremité terminale une arborisation allongée qui s'applique sur le corps et les branches principales des cellules de Purkinje et semble grimper sur ces parties comme les lianes ou le lierre sur les arbes." (Cajal 1955, p. 64)

Further details regarding the structure of these fibres were described by Cajal and Illera (1907). Cajal (1911) published a comprehensive report of his observations of the climbing fibres, in which he described them as afferents of extra-cerebellar origin that course undivided through the white matter of the folia. The fibres traverse the granular layer by a sinuous course to reach the Purkinje cell layer. Each climbing fibre then attaches itself to a Purkinje cell body, where it divides, its branches following closely the primary and secondary dendritic branches of the

Purkinje neuron (Fig. 1).

Many of the early investigators concerned with the problem of the origin of these fibres, studied various extra-cerebellar fibre systems. Cajal had suggested that the fibres originated from either the pontine nucleus or the vestibular nuclei. Subsequent degeneration studies done by Miskolczy (1931, 1934), Snider (1936) and Rosiello (1937), showed degeneration only of mossy fibres.

The experiments of Miskolczy (1931, 1934) led him to the conclusion that both the spino-cerebellar and olivo-cerebellar fibres terminate as mossy fibres. His first study involved a hemi-section of the spinal cord and in his second study he created a lesion in the restiform body, both inferior olivary nuclei and possibly the lateral reticular nucleus. His conclusions were confirmed by Rosiello (1937).

Snider (1936) made lesions of the brachium pontis and found alterations of mossy terminals but no degenerative changes of climbing fibres. Mettler and Lubin (1942) also made lesions of the brachium pontis in cats. They found loss of mossy fibres in the homo-lateral cerebellar cortex and to a lesser extent on the contra-lateral side. While all these studies established the sources of the mossy fibres, the origin of the climbing fibres remained obscure.

Szentágothai (1939) suggested that the failure of such studies to reveal climbing fibres might be due to

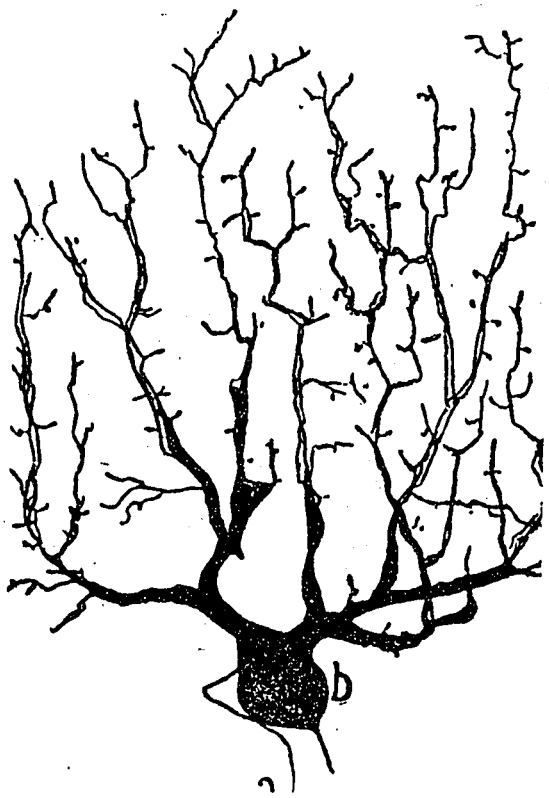

Figure 1: Photograph of a climbing fibre arborizing along the dendritic tree of a Purkinje cell. Taken from a drawing of Golgi-Cox stained material a, climbing fibre; b, Purkinje cell (Cajal 1955, p. 66).

technical difficulties in staining degenerative fragments of axons in the molecular layer. He postulated an olivary origin for these fibres, because he could trace degenerated axons up to the level of the origin of the main dendrites of the Purkinje cell after lesions involving the inferior olive. He also observed degenerated horizontal and descending side branches arising from the main fibres at the level of the Purkinje cell body. This finding was contradictory to the classical description of Cajal which had stated that climbing fibres had no side branches at this level.

One possible source of climbing fibres that was never mentioned in these earlier studies was the cerebellar nuclei. Carrea, Reissig and Mettler (1947) introduced this concept. They placed lesions in the cerebellar nuclear complex in monkeys and cats and lesions in the olivary nucleus in cats. The survival times used ranged from 7-265 days. From their results, they concluded that olivo-cerebellar fibres terminate as mossy fibres. They found clear evidence of the presence of degenerating fibres, which they interpreted as climbing fibres, after lesions in the cerebellar nuclei.

On the basis of the appearance of these fibres, the authors classified them into two types. Type A fibres were characterized either by the formation of fusiform spheres, ovoids and reniform masses or by a conglomeration of three or four spheres on the axis cylinder. Type B fibres showed

fusiform thickenings, some of which were homogenous, while others displayed different degrees of vacuolation. Carrea et al (1947) suggested that the climbing fibres were not extra-cerebellar afferents as previously thought, but were either recurrent collaterals of the cerebello-fugal fibres emerging from the deep nuclei or primary axons of small nuclear neurons.

This study was severely criticized for its elaborate surgical procedure and inappropriate interpretations by Ule (1957) who observed intact climbing fibres in several cases of human pathological material which had almost complete cellular degeneration of the cerebellar nuclei. Szentágothai and Rajkovits (1959) also discredit Carrea et al's interpretation on the grounds that the characteristic signs of axis cylinder degeneration, mainly, fragmentation and fluffy vacuolation were not observed in the study. Szentágothai pointed out, in addition, that the knobs and spheres of their type A fibres have been observed in several regions of the central nervous system in normal brain tissue. The hypertrophic changes observed in the type B fibres could not be considered to be manifestations of secondary degeneration. Finally, on the basis of the long survival times used, these changes were not considered representative of phenomena of secondary degeneration.

Until about 1950 neuro-anatomical studies used various techniques: Modifications of Cajal's reduced silver

method and the Marchi technique which is known to be restricted to the demonstration of myelinated fibres were amongst these.

Subsequent to the availability of the more specific Nauta-Gygax (1954) staining procedure for degenerating nerve fibres and pre-terminals and the new Golgi data by Scheibel and Scheibel (1954) (see below) on the intracortical relations of the climbing fibres, Szentagothai working with Rajkovits, resumed his study of the origin of the climbing fibres (1959).

The investigations of Szentagothai and Rajkovits (1959) have been more favourably received. They placed lesions in cats in: the inferior olive, the corpus restiforme, the pons, different regions of the reticular formation of the pons and midbrain, the nucleus vestibularis and the spinocerebellar tract. The animals were allowed to survive for 4-5 days, after which 20 μ sections were stained with the Nauta-Gygax (1954) and Gros-Bielschowsky (1959) methods. From these materials the majority of their results revealed degeneration of mossy fibres in the granular layer. Only in animals with lesions of the restiform body and the inferior olive did they observe stained fragments in the molecular layer which was interpreted as degeneration of climbing fibres.

In animals with isolated lesions in the inferior olive, fine degenerated fibres were observed in the lowermost portion of the molecular layer accompanying the primary dendrites of the Purkinje neurons. With lesions of the restiform body, the

characteristic degeneration of mossy fibres (belonging to the spino-cerebellar tract) was seen in addition. When the lesion of the inferior olive extended deeper into the adjacent reticular formation, mossy fibre degeneration was also observed. All lesions in the reticular formation especially in the nucleus reticularis medullae oblongatae led to degeneration of mossy fibres. In some cases degeneration of climbing fibres was observed in the contra-lateral flocculus after lesions in the brachium pontis. The exact origin of such degenerating fibres could not be ascertained.

In an ultrastructural study in the cat, Smith et al (1966) transected the brachium pontis and the restiform body. They also made stab wounds in the cerebellar cortex, thereby isolating a few folia. In examining cases with peduncular lesions, they found no degenerative changes in the molecular layer. In animals with isolated folia, they found degenerating Purkinje dendrites in the molecular layer. No axonal degeneration was observed, even in areas where the underlying white matter showed heavy degeneration. On the other hand, mossy fibre terminal degeneration was observed in all cases and was heaviest in the isolated cortex. The suggestion from these results is that all extrinsic afferents to the cerebellum terminate as mossy fibres.

Using ultrastructural and Golgi methods, O'Leary

et al (1968) studied terminal degeneration in the cerebellum of rats. They placed lesions in the inferior olivary nuclei, the brachium pontis and the cerebellar nuclei.

Following the olivary and pontine lesions, mossy fibre degeneration was observed both at the ultrastructural and light microscopic levels. Scanty evidence of degenerating climbing fibre strands was observed in the ultrastructural material, but significant numbers of normal climbing fibres were also present. The Golgi material yielded less evidence of climbing fibre degeneration.

Subsequent to the large electrolytic lesions in the cerebellar nuclei, climbing fibre degeneration was noted in areas immediately adjacent to the lesions, in the Nauta and Fink-Heimer preparations. This degeneration became progressively less evident in areas farther removed from the margin of the lesion. In corresponding Golgi material, some climbing fibre degeneration was observed only in folia that had been accidentally isolated during the surgical procedure.

These findings support the original observations of Snider (1936) and Mettler and Lubin (1942) with regard to mossy fibre origins from both the pontine and olivary nuclei. In the case of the cerebellar nuclear lesions, the results could neither support nor refute the theory of an intra-cerebellar origin of climbing fibres as suggested by Carrea et al (1947), since both normal and scattered strands of degenerating climbing

fibres were found. The point is made, however, that in the rat, the olivo-cerebellar tract runs in close juxtaposition to the rostral margin of the medial cerebellar nuclei and could have been damaged in these central nuclear lesions.

Other sources of climbing fibres have been investigated. Inter-folial cortical association fibres, which are considered to be axon collaterals of Purkinje cells, have been implicated as the possible source of the numerous climbing fibres in the cerebellar cortex. No effort was made to investigate this possibility through experiment, except for the incidental observations of Snider (1936) who while studying ponto-cerebellar projections, observed degenerating climbing fibres in the folia adjacent to the knife punctures of the cortex.

In a later study of the mode of termination of association fibres, Eager (1965) found degenerative changes resembling those of climbing fibres. In his experiment, some of the degenerated fibres seen in the molecular layer showed preterminal relationships with primary dendritic branches of Purkinje cells, suggesting that they might be climbing fibres, though the actual terminations of most of the degenerating elements were not clearly discernible. Eager made small lesions by means of needle scratches to the cerebellar cortex and stained his material with the Nauta-Gygax technique (1954). This interpretation has not been accepted (see discussion)

and has not been supported by a Golgi study of intra-folial Purkinje axon collaterals, (Ha 1970).

A vestibular origin for climbing fibres was considered by Ariëns Kappers (1921). Subsequent investigations were not in agreement as to the type of ending for these fibres. Snider (1936) in studying ponto-cerebellar connections accidentally injured the eighth cranial nerve in some of his animals and he demonstrated only terminal degeneration of mossy fibres.

The work of Brodal and Høivik (1964) has now conclusively demonstrated that the primary vestibular fibres terminate as mossy fibres. In this study, the vestibular nerve was sectioned and the cerebellar cortex examined with the Nauta-Laidlaw (1957), Nauta-Gygax (1954) and Reumont-Lhermitte (1948) techniques. Mossy fibre terminals were observed in the granular layer with each staining technique used. The degeneration is described as being similar to those seen in the anterior lobe following section of the spino-cerebellar fibres, (Brodal and Grant 1962). No evidence of degeneration of climbing fibres was observed in the molecular layer.

The most convincing evidence for an olivary origin of climbing fibres is to be found in the physiological data (see discussion) and the study of Szentágothai and Rajkovits (1959). The inferior olivary nuclei are at present considered to contribute an afferent system to the cerebellar cortex whose fibres terminate as climbing fibres. There is no doubt

that the inferior olive projects to the cerebellum and does so in a topographical manner, as has been demonstrated by the modified Gudden technique (Brodal 1940).

It is evident from a review of the literature of the anatomical data, that the mode of termination of the projection from the inferior olive to the cerebellum still remains controversial. The projection of other cerebellar afferent systems terminating as mossy fibres seems more firmly established by both anatomical and physiological studies.

The morphology of climbing fibres has been re-investigated using Golgi methods and electron microscopic analysis. Scheibel and Scheibel (1954) undertook an elaborate Golgi study of the cortical relations of the climbing fibres. Their observations revealed that the climbing fibres have more extensive synaptic relations in the cerebellar cortex than was previously believed.

Small recurrent collaterals were often seen leaving the parent fibre at the level of the Purkinje cell body, to re-enter the granule cell layer, where they were lost in the neuropil of that layer. Rarely, these collaterals were seen to end in simple large nodules. These descending recurrent collaterals had been observed by Szentágothai (1939) to contact the cell body and proximal parts of the dendrites of the Golgi cells in the sub-ganglionic layer.

Many fine collaterals were observed leaving the

climbing fibre in the initial part of its course along the primary dendrite of the Purkinje cells. They terminate on the neck, soma and primary dendrites of adjacent Purkinje neurons. A single climbing fibre was observed contacting as many five to ten adjacent Purkinje cells.

Further observations by these authors led to the suggestion that the climbing fibre contacts basket and stellate cells in the molecular layer and establishes axo-axonic relations with these neurons as well as with parallel fibres.

On the basis of this study, further efforts were made to identify the climbing fibre and its synaptic contacts in the cerebellar cortex with the use of the electron microscope. Hamori and Szentagothai (1966) were able to confirm some of the observations of Scheibel and Scheibel (1954).

Two types of axonal profiles were seen in their ultrastructural material, making contacts with primary and secondary Purkinje dendrites. One type consisted of preterminal fibres running parallel to the Purkinje dendrites and making contacts 'de passage' with their surface. These fibres were densely packed with coarse neurofilaments and had only a few synaptic vesicles at the sites of contact. The other type of axonal profile was made up of fibres with knob-shaped synaptic

contacts which contained abundant vesicles and no neurofilaments.

Since more than one axonal profile in the molecular layer has neurofilaments, it was necessary to do experimental studies, in order to be able to identify any given profile containing neurofilaments. Certain folia of the cerebellar cortex were chronically isolated, that is deafferented, by the technique of undercutting. The isolated folia were then examined. It was observed that in these folia, the neurofilamentous profiles were no longer present. The only terminals remaining in contact with the Purkinje dendrites were of the vesicular type. Since the only extra-cerebellar afferents arrive via the mossy and climbing fibre systems and since the latter is the only afferent system to reach the molecular layer, the neurofilamentous profiles in contact with Purkinje dendrites, which disappeared in the isolated folia, were identified as belonging to the climbing fibres.

By similar methods, climbing fibre contacts with Golgi and basket cells were established. These results support the Golgi study of Scheibel and Scheibel with regard to climbing fibre contacts with Purkinje dendrites and basket cell bodies. However, they could find no evidence of either axo-dendritic contacts between climbing fibres and basket cell dendrites or of axo-axonic synapses with parallel fibres, basket and stellate cell axons as reported in the

Golgi study.

Fox et al (1967) demonstrated climbing fibres by Golgi and electron microscopic techniques in the monkey. Their Golgi illustrations are identical to all previous work on the cerebellar cortex. In comparing this normal (Golgi) picture of the climbing fibre and the studies using degenerating staining techniques, one is impressed with the fact that in the latter case, the climbing fibre has never been shown in its entirety, after lesions in any part of the central nervous system.

Afferents to the deep cerebellar nuclei have now assumed a critical importance due to the recent data on the circuitry of the cerebellum, (Eccles et al 1967). All the afferent systems to the cerebellar cortex have been reported to send collaterals to the deep cerebellar nuclei, although the Purkinje axons are the predominant source of the afferents to these nuclei.

Brodal (1940) (see also Jansen and Brodal 1954) in his detailed study of the topographical projection from the inferior olive to the cerebellum, reported that all parts of the cerebellar cortex and nuclei receive fibres from this nucleus. Though the projection to the cerebellar nuclei is very orderly and related topographically to specific parts of

the inferior olive, the borders between the projection areas are not as clearly demarcated as in the cerebellar cortex.

Secondary vestibular fibres have also been thought to give collaterals to the medial (fastigial) nucleus. Brodal and Torvik (1957) confirmed this and found these fibres to originate in the lower part of the nucleus descendens of the vestibular nuclear group and cell group X.

Eccles et al (1967) using the Nauta procedure reported that the spino-cerebellar system gave a fair amount of collaterals to the medial (fastigial) nucleus, a little less to the intermediate (interposital) nucleus and still less to the lateral (dentate) nucleus. They also found evidence of abundant collaterals from the ponto-cerebellar fibres, olivo-cerebellar and possibly the reticulo-cerebellar systems to the deep nuclei.

Electrophysiological studies have substantiated the existence of these collaterals (see discussion). However, the anatomical evidence using degeneration staining techniques is still meagre. In particular the projection from the inferior olive to the cerebellar nuclei has not been thoroughly investigated.

Problem Formulation

The existence of climbing fibres and their synaptic relations with the Purkinje dendrites in the molecular layer of the cerebellar cortex, have been unequivocally demonstrated by Golgi studies and electron microscopic examination. A review of the literature clearly indicates that the question of the origin of the climbing fibre remains unsettled.

Degeneration studies have suggested various sources of these climbing fibres. On the basis of recent anatomical and physiological studies, the most likely source for climbing fibres is the inferior olive and the deep cerebellar nuclei.

The anatomical evidence supporting an olivary origin has not been overwhelming. The work of Szentagothai and Rajkovits (1959) using the Nauta-Gygax and the Gros-Bielschowsky methods, is the most recent experimental study with silver impregnation techniques, to suggest climbing fibre degeneration, following a lesion in the inferior olive in cats. Other studies have not supported this report and seem to indicate that the inferior olive projects as mossy fibres, (O'Leary et al 1968 and Smith et al 1966).

The physiological evidence on the other hand has been most convincing and supports the olivary origin of climbing fibres (Eccles et al 1966), (Voorhoeve 1967), (Precht and Llinas

1969). It has been suggested that the failure to demonstrate degenerated climbing fibres in the molecular layer, could be due to inadequacies in the staining techniques used. Improvements of the techniques for staining degenerating fibres and preterminals light microscopically are now available, Viz: Nauta-Laidlaw (1957), Fink-Heimer I and II (1967) and Wiitanen (1969). This fact is of considerable importance, since the climbing fibre loses its myelin sheath at the level of the Purkinje cells. Since the improved techniques are more specific and are thought to stain unmyelinated portions of a nerve fibre, including preterminals and possibly terminals, it was thought to be desirable to re-investigate the projection of the inferior olive to the cerebellar cortex in the cat.

Secondly, anatomical and physiological studies have demonstrated evidence of collateral distribution from all cerebellar afferent systems to the cerebellar nuclei. This investigation will also re-examine the question of collateral projection from the inferior olivary nuclei to the cerebellar nuclei in the cat.

CHAPTER II

MATERIAL AND METHODS

This investigation is based on findings in the brain of 23 adult cats weighing 1.8-3.6 kg. In each case the animal was anesthetized with an intraperitoneal injection of nembutal. (35 mg./kg. body weight)

In a majority of the animals an electrolytic lesion was placed in the inferior olivary nuclear complex. The spino-cerebellar tract was interrupted in one cat and in six other cats, an electrolytic lesion was placed in the white matter of the cerebellar hemispheres. Finally, surgical lesions were made in the cerebellum of two additional cats. In all electrolytic lesions, a 26 gauge insulated stainless steel bi-polar electrode was used and electrocoagulation was carried out by means of a stoelting lesion generating device applying a DC current of 3 ma. for 5-10 seconds.

Olivary Lesions

The inferior olivary nuclear complex was impaled from a posterior approach. The co-ordinates used were pre-determined with the aid of Verhaart's stereotactic atlas of the brainstem of the cat (1964).

Stereotaxic Co-ordinates:

Frontal:	Posterior	10.0
Vertical:		-10.0
Lateral:		1.5

For each cat the electrode was adjusted on the zeroing device prior to the operation using an angle of 70°, necessary for a posterior approach. It was found by preliminary trials that the final electrode position for placing a lesion in the inferior olive, necessitated an additional 1.5 mm. advance.

An incision was made along the ligamentum nuchae in the midline and the muscles displaced laterally until the posterior atlanto-occipital membrane was in view. This membrane as well as the dura were slit open exposing the cistern magna. The electrode was introduced into the medulla oblongata through the foramen magnum, entering it below the nuclei cuneatus and gracilis. After the lesion was made, the opening was closed over with gelfoam, the muscles and fascia were sutured and the skin incision clipped together. An intramuscular injection of 1 cc. of Ayercillin (penicillin) was given to each animal post-operatively.

White Matter Lesions

The electrolytic lesions in the white matter were made with the aid of Berman's stereotaxic atlas of the brainstem of the cat (1968).

a) In the first two animals with this lesion, the electrode was introduced into the white matter directly through the folia, on the same side as the lesion.

Stereotaxic Co-ordinates:

Frontal:	Posterior	10.8
Vertical:		2.0
Lateral:		8.0
Angle:		39°

This procedure created an electrode tract in the folia to be studied. The resulting damage to the cortex caused a local traumatic reaction which complicated the interpretation of the results obtained.

b) In the subsequent animals in this series, a contra-lateral approach was used thus leaving the folia to be studied undisturbed. The electrode was introduced at an angle of 46° determined with the aid of a cat skull, such that the electrode would pass beneath the bony tentorium and above the superior nuchal line. The carrier was rotated 80° to obtain the desired electrode placement.

Stereotaxic Co-ordinates:

Frontal:	10.0
Vertical:	1.5
Lateral:	8.0

In both approaches, the procedures for obtaining the final co-ordinates for each cat was identical to that described previously for inferior olivary lesions using the zeroing device.

Surgical Lesions

In two cats, surgical lesions were made in the cerebellar cortex. A large bore hole was made in the skull of one cat and a straight-edge sterilized blade was introduced in such a manner as to isolate several folia by undercutting. The lesion extented from the (superior) rostral to the (inferior) caudal surfaces on one side of the cerebellar hemisphere.

In the second cat, bore holes were made bilaterally in the skull between the superior nuchal line and the bony tentorium and the dura was incised. Knife punctures were then made in the exposed paravermal folia of the cerebellar cortex. The intention was to introduce lesions only in the molecular layer. On both sides, however, the lesions extended deeper. All skull openings were closed over with gelfoam and the muscle and skin sutured.

Spino-Cerebellar Lesion

The spinal cord of one cat was exposed by an incision in the skin and muscles at the back of the neck, similar to that made for olivary lesions. The first cervical nerve was identified and a sterilized blade was used to make a lesion in the lateral funiculus of the cord just rostral to this nerve.

A summary of the olivary and non-olivary lesions including survival times is presented in tables I and II respectively.

Fixation

The cats were deeply anesthetized with nembutal and perfused with 300-400 cc. of normal saline solution introduced into the left ventricle and followed by 300-400 cc. of 10% neutral formalin. The cerebellum with the brainstem attached was removed and stored in 10% neutral formalin for periods of 1 week to 1 month. The brains were then put in a 30% sucrose solution for 3-4 days. This step was recommended to facilitate sectioning on the freezing microtome.

Staining

The cerebellum was detached from the medulla oblongata. The latter was sectioned transversely at 30 u and the extent of the olivary lesion visualized. The cerebellum of these animals was sectioned at 25 μ or 30 μ in the frontal, horizontal or para-sagittal planes. In the cat with the knife punctures in the cortex, an attempt was made to section the folia involved along their longitudinal axes. All other brains with cerebellar lesions were sectioned at 30 μ para-sagittally or horizontally. The cerebellum of the cat with a lesion of the spino-cerebellar tract in the lateral funiculus of the spinal cord was sectioned para-sagittally at 30 μ and the spinal cord sectioned transversely. (see tables I and II)

The sections were collected in 10% and 2% neutral formalin. These sections collected in 10% neutral formalin were stained by:

a) The Nauta-Laidlaw technique (Nauta 1957) using both the phosphomolybdic acid and Uranyl Nitrate variations.

b) Both procedures I and II of the Fink-Heimer technique (Fink and Heimer 1967).

Sections collected in 2% neutral formalin were stained with the Wiitanen technique (Wiitanen 1969).

CHAPTER III

OBSERVATIONS

The data presented here are based on observations made in twenty-three adult cats processed with four silver impregnation techniques for degenerating nerve fibres. Two main types of lesions were made:

A. Inferior Olivary Nuclear Lesions

B. Non-Olivary Lesions

A. Inferior Olivary Nuclear Lesions

An electrolytic lesion was introduced in the left inferior olivary nuclear complex in twenty cats. Of these, the lesion in fourteen cats was successfully placed within the nuclear complex. The best nine of these lesions have been selected for this report. The cats survived for periods of 2-13 days post-operatively and have been divided into three sub-groups on the basis of their survival times (see table I).

The inferior olive is an elongated nuclear mass in the medulla oblongata composed of a principal olive, a medial accessory olive, a dorsal accessory olive and other smaller cellular sub-groupings (see Jansen and Brodal 1954). The extent of the lesion in each animal varied and the lesions were classified as small, moderate, large or extensive. The lesion did not involve the entire rostro-caudal extent of the nuclear mass in any of the cats. On examining the lesions in

TABLE I

Inferior Olivary Lesions

SUB-GROUP	CAT NUMBER	SURVIVAL TIME IN DAYS	PLANE OF SECTION
(a)	17	2	Parasagittal
	26	3½	Parasagittal (Right cerebellar hemisphere)
			Frontal (Vermis)
			Parasagittal (Left cerebellar hemisphere)
	27	3½	Horizontal
(b)	10	5	Frontal
	16	5	Parasagittal
	20	7½	Horizontal
	21	5	Frontal
(c)	8	11	Horizontal
	18	13	Parasagittal

transverse sections, they were seen to involve only parts of the total nuclear complex. In a few cats, all of the component cellular subdivisions were involved, as well as fibres leaving the hilus of the nucleus.

According to Brodal (1940), all cells of the inferior olive in the cat project to the cerebellum and the fibres are almost completely crossed. A unilateral lesion could possibly interrupt those fibres decussating from the contra-lateral nuclear group. It was not surprising therefore, that degenerated fibres were observed more or less bilaterally in the cerebellar cortex.

Since the main aim of this investigation was to study the mode of termination of the afferent projection to the cerebellar cortex from the inferior olive, little attempt was made to identify precisely, the lobes and lobules in which degenerated fibres were seen. Quantitatively, the amount of degeneration observed seemed to correlate more closely with the size of the lesion, than with the various survival times. In each animal degenerated fibres were observed in the white matter of the hemisphere, within the white matter of some folia and in the granular layer of these folia. The only exception was after a two day survival time (cat 17) in which no degeneration was observed anywhere in the cerebellum.

Sub-Group (a)

Cat 17 (2 day survival):

The lesion in this animal was large. It involved almost all of the component cell groups of the inferior olive. In parasagittal sections stained with each of the four techniques, no degenerated fibres were observed in the cerebellar cortex.

Cats 27 and 26 ($3\frac{1}{2}$ day survival):

In cat 27, the lesion was also large. It involved the principal olive, the dorsal accessory olive and the ventro-lateral parts of the medial accessory olive, as seen in a transverse section (Fig. 2). It also encroached on the lateral vestibulo-spinal tract. Inspite of the size of the lesion, degeneration was relatively scanty in many of the folia of the hemispheres; still less was observed in the vermis of the anterior lobe. The lesion in cat 26 was of small size and involved portions of the dorsal and medial accessory olive and the hilus of the nucleus.

Long streams of degenerated fibres were observed in the white matter of the cerebellum (Fig. 3) and could be followed into the white matter of the folia (Fig. 4), with both the Nauta phosphomolybdic acid staining procedure and the Wiitanen technique. From the folial white matter, some degenerated fibres entered the granular layer. Some of the

fibres in the basal parts of this layer continued in the longitudinal plane, parallel to the course of the fibres within the white matter. More frequently the fibres ascended through the granular layer in various directions (Fig. 5). A few fibres reach the most superficial parts of the granular layer (Fig. 6, arrows), (Fig. 7, arrow). Even though the lesion was smaller, the degeneration observed in cat 26 was considerably more marked, presumably because the lesion involved fibres at the hilus.

Degenerated fibres were observed to stain to a greater or lesser extent with the different staining methods used. In our experience, the Nauta phosphomolybdic acid modification usually brought out maximum staining of degenerated fibres in the white matter, in comparison to the Wiitanen technique. The Nauta uranyl nitrate modification was difficult to assess as the great number of normal fibres stained masked the staining of the degenerated fragments. In the granular layer, the Nauta phosphomolybdic acid method often stained a moderate amount of degenerated fibres, whereas the Wiitanen procedure was more effective in this layer. In addition, the latter was the only technique which stained degenerated fragments in the molecular layer (see non-olivary lesions). The Fink-Heimer procedure II did not always stain degenerated fibres in the granular layer. In cat 26, this technique failed to reveal any degenerated fibres, even

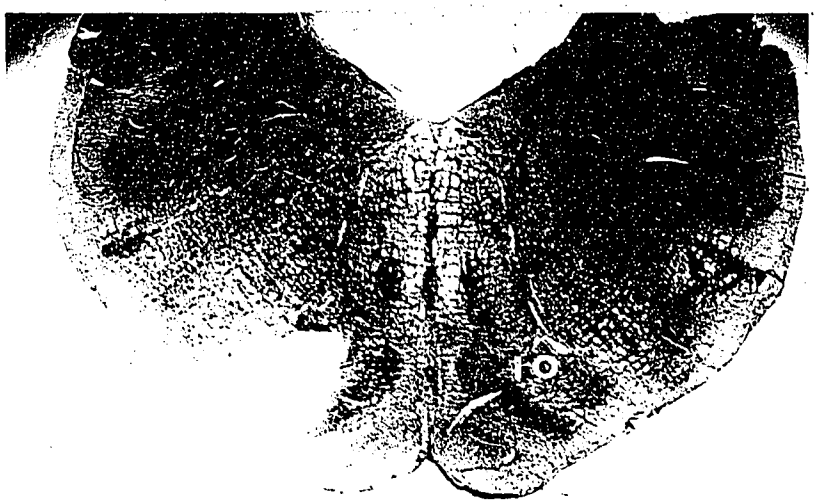

Figure 2: Brainstem of cat 27 showing lesion in the left inferior olivary nuclear complex. Nauta Phosphomolybdic, mag. 7X.

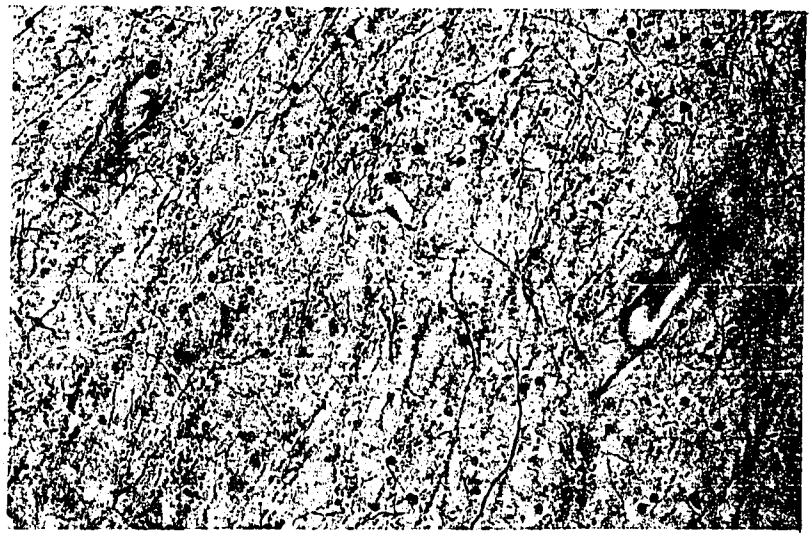

Figure 3: Degeneration in white matter at the base of a folium. Cat 26, 3½ days survival. Nauta Phospho-molybdic, mag. 240X.

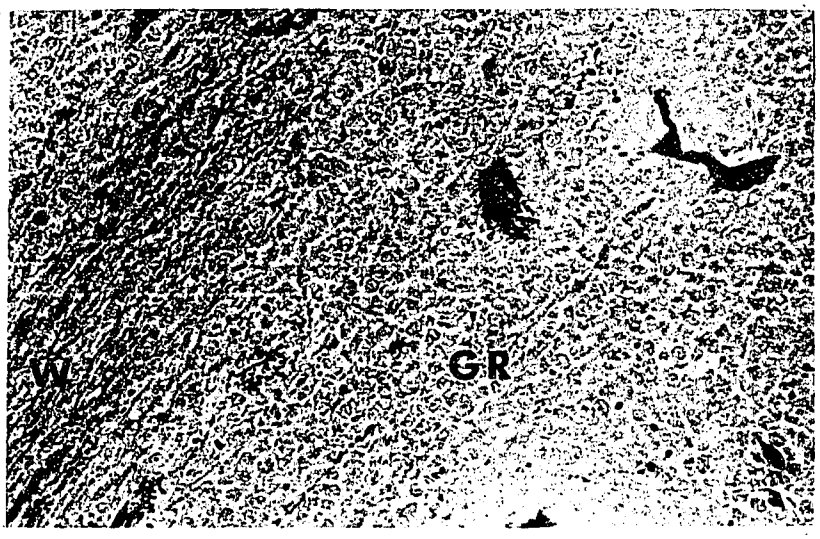

Figure 4: Degeneration in white matter and granular layer. Cat 27, 3½ days survival. Wiitanen Technique, mag. 375X.

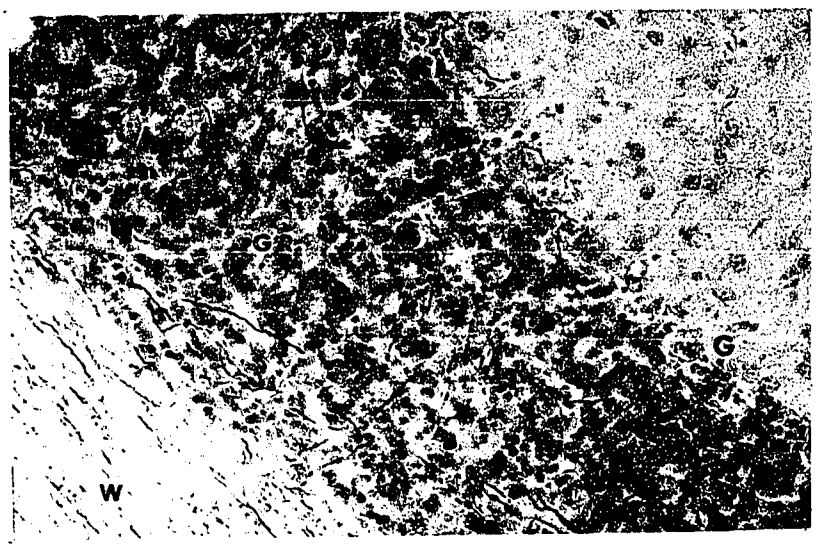

Figure 5: Degenerating fibres are seen throughout the granular layer reaching the base of the ganglionic layer. Cat 26, 3½ days survival. Nauta Phospho-molybdic, mag. 240X.

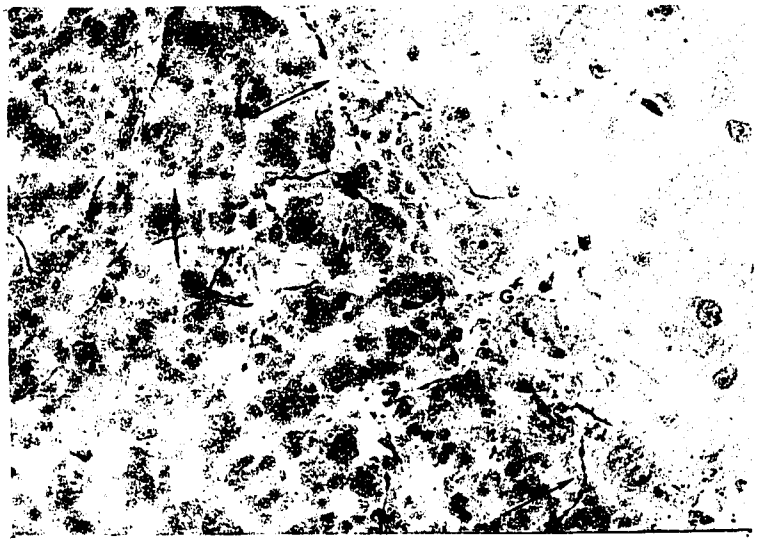

Figure 6: Higher magnification of the same material as Fig. 5 showing degeneration at the base of the ganglionic layer (arrows). Nauta Phospho-molybdic, mag. 375X.

Figure 7: Degeneration in the white matter and granular layer of the vermis. A degenerated fragment is seen in the most superficial part of the granular layer (arrow). Cat 26, 3½ days survival. Wiitanen Technique, mag. 240X.

in the white matter, where the presence of degenerated fibres had been clearly demonstrated with the Nauta and Wiitanen techniques. None of the techniques revealed any staining of degenerated fragments above the level of the Purkinje cell body (i.e. molecular layer), after inferior olivary lesions, with survival times of 2 and $3\frac{1}{2}$ days.

Sub-Group (b)

Cats 16, 21 and 10 (5 day survival):

The lesion in cat 16 was of moderate size. It involved the medial accessory olive, the dorso-medial half of the principal olive and the hilus of the nucleus (Fig. 8). An extensive lesion in cat 21 destroyed the entire left inferior olivary nuclear complex at one transverse level and encroached on the vestibulo-spinal and cortico-spinal tracts and the reticular formation. In cat 10, the lesion was large. It involved all of the principal and medial accessory olive as well as the dorso-medial portion of the dorsal accessory olive and the hilus of the nucleus.

Marked degeneration was seen in the white matter of the folia, with the Nauta (Fig. 9), Wiitanen (Fig. 10a and Fig. 10b) and the Fink-Heimer II (Fig. 11) methods. Only a few degenerated fibres could be observed in the granular layer in cat 10 (Fig. 9 and Fig. 14), with the Nauta stain, whereas in cat 16, a more significant number of degenerated

fibres was observed to spread into the granular layer from the white matter, with the Wiitanen (Fig. 10a) and Fink-Heimer II (Fig. 11 and Fig. 12) methods. The degenerated fibres assumed various directions in their course through the granular layer, but more frequently they entered and ascended in this layer at an angle perpendicular to the white matter (Fig. 10a and Fig. 13).

In some folia, a few degenerated fibres were seen to ascend through the granular layer, as far as the base of the ganglionic cell layer (Fig. 11) and in other instances, only isolated strands of degenerated fibres could be seen at this level (Fig. 15 and Fig. 16, arrows). Occasionally a degenerated fibre strand was seen to course toward the Purkinje cell layer and subsequently appeared to curve back a short distance into the granular layer (Fig. 17).

Cat 20 ($7\frac{1}{2}$ day survival):

The lesion in this animal was extensive and was similar in location and extent to the lesion obtained in cat 21. A moderate amount of very long degenerated fibres was observed in the white matter of the folia, from where they entered into and traversed almost the entire expanse of the granular layer, in Wiitanen stained sections (Fig. 18). In a Nauta preparation a few scattered long degenerated fibres were observed in the white matter and granular layer. Degenerated fragments were also seen, in the most superficial part of the granular layer (Fig. 19, arrows).

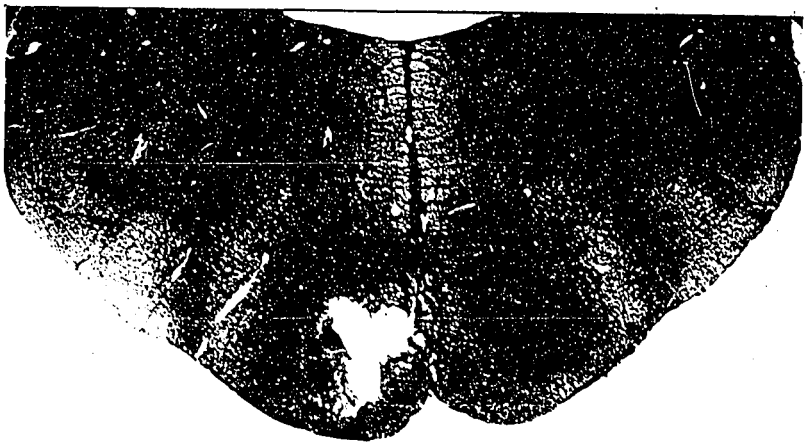

Figure 8: Brainstem of cat 16 showing a lesion in the left inferior olivary nuclear complex. Nauta Phosphomolybdic, mag. 7X.

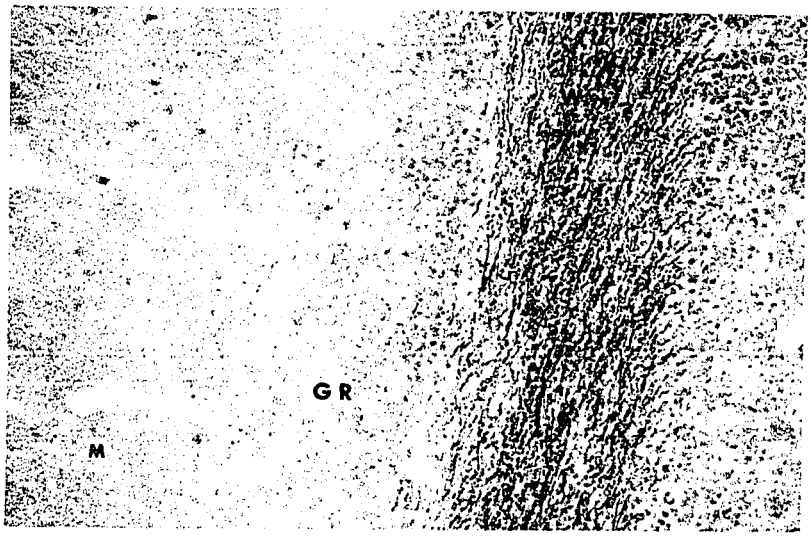

Figure 9: Abundant degeneration is seen restricted almost exclusively to the white matter of a folium. Cat 10, 5 days survival. Nauta Phosphomolybdic, mag. 150X.

Figura 10A: Degenerated fibres are seen leaving the white matter to ramify throughout the granular layer. Cat 16, 5 days survival. Wiitanen Technique, mag. 150X.

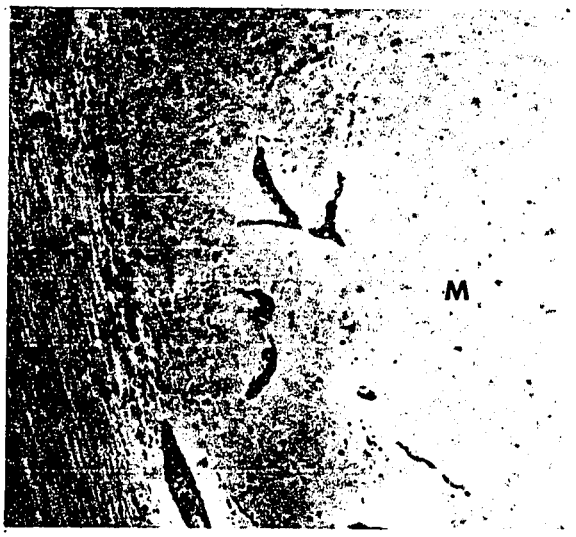

Figure 10B: Degenerated fibres are seen predominantly within the white matter. A few fragments are also seen in the granular layer. Cat 16, 5 days survival. Wiitanen Technique, mag. 240X.

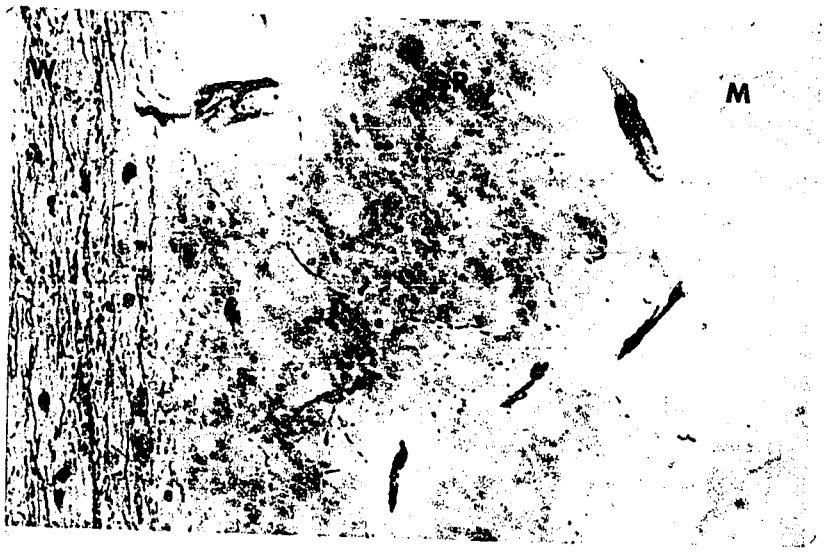

Figure 11: Degenerated fibres extending through the granular layer. Cat 16, 5 days survival. Fink-Heimer Procedure II, mag. 240X.

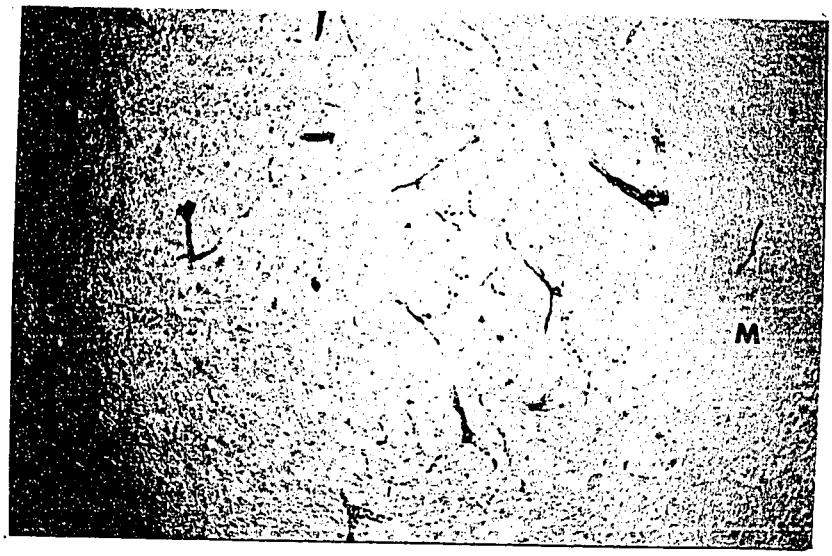

Figure 12: Degenerated fragments ramifying in the granular layer. Cat 10, 5 days survival. Fink-Heimer Procedure II, mag. 150X.

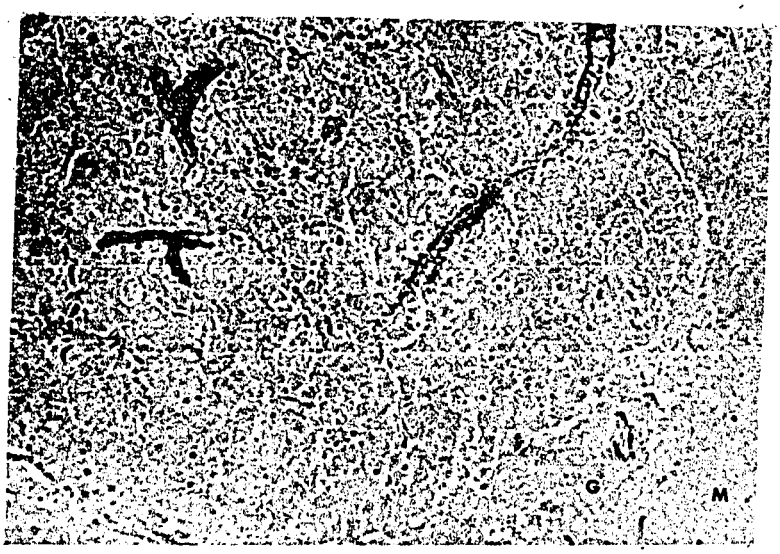

Figure 13: Degenerated fibres ascend to the midpoint of the granular layer. Cat 20, 5 days survival. Wiitanen Technique, mag. 375X.

Figure 14: Another example of fibre degeneration in the granular layer as seen with the Nauta stain. Cat 10, 5 days survival, mag. 240X.

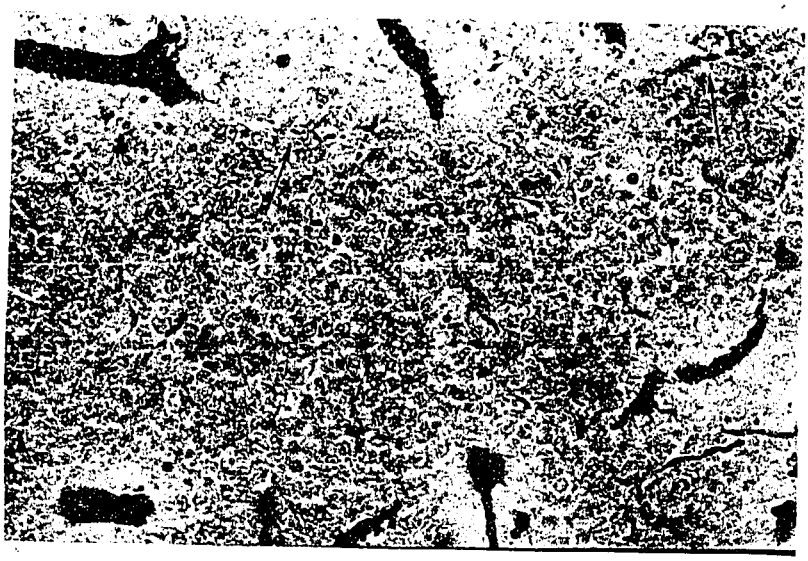

Figure 15: Degeneration can be seen to reach the base of Purkinje cell body (arrows). Cat 16, 5 days survival. Wiitanen Technique, mag. 375X..

Figure 16: A lengthy stretch of a degenerated fibre is seen coursing at the level of the ganglionic layer (arrow). Cat 21, 5 days survival. Wiitanen Technique, mag. 240X.

Figure 17: A degenerated fibre is seen to course toward the Purkinje cell layer and subsequently appears to curve back into the granular. Cat 21, 5 days survival. Wiitanen Technique, mag. 375X.

Figure 18: Abundant degeneration is seen at the top of the folium. The fibres leave the white matter extend throughout the granular layer to reach the base of the ganglionic layer. Cat 20, 7½ days survival. Wiitanen Technique, mag. 240X.

Figure 19: Degenerated fragments at the level of the Purkinje cell body (arrows). Cat 20, 7½ days survival. Nauta Phosphomolybdic, mag. 375X.

There was usually less degeneration observed in the ipsi-lateral cerebellar cortex, than in the contra-lateral side, except in animals in which the lesion had involved the fibres at the hilus of the nucleus, or presumably those decussating from the contra-lateral nuclear group. The fibres seemed to be unbranched and generally followed a winding course through the granular layer. No degeneration was obtained in the molecular layer, with any of the staining techniques used, following lesions in the inferior olivary nucleus, at survival times of five and seven and a half days.

Sub-Group (c)

Cats 18 and 8 (13 and 11 day survival respectively):

The lesion in cat 18 was extensive. It destroyed all of the component cellular groupings of the nucleus, the fibres at the hilus and encroached on the lateral vestibulo-spinal tract at one transverse level (Fig. 20). There was also an extensive lesion in cat 8. It involved all of the left inferior olivary nucleus, the fibres at the hilus and part of the pyramidal tract on that side.

Inspite of the long survival times, pronounced degeneration was observed in the white matter of the folia in these animals, in the Nauta phosphomolybdic acid, the

Fink-Heimer II (Fig. 22) and the Wiitanen techniques (Fig. 21). In the former two techniques, however, an excessive staining of normal fibres masked clear visibility of the degenerated fibres (Fig. 22). At the top and the sides of the folia degenerated fibres were seen to splay into the granular for various distances, with the Wiitanen (Fig. 21) and Fink-Heimer II (Fig. 23) techniques. A few scattered degenerated fibres were observed in the granular layer, as far as the base of the Purkinje cell bodies, with the Fink-Heimer II (Fig. 22) and Nauta phosphomolybdic (Fig. 24) techniques. At these survival times of thirteen and eight days degeneration of fibres in the molecular layer, following inferior olivary lesions, could not be demonstrated with the various staining methods used.

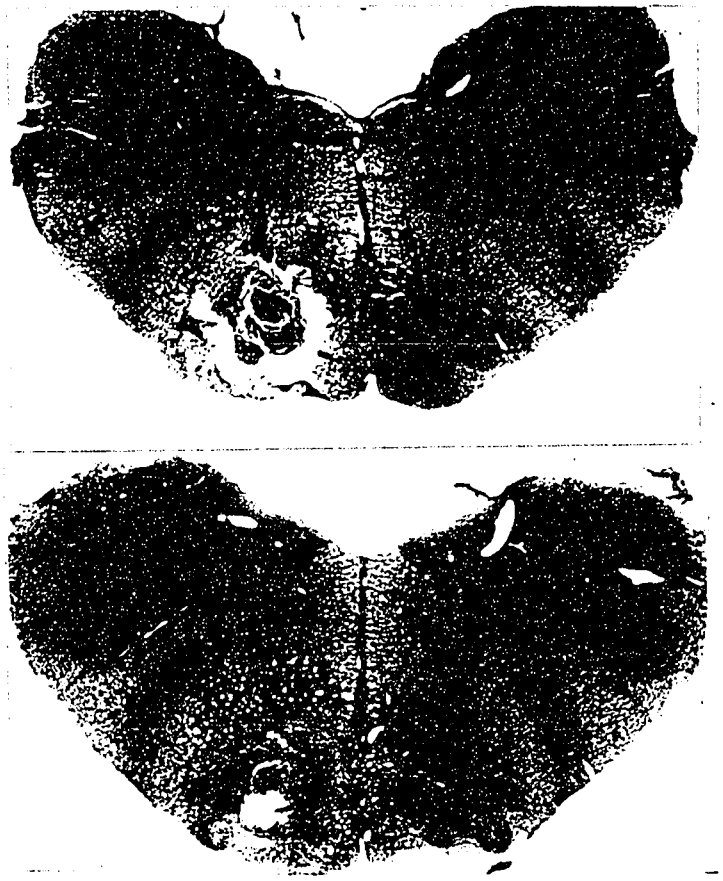

Figure 20: An extensive electrolytic lesion in the left inferior olivary nuclear complex. Cat 18, 13 days survival. Nauta Phosphomolybdic, mag. 7X.

Figure 21: Degenerated fibres leaving the white matter to enter the granular layer at the top of a folium. Cat 18, 13 days survival. Wiitanen Technique, mag. 240X.

Figure 22: Degenerated fibres coursing randomly in the granular layer. Cat 8, 11 days survival. Fink-Heimer Procedure II, mag. 240X.

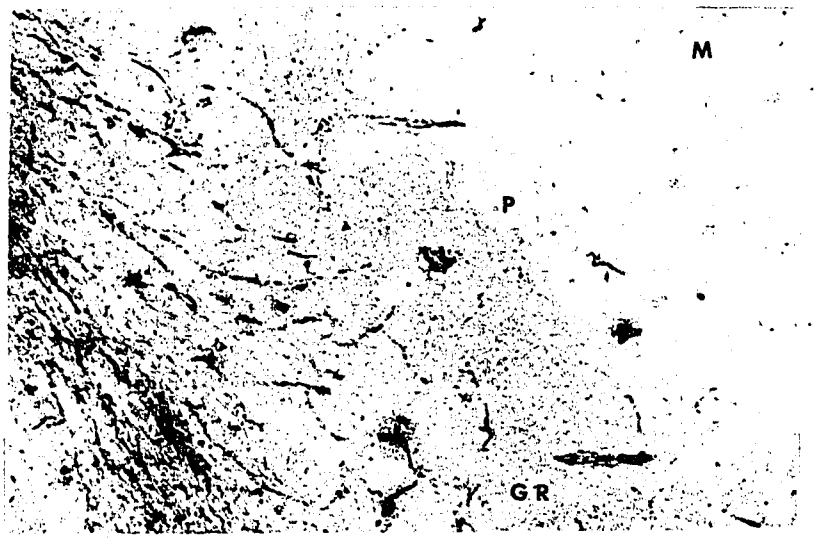

Figure 23: Degenerated fibres ascending in the granular layer. Cat 18, 13 days survival. Fink-Heimer Procedure II, mag. 240X.

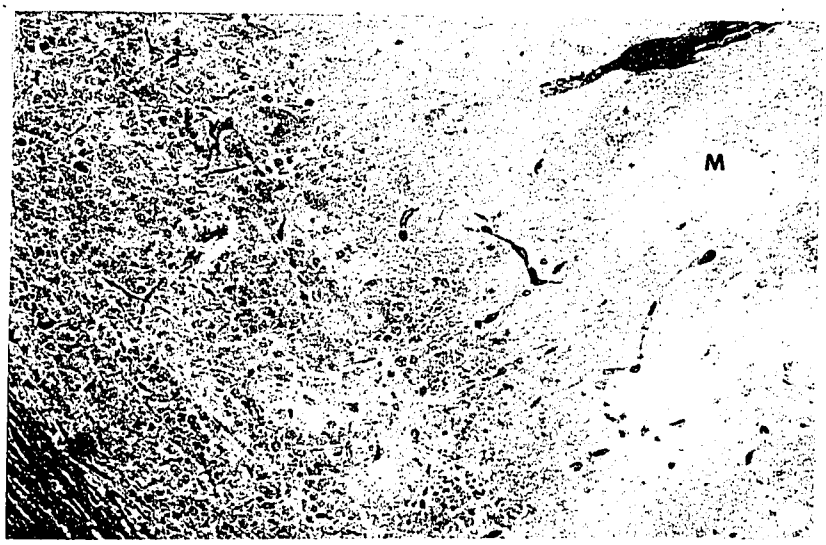

Figure 24: Degenerated fragments seen in the outer part of the granular layer. Cat 18, 13 days survival. Nauta Phosphomolybdic, mag. 240X.

B. Non-Olivary Lesions

The results obtained following lesions in the inferior olivary nuclear complex failed to show any evidence of climbing fibre degeneration in the molecular layer of the cerebellar cortex. In an attempt to investigate these negative findings further, on the premise that either the inferior olive does not project as climbing fibres, or that the fibres simply cannot be stained in the molecular layer by the current techniques, it was decided to interrupt these fibres within the cerebellar cortex. Lesions were made in the white matter of the cortex where the two afferent systems to the cerebellum, the mossy and climbing fibre systems would be interrupted.

Two types of lesions were made (see table II): A) electrolytic lesions in the white matter by an ipsi-lateral and a contra-lateral approach; B) surgical lesions in the cortex. A third type of lesion was done; C) section of the spino-cerebellar tract. This third lesion was made to serve as a means of comparison between the type of degeneration obtained after olivary and cerebellar lesions (non-olivary lesions) and that obtained after interruption of the spino-cerebellar system coursing in the lateral funiculus of the spinal cord. It has been well established that the spino-cerebellar fibre system terminates as mossy fibres in the cerebellar cortex (Grant 1962), (Brodal and Grant 1962).

TABLE II

Non-Olivary Lesions

GROUP	TYPE OF LESION	CAT NUMBER	SURVIVAL TIME IN DAYS	PLANE OF SECTION
A	Electrolytic lesion in white matter:			
	Ipsi-lateral approach	28	3½	Horizontal
		29	4½	Parallel to the longitudinal axes of folia
	Contra-lateral approach	32	3½	Horizontal
		33	3½	Horizontal
		34	7½	Horizontal
		35	12	Horizontal
B	Surgical lesions:			
	Knife punctures	31	3½	Parallel to the longitudinal axes of folia involved
	Isolated folia	25	5	Parasagittal
C	Section of the spino-cerebellar tract	30	3½	Parasagittal

Two approaches were used in making the electrolytic lesions in the white matter. In the first approach (cats 28 and 29), the electrode was introduced through the cortex on the same side in which the lesion was made. This procedure created local traumatic reactions in the molecular layer of the folia involved (see below). A contra-lateral approach was used in the subsequent animals (cats 32-35) to avoid this complication.

Cat 32 and 33

In cat 32 (Fig. 25), the lesion was not properly located in the white matter. It was placed at the base of two folia where it also damaged the cortical layers in these folia. A more successfully located lesion was obtained in cat 33 (Fig. 26). The lesion here was located predominantly in the white matter of the hemisphere. A slight encroachment on the cortical layers near the base of one folium was observed.

The folia selected for study were those in which degeneration was seen in the white matter leading from the lesion. In such folia, the cortical layers adjacent to and distal to the lesion were examined.

In locations adjacent to the lesion, both the granular and molecular layers showed abundant degeneration. In the molecular layer the argyrophilic fragments were displayed in columns of varying thickness which extended across the whole width of that layer. Each column appeared to be composed

of coarse degenerated fragments and each column was generally of uniform size in its entire length from the deep parts of the molecular layer to the pial surface (Fig. 27). Abutting on these columns and sometimes even extending through them, a second type of molecular layer degeneration was observed in areas still adjacent to the lesion. This was composed of fine grains of degenerated fragments that appeared to run in a plane parallel to the longitudinal axis of the folium in folia that were sectioned along this axis (Fig. 27 and Fig. 28). In folia that were not sectioned along their longitudinal axes, these fine degenerated fragments were seen to be randomly dispersed throughout the molecular layer (Fig. 29). These fine degenerated fibres extended for various distances and gradually disappeared from the molecular layer in locations farther removed from the lesion. This degeneration also extended further from the site of cortical damage than the columns (Fig. 27). A third type of degenerated fibres was observed in the molecular layer. These appeared as coarse fragments that ran for various distances in a plane parallel to the longitudinal axis of the folium and were located in the deep stratum of the molecular layer, above the Purkinje cell bodies (Fig. 29, arrow). This is the location and orientation of the supraganglionic plexus. The fragments were also seen only in areas of the cortex adjacent to the lesion.

In locations more distal to the lesions, degenerated

fibres were observed in the white matter of the folium and the granular layer, but no degeneration of any of the three types mentioned above was seen in the molecular layer (Fig. 30 and Fig. 31). In figure 30, some of the degenerated fragments in the granular layer appeared to be in small clumps. These clumps could be due to mossy fibre terminal degeneration as the mossy fibre system was also interrupted in these lesions. Brodal and Grant (1962) mentioned this type of degenerating clumps in their study of the spino-cerebellar system. On the other hand, figure 31 shows predominantly longer degenerated fragments which suggest that they may be fibres of passage through the granular layer.

Since there was only minimal electrolytic damage to the cortex in cat 33 no degenerating elements were seen in the molecular layer in most of the folia examined (Fig. 32 and Fig. 33), except in the small portion of the folium to which the damage was done.

Degenerated fibres were seen coursing in the white matter of the folia and in the granular layer (Fig. 32 and Fig. 33). A few degenerated fragments reached the base of the Purkinje cell bodies (Fig. 33).

Cats 28 and 29, already mentioned, will be reported on later. Cats 34 and 35 had lesions introduced from a contra-lateral approach, similar to cats 32 and 33, but their survival times were seven and twelve days respectively and

Figure 25: An electrolytic lesion made from a contralateral approach and located at the base of two folia. Arrows indicate location of areas represented in subsequent figures. Frontal plane. Cat 32, 3½ days survival. Nauta Phosphomolybdic, mag. 7X.

Figure 26: An electrolytic lesion made from a contralateral approach and located in the white matter of the hemisphere. Horizontal plane. Cat 33, 3½ days survival. Nauta Phosphomolybdic, mag. 7X.

Figure 27: Argyrophilic columns which stretch across the molecular layer in an area immediately adjacent to the lesion (arrow 1, Fig. 25). Fine degenerated fibres can also be seen running at right angle to these columns along the longitudinal plane of the folium. Cat 32, 3½ days survival. Wiitanen Technique, mag. 240X.

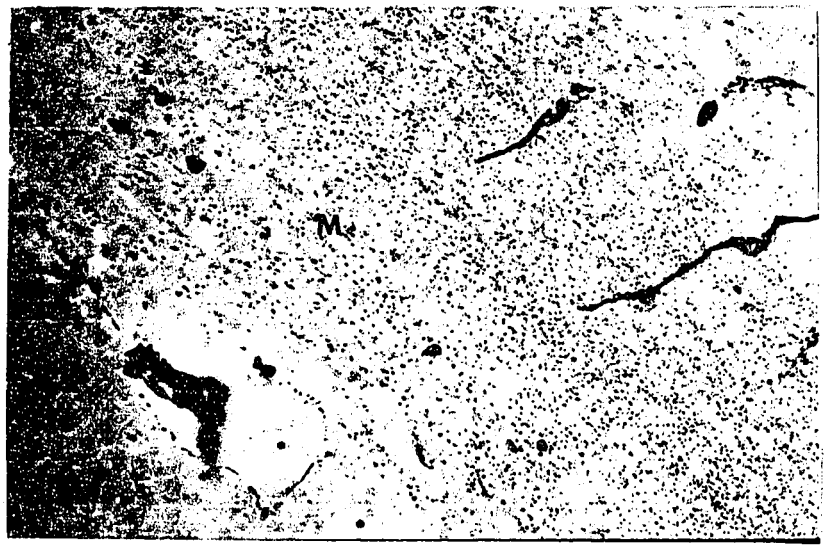

Figure 28: Higher magnification of fine longitudinally running fibres seen in Fig. 27. Cat 32. Wiitanen Technique, mag. 375X.

Figure 29: Fine degenerated fibres in the molecular layer of another folium, from a location proximal to the lesion (arrow 2, Fig. 25). Degenerated fibres can also be seen coursing in a longitudinal plane in the supraganglionic plexus (arrow). Cat 32, 3½ days survival. Wiitanen Technique, mag. 240X.

Figure 30: Degenerated fibres in the granular layer. This photograph is taken from an area quite distal to the lesion (arrow 3, Fig. 25). Note that no degeneration of any kind is seen in the molecular layer. Cat 32, 3½ days survival. Wiitanen Technique, mag. 150X.

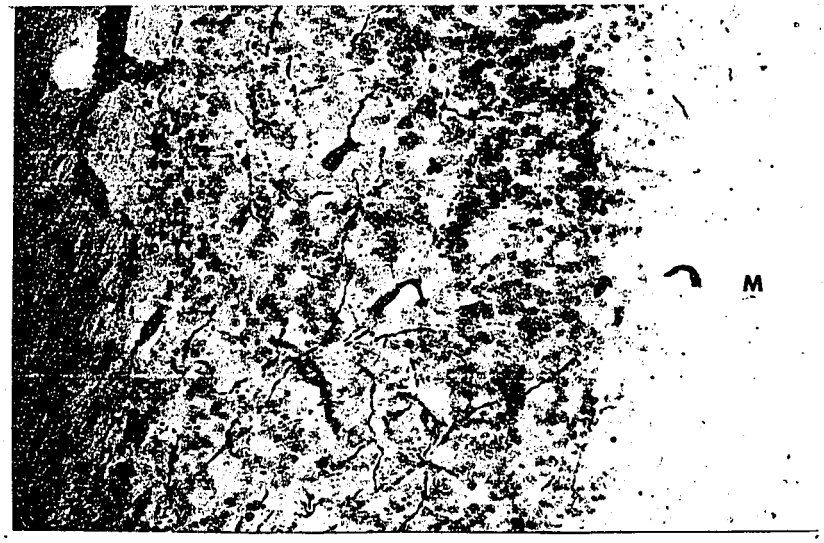

Figure 31: Another area distal to the lesion, showing granular layer degeneration at higher magnification. Cat 32, Wiitanen Technique, mag. 240X.

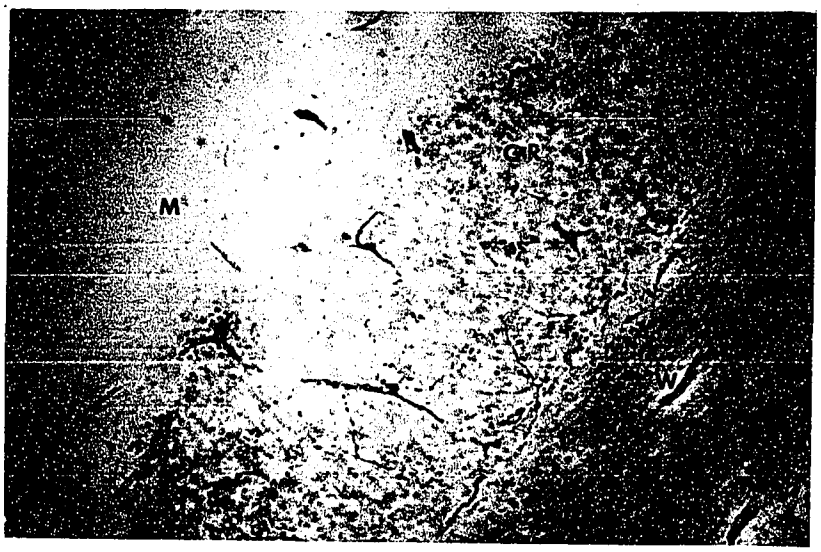

Figure 32: Degenerated fibres in the granular layer distal to the lesion as seen with the Nauta Phospho-molybdic acid stain. Cat 33, 3½ days survival, mag. 150X.

Figure 33: Degenerated fibres coursing in the white matter (bottom left of picture). Some of the fibres ascend perpendicularly into the granular layer, others take diverse courses. Cat 33, 3½ days survival. Wiitanen Technique, mag. 375X.

will also be reported on later.

The molecular degeneration observed in cat 32, namely, the argyrophilic columns, the fine parallel-running degeneration and the coarse degeneration in the supraganglionic layer, prompted further investigation of these observations. The question was raised as to whether these argyrophilic fragments and degenerated fibre types were indeed due to damage caused in the cortical layers. It was decided that a lesion involving only the cortical layers and not interrupting the afferent fibre systems, running in the white matter of the hemispheres, might clarify this question. On this basis, surgical lesions were made by knife punctures to the cerebellar cortex in one cat (cat 31). The intention was to nick only the molecular layer but as can be observed, the lesions extended deeper into granular layer (Fig. 34, double arrows). However, there seemed to be no white matter involvement.

The results obtained following these lesions were similar to those described for cat 32 in the areas adjacent to the electrolytic lesion. In these areas, the argyrophilic columns were again observed in the molecular layer (Fig. 35 and Fig. 36). Some degenerated fibres were seen in the granular layer in figure 35, but these were apparently due to the fact that the granular layer had also been lesioned. The fine dust-like particles seen among these degenerated fibres in the granular layer in figure 35 are artifacts,

frequently obtained with the Wiitanen technique. They are not present in the granular layer in figure 36. Degenerated fibres were also absent in the granular layer in this figure. In the molecular layer, fine parallel-running degenerated fibres were again observed in this cat, located just beyond the margin of the columns. In addition the coarse type of degenerated fibres in the supraganglionic plexus was observed (Fig. 37). Argyrophilic columns were not necessarily observed superficial to this supraganglionic layer degeneration, though one such column could be seen in the molecular layer in figure 37, suggesting that these three types of stained elements were occurring independently of each other.

In cats 28 and 29, in which an ipsi-lateral stereotaxic approach was used, the lesion was considerably displaced (Fig. 38), being located not in the white matter of the hemisphere, but between two folia. It involved the cortical layers in these folia and extended into the white matter of folium b (seen in Fig. 38). The lesion in cat 29 could not be visualized as the cerebellar hemisphere was sectioned in a plane parallel to its folia. In these two animals, three factors had to be accounted for in interpreting the results; the electrode tract which penetrated through the folia to be studied, the involvement of the white matter of folium b and the involvement of the cortical layers in both folia a and b

(Fig. 38) due to the lesion. Because of all these factors, the assessment of the results was dependent upon more specific lesions in the white matter (cats 32 and 33) and in the molecular layer (cat 31).

Extensive degeneration was observed in the white matter and granular layers in both folia a and b, in locations adjacent to the lesion. A little farther removed from the lesion, towards the top of the folium, the degeneration in the granular was not as profuse though quite evident (Fig. 39), with a few degenerated fragments reaching the superficial portion of this layer. A degenerated fibre strand could be seen in the supraganglionic layer in this figure also. No other evidence of degeneration was observed in the molecular layer, in similar locations that were distal to the lesion.

In locations adjacent to the lesion, in both folia, in cat 28 and also in cat 29, argyrophilic columns and degenerated fibres were again observed in the molecular layer. The argyrophilic columns could be seen even at low magnification (Fig. 38, unnumbered arrows). These columns could be seen with little or no degeneration occurring in the subjacent granular layer (Fig. 40) and in other instances, the granular layer below the columns showed profuse degeneration (Fig. 41).

The lesions in cats 34 and 35 were also made by a contra-lateral approach. In each cat, it was located in the white matter of the hemisphere, but in cat 35, there was

Figure 34: Shows the lesion in cat 31 (double arrows), made by a surgical incision in the cortex. The single arrows will be referred to in subsequent figures. Sectioned in the longitudinal plane of the folium lesioned. 3½ days survival. Wiitanen Technique, mag. 7X.

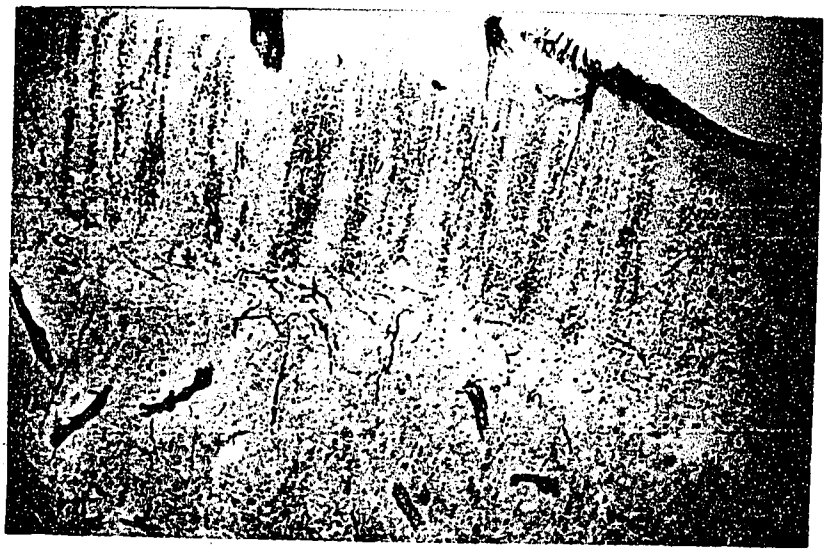

Figure 35: Argyrophilic columns seen extending across the molecular layer. Taken at a location proximal to the lesion (arrow 4, Fig. 34). Note the uniformity along the whole extent of each column. Cat 31, 3½ days survival. Wiitanen Technique, mag. 150X.

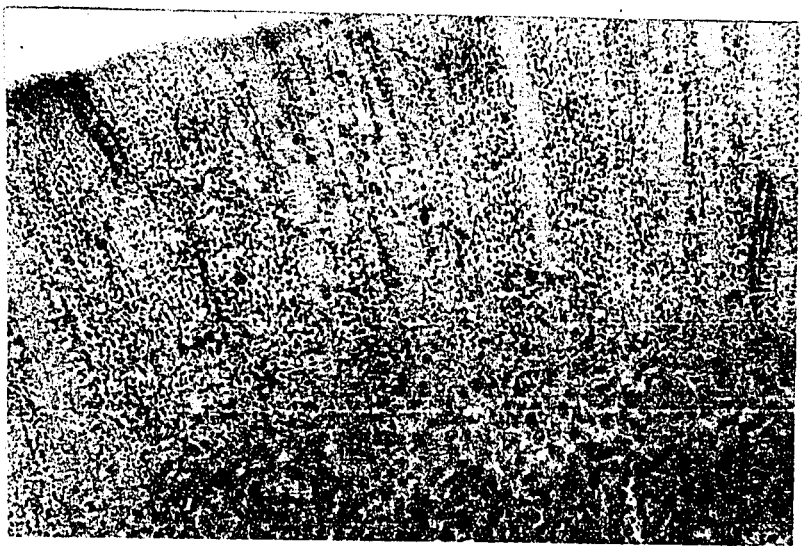

Figure 36: Higher magnification of argyrophilic columns in the molecular layer. Cat 31, 3½ days survival. Wiitanen Technique, mag. 240X.

Figure 37: Degenerated fibres coursing in the supraganglionic plexus. A degenerated column is seen in the molecular layer (location at arrow 5, Fig. 34). Cat 31, 3½ days survival. Wiitanen Technique, mag. 375X.

Figure 38: An electrolytic lesion made by an ipsi-lateral approach and located between two folia. It involved the cortical layers and encroached on the white matter in folium b. The degenerated columns can be seen at this low magnification (unnumbered arrows). Cat 28, 3½ days survival. Wiitanen Technique, mag. 7X.

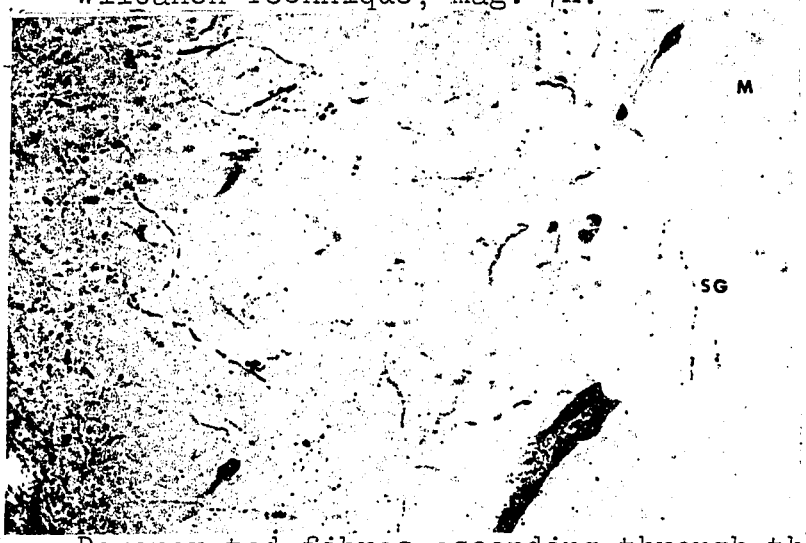

Figure 39: Degenerated fibres ascending through the granular layer to reach the base of the Purkinje cell body. Taken from an area lateral to the lesion (arrow 6, Fig. 38). A degenerated fibre strand is seen in the supraganglionic layer. Cat 28, 3½ days survival. Wiitanen Technique, mag. 240X.

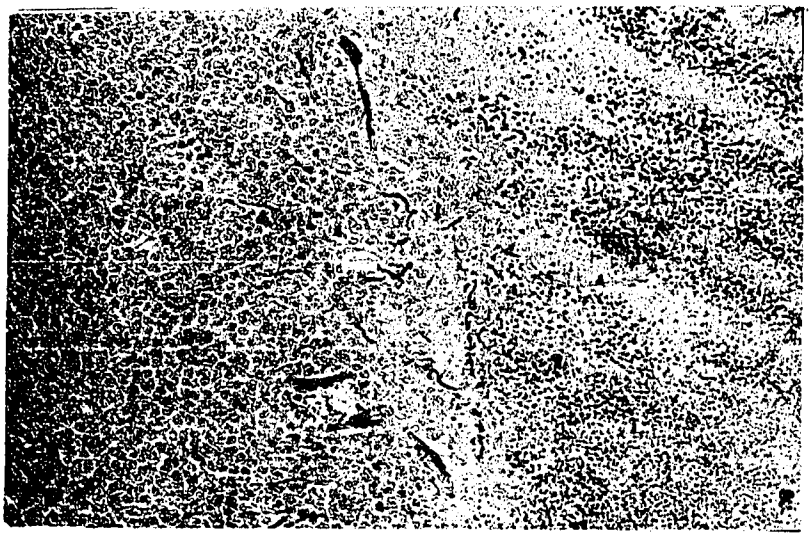

Figure 40: Argyrophilic columns (seen at unnumbered arrows in Fig. 38) in the molecular layer proximal to the lesion. A few degenerated fibre strands can be seen in the granular layer. Cat 28, 3½ days survival. Wiitanen Technique, mag. 240X.

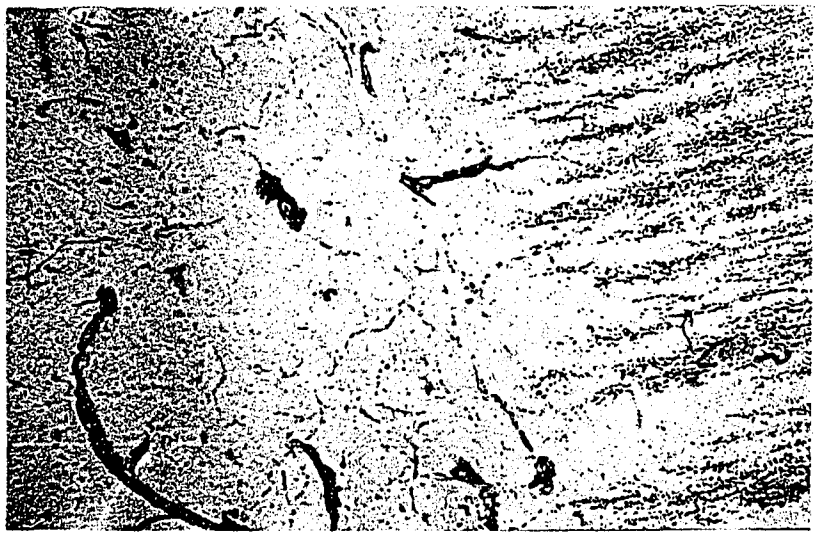

Figure 41: Argyrophilic columns seen in the molecular layer in cat 29 which had a similarly located lesion as cat 28. These columns can be seen with or without granular layer degeneration. Profuse fibre degeneration is observed in the granular layer here, (compare with Fig. 40). Cat 29, 4½ days survival. Wiitanen Technique, mag. 240X.

also a slight involvement of the cortical layers at the base of one folium. The survival times were seven and twelve days respectively. Degenerated fibres were observed in the white of the folia located distal from the lesion and in the granular layer. In the granular layer of cat 34, degenerated fibres were seen to course in all directions (Fig. 42 and Fig. 43), and a few fibres reach the most superficial part of the granular layer (Fig. 43, arrows). A similar display of degenerated fibres was observed in the granular layer in cat 35, approaching the base of the ganglionic layer even in a Nauta-stained section (Fig. 44).

No degeneration was seen in the molecular layer in these animals (Fig. 42,43,44 and 46), except in the folium in which there was some damage to the cortical layer. Adjacent to the lesion in this folium, a few argyrophilic columns were present (Fig. 45) in the molecular layer and there was evidence of some degeneration in the supraganglionic plexus also (Fig. 45).

Another method by which the afferent fibre systems coursing in the white matter of the cerebellar hemispheres could be interrupted was by surgically undercutting some folia. This procedure was used in making the lesion in cat 25, which survived for five days. The lesion extended from the superior to inferior surfaces (Fig. 47, arrow).

The results obtained with this lesion were similar to those described in the preceding lesions. Molecular layer degeneration was observed in folia which had been damaged by the lesion. This consisted of columns, fine fragments dispersed in the molecular layer and degenerated fibres in the supraganglionic layer. In one folium (Fig. 47, arrow 7) which appeared to have no cortical damage, a molecular layer degeneration was absent. The white matter of this folium and the granular layer showed degenerating fibres (Fig. 48), some of which ascended up to the Purkinje cell body layer.

As a result of the various types of lesions, the degenerating elements observed in the molecular layer particularly with the Wiitanen technique, may be interpreted at this point. The coarse degeneration in the supraganglionic plexus is the consequence of interruption of fibres by a lesion involving this plexus, or fibres entering this plexus. The finely dispersed degeneration sometimes seen coursing in the plane of the longitudinal axis of the folium is thought to be due to parallel fibres, when these axons are interrupted by the lesion. The third type, namely the columns, are attributed to staining of glial elements. Since this event occured in areas which were traumatized, it could be considered that a glial reaction, induced by the trauma, allowed staining of a non-neuronal component. It should be emphasized that climbing fibre degeneration could not be identified as a result of these various lesions.

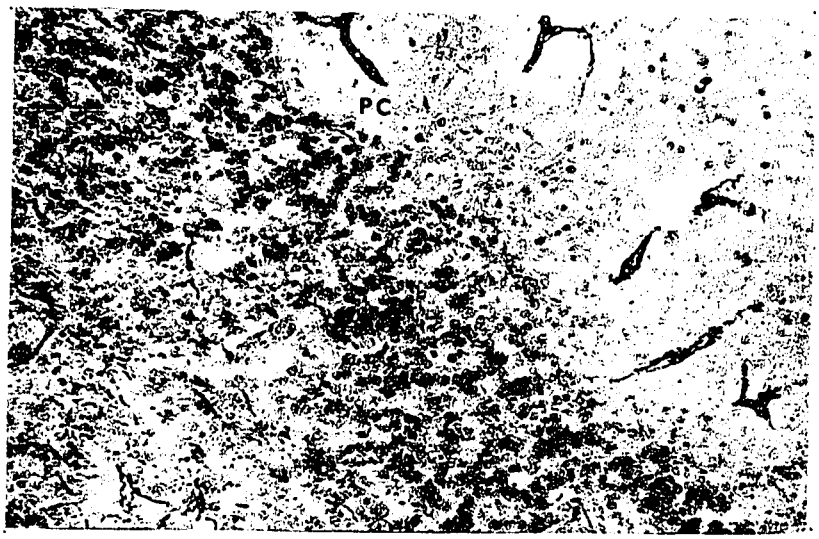

Figure 42: Degenerated fibres spread in various directions in the granular layer. A few degenerated fibres reach the base of the Purkinje cell body. Cat 34, 7 days survival. Wiitanen Technique, mag. 240X.

Figure 43: Another example of granular layer degeneration at a lower magnification to show the profuse amount of these degenerated fibres. Degenerated fibre strands can be seen to partially surround the base of a few Purkinje cell bodies (arrows). Cat 34, 7 days survival. Wiitanen Technique, mag. 150X.

Figure 44: Degenerated fibres spreading into the granular from the white matter. Some degenerated fragments reach the ganglionic layer. Cat 35, 12 days survival. Nauta Phosphomolybdic, mag. 240X.

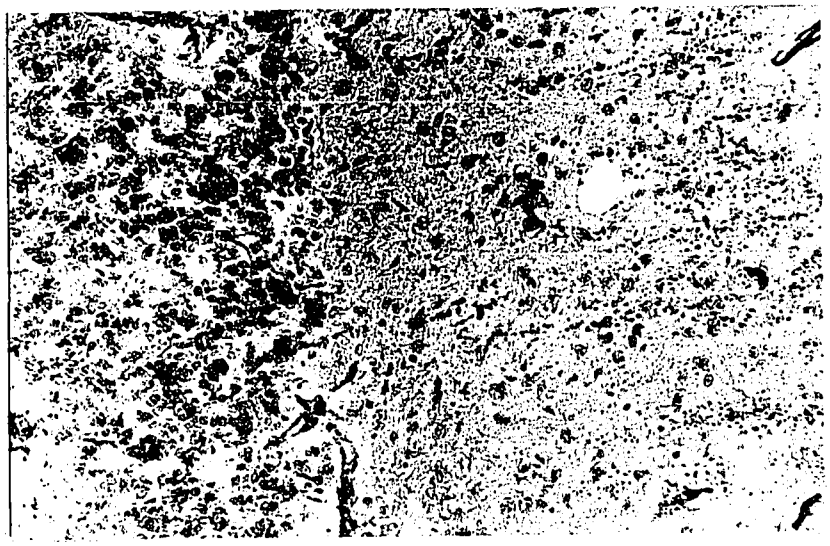

Figure 45: A few argyrophilic strands are observed in the molecular layer of one folium. The lesion in this animal presumably encroached very slightly on the cortex in this folium. Cat 35, 12 days survival. Wiitanen Technique, mag. 240X.

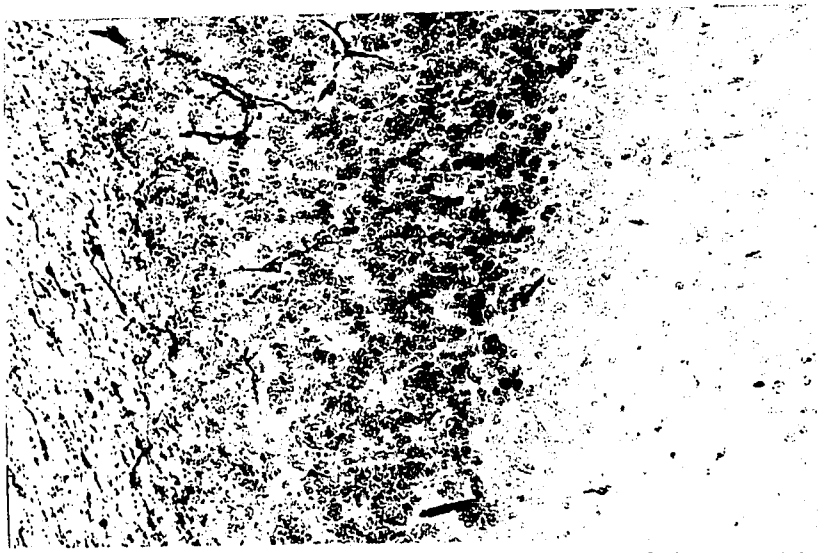

Figure 46: Degeneration can be seen in the white matter and granular layer. No degeneration is seen in the molecular layer. Cat 35, 12 days survival. Wiitanen Technique, mag. 240X.

Figure 47: A parasagittal section showing the surgical lesion made by the technique of under cutting (arrow). Cat 25, 5 days survival. Nauta Phosphomolybdic, mag. 7X.

Figure 48: Degenerated fibres in the granular layer at the top of a folium (arrow 7, Fig. 47). Cat 25, 5 days survival. Fink-Heimer Procedure II, mag. 240X.

A lesion was placed in the lateral funiculus of the spinal cord just rostral to the first cervical nerve in cat 30 (Fig. 49), thereby interrupting the spino-cerebellar fibre system. Following this lesion, profuse degeneration was observed throughout the white matter and granular layer of the anterior lobe of the cerebellar cortex (Fig. 50 and Fig. 51). By careful examination of the stained sections, evidence of branching axons could be seen in the granular layer. Since this occured at different focal planes in the section, a photographic example could not be obtained. These findings were consistent with those of other authors (Brodal and Grant 1962; Grant 1962) in studies of mossy fibres and terminals. No degeneration was seen in the molecular layer.

Figure 49: A transverse section in the lateral funiculus of the spinal cord just rostral to the first cervical nerve. Cat 30, 3½ days survival. Nauta Phosphomolybdic, mag. 4X.

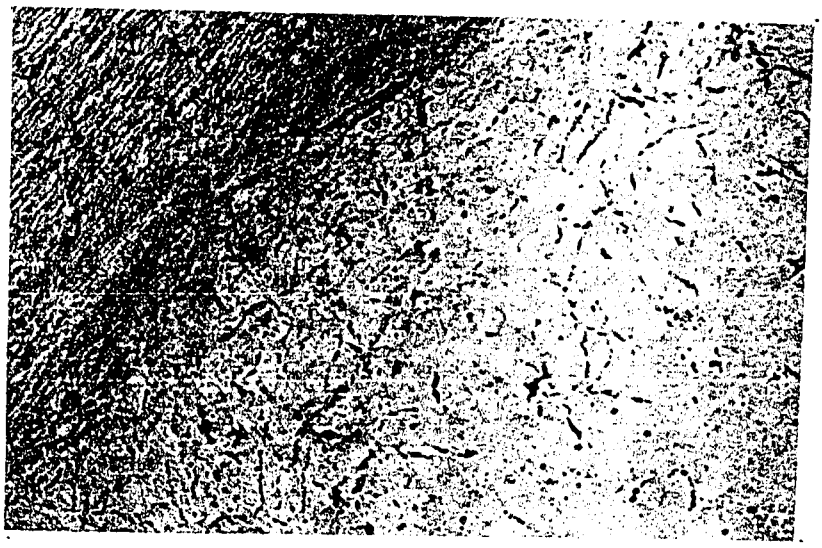

Figure 50: Degenerated fibres in the white matter. Abundant degeneration is also seen in the granular layer in the cortex of the anterior lobe. Parasagittal plane. Cat 30, 3½ days survival. Wiitanen Technique, mag. 240X.

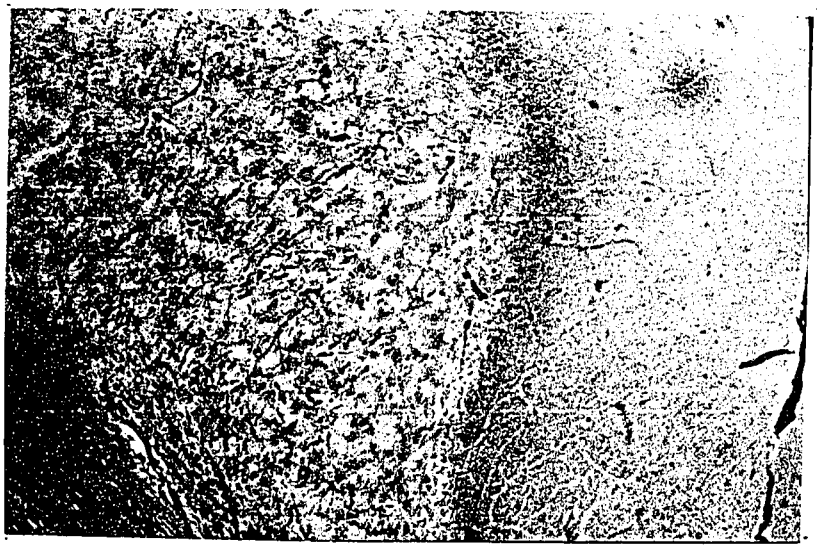

Figure 51: Degenerated fibres are seen to branch from the white matter and ascend perpendicularly through the granular layer. The degeneration does not quite reach the base of the Purkinje cell bodies. Cat 30, 3½ days survival. Wiitanen Technique, mag. 150X.

Deep Cerebellar Nuclei

In addition to the cortical projection, the cerebellar nuclei were also examined for evidence of degeneration, following the inferior olivary lesions. Moderate to marked degeneration was observed in different parts of the cellular groups, with all the survival periods, except in the case of the two day survival time (cat 17), in which no degeneration was observed. Degeneration was observed bilaterally, but the contra-lateral projection was usually more pronounced.

No special attempt was made, using cell stains, to identify the nuclear components precisely. i.e. Lateral (dentate), intermediate (interposital), medial (fastigial) nuclei. In horizontal sections, each of the three nuclear masses could be visualised, but in parasagittal planes these cell groups could not be identified with certainty. Heavily degenerated streams of fibres were observed coursing around and between the nuclear masses. From these fascicles, degenerated fibres were sometimes seen to branch into the adjacent nuclear mass.

In the group of animals with a $3\frac{1}{2}$ day survival period (cats 26 and 27), degeneration was observed in all three nuclei. In cat 27 many degenerated fibres of passage were seen coursing through the cell groups of the contra-lateral hemisphere with the Nauta technique. Degenerated fibres and pre-terminals were observed mainly among the cell bodies of the lateral nucleus, with the Wiitanen technique,

but the intermediate and medial cell groups also showed some evidence of degeneration. In cat 26 degeneration was more marked in the fascicles coursing around and within the nuclear groups than in cat 27. The nuclear components could not be identified in this animal because of the plane of section (see table I). In a section of the vermis however, which presumably contained the medial nucleus and possibly portions of the intermediate nucleus, a few degenerated fragments were seen. In general there was less degeneration observed in these cell groups than was observed in sections of the lateral part of the cerebellum. In one nuclear mass of the contra-lateral hemisphere, only part of the cell group showed degenerated fibres and pre-terminals and possibly terminal degeneration (Fig. 52), the other part of this group being conspicuously free of any degenerated fragments.

In cats 10, 16 and 21, each with 5 day survival times, there was also clear evidence of degenerated fibres of passage, pre-terminals and terminals, in the cerebellar nuclei. Degenerated fibres were seen to come in close contact with the cell bodies of the nuclear neurons and often surrounded them (Fig. 53). Degeneration was also more heavily concentrated in the lateral nucleus than in the other nuclear groups. In cat 16, straight unbranched degenerated fibres were observed coursing between the nuclei. A few fibres could be seen ramifying among the cells (Fig. 54).

Similar results were obtained in cat 21. Degenerating fibres were again more numerous in the lateral nucleus of the contralateral hemisphere in this animal. The observations made in cat 20, which survived for 7½ days, were not significantly different from those described above.

In the two animals with longer survival times (cats 18 and 8), degeneration was very profuse in the deep nuclei. The lateral cell group again showed the predominant amount of degenerated fibres (Fig. 55), although a fair number were seen in the medial nucleus and still some in the intermediate nucleus.

The deep cerebellar nuclei were also examined in animals with non-olivary lesions. Following the interruption of the spino-cerebellar fibre system, degeneration was observed in these nuclei (Fig. 56). Degenerated fibres were also observed in the cerebellar nuclei, in animals which had white matter lesions (cat 32-35). This degeneration was due to the interruption of Purkinje axons which project to the cerebellar nuclei.

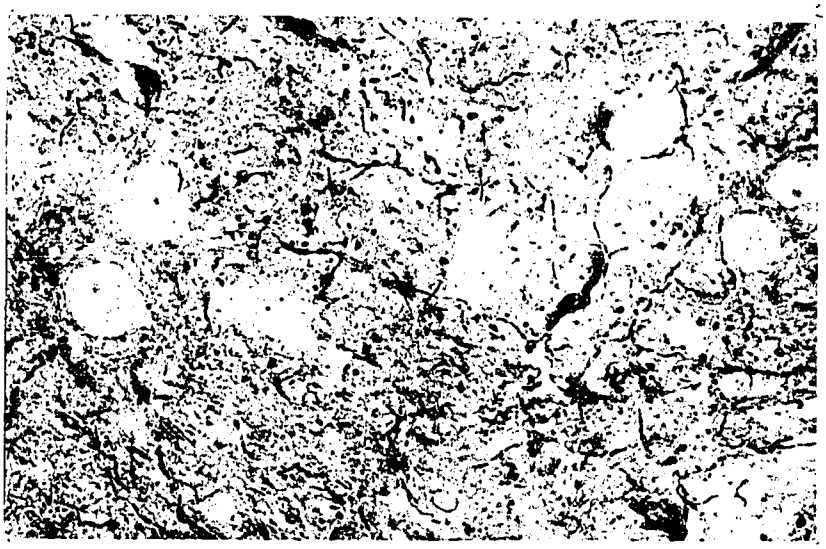

Figure 52: Preterminal and possibly terminal degeneration seen in the lateral (dentate) cerebellar nuclear group. Inferior olivary lesion. Cat 26, 3½ days survival. Wiitanen Technique, mag. 375X.

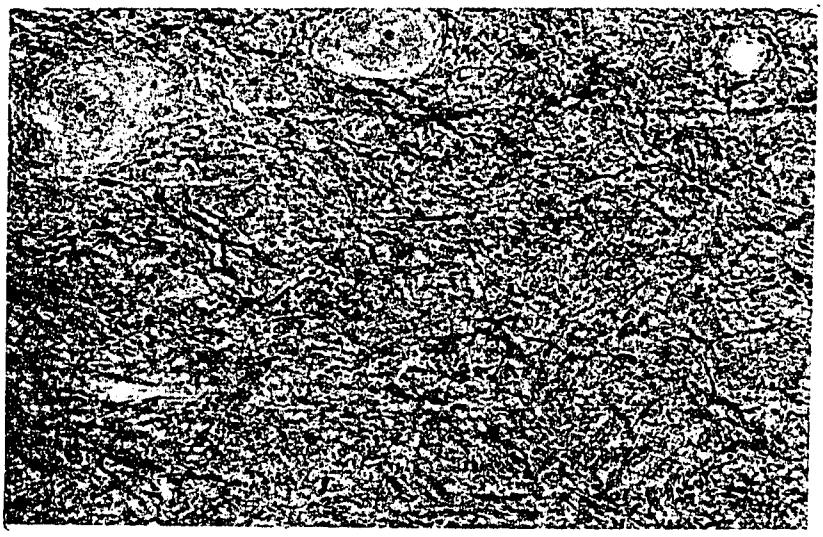

Figure 53: Degenerated fibres can be seen to ramify among the cells of lateral (dentate) cerebellar nuclear group. Inferior olivary lesion. Cat 10, 5 days survival. Nauta Phosphomolybdic, mag. 375X.

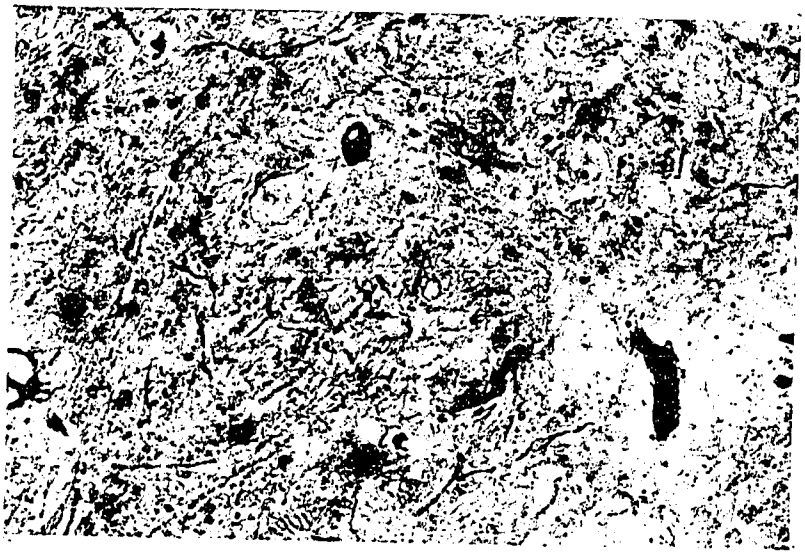

Figure 54: Degenerated fibres and preterminals in one of the deep cerebellar nuclei. Inferior olivary lesion. Cat 16, 5 days survival. Wiitanen Technique, mag. 375X.

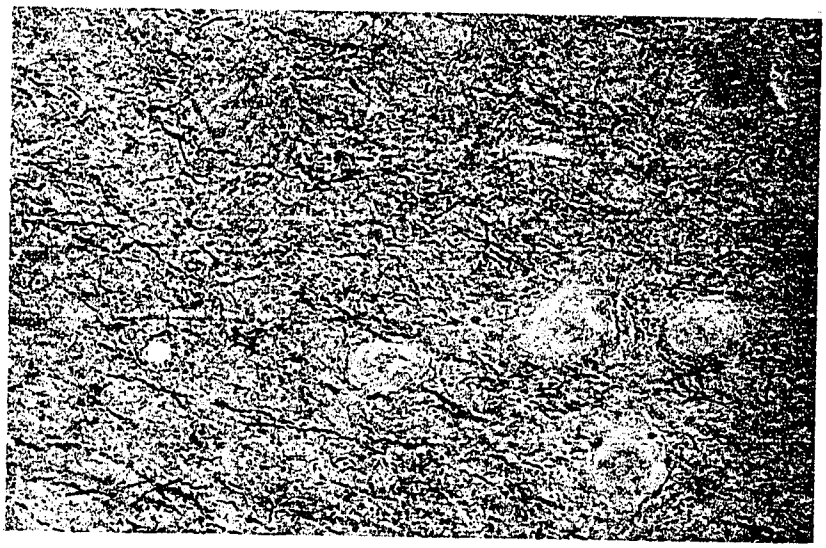

Figure 55: Degenerated fibres ramifying among the cells of the lateral (dentate) nucleus. Inferior olivary lesion. Cat 8, 11 days survival. Nauta Phospho-molybdic, mag. 240X.

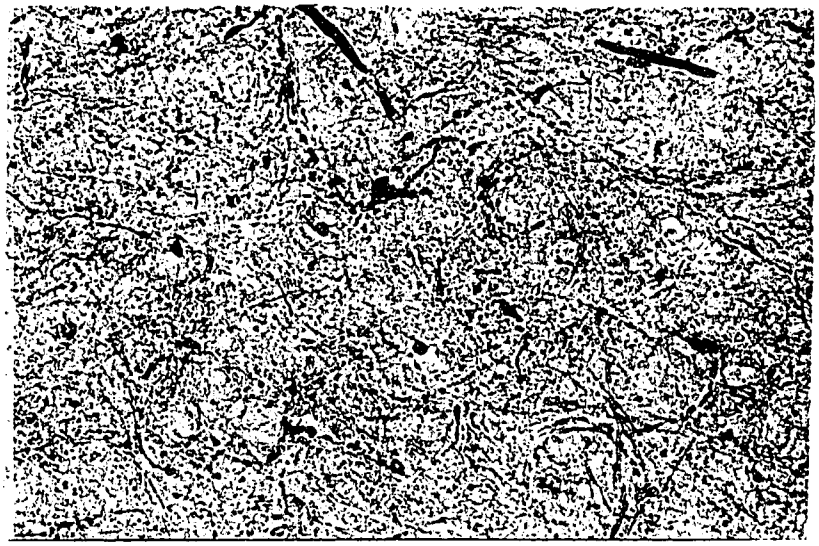

Figure 56: A few degenerated fragments are seen in the intermediate (interpositus) cerebellar nucleus after interruption of the spinocerebellar tract. Cat 30, 3½ days survival. Nauta Phospho-molybdic, mag. 375X.

Abbreviations Used in Figures

G	-	Ganglionic layer (This layer has also been referred to as Purkinje cell layer - P)
GR	-	Granular layer
IO	-	Inferior olivary nucleus
M	-	Molecular layer
PC	-	Purkinje cell body
P	-	Purkinje cell layer
SG	-	Supraganglionic layer
W	-	White matter

CHAPTER IV

DISCUSSION

The results obtained in this investigation failed to demonstrate degeneration of climbing fibres in the molecular layer after lesions of the inferior olive, even though degeneration was seen throughout the white matter and granular layer. In some animals degeneration was seen to ascend as far as the base of the Purkinje cell body and in most cases showing cortical degeneration, the deep cerebellar nuclei also showed degenerated fibres.

These negative findings with respect to climbing fibre degeneration may be accounted for by one of three explanations: (i) that climbing fibres do not exist; (ii) that climbing fibres must originate from a source or sources other than the inferior olivary nuclei; (iii) that climbing fibres are incapable of being stained in the molecular layer by all the current light microscopic silver impregnation techniques.

The first of these three alternatives is highly unlikely. The existence of climbing fibres and their extensive synaptic relationship with Purkinje cell dendrites and other elements in the molecular layer have been clearly demonstrated in Golgi studies (see introduction). Climbing fibres have also been identified in the molecular layer making axo-dendritic contacts with Purkinje neurons in ultra-structural studies (Hámori and Szentágothai 1966).

The second alternative though unlikely from evidence of other studies, is not improbable. Other sources for climbing fibres have been suggested. The deep cerebellar nuclei were suggested by Carrea et al (1947) as the source of climbing fibres. These authors found no evidence of climbing fibre degeneration with olivary lesions, but obtained degenerating elements in the molecular layer which were related to main Purkinje dendrites after cerebellar nuclear lesions. These degenerated fragments were interpreted as climbing fibres. This interpretation however has not been favourably received (see introduction).

Inter-cortical association fibres were proposed to end as climbing fibres (Eager 1965). On re-examination of Eager's photographs, Eccles, Ito and Szentagothai (1967) concluded that the degenerating fragments that appeared to be running parallel with the large Purkinje dendrites were really crossing over these dendrites. In a review of the pertinent literature, Eccles et al refer to the work of Frezik (1963). The latter author interpreted the terminations of inter-cortical association fibres as being due to degeneration of the infraganglionic and supraganglionic plexuses. These plexuses are composed of association and recurrent Purkinje axon collaterals which are myelinated axons. Eager explained his good results with the Nauta stain (1954) as probably due to the fact the fibres were myelinated. It should be noted that

climbing fibres lose their myelin sheath at the level of the Purkinje cell body.

On comparing the work of Eager (1965) with that of Frezik (1963). Eccles et al (1967) concluded that both were evidences of degeneration of the ganglionic plexuses. In addition, Frezik's photographs were thought to correspond to the classical description of recurrent Purkinje axon collaterals and were not similar to the degenerated fragments of climbing fibres demonstrated by Szentagothai and Rajkovits (1959).

Finally, Golgi studies of both association and recurrent Purkinje axon collaterals (cat) show that they ramify at the level of the Purkinje soma and initial portion of their dendrites (Ha 1970). Therefore, Purkinje axon collaterals do not seem to be a potential source of climbing fibres.

Escobar, Sampedro and Dow (1968) made cell counts of the inferior olive nuclear neurons in man, cat and vampire. They found a discrepancy between the number of the neurons in both inferior olivary complexes and the accepted number of Purkinje cells in the cerebellar cortex. In the case of the inferior olive in human specimens, their calculations, as well as those of Moatameds (1966), gave figures which are about one-fifteenth the estimated Purkinje cell population as given by Braitenberg and Atwood (1958).

If the inferior olive exclusively projects to the cerebellar cortex by means of climbing fibres and if one

assumes a one to one relationship between climbing fibre and Purkinje cell, the discrepancy in these cell counts must be explained. Three possible explanations were suggested: (i) that the number of Purkinje cells in the human cerebellar cortex was incorrectly over-estimated; (ii) that each axon contacts more than one Purkinje cell or (iii) that there is another important source of climbing fibres.

In the cat the anatomical evidence for an olivary origin for climbing fibres has been strengthened by physiological findings. Eccles, Llinás and Sasaki (1966) (see also Eccles et al 1967) demonstrated the powerful all-or-none synaptic activation of Purkinje cells, believed to be typical of climbing fibre activity while stimulating the accessory inferior olivary nucleus. This finding correlates well with the anatomical description of an extensive synaptic contact between climbing fibres and Purkinje dendrites. The physiological findings also support the claim of Szentágothai and Rajkovits (1959) that the olivo-cerebellar pathway is composed exclusively of climbing fibres, as pure climbing fibre responses could be recorded by repeated stimulation of the inferior olive.

Eccles et al (1966) also demonstrated that with rare exception, a one-to-one relationship exists between the climbing fibre and the Purkinje dendrite. This is contrary to the Golgi evidence (Scheibel and Scheibel 1954) which showed a multiple innervation of more than one Purkinje cell

by a single climbing fibre. Such a distribution should produce, in the all-or-nothing response, spike potentials from two Purkinje cells when stimulated at the same threshold. This phenomenon was never observed in the recordings of Eccles and his co-workers. They, however, did not exclude such a multiple innervation on the basis of their results, as their extra-cellular recording electrode was rarely placed so that they could record from two adjacent Purkinje cells. There may in fact be no discrepancy between the physiological data, the Golgi studies and the data obtained from cell counts.

The climbing fibre response is physiologically characteristic and has been differentiated from that obtained by activation of other fibre systems in the cerebellar cortex (Eccles et al 1967). This typical climbing fibre response has also been observed in the cat following stimulation of the contra-lateral inferior olivary nucleus (Voorhoeve 1967), (Precht and Llinas 1969).

In studies using animals other than the cat, other fibre systems have been found to terminate as climbing fibres. In the frog auricular lobe, climbing fibre activation of Purkinje cells with stimulation of the vestibular nerve has been demonstrated (Precht and Llinas 1969). These authors also obtained the graded repetitive response known to be mediated by the mossy fibre system. Anatomically climbing fibres have been observed following transection of the

vestibular nerve. Hillman (1969) studying bull frogs could stain with the Nauta (1967) method degenerated fragments in the molecular layer of the ipsi-lateral cerebellum that resemble climbing fibres, as well as mossy fibre degeneration in the granular layer, after transection of the vestibular nerve. Ultrastructurally, he could also show degenerated myelinated fibres in the molecular layer, which after losing their myelin sheath, branch and make contacts with large Purkinje cell dendrites.

These contacts were made via boutons which contained large round vesicles. This type of bouton has been interpreted as evidence of an excitatory synaptic terminal (see Halász and Csillik 1969). The results obtained in Hillman's study confirm the electrophysiological findings of Precht and Llinás (1969) which indicated that direct vestibular nerve fibres in the frog project as both mossy and climbing fibres. However, in cats, vestibular fibres have been shown to terminate exclusively as mossy fibres (Brodal and Høivik 1964).

In the rat, O'Leary et al (1968) found degenerated climbing fibres in folia which had been isolated while lesioning the central cerebellar nuclei. In addition, they found little evidence of degenerating climbing fibres following lesions of the inferior olive, whereas degeneration of mossy fibres and terminals in the granular layer was more frequently observed. No other source of climbing fibres has been documented in recent

experimental studies using either light or electron microscopic techniques.

Although the anatomical data for the source of climbing fibres to the cerebellar cortex has not been conclusively established, the physiological data (particularly in the cat) firmly support the inferior olive as the exclusive source of these fibres.

The third alternative that might explain the lack of degenerating climbing fibres in the molecular layer in this study, is that the techniques are still inadequate for demonstrating these axons in the degenerated state. On the basis of results obtained in this investigation we are more inclined to accept this explanation. Our findings are in complete agreement with those of Eager (1970). Eager used modifications of the Nauta-Laidlaw and Fink-Heimer methods and could not obtain any degenerated climbing fibres in the molecular layer, after lesions of the spinal cord, pons and inferior olive.

On what basis could this lack of staining reaction be explained? One possibility is fibre size, another is some unknown characteristic of the molecular layer itself. However, we have demonstrated that degenerated fragments other than those due to climbing fibres can be stained in the molecular layer. Three types of degenerating elements have been mentioned in the observations. The first of these which appeared as argyrophilic columns that extended the whole width

of the molecular layer was attributed to glial reaction stimulated by local trauma to the cortical layers.

The morphology of the columns resemble that of Bergmann fibres described by Fox (1967) and Sotelo (1967). They are described as a special type of astroglia indigenous to the molecular layer, whose soma, the Golgi epithelial cells, are located between the perikarya of the Purkinje cell bodies. These Golgi epithelial cells give off two or three long processes that extend perpendicularly across the molecular layer. The processes form straight palisades and terminate in conical swellings whose bases are applied to the pia mater. The argyrophilic columns were always seen in this study, only in the immediate vicinity of the traumatized cortex. They are comparable to those described by O'Leary et al (1968) in the rat, a phenomenon which these authors interpreted to be climbing fibre degeneration.

The finely dispersed type of degeneration was observed in areas slightly removed from the damaged cortex. In some animals these fibres were seen to have a distinct parallel orientation with the longitudinal axis of the folium. This type of degeneration was attributed to parallel fibre degeneration. From electron microscopic observations the diameter of the parallel fibres varies from slightly more than 1 μ in the largest fibres located in the deep layers of the molecular layer to 0.1 μ in the smallest most superficially located fibres (Fox et al 1964).

The fibres were not seen with the Nauta-Laidlaw (1957) technique; were poorly seen with the Fink-Heimer II procedure; but were well demonstrated with the Wiitanen method. There has not been any previous report of degeneration of parallel fibres in the cerebellum using light microscopic silver impregnation techniques. If our interpretation of this type of degeneration is correct, then it is clear that non-myelinated fibres of small dimensions can be stained in the molecular layer with the Wiitanen technique. Therefore the inability of this staining technique to demonstrate degenerated climbing fibres in our experiments, can neither be due to fibre size nor to some peculiar characteristic of the molecular layer.

The third type of molecular layer fibre degeneration we observed was located in the supraganglionic plexus. The fibres were rather coarse and could be traced for various distances and coursed in the longitudinal plane of the folium. This type of degeneration was demonstrated in all the staining procedures used and has been reported previously by other authors (Frezik 1963). In addition, as mentioned earlier, Eager's (1965) study has been re-interpreted as evidence of degeneration of associational Purkinje axon collaterals. These coarse fibres we suggest are due to degenerating Purkinje axon collaterals, which according to Cajal's (1911) description, assume this longitudinal orientation in the supraganglionic plexus. Ha (1970) in a Golgi study, identified two types of Purkinje axon collaterals; the recurrent type that divides

repeatedly to form a varicose plexus at the Purkinje cell body level or deep molecular layer and an associational type that projects for long distances, to synapse with Purkinje cells of adjacent folia.

In animals with lesions in the white matter of the cerebellar cortex, in which the contra-lateral approach was used (see materials and methods) to avoid local damage to the cortex, the lesions should have interrupted both mossy and climbing fibres to certain folia. No climbing fibre degeneration was demonstrated distal to these lesions, even with the Wiitanen technique. Since climbing fibres are thought to come from outside the folium and since Purkinje axon collaterals are not a possible source of climbing fibres, one must conclude that climbing fibre degeneration in the molecular layer cannot be identified with these techniques.

That fibres could be stained in the granular layer after inferior olivary lesions must be explained. Climbing fibres are myelinated in this layer and are possibly of larger dimension at this level than they are near their terminals. The fibre diameter of the olivo-cerebellar system has been measured to be 1-5 μ (Eccles et al 1967). However, on the basis of data obtained with the Wiitanen technique the fact that the climbing fibre is unmyelinated in the molecular layer and may be of small dimension, should not preclude their identification with this technique

i.e. parallel fibre (0.1-1.0 μ) degeneration has been identified. On the basis of electron micrographs (Fox et al 1967), (Hámori and Szentágothai 1966), the climbing fibre in the molecular layer measures approximately 0.5 μ along the basal portions of the Purkinje dendrite. The Nauta-type staining technique could conceivably stain only the white matter and granular layer portions of the climbing fibre.

The most likely possibility that might explain the lack of the stainability of these fibres must be some peculiar feature of the climbing fibre terminal degeneration in the molecular layer, that makes them refractory to all the currently available techniques. The absence of staining in the molecular layer constitutes a negative finding and as such precludes any interpretation on the basis of these techniques.

The spinal cord lesion (one cat) was done to interrupt the spino-cerebellar tract which is known to terminate as mossy fibres. It was considered that this might serve as a possible basis for comparison with the morphology of degenerating fibres in the granular layer, obtained after inferior olivary lesions. An attempt was also made to see whether two distinct types of degenerating fibres could be identified in the granular layer in animals with white matter lesions of the cerebellum, where both mossy and climbing fibre systems were presumably interrupted.

After detailed study of the sections and photographic

material, it was not possible to identify two distinct types of degenerating fibres in the granular layer. Where a branching fibre was clearly discernible, the fibre was identifiable as a mossy fibre, but this was seen only occasionally after the spinal lesion, rarely, with the white matter cerebellar lesions and never after inferior olivary lesions. In the absence of obvious branching all degenerating fibres in the granular layer appeared to be similar.

On the basis of the current results, particularly with lesions located in the cerebellar white matter using a contra-lateral stereotaxic approach it is concluded that degenerating climbing fibres cannot be stained in the cat molecular layer. Of necessity this conclusion must also be extended to other studies in the cat cerebellum, as have been referred to in the introduction, leading to the general statement, that studies of the climbing fibre (cat) cannot correctly determine the presence or absence of climbing fibres on the basis of the Nauta (or modified Nauta) staining techniques.

Finally, a further question raised by several authors concerns the existence of collaterals from the cerebellar afferent systems to the deep cerebellar nuclei. As has been mentioned in the introduction, a few authors have reported anatomical evidence of the presence of such collaterals from various afferent fibre systems. Electrophysiological

studies have also reported evidence of collateral input to the deep nuclei.

The cerebellar nuclei have been reported to exhibit a rapid "spontaneous" firing (Russel and Barrat 1969). Since the Purkinje cells are quantitatively the main afferent input to the nuclear cells (Jansen and Brodal 1954a); (Voogd 1964) and since these Purkinje axons are now known to have a powerful inhibitory effect on these cells (Ito et al 1964); (Eccles et al 1967), it would be expected that the deep nuclear cells would have to be activated by a powerful excitatory input. It is conceivable therefore that collaterals from all afferent systems including those from the inferior olive could fulfill this function. Brodal (1940), demonstrated that the olivo-cerebellar fibres project in an orderly manner to the deep cerebellar nuclei and Eccles et al (1966), have reported that the olivo-cerebellar fibres have a powerful excitatory action. It was also postulated that if a fibre is excitatory all its collaterals are also excitatory (Eccles et al 1966a); (Fox et al 1967). It is conceivable therefore that the olivo-cerebellar fibres would drive the deep cerebellar nuclei and then continuing on, possibly as climbing fibres, stimulate the Purkinje cells. The inhibitory activity of Purkinje axons on the intra-cerebellar nuclei would then be continuously modified by the activity of mossy fibres arriving from their various nuclei of origin and by climbing fibres.

In this investigation the degenerated elements observed in the intra-cerebellar nuclei revealed that a substantial input of fibres is provided to these nuclei by the olivo-cerebellar system. In some instances, the observations would support the contention that these fibres were collaterals. It was difficult to assess the number of these collaterals actually terminating in the nuclear cell groups, because of the abundant number of fibres of passage that were observed coursing around the cell groups and among the cells. It was quite evident, however, from the appearance of the degenerated fragments, particularly with the Wiitanen technique and also with the Nauta phospho-molybdic acid procedure, that a significant number of these degenerated fragments were preterminals and terminals.

Eccles et al (1967) mentioned that the synaptic relationship of the collaterals of the afferent systems, with the nuclear neurons, was mainly by axo-dendritic synapses and that the cell bodies did not appear to be contacted by these collaterals. In our material, degenerated fragments could be observed in contact with the surface of cell bodies. Some of the degenerated fragments surrounding the cells were not in close juxtaposition to the surface of the cell bodies, some others seemed to be in contact with prolongations from the cell bodies. These prolongations were presumed to be the dendritic branches of the cells. Many of the degenerated

fragments however, appeared to be randomly dispersed in the neuropil of the nucleus.

Eager (1968) in an ultrastructural study of cerebellar nuclei in the cat observed both axo-somatic and axo-dendritic terminals on large and small neurons. Since this study was done in normal animals the author could not identify which of the several pathways terminating in the cerebellar nuclei made axo-somatic or axo-dendritic contacts with the nuclear neurons. Whether the close apposition of some of the degenerated fragments to the cell body surfaces, which were seen in this study, represent true synaptic contacts cannot be ascertained.

However, the present study corroborates the existing anatomical evidence, that the olivo-cerebellar system does project fibres and possibly collaterals to the intra-cerebellar nuclei, particularly to the lateral nuclear groups, as was suggested in our results, and also lends added anatomical support to the physiological evidence for the existence of such a collateral input.

It is interesting to note that the staining techniques used were capable of demonstrating what has been interpreted as preterminal and terminal degeneration in the deep cerebellar nuclei. On the other hand, these same techniques were not sufficient to identify what is presumably the same type of terminal degeneration in the molecular layer of the cortex, presuming that the olivo-cerebellar fibres do project to the cortex as climbing fibres.

CHAPTER V

SUMMARY

1. Electrolytic lesions were placed in the left inferior olivary nucleus of adult cats and the fibre projection to the cerebellum was studied, after survival times of two to thirteen days.
2. Four silver impregnation techniques for demonstrating degenerated fibres, preterminals and terminals were utilized in this study.
3. The degeneration observed in all cases was restricted to the white matter and granular layer of the cortex. In no case did the degeneration extend into the molecular layer.
4. It was concluded that either the olivo-cerebellar system did not project as climbing fibres to the molecular layer, or, that the techniques utilized were not capable of staining these fibres in the molecular layer.
5. In order to explore these alternatives, lesions were introduced within the cerebellum affecting fibres in the white matter and/or damaging the cortical layers.
6. Applying the same staining techniques degeneration was observed within the molecular layer only with

the Wiitanen technique and only in cats which had a demonstrable lesion affecting the cortex.

7. The molecular layer degeneration was interpreted to be due to parallel fibres, Purkinje axon collaterals and reactive Bergmann glial astrocytes.

8. After lesions interrupting fibres in the white matter, degeneration was again restricted to the granular layer of the affected folia. No other type of degeneration was seen in the molecular layer.

9. It is concluded that climbing fibres within the molecular layer cannot be stained in an experimental degenerative study with the techniques used in this investigation.

10. A lesion of the spino-cerebellar tract was performed in one cat to demonstrate the degeneration of mossy fibres within the granular layer.

11. Degeneration was seen within the deep cerebellar nuclei after inferior olivary nuclear lesions.

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