THE EFFECT OF CEREBELLAR PEDUNCLOTOMY ON THE NORADRENERGIC AND OLIVARY AFFERENTS

CHAPTER SIX

CATECHOLAMINE FLUORESCENCE

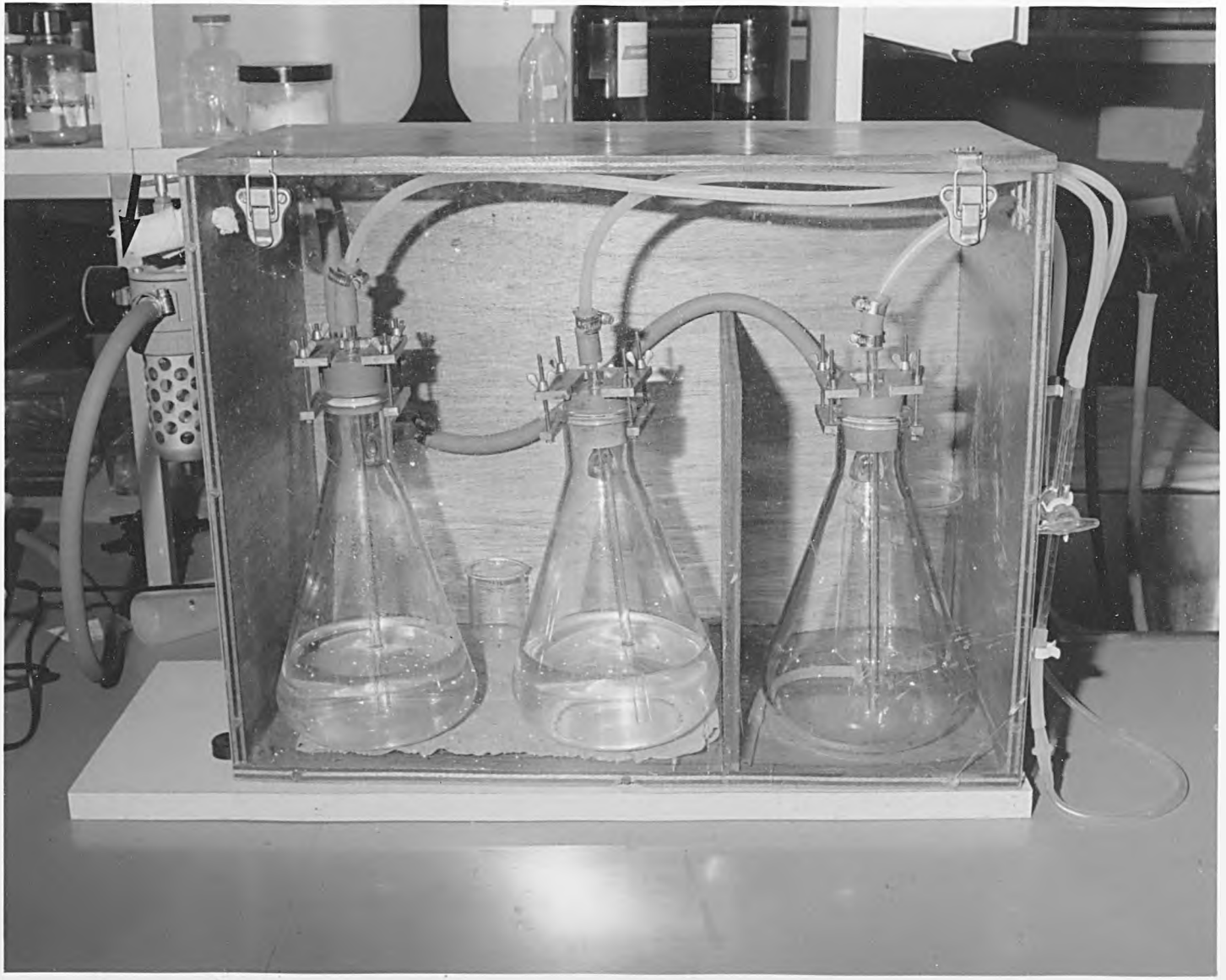
INTRODUCTION

The catecholaminergic afferents to the cerebellum have been described in Chapter 1. Because noradrenalin is synthesised prenatally (Lauder and Bloom, 1975) and will produce a Purkinje cell response before synaptogenesis (Woodward et al., 1971), it is possible that it is important in neural development (Lawrence and Burden, 1973). Also noradrenergic fibres have been shown to regenerate following both chemical (Schmidt and Bhatnagar, 1980; Schmidt et al., 1980) and surgical (Bjorklund and Stenevi, 1971; Katzman et al., 1971; Pickel et al., 1973) injury, and it has been proposed that noradrenalin plays an important role in neural plasticity (Kasamatsu et al., 1979). Therefore it is important to know how the cerebellar noradrenergic afferents respond to pedunclotomy at different ages.

Catecholamine fluorescence is produced by the condensation of catecholamines by formaldehyde into the fluorophore 6,7-dihydroxy-3,4-dihydroisoquinoline (Corrodi and Jonsson, 1967). The original technique of Falck et al. (1962) was not very sensitive. However the newer techniques are considerably more sensitive and are suitable for the small concentrations of catecholamines in the neonatal brain.

Figure 6.1

The specialised apparatus for perfusion at a pressure of 2 bar. This is modified from Bower (1981) and Loren et al. (1980). The pressure delivered by compressed air is measured by the gauge (arrow).



METHODS

Cerebellar catecholamine fluorescence was studied using the highly sensitive aluminium-formaldehyde technique of Loren et al. (1980) and its modification for neonatal rats (Loren et al., 1982).

The animals in the study underwent left cerebellar pedunclotomy, as described in Chapter 2, aged 3, 7, 10 or 22 days and survived 35 days post operatively. Also, neonatal animals aged 3 or 10 days at operation were allowed to survive for 4 days in order to observe any acute changes in the noradrenergic fibres.

A special perfusion apparatus was required to allow high pressure perfusion driven by compressed air. The apparatus used was a modification of that described by Bower (1981) using high pressure teflon tubing, vacuum taps and placed in a safety box (Fig. 6.1) and was similar to the one recommended by Loren et al. (1980).

PERFUSION

The perfusion took place in two stages, a preperfusate of modified Tyrode's buffer pH 7.2 given at low pressure followed by an aluminium-formaldehyde perfusate pH 3.8 delivered at high pressure. Both solutions must be freshly made (Appendix IX). The modification of Tyrode's buffer is different for the neonatal and adult groups but the perfusate is the same for both.

All animals were pretreated with a monoamine oxidase inhibitor, pargyline hydrochloride (Sigma, 150 mg/kg I.P., 2-3 hours), to maximise the catecholamines in the nerve terminals, and heparin (50-200 units I.P., 2-10 minutes) to improve the perfusion efficiency. They were anaesthetised with ether and a Jelco I.V. catheter placement unit was inserted transcardially into the ascending aorta. The thoracic descending aorta was clamped and the right atrium removed.

For neonates less than 12 days old the preperfusate of Tyrode's buffer was modified with 2g $MgSO_4.7H_2O$ and 1g procaine chloride per litre to minimise vasoconstriction. This was delivered at room temperature through a 22G cannula at 0.5 bar for 30 seconds, which gave a volume of approximately 150 ml. This was immediately followed by ice cold aluminium-formaldehyde perfusate and the pressure was rapidly increased to 2 bar. The animal was perfused for 45 seconds giving approximately 300 ml of perfusate.

Adult animals were perfused through an 18G cannula in the same manner as the neonates, the only difference being that the preperfusate was an ice cold solution of 2% glyoxylic acid in Tyrode's buffer.

The perfusion times were found by the time needed for the required volumes (150 and 300 ml) to flow into a graduated flask at the pressures used. This can

only give an approximate measurement of the actual perfusion volumes because the flow does not have to overcome the resistance of the animal's vascular system. However the graduations on the reservoirs showed that the volumes perfused were approximately those stated.

As the aluminium sulphate ions in the perfusate precipitate blood (Loren et al., 1982) the system had to be completely cleared of perfusate between animals. To facilitate this the perfusate was coloured with 0.1% azure B (aq). This dye was chosen because it is a basic dye which will not conjugate the aluminium ions (Al^{3+}) also, neither in solution nor on an electrophoresis gel plate did the dye fluoresce when excited by light of wavelength 355-425 nm (the wavelength used for monoamine fluorescence).

TISSUE PROCESSING

Freeze Drying

The perfused brains were quickly dissected to remove the forebrain and superior colliculi. The remaining cerebellum and brainstem of neonates, in the experiment with acute pedunclotomy, were treated intact and those of older animals, which had had chronic pedunclotomy, were halved sagittally. They were then placed on labelled card, wrapped in cotton gauze and quenched in isopentane cooled with liquid nitrogen. The tissue

was dried in an Edwards Tissue Dryer ETD4 on a cold stage set to -60°C with pre-dried molecular sieve type 4A ($^{1}/_{16}^{th}$ inch pellets of sodium alumino-silicate) as a water trap. After one week the cold plate was heated to at least room temperature before the vacuum was broken to minimise water condensation on the extremely hygroscopic tissue.

The drying time was found from a pilot study in which different drying times were tried. It is in accordance with some results (Eranko, 1967; Moore, 1981) though longer than most (Falck and Owman, 1965; Heym, 1981) possibly because the specimens are large and neural tissue is known to take longer to dry than other tissue types (Eranko, 1967). Also the cold plate is kept below the routinely used -40°C because the uneven tissue surface makes poor contact and this lower drying temperature, while possibly giving better results, slows the drying process (Eranko, 1967).

Exposure to Formaldehyde Vapour

It is formaldehyde vapour which produces the reaction that changes biogenic monoamines into a fluorophore (Falck et al., 1962; Corrodi and Jonsson, 1967). The humidity in which the reaction occurs will affect the result, low humidity decreases the condensation reaction but keeps the catecholamines within their nerve terminals, while high humidity will produce a

brighter fluorescence but increases the possibility of diffusion of the product into the tissue. A compromise of 50-70% humidity is the best range for biogenic amines (Falck and Owman, 1965; Hamberger et al., 1965; Eranko, 1967; Heym, 1981; Moore, 1981) and a pilot study of each humidity revealed that 50% gave the best results. Using tables of the relative humidity of air over different concentrations of sulphuric acid, which are calculated from chemical and physical data (Eranko, 1967; Heym, 1981), the required volumetric concentration of sulphuric acid was calculated. A 24.5% solution of concentrated sulphuric acid was made and paraformaldehyde, predried over molecular sieve type 4A, was equilibrated for at least 7 days.

The freeze-dried tissue was transferred to foil boats which were placed in 100 ml specimen jars containing an excess of equilibrated paraformaldehyde. With the lid tightly closed the jars were heated in an oven to 80°C for 1 hour.

Embedding and Sectioning

Tissue exposed to formaldehyde vapour was immediately immersed in degassed paraffin wax and embedded under vacuum for 3 hours. The cotton gauze and identification cards were removed before blocking out in paraffin wax.

Although tissue blocks can be stored at -20°C for many months with little or no loss of fluorescence

(Loren et al., 1976 and 1982; Moore, 1981) the tissue was sectioned and analysed as soon as possible because central nervous system catecholamines are relatively unstable (Bjorklund et al., 1972).

Sagittal sections 10 µm thick were taken every 200 µm across the cerebellum. They were gently straightened onto clean glass slides and heated to 60°C on a hotplate to melt the wax and expand the section. When the slides had cooled and the wax rehardened they were mounted in a mixture of 2:3 Fluorolite (R. A. Lamb, London) and xylene and returned to the hotplate so the wax melted into the mounting medium. This is a different ratio from that suggested by Loren et al. (1982) of 4:1 Fluorolite to xylene, which was too thick giving a rather blurred section and into which the wax did not completely dissolve before the medium dried hard.

Microscopy

Using a Leitz MPV2 microscope with excitation wavelength of 355-425 nm and a barrier filter of 460 nm, the sections were analysed as soon as possible to prevent the fluorophore fading.

RESULTS

Any chronic changes in the noradrenergic afferents to the cerebellar cortex were observed in those animals which survived 35 days post operatively. However, the

results were the same in all groups irrespective of the operative age and will therefore be described together. In addition, those animals aged 10 days at operation which survived 4 days post operatively have also been included since these results were similar to those of the adults.

Fluorescent fibres were seen in the central white medulla and all three layers of the cortex. The fibres were very fine tenuous threads with intermittent varicosities which gave them the beaded appearance described in the literature (Hökfelt and Fuxe, 1969; Pickel et al., 1973). The fibres passed from the white matter into the granular layer in which they formed a rather disorganised plexus. The path of the fibres though the granular layer was tortuous except in the more apical areas of the folium where they had a much straighter course from the white matter towards the Purkinje cells. At the Purkinje cell layer fluorescent fibres were seen to branch and run horizontally at the level of the infraganglionic plexus and there were many terminals around the Purkinje cell somata. In the molecular layer there were two types of fibres both of which retained the typical beaded appearance and ran fairly straight courses, but they either ascended vertically through the layer to the pia mater or divided in a T-shape to run horizontally parallel to the Purkinje cell layer. The horizontal fibres were more common in the lower half

of the molecular layer. Since these fibres were seen in sagittal sections they run perpendicular to the parallel fibres of granule cells.

Noradrenergic fibres were seen in all regions of the cerebellum but their distribution was patchy, probably because of uneven dewaxing of the sections. Because of this it was difficult to assess the density of fibres and make comparisons between the control and experimental groups. However a subjective impression was that there were slightly fewer fibres in the left hemicerebella of the experimental groups than in either the contralateral hemicerebella or the control animals.

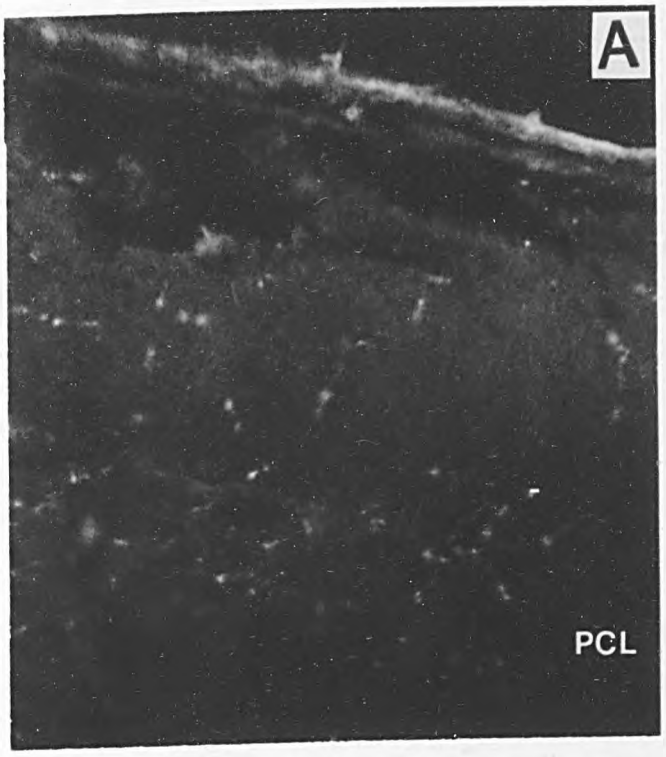
The fluorescent fibres in the cerebella of animals which underwent pedunclotomy aged 3 days were brighter and appeared thicker than in the adult animals. They were also present in the white matter and traversed the developing internal granular layer. The plexus of fibres was most dense at the Purkinje cell layer and fibres were seen ascending through the molecular layer to the external granular layer. However, very few fibres were seen in this layer. As with the chronically lesioned animals the noradrenergic fibres were found in all regions of the cerebellum. Also there appeared less fibres in the hemicerebellum ipsilateral to the pedunclotomy although the differences between experimental and control groups were minimal.

Figure 6.2

Fluorescent Noradrenergic Fibres in
the Right Hemisphere of a Normal 38 day
Cerebellum

A. Fibres ascend from the Purkinje cell layer (PCL) into the lower half of the molecular layer. (x 296)

B. Fluorescent terminals are present around the Purkinje cells and ascend onto the lower dendrites in the molecular layer (ML). (x 296)


A
PCL

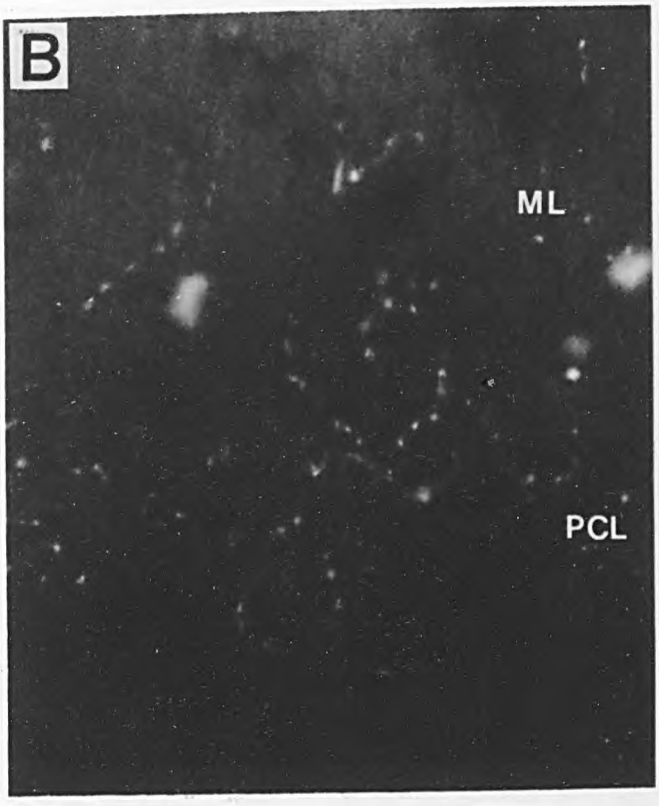

B
ML
PCL

Figure 6.3

Noradrenergic Fibres in the Cerebellum from a Rat Pedunclotomised on Day 3 and Surviving 35 Days

A. A single fibre with the typical beaded appearance ascending vertically in the molecular layer from the Purkinje cell layer. (x 296)

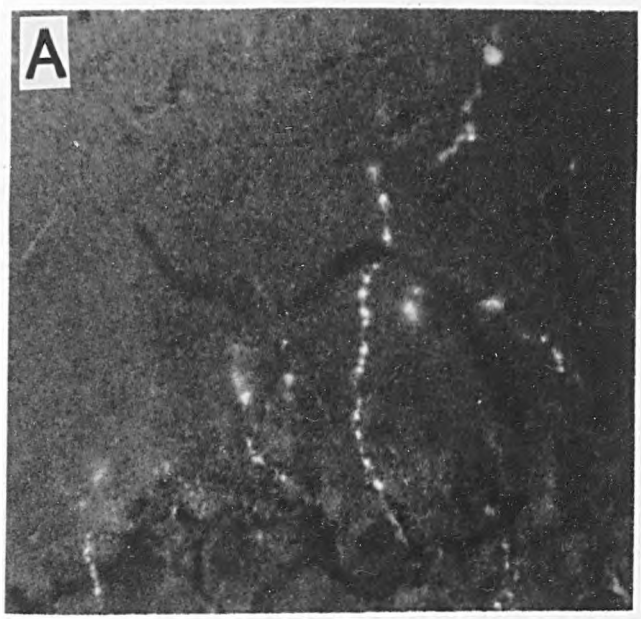
B. Fluorescent fibres ascend from the granular layer (GL) around the Purkinje cells (P) into the molecular layer (ML). (x 296)

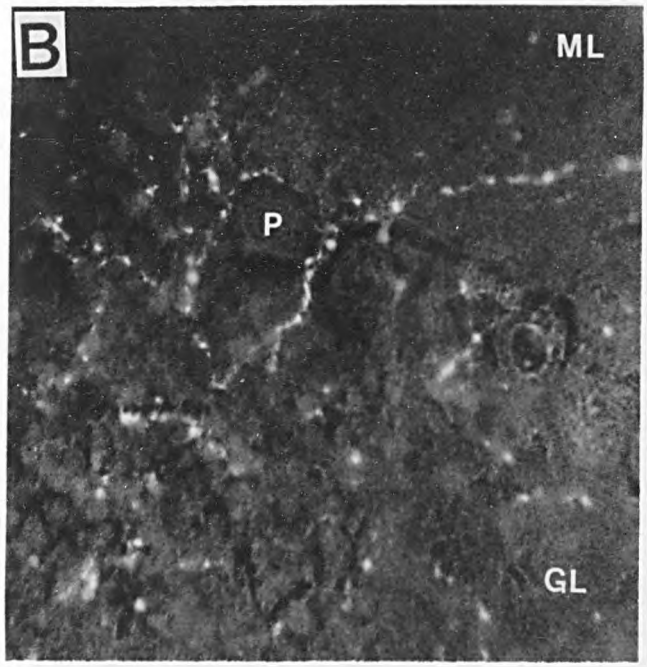
C. A horizontal fibre running parallel to the Purkinje cell layer towards the apex of the folium (A). (x 296)

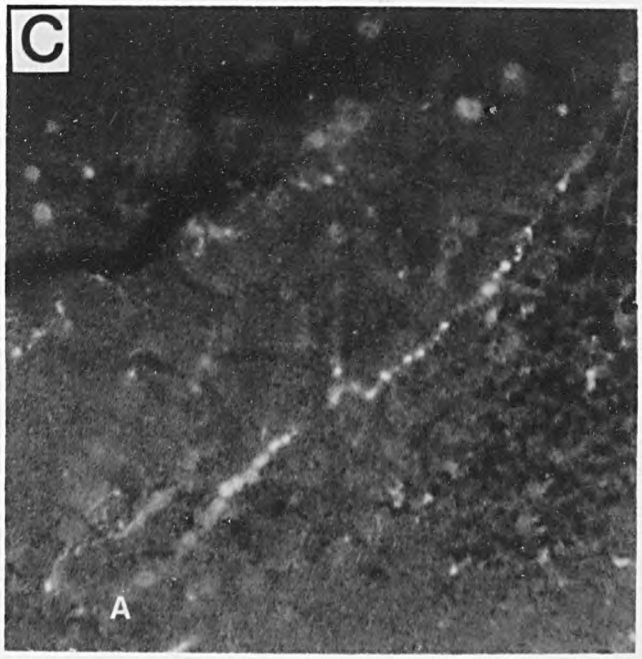
D. A fibre ascending obliquely to the Purkinje cell layer and giving terminals onto the Purkinje cell perikarya. (x 296)

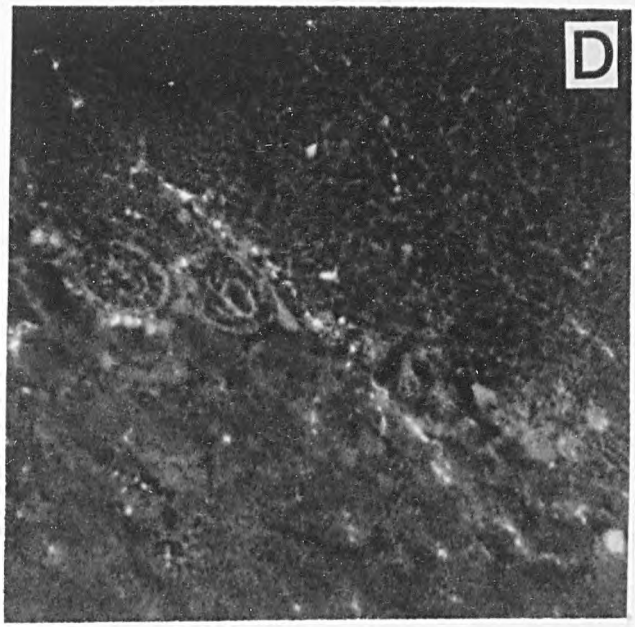
E. Fluorescent preterminal fibres in the infraganglionic plexus ascending to a Purkinje cell (P). (x 296)

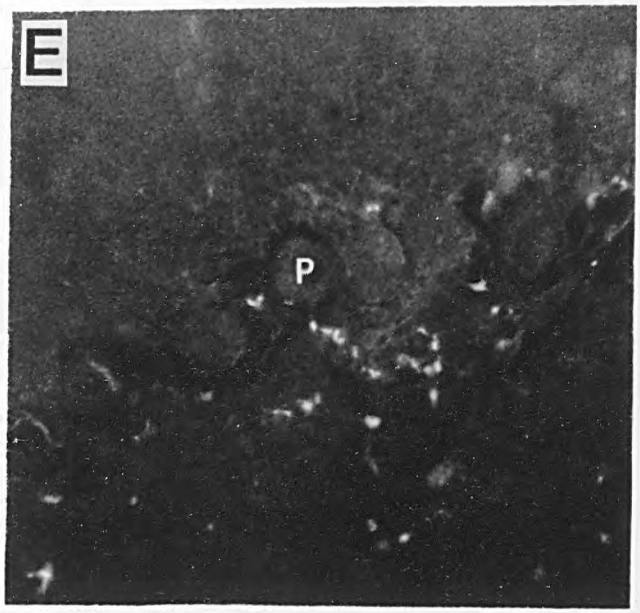
F. This tangential section of part of the Purkinje cell layer shows the fluorescent fibres encircling the Purkinje cell perikarya. (x 296)


A


B
ML
P
GL


C
A


D


E
P

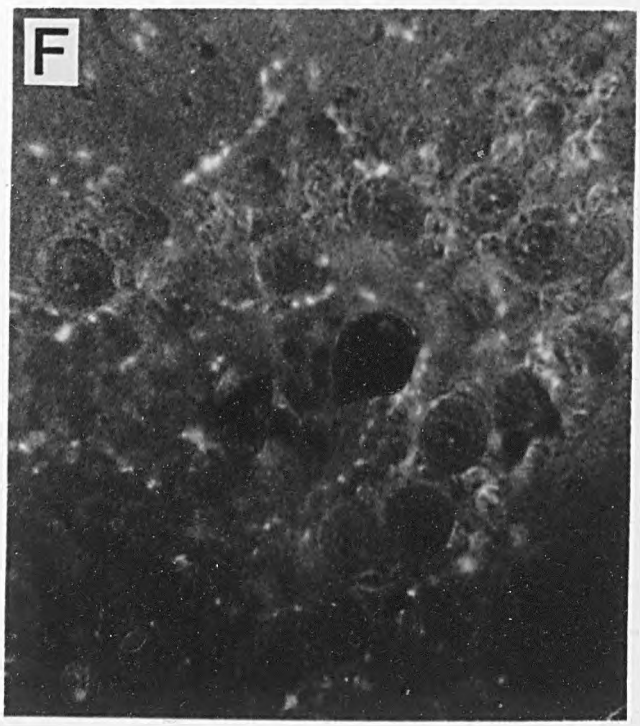

F

Figure 6.4

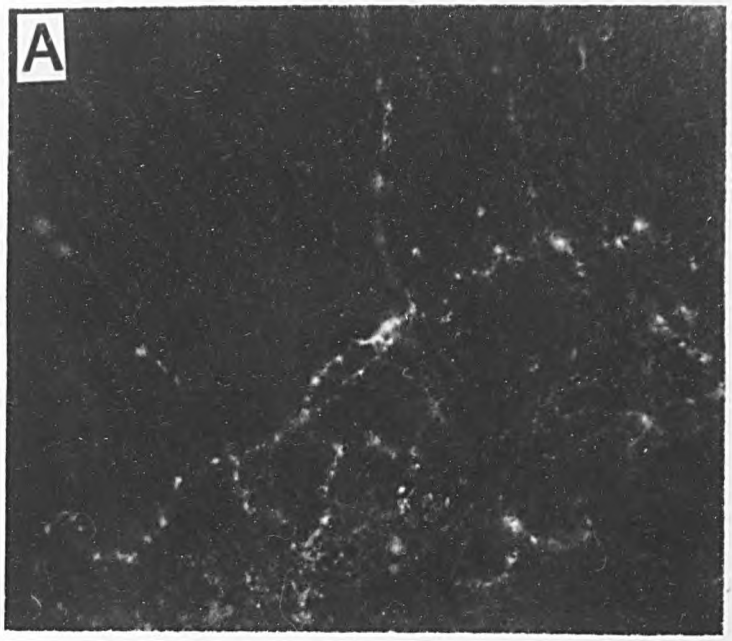
Fluorescent Noradrenergic Fibres in a
Control Cerebellum from a 42 Day Old Rat

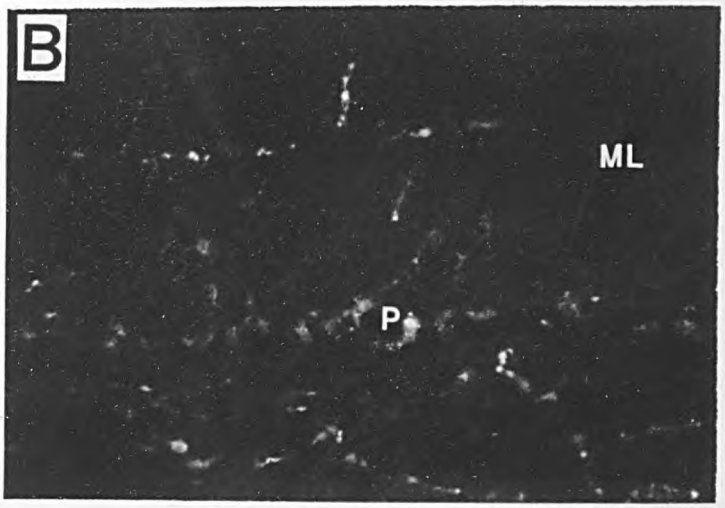
A. An H-like formation of fluorescent fibres in the lower part of the molecular layer. (x 296)

B. Fibres from the granular layer give terminals to the Purkinje cells (P) and continue into the molecular layer (ML) and run either vertically or horizontally. (x 296)

C. Vertically ascending fibres in the molecular layer. (x 296)

D. The plexus of noradrenergic fibres seen in the granular layer. (x 296)


A


B
ML
P


C

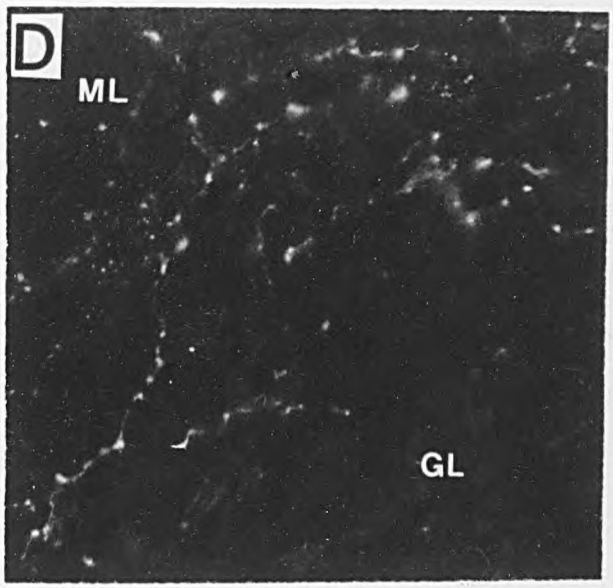

D
ML
GL

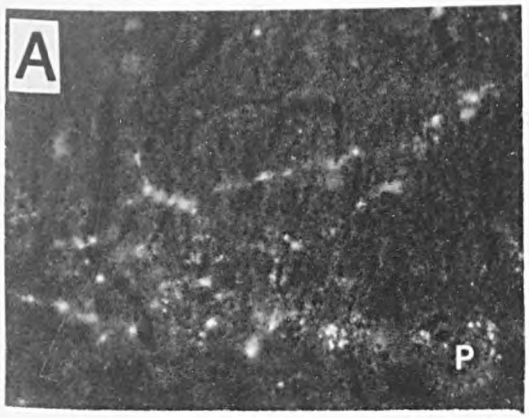
Figure 6.5

Noradrenergic Fibres in the Cerebellum
35 Days after Neonatal Pedunclotomy
on Day 7

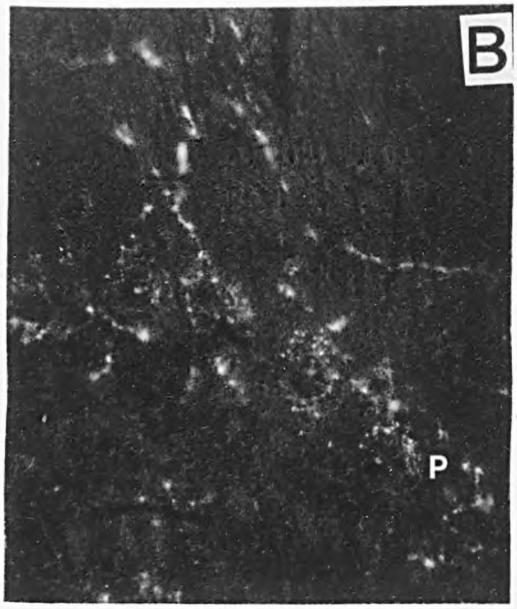
A and B. Fluorescent terminals are present around the Purkinje cell somata in the Purkinje cell layer (P) and run obliquely (A) and horizontally (B) in the lower molecular layer. (x 296)

C. Vertically running fibres in the molecular layer. (x 296)

D and E. Fluorescent fibres running through the granular layer (GL) to the Purkinje cell layer and to the lower part of the molecular layer. (x 296)


A
P


C


B
P


E
GL

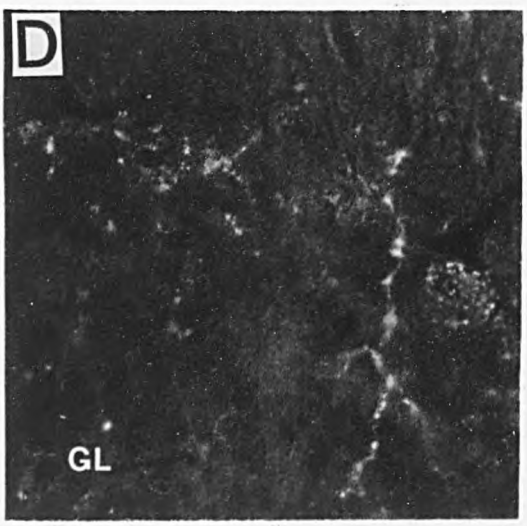
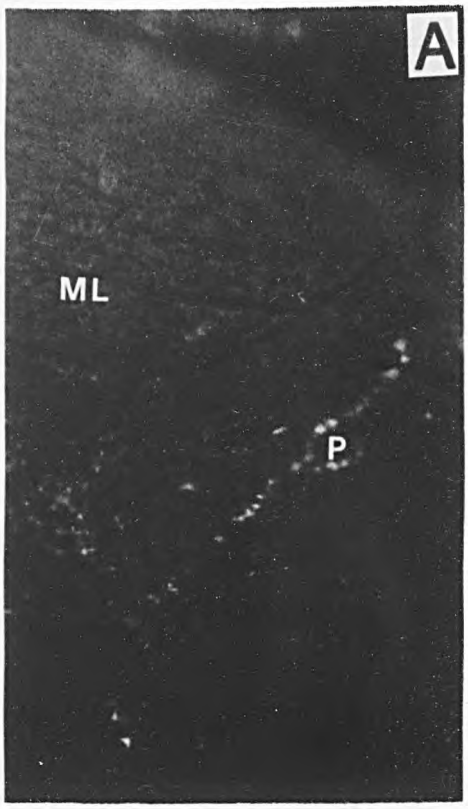

D
GL

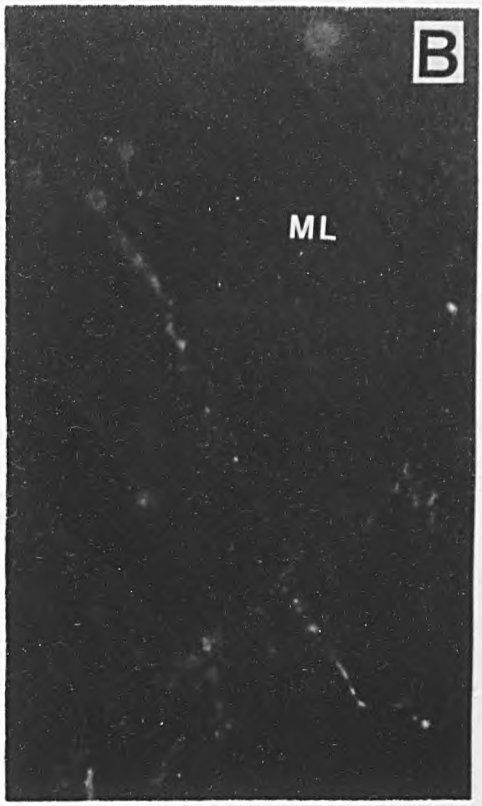
Figure 6.6

The Catecholaminergic Fibres Seen in the Cerebellum of an Animal Pedunclotomised Aged 10 Days which Survived for 35 Days

A and B show fluorescent fibres ascending from the granular layer through to the molecular layer (ML). In A the fibre splits around a Purkinje cell (P). (x 116)

C. The horizontal plexus of fibres running along the Purkinje cell layer (x 296) and giving a few ascending branches into the molecular layer.


A
ML
P


B
ML

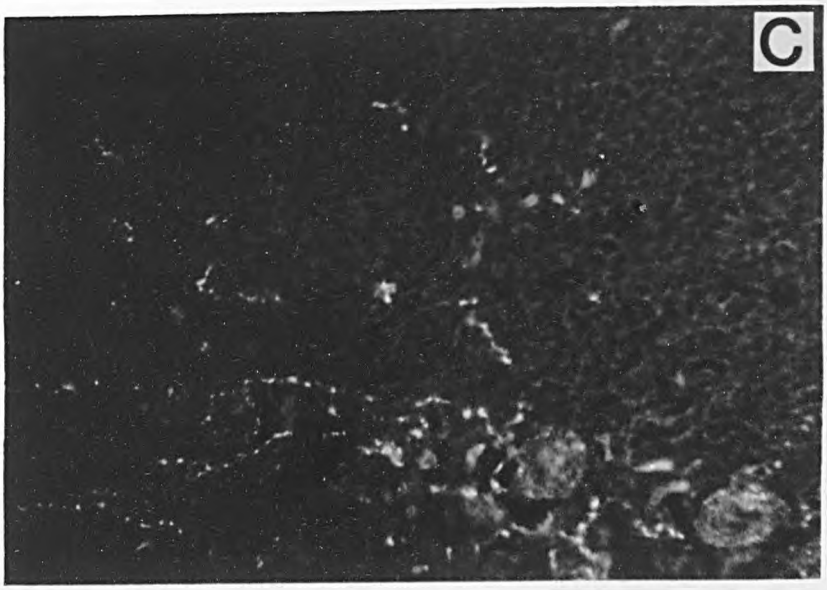

C

Figure 6.7

Fluorescent Noradrenergic Fibres in
a Normal 7 Day Cerebellum

A. Short beaded fibres in the lower part of the molecular layer at the base of a sulcus. (x 296)

B and C. Fluorescent fibres ascending through the granular layer to the Purkinje cell layer (PL). (x 296)

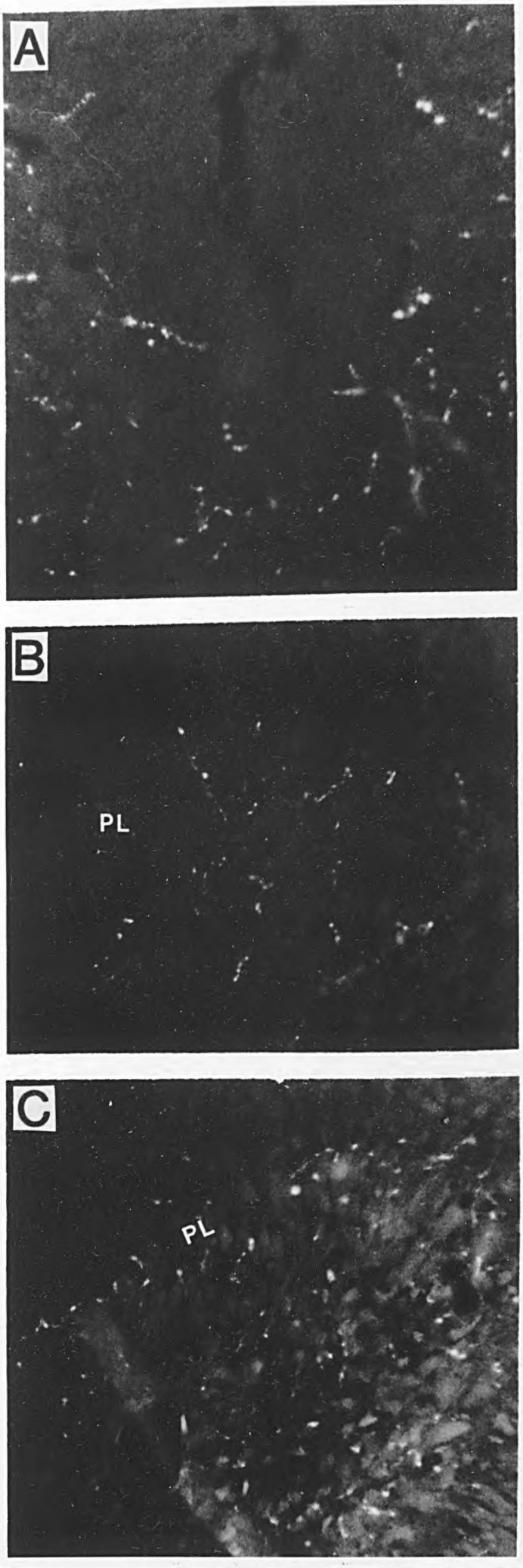

A
B
PL
C
PL

Figure 6.8

The Noradrenergic Fibres in the Cerebellum of a Neonatal Rat 4 Days After Pedunclotomy on Day 10

A. The brightly fluorescent neurons of the locus coeruleus in a control brainstem. (x 116)

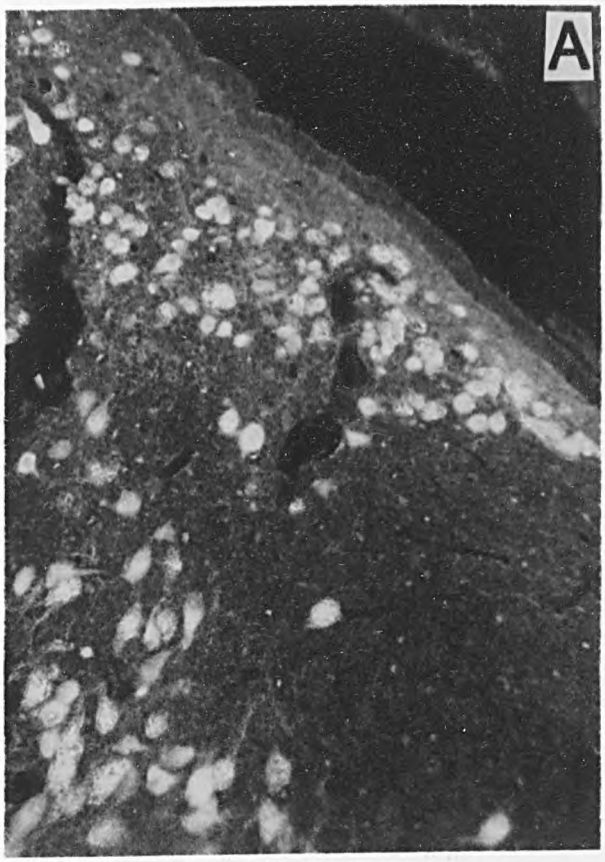
B. A low power micrograph showing fluorescent fibres in the molecular, Purkinje cell and granular layers of a folium. (x 116)

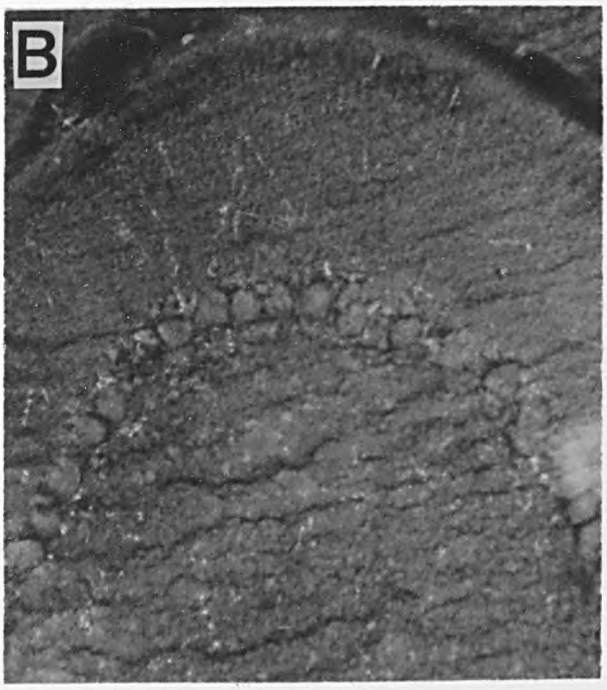
C and D. Horizontal fluorescent fibres in the Purkinje cell layer with collaterals ascending into the molecular layer (ML). (x 296)

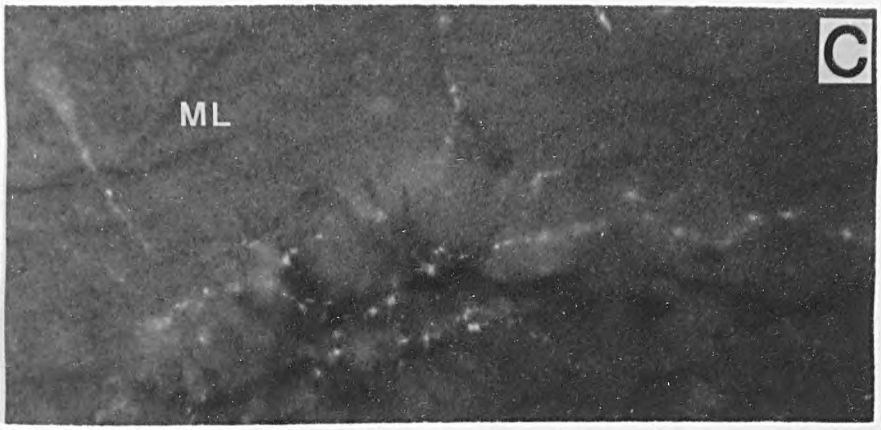
E. Fibres in the granular layer and a few terminals around the Purkinje cells (P). (x 296)

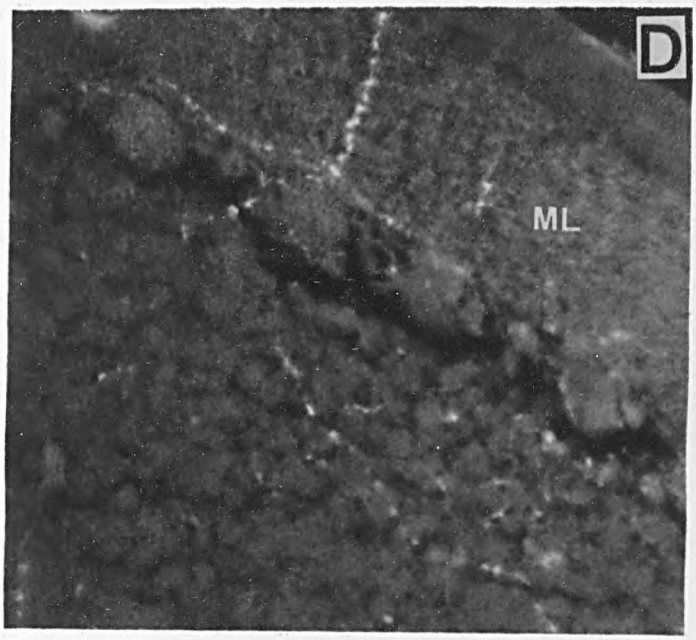
F and G. Fibres from the molecular layer can be followed right into the external granular layer (EL). (x 296)

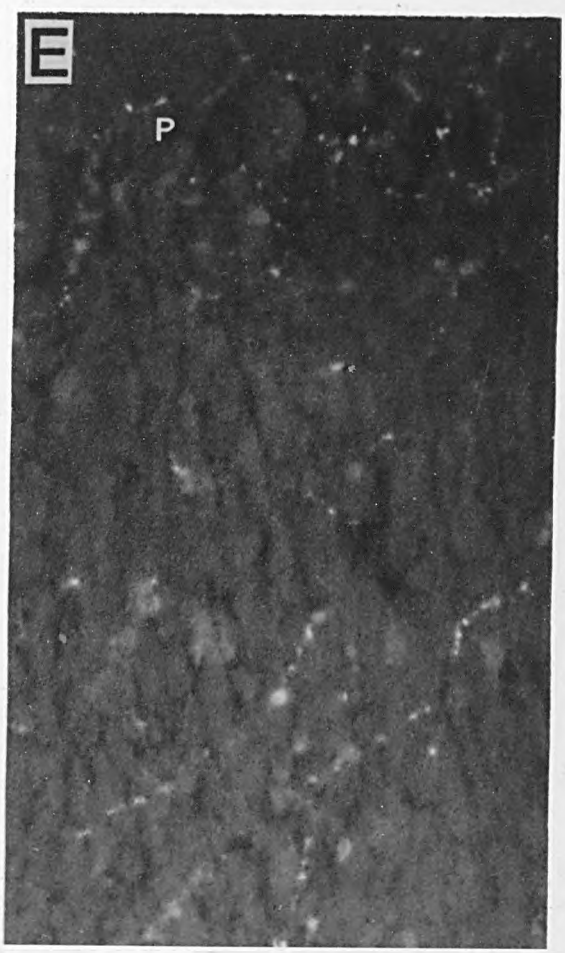
H and I. Fluorescent fibres ascending vertically (H) and obliquely (I) through the molecular layer from the Purkinje cell layer (PL). (x 296)

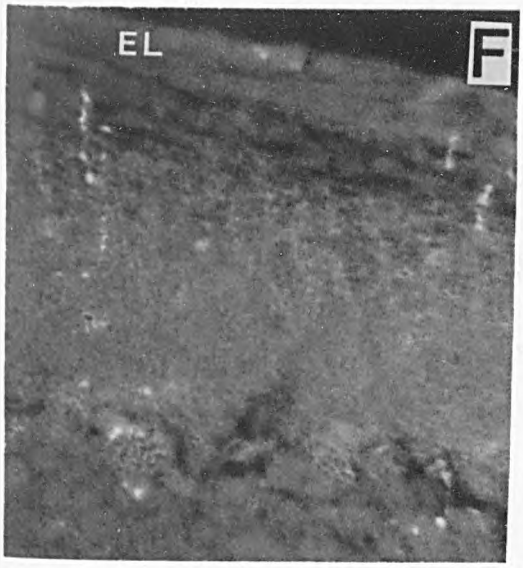

A

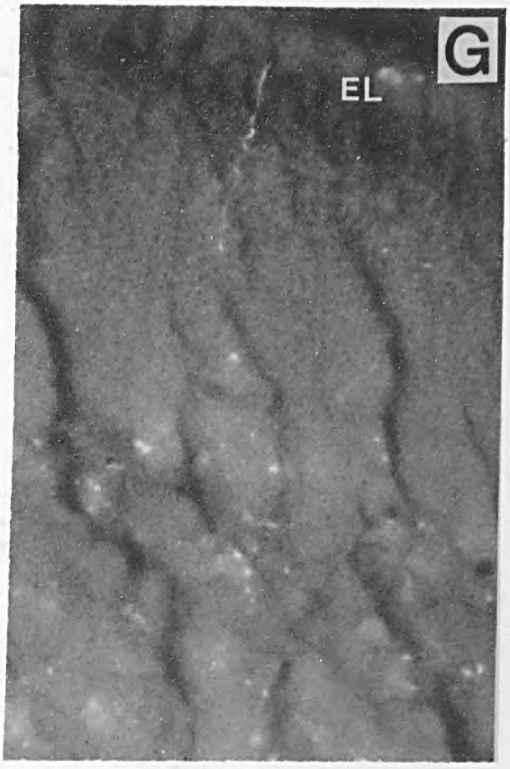

B

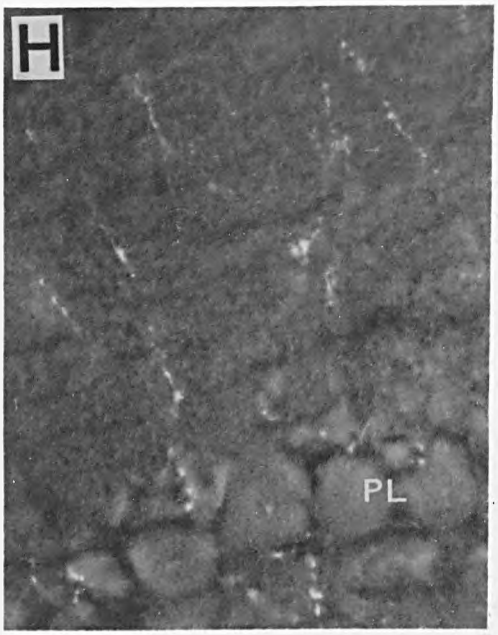

C
ML

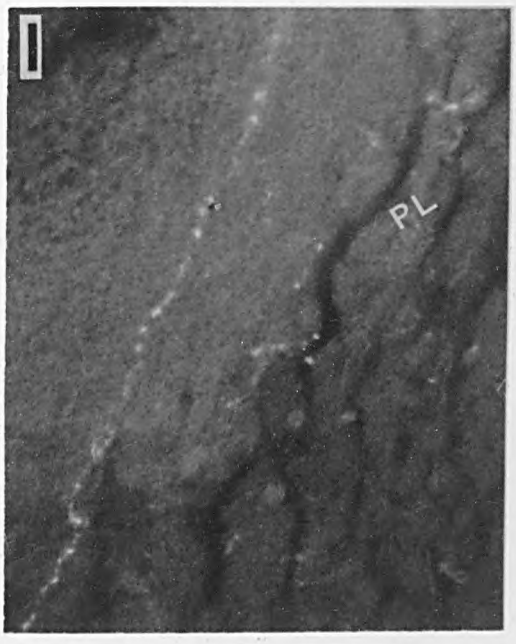

D
ML


E
P


EL
F


EL
G


H
PL


I
PL

CHAPTER SEVEN

AN AUTORADIOGRAPHIC INVESTIGATION OF THE OLIVOCEREBELLAR PATHWAY

INTRODUCTION

The olivocerebellar projection of climbing fibres has been described in chapter 1. The cerebellum is known to exhibit plasticity in response to surgical trauma both with its efferents (Lim and Leong, 1975; Castro, 1978; Kawaguchi et al., 1979) and its afferents (Payne and Bower, 1983). It was decided to investigate whether the intact inferior olive (ipsilateral to the pedunclotomy) could reinnervate the deafferented hemicerebellum, a phenomenon since found by Angaut et al. (1982) and Alvarado-Mallart et al. (1983). In order to try and discover whether or not these fibres were present in the immature brain, the neonatal olivocerebellar projection was also investigated both with and without a pedunclotomy. This study was also important because at the time there was only one brief anatomical study of this pathway (Dupont et al., 1981) however Sotelo et al. (1984) have recently made a detailed study of it in neonates aged 1 and 4 days. Therefore neonatal rats, aged 3, 7 or 10 days, received tritiated leucine in either inferior olive and underwent an acute left cerebellar pedunclotomy. Also animals which had undergone the pedunclotomy at the above ages and survived for 35 days had the isotope injected into the left inferior olive to study any aberrant cerebellar projections.

The technique used is based on procedures known to work in the nervous system (Cowan et al., 1972; Beckstead and Norgreen, 1979; Norman and Bower, 1982).

METHODS

PREPARATION OF THE ISOTOPE

The commercially available L-[4,5-^{3}H]leucine (Radiochemical Centre, Amersham) must be concentrated so that a sufficient specific activity can be injected in the small volumes required. To concentrate the L-[4,5-^{3}H]leucine, obtained with a specific activity of 1 mCi/ml, 1 ml is freeze dried using an electric vacuum pump until the residue is just still visible. The isotope can be stored in this form overnight in a deep freeze.

Just prior to use the isotope is rediluted in 0.05 ml of 0.9% saline, to give a specific activity of 20 mCi/ml.

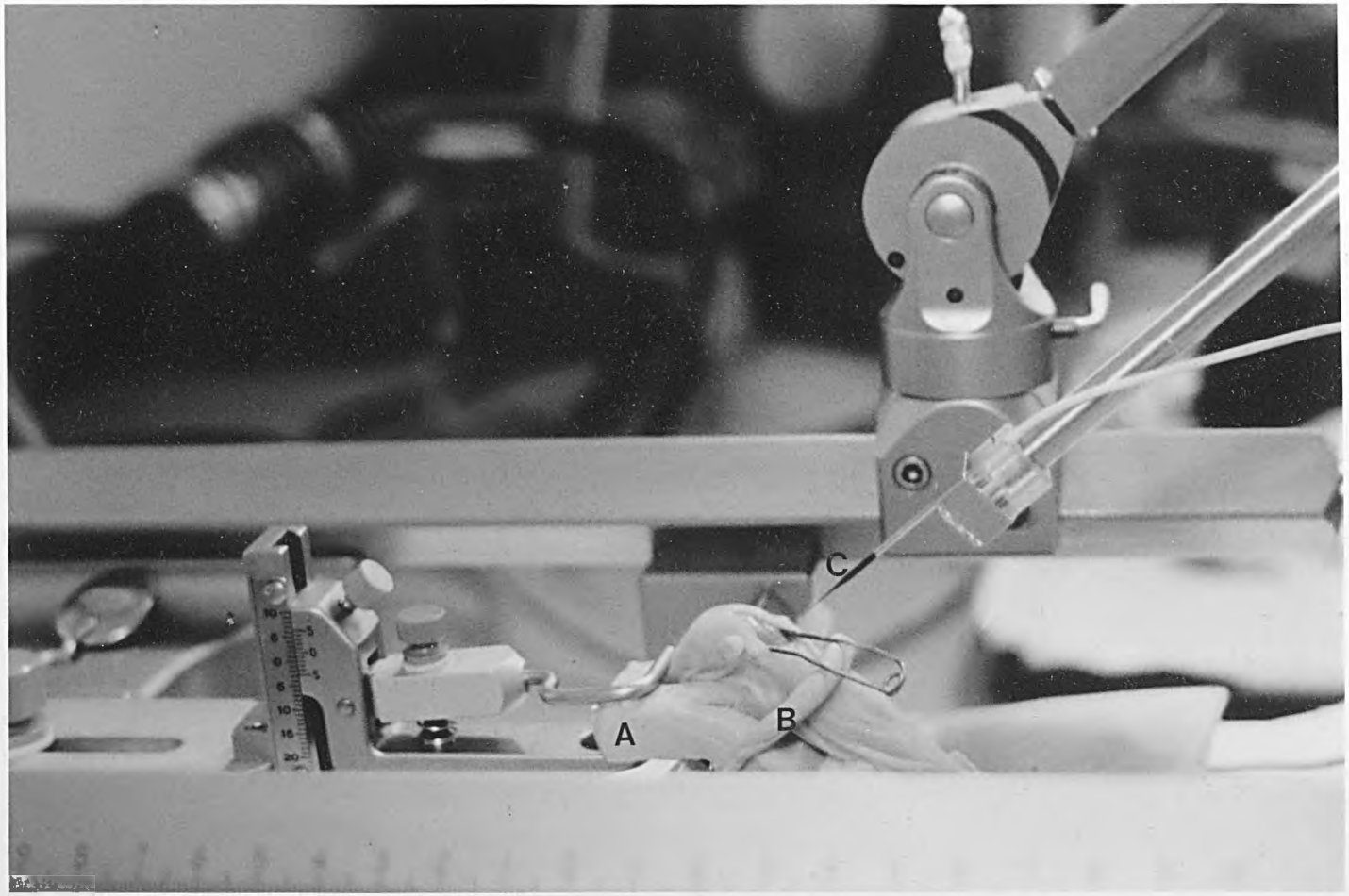
INJECTION INTO THE BRAINSTEM

Anaesthesia

The anaesthetic procedure used was different for the two groups of animals injected. Those undergoing injection with acute pedunclotomy could only be anaesthetised with ether because a weight-related dose of ketamine hydrochloride and pentabarbitone sodium was lethal. Because ether is short acting the neonates were returned to the ether jar as necessary during the operation. Those animals being injected following chronic pedunclotomy were anaesthetised with ketamine hydrochloride and pentabarbitone sodium as described in chapter 2.

Figure 7.1

These two photographs illustrate the method of holding neonatal rats in the stereotaxic frame for injection of radiolabelled material into the brainstem. The rats are held in a plastic mould (A) with Blue Tac (B) and the micropipette (C) (containing blue dye to improve its visibility in the photograph) is orientated at 50° to the vertical. The dissecting microscope (D) is used to position the micropipette accurately in the brainstem.


C
A
B

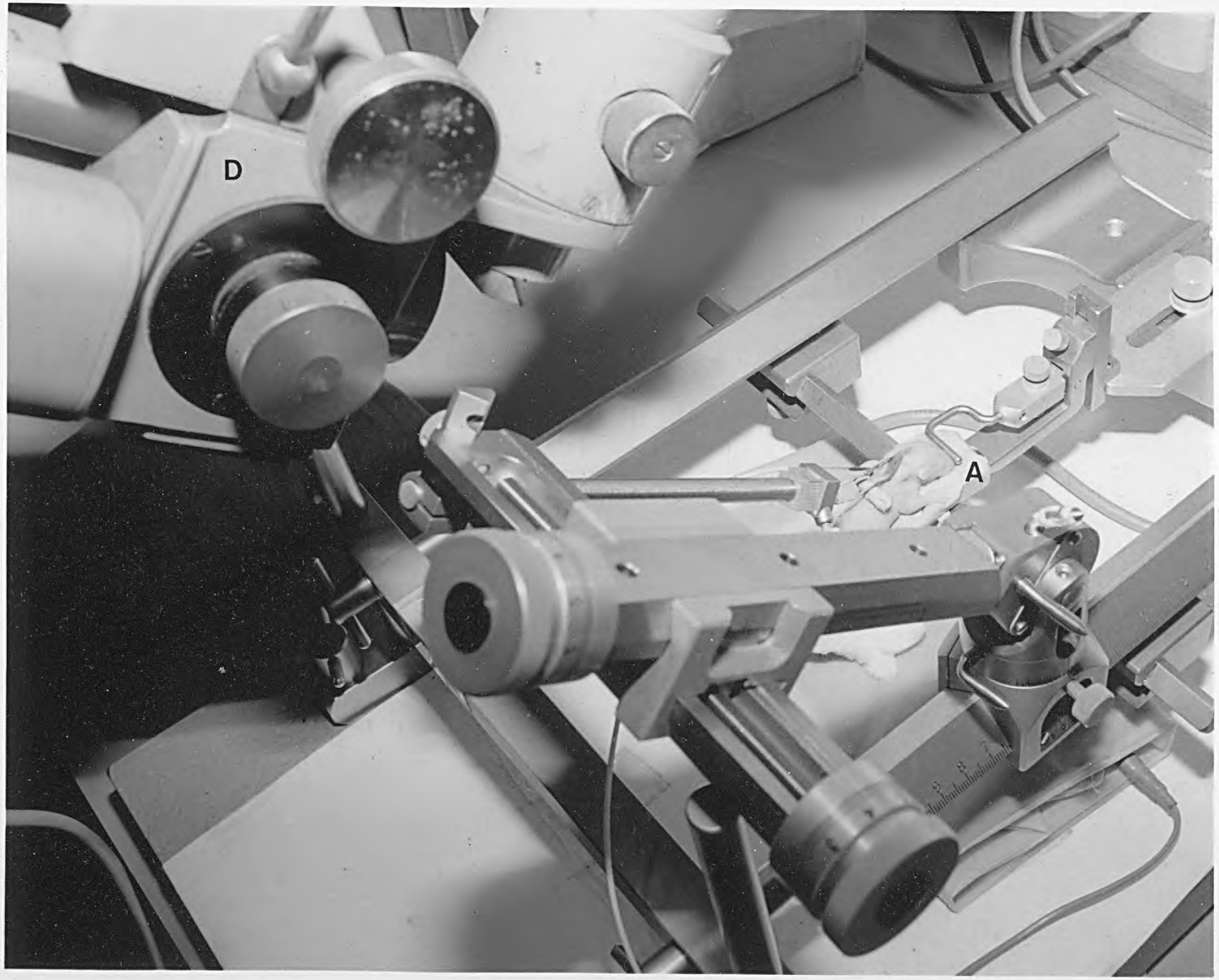
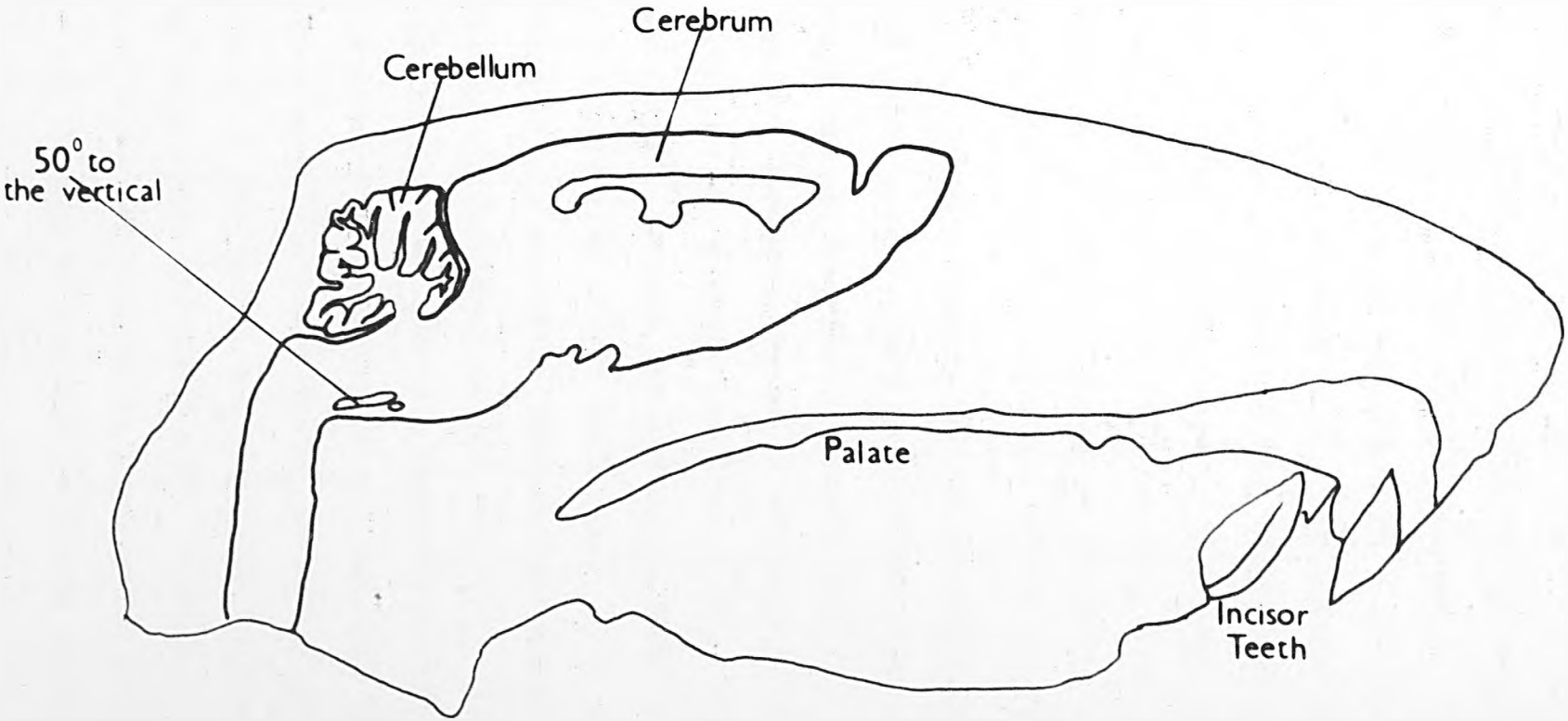

D
A

Figure 7.2

A sagittal section through a rat's head to illustrate the angle of micropipette insertion through the foramen magnum and brainstem to the inferior olive (Zeman and Innes, 1963).


Cerebrum
Cerebellum
50° to
the vertical
Palate
Incisor
Teeth

Injection

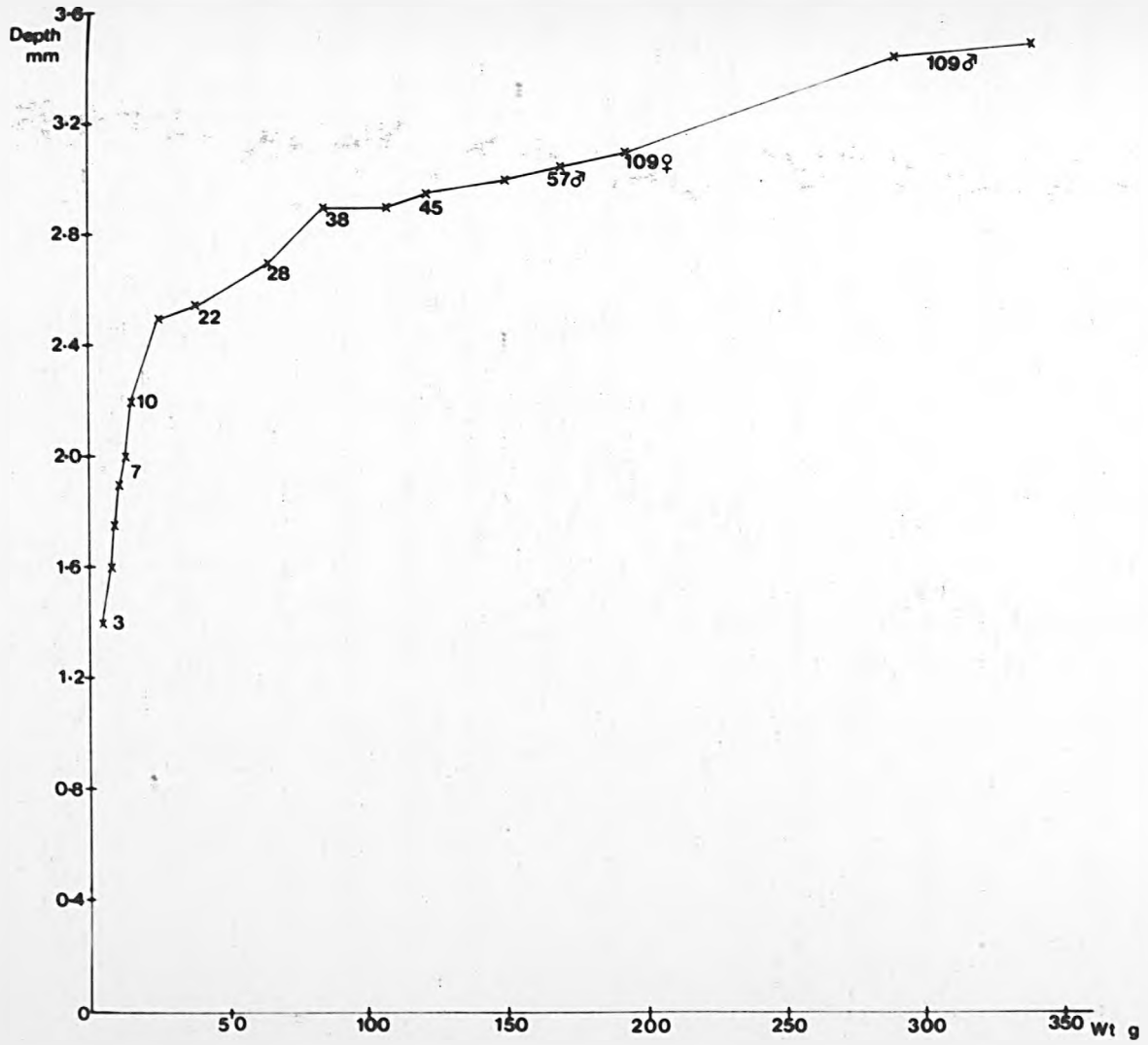
The animals are initially treated in the same manner as described for the pedunclotomy in Chapter 2. However for this procedure the neck muscles have to be more fully retracted using a small retractor. The atlanto-occipital membrane is fully cleared so that the underlying obex and dorsal columns can be distinguished. With the aid of a low power binocular (Nachet, NS 50) and cold light illumination the membrane is removed to expose the obex, both dorsal columns and the posterior part of the vermis.

The animal is then transferred to a Kopf stereotaxic frame. The older animals with chronic pedunclotomy can be fixed using the ear and upper incisor bars in the usual manner. However the young neonates are held in plastic moulds attached to the incisor bar (Fig. 7.1). The animal is orientated so that the top of its head is horizontal and its body lies almost vertical. This leaves the dorsal surface of the brainstem roughly horizontal and a point of flexion at the level of the obex (Fig. 7.2). Using a cold illumination Vicker's stereotaxic dissecting microscope a glass micropipette, of tip diameter 50-60 µm, is carefully orientated to penetrate the brainstem at 50° to the vertical on the dorsal column medial to the artery, 0.5 mm from the midline and level with the obex. The depth through which the pipette is inserted is measured from the dorsal surface of the brainstem and depends on the animal's weight. The values required can be found

Figure 7.3

This graph shows the relationship between body weight and the distance through the brainstem from the surface of the dorsal column nuclei to the midrostral region of the inferior olive. The measurements were made with the animal's neck flexed approximately at the obex, the floor of the 4th ventricle horizontal and the angle of insertion being 50° to the vertical.

The numbers lying on the curve show the age (in days) of animals with the relevant body weight.


Depth mm
3·6
3·2
2·8
2·4
2·0
1·6
1·2
0·8
0·4
0
5·0
100
150
200
250
300
350
Wt g
3
7
10
22
28
38
45
57♂
109♀
109♂

in Fig. 7.3. Between 50-100 nL (1.0-2.0 μCi) of isotope is slowly injected over 1·5 – 2 minutes using air compression.

Rats in the chronic pedunclotomy group have L-[4,5-^{3}H]leucine injected into the left inferior olive while the neonates undergoing acute pedunclotomy are injected into either the right or left inferior olive.

Postinjection Procedure

After the injection the young rats are operated upon for a cerebellar pedunclotomy in the manner previously described. However because the atlanto-occipital membrane has been removed in neither group of rats is the antibiotic sprayed into the wound because the cold shock to the unprotected brain was lethal. The skin is sutured with silk and sprayed with plastic dressing.

Both groups of rats were kept warm in boxes under a lamp until they had recovered from the anaesthetic, at which time the neonates are returned to their mother.

Discussion

Autoradiography, an anterograde tract-tracing technique, is used in this study because it will reveal all the terminals arising from the labelled inferior olivary neurons including any aberrant projections, which may be missed with a retrograde tracing technique. Autoradiography is preferable to using anterograde degeneration because climbing fibres are known to degenerate very quickly and be

difficult to stain (Desclin, 1974). Also lesions to an inferior olive will inevitably damage the climbing fibre projection from the contralateral side (this is only important in the neonatal animals) and consequently an apparently erroneous projection may be disregarded.

Tritiated leucine is chosen as the tracer because it is an aminoacid commonly incorporated into protein and is therefore likely to label all axon paths, unlike proline which has been shown not to label some neuronal connections (Kunzle and Cuénod, 1973). Tritium is a low activity isotope which gives high resolution for both climbing fibre terminals and inferior olivary neuron labelling.

Because of the relatively caudal position of the inferior olive within the brain, a foramen magnum approach was chosen in preference to either the burr-hole or retro-pharyngeal techniques since it is far less traumatic for the animals. Stereotaxic coordinates for any specific nucleus are available for adult animals of a particular weight and orientated in a particular manner (the incisor bar 5 mm above the level of the ear bars and the animal lying almost flat). The coordinates of the obex, the rostral and caudal limit of the inferior olive and the dorsal and ventral surfaces of the brainstem were obtained from a stereotaxic atlas (Pellegrino et al., 1979). The angle at the obex subtended by the rostral and caudal limits of the inferior olive was calculated by geometry. From this the angle and depth required to reach the

midpoint of the olive was deduced and allowing for rotation of the head this was expressed relative to the vertical axis - 29° posterior to the vertical and 3.1 mm deep. However this value could only be an approximate starting point because the distance is calculated for an adult animal and the geometry makes no allowance for any distortions in the brainstem incurred by flexion of the neck. An initial trial injecting 0.1% azure B (aq) revealed that 29° was too steep an angle of approach. A sagittal section through a whole rats head (Zeman and Innes, 1963) shows that the angle of approach should be between 45° and 50° to the verticle because when the neck is flexed the obex changes its position relative to both the foramen magnum and the inferior olive. Including this angle (50°) in the geometric calculations gives a rough estimate of the depth the micropipette must penetrate.

A pilot study was made injecting 0.1% azure B (aq) into rats of different ages to obtain the depth the micropipette had to be inserted to label the inferior olive. The weight/depth ratio is seen in Fig. 7.3.

The isotope is injected as slowly as possible because this increases its availability for uptake and minimizes any tissue damage (Cowan et al., 1972). This is particularly important for the young neonate because of the small amount of myelination in the immature brainstem. This reduces the tissue "grip" around the micropipette and allows massive backflow of the isotope, especially if it is not injected extremely slowly.

TISSUE PROCESSING

The animals were allowed to survive for up to 4 days and were then re-anaesthetised with ether and perfused with fixative. This survival time correlates with other autoradiographic studies of the olivocerebellar projection (Chan-Palay et al., 1978; Armstrong et al., 1982). Also three days is sufficient time for the whole path to be labelled by slow axon transport at a rate of 5 mm/day (Cowan et al., 1972). A Jelco I.V. catheter placement unit, either size 16G or 20G depending on the animal's age, was inserted through the left ventricle into the ascending aorta. The animals were perfused at 0.25 bar with 0.9% saline followed by 10% buffered formalin until at least 200 ml fixative had been used. The brains were rapidly removed from the skull and stored in fixative for at least a week.

Just before processing the brains were dissected to remove the forebrain and the superior colliculi. The remaining cerebellum and brainstem was dehydrated, cleared and embedded in paraffin wax with a Shandon-Southern 2L Processor. When the tissue is blocked out it is orientated so that coronal sections can be cut.

Sections 10 µm thick were taken every 200 µm from the caudal end of the inferior colliculus. They were expanded on distilled water and dried onto 'subbed' slides (Appendix III) warmed to 45°C in order to adhere the sections to the slide. Subbed slides are used in preference to clean

slides smeared with glycerin albumin because the albumin can react with the emulsion and because it improves the adherence of the emulsion to the slide and reduces lateral displacement, with resultant stress artefacts, during drying (Rogers, 1979 p 15).

PREPARATION OF AUTORADIOGRAPHIC SLIDES

The sections were dewaxed in at least two changes of xylene, preferably overnight, then rehydrated through alcohols to distilled water. The slides must drain thoroughly because any excess water on them would over-dilute the nuclear emulsion coating.

The emulsion preparation, slide coating and developing were all carried out in a dark room using a Kodak Safelight filter No. 2.

Emulsion Preparation

The nuclear emulsion, L4 (Ilford Ltd., U.K.), was bought in a gel form and was melted in a water bath at 43°C and diluted 1:1 with prewarmed 1% aqueous glycerol. The glycerol is important to maintain the emulsion's plasticity so it will not crack during drying. The mixture was gently stirred to ensure an even mix and allowed to stand for 10-15 minutes to let any air bubbles rise to the surface.

Section Coating

Before coating tissue sections with the emulsion it is advisable first to test the consistency of the emulsion by

coating a clean glass slide.

Slides placed back to back were coated with nuclear emulsion by being dipped vertically into the liquid and slowly withdrawn at a steady rate to ensure an evenly thick emulsion layer. The slides were separated, turned horizontal, the backs wiped clean, and placed on a cold plate to gel for 5 minutes. After gelling the slides were packed in trays and left to dry for a further 2 hours in a humid atmosphere, which prevents the emulsion from cracking as it dries. Trays of dry slides were stored with a desiccant, anhydrous calcium chloride, in light-tight boxes at 4°C during the exposure period. A desiccant is important to reduce latent image fading through the presence of excess water vapour.

Slides were exposed for 3-5 days for the injection site and 3-4 weeks for the projection site because this will be less heavily labelled.

Control Chemography

Positive and negative chemography controls are essential to ensure that any results are due to the isotope and not to a reaction between the tissue and the emulsion, which may be either falsely positive or negative (Rogers, 1979).

Positive Chemography. Sections of cold tissue were processed for autoradiography exactly as described above. If there is a reaction between the tissue and the emulsion (positive chemography) then there will be a higher grain

density over the section than on other areas of the slide.

Negative Chemography. Radioactive material identical to that used in the experiment is processed as normal but just before it is stored for exposure, it is fogged with light. Any light patches overlying the tissue on what should be a completely black slide indicate that the tissue may leach out silver ions (negative chemography).

Developing

After exposure the slides were restacked in staining racks. They were immersed for 4 minutes in Kodak D19 diluted 1:1 and maintained at 18°C. The slides were left undisturbed to minimize the variation of image formation during developing. The slides were rinsed in distilled water then fixed in freshly made 30% sodium thiosulphate for 4.5 minutes with intermittent agitation.

Sections must be rinsed for at least 30 minutes in running tap water before counterstaining with cresyl fast violet (Appendix IV).

Microscopy

Autoradiographs were viewed with a Leitz MPV2 microscope using both incident and transmitted light.

Figure 7.4

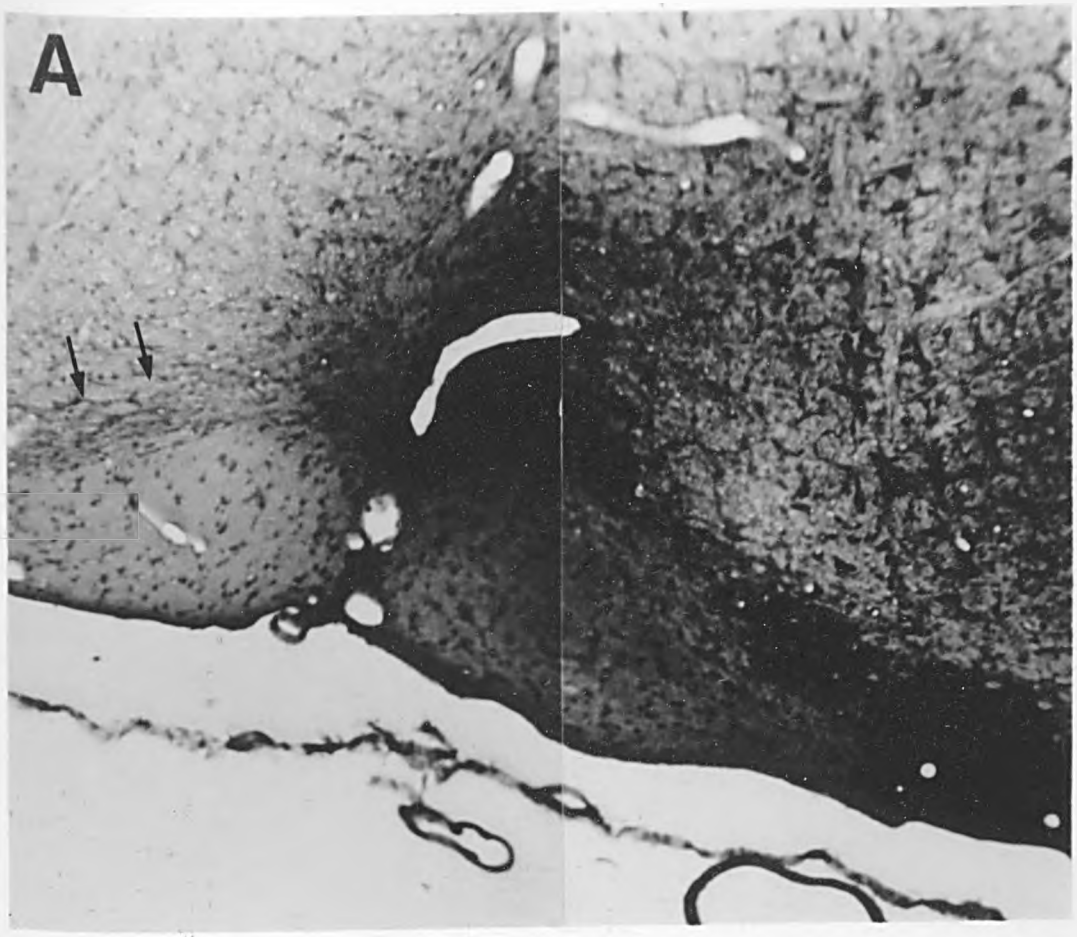
A. The injection site into the left inferior olive 35 days after pedunclotomy on day 10. Although the injection site does spill across the midline there are no surviving inferior olivary neurons to be labelled. The efferent fibres from the labelled inferior olive can be seen traversing the site of the degenerated right olive (arrows). (x 74)

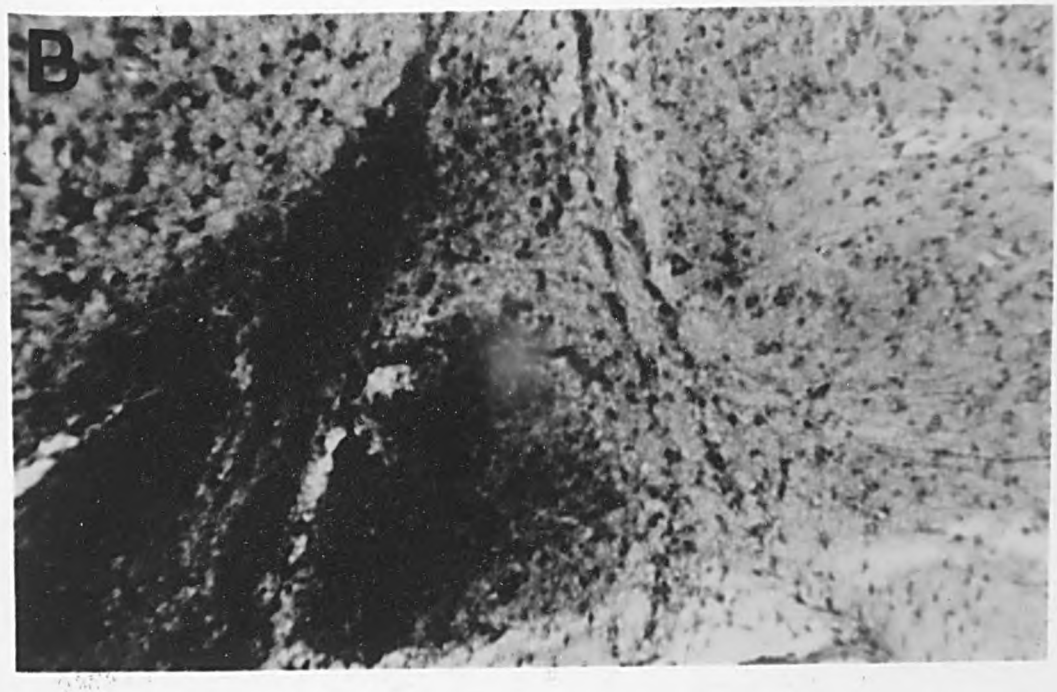
B. An injection site in the right inferior olive two days after injection and pedunclotomy on day 3, which clearly has not crossed the midline. The density of labelling is caused by the long 3 week exposure time. (x 116)

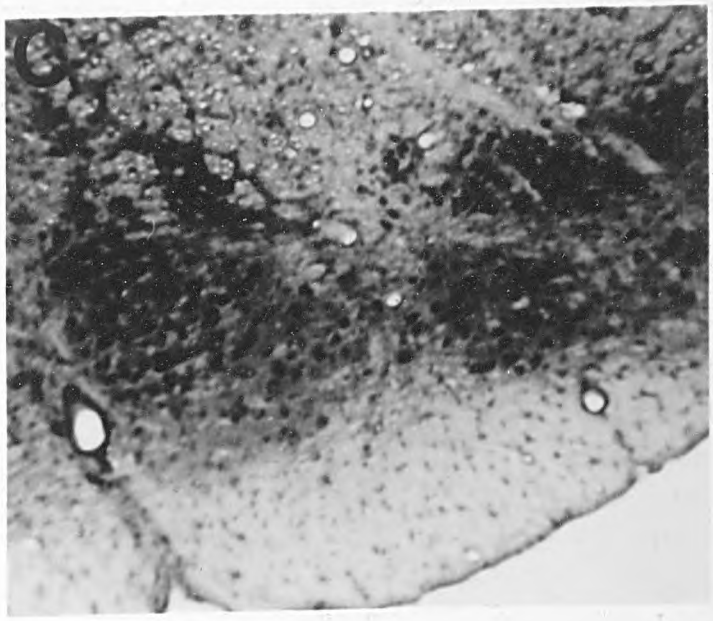
C. The injection site in the left inferior olive 35 days after pedunclotomy on day 3, which is well confined to the inferior olivary nucleus. (x 74)

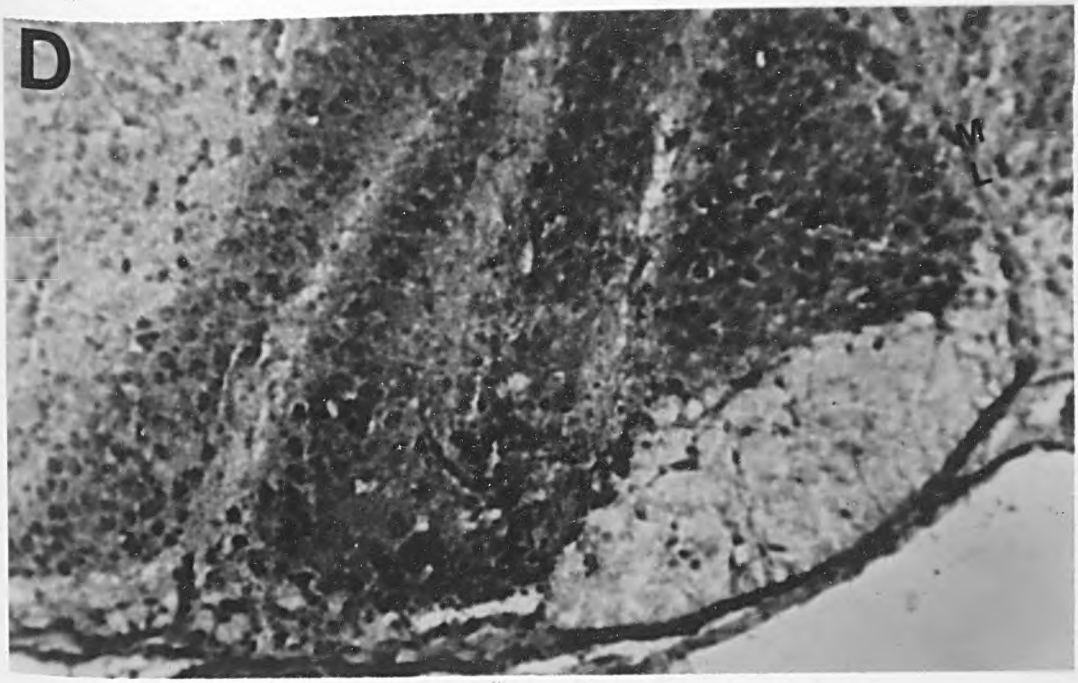
D. This injection site in the right inferior olive one day after injection and pedunclotomy on day 3 demonstrates how extensively the nucleus can be labelled even with a small injection (≃ 70nl). Even after three days exposure the neurons are more densely labelled than the neuropil and there is no evidence of a spill across the midline (ML). (x 116)

E. An injection site in the right inferior olive four days after pedunclotomy and injection on day 7. The individual neurons are difficult to distinguish especially when compared with their appearance one day after pedunclotomy (D). The exposure time for this injection site was 3 weeks and the silver grains on the left of the midline overlie the numerous efferent fibres from the right inferior olive and there is no evidence of neuronal labelling. (x 116)


A


B


C


D
W
L


E

Figure 7.5

This montage shows the injection site in the right caudal medial accessory olive (equivalent to caudo-rostral level 560 from Gwyn et al., 1977) from a neonate which received both left pedunclotomy and radioisotope injection on postnatal day 3. Despite a small injection (≈ 50nl) and a 3 day exposure labelled neurons (e.g. arrowed) are covered with a greater density of silver grains than is the surrounding tissue. In addition the injection site does not stray across the midline (ML) into the contralateral inferior olive. (x 296)

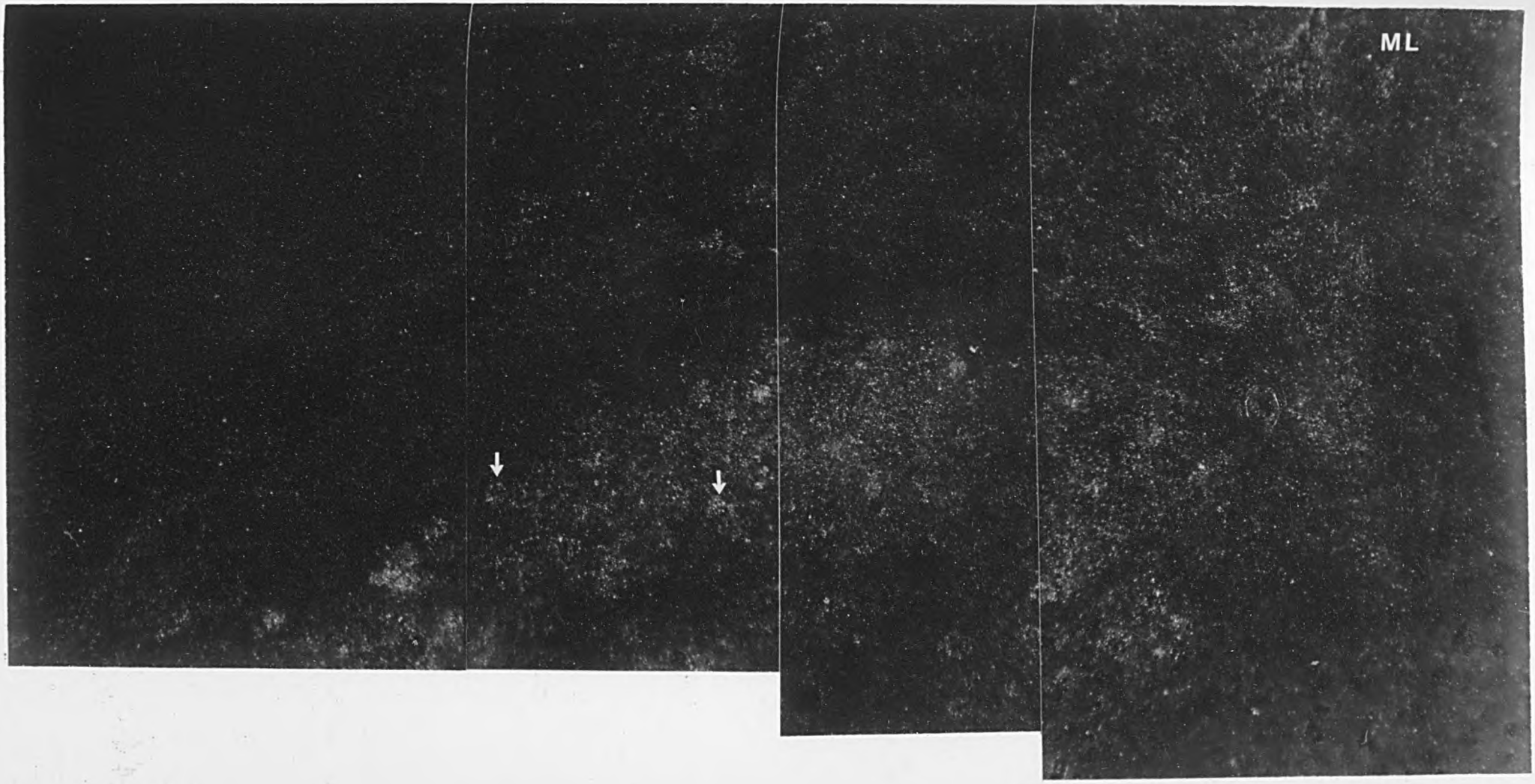
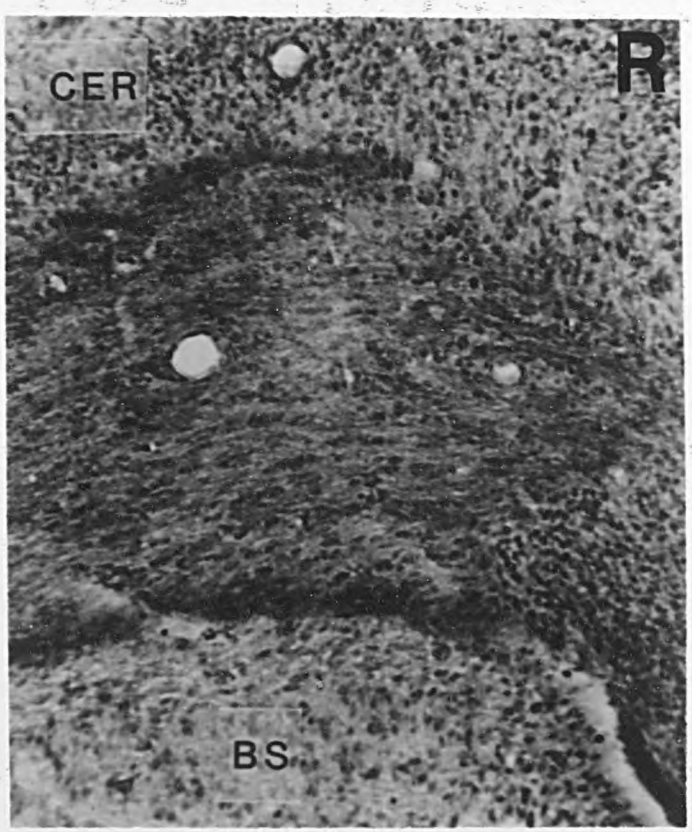
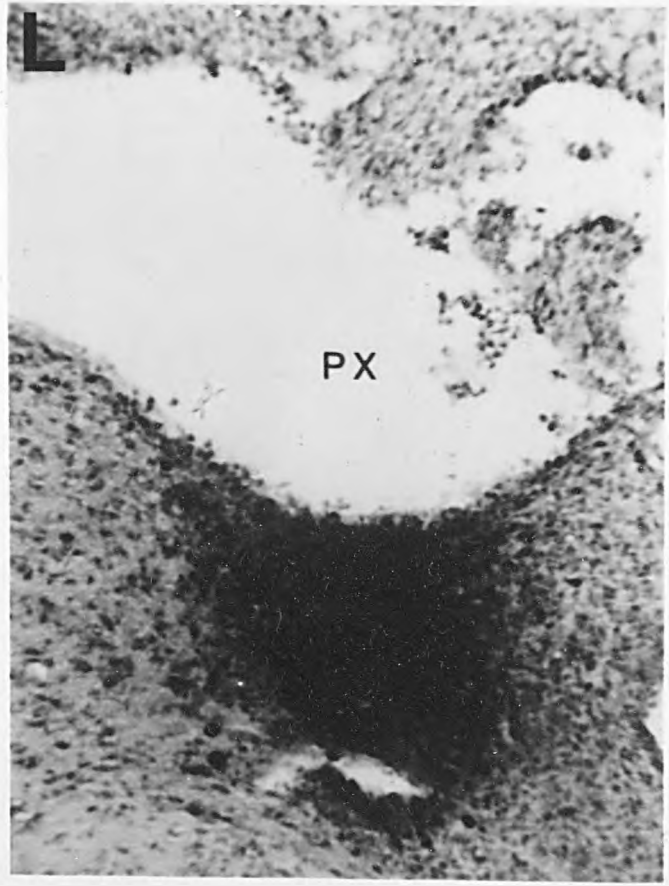

ML

Figure 7.6

These two micrographs are taken from a neonate which underwent left pedunclotomy and right inferior olivary injection on day 7. On the right side (R) the inferior peduncle containing silver-labelled axons can be traced from the brainstem (BS) through to the cerebellum (CER). But on the left (L) the inferior peduncle is completely destroyed (PX) and an excess of label has built up in the axons proximal to the lesion. (x 74)


CER
R
BS


L
PX

RESULTS

CRITERIA FOR INCLUDING DATA IN THE RESULTS

Because autoradiography requires the observation of silver grains formed in an emulsion overlying radioactively labelled tissue a set of criteria have to be established to ensure that any labelling is relevant to the experiment and not due to non-specific reactions within or upon the emulsion.

If after four days exposure, cells within the injection site were covered by a greater density of silver grains than was found overlying the neuropil then they were considered to have actively taken up the tracer. In addition, the injection sites were checked in autoradiographs which had exposed for 3 weeks. For animals in the chronic experiments which had survived 35 days after pedunclotomy, in addition to labelling the left inferior olive, the left lateral reticular nucleus and the right inferior olive had to have degenerated. In neonatal animals, which received a left cerebellar pedunclotomy, the injection must not have crossed the midline and thus labelled neurons in the contralateral inferior olive. Neurons of the right inferior olive also had to be degenerating since not all the neurons had disappeared by 4 days post pedunclotomy. However, there was one set of exceptions to the criterion for labelled cells within an injection site. In the acute experiments which had a 4 day post operative survival time the majority of the neurons of the right inferior olive had degenerated. Therefore in those

animals in which the right inferior olive was injected the nucleus was considered to be successfully labelled if the area in the ventral brainstem usually occupied by it was densely covered with silver grains. The glial cells which infiltrated the degenerating nucleus did not take up the tracer since they were not related to any increased density of silver grains.

Since a nuclear emulsion will always contain some silver grains it is important to recognise genuinely labelled climbing fibre terminals from artifact. Also they must be distinguished from any mossy fibres which have been labelled by the inclusion of reticular formation neurons within the injection site. In the adult cerebellum the climbing fibres have a specific structure following the Purkinje cell dendritic tree. In coronal sections they lie as thin vertical bands in the lower two-thirds of the molecular layer and therefore the distribution of silver grains overlying labelled terminals must have the same appearance. In distinction, radioactive labelling of mossy fibre rosettes produces discrete clumps of silver grains over the glomerular islands in the granular layer. In neonatal animals the autoradiographic appearance of labelled climbing fibres can be deduced from the developmental histology. Between 5 and 10 days post partum the immature climbing fibres synapse upon the Purkinje cell perisomatic processes before ascending to the primary dendrites by day 12.

Figure 7.7

Developmental Climbing Fibre Histology

A. By postnatal day 5 the climbing fibres have reached the Purkinje cells (PC) and are beginning to grow round the somata. Therefore the labelled fibres are seen as cups of silver grains around the bases of the cells (arrows) and there is no labelling towards the external granular layer (EGL). (x 296)

B. By day 9 the climbing fibres have completely surrounded the Purkinje cell perikarya (arrowed) but have not ascended into the molecular layer (ML). (x 296)

C. On postnatal day 11 the climbing fibres are starting to ascend to the developing primary dendrites of the Purkinje cell and can be seen both around the perikarya and in the lower molecular layer (ML). (x 592)

D. In the adult cerebellar cortex the labelled climbing fibres ascend vertically through the molecular layer following the Purkinje cell dendritic tree. (x 476)

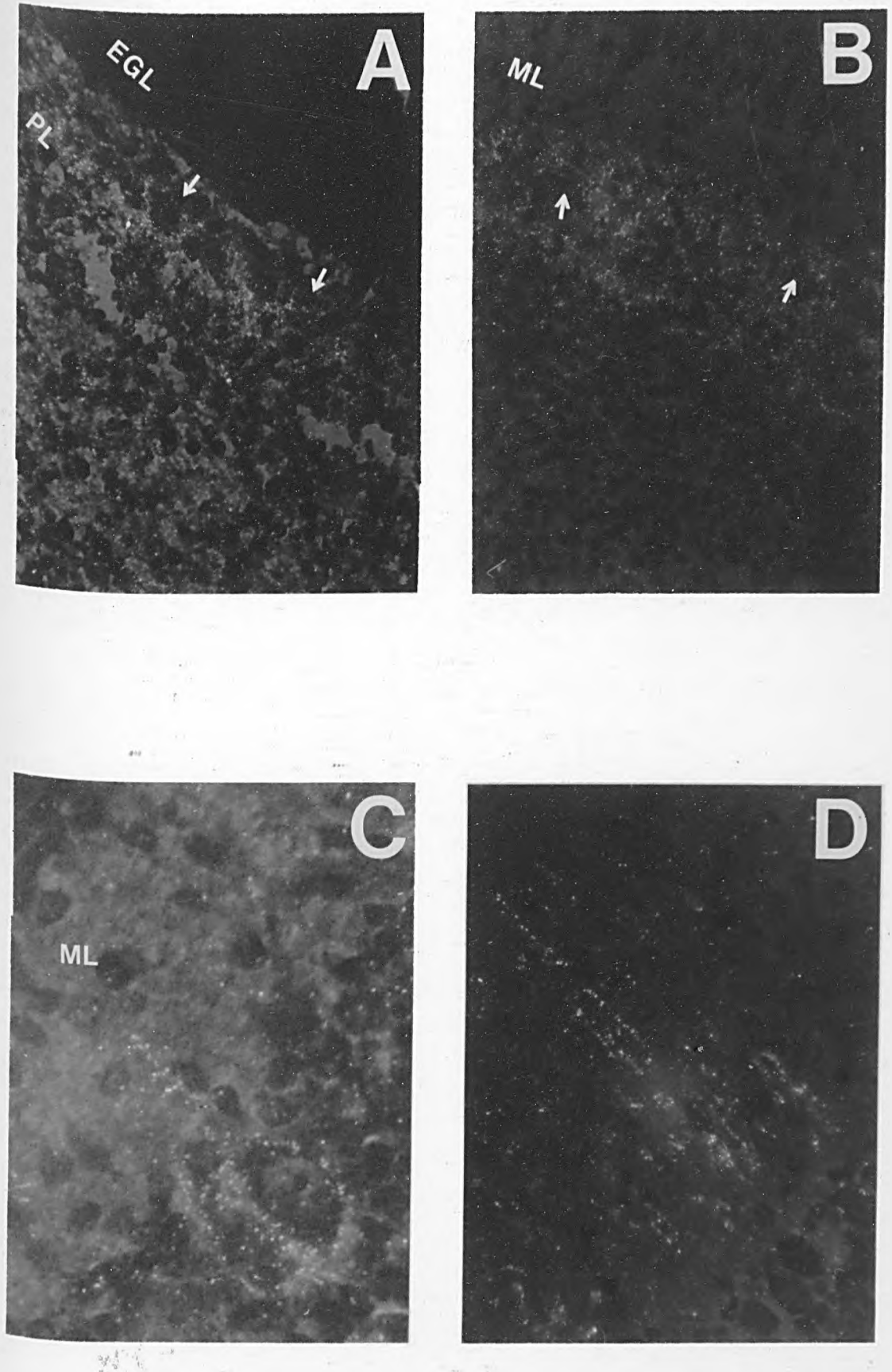
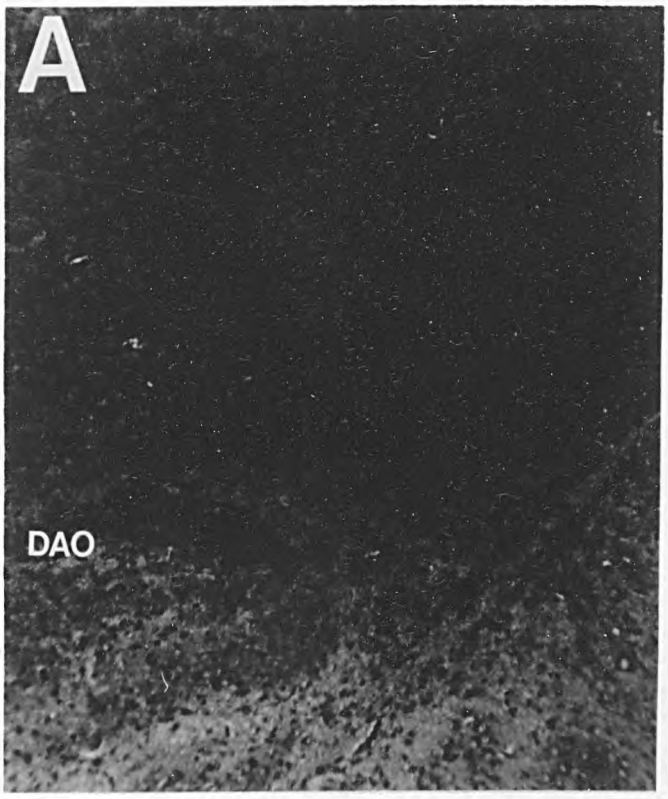
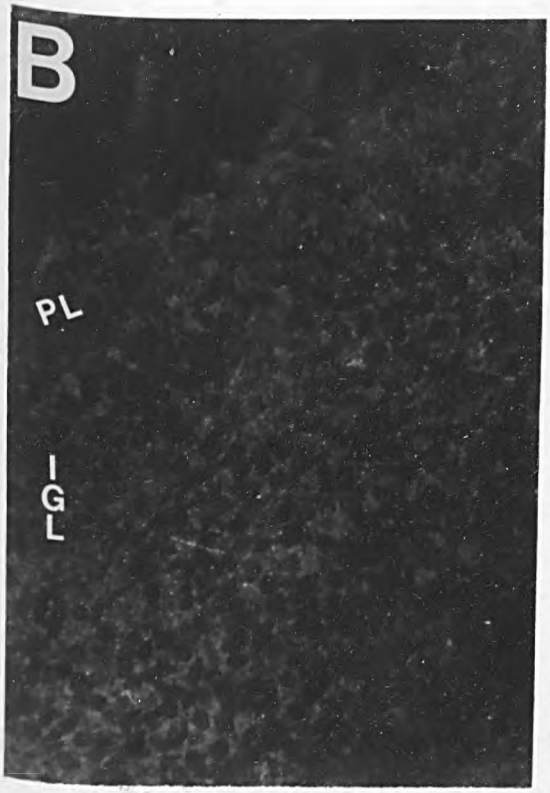

A
EGL
PL
B
ML
C
ML
D

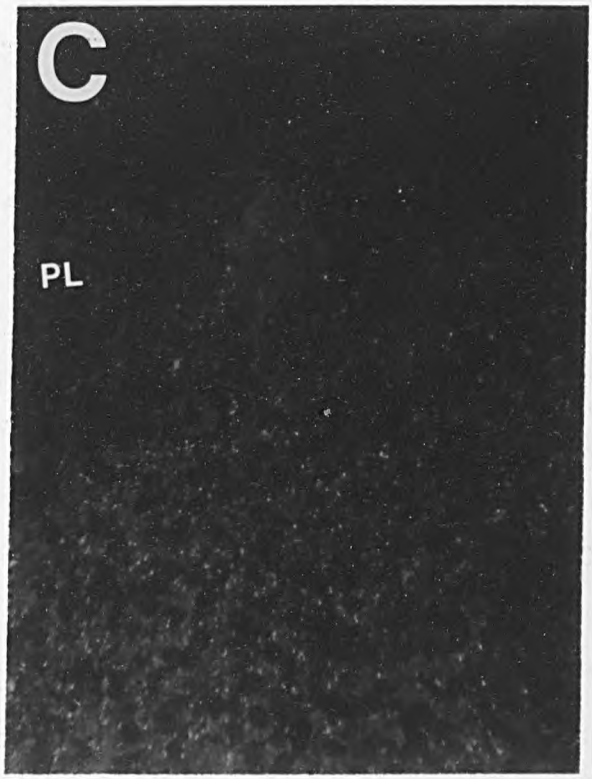
Figure 7.8

These micrographs show that even in young neonates aged 5 days the appearance of labelled climbing fibres which have reached the Purkinje cells (see Fig. 7.7) can be distinguished from that of mossy fibres.

The injection site (A x 74) is seen to be mainly in the reticular formation and just includes the medial right dorsal accessory olive (DAO). In the cerebellar cortex (B and C x 296) the labelled afferents arising in the reticular formation terminate in the white matter and internal granular layer (IGL) and do not extend up to the Purkinje cells which have almost formed a monolayer (PL).


A
DAO


B
PL
IGL


C
PL

Therefore the silver grains overlying labelled terminals form rings around the Purkinje cells (Dupont et al., 1981; Sotelo et al., 1984). In neonates aged 3 days the developing climbing fibres have barely reached the Purkinje cells and therefore the distribution of the silver grains lies more over the internal granular layer and just reaches the Purkinje cell layer. Unfortunately at this age mossy fibres have not developed their rosettes, therefore any mossy fibre labelling has a very similar appearance to the climbing fibres except that the silver grains stop short of the Purkinje cell layer. In these very young animals the results are difficult to interpret, however silver grains overlying the cerebellar cortex were considered to be produced by labelled climbing fibres if the silver reached the Purkinje cells and the injection site included a substantial part of the inferior olive.

CHRONIC EXPERIMENTS

Any changes in the olivocerebellar projection following neonatal cerebellar pedunclotomy were studied in animals which had undergone the pedunclotomy aged 3, 7 and 10 days and survived for 35 days, at which time the left inferior olive was injected with [^{3}H]leucine.

Histology

In all animals from each age group a large part of the left inferior olive was labelled and in the majority of cases the injection site encroached upon the reticular

Figure 7.9

Climbing Fibres from an Animal
Pedunclotomised on Day 7 and Injected
into the Left Inferior Olive after 35 Days

A. The normal histological appearance of climbing fibres ascending vertically through the molecular layer of the right paramedian lobule. (x 296)

B. This micrograph illustrates a single labelled climbing fibre which has a totally different appearance from the labelled mossy fibre rosette (MF). (x 296)

C. The climbing fibres in the left hemicerebellum are also heavily labelled although they have a more plexus-like morphology and do not reach the whole depth of the molecular layer. (x 296)

D. These labelled climbing fibres are seen in the left paramedian lobule and their differing histological appearance can be directly compared with those seen in the contralateral side (A). (x 296)

E. This micrograph shows labelling in both the white matter and molecular layer in the left lateral vermis. However there is almost no labelling in the granular layer, which demonstrates that it is the climbing fibres which have been labelled. (x 296)

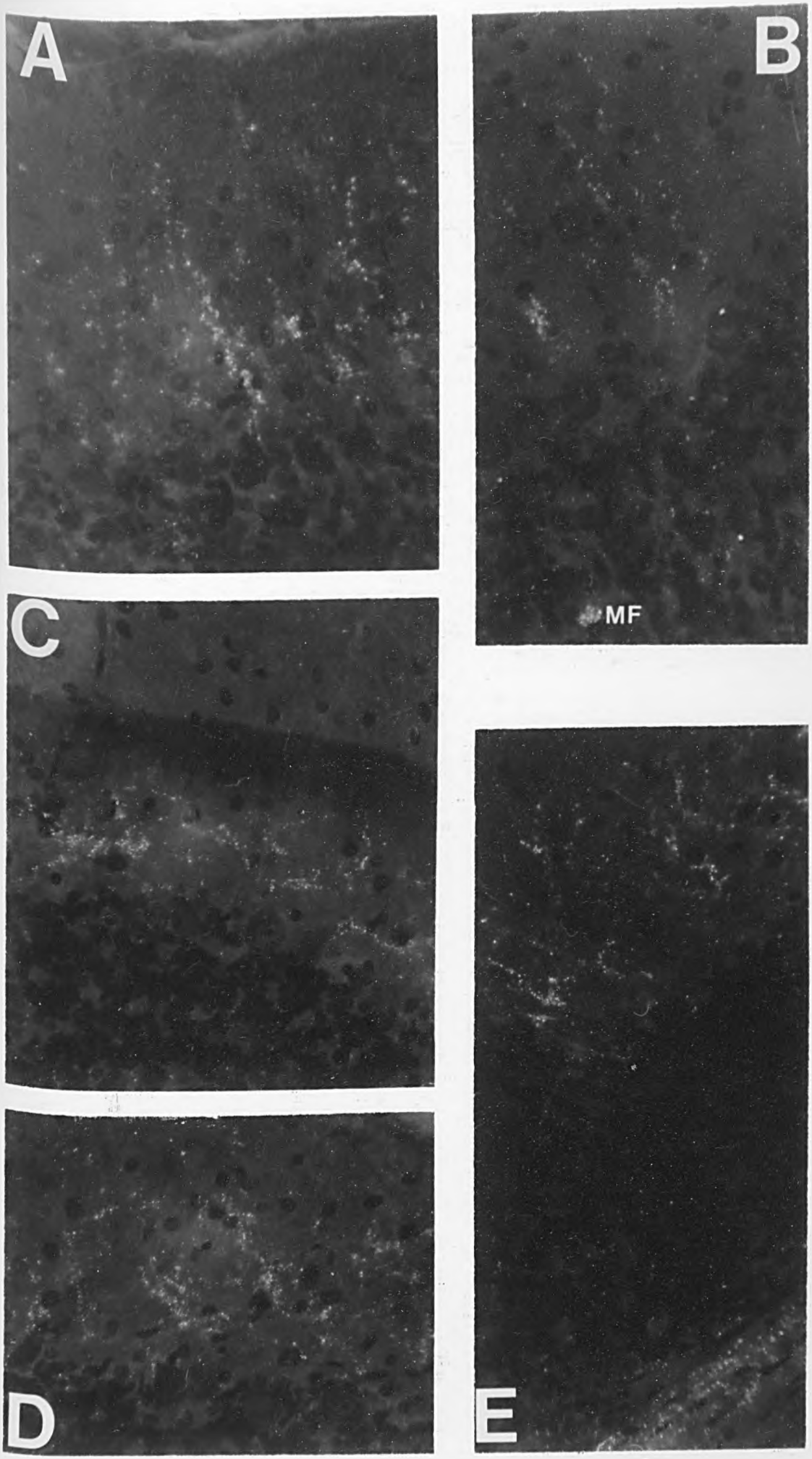

A
B
MF
C
D
E

Figure 7.10

These three micrographs (x 476) illustrate the labelled climbing fibre histology in animals injected 35 days after pedunclotomy on day 10. Since these fibres were only seen in the right hemicerebellum the normality of their appearance was expected.

formation. In some animals a few neurons at the centre of the injection site had been destroyed. Axons from the inferior olive crossed the midline and passed through the site of the degenerated right inferior olive to converge at the lateral edge of the brainstem. The bundle of fibres turned dorsally and travelled in a dorsorostral direction to enter the cerebellum through the right inferior peduncle. Labelled fibres passed around the deep cerebellar nuclei in their course towards the cortex. Discrete labelled mossy fibre rosettes were often present in the granular layer but most of the cortical labelling was found in the molecular layer. The labelled climbing fibres appeared as thin rows of silver ascending vertically through the molecular layer. The labelled fibres were grouped together into distinct bands which were continuous through all lobules in a single sagittal plane. These labelled strips of cortex alternated with strips which were either not labelled or only very faintly labelled. However they were not a uniform width, in some lobules almost the whole mediolateral extent was positively labelled and in others the labelling was found in thinner clearly alternating bands.

Olivocerebellar Projection

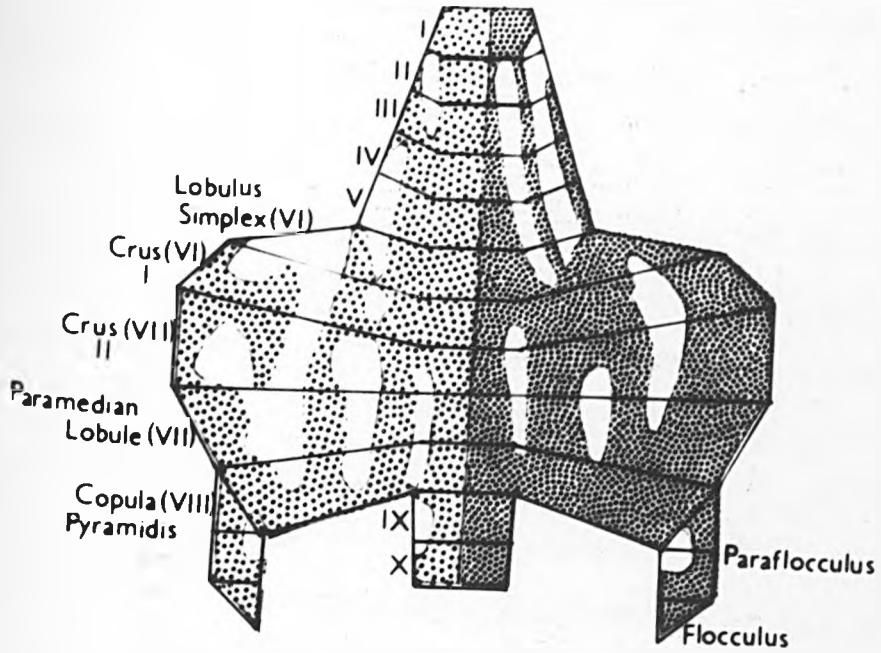
Although the histological appearance of labelled climbing fibres was the same in control and experimental animals, irrespective of the age at operation, the general topography of the olivocerebellar projection was not.

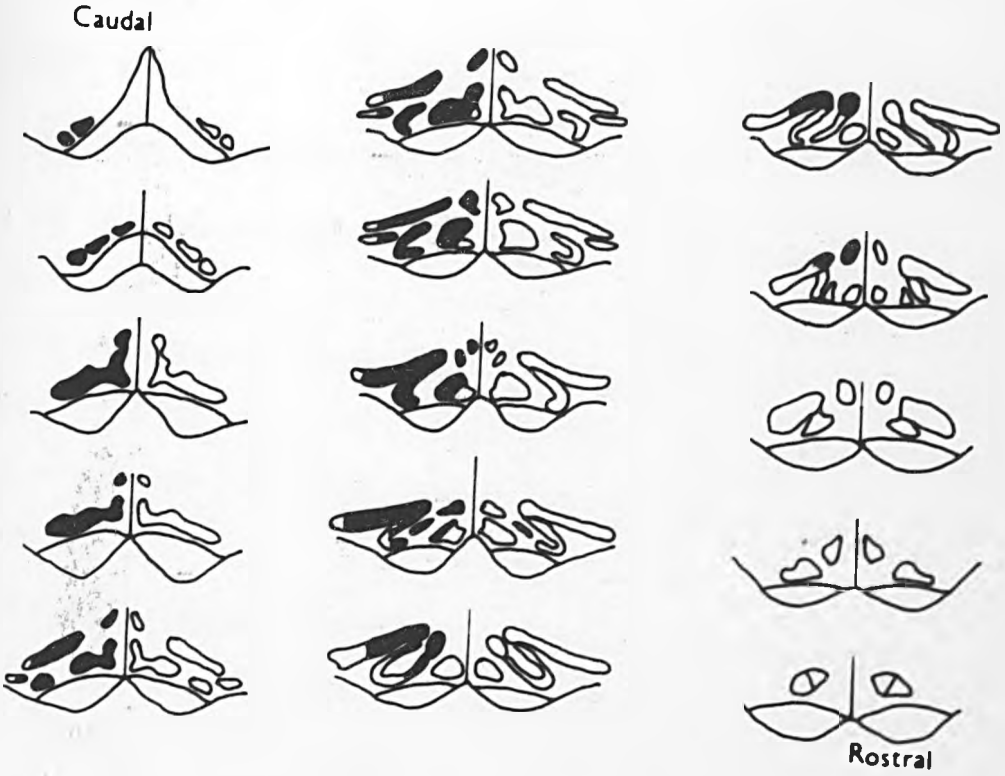
Figure 7.11

The Olivocerebellar Projection of an Animal Pedunclotomised on Day 3 and Injected After 35 Days

In the cerebellum the climbing fibre labelling was heavy in the right hemicerebellum (heavy stippling). The unshaded areas did not contain labelled climbing fibres.

In the diagram of the inferior olive the shaded areas show the extent of the injection site within the inferior olive. In the pedunclotomised rats the right inferior olive is missing.


I
II
III
IV
V
Lobulus Simplex (VI)
Crus (VI) I
Crus (VII) II
Paramedian Lobule (VII)
Copula (VIII) Pyramidis
IX
X
Paraflocculus
Flocculus


Caudal
Rostral

Because a large part of the inferior olive was labelled in most animals, the fine detail of the olivocerebellar topography could not be analysed.

Control Animals

In the control animals aged 38, 42 or 45 days the regional labelling within the cerebellum was highly consistent considering in no two animals was the injection site identical. If the injection site was confined to a single inferior olive then the climbing fibre labelling within the cerebellum was solely contralateral. Any molecular layer labelling seen ipsilateral to the injection site could always be accounted for by encroachment of the injection site into the medial part of the opposite inferior olive.

Animals Pedunclotomised Aged 3 Days

In this experimental group four animals fulfilled the criteria for a successful inferior olivary injection and unilateral pedunclotomy. In all these animals the left cerebellar hemisphere was smaller than the right hemisphere and the regions labelled within the cerebellum were very similar.

After an injection of [^{3}H]leucine into the left inferior olive extensive climbing fibre labelling was found in the right hemisphere and the right half of the vermis. These fibres were of the same histological appearance as was seen in control animals and in some

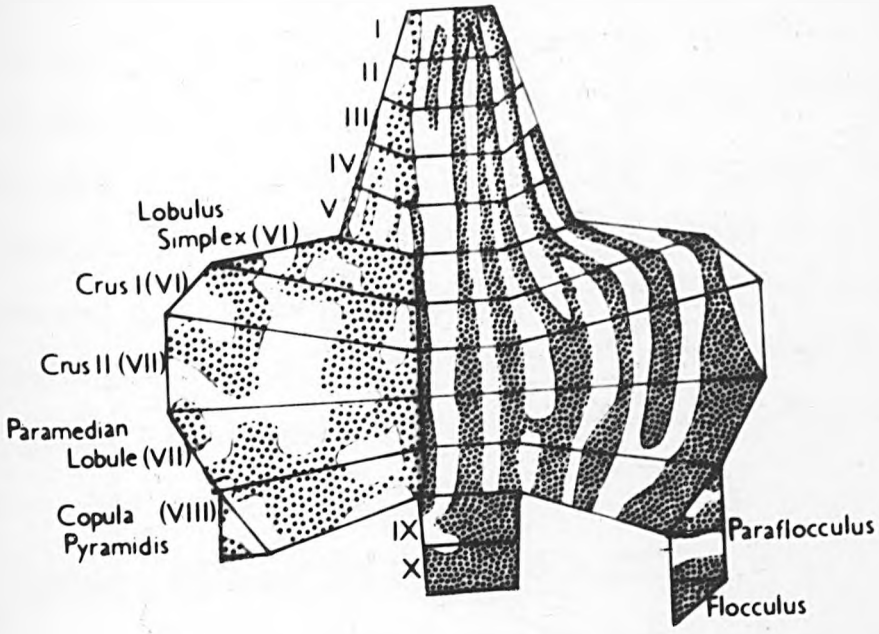
Figure 7.12

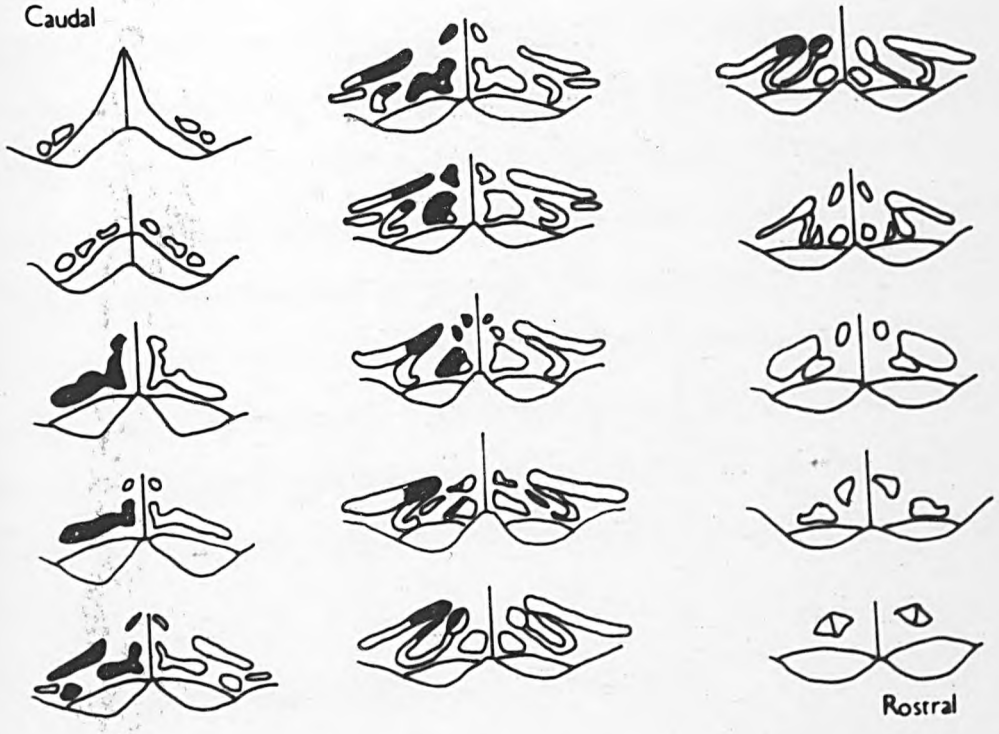
The Olivocerebellar Projection from a Rat Pedunclotomised on Day 7 and Injected After 35 Days

The cerebellar cortical labelling is very precisely organised into sagittal bands in the right hemicerebellum but this is much less heavy and less clearly seen in the left hemicerebellum.

The ventral paraflocculus and flocculus on the left side were often missing in the experimental animals probably through damage during the pedunclotomy.

The conventions are the same as those in figure 7.11.


I
II
III
IV
V
Lobulus Simplex (VI)
Crus I (VI)
Crus II (VII)
Paramedian Lobule (VII)
Copula (VIII)
Pyramidis
IX
X
Paraflocculus
Flocculus


Caudal
Rostral

lobules were grouped into sagittal bands. In addition to this, molecular layer labelling was also found in the left hemicerebellum as far lateral as the paraflocculus. These fibres had the appearance of lightly labelled climbing fibres seen both in control animals and the contralateral hemicerebellum. Not only was this labelling in the left hemicerebellum lighter than on the right but the pattern of labelling was almost a mirror-image of that seen in the right hand cortex, the major difference was that the sagittal bands were less obvious.

Animals Pedunclotomised Aged 7 Days

Four animals fulfilled the criteria to be included in this group. A left inferior olivary injection produced extensive climbing fibre labelling in all regions of the right hemicerebellum which was clearly organised in distinct sagittal zones. As was found in the 3 day pedunclotomy group, labelled climbing fibres were also found throughout the left hemicerebellum although the density of silver grains was much lower than in the right hemicerebellum. Also, unlike the animals pedunclotomised at 3 days, the areas of labelling and the sagittal banding in the left hemisphere were not symmetrical to those seen in the right hemisphere.

Animals Pedunclotomised Aged 10 Days

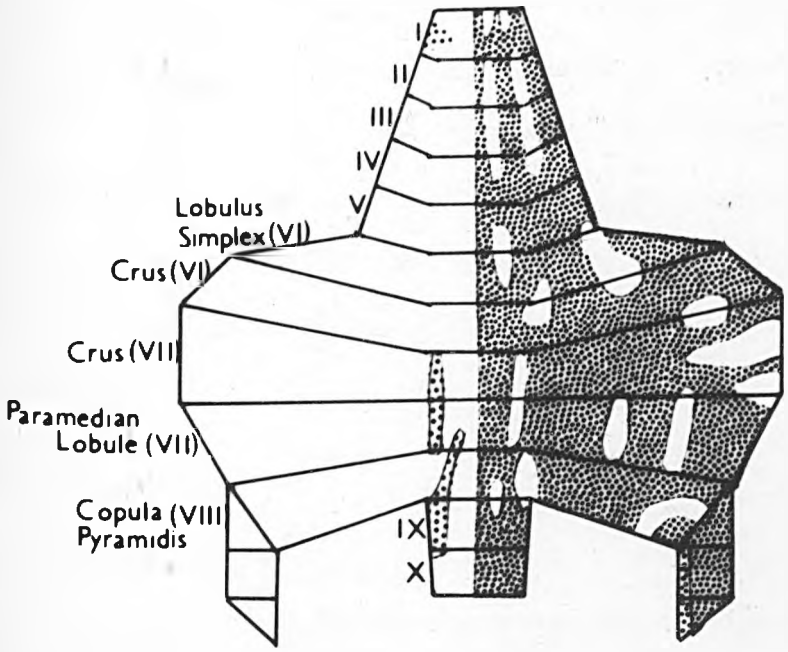
Four experimental animals were included in this group. The olivocerebellar projection in these animals was almost

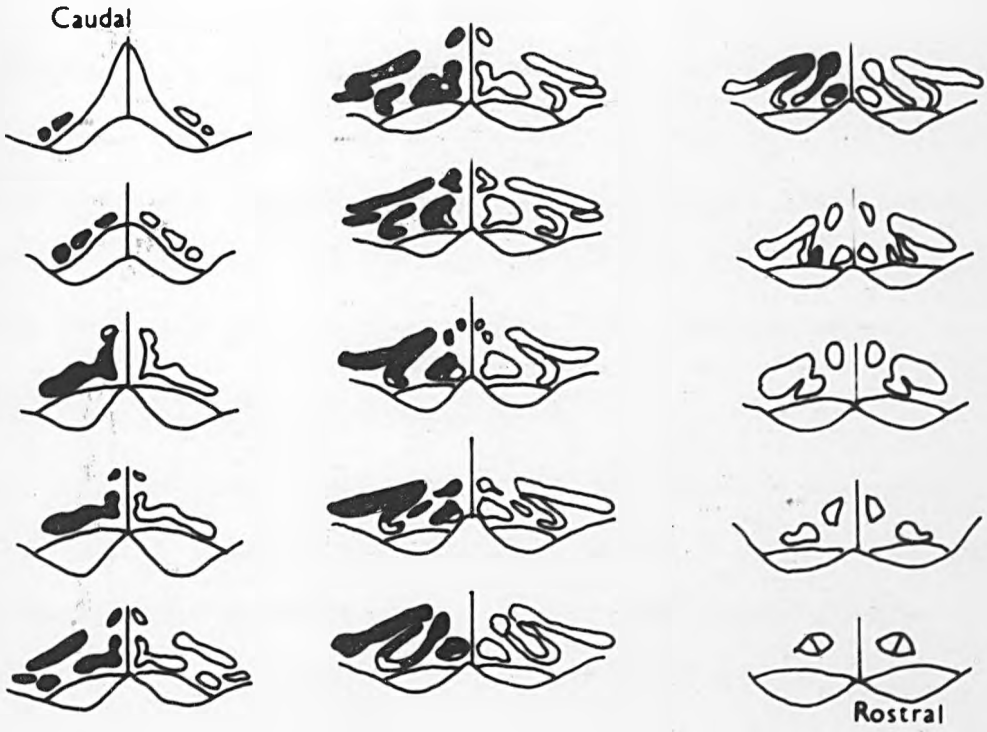
Figure 7.13

The Olivocerebellar Projection from a Rat Pedunclotomised on Day 10 and Injected After 35 Days

Following an extensive olivary injection site the right hemicerebellum was substantially filled with labelled climbing fibres and with a very small exception this did not continue into the left hemicerebellum.

The conventions follow those of figure 7.11.


I
II
III
IV
V
Lobulus Simplex (VI)
Crus (VI)
Crus (VII)
Paramedian Lobule (VII)
Copula (VIII)
Pyramidis
IX
X


Caudal
Rostral

the same as in control animals. From the injected left inferior olive, labelled climbing fibres were found in all areas of the right hemicerebellum and in some lobules, especially the anterior vermis, sagittal banding occurred. However the labelling of climbing fibres ceased exactly at the midline and only in one animal was a very small number of labelled climbing fibres found in lobules VI-IX of the left hemivermis.

ACUTE EXPERIMENTS

In these experiments the acute effects of unilateral cerebellar pedunclotomy on the neonatal olivocerebellar projection were studied. Neonatal rats aged 3, 7 or 10 days underwent pedunclotomy and at the same time [^{3}H]leucine was injected into the relevant inferior olive and the pups were allowed to survive for up to 4 days. The histological appearance and topographical organisation varied with each age group because of the different stages of both Purkinje cell and climbing fibre development.

Animals Pedunclotomised Aged 3 Days

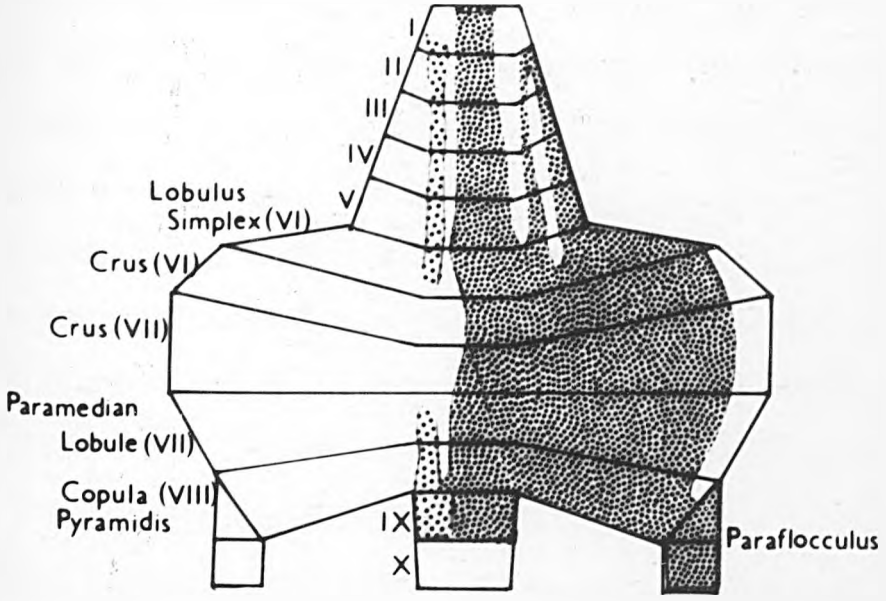
In this experimental group the olivocerebellar path was studied in animals aged between 4 and 7 days. By day 4 the developing climbing fibres have just reached the Purkinje cell layer therefore the labelled fibres were seen as crescents of silver around the base of the Purkinje cells. However, by 7 days of age the climbing fibres have formed well established contacts with the

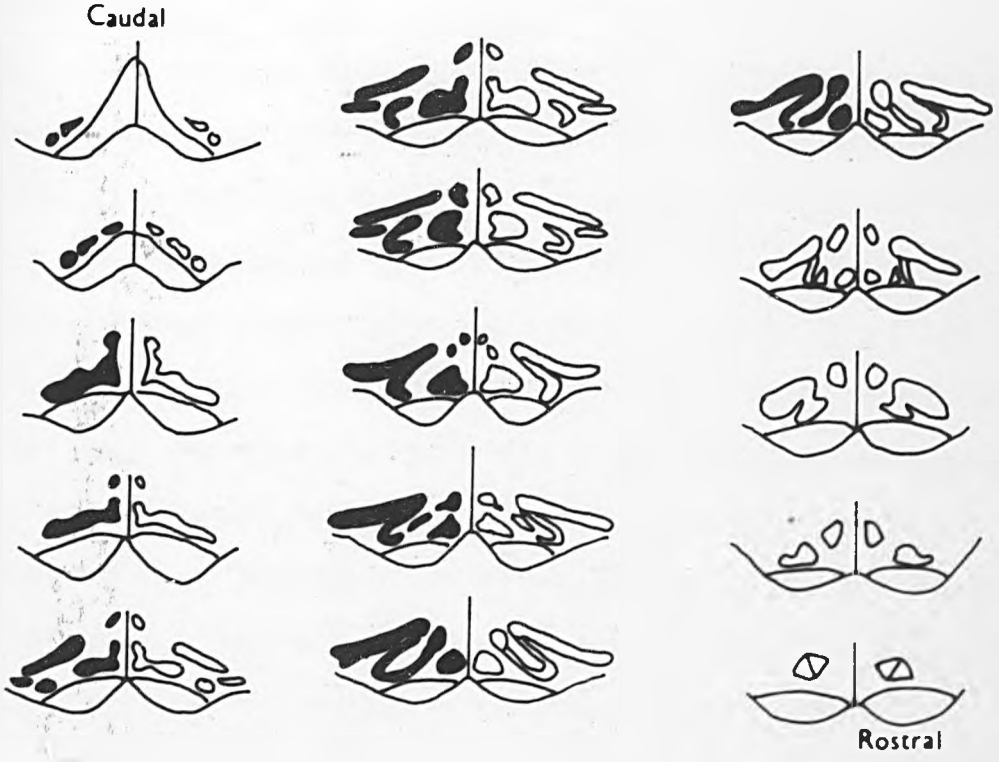
Figure 7.14

The Olivocerebellar Projection from a Neonatal Rat Pedunclotomised and Receiving an Injection into the Left Inferior Olive on Day 3 and Surviving for 4 Days

After extensive inferior olivary labelling the right hemisphere and the vermis were extensively labelled with almost no evidence of sagittal banding. The left hemivermis was less heavily labelled (light stippling) than the right hemicerebellum (heavy stippling).

In the neonatal rat the inferior olive is smaller than in the adult, therefore a small injection will extensively label the nucleus which is shown by the shaded areas. Also the rostrocaudal levels described by Gwyn *et al.* (1977) (see Fig. 1.3) do not correspond with those of the smaller neonatal inferior olive.


I
II
III
IV
V
Lobulus
Simplex (VI)
Crus (VI)
Crus (VII)
Paramedian
Lobule (VII)
Copula (VIII)
Pyramidis
IX
X
Paraflocculus


Caudal
Rostral

Purkinje cell perisomatic processes and the labelled fibres could be clearly seen surrounding the Purkinje cell somata. Because the Purkinje cell dendrites have not developed by these ages the density of silver grains overlying the molecular and external granular layers was not greater than background. The organisation of the labelled climbing fibres into sagittal bands was less precisely defined than in the adult animals. Large areas of the Purkinje cell layer were labelled and they formed broad longitudinal zones over several lobules.

Left Pedunclotomy with Left Inferior Olive Injection

With post-operative survival times of 1, 2 or 4 days, 4 animals fulfilled the criteria to be included in this group. The results were consistent between animals and were not dependent upon the survival time. In each brainstem the left inferior olive was extensively injected and from it labelled fibres crossed the midline, passed through the degenerating right inferior olive to converge at the lateral edge of the brainstem. The fibres then travelled dorsally and rostrally through the right inferior peduncle into the cerebellar medulla. Almost immediately a tract of fibres turned laterally to enter the paraflocculus and the flocculus. Labelled climbing fibres were found in the white matter and the Purkinje cell layer throughout the right hemicerebellum but there was also consistent light climbing fibre labelling in the left hemivermis as far laterally as the paravermal zone.

Figure 7.15

Climbing Fibre Labelling in a Neonate which Underwent Left Pedunclotomy and Inferior Olivary Injection on Postnatal Day 3

A and B. After injection into the left inferior olive the labelled climbing fibres around the Purkinje cell perikarya were denser in the right hemisphere (B) than in the lateral part of the left hemivermis (A). (x 296)

C and D. Following an injection into the right inferior olive an ipsilateral pathway can be seen. Interestingly the climbing fibre labelling was greater in the left hemisphere (C x 296) than in the right hemisphere (D x 476).

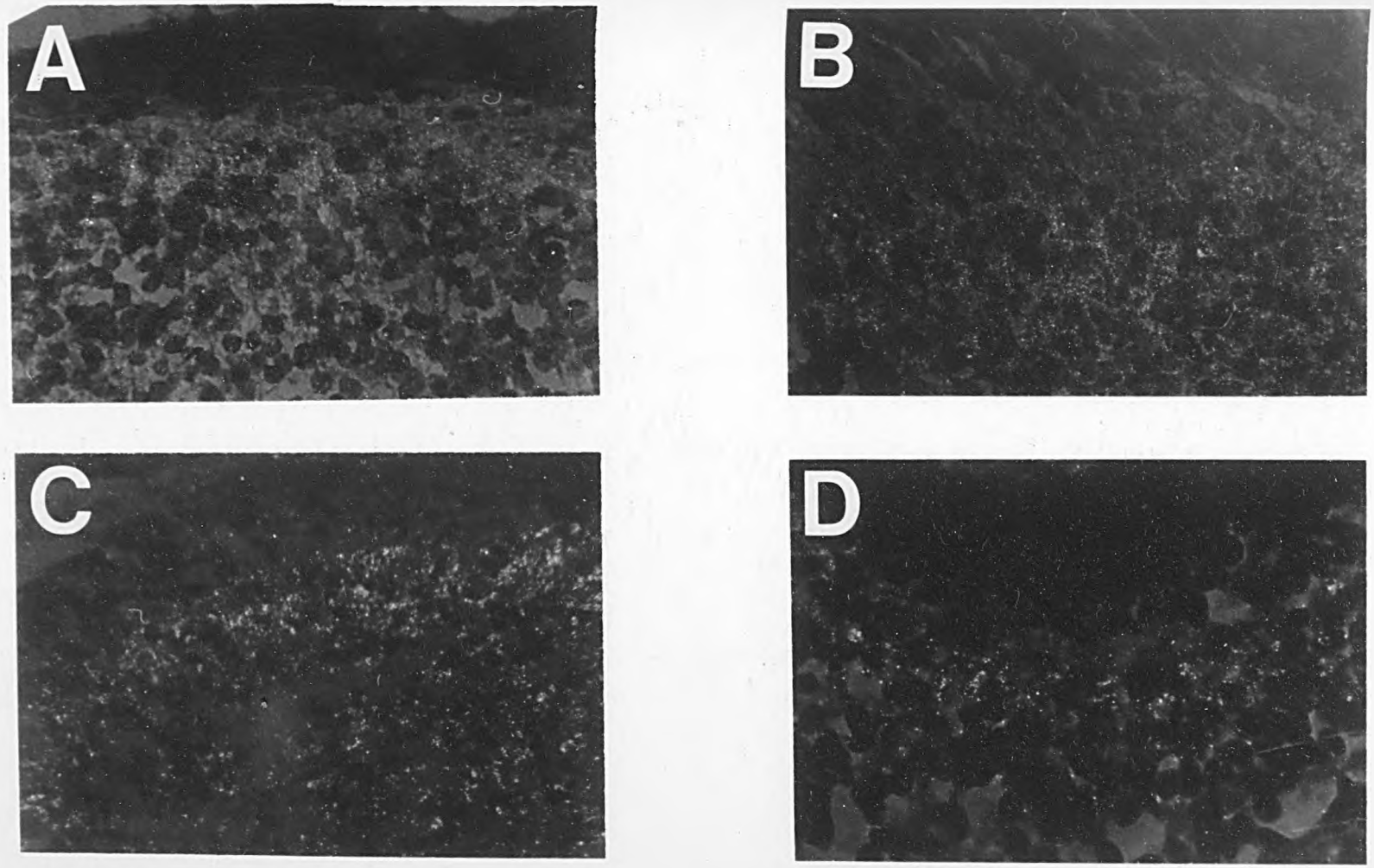
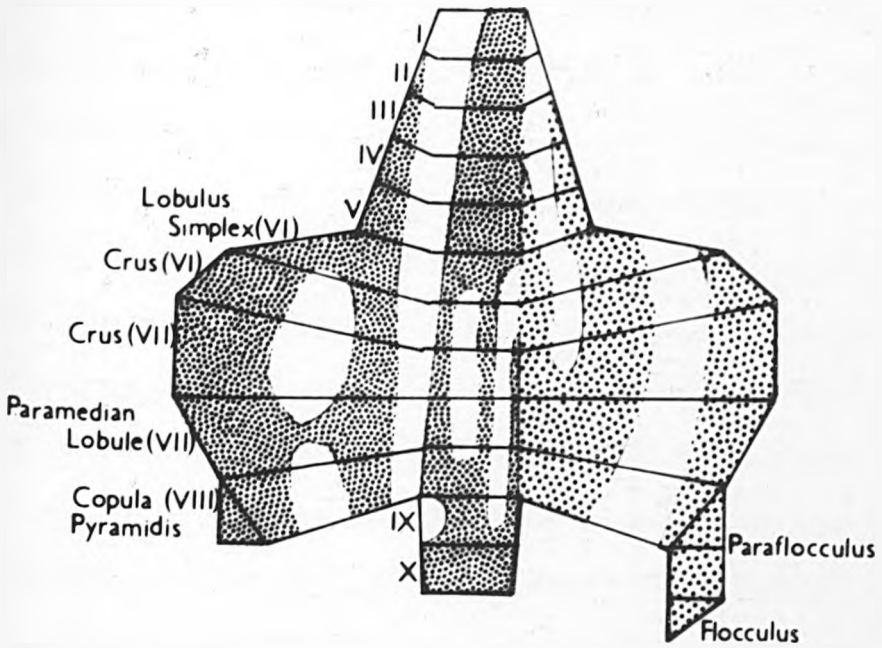

A
B
C
D

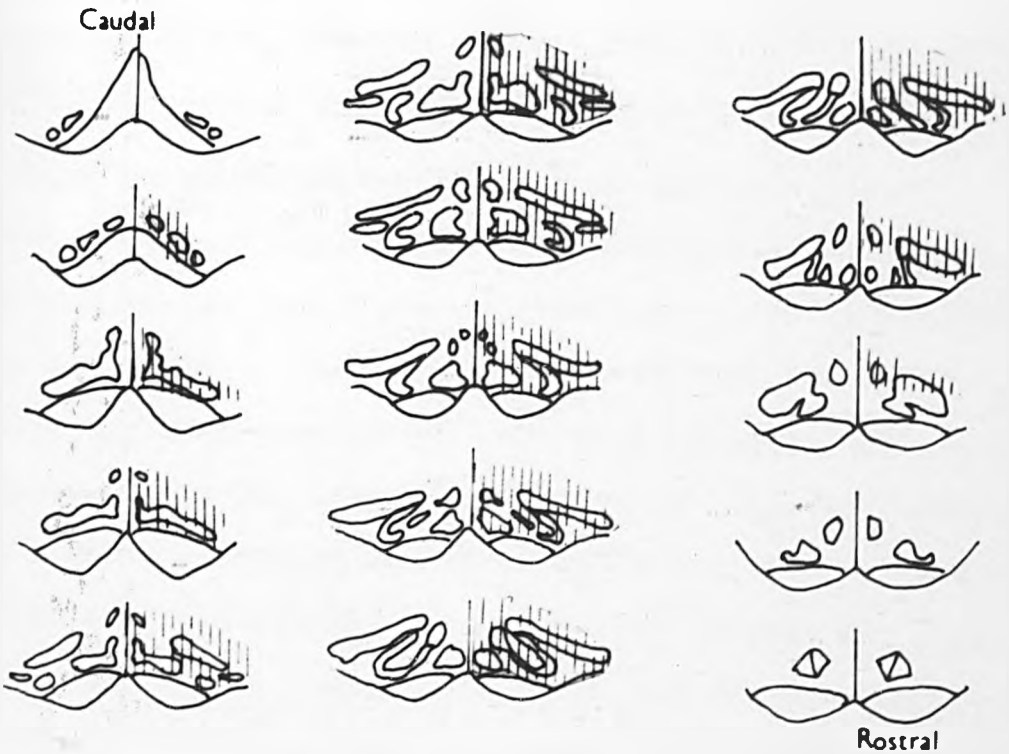
Figure 7.16

The Olivocerebellar Projection from a Neonatal Rat Pedunclotomised and Receiving an Injection into the Right Inferior Olive on Day 3 and Surviving for 2 Days

In the cerebellar cortex the climbing fibre labelling was heavier in the left hemisphere and vermis (heavy stippling) than the right hemisphere (light stippling) and there was only rather crude broad sagittal banding.

The right inferior olivary hatching represents the area of dense silver accumulation which was considered to be the injection site. However due to the degenerative changes in the olivary neurons a more precise map could be incorrect and misleading. As with figure 7.14 the olivary map does not fit the adult rostrocaudal levels shown in figure 1.3.


I
II
III
IV
V
Lobulus Simplex(VI)
Crus(VI)
Crus(VII)
Paramedian Lobule(VII)
Copula (VIII) Pyramidis
IX
X
Paraflocculus
Flocculus


Caudal
Rostral

Left Pedunclotomy with Right Inferior Olive Injection

In this experimental group 4 animals fulfilled the criteria and all of them had survived 2 days post-operatively. In no animal did the injection site encroach upon the left inferior olive. The degree of neuronal labelling within the right inferior olive was difficult if not impossible to assess because the neurons were undergoing rapid retrograde degeneration. Almost all the neurons of the dorsal and medial accessory olives had disappeared while those remaining within the principal olive did not look normal and possessed ill-defined nuclear and somatic edges. Also most of this region was infiltrated by small dark glial cells. Therefore the extent of the injection site within the right inferior olive was assessed in each section using the intact contralateral olive as a guide.

From the injected inferior olive labelled fibres crossed the midline and traversed the contralateral inferior olive to form the left inferior peduncle on the lateral edge of the brainstem. These fibres were stopped where the peduncle had been transected. As well as these, fibres also passed from the lateral aspect of the injected inferior olive to form a similar bundle at the right lateral edge of the brainstem and entered the cerebellum through the right inferior peduncle. This ipsilateral pathway was always less heavily labelled than the contralateral side. Labelled fibres passed through the deep nuclei to the cortex throughout the cerebellum and terminated in broad poorly defined

sagittal zones along the Purkinje cell layer. Also, some fibres could be traced right across the vermis into the left hemisphere. Although the white medulla around the deep nuclei of the left hemisphere was only lightly labelled the climbing fibre terminals along the Purkinje cell layer were always more intensely labelled than those in the right hemisphere.

Animals Pedunclotomised Aged 7 Days

In the animals of this operative age group the cerebellar climbing fibres were studied in animals aged 8 to 11 days. The histological appearance of labelled climbing fibres was the same for each age and each experimental group. Because the climbing fibres still terminate on the Purkinje cell perisomatic processes their labelling was seen as dense accumulations of silver around the Purkinje cells. While the labelling of the developing molecular layer and the external granular layer was never above background the internal granular layer was usually more densely labelled including distinct 'rosette-like' structures because of some involvement of the reticular formation in the injection sites. The labelled climbing fibres were organised into broad longitudinal zones in which thinner sagittal bands could be seen.

No Pedunclotomy but Right Inferior Olivary Injection

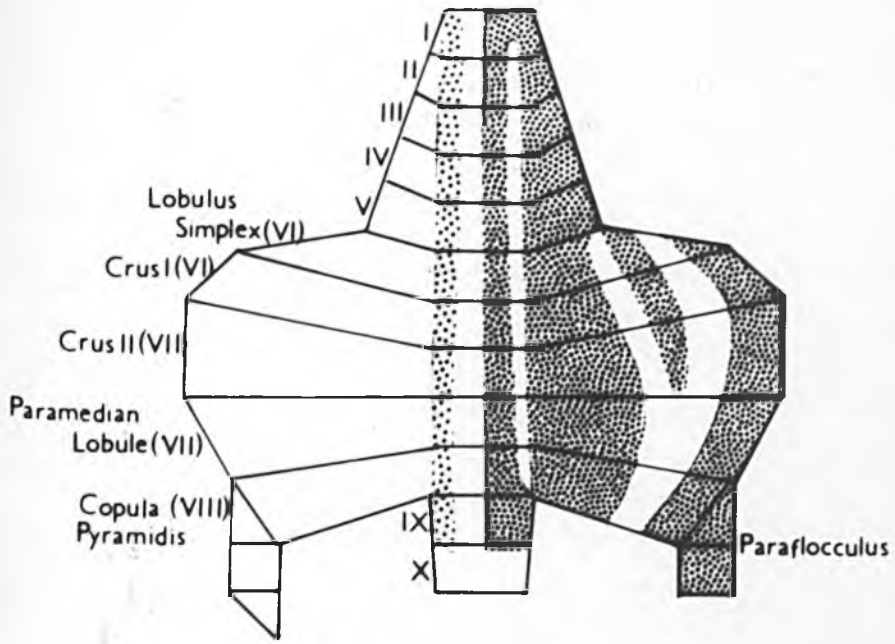
After survival times of 1 and 4 days, 4 control animals were included in this group although in only one animal was

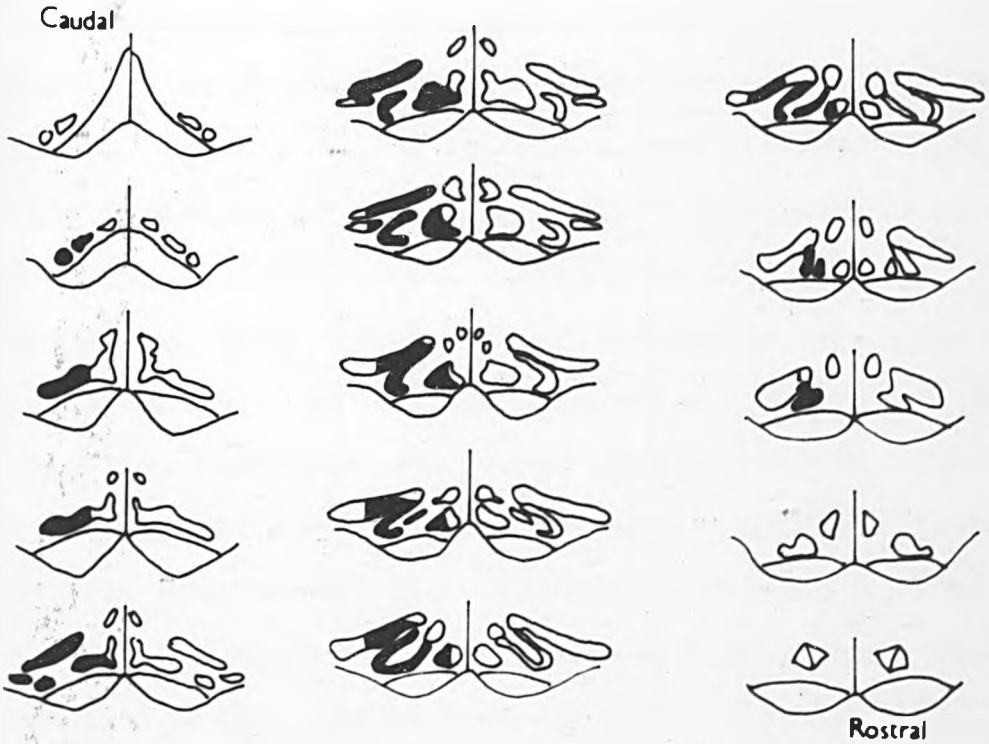
Figure 7.17

The Olivocerebellar Projection from a Neonatal Rat Pedunclotomised and Injected into the Left Inferior Olive on Day 7, which Survived for 2 Days

In the cerebellum there was heavy climbing fibre labelling (heavy stippling) in most regions of the right hemicerebellum to the midline and lighter labelling (light stippling) in the left vermis.

The conventions applied to the inferior olivary labelling are the same as those seen in figure 7.14.


I
II
III
IV
V
Lobulus
Simplex(VI)
Crus I(VI)
Crus II(VII)
Paramedian
Lobule(VII)
Copula (VIII)
Pyramidis
IX
X
Paraflocculus


Caudal
Rostral

there no involvement in the injection site of the medial edge of the left inferior olive. Labelled fibres crossed the midline, traversed the left inferior olive and passed round the lateral edge of the brainstem to enter the cerebellum through the left inferior peduncle.

Climbing fibre labelling was found around the Purkinje cells throughout the left hemicerebellum and this stopped at the midline. Where the injection site had encroached into the left inferior olive small numbers of labelled climbing fibres were found in the right hemisphere and vermis.

Left Pedunclotomy with Left Inferior Olivary Injection

With survival times of 1, 2 and 4 days 7 animals were included in this group and the results were not dependent on the survival time. In all animals a major portion of the left inferior olive had been injected. Fibres could be traced across the midline and through the degenerating inferior olive, round the lateral brainstem to the right inferior peduncle. Within the cerebellum the central white medulla of the right hemisphere was heavily labelled and tracts of labelled fibres passed to all areas of the cortex in the right hemicerebellum. The climbing fibre terminal labelling in the Purkinje cell layer was much heavier than the amount of silver grains seen overlying the internal granular layer and the molecular and external granular layers, which were not labelled above background. Heavy

Figure 7.18

Labelled Climbing Fibres in a Neonate which Underwent Pedunclotomy and Inferior Olivary Injection on Postnatal Day 7

A and B. After injection into the left inferior olive there is extensive labelling in the right hemicerebellum (B) but the labelling across the midline to the left hemivermis is much sparser (A). (x 296)

C and D. Following an injection into the right inferior olive an aberrant ipsilateral pathway is demonstrated but the climbing fibre labelling around the Purkinje cells is much fainter in the left hemisphere (C) than in the right hemisphere (D). (x 296)

ML = molecular layer

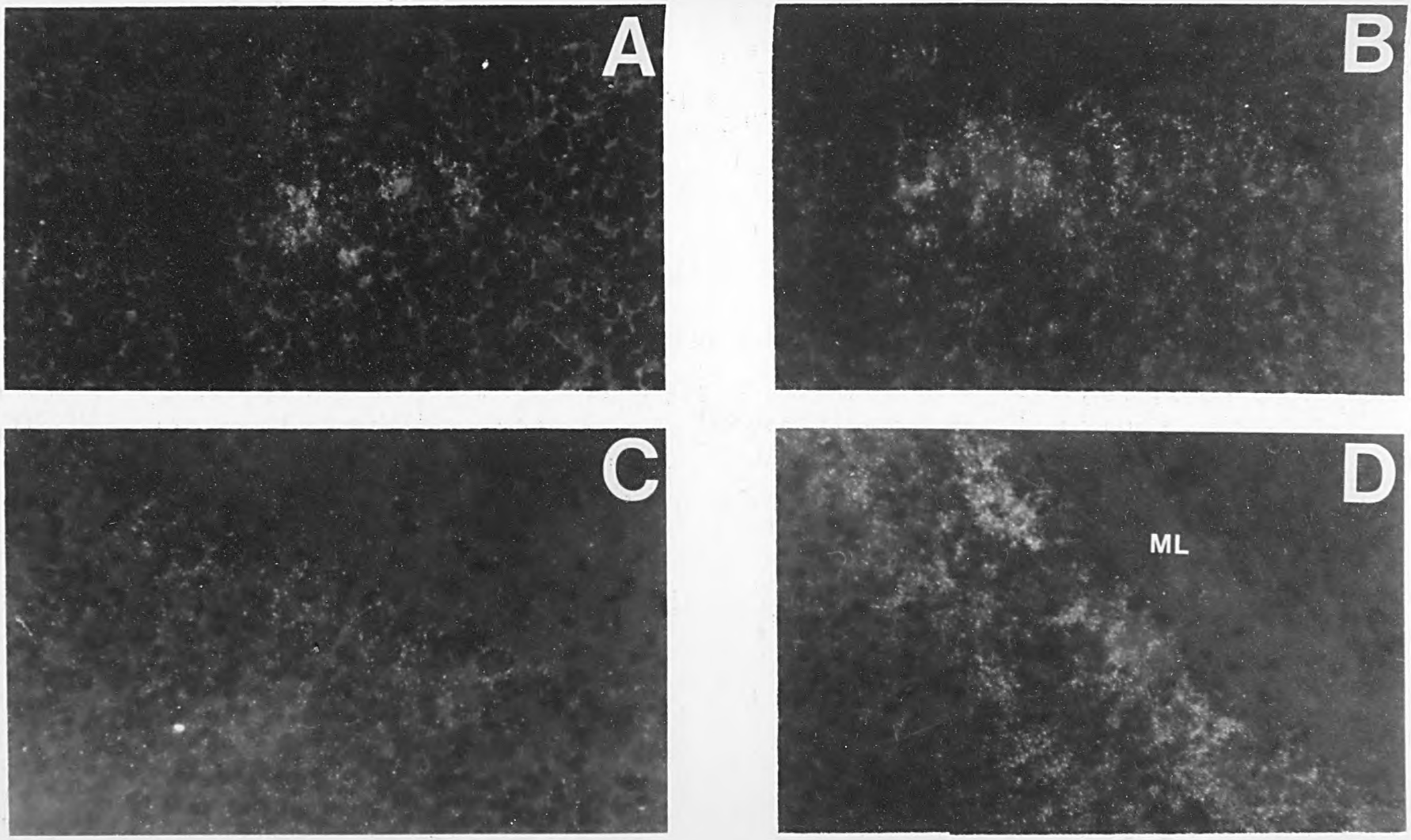
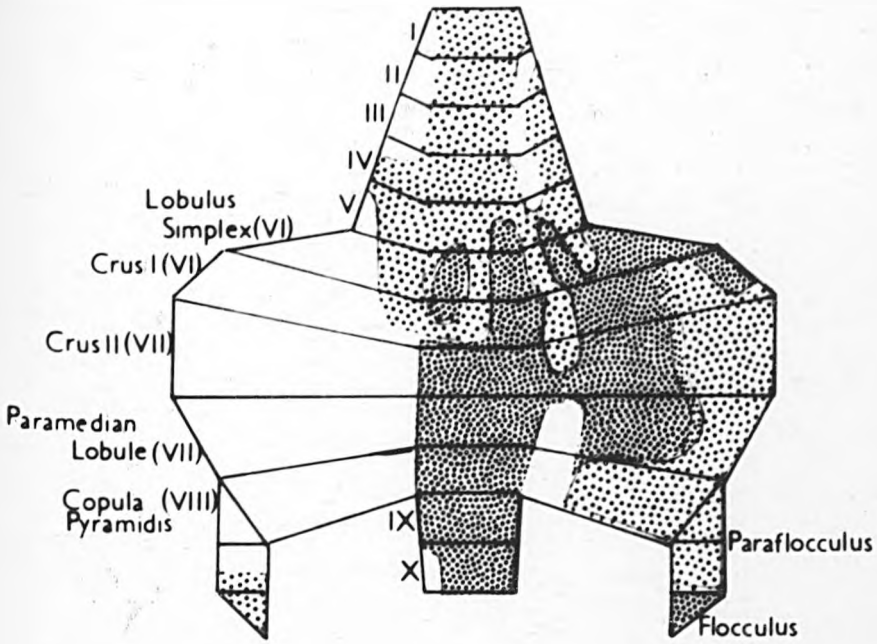

A
B
C
D
ML

Figure 7.19

The Olivocerebellar Projection from a Neonatal Rat Pedunclotomised and Injected into the Right Inferior Olive on Day 7, which Survived for 4 Days

The labelled climbing fibres in the cerebellar cortex were seen throughout the right hemicerebellum and the left hemivermis. While the anterior lobe and posterolateral right hemisphere were generally less heavily labelled (light stippling) there was no obvious sagittal banding.

The injection site has been generally outlined (vertical hatching) for the reasons explained in figure 7.16.


I
II
III
IV
V
Lobulus
Simplex(VI)
Crus I (VI)
Crus II (VII)
Paramedian
Lobule (VII)
Copula (VIII)
Pyramidis
IX
X
Paraflocculus
Flocculus

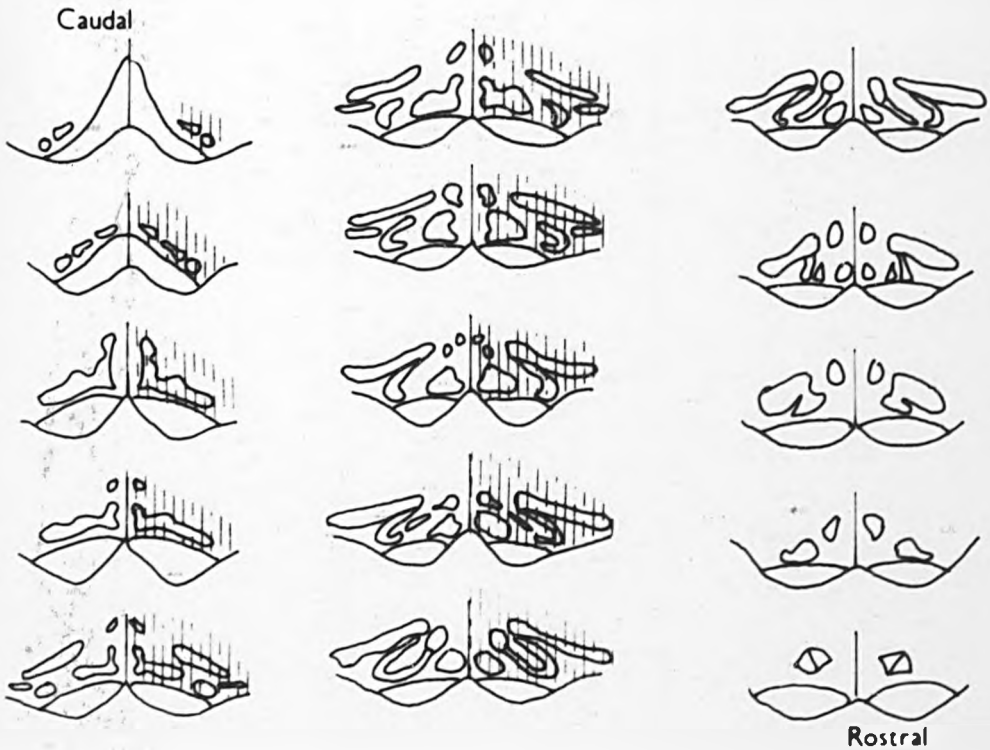
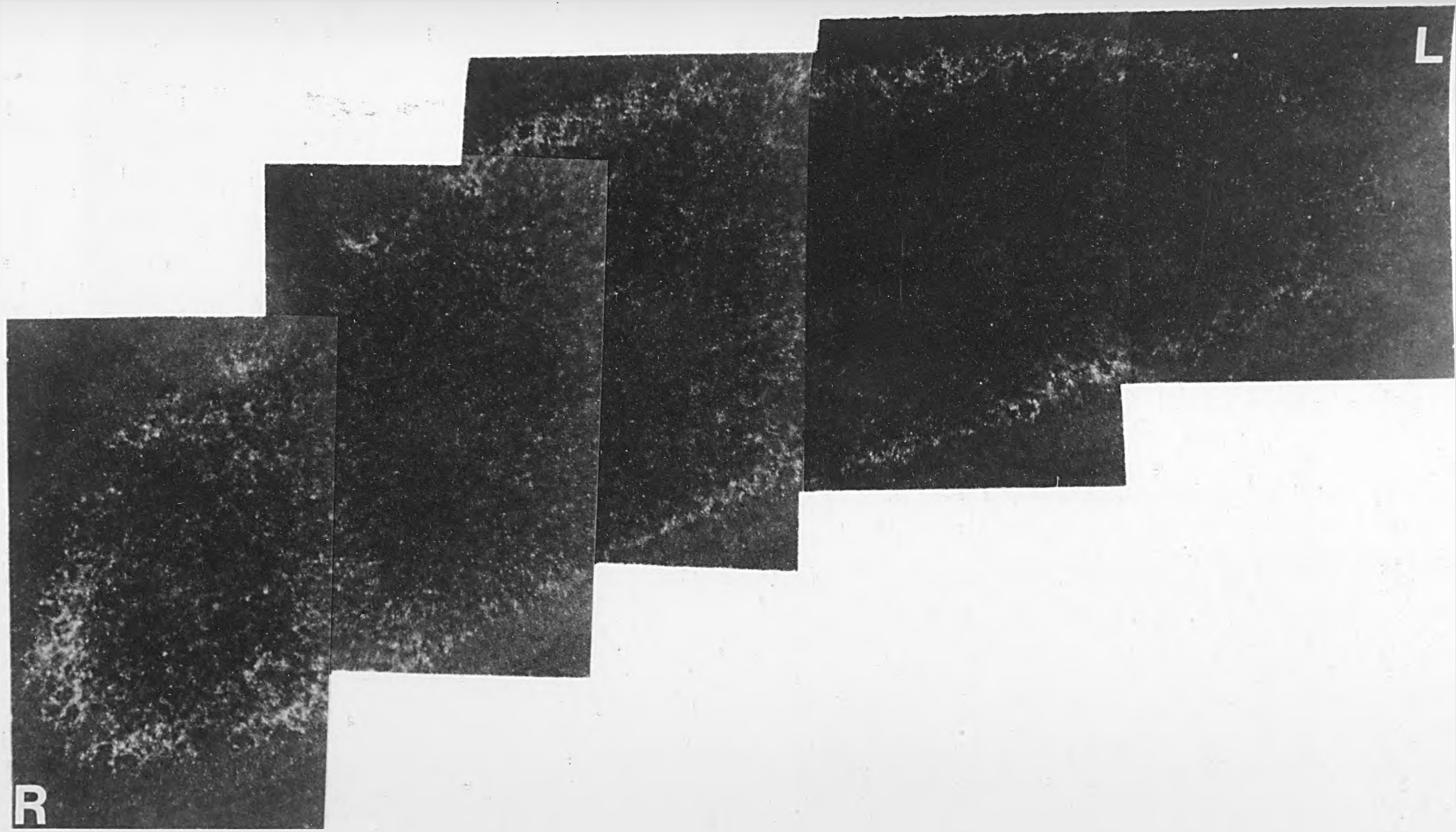
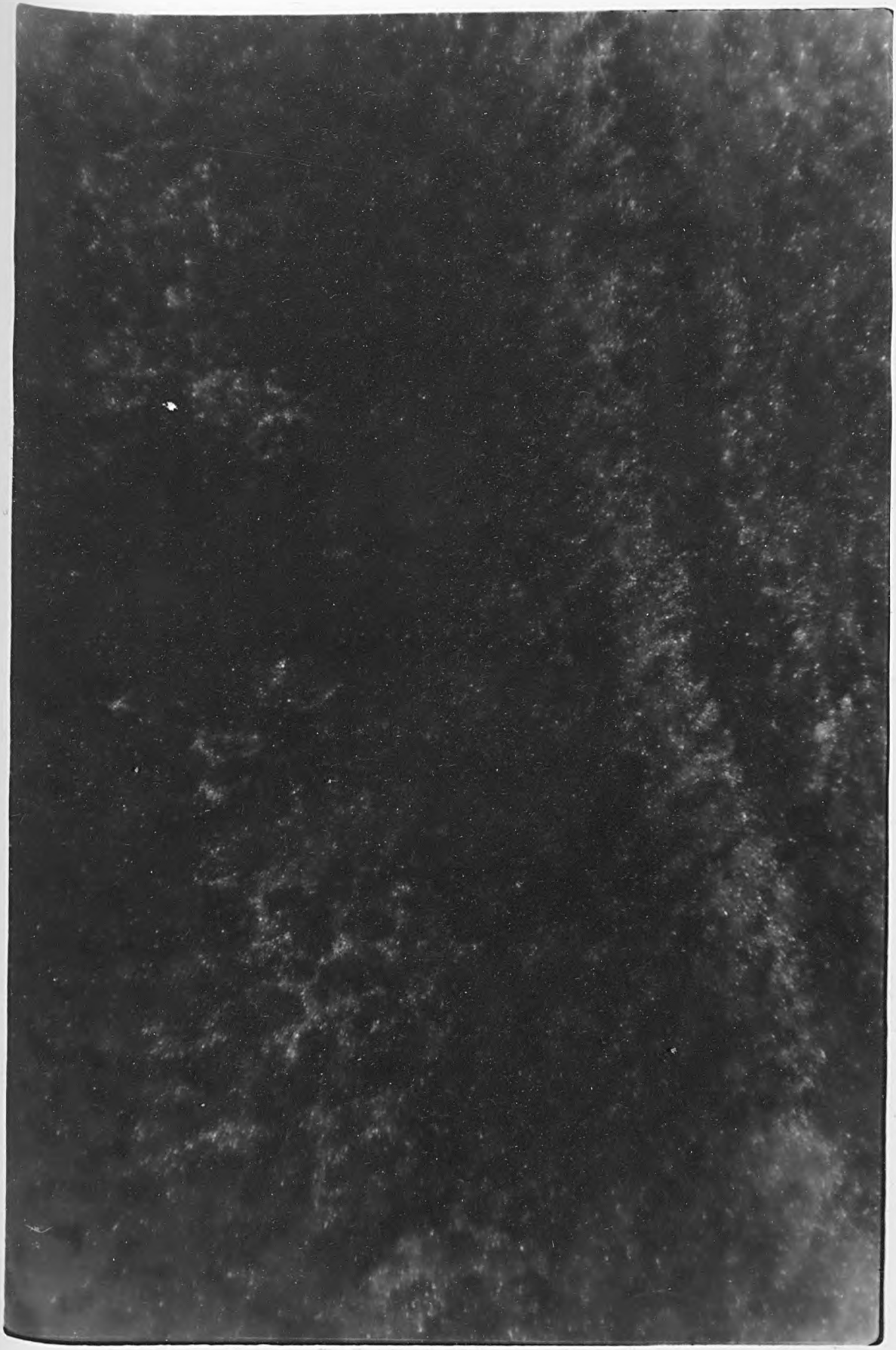

Caudal
Rostral

Figure 7.20

This montage (x 204) of the climbing fibre labelling in vermal lobule VII illustrates the breadth of the olivocerebellar projection, with virtually no sagittal banding, throughout a whole lobule. This appearance was found extensively in the developing cerebella. The micrographs are taken from a neonate receiving [^{3}H]leucine into the right inferior olive and a left pedunclotomy on day 7 and surviving 4 days and demonstrates that the aberrant ipsilateral pathway is extensive. This widespread climbing fibre labelling should be compared with the appearance of sagittal banding in a different vermal lobule, the right half of IX, from the same animal (overleaf, x 481).

L = left hand side of the vermis
R = right hand side of the vermis


L
R



climbing fibre labelling stopped at the midline, however a small consistent projection of lightly labelled climbing fibre terminals was also found in the left hemivermis and it extended almost to the lateral edge of the vermis.

Left Pedunclotomy with Right Inferior Olive Injection

Four animals, which had survived either 2 or 4 days post-operatively, fulfilled the criteria for a successful injection of the right inferior olive with no encroachment of the left inferior olive. After 2 days survival a few principal olivary neurons remained but they appeared degenerating with poorly defined edges of the nuclei and perikarya, but by 4 days post-pedunclotomy the whole right inferior olive had disappeared. Therefore the area of injected inferior olive was deduced using the intact left inferior olive.

From the injection site, heavily labelled fibres crossed the midline traversed the left inferior olive and passed round the lateral rim of the brainstem to where the left inferior peduncle had been transected. Fibres also emerged from the lateral side of the injection site and followed a similar course around the rim of the brainstem to the right inferior cerebellar peduncle. These fibres ipsilateral to the injection site were less heavily labelled than the contralateral path. Labelled climbing fibres entered the cerebellum through the right inferior peduncle and passed mainly around but also through the deep

nuclei on their course to the cortex. Heavy terminal labelling overlay the Purkinje cell layer throughout the right hemisphere and the whole vermis and it was organised into sagittal zones. The labelling ceased at the left edge of the vermis, and the cortex and the central white medulla of the left hemisphere was not labelled greater than background.

Animals Pedunclotomised Aged 10 Days

In this experimental group the olivocerebellar projection was studied in neonatal animals aged 11 to 14 days. At 11 days of age the climbing fibre terminals still terminate on the Purkinje cell perisomatic processes therefore any labelling appeared as accumulations of silver grains over the Purkinje cell layer. By day 14 the climbing fibres have ascended to the Purkinje cell primary dendrites and labelled terminals were found around the apices of the Purkinje cells and as fine threads of silver in the lower third of the molecular layer. The distribution of terminals was clearly in sagittal bands and appeared very similar to those seen in the adult cortex. There was no labelling in the external granular layer greater than background, while in the internal granular layer any labelling was confined to discrete accumulations of silver over the mossy fibre rosette glomerular islands.

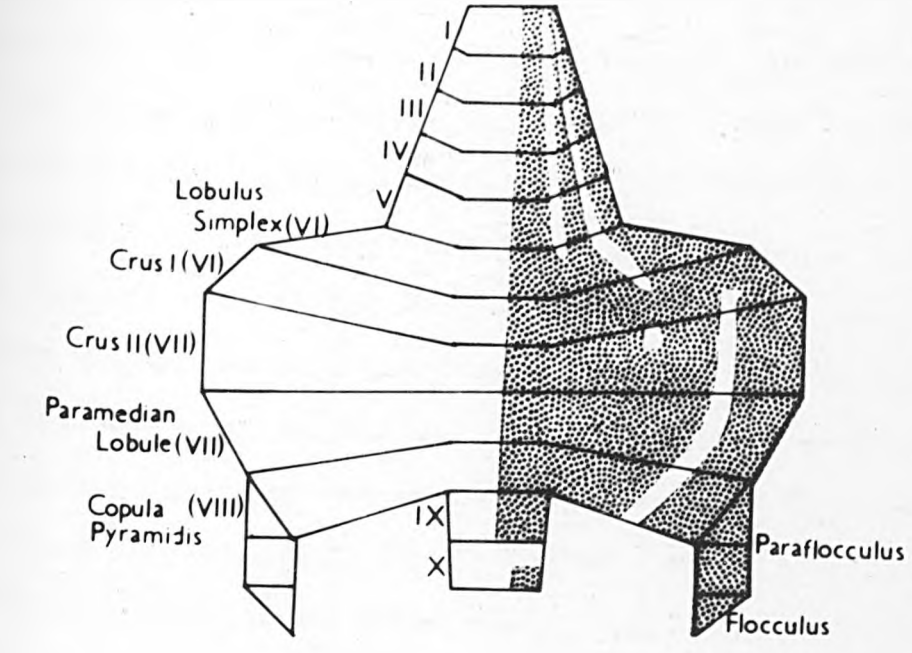
No Pedunclotomy but Right Inferior Olivary Injection

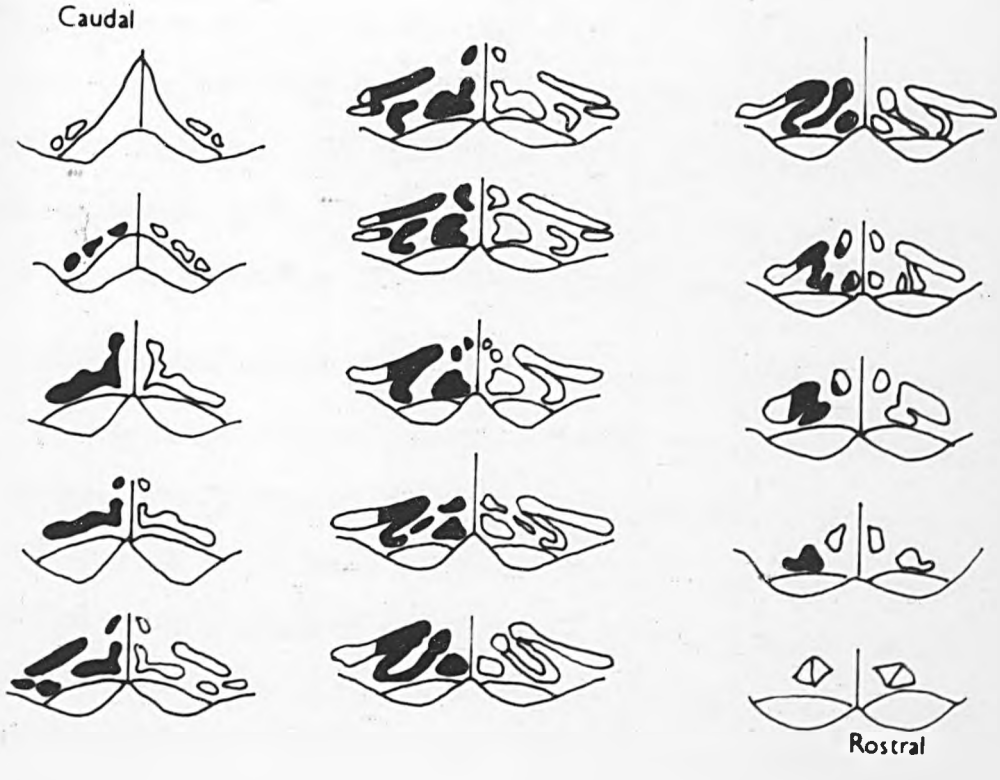
Five control animals with survival times of 1 or 4 days fulfilled the criteria to be included in this group

Figure 7.21

The Olivocerebellar Projection in a Neonatal Rat Pedunclotomised and Injected into the Left Inferior Olive on Day 10 and which Survived for 4 Days

The injection site included the majority of the inferior olivary nucleus and therefore the right hemicerebellum was extensively labelled in accordance with the topography illustrated in figure 1.3.


I
II
III
IV
V
Lobulus
Simplex(VI)
Crus I(VI)
Crus II(VII)
Paramedian
Lobule(VII)
Copula (VIII)
Pyramidis
IX
X
Paraflocculus
Flocculus


Caudal
Rostral

and in only one animal did the injection site encroach upon the left inferior olive. In each animal a major part of the right inferior olive was injected and the ventral part of the reticular formation was also included. From the injected inferior olive labelled fibres could be traced across the midline and through the left inferior olive to the lateral edge of the brainstem. From here the fibres passed dorsorostrally and entered the cerebellum through the left inferior peduncle. The central white medulla of the left hemisphere was heavily labelled and tracts of labelled fibres passed to the cortex. The climbing fibre terminal labelling in the Purkinje and lower molecular layers was organised into discrete sagittal bands in all areas of the left hemisphere and the left hemivermis. The labelling stopped exactly at the midline except in the one animal in which the injection site was slightly bilateral where small areas of climbing fibre labelling were also found in the right hemicerebellum.

Left Pedunclotomy with Left Inferior Olive Injection

In this experimental group all the four animals which were successfully injected had a post-operative survival time of 4 days. In each animal the left inferior olive was extensively injected and from it heavily labelled fibres traversed the midline and through the contralateral inferior olive to form the right inferior peduncle on the lateral edge of the brainstem. The labelled climbing fibres entered the cerebellum through this peduncle and

passed around the deep nuclei on their path to the cortex. In the cortex the Purkinje cell layer and the lower third of the molecular layer of the right hemicerebellum were labelled in longitudinal zones. The labelling stopped at the midline with no additional labelled climbing fibres in either the left vermis or hemisphere.

CHAPTER EIGHT

DISCUSSION ON THE RESULTS OF NORADRENERGIC FLUORESCENCE AND OLIVOCEREBELLAR AUTORADIOGRAPHY

NORADRENERGIC AFFERENTS

The noradrenergic afferents to the cerebellum were studied following neonatal unilateral cerebellar pedunclotomy in order to try and assess whether they would regenerate and hyperinnervate the deafferented cortex, a response which has been shown in adults (Pickel et al., 1973 and 1974b) and following perinatal treatment with 6-hydroxydopamine (6OHDA) (Sachs and Jonsson, 1975; Bendeich et al., 1978; Schmidt and Bhatnagar, 1980; Schmidt et al., 1980; Sievers et al., 1980; Schmidt et al., 1981; Sievers et al., 1981; Harston et al., 1982; Jonsson and Sachs, 1982; Kostrzewa et al., 1982; Lovell, 1982).

The histological appearance of the noradrenergic fibres in this study is in agreement with that found in the literature (Hokfelt and Fuxe, 1969; Chan-Palay, 1977; Yamamoto et al., 1977; Moore and Bloom, 1979). The density of fibres in those animals pedunclotomised aged 3 days and surviving 4 days is compatible with their known development as is the lower adult density in the 10 day pedunclotomy group (Yamamoto et al., 1977; Waddington and Banks, 1981). In the neonatal animals noradrenergic fibres were seen ascending through the molecular layer to the external

granular layer although very few fibres were seen within this layer. This result is in agreement with Sievers and Klemm (1982) but it differs from Schmidt et al. (1981) who found noradrenergic fibres in the external granular layer of ventrally placed lobules, an innervation which was maximum at 5 days of age.

The results from this experiment were not very satisfactory because of the patchiness of visible fluorescent fibres within the sections. This patchiness could be caused by either uneven perfusion or uneven dewaxing of the sections. In the description of the technique Loren et al. (1980) say that if the brain looks mottled then the perfusion was uneven and this could be caused by a slight drop of the perfusion pressure or vasoconstriction in the small blood vessels. Since the preperfusate used in adult animals is ice-cold it is not unlikely that spasm of the small blood vessels in the brain will occur and consequently induce unevenness in the catecholamine fluorescence. Since only a few brains had this mottled appearance this is unlikely to be the cause of the patchy appearance of the sections. Because wax masks the fluorescence a more likely explanation is uneven dewaxing of the sections. Since the fragile freeze-dried tissue is supported by the impregnated wax, it disintegrates if the wax is totally removed. Therefore, a compromise has to be made between the degree of dewaxing and the integrity of the tissue morphology.

The paucity of fluorescent fibres in these cerebella makes a comparison between the experimental and control animals extremely difficult but the results can be viewed with reference to other findings described in the literature. It is known that transected noradrenergic axons can sprout new fibres (Olson and Malmfors, 1970; Katzman et al., 1971) and this has been demonstrated among the noradrenergic afferents to the cerebellum of the adult rat (Pickel et al., 1973 and 1974b). In addition, the locus coeruleus is known to have a great capacity for plasticity (Kerr, 1975) and will even grow fibres into transplants of smooth muscle (Bjorklund and Stenevi, 1971) and foetal brain tissue (Sievers and Klemm, 1982). Also transplanted foetal locus coeruleus neurons will correctly innervate host tissue (Bjorklund et al., 1976; Bjorklund et al., 1979). This capacity for transected axons of the central nervous system to regenerate has also been found in some other non-adrenergic systems (Graziadei and Monti-Graziadei, 1978; Lund, 1978) but not all of them (Miller and Lund, 1975). If the ascending noradrenergic fibres are cut the ipsilateral forebrain is deprived of its input and this does not regenerate (Jonsson and Sachs, 1982) nor is there collateral sprouting from the contralateral noradrenergic axons (Maeda et al., 1974). The transected fibres do sprout, as is mentioned above, but the regenerated fibres are confined to the site of the lesion (Katzman et al., 1971). However, collateral sprouting from the spared fibres of a bilateral

innervation has been seen in the septal nuclei (Raisman, 1977). From these findings it would seem quite likely that the deprived hemicerebellum could be reinnervated with noradrenergic fibres either by regrowth of the transected axons or by collateral sprouting from the intact fibres which arise in the contralateral locus coeruleus. Therefore the similarity in density of fluorescent fibres in both the chronically pedunclotomised and control cerebella would indicate reinnervation, probably by collateral sprouting. However, it is impossible to be certain of this by just studying the sections. If the noradrenergic fibres had not reinnervated the left hemicerebellum then the whole cerebellar cortex in pedunclotomised animals would contain considerably fewer fluorescent fibres than the control brains because the catecholaminergic innervation is fairly evenly bilateral.

Because there was no evidence of hyperinnervation in the pedunclotomised cerebella these results are not in agreement with those of Pickel et al. (1973 and 1974b), who do find a hyperinnervation following pedunclotomy in adults. There are two problems with this result. The first is that they believed the locus coeruleus projection to the cerebellum to be ipsilateral, therefore a certain amount of 'regrown' fluorescent fibres may have been normal innervation from the contralateral locus coeruleus. In addition to this the density of innervation was quantified using autoradiographic labelling with tritiated proline, which is

known to be differentially transported by different nuclear groups (Kunzle and Cuenod, 1973). It is possible that neurons regenerating lesioned axons will take up and transport labelled proline more readily than undamaged neurons which in turn could give a false impression of hyperinnervation.

Noradrenergic hyperinnervation is also found following neonatal injury with 6OHDA (Bendeich et al., 1978; Schmidt and Bhatnagar, 1980; Schmidt et al., 1980: Schmidt et al., 1981; Kostrzewa et al., 1982; Sievers and Klemm, 1982), which may be caused by the direct action of a neurotoxin that is known to have many extraneuronal effects (Hedreen and Chalmers, 1972; Sievers et al., 1980: Sievers et al., 1981). This hyperinnervation may also be due to an excess of transmitter transported along the cerebellar collaterals since the ascending collaterals supplying the forebrain do not regenerate (Sachs and Jonsson, 1975; Schmidt and Bhatnagar, 1980; Sievers et al., 1980), a phenomenon which also occurs after the ascending noradrenergic fibres are transected (Jonsson and Sachs, 1982; Kostrzewa et al., 1982). However, because the ascending noradrenergic fibres have not been interrupted in this study, this may explain why the cerebellar reinnervation was not in excess of the control values.

For those animals which have only survived 4 days post pedunclotomy the similar density of fluorescent fibres in

both the experimental and control cerebella indicates that the rate of reinnervation is extremely rapid. After injury with 6OHDA in perinatal rats fluorescent fibres are found in the base of the superior peduncles after 24 hours (Schmidt et al., 1980) and the cerebellar cortical innervation has returned to control levels after 3 days (Harston et al., 1982). In adult animals noradrenergic fibres begin to invade transplanted tissue after 7 days (Bjorklund and Stenevi, 1971; Katzman et al., 1971) and septal cholinergic fibres have been found entering hippocampal implants 3-6 days after the transplant (Kromer et al., 1980). Generally, neonatal neural tissue reacts more quickly than fully mature tissue and if neonatal noradrenergic fibres redevelop at 2.4 mm/day, the rate of peripheral nerve regeneration (Lund, 1978), then it is possible that the locus coeruleus could reinnervate the ipsilateral cerebellum in 4 days.

The morphology of the cerebellar cortex following neonatal pedunclotomy is qualitatively normal, possibly because after unilateral pedunclotomy almost half the noradrenergic innervation is retained and there is rapid reinnervation to approximately control levels. It is known that noradrenaline is important for the normal maturation of neurons (Maeda et al., 1974) and the capacity of developing neural tissue to plasticise (Kasamatsu and Pettigrew, 1979; Kasamatsu et al., 1979).

CLIMBING FIBRE AUTORADIOGRAPHY

The olivocerebellar projection was studied following neonatal cerebellar pedunclotomy because climbing fibres can activate the Purkinje cells by postnatal day 3 (Crepel, 1971; Crepel et al., 1981; Mariani and Changeux, 1981; Crepel, 1982). Also, they have a capacity for plastic adaptation; in the adult they are capable of sprouting to innervate embryonic neural implants (Hallas et al., 1980; Oblinger et al., 1980) and there is some reinnervation of the pedunclotomised hemicerebellum by climbing fibres from the contralateral side (Angaut et al., 1982; Alvarado-Mallart et al., 1983).

CRITERIA FOR LABELLING

To analyse the size of the injection site a 4 day exposure was chosen to try and obtain genuine neuronal labelling. With longer exposure times those lightly labelled neurons, which have not taken up sufficient tracer to produce visible terminals in the cerebellar cortex, will also appear to be labelled (Kunzle, 1975a). The high specific activity of the injection site induces sufficient latent image formation that with a long exposure time the whole area is densely covered with silver grains such that the criterion for neuronal labelling cannot be enforced. Also, the extra silver grains produced with increased time by the efferent fibres passing through the contralateral

olive can appear to overlay both neurons and neuropil. Therefore a short exposure period was chosen so the size of the injection site was not overestimated (Cowan et al., 1972).

Climbing fibre labelling within the adult cerebellar cortex was easily recognised from the histological description of these fibres and the criteria defined by Campbell and Armstrong (1983a). In the neonatal cortex terminal labelling was identified as coming from climbing fibres because it has the same structure as the developing climbing fibres (O'Leary et al., 1971; Altman, 1972b). However, Sotelo et al. (1984) describe a more advanced climbing fibre histology than is seen in this study. They describe terminal labelling around the Purkinje cells at 5 days of age, while in this study the accumulations of silver were cup-shaped at day 5 and did not completely surround the Purkinje cells until 7 days post partum. This difference may be caused by uncertainty, of as much as 12 hours, about the neonates' age. Another probable explanation is that some terminals had grown all around the Purkinje cell somata by day 5 but the considerably lower specific activity of the tracer used in this experiment did not label them above background, while in the study of Sotelo et al. (1984) they were lightly labelled. In the older neonatal cerebella the climbing fibre terminal labelling was identical to that described by Dupont et al. (1981) and Sotelo et al. (1984).

CHRONIC EXPERIMENTS

In those animals which were pedunclotomised aged 3 or 7 days and survived 35 days post operatively an extra climbing fibre innervation was found in the left hemicerebellum after a left inferior olivary injection. These fibres had the histological appearance of climbing fibres although both the labelling and the density of terminals was much less than was found in the contralateral cerebellar cortex. This afferent path into the deafferented hemicerebellum has since been described in animals which underwent unilateral inferior pedunclotomy between birth and 2 days of age and were subsequently allowed to grow to adulthood (Angaut et al., 1982; Alvarado-Mallart et al., 1983). This study gives further information that the extra climbing fibre afferents to the deafferented hemicerebellum are already present 35 days after the pedunclotomy and that between the ages of 7 and 10 days the capacity for their development ceases.

Alvarado-Mallart et al. (1983) found that the areas of autoradiographic labelling within the deafferented hemicerebellum were a mirror-image of the sagittal banding found in the right hemicerebellum. However, in this study the pattern of climbing fibre labelling in the left hemicerebellum is not quite a mirror-image of the contralateral side. In those animals which underwent pedunclotomy on day 3, the sagittal banding within the climbing fibre labelling on the

left side was less clear than that seen in the right hand cortex, while in those animals pedunclotomised aged 7 days the difference between the two hemicerebella is clearly marked (Fig. 7.12). It was also noticed in both experimental age groups that the left hemicerebellar labelling was organised in broader sagittal bands, which resembled those seen in the neonatal climbing fibre projection. Both Angaut et al. (1982) and Alvarado-Mallart et al. (1983) describe this aberrant projection as sprouted collaterals which will correctly reinnervate the region corresponding to their normal termination zone rather than fill adjacent denervated sites as has been found in different regions of the nervous system (Lynch et al., 1972; Lynch et al., 1973 a & b; Zimmer, 1973 a & b; Hickey, 1975; Raisman, 1977). Reinnervation of an area by fibres with the correct topography has been described (Miller and Lund, 1975) but this is due to regeneration of transected axons, which cannot have occurred in this case as is evident by the degenerated right inferior olive. In the hippocampus, if the entorhinal afferents to the superficial part of the dentate gyrus molecular layer are removed, fibres with known capacity to plasticise cannot sprout from the inner zone through the intact intermediate part of the molecular layer to reinnervate the deafferented outer zone (Zimmer, 1974). Therefore it seems unlikely that climbing fibres in the right hemisphere will be able to develop collaterals through the normally innervated ipsilateral vermis and the

deafferented contralateral vermis to terminate in the correct sagittal zone of the left hemisphere. However, there is evidence that neonatal projections can reinnervate areas which are equivalent although contralateral to their normal termination and with the correct topography (Leong and Lund, 1973; Leong, 1976; Mustari and Lund, 1976), and that this is not due to the retention of a developmental bilateral projection (Nah et al., 1980; Mihailoff et al., 1984).

The evidence indicates that the aberrant transcommissural fibres develop in response to denervation of the contralateral hemicerebellum and that immature climbing fibres have a remarkable capacity to adapt to an altered environment. However, there are some points which might indicate that these fibres could be persistent neonatal collaterals. Both Angaut et al. (1982) and Alvarado-Mallart et al. (1983) find in electron microscopic examination of the pedunclotomised hemicerebellum that only a proportion of the Purkinje cells were reinnervated by climbing fibres. If the intact climbing fibres have grown collaterals to the corresponding sagittal zone of the contralateral hemicerebellum why do they then not synapse upon so many of the vacated Pukinje cells? Also, if the signal for collateral sprouting was the presence of degenerating axons around the left inferior olive (the stimulus of adjacent vacated postsynaptic sites would only apply to those climbing fibres of the medial vermis) how do the sprouted collaterals know, over a distance of as much as 5 mm, where to go and where to form connections? While

this factor also applies to the collaterals of corticotectal fibres, it is interesting that among corticofugal fibres which normally terminate laterally within the superior colliculus the topographic precision of collaterals was poor (Lund, 1978). This was not the finding in this study, the lateral lying flocculus and paraflocculus, when present, were consistently labelled symmetrically to the contralateral side. A reduction in the topographical precision appeared to be correlated with age rather than laterality which might suggest the preservation of an increasingly degenerated path. Collaterals which form aberrant projections in the neonate and then later retract have been described (e.g. Innocenti, 1979; Stanfield, 1984) including abnormal cerebellar afferents (Tolbert and Penneton, 1983).

ACUTE EXPERIMENTS

Control Animals

Since, until recently, there was only one sparse study of the neonatal olivocerebellar projection (Dupont et al., 1981), normal non-pedunclotomised animals were studied to provide further information about the immature path and a reference for the results in experimental animals. The climbing fibre labelling seen in normal cerebella had the same general appearance as that already described in the literature (Dupont et al., 1981; Sotelo et al., 1984). The projection is totally crossed and forms broad sagittal

zones within which thinner bands could be seen, this has also been described by Dupont et al. (1981). The topography was difficult to deduce because even small injections labelled a large amount of the neonatal inferior olive which is much smaller than in the adult.

Left Pedunclotomy with Left Inferior Olive Injection

This experimental group was investigated to see whether the aberrant collateral path found in animals with chronic pedunclotomy was present in neonatal animals and subsequently did not degenerate. In the neonatal animals pedunclotomised aged 3 and 7 days climbing fibre labelling after left inferior olivary injection was seen in the right hemicerebellum and in the left vermis. The labelling in the left hemivermis was lighter in those animals pedunclotomised on day 7 than in those which underwent surgery on day 3. In the group pedunclotomised aged 10 days the climbing fibre labelling from the left inferior olive filled the right hemicerebellum and stopped at the midline. Since the injection site did not cross the midline in any of these animals the olivocerebellar projection following acute pedunclotomy at either 3 or 7 days of age is different from normal. The climbing fibre labelling across the midline in the 3 and 7 day pedunclotomy groups, which is not seen in the 10 day operative age group, is in keeping with the results found after chronic pedunclotomy. That the extra climbing fibre labelling stops at the paravermal zone and

does not extend into the hemisphere may indicate three possibilities. The first is that these fibres form part of the normal neonatal terminal field which is larger than that seen in adult animals and are not connected to the climbing fibre plasticity. This phenomenon has been shown in the developing basal pontine nucleus (Mihailoff et al., 1984) and is demonstrated in the normal cerebellar climbing fibre system as the multiple innervation of Purkinje cells. The reason why these fibres are not normally labelled will be discussed in the next section. The second is that these fibres have grown and are the first of those seen in the chronically pedunclotomised cerebella to develop. However, it seems unlikely that they can have grown in 24 hours (the shortest survival time) and if they had it would be inexplicable for no subsequent growth to be seen in those animals which survived 4 days post injection. The third possibility is that the transcommissural collaterals seen in the chronic experiments are present in the neonatal cerebellum but in these experiments they were too lightly labelled to be distinguished from background. In this experimental group for all operative ages extremely small injections were given to try to confine the injection site to the inferior olive and prevent mossy fibre labelling, which can make lightly labelled climbing fibres, especially in the youngest neonates (4-5 days), difficult to identify. The consequence of a small injection volume is a low

specific activity and all the climbing fibre labelling in these animals was rather faint. Too low specific activity is another reason, along with uneven uptake of the amino-acid and negative chemography, for obtaining a falsely negative result (Kunzle and Cuenod, 1973). Uneven uptake of the aminoacid has been minimized because leucine was used and this is extensively transported throughout the nervous system (Kunzle and Cuenod, 1973). Also, control slides for negative chemography were taken and there was no evidence that the cerebellum or brainstem exhibited this phenomenon.

Left Pedunclotomy with Right Inferior Olive Injection

This study group was initially undertaken to provide further evidence of total pedunclotomy and to increase the sparse knowledge of the neonatal olivocerebellar projection. The ipsilateral path which was demonstrated could have arisen from either the inferior olive or the reticular formation which was always labelled because of the ease with which the tracer spread in the neonatal brainstem, especially along the micro-pipette track. The ipsilateral labelling could not have arisen in the reticular formation because there is no evidence that it is a source of climbing fibres (Campbell and Armstrong, 1983a) and mossy fibre labelling is clearly different from that of climbing fibres even in neonatal animals (see Chapter 7, Results - Criteria for Including Data). It is possible that some

labelling within the Purkinje cell layer could have arisen in the reticular formation due to the temporary mossy fibre synapses on the Purkinje cell perisomatic processes (Altman, 1972b). But it is unlikely that these would account for the density of Purkinje cell labelling which, moreover, is the same as the climbing fibre histology (O'Leary et al., 1971) and the descriptions of Dupont et al. (1981) and Sotelo et al. (1984).

It is of interest to note that following axonal transection the neonatal right inferior olivary neurons do not all degenerate at the same rate. After 24 hours the whole inferior olive is still visible but the neurons are degenerating and have indistinct borders. By 48 hours after axotomy most of the medial and dorsal accessory olives had disappeared and most of the principal olivary neurons no longer contained nuclei. Only a few of these neurons remained by 4 days post axotomy and by 5 days the whole inferior olive had disappeared (see Chapter 3). Despite the rapid disappearance the neurons still had sufficient time to take up, metabolise and transport the tracer (Droz and Leblond, 1963) before they degenerated. Further evidence for this is the high density of silver grains overlying the left inferior cerebellar peduncle proximal to the lesion, due to the build-up of transported products along the inferior olivary neurons' axons.

The labelled climbing fibres in the cerebellum did not arise in the left inferior olive because the injection site

did not spill across the midline in either exposure time and the left inferior olivary neurons did not fulfil the criteria for active tracer uptake (see Chapter 7, Results - Criteria for Including Data). Also, the climbing fibre axons of passage traversing the right inferior olive do not take up and transport aminoacids (Cowan et al., 1972). If the tracer had been transported by the left inferior olivary neurons the pattern of labelling within the cerebellum is difficult to explain. In those neonates pedunclotomised aged 3 days the result would confirm that found in the long term survival animals and indicate that a neonatal pathway had been retained after pedunclotomy. But the distribution of cerebellar cortical labelling within the acute and chronic experiments after pedunclotomy on day 7 is different and cannot be explained. Therefore the origin of the labelled climbing fibres is highly unlikely to be the left inferior olive.

Within the cerebellar cortex of those neonates aged 3 days at pedunclotomy, the climbing fibre labelling in the left hemisphere was heavier than in the right hemisphere. Since the left cerebellar peduncles had been cut, this result would imply that there is some inequality in either the number of collaterals to each hemicerebellum or the distribution of transported materials down axon collaterals. If there are two populations of inferior olivary neurons which project to opposite sides of the cerebellum then the density of terminals might be expected to be equal, also their cerebellar projection would be seen in control animals. Therefore, the

ipsilateral cerebellar climbing fibres must be collaterals of normal inferior olivary neurons. It is improbable that they have sprouted in response to axotomy for two reasons. The first is that if they grow at the same rate as normal foetal retinal ganglion cell axons, 80-100 μm/hour (Lund, 1978), and the trauma of axotomy to the main axon does not delay the onset of collateral growth, then it is just possible that in 2 days the developing axons may have reached the vermal midline but it does not seem likely that in the time allowed they would have developed topographically organised terminals throughout the whole bilateral cerebellar cortex. The second reason is that it would seem unlikely that a rapidly degenerating olivary neuron is capable of producing a fast growing new axon, which is neither an extension of nor even in the same direction as the lesioned neurite.

The presence of extra collaterals in the immature nervous system which degenerate during development is not unknown (see review Stanfield, 1984) therefore it is possible that they also exist in the developing olivocerebellar projection. But if the presence of transient collaterals is the explanation for this neonatal ipsilateral pathway it raises some difficult questions. The transient collaterals which have been described in other systems can be demonstrated in the normal neonatal animal whereas both this ipsilateral path and the extra recrossed fibres described in the previous section require an ipsilateral pedunclotomy before they can

be seen. While 'silent-synapses', which become active after the normal path has been removed, have been proposed (Wall, 1977) the concept of silent axons seems rather difficult to accept. Added to this, collaterals are known to be able to maintain the neuron after the main axon has been cut (Fry and Cowan, 1972) but the right inferior olivary neurons were not maintained. It is possible that, since the olivocerebellar fibres enter the cerebellar primordium on embryonic day 17 (E17) (Altman and Bayer, 1978b), the ipsilateral collaterals would be demonstrated following inferior olivary injection on E20 and that they cease functioning prenatally rather than in the second postnatal week, which is when the transient neocortical collaterals cease to be demonstrated (Stanfield, 1984). It is quite probable that a neuron can change the transport of materials down a particular collateral because the perikaryon monitors several functions in relation to intracellular transport: e.g. 1) It alters the rate of axon transport during development (Levine et al., 1982); 2) It controls both retrograde (Bisby, 1982) and several rates of anterograde transport (Brady and Lasek,,1982) simultaneously within a single axon; and 3) It can regulate more than one type of transmitter (Hökfelt et al., 1980). Also, it has been shown that in response to transection of one collateral the components of fast axon transport are rapidly rerouted to the remaining collateral and it has been proposed that it is information from the terminal synapses which directs the

distribution of rapidly transported macromolecules (Aletta and Goldberg, 1984). The perinatal olivocerebellar fibres are still developing and altering their connections, as is evidenced by the multiple innervation of Purkinje cells up to day 15. Therefore, the loss of function in the ipsilateral pathway is quite possibly caused by a reduction, rather than a sudden cessation, of rapid axonal transport along the collateral. Since proteins of even the fast component of axonal transport are retained along the axon this would drastically reduce the supply of constituents to the terminals. Consequently the terminals will degenerate before the parent axon (Munoz-Martinez, 1982) giving the appearance that the collateral is dying back, a phenomenon which would explain the difference between the bilateral projection of the 3 day experimental group and the more restricted termination of the 7 day pedunclotomy group. However, this concept of dying back may not fit with the specialised glial cells found in the developing brain which remove transient collaterals (Ling, 1976 and 1977; Ivy and Killackey, 1978). But there is no evidence of what the signal is to these glia which stimulates them to ingest the collateral axons (Killackey, 1984), perhaps the degenerating terminals of a 'dying-back' axon is one. If the distal branches and terminals of the ipsilateral path are non-functioning and in a poor metabolic state it may explain why these collaterals are not labelled in the control animal and the presence of two malfunctioning collaterals might

explain why the inferior olivary neurons degenerate rather than survive.

SUMMARY OF AFFERENT PLASTICITY

The evidence suggests that the noradrenergic afferents reinnervate the cerebellum following neonatal pedunclotomy. Although noradrenergic fibres have been shown to sprout following axotomy as is discussed above, it seems more likely that the reinnervation is brought about by the collateral sprouting of the remaining fibres which arise in the contralateral locus coeruleus. This rapid return to normal levels of the noradrenergic afferents may help to explain the comparative normality of the cortical histology since noradrenaline is known to be important for normal development (Lawrence and Burden, 1973; Maeda *et al*., 1974).

The aberrant olivocerebellar pathway seen in the animals with chronic pedunclotomy is possibly a retained neonatal path. Although there is very little evidence to support this the climbing fibre labelling in the neonatal experimental group with a left inferior olive injection is clearly different from that seen in controls and cannot be accounted for as a large immature terminal field. It is possible that with injections of higher specific activity and a longer survival time the bilateral innervation found in the chronic animals may be demonstrated in the acutely pedunclotomised animals. Another indication that the

bilateral innervation of the cerebellar cortex seen in the chronically lesioned animals is a persistent neonatal path is the presence of an ipsilateral projection in the neonatal rat. The evidence seems to suggest that the ipsilateral fibres from the inferior olive to the cerebellum are remaining, although degenerating, normal collaterals because they do not maintain the inferior olivary neurons after pedunclotomy and there is now much evidence of transient collaterals in the developing brain.

CONCLUDING DISCUSSION

CHAPTER NINE

CONCLUDING DISCUSSION

CEREBELLAR PEDUNCLOTOMY COMPARED TO OTHER EXPERIMENTAL MODELS

The normality of the cerebellar cortex to the level at which it has been studied indicates that surgical pedunclotomy is a different model from most of the other experimental methods employed for studying cerebellar development. Severe degranulation by either drugs or long term X-irradiation as well as the mutant mice produce a highly disrupted cortical organisation which is extremely difficult to compare with normal development. As Sotelo (1982) concluded, these experiments imply that in abnormal circumstances Purkinje cells will form synapses with whatever afferents are available and developing neurons (particularly the granule cells) need functioning synapses to stabilize their population. However, this makes no allowances for the non-specific effects of antimitotic drugs, repeated X-irradiation or the locus of an abnormal chromosome upon the whole developing system, neuronal or non-neuronal. An indicator of these non-specific effects is found in those experiments inducing mild degranulation. If it is induced by mossy fibre reduction (Hamori, 1969) or malnutrition (Bedi *et al.*, 1980; Chen and Hillman, 1980; Hillman and Chen, 1981a; Borges and Lewis, 1983; Dvergsten *et al.*, 1983) the cortical histology appears unaltered and the

Purkinje cells have a normal although smaller dendritic tree (McConnell and Berry, 1978a; Chen and Hillman, 1980). But after X-irradiation, drug- or viral-induced partial external granular layer destruction the parallel fibres become misaligned (Altman, 1973; Herndon and Oster-Granite, 1975) and the Purkinje cell dendritic tree becomes "weeping-willow" shaped (Bradley and Berry, 1976a, 1978b; Crepel et al., 1980). Since Angaut et al. (1982) found that the Purkinje cell dendritic trees in pedunclotomised hemispheres were relatively normal with only some cells possessing spines on the primary dendritic trunks, a result also seen in a few preliminary Golgi impregnations undertaken for this study, and the reduced Purkinje cell somatic diameter probably reflects a smaller dendritic tree, it would appear that pedunclotomy produces a similar effect on cerebellar development as malnutrition. But malnutrition will reduce the substrates required for normal neuronal proliferation and growth while deafferentation possibly reduces the stimulus for such growth. Also, any of the effects of pedunclotomy being due to malnutrition have been removed because only those animals of normal body weight were included.

The normal histology found after pedunclotomy is similar to that seen after climbing fibre ablation (Kawaguchi et al., 1975; Bradley and Berry, 1976a; Sotelo and Arsenio-Nunes, 1976), which decreases the size of the Purkinje cell dendritic tree, if the Purkinje cell neuronal

diameter is a reflection of the dendritic tree size. But in the previous experiments no mention has been made of a reduced cerebellar size. However, a reduction in cerebellar volume and granule cell numbers has been described if the mossy fibre afferents are reduced (Hamori, 1969), which would suggest that the majority of the weight loss after pedunclotomy is due to a reduction in the number of mossy fibres. But it is difficult to understand why the mossy fibres should have such an important neurotrophic role when they do not arrive in the cortex until postnatal day 7 and are even then some distance from their presumptive target cells, at that time still undifferentiated and in the external granular layer. Also the mossy fibre projection is known to be bilateral (e.g. Burne et al., 1978 a & b; Llinas and Simpson, 1981; Mihailoff et al., 1981) with either an ipsilateral (lateral reticular nucleus) or contralateral (basal pontine and pontine tegmental reticular nuclei) predominance, therefore unilateral pedunclotomy does not totally remove the mossy fibres from an hemicerebellum. Because the mossy fibre afferents are only reduced and not abolished this may not explain the slower growth rate following pedunclotomy especially since the degree of bilaterality among lateral reticular mossy fibres increases after pedunclotomy (Payne and Bower, 1983). Unfortunately following mossy fibre reduction the number and size of Purkinje cells has not been studied.

Because of the extensive non-specific toxicity of 6-OHDA it is very difficult to compare previous studies on the effect of noradrenaline on cerebellar development with cerebellar pedunclotomy. Because the noradrenergic innervation, like that of the mossy fibres, is predominantly bilateral, unilateral pedunclotomy will only reduce the innervation by just over 50% but because noradrenaline is known to be important in neuronal development (Lawrence and Burden, 1973), maturation (Maeda et al., 1974) and plasticity (Kasamatsu and Pettigrew, 1979; Kasamatsu et al., 1979) it is not impossible that even a 50% loss of noradrenergic innervation will disrupt the cerebellar growth, especially of the external granular layer. Although it is impossible to assess to what extent noradrenergic denervation is responsible for the effects of pedunclotomy it would seem unlikely that its role in postnatal cerebellar development is as minor as was suggested by Berry et al. (1980 a & b). An indication of the importance of noradrenaline in postnatal cerebellar development is found in the result after pedunclotomy on day 1 when noradrenergic fibres are the only afferents being removed since mossy and climbing fibres have not grown further than the central medulla. Although the lesion would delay the subsequent arrival of mossy and climbing fibres into the cerebellar cortex this would also occur following pedunclotomy on day 3, yet the cerebellum is more severely affected by pedunclotomy at the younger age. This possibly indicates that noradrenaline is important for the rapid proliferation within the external granular layer.

THE EFFECT OF PEDUNCLOTOMY ON CEREBELLAR DEVELOPMENT

Pedunclotomy on Day 3

Unilateral cerebellar pedunclotomy on day 3 appears to have a relatively small effect on the cerebellar cortical development because the left hemispheric weight is not significantly below normal and neither the Purkinje cell numbers nor size is reduced. This result is quite possibly due to the transection of the noradrenergic afferents when they are morphologically immature rapidly growing fibres. Since the density of noradrenergic fibres is still increasing on day 3 (Yamamoto *et al.*, 1977), it seems likely that the remaining preterminal axons will continue growing to replace those which have degenerated, therefore minimizing any noradrenergic deprivation resulting from the pedunclotomy. In addition to this, the climbing fibres are removed before they have entered the developing cortex from the central white medulla and 4 days before the majority of Purkinje cells have received synaptic connections (Altman, 1972b). The autoradiographic results from the chronically pedunclotomised animals reveal an extensive bilateral projection from the intact left inferior olive. If this pathway is present in the neonatal rat and therefore retained then the cerebellar cortex can continue to develop with a functional, albeit abnormal, olivary input. But if the bilateral path has grown by collateral sprouting then pedunclotomy on day 3 allows it to have been growing for 4 days before the majority of cortical components will have a deficient

synaptic input. If mossy fibres, like noradrenergic fibres (Schmidt et al., 1980), have a greater adaptive capacity in younger animals then it is likely that they too are able to offset some of the afferent deficit through collateral sprouting. Therefore, it would appear that it is the immaturity of the 3 day cerebellum and its afferents which make it so resistant to unilateral pedunclotomy.

Pedunclotomy on Days 5 and 10

The cerebellar response to unilateral pedunclotomy on days 5 and 10 gives an interesting insight into its development. Following unilateral pedunclotomy at these two ages the cerebellar growth curves are remarkably similar (with the exception of the initial weight loss of the left hemisphere after pedunclotomy on day 10) as are the Purkinje cell numbers in the left hemisphere. But the Purkinje cells are smaller than normal after pedunclotomy on day 10 but not on day 5. Despite the similarities the cerebellar developmental stages at the two ages are quite different. By postnatal day 5 the external granular layer cells are continuing to proliferate rather than differentiate, the Purkinje cells are spreading out into a monolayer and the climbing fibres are beginning to form synapses on the Purkinje cell perisomatic processes while the noradrenergic fibres are still growing. Therefore the noradrenergic afferents will rapidly reinnervate the denervated cortex and, because there is reinnervation in

both the 3 and 7 day pedunclotomy groups, the climbing fibres will also be retained, or develop, although probably not quite as extensively as in the 3 day pedunclotomy group. The reduced left hemispheric size is probably caused by a post-traumatic pause in the normal development followed by an initially slower growth rate, which can be seen in the growth curve (Fig. 3.9A), therefore resulting in a smaller cerebellum by day 15, when microneuron production ceases (Altman, 1972c). However, by 10 days of age the cortex is more stable because the basket cells have been formed and are starting to develop synapses onto the Purkinje cell somata and many granule cells have differentiated and migrated to the internal granular layer. Also, the Purkinje cell monolayer is well established and the neuronal polarity is determined by the large apical growth cone towards which the climbing fibres are starting to move. But the cerebellum is as severely affected by pedunclotomy on day 10 as at day 5 because, although the by now regressing noradrenergic fibres do reinnervate the left hemicerebellum, the climbing fibres do not and there is no evidence of a neonatal path which is retained. But this is despite the retention of sufficient climbing fibre 'plasticity' to reorganise the multiple innervation of Purkinje cells even as late as day 15 (Crepel et al., 1976; Mariani and Changeux, 1981).

Although the left hemisphere weight and Purkinje cell numbers are almost identical for both experimental age

groups the cortical circuitry will inevitably be different because pedunclotomy on day 5 is more likely to affect the basket and early granule cells, while on day 10 the stellate and later formed granule cells are more likely to be affected. Added to this, after pedunclotomy on day 10 the left hemicerebellum does not contain any climbing fibres. Therefore it seems likely that the number of Purkinje cells surviving pedunclotomy is dependent on the volume (i.e. the weight) of the cerebellar cortex at a crucial stage of development presumably between the formation of the monolayer (days 4-5) and synaptogenesis (days 21-30) and that there is a critical packing density greater than which the Purkinje cells cannot survive. This conclusion is consistent with the electron microscopic evidence, despite the light microscope appearance to the contrary, that the Purkinje cell perikarya are closely packed and only separated by thin glial sheets (Palay and Chan-Palay, 1974). The apparently inconsistent result obtained after pedunclotomy on day 22, that the number of Purkinje cells is normal despite a reduced hemisphere, is probably explained by the considerably smaller somatic diameter and the capability of the basket and parallel fibre synapses to maintain the cell. The smaller Purkinje cell diameters 35 days after pedunclotomy on day 10 probably indicate that the climbing fibres do have some trophic effect on the developing dendritic tree, which was proposed by Kornguth and Scott (1972) and is in agreement

with the smaller Purkinje cell dendritic tree found after climbing fibre ablation (Bradley and Berry, 1976a; Sotelo and Arsenio-Nunes, 1976).

Pedunclotomy on Day 7

Unilateral pedunclotomy has been shown to have a severe effect on the cerebellar development. At this age four major events take place in the developing cortex:- the mossy fibres reach the internal granular layer; the climbing fibres have synapsed upon the Purkinje cell perisomatic processes; the noradrenergic fibre plexus has reached its maximum density and thereafter starts to regress; and the external granular layer cells change from predominantly proliferation to differentiation of the granule cells. Therefore, unilateral pedunclotomy will drastically reduce the noradrenergic input to the external granular layer at a time when the primary function is changing from proliferation to differentiation, and while the noradrenergic input to this layer is probably fairly small (Schmidt et al., 1981; Sievers and Klemm, 1982) the noradrenergic fibres which terminate throughout the developing molecular layer are postulated to have a neurohumoral effect (Schulman, 1983). Also, pedunclotomy will denervate the Purkinje cells just as they have formed their first functional synaptic contacts and before their input can be replaced by either basket cell axons or parallel fibres. Although the autoradiographic results

from the chronically pedunclotomised animals show that there is an additional climbing fibre input it is less well organised even within the considerably reduced left cerebellar hemisphere than the innervation seen in the group pedunclotomised on day 3. If this chronic pathway is present in the neonatal cerebellum it is likely to be more degenerate by day 7 than on days 3 or 5 and therefore less extensively distributed throughout the hemisphere and less capable of forming synapses and supporting the Purkinje cells. Alternatively, if the path is formed by collateral sprouting in response to pedunclotomy then the Purkinje cells will be without climbing fibre innervation for longer than those in the 3 and 5 day experimental groups.

It is not possible to say whether the extensive Purkinje cell loss is due to deafferentation at a crucial age or whether the pedunclotomy so affects the granule cell production, and consequently the hemispheric volume, that the Purkinje cells become too closely packed to be able to survive. However, it seems quite possible that the extensive Purkinje cell loss is a result of more than one factor. Since an enormous number of cells die within the first five post operative days, this could be because some neurons, either further or less advanced than the 'average', are more susceptible to sudden deafferentation combined with the minimal growth (3.5 mg instead of the normal 24.5 mg) taking place which prevents the Purkinje cell density from decreasing to the expected level. There is

a further substantial Purkinje cell loss between days 22 and 42, possibly during synaptogenesis a time when inappropriately connected neurons are known to die (Kelly and Cowan, 1972), and is also likely to be caused by overcrowding because 1) by this age the surviving Purkinje cells will receive at least some parallel fibre synapses, 2) Purkinje cells do survive after perinatal climbing fibre ablation (Bradley and Berry, 1976a; Sotelo and Arsenio-Nunes, 1976) and 3) Angaut et al. (1982) found Purkinje cells both with and without climbing fibre synapses in adult animals which underwent pedunclotomy at birth. This result would also be in accordance with the hypothesis that the number of Purkinje cells is dependent upon the cortical volume. An exception to this would be the small agranular cerebella found in mutant mice (Jacobson, 1978) in which there is no evidence of Purkinje cell loss but in this type of disorganised cortex the Purkinje cells are not constrained to occupy a monolayer.

Pedunclotomy on Day 22

Unilateral cerebellar pedunclotomy on day 22 causes the ipsilateral hemisphere to consistently lose weight over the subsequent 35 days. By day 22 the cerebellar microneurons are formed and have migrated to their normal positions. During the 4th postnatal week synaptogenesis and glial proliferation take place. Although the left hemisphere loses weight the number of Purkinje cells is

not affected, however their somatic diameters are significantly reduced.

Since there is no climbing fibre regeneration after pedunclotomy on day 10 it is highly unlikely that there will be any after pedunclotomy on day 22 and it has been assumed that there was none. However the noradrenergic innervation was shown to have returned to control levels even after transection at this late age. This response is most probably due to terminal sprouting of the intact bilateral innervation from the contralateral locus coeruleus, a phenomenon demonstrated in other central nervous systems and discussed in chapter 8. It also illustrates that 6-OHDA does directly damage the locus coeruleus neurons since they will not regenerate their axons to the cerebellum if they are injured after 12 days post partum (Schmidt et al., 1980).

Because of the hemispheric weight loss and the retention of the normal number of Purkinje cells there must have been a reduction of the number of granule cells. This has been described in chapter 4 - Cerebellar Histology/Adult Cerebella (Fig. 4.5). The granule cell loss was almost certainly a transneuronal degeneration in response to substantial mossy fibre deafferentation just prior to the normal age of synaptogenesis (Kelly and Cowan, 1972). This in turn will reduce the number of parallel fibres therefore the width of the molecular layer and the size of the Purkinje cell dendritic tree. This transneuronal

atrophy of the Purkinje cell dendrites due to a loss of parallel fibre afferent input is almost certainly reflected in the small perikarya.

CONCLUSIONS

This study on the effect of unilateral pedunclotomy on cerebellar development shows that its subsequent growth is differentially affected depending on the age at which the neonate undergoes the operation. This is probably due to a combination of the stage of cerebellar development at the time of the lesion and the capacity of the afferent systems to plasticise. The number of Purkinje cells in the pedunclotomised cerebella of different experimental groups indicates that the population size is dependent upon cortical volume and that there may be a critical packing density during development above which the Purkinje cells cannot survive. Also, the establishment of the monolayer is sufficiently rigidly programmed that Purkinje cells will degenerate rather than the monolayer be disrupted (see Figs. 4.2, 4.6 and 4.9). The reduced diameters of the surviving Purkinje cells probably reflect a smaller dendritic tree, which suggests that the normal complement of afferents, both climbing fibres (abolished in the 10 and 22 day pedunclotomy groups) and parallel fibres (greatly reduced after pedunclotomy on days 7 and 22), are important for the growth and maintenance of dendrites.

Noradrenergic fibres are known to be capable of local regeneration after axotomy and in this study are seen to exhibit a form of collateral sprouting found elsewhere in the nervous system. It also seems probable that they have an important role in postnatal cerebellar development which indicates that the non-specific toxicity of 6-OHDA masks any developmental response to noradrenergic denervation. As has been indicated by Angaut et al. (1982) and Alvarado-Mallart et al. (1983) the olivocerebellar projection will demonstrate plasticity, a capacity which has been shown in this study to be lost between the ages of 7 and 10 days post partum. However, it seems likely that this is due to the retention of an aberrant neonatal projection rather than denervation-induced collateral sprouting. In addition to this an extra aberrant ipsilateral pathway has been discovered in neonates up to the end of the first postnatal week.

APPENDICES AND BIBLIOGRAPHY

Titles of abstracts of communications given during the course of this thesis

Bower, A. J. and Sherrard, R. M. (1982)

"Observations on the effect of unilateral cerebellar pedunclotomy in the developing cerebellum of the rat."

Neurosci Lett Suppl 10: S37

Sherrard, R. M. and Bower, A. J.

"A bilateral olivocerebellar projection via the ipsilateral inferior cerebellar peduncle in the neonatal rat: An autoradiographic study."

J. Anat in press

APPENDIX I

10% BUFFERED FORMALIN

40% formaldehyde	100 ml
Distilled water	900 ml
Acid sodium phosphate (NaH_2PO_4)	4 g
Anhydrous disodium phosphate (Na_2HPO_4)	6.5 g

Mix together with stirring until dissolved, 15 minutes.

APPENDIX II

1% TOLUIDINE BLUE

Materials

Toluidine blue	1 g
Distilled water	100 ml

Dissolve the stain in the water and filter before use.

Procedure

1. Place sections in stain for 2 minutes.
2. Rinse in distilled water.
3. Differentiate in running tap water for 5 minutes.
4. Allow to dry overnight and mount in DPX polymount.

Results

Nissl substance and nucleoli	deep purple/blue
Nuclei	light blue
Background	unstained.

APPENDIX III

SUBBED SLIDES

Wash slides in Tepol or Pyroneg for at least 2 hours.
Rinse in running tap water overnight.
Make up a gelatin mixture:-

Gelatin	10 g
Chromium potassium sulphate	10 g
Distilled water	2 l

Dissolve the chrome alum in a small quantity of water.
Add the rest of the water.
Slowly add the gelatin with stirring.
Warm to 40°C until clear.

Immerse the clean slides in the gelatin solution then drain vertically to dry.

APPENDIX IV

1% CRESYL FAST VIOLET

Materials

Cresyl Fast Violet	1 g
Distilled water	100 ml

Dissolve stain and filter prior to use.

Procedure

1. Dewax and rehydrate sections with water.
2. Place in stain 1 minute.
3. Rinse in distilled water.
4. Differentiate in 96% alcohol.
5. Dehydrate in two changes of absolute alcohol and clear in xylene.
6. Mount in DPX polymount.

Results

Nissl substance and nuclei	deep purple
Background	v. pale pink

APPENDIX V

A comparison of weight data and Purkinje cell diameters in perfusion and infiltration fixed brains from animals aged 44 days.

	Weight mg ± SD	
	Perfusion (5)	Infiltration (4)
Brain	1812.5 ± 76.9	1840 ± 40.3
Cerebellum	273.8 ± 19.1	284.6 ± 7.9
L. Hemisphere	78.2 ± 5.3	83.3 ± 5.4
Vermis	106.0 ± 9.2	109.2 ± 4.6
R. Hemisphere	87.5 6.2	90.8 ± 5.2

None of the differences between the two methods of fixation are statistically significant.

Diameter size µm		14	15	16	17	18	19	20	21	22	23	24	25	26	27
% of Cells	P	0.5	0.2	3.6	6.5	17.4	18.7	19.2	19.3	8.6	5.2	1.4	0.4	-	-
	In	0.1	1.0	4.2	8.4	15.3	16.2	16.1	18.9	9.4	5.8	1.8	1.5	1.0	0.3

APPENDIX VI

The body weights of animals at the time of sacrifice.

A. Data from animals which survived 35 days post operatively.

B. Data from animals in the time-course experiments.

A.

Age at Operation (Days)	Sex	Weight (g)			
		Op		C	
		$\bar{x}$	SD	$\bar{x}$	SD
1	M	97.9	4.8	104.8	1.3
	F	90.8	0	84.1	8.1
3	M	94.0	1.9	97.9	12.1
	F	93.6	4.4	86.8	4.7
4	M	104.8	7.2	107.0	8.8
	F	90.7	7.7	94.4	10.4
5	M	123.2	4.0	117.2	5.9
	F	95.0	7.9	97.6	15.1
6	M	121.5	3.9	117.8	9.9
	F	122.3	0	100.5	11.9
7	M	106.3	20.7	119.2	15.5
	F	95.5	13.1	115.0	7.4
8	M	118.1	8.7	122.3	10.9
	F	96.8	13.2	109.3	12.9
9	M	122.1	6.7	150.4	16.1
	F	98.9	10.9	119.3	10.7
10	M	122.0	17.1	129.8	12.5
	F	102.9	11.4	113.9	8.2
15	M	154.2	14.5	153.8	7.1
	F	-	-	-	-
22	M	182.4	7.7	183.9	32.7
	F	155.4	15.7	128.3	12.8
30	M	216.4	18.9	197.6	26.2
	F	171.4	7.8	175.6	11.1

B.

Age at Operation (Days)	Sex	Post operative Survival Time (Days)											
		5		10		15		20		25		30	
		Op	C	Op	C	Op	C	Op	C	Op	C	Op	C
5	M	17.3 ±1.7	18.0 ±1.1	24.5 ±3.1	25.5 ±2.9	31.5 ±3.4	31.3 ±3.5	47.4 ±4.3	55.9 ±5.3	66.1 ±3.0	68.6 ±3.3	71.0 ±2.5	93.7 ±3.2
	F											75.8 ±4.6	81.0 ±16.7
7	M	19.3 ±2.0	20.4 ±1.0	27.6 ±3.1	28.0 ±0	30.9 ±6.3	36.2 ±0.9	55.5 ±2.4	55.5 ±2.2	74.9 ±4.4	67.8 ±7.9	95.9 ±7.7	87.9 ±0
	F											89.9 ±8.3	87.6 ±5.3
10	M	22.4 ±3.4	25.5 ±2.9	29.4 ±3.5	31.3 ±3.5	52.6 ±2.7	55.9 ±5.2	65.1 ±2.3	68.6 ±3.3	101.4 ±8.4	93.7 ±3.2	91.1 ±0	117.2 ±5.9
	F									94.0 ±5.5	81.0 ±16.7	82.9 ±6.8	97.6 ±15.1
22	M	49.9 ±6.6	55.5 ±2.2	77.2 ±7.7	67.8 ±7.7	73.4 ±6.6	87.9 ±0	110.2 ±0.8	119.2 ±15.5	126.4 ±4.9	137.4 ±11.1	-	-
	F					86.5 ±2.8	87.6 ±5.3	109.6 ±2.1	115.0 ±7.4	113.1 ±0	114.4 ±0	144.1 ±0.5	119.4 ±8.4

APPENDIX VII

QUANTITATION

A typical example of the sagittal length of the Purkinje cell layer and the Purkinje cell linear density from an adult animal.

Distance from medial edge mm	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.2	1.3	1.4	1.5	1.6
Sagittal length mm	28.3	32.6	35.9	39.3	37.8	37.6	38.1	38.3	38.9	38.9	40.1	40.1	40.7	37.3	37.8	36.9	35.8
PC Density cells/mm	16.53	17.3	18.32	15.96	16.51	16.84	16.82	16.34	16.72	16.2	15.23	15.53	15.09	17.28	16.22	17.3	17.52
Distance from medial edge mm	1.7	1.8	1.9	2.0	2.1	2.2	2.3	2.4	2.5	2.6	2.7	2.8	2.9	3.0	3.1	3.2	
Sagittal length mm	34.3	34.2	33.9	32.9	32.7	32.4	26.7	24.4	24.1	20.6	19.1	17.1	11.8	8.3	6.8	5.1	
PC Density cells/mm	17.76	17.01	17.08	15.64	17.17	16.51	15.92	18.26	17.25	16.79	17.0	19.05	18.98	18.92	16.45	15.22	

This table shows that there is no mediolateral variation in the linear Purkinje cell density. There is a maximum of 2% difference between the number of Purkinje cells if the calculations involve every section, alternate sections or just two sections.

	Linear PC density/mm	PC Layer area mm^2	PC Number
Sections every 100 µm	16.87 ± 1.03	99.9	84,249
Sections every 200 µm	16.84 ± 1.19	101.7	85,606
2 sections from those taken every 200 µm	16.91		85,962

APPENDIX VIII

The mean logarithm of Purkinje cell diameters for animals pedunclotomised at 3, 5, 7, 10 or 22 days of age which survived up to 35 days post operatively. Within each group a mean diameter and standard deviation is obtained for each animal. These standard deviations are logged and a new mean and standard deviation of these logarithms was calculated for each group. The resultant numbers are in this table. A comparison between the experimental and control values analyses whether the shape of the original Purkinje cell diameter distribution for each group is different between experimental and control animals. There is a 5% probability of any difference being due to chance, therefore, the one significant difference seen in this table (10-10, $p < 0.01$) is probably not relevant.

† = $p < 0.01$

Age at Operation (Days)		Mean Logarithm of Purkinje Cell Diameter Standard Deviations														
		Post Operative Survival Time (Days)														
		5			10			15			25			35		
		n	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD
3	Op													6	-6.01187	0.20273
	C													6	-5.87672	0.151109
5	Op	6	-6.21461	0				5	-5.80914	0	6	-5.94429	0.19114	6	-6.01187	0.20273
	C	6	-6.21461	0				4	-6.12112	0.18058	5	-6.13352	0.16219	4	-6.21461	0.49013
7	Op	6	-6.07945	0.19114				5	-5.97133	0.19864				6	-6.01187	0.20273
	C	5	-6.21461	0				4	-6.01187	0.20273				6	-6.07945	0.19114
10	Op				6	-5.80914†	0							6	-6.01187	0.20273
	C				4	-6.12112	0.18058							5	-6.21461	0
22	Op													3	-5.94429	0.19114
	C													5	-5.89023	0.16219

APPENDIX VIIIA

PURKINJE CELL DIAMETER DISTRIBUTION

5-5

Diameter size µm		10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	Total numbee cells
Number of cells	C	1	4	8	38	68	153	182	180	134	68	39	22	6	2	1	906
	Op	2	13	23	80	113	133	117	77	47	31	9	4	0	1	-	650

5-15 & 10-10

Diameter size µm		12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	Total number cells
Number of cells	C	-	3	8	17	28	52	71	65	90	97	81	56	32	19	8	4	-	-	-	631
	Op5	3	11	28	55	71	93	90	95	120	90	46	35	26	16	10	3	1	0	1	794
	Op10	3	5	24	43	57	68	80	94	101	106	71	62	34	14	12	7	2	1	-	784

5-25

Diameter size µm		12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	Total number cells
Number of cells	C	-	-	-	8	26	46	98	118	175	183	166	161	92	62	25	12	2	2	1	-	-	-	-	1177
	Op	1	2	6	16	44	71	110	111	141	109	96	74	44	27	15	14	3	3	0	0	0	0	1	888

7-5

Diameter size µm		10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	Total number cells
Number of cells	C	-	1	2	15	40	106	171	205	279	193	174	102	42	20	15	2	0	0	0	1	1368
	Op	1	3	18	36	77	122	105	95	72	50	40	13	5	4	0	0	0	1	-	-	642

7-15

Diameter size µm		12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	Total number cells
Number of cells	C	-	1	3	13	32	61	81	81	93	90	63	58	27	16	9	3	2	2	-	665
	Op	1	3	11	27	29	63	63	66	53	55	36	29	15	10	3	0	1	0	1	466

7-35

Diameter size µm		12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Total number cells
Number of cells	C	-	-	2	4	29	64	129	186	199	186	126	91	46	35	23	10	5	1	2	1	1139
	Op	4	22	47	95	126	127	137	93	62	45	28	18	7	5	2	1	-	-	-	-	819

5-35

Diameter size µm		12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	Total number cells
Number of cells	C	-	3	12	53	108	126	161	149	92	60	38	37	33	14	8	5	2	1	902
	Op	1	5	27	36	68	98	120	115	93	89	58	31	29	10	6	2	1	-	789

10-35

Diameter size µm		11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	Total number cells
Number of cells	C	-	-	-	1	7	33	65	119	126	125	147	73	45	14	12	8	2	777
	Op	1	5	19	63	124	141	144	128	85	65	45	34	24	12	8	4	3	905

3-35

Diameter size µm		14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	Total number cells
Number of cells	C	6	17	56	82	89	87	81	67	60	48	36	10	8	3	3	653
	Op	6	18	51	84	113	124	99	76	47	43	22	16	4	2	2	707

22-35

Diameter size µm		12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	Total number cells
Number of cells	C	-	1	2	14	29	62	96	110	122	97	88	57	41	21	9	7	0	2	759
	Op	1	5	12	30	53	68	53	40	41	38	21	9	4	3	1	-	-	-	378

APPENDIX IX

ALUMINIUM-FORMALDEHYDE PERFUSION FOR CATECHOLAMINE FLUORESCENCE

Preperfusate

A modified Tyrode's buffer pH 7.2 contains per litre of distilled water:-

NaCl	8.0 g
KCl	0.2 g
$CaCl_2.6H_2O$	0.4 g
$MgCl_2.H_2O$	0.1 g
$NaHCO_3$	1.0 g
$NaH_2PO_4.2H_2O$	0.05g
D-Glucose	1.0 g

For Adult Animals:-

Add 20 g glyoxylic acid monohydrate/L
Add NaOH pellets until pH 7
Cool on ice.

For Neonatal Animals:-

Add 2 g $MgSO_4.7H_2O$ and 1 g procain chloride/L
Use at room temperature.

Perfusate

20 g paraformaldehyde is dissolved in 500 ml distilled water by heating to 60°C.
Add 4-6 drops 1N NaOH and cool.
Add 500 ml ice cold double-strength Tyrode's buffer.
Add 100 g $Al_2(SO_4)_3.18H_2O$/L
Adjust pH to 3.8 with 3.8 g $Na_2B_4O_7.10H_2O$/L.
Add 0.1% azure B to a deep royal blue colour and keep on ice.

ABERCROMBIE, M. (1946). Estimation of nuclear population from microtome sections.
Anat. Rec. 94: 239-247.

ADDISON, W.H.F. (1911). The development of the Purkinje cells and of the cortical layers in the cerebellum of the albino rat.
J. Comp. Neurol. 21: 459-487.

ALETTA, J.M. & D.J. GOLDBERG (1984). Routing of transmitter and other changes in fast axonal transport after transection of one branch of the bifurcate axon of an identified neuron.
J. Neurosci. 4: 1800-1808.

ALTMAN, J. (1969). Autoradiographic and histological studies of postnatal neurogenesis III. Dating the time of production and onset of differentiation of cerebellar microneurons in rats.
J. Comp. Neurol. 136: 269-294.

ALTMAN, J. (1972a). Postnatal development of the cerebellar cortex in the rat. I. The external granular layer and the transitional molecular layer.
J. Comp. Neurol. 145: 353-398.

ALTMAN, J. (1972b). Postnatal development of the cerebellar cortex in the rat. II. Phases in the maturation of Purkinje cells and of the molecular layer.
J. Comp. Neurol. 145: 399-464.

ALTMAN, J. (1972c). Postnatal development of the cerebellar cortex in the rat. III. Maturation of the componants of the granular layer.
J. Comp. Neurol. 145: 465-514.

ALTMAN, J. (1973). Experimental reorganisation of the cerebellar cortex in the rat. IV. Parallel fibre reorientation following regeneration of the external germinal layer.
J. Comp. Neurol. 149: 181-192

ALTMAN, J. (1975). Postnatal development of the cerebellar cortex in the rat. IV. Spatial organisation of bipolar cells, parallel fibres and glial pallisades.
J. Comp. Neurol. 163: 427-448.

ALTMAN, J. (1982). Morphological development of the rat cerebellum and some of its mechanisms.
In "The Cerebellum - New Vistas" Exp. Brain Res. Suppl. 6 S.L. Palay & V. Chan-Palay (Eds.), Springer-Verlag Berlin, Heidelberg, New York.

ALTMAN, J. & W.J.ANDERSON (1972). Experimental reorganisation of the cerebellar cortex. I. Morphological effects of elimination of all microneurons with prolonged X-irradiation started at birth.
J. Comp. Neurol. 146: 355-406.

ALTMAN, J. & S.A. BAYER (1978a). Prenatal development of the cerebellar system in the rat. I. Cytogenesis and histogenesis of the deep nuclei and the cortex of the cerebellum.
J. Comp. Neurol. 179: 23-48.

ALTMAN, J. & S.A. BAYER (1978b). Prenatal development of the cerebellar system in the rat. II. Cytogenesis and histogenesis of the inferior olive, pontine grey and precerebellar reticular nuclei.
J. Comp. Neurol. 179: 49-76.

ALTMAN, J. & G.D. DAS (1970). Postnatal changes in the concentration and distribution of cholinesterase in the cerebellar cortex of rats.
Exp. Neurol. 28: 11-34.

ALTMAN, J. & A.T. WINFREE (1977). Postnatal development of the cerebellar cortex in the rat. V. Spatial organisation of Purkinje cell perikarya
J.Comp.Neurol. 171: 1-16.

ALVARADO-MALLART, R.M., ANGAUT, P. & C. SOTELO (1983). Transcommisural climbing fibres in the adult rat cerebellum following unilateral neonatal pedunculotomy.
J. Physiol. 345: 164P.

ALVARADO-MALLART, R.M. & C. SOTELO (1982). Differentiation of cerebellar analge heterotypically transplanted to adult rat brain: A light and electron microscopic study.
J. Comp. Neurol. 212: 247-267.

ANGAUT, P., ALVARADO-MALLART, R.M. & C. SOTELO (1982). Ultrastructural evidence for compensatory sprouting of climbing and mossy afferents to the cerebellar hemisphere after ipsilateral pedunculotomy in the newborn rat.
J. Comp. Neurol. 205: 101-111.

ANGAUT, P. & C. SOTELO (1975). Diversity of mossy fibres in the cerebellar cortex in relation to different afferent systems: an experimental electron microscopic study in the cat.
Brain Res. 95: 179-189.

ARIENS KAPPERS, C.U., HUBER, G.C. & E.C. CROSBY (1936). "The comparative Anatomy of the Nervous System of Vertebrates, Including Man." Hafner Publishing Company, New York.

ARMSTRONG, D.M., CAMPBELL, N.C., EDGLEY, S.A., SCHILD R.F. & J.R. TROTT (1982). Investigations of the olivo cerebellar and spino-olivary pathways. In " The Cerebellum - New vistas." Exp. Brain Res. Suppl. 6. S.L. Palay & V. Chan-Palay (Eds.), Springer-Verlag, Berlin, Heidleberg and New York.

ARMSTRONG, D.M., HARVEY, R.J. & R.F. SCHILD (1971). Distribution in the anterior lobe of the cerebellum of branches from climbing fibres to the paramedian lobule. Brain Res. 25: 203-206.

ARMSTRONG, D.M., HARVEY, R.J. & R.F. SCHILD (1973a). Branching of inferior olivary axons to terminate in different folia or lobes of the cerebellum. Brain Res. 54: 365-371.

ARMSTRONG, D.M., HARVEY, R.J. & R.F. SCHILD (1973b). Cerebello-cerebellar responses mediated via climbing fibres Exp. Brain Res. 18: 19-39.

ARMSTRONG, D.M., HARVEY, R.J. & SCHILD R.F. (1973c). The spatial organisation of climbing fibre branching in the cat cerebellum. Exp. Brain Res. 18: 40-58.

ARMSTRONG, D.M., HARVEY, R.J. & R.F. SCHILD (1974). Topographical localisation in the olivo-cerebellar projection: an electrophysiological study in the cat. J. Comp. Neurol. 154: 287-302.

ARMSTRONG, D.M. & R.F. SCHILD (1970). A quantitative study of the Purkinje cells in the cerebellum of the albino rat. J. Comp. Neurol. 139: 449-456.

ARMSTRONG, D.M. & R.F. SCHILD (1978a). An investigation of the cerebellar cortico-nuclear projections in the rat using an autoradiographic tracing method.I. Projections from the vermis. Brain Res. 141: 1-19.

ARMSTRONG, D.M. & R.F. SCHILD (1978b). An investigation of the cerebellar cortico-nuclear projections in the rat using an autoradiographic tracing method. II. Projections from the hemisphere.
Brain Res. 141:235-249.

AZIZI, S.A., MIHAILOFF, G.A., BURNE, R.A. & D.J. WOODWARD (1981). The pontocerebellar system in the rat: An HRP study. I. Posterior vermis.
J. Comp. Neurol. 197: 543-558.

BASCO, E., HAJOS, F. & Z. FULOP (1977). Proliferation of Bergmann-glia in the developing rat cerebellum.
Anat. Embryol. 151: 219-222.

BATINI, C., CORVISIER, J., DESTOMBES, J., GIOANNI, H. & J. EVERETT (1976). The climbing fibres of the cerebellar cortex, their origin and pathways in the cat.
Exp. Brain Res. 26: 407-422.

BECKSTEAD R.M. & R. NORGREN (1979). An autoradiographic examination of the central distribution of the trigeminal, facial, glossopharyngeal and vagal nerves in the monkey.
J. Comp. Neurol. 184: 455-472.

BEDI, K.S., HALL, R., DAVIES, C.A. & J DOBBING (1980). A stereological analysis of the cerebellar granule and Purkinje cells of 30-day old and adult rats undernourished during early postnatal life.
J. Comp. Neurol. 193: 863-870.

BENDEICH, E.G., KONKOL, R.J., KRIGMAN, M.R. & G.R. BREESE (1978). Morphological evidence for 6-hydroxydopamine induced sprouting of noradrenergic neurons in the cerebellum.
J. Neurol. Sci. 38: 47-57.

BERRY, M & P. BRADLEY (1976a). The growth of the dendritic trees of Purkinje cells in the cerebellum of the rat.
Brain Res. 112: 1-35.

BERRY, M & P. BRADLEY (1976b). The growth of the dendritic trees of Purkinje cells in irradiated agranular cerebellar cortex.
Brain Res. 116: 361-387.

BERRY, M., SIEVERS, J. & H.G. BAUMGARTEN (1980a). The influence of afferent fibres on the development of the cerebellum.
In "A Multidisiplinary Approach to Brain Development." C. di Benedetta (Ed.), Elsevier North Holland, The Netherlands.

BERRY, M., SIEVERS, J. & H.G. BAUMGARTEN (1980b). Adaptation of the cerebellum to deafferentation.
In "Adaptive Capabilities of the Nervous System" Prog. Brain Res. 53 McConnell, Boer, Komiti, Van der Poll & Corner(Eds.), Elsevier North Holland The Netherlands .

BEYERL, B.D., BORGES, L.F., SWEARINGEN, B. & R.L. SIDMAN (1982). Parasagittal organisation of the olivocerebellar projection in the mouse.
J. Comp. Neurol. 209: 339-346.

BISBY, M.A. (1982). Retrograde axonal transport of endogenous proteins
In "Axoplasmic Transport." D.G. Weiss (Ed.), Springer-Verlag, Berlin, Heidelberg, New York.

BJORKLUND, A., FALCK, B. & C.OWMAN (1972). Fluorescence microscopic and microspectrofluorometric techniques for the cellular localisation and charactarization of biogenic amines.
In "Methods in Investigative and Diagnostic Endocrinology. Vol.1. The Thyroid and Biogenic Amines." J.E. Rall & I.J. Kopin (Eds.), North Holland Publishing Co. Amsterdam.

BJORKLUND, A., SEGAL,M. & U. STENEVI (1979). Functional reinnervation of rat hippocampus by locus coeruleus implants.
Brain Res. 170: 409-426.

BJORKLUND, A. & U.STENEVI (1971). Growth of central catecholamine neurons into smooth muscle grafts in the rat mesencephalon.
Brain Res. 31: 1-20

BJORKLUND, A., STENEVI, U. & N.A. SVENDGAARD (1976). Growth of transplanted monoaminergic neurons into the adult hippocampus along the perforant path.
Nature 262: 787-790.

BLACK, I.B., HENDRY, I.A & L.L. IVERSEN (1972). Effects of surgical decentralisation and nerve growth factor on the maturation of adrenergic neurons in a mouse sympathetic ganglion.
J. Neurochem. 19: 1367-1377.

BLANK, N.K. & F.J. SEIL (1982). Mature Purkinje cells in cerebellar tissue cultures: an ultrastructural study.
J. Comp. Neurol. 208: 169-176.

BLANKS, R.H.I., PRECHT, W. & Y TORIGOE (1983). Afferent projections to the cerebellar flocculus in the pigmented rat demonstrated by retrograde transport of horseradish peroxidase.
Exp. Brain Res. 52: 293-306.

BLOOM, F.E., HOFFER, B.J. & G.R. SIGGINS (1971). Studies on norepinephrine containing afferents to Purkinje cells of rat cerebellum. I. Localisation of the fibres and their synapses.
Brain Res. 25: 501-521.

BORGES, S. & P.D. LEWIS (1983). Effects of ethanol on postnatal cell aquisition in the rat cerebellum.
Brain Res. 271: 388-391.

BOWER, A.J. (1981). A simple, inexpensive apparatus to allow sequential perfusion of an animal with several different fluids without an intervening drop in perfusion pressure.
J. Microsc. 122: 103-104.

BOWER, A.J., MOLONY, V. & C.M. BROWN (1975). An autoradiographic method for the demonstration of vagal afferent fibres in the lower respiratory tract of the chicken (Gallus gallus domesticus).
Experientia 31: 620-622.

BOWER, A.J. & G. WADDINGTON (1981). A simple operative technique for chronically severing the cerebellar peduncles in neonatal rats.
J. Neurosci. Meths. 4: 181-188.

BRADLEY, P & M. BERRY (1976a). The effects of reduced climbing fibre and parallel fibre input on Purkinje cell dendritic growth.
Brain Res. 109: 133-151.

BRADLEY, P. & M. BERRY (1976b). Quantitative effects of climbing fibre deafferentation on the adult Purkinje cell dendritic tree.
Brain Res. 112: 133-140.

BRADLEY, P. & M. BERRY (1978a). The Purkinje cell dendritic tree in mutant mouse cerebellum. A quantitative Golgi study of weaver and staggerer mice. Brain Res. 142: 135-141.

BRADLEY, P. & M. BERRY (1978b). Quantitative effects of methylazoxymethanol acetate on Purkinje cell dendritic growth.
Brain Res. 143: 499-511.

BRADY, S.T. & R.J. LASEK (1982). The slow components of axonal transport: movements, compositions and organisation.
In "Axonal Transport." D.G. Weiss (Ed.), Springer-Verlag, Berlin, Heidelberg, New York.

BRAITENBERG, V. & R.P. ATWOOD (1958). Morphological observations on the cerebellar cortex.
J. Comp. Neurol. 109: 1-33.

BRODAL, A. (1940). Experimentelle Untersuchungen uber die olivo-cerbellare Lokalisation.
Z. Ges. Neurol. Pyschiat. 169: 1-153.

BRODAL, A. (1952). Experimental demonstration of cerebellar connections from the perihypoglossal nuclei (nucleus intercalatus, nucleus praepositus hypoglossi and nucleus of Roller) in the cat.
J. Anat. 86: 110-120.

BRODAL, A. (1953). Reticulo-cerebellar connections in the cat. An experimental study.
J. Comp. Neurol. 98: 113-153.

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

BRODAL, A. & P.A. DRABLOS (1963). Two types of mossy fibre terminals in the cerebellum and their regional distribution.
J. Comp. Neurol. 121: 173-187.

BRODAL, A. & B. HOIVIK (1964). Site and mode of termination of primary vestibulocerebellar fibres in the cat. An experimental study with silver impregnation methods.
Arch. Ital. Biol. 102: 1-21.

BRODAL, A & J. JANSEN (1946). The pontocerebellar projection in the rabbit and cat: experimental investigations.
J. Comp. Neurol. 84: 31-118.

BRODAL, A. & K. KAWAMURA (1980). Olivocerebellar projection: A review.
Adv. Anat. Embryol. Cell Biol. Vol.64.

BRODAL, A. & F. WALBERG (1977a). The olivocerebellar projection in the cat studied with the method of retrograde axonal transport of horseradish peroxidase. IV. The projection to the anterior lobe.
J. Comp. Neurol. 172: 85-108.

BRODAL, A. & F. WALBERG (1977b). The olivocerebellar projection in the cat studied with the method of retrograde axonal transport of horseradish peroxidase. VI. The projection onto longitudinal zones of the paravermal lobule.
J. Comp. Neurol. 176: 281-294.

BRODAL, A., WALBERG, F., BERKLEY, K.J. & A. PELT (1980). Anatomical demonstration of branching of olivocerebellar fibres by means of a double retrograde labelling technique.
Neuroscience 5: 2193-2202.

BROWN, P.A. (1980). The inferior olivary connections to the cerebellum in the rat studied by retrograde axonal transport of horseradish peroxidase.
Brain Res. Bull. 5: 267-275.

BURNE, R.A., ERIKSSON, M.A., SAINT-CYR, J.A. & D.J. WOODWARD (1978a). The organisation of the pontine projections to lateral cerebellar areas in the rat: dual zones in the pons.
Brain Res. 139: 340-347.

BURNE, R.A., MIHAILOFF, G.A. & D.J. WOODWARD (1978b). Visual corticopontine input to the paraflocculus: a combined autoradiographic and horseradish peroxidase study.
Brain Res. 143: 139-146.

CALVET, M-C., LEPAULT, A-M. & J. CALVET (1976). A procion yellow study of cultured Purkinje cells.
Brain Res. 111: 399-406.

CAMPBELL, N.C. & D.M. ARMSTRONG (1983a). The olivocerebellar projection in the rat: an autoradiographic study.
Brain Res. 275: 215-233.

CAMPBELL, N.C. & D.M. ARMSTRONG (1983b). Topographical localisation in the olivocerebellar projection in the rat: an autoradiographic study.
Brain Res. 275: 235-249.

CARREA, R.M.E., REISSIG, M. & F.A. METTLER (1947). The climbing fibres of the simian and feline cerebellum: an experimental inquiry into their origin by lesions of the inferior olive and the deep cerebellar nuclei.
J. Comp. Neurol. 87: 321-365.

CASTRO, A.J. (1978). Projections of the superior cerebellar peduncle in rats and the development of new connections in response to neonatal hemicerebellectomy.
J. Comp. Neurol. 178: 611-628.

CHAN-PALAY, V. (1971). The recurrent collaterals of Purkinje cell axons: a correlated study of the rats cerebellar cortex with electron microscopy and the Golgi method.
Z. Anat. Entwickl.-Gesch. 134: 200-234.

CHAN-PALAY, V. (1973). Early stages in the degeneration of mosy fibres after hemi-section of the spinal cord. Cited in "Cerebellar Cortex, Cytology and Organisation." Palay and Chan-Palay. Springer-Verlag, Berlin, Heidelberg and New York.

CHAN-PALAY, V. (1975). Fine structure of labelled axons in the cerebellar cortex and nuclei of rodents and primates after intraventricular infusions with tritiated serotonin.
Anat. Embryol. 148: 235-265.

CHAN-PALAY, V. (1977). Cerebellar Dentate Nucleus Organisation, Cytology and Transmitters.
Springer-Verlag, Berlin, Heidelberg, New York.

CHAN-PALAY, V. & S.L. PALAY (1970). Interrelations of basket cell axons and climbing fibres in the cerebellar cortex of the rat.
Z. Anat. Entwickl.-Gesch. 132: 191-227.

CHAN-PALAY, V. & S.L. PALAY (1971a). Tendril and glomerular collaterals of climbing fibres in the granular layer of the rats cerebellar cortex.
Z. Anat. Entwickl.- Gesch. 133: 247-273.

CHAN-PALAY, V. & S.L. PALAY (1971b). The synapse en marron between Golgi II neurons and mossy fibres in the rats cerebellar cortex.
Z. Anat. Entwickl.-Gesch. 133: 274-287.

CHAN-PALAY, V., PALAY, S.L., BROWN, J.T. & C. VAN ITALLIE (1978). Sagittal organisation of olivocerebellar and reticulocerebellar projections: autoradiographic studies with S-methionine.
Exp. Brain Res. 30: 561-576.

CHEN, S. & D.E. HILLMAN (1980). Giant spines and enlarged synapses induced in Purkinje cells by malnutrition.
Brain Res. 187: 487-493.

CORRODI, H. & G. JONSSON (1967). The formaldehyde fluorescence method for the histological demonstration of biogenic monamines. A review on the methodology.
J. Histochem. Cytochem. 15: 65-78.

COURVILLE, J. (1975). Distribution of olivocerebellar fibres demonstrated by a radioautographic tracing method.
Brain Res. 95: 253-263.

COURVILLE, J. & F. FARACO-CANTIN (1978). On the origin of the climbing fibres of the cerebellum. An experimental study in the cat with an autoradiographic tracing method.
Neuroscience. 3: 797-809.

COURVILLE, J., FARACO-CANTIN, F. & N. DIAKIW (1974). A functionally important feature of the distribution of the olivocerebellar climbing fibres.
Can. J. Physiol. Pharmacol. 52: 1212-1217.

COWAN, W.M. (1978). Aspects of neural development.
Int. Rev. Physiol. 17: 148-191.

COWAN, W.M., GOTTLIEB, D.I., HENDRICKSON, A.E., PRICE, J.L. & T.A. WOOLSEY (1972). The autoradiographic demonstration of axonal connections in the central nervous system.
Brain Res. 37: 21-51.

CRAIGIE, E.H. (1926). Notes on the morphology of the mossy fibres in some birds and mammals.
Trab. Lab. Invest. Biol. (Madrid) 24: 319-331.

CREPEL, F (1971). Maturation of climbing fibre responses in the rat.
Brain Res. 35: 272-276.

CREPEL, F. (1982). Regression of functional synapses in the immature mammalian cerebellum.
Trends Neurosci. 5: 266-269.

CREPEL, F., DELHAYE-BOUCHAUD, N. & J.L. DUPONT (1981). Fate of the multiple innervation of cerebellar Purkinje cells by climbing fibres in immature control, X-irradiated and hypothyroid rats. Dev. Brain Res. 1: 59-71.

CREPEL, F., DELHAYE-BOUCHAUD, N., DUPONT, L.J. & C. SOTELO (1980). Dendritic and axonic fields of Purkinje cells in developing and X-irradiated rat cerebellum using intracellular staining with HRP. Neuroscience. 5: 333-347.

CREPEL, F. & J. MARIANI (1976). Multiple innervation of Purkinje cells by climbing fibres in the cerebellum of the weaver mutant mouse. J. Neurobiol. 7: 579-582.

CREPEL, F. MARIANI, J. & N. DELHAYE-BOUCHAUD (1976). Evidence for multiple innervation of Purkinje cells by climbing fibres in the immature rat cerebellum. J. Neurobiol. 7: 567-578.

CUPEDO, R.N.J. (1965). A trigeminal midbrain - cerebellar fibre connection in the rat. J. Comp. Neurol. 124: 61-70.

DAS, G.D. & H.O. NORNES (1972). Neurogenesis in the cerebellum of the rat: an autoradiographic study. Z. Anat. Entwickl.-Gesch. 138: 155-165.

DE CAMILLI, P., MILLER, P.E., LEVITT, P., WALTER, U. & P. GREENGARD (1984). Anatomy of cerebellar Purkinje cells in the rat determined by a specific immunohistochemical marker. Neuroscience. 11: 761-817.

DELHAYE-BOUCHAUD, N., MORY, G. & F. CREPEL (1978). Differential role of granule cells in the specification of synapses between climbing fibres and cerebellar Purkinje cells in the rat. Neurosci. Letts. 9: 51-58.

DESCLIN, J.C. (1974). Histological evidence supporting the inferior olive as the major source of cerebellar climbing fibres in the rat. Brain Res. 77: 365-384.

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

DOBBING, J., HOPEWELL, J.W. & A. LYNCH (1971). Vulnerability of developing brain. VII. Permanent deficit of neurons in cerebral and cerebellar cortex following early mild undernutrition.
Exp. Neurol. 32: 439-447.

DOW, R.S. (1936). The fibre connections of the posterior parts of the cerebellum in the rat and the cat.
J. Comp. Neurol. 63: 527-548.

DOW, R.S. (1942). The evolution and anatomy of the cerebellum.
Biol. Rev. 17: 179-220.

DROZ, B. & C.P. LEBLOND (1963). Axonal migration of proteins in the central nervous system and peripheral nerves as shown by autoradiography.
J. Comp. Neurol. 64: 285-307.

DUPONT, J.L., DELHAYE-BOUCHAUD, N. & F. CREPEL (1981). Autoradiographic study of the distribution of olivocerebellar connections during the involutions of the multiple innervation of Purkinje cells by climbing fibres in the developing rat.
Neurosci. Letts. 26: 215-220.

DVERGSTEN, C.L., FOSMIRE, G.J., OLLERICH, D.A. & H.H. SANDSTEAD (1983). Alterations in the postnatal development of the cerebellar cortex due to zinc deficiency. I. Impaired aquisition of granule cells.
Brain Res. 271: 217-226.

ECCLES, J.C., ITO, M. & J. SVENTAGOTHAI (1967). The Cerebellum as a Neuronal Machine.
Springer-Verlag, Berlin, Heidelberg and New York.

ECCLES, J.C., LLINAS, R. & K. SASAKI (1964). Excitation of cerebellar Purkinje cells by the climbing fibres.
Nature 203: 245-246.

EISENMAN, L.M. (1976). Horseradish peroxidase studies of brainstem projections to the vermis and paramedian lobule of the cerebellar cortex in the rat.
Anat. Rec. 184: 396.

EISENMAN, L.M. (1980). Pontocerebellar projections to the paraflocculus in the rat.
Brain Res. 188: 550-554.

EISENMAN, L.M. (1981a). Olivocerebellar projections to the pyramis and copula pyramidis in the rat: differential projection to parasagittal zones.
J. Comp. Neurol. 199: 65-76.

EISENMAN, L.M. (1981b). Pontocerebellar projections to the pyramis and copula pyramidis in the rat: evidence for a mediolateral topography
J. Comp. Neurol. 199: 77-86.

EISENMAN, L.M. (1982). The reticulocerebellar projection to the pyramis and copula pyramidis in the rat: an experimental study using retrograde transport of horseradish peroxidase.
J. Comp. Neurol. 210: 30-36.

EISENMAN, L.M. (1984). Organisation of the olivocerebellar projection to the uvula in the rat.
Brain Behav. Evol. 24: 1-12.

EISENMAN, L.M. & G.P. GORACCI (1983). A double-label retrograde tracing study of the olivocerebellar projection to the pyramis and uvula in the rat.
Neurosci. Letts. 41: 15-20.

EISENMAN, L.M. & C.R. NOBACK (1980). The pontocerebellar projection in the rat: differential projections to sublobules of the uvula.
Exp. Brain Res. 38: 11-17.

ELIAS, H., HENNIG, A. & D.E. SCHWARTZ (1971). Stereology: Applications to biomedical research.
Physiol. Rev. 51: 158-200.

ELLENBERGER, C., HANAWAY, J. & M.G. NETSKY (1969). Embryogenesis of the inferior olivary neurons in the rat: a radioautographic study and a re-evaluation of the rhombic lip.
J. Comp. Neurol. 137: 71-88.

ERANKO, O. (1967). The practical histochemical demonstration of catecholamines by formaldehyde induced fluorescence.
J. Roy. Micro. Soc. 87: 259-276.

ESCOBAR, A., SAMPEDRO, E.D. & R.S. DOW (1968). Quantitative data on the inferior olivary nucleus in man, cat and vampire bat. J. Comp. Neurol. 132: 397-404.

FABER, D.S. & J.T. MURPHY (1969). Axonal branching in the climbing fibre pathway to the cerebellum. Brain Res. 15: 262-267.

FALCK, B., HILLARP, N.A., THIEME, G. & A. TORP (1962). Fluorescence of catecholamines and related compounds condensed with formaldehyde. J. Histochem. Cytochem. 10: 348-354.

FALCK, B. & C. OWMAN (1965). A detailed methodological description of the fluorescence method for the cellular demonstration of biogenic amines. Acta. Univ. Lund. Sect. 11 No. 7.

FAULL, R.L.M. (1977). A comparative study of the cells of origin of cerebellar afferents in the rat, cat and monkey studied with the horseradish technique. I. The non-vestibular brainstem afferents. Anat. Rec. 187: 577.

FERRARO, A. & S.E BARRERA (1936). The effects of lesions of the superior cerebellar peduncle in the macacus rhesus monkey. Bull. Neurol. Inst. (NY) 5: 165-179.

FOX, C.A. (1959). The intermediate cells of Lugaro in the cerebellar cortex of monkey. J. Comp. Neurol. 112: 39-51.

FOX, C.A. & J.W. BARNARD (1957). A quantitative study of the Purkinje cell dendritic branchlets and their relationship to afferent fibres. J. Anat. 91: 299-313.

FOX, C.A., SIEGESMUND, K.A. & C.R. DUTTA (1964). The Purkinje cell denndritic branchlets and their relation with the parallel fibres: light and electron microscopic observations. In "Morphological and Biochemical Correlates of Neural Activity." M.M. Cohen & R.S. Snider(Eds.), Harper & Row, New York.

FRITZSCH, B. (1981). Transneuronal vestibular afferent influence on the nodular molecular layer synaptogenesis. Anat. Embryol. 162: 199-208.

FRY, F.J. & W.M. COWAN (1972). A study of retrograde cell degeneration in the lateral mammillary nucleus of the cat, with special reference to the role of axonal branching in the preservation of the cell.
J. Comp. Neurol. 144: 1-24.

FURBER, S.E. & C.R.R. WATSON (1983). Organisation of the olivocerebellar projection in the rat.
Brain Behav. Evol. 22: 132-152.

FUXE, K (1965). Evidence for the existance of monoamine containing neurons in the central nervous system. IV. The distribution of monoamine terminals in the central nervous system.
Acta. Physiol. Scand. 64 suppl. 247: 39-85.

GAREY, L. & C. BLAKEMORE (1977). Monocular deprivation: morphological effects on different classes of neurons in the lateral geniculate nucleus.
Science 195: 414-416.

GOODMAN, D.C., HALLET, R.E. & R.B. WELCH (1963). Patterns of localisation in the cerebellar corticonuclear projections in the albino rat.
J. Comp. Neurol. 121: 51-63.

GOULD, B.B. (1980). Organisation of afferents from the brainstem nuclei to the cerebellar cortex in the cat.
Adv. Anat. Embryol. Biol. 62.

GOULD, B.B. & A.M. GRAYBIEL (1976). Afferents to the cerebellar cortex in the cat: evidence for an intrinsic pathway leading from the deep nuclei to the cortex.
Brain Res. 110: 601-611.

GRANT, G. (1962a). Spinal course and somatotopically localised termination of the spinocerebellar tracts: An experimental study in the cat.
Acta. Physiol. Scand. 56 (suppl. 193): 5-45.

GRANT, G. (1962b). Projection of the external cuneate nucleus onto the cerebellum in the cat: An experimental study using silver methods.
Exp. Neurol. 5: 179-195.

GRANT, G. (1970). Demonstration of degenerating climbing fibres in the molecular layer of the cerebellum.
Brain Res. 22: 236-242.

GRAY, E.G. (1961). The granule cells, mossy synapses and Purkinje spine synapses of the cerebellum: Light and electron microscopic observations.
J. Anat. 95: 345-356.

GRAZIADEI, P.P.C. & G.A. MONTI GRAZIADEI (1978). The olfactory system: a model for the study of neurogenesis and axonal regeneration in mammals.
In "Neuronal Plasticity" C.W.Cotman (Ed.), Raven Press, New York.

GROENEWEGEN, H.J. & J. VOOGD (1977). The parasagittal zonation within the olivocerebellar projection. I. Climbing fibre distribution in the vermis of the cat cerebellum.
J. Comp. Neurol. 174: 417-488.

GROENEWEGEN, H.J., VOOGD, J. & S.L. FREEDMAN (1979). The parasagittal zonation within the olivocerebellar projection II. Climbing fibre distribution in the intermediate and hemispheric parts of the cat cerebellum.
J. Comp. Neurol. 183: 551-602.

GWYN, D.G., NICHOLSON, G.P. & B.A. FLUMERFELT (1977). The inferior olivary nucleus of the rat: a light and electron microscopic study.
J. Comp. Neurol. 174: 489-520.

HAAS, R.J. & J. WERNER (1973). Cytokinetics of neonatal cerebellar neurons in rats as studied by the "Complete H-thymidine labelling" method.
Exp. Brain Res. 18: 59-68.

HAINES, D.E., PATRICK, G.W. & P. SATRULEE (1982). Organisation of cerebellar corticonuclear systems.
In "Cerebellum - New Vistas." Expl. Brain Res. Suppl. 6. S.L. Palay & V. Chan-Palay.(Eds.), Springer -Verlag, Berlin, Heidelberg, New York.

HALLAS, B.H., OBLINGER, M.M. & G.P. DAS (1980). Heterotopic neural transplants in the cerebellum of the rat: their afferents.
Brain Res. 196: 242-246.

HAMORI, J. (1969). Development of synaptic organisation in the partially agranular and in the transneuronally atrophied cerebellar cortex.
In "Neurobiology of Cerebellar Evolution and Development" R. Llinas.(Ed.), American Medical Association, Chicago.

HAMORI, J., MEZEY, E. & J. SZENTAGOTHAI (1981). Electron microscopic identification of cerebellar nucleo-cortical mossy terminals in the rat. Exp. Brain Res. 44: 97-100.

HAMORI, J. & J. SOMOGYI (1983b). Differentiation of cerebellar mossy fibre sysnapses in the rat: a quantitative electron microscopic study. J. Comp. Neurol. 220: 365-377.

HARSTON, C.T., CLARK, M.B., HARDIN, J.C. & R.M. KOSTRZEWA (1982). Developmental localisation of noradrenergic innervation to the rat cerebellum following neonatal 6-hydroxydopa and morphine treatment. Dev. Neurosci. 5: 252-262.

HEDREEN, J.C. & J.P. CHALMERS (1972). Neuronal degeneration in rat brain induced by 6-hydoroxydopamine; a histological and biochemical study. Brain Res. 47: 1-36.

HEINSEN, H. (1977). Quantitative anatomical studies on the postnatal development of the cerebellum of the albino rat. Anat. Embryol. 151: 201-218.

HEINSEN, H. (1978). Postnatal quantitative changes in the cerebellar uvula of albino rats. Anat. Embryol. 154: 285-304.

HERNDON, R.M., MARGOLIS, G. & L. KILHAM (1971a). The synaptic organisation of the malformed cerebellum induced by perinatal infection with the feline panleucopenia virus: I. Elements forming the cerebellar glomeruli. J. Neuropath. Exp. Neurol. 30: 196-205.

HERNDON, R.M., MARGOLIS, G. & L. KILHAM (1971b). The synaptic organisation of the malformed cerebellum induced by perinatal infection with the feline panleucopenia virus:II. The Purkinje cell and its afferents. J. Neuropath. Exp. Neurol. 30: 557-569.

HERNDON, R.M. & M.L. OSTER-GRANITE (1975). Effect of granule cell destruction on development and maintenance of the Purkinje cell dendrite. In "Physiology and Pathology of Dendrites" Adv. Neurol. Vol. 12 G.W. Kreutzberg.(Ed.), Raven Press, New York.

HESS, D.T. (1982a). Cerebellar nucleo-cortical neurons projecting to the vermis of lobule VII in the rat. Brain Res. 248: 361-366.

HESS, D.T. (1982b). The tecto-olivo-cerebellar pathway in the rat. Brain Res. 250: 143-148.

HEYM, C. (1981). Fluorescence histochemistry of biogenic monoamines. In "Techniques in Neuroanatomical Research". C. Heym & W.G. Forsmann (Eds.), Springer-Verlag, Berlin, Heidelberg and New York.

HICKEY, T.L. (1975). Translaminar growth of axons in the kitten dorsolateral geniculate nucleus following removal of one eye. J. Comp. Neurol. 161: 359-382.

HILLMAN, D.E. & S. CHEN (1981a). Vulnerability of cerebellar development in malnutrition -I. Quantitation of layer volume and neuron numbers. Neuroscience. 6: 1249-1262.

HILLMAN, D.E. & S. CHEN (1981b). Vulnerabilty of cerebellar development in malnutrition-II. Intrinsic determination of total synaptic area on Purkinje cell spines. Neuroscience. 6: 1263-1275.

HIRANO, A., DEMBITZER, H. & M. JONES (1972). An electronmicroscopic study of cycasin-induced cerebellar alterations. J. Neuropath. Exp. Neurol. 31: 113-125.

HOFFER, B.J., SIGGINS, G.R. & F.E. BLOOM (1971). Studies on norepinephrine containing afferents to Purkinje cells of rat cerebellum II. Sensitivity of Purkinje cells to norepinephrine and related substances administered by microiontophoresis. Brain Res. 25: 523-534.

HOFFER, B.J., SIGGINS, G.R., OLIVER, A.P. & F.E. BLOOM (1973). Activation of the pathway from locus coereleus to rat cerebellar Purkinje neurons: pharmacological evidence of noradrenergic central inhibition. J. Pharmacol. Exp. Ther. 184: 553-569.

HOKFELT, T. & K. FUXE (1969). Cerebellar monoamine nerve terminals, a new type of afferent fibre to the cortex cerebelli. Exp. Brain Res. 9: 63-72.

HOKFELT, T., JOHANSSON, O., LJUNGDAHL, A., LUNDBERG, J.M. & M. SCHULZBERG (1980). Peptidergic neurons. Nature 284: 515-521

HOLMES, G. & T.G. STEWART (1908). On the connection of the inferior olives with the cerebellum in man. Brain 31: 125-137.

HRYCYSHYN, A.W., FLUMERFELT, B.A. & W.A. ANDERSON (1982). A horseradish peroxidase study of the projections from the lateral reticular nucleus to the cerebellum in the rat. Anat. Embryol. 165: 1-18.

HUANG, C.M., LIU,G. & R. HUANG (1982). Projections from the cochlear nucleus to the cerebellum. Brain Res. 244: 1-8.

INNOCENTI, G.M. (1979). Two types of brain plasticity? In "Development and Chemical Specificity of Neurons." Progress in Brain Research Vol. 51. Cuenod, Kreutzberg, & Bloom.(Eds.), Elsevier North Holland Biomedical Press, The Netherlands.

INUKAI, T. (1928). On the loss of Purkinje cells, with advancing age, from the cerebellar cortex of the albino rat. J. Comp. Neurol. 45: 1-32.

IVY, G.O. & H.P. KILLACKEY (1978). Transient populations of glial cells in developing rat telencephalon revealed by horseradish peroxidase. Brain Res. 158: 213-218.

JACOBSON M. (1978). "Developmental Neurobiology" 2nd. Ed. Plenum Press, New York.

JENESKOG, T. (1981). On climbing fibre projections to the cerebellar paramedian lobule activated from the mesencephalon in the cat. Brain Res. 211: 135-140.

JONSSON, G. & C. SACHS (1982). Changes in the development of central noradrenaline neurons after neonatal axon lesions. Brain Res. Bull. 9: 641-650.

KASAMATSU, T. & J.D. PETTIGREW (1979). Preservation of binocularity after monocular deprivation in the striate cortex of kittens treated with 6-hydroxydopamine. J. Comp. Neurol. 185: 139-162.

KASAMATSU, T., PETTIGREW, J.D. & M. ARY (1979). Restoration of visual cortical plasticity by local microperfusion of norepinephrine. J. Comp. Neurol. 185: 163-182.

KATZMAN, F., BJORKLAND, A., OWMAN, C.H., STENEVI, V. & K.A. WEST (1971). Evidence for regenerative axon sprouting of central catecholamine neurons in the rat mesencephalon following electrolytic lesions. Brain Res. 25: 579-596.

KAWAGUCHI, S., YAMAMOTO, T., MIZUNO, N. & N. IWAHORI (1975). The role of climbing fibres in the development of Purkinje cell dendrites. Neorsci. Letts. 1: 301-304.

KAWAGUCHI, S., YAMAMOTO, T., SAMEJIMA, A., ITOH, K. & N. MIZUNO (1979). Morphological evidence for axonal sprouting of cerebellothalamic neurons in kittens after neonatal hemicerebellectomy. Exp. Brain Res. 35: 511-518.

KELLY, J.P. & W.M. COWAN (1972). Studies on the development of the chick optic tectum. III. Effects of early eye removal. Brain Res. 42: 263-288.

KERR, F.W.L. (1975) Structural and functional evidence of plasticity in the CNS. Exp. Neurol. 48: 16-31.

KILLACKEY, H.P. (1984). Glia and the elimination of transient cortical projections. Trends. Neurosci. 7: 225-226.

KIMOTO, Y., SATOH, K., SAKUMOTO, T., TOHYAMA, M. & N. SHIMIZU (1978). Afferent fibre connections from the lower brainstem to the rat cerebellum by the horseradish peroxidase method combined with monoamine oxidase staining, with special reference to noradrenergic neurons. J. Hirnforsch. 19: 85-100.

KOBAYASHI, R.M., PALKOVITS, M., KOPIN, D. & D.M. JACOBOWITZ (1974). Biochemical mapping of noradrenergic neurons arising from the rat locus coereleus. Brain Res. 77: 269-279.

KOPPEL, H., LEWIS, P.D. & J.S. WIGGLESWORTH (1982). A study of the vascular supply to the external granular layer of the postnatal rat cerebellum.
J. Anat. 134: 75-84.

KORNELIUSSEN, H.K. (1968a). Comments on the cerebellum and its divisions.
Brain Res. 8: 229-236.

KORNELIUSSEN, H.K. (1968b). On the morphology and subdivisions of the cerebellar nuclei of the rat.
J. Hirnforsch. 10: 109-122.

KORNELIUSSEN, H.K. (1968c). On the ontogenetic development of the cerebellum (nuclei, fissures and cortex) of the rat, with special reference to regional variations in corticogenesis.
J. Hirnforsch. 10: 379-413.

KORNELIUSSEN, H.K. (1969). Cerebellar organisation in the light of cerebellar nuclear morphology and cerebellar corticogenesis.
In "Neurobiology of Cerebellar Evolution and Development." R. Llinas (Ed.) Institute of Biomedical Research, USA.

KORNGUTH, S.E. & G. SCOTT (1972). The role of climbing fibres in the formation of Purkinje cell dendrites.
J. Comp. Neurol. 146: 61-82.

KOSTRZEWA, R.M., HARSTON, C.T., FUKUSHIMA, H. & R. BRUS (1982). Noradrenergic fibre sprouting in the cerebellum.
Brain Res. Bull. 9: 509-517.

KOTCHABHAKDI, N., HODDEVIK, G.H. & F. WALBERG (1978a). Cerebellar afferent projections from the perihypoglossal nuclei: an experimental study with the method of retrograde axonal transport of horseradish peroxidase.
Exp. Brain Res. 31: 13-29.

KOTCHABHAKDI, N., WALBERG, F. & A. BRODAL (1978b). The olivocerebellar projection in the cat studied with the method of retrograde axonal transport of horseradish peroxidase. VII. The projection to lobulus simplex, crus I and crus II.
J. Comp. Neurol. 182: 293-314.

KROMER, L.T., BJORKLUND, A. & U. STENEVI (1980). Innervation of embryonic hippocampal implants by regenerating cholinergic septal neurons in the adult rat.
Brain Res. 210: 153-171.

KUNZLE, H (1975a). Bilateral projections from precentral motor cortex to the putamen and other parts of the basal ganglia. An autoradiographic study in Macaca fasicularis.
Brain Res. 88: 195-209.

KUNZLE, H. (1975b). Autoradiographic tracing of the cerebellar projections from the lateral reticular nucleus in the cat.
Exp. Brain. Res. 22: 255-266.

KUNZLE, H. & M. CUENOD (1973). Differential uptake of H-proline and H-leucine by neurons: its importance for the autoradiographic tracing of pathways.
Brain Res. 62: 213-217.

LANGE, W. (1982). Regional differences in the cytoarchitecture of the cerebellar cortex.
In " Cerebellum-New Vistas." Palay & Chan-Palay.(Eds.), Experimental Brain Research Suppl. 6. Springer-Verlag, Berlin, Heidelberg, New York.

LARRAMENDI, L.M.M. & T. VICTOR (1967). Synapses on the Purkinje cell spines in the mouse. An electron microscopic study.
Brain Res. 5: 15-30.

LARSELL, O. (1952). The morphogenesis and adult pattern of the lobules and fissures of the cerebellum of the white rat.
J. Comp. Neurol. 97: 281-356.

LARSELL, O. & J. JANSEN (1972). "The Comparative Anatomy and Histology of the Cerebellum: The Human Cerebellum, Cerebellar Connections and Cerebellar Cortex".
University of Minnisota Press, Minneapolis.

LAUDER, J.M. & F.E. BLOOM (1974). Ontogeny of monoamine neurons in the locus coereleus, raphe nuclei and substantia nigra of the rat I. Cell differentiation.
J. Comp. Neurol. 155: 469-482.

LAUDER, J.M. & F.E. BLOOM (1975). Ontogeny of monoamine neurons in the locus coereleus, raphe nuclei and the substancia nigra of the rat. II. Synaptogenesis.
J. Comp. Neurol. 163: 251-264.

LAWRENCE, I.E. & H.W. BURDEN (1973). Catecholamines and morphogenesis of the chick neural tube and notochord. Am. J. Anat. 137: 199-208.

LEONG, S.K. (1976). An experimental study of the corticofugal system following cerebral lesions in the albino rat.
Exp. Brain Res. 26: 245-247.

LEONG, S.K. (1977). Plasticity of cerebellar afferents after neonatal lesions in albino rats.
Neurosci. Letts. 7: 281-289.

LEONG, S.K. & R.D. LUND (1973). Anomalous bilateral corticofugal pathways in albino rats after neonatal lesions.
Brain Res. 62: 218-221.

LEVINE, J., SIMON, C. & M. WILLARD (1982). Mechanistic implications of the behaviour of axonally transported proteins.
In "Axoplasmic Transport." D.G. Weiss (Ed.), Springer-Verlag, Berlin, Heidelberg, New York.

LIM, K.H. & S.K. LEONG (1975). Abberent bilateral projections from the dentate and interposed nuclei in albino rats after neonatal lesions.
Brain Res. 96: 306-309.

LINDVALL, O. & A. BJORKLAND (1983). Dopamine- and norepinephrine-containing neuron systems: their anatomy in the rat brain
In "Chemical Neuroanatomy". P.C. Emson (Ed.), Raven Press, New York.

LING, E.A. (1976). Some aspects of amoeboid microglia in the corpus callosum and neighbouring regions of neonatal rats.
J. Anat. 121: 29-45.

LING, E.A. (1976). Light and electron microscopic demonstration of some lysosomal enzymes in the amoeboid microglia of neonatal rat brain.
J. Anat. 123: 637-648.

LLINAS, R.R. & J.I. SIMPSON (1981). Cerebellar control of movement.
In "Handbook of Behavioural Neurobiology. Vol. 5 Motor Coordination." A.L. Towe & E.S. Luschei,(Eds.), Plenum Press, New York, London.

LOIZOU, L. (1969). The development of monomanine containing neurons in the brain of the albino rat. J. Anat. 104: 588.

LOIZOU, L.A. (1972). The postnatal ontogeny of Monoamine containing neurons in the central nervous system of the albino rat. Brain Res. 40: 395-418.

LOIZOU, L. & P. SALT (1970). Regional changes in monoamines of the rat brain during postnatal development. Brain Res. 20: 467-470.

LOREN, I., BJORKLUND, A., FALCK, B. & O. LINDVALL (1976). An improved histofluorescence procedure for freeze-dried paraffin-embedded tissue based on combined formaldehyde-glyoxilic acid perfusion with high magnesium content and acid pH. Histochemistry 49: 177-192.

LOREN, I., BJORKLUND, A., FALCK, B. & O. LINDVALL (1980). The aluminium-formaldehyde (ALFA) histofluorescence method for improved visualisation of catecholamines and indolamines. I. A detailed account of the methodology for central nervous tissue using paraffin, cryostat or vibratome sections. J. Neurosci. Meth. 2: 277-300.

LOREN, I., BJORKLUND, A., LINDVALL, O. & R.H. SCHMIDT (1982). Improved catecholamine histofluorescence in the developing brain based of the magnesium and aluminium (ALFA) perfusion techniques: methodology and anatomical observations. Brain Res. Bull. 9: 11-62.

LOVELL, K.L. (1982). Effects of 6- hydroxydopamine - induced noprepinephrine depletion on cerebellar development. Dev. Neurosci. 5: 359-368.

LOVELL, K.L. & M.Z. JONES (1980b). Partial external granular regeneration in the cerebellum following methyazoxymethanol administration: effects on Purkinje cell dendritic spines. J. Neuropath. Exp. Neurol. 39: 541-548.

LUND, R.D. (1978). "Development and Plasticity of the Brain. An introduction." OUP. New York.

LYNCH, G., MATTHEWS, D.A., MOSKO, S., PARKS, T. & C. COTMAN (1972). Induced acetylcholinesterase-rich layer in rat dentate gyrus following entorhinal lesions. Brain Res. 42: 311-318.

LYNCH, G., MOSKO, S., PARKS, T. & C. COTMAN (1973a). Relocation and hyperdevelopment of the dentate gyrus commissural system after entorhinal lesions in immature rats. Brain Res. 50: 174-178.

LYNCH, G., STANFIELD, B. & C.W. COTMAN (1973b). Developmental differences in post-lesion axonal growth in the hippocmpus. Brain Res. 59: 155-168.

MAEDA, T., TOMYAMA, M. & N. SHIMIZU (1974). Modification of postnatal development of neocortex in rat brain with experimental deprivation of the locus coereleus. Brain Res. 70: 151-520.

MARCHAND, R. & L. POIRIER (1982). Autoradiographic study of the neurogenesis of the inferior olive, red nucleus and cerebellar nuclei of the rat brain. J. Hirnforsch. 23: 211-224.

MARIANI, J. & J.-P. CHANGEUX (1981). Ontogenesis of olivocerebellar relationships. I. Studies by intracellular recordings of the multiple innervation of Purkinje cells by climbing fibres in the developing rat cerebellum. J. Neurosci. 1: 696-702.

MASON, S.T. & H.C. FIBIGER (1979). Regional topography within noradrenergic locus coereleus as revealed by retrograde transport of horseradish peroxidase. J. Comp. Neurol. 187: 703-724.

MAYHEW, T.M. (1978). Quantitative methods for the ultrastructural charactarisation of synapses. J. Anat. 127: 221.

MAYHEW, T.M. (1979). Stereological approach to the study of sysnapse morphology with particular regard to estimating numbers in a volume and on a surface. J. Neurocytol. 8: 121-138.

MAYHEW, T.M. & C.K. MOMOH (1973). Contribution to the quantitative analysis of neuronal parameters: The effects of biased sampling procedures on estimates of neuronal volume, surface area and packing density. J. Comp. Neurol. 148: 217-228.

McCONNELL, P. & M. BERRY (1978a). The effects of malnutrition on Purkinje cell dendritic growth in the rat.
J. Comp. Neurol. 177: 159-172.

MEHLER, W.R. (1977). A comparative study of the cells of origin of cerebellar afferents in the rat, cat and monkey studied with the horseradish peroxidase technique:II. The vestibular nuclear complex.
Anat. Rec. 187: 653.

MIHAILOFF, G.A. (1983). Intra- and interhemispheric collateral branching in the rat pontocerebellar system, a fluorescence double -label study.
Neuroscience. 10: 141-160.

MIHAILOFF, G.A., ADAMS, C.E. & D.J. WOODWARD (1984). An autoradiographic study of the postnatal development of sensorimotor and visual components of the corticopontine system
J. Comp. Neurol. 222: 116-127.

MIHAILOFF, G.A., BURNE, R.A., AZIZI, S.A., NORELL, G. & D.J. WOODWARD (1981). The pontocerebellar system in the rat: an HRP study. II. Hemispheral componants.
J. Comp. Neurol. 197: 559-577.

MILLER, B.F. & R.D. LUND (1975). The pattern of retinotectal connections in albino rats can be modified by foetal surgery.
Brain Res. 91: 119-125.

MOORE, R.Y. (1981). Fluorescence histochemical methods, neurotransmitter histochemistry.
In "Neuroanatomical Tract- Tracing Methods". L. Heimer & M.J. Roberts (Eds.), Raven Press, New York.

MOORE, R.Y. & F.E. BLOOM (1979). Central catecholamine neuron systems: anatomy and physiology of the norepinephrine and epinephrine systems.
Ann. Rev. Neurosci. 2: 113-168.

MUNOZ-MARTINEZ, E.J. (1982). Axonal retention of transported material and the lability of nerve terminals.
In "Axoplasmic Transport." D.G. Weiss (Ed.), Springer-Verlag, Berlin, Heidelberg, New York.

MURPHY, M.G., O'LEARY, J.L. & D. CORNBLATH (1973). Axoplasmic flow in cerebellar mossy and climbing fibres.
Arch. Neurol. 28: 118-123.

MUSTARI, M.J. & R.D. LUND (1976). An aberrant crossed visual corticotectal pathway in albino rats. Brain Res. 112: 37-42.

NAGAI, T., SATOH, K., IMAMOTO, K. & T. MAEDA (1981). Divergent projections of catecholamine neurons of the locus coereleus as revealed by fluorescent retrograde double labelling technique. Neurosci. Letts. 23: 117-123.

NAH, S.H., ONG, L.S. & S.K. LEONG (1980). Is sprouting the result of a persistant neonatal connection. Neurosci. Letts. 19: 39-44.

NATHANSON, N., COLE, G.A. & H. VAN DER LOOS (1969). Heterotopic cerebellar granule cells following administration of cyclophosphamide to suckling rats. Brain Res. 15: 532-536.

NORMAN, P. & A.J. BOWER (1982). An autoradiographic study of the brainstem projections of the vagal visceral afferent fibres in the domestic hen. J. Anat. 134: 583-589.

OBLINGER, M.M., HALLAS, B.H. & G.D. DAS (1980). Neocortical transplants in the cerebellum of the rat: their afferents and efferents. Brain Res. 189: 228-232.

O'LEARY, J.L., DUNSKER, S.B., SMITH, J.M., INUKAI, J. & M. O'LEARY (1970). Termination of the olivocerebellar system in the cat. Arch. Neurol. 22: 193-206.

O'LEARY, J., INUKAI, J. & J.M. SMITH (1971). Histogenesis of the cerebellar climbing fibres in the rat. J. Comp. Neurol. 142: 377-392.

O'LEARY, J.L., PETTY, J., SMITH, J.M., O'LEARY, M. & J. INUKAI (1968). Cerebellar cortex of rat and other animals, a structural and ultrastructural study. J. Comp. Neurol. 134: 401-432.

OLSON, L. & K. FUXE (1971). On the projections from the locus coereleus noradrenergic neurons: the cerebellar innervation. Brain Res. 28: 165-171.

OLSON, L. & T. MALMFORS (1970). Growth charactaristics of adrenergic nerves in the adult rat. Acta. Phys. Scand. Suppl. 348: 1-112.

OLSON, L. & A. SEIGER (1972). Early prenatal ontogeny of central monoamine neurons in the rat: Fluorescence histochemical observations
Z. Anat. Entwickl.-Gesch. 137: 301-316.

OSCARSSON, O. (1976). Spatial distribution of climbing and mossy fibre inputs into the cerebellar cortex. In "Afferent and Intrinsic Organisation of Laminated Structures in the Brain". Exp. Brain Res. Suppl. 1. O. Creudzfeldt (Ed.), Springer-Verlag, Berlin, Heidelberg and New York.

PALAY, S.L. & V. CHAN-PALAY (1974). Cerebellar Cortex, Cytology and Organisation
Springer Verlag, Berlin, Heidelberg and New York.

PALAY, S.L. & V. CHAN-PALAY (Eds.). " The Cerebellum- New Vistas." Exp. Brian Res. Suppl. 6 Springer-Verlag, Berlin, Heidelberg, New York.

PALKOVITS, M., MAGYAR, P. & J. SZENTAGOTHAI (1971a). Quantitative histological analysis of the cerebellar cortex in the cat. I. Number and arrangement in space of Purkinje cells.
Brain Res. 32: 1-13.

PALKOVITS, M., MAGYAR, P. & J. SZENTAGOTHAI (1971c). Quantitative histological analysis of the cerebellar cortex in the cat. III. Structural organisation of the molecular layer.
Brain Res. 34: 1-18.

PAPADOPOULOS, N.J., ALBERT, E.N. & M.F. SHERIF (1981). Some quantitative histological observations on the growing cerebellum of the albino rat (dimensions of the cortical layers and white core and relative numbers of Purkinje cells).
Anat. Anz. 150: 485-497.

PASQUIER, D.A., GOLD, M.A. & D.M. JACOBOWITZ (1980). Noradrenergic perikarya (A5-A7, subcoereleus) projections to the rat cerebellum.
Brain Res. 196: 270-275.

PAYNE, J.N. (1983a). The cerebellar nucleo-cortical projection in the rat studied by the retrograde fluorescent double-labelling method.
Brain Res. 271: 141-144.

PAYNE, J.N. (1983b). Axonal branching in the projections from precerebellar nuclei to the lobulus simplex of the rat's cerebellum investigated by retrograde fluorescent double labelling.
J. Comp. Neurol. 213: 233-240.

PAYNE, J.N. & A.J. BOWER (1983). Rat cerebellar afferents after unilateral pedunculotomy. A retrograde fluorescent double-labelling study.
Dev. Brain Res. 11: 124-127.

PEARSON, A.A. (1949). Further observations on the mesencephalic root of the trigeminal nerve.
J. Comp. Neurol. 91: 147-194.

PELLEGRINO, L.J., PELLEGRINO, A.S. & A.J. CUSHMAN (1979). A Stereotaxic Atlas of the Rat Brain.
Plenum Press, New York.

PICKEL, V.M., KREBS, H. & F.E. BLOOM (1973). Proliferation of norepinephrine-containing axons in rat cerebellar cortex after peduncle lesions.
Brain Res. 59: 169-179.

PICKEL, V.M., SEGAL, M. & F.E. BLOOM (1974a). A radioautographic study of the afferent pathways of the nucleus locus coereleus.
J. Comp. Neurol. 155: 15-42.

PICKEL, V.M., SEGAL, M. & F.E. BLOOM (1974b). Axonal proliferation following lesions of cerebellar peduncles. A combined fluorescence microscopic and autoradiographic study.
J. Comp. Neurol. 155: 43-60.

PRIVAT, A. & M.J. DRIAN (1976). Postnatal maturation of rat Purkinje cells cultivated in the absence of two afferent systems: an ultrastructural study.
J. Comp. Neurol. 166: 201-244.

RAISMAN, G. (1977). Formation of synapses in the adult rat after injury: similarities and differences between a peripheral and a central nervous site.
Phil. Trans. R. Soc. Lond. B 278: 349-359.

RAKIC, P. (1971). Neuron-glia relationship during granule cell migration in developing cerebellar cortex. A Golgi and electron microscope study in Macacus Rhesus.
J. Comp. Neurol. 141: 283-312.

RAMON Y CAJAL, S.(1888). Estructura de los centros nerviosos de las aves.
Rev. Trimestr. Histol. 1: 1-10.

RAMON Y CAJAL, S. (1911). "Histologie du Systeme Nerveux de L'homme et des Vertebres".
Maloine, Paris.

RAMON Y CAJAL, S. (1960). Studies on Vertebrate Neurogenesis.
Springfield: Thomas.

ROGERS, A.W. (1979). Techniques of Autoradiography 3rd. Ed.
Elsevier/North Holland Biomedical press, Amsterdam/ New York/Oxford.

ROOM, P., POSTEMA, F. & J. KORF (1981). Divergent axon collaterals of rat locus coereleus neurons: demonstration by a fluorescent double-labelling technique.
Brain Res. 221: 219 230.

SACHS, C. & G. JONSSON (1975). Effects of 6-hydroxydopamine on central noradrenergic neurons during ontogeny.
Brain Res. 99: 277-291.

SCHEIBEL, A.B. (1977). Sagittal organisation of mossy fibre terminal systems in the cerebellum of the rat: a Golgi study.
Exp. Neurol. 57: 1067-1070.

SCHEIBEL, M.E. & A.B. SCHEIBEL (1954). Observations on the intracortical relations of the climbing fibres of the cerebellum.
J. Comp. Neurol. 101: 733-763.

SCHERINI, E., BOLCHI, F., BIGGIOGERA, M. & G. BERNOCCHI (1981). Further evidence of different morphological aspects in the Purkinje cell population of adult rat cerebellum. An ultrastructural study.
J. Submicrosc. Cytol. 13: 17-29.

SCHILD, R.F. (1970). On the inferior olive of the albino rat.
J. Comp. Neurol. 140: 255-260.

SCHILD, R.F (1980). Length of the parallel fibres in rat cerebellar cortex.
J. Physiol. 303: 25P.

SCHMIDT, R.H. & R.K. BHATNAGAR (1980). Intracisternal dose-response analysis of 6-hydroxydopamine induced noradrenergic sprouting in the neonatal rat cerebellum.
J. Pharmacol. Exp. Ther. 212: 456-461.

SCHMIDT, R.H., BJORKLUND, A. & I LOREN (1981). Neuron -target interactions in the development of central catecholamine systems.
In "Development in the Nervous System". 5th Symposium of the British Society for Developmental Biology. D.R. Garrod & J.D. Feldman.(Eds.), O.U.P.

SCHMIDT, R.H., KASIK, S.A. & R.K. BHATNAGAR (1980). Regenerative critical periods for locus coereleus in postnatal rat pups following intracisternal 6-hydroxydopamine: a model of noradrenergic development.
Brain Res. 191: 173-190.

SCHULMAN, J.A. (1983). Chemical neuroanatomy of the cerebellar cortex.
In "Chemical Neuroanatomy". P.C. Emson (Ed.), Raven Press, New York.

SCOTT, Th. G. (1964). A unique pattern of localisation within the cerebellum of the mouse.
J. Comp. Neurol. 122: 1-8.

SEGAL, M., PICKEL, V. & F.E. BLOOM (1973). The projections of the nucleus locus coereleus: an autoradiographic study.
Life Sci. 13: 817-821.

SEIGER, A & L. OLSON (1973). Late prenatal ontogeny of central monoamine neurons in the rat: fluorescence histochemical observations.
Z. Entwickl.-Gesch. 140: 281-318.

SHINNAR, S., MACIEWICZ, R.J. & R.J. SHOFFER (1973). A raphe projection to the cat cerebellar cortex.
Brain Res. 97: 139-143.

SIEVERS, J., BERRY, M. & H. BAUMGARTEN (1981). The role of noradrenergic fibres in the control of postnatal cerebellar development.
Brain Res. 207: 200-208.

SIEVERS, J. & H.P. KLEMM (1982). Locus coerleus-cerebellum: interaction during development.
Biblthca. Anat. 23: 56-75.

SIEVERS, J., KLEMM, H.P., JENNER, S., BAUMGARTEN, H.G. & M.BERRY (1980). Neuronal and extraneuronal effects of intracisternally administered 6-hydroxydopamine on the developing rat cerebellum.
J. Neurochem. 34: 765-771.

SMOLYANINOV, V.V. (1971). Some special features of organisation of the cerebellar cortex.
In "Models of the Structural - Functional Organisation of Certain Biological Systems". I.M. Gelfand, V.S. Garfand, S.V. Fomin & M.L. Tsetlin (Eds.), p250-325. Cambridge, M.I.T. Press.

SNYDER, R.L., FAULL, R.L.M. & W.R. MEHLER (1978). A comparative study of the neurons of origin of the spinocerebellar afferents in the rat, cat and squirrel monkey based on the retrograde transport of horseradish peroxidase.
J. Comp. Neurol. 181: 833-852.

SOMANA, R. & F. WALBERG (1978). Cerebellar afferents from the paramedian reticular nucleus studied with retrograde transport of horseradish peroxidase.
Anat. Embryol. 154: 353-368.

SOTELO, C. (1981). Development of synaptic connections in genetic and experimentally induced cerebellar malformations.
In "Development in the Nervous System". 5th symposium of the British Society for Developmental Biology. D.R. Garrod & J.D. Feldman (Eds.), O.U.P.

SOTELO, C. (1982). Synaptic remodelling in agranular cerebella.
In " The Cerebellum-New Vistas." Exp. Brain Res. Suppl. 6. S.L. Palay & V. Chan-Palay (Eds.), Springer-Verlag, Berlin, Heidelberg, New york.

SOTELO, C. & M.L. ARSENIO-NUNES (1976). Development of Purkinje cells in absence of climbing fibres.
Brain Res. 111: 389-395.

SOTELO, C., BOURRAT, F. & A. TRILLER (1984). Postnatal development of the inferior olivary complex in the rat. II. Topographic organisation of the immature olivocerebellar projection.
J. Comp. Neurol. 222: 177-199.

STANFIELD, B.B. (1984). Postnatal reorganisation of cortical projection: the role of collateral elimination.
Trends Neurosci. 7: 37-41.

STENT, G.S. (1980). The genetic approach to developmental neurobiology.
Trends. Neurosci. 3: 49-51.

SZENTAGOTHAI, J. & V. RAJKOVITS (1959). Uber den Ursprung der Klitterfasern des Kleinhirns.
Z. Entwickl.-Gesch. 121: 130-141.

TOLBERT, D.L. (1982). The cerebellar nucleocortical pathway.
In "The Cerebellum-New vistas." Exp. Brain Res. Suppl. 6. S.L. Palay & V. Chan-Palay (Eds.), Springer-Verlag, Berlin, Heidelberg, New York.

TOLBERT, D.L., BANTLI, H. & J.R. BLOEDEL (1976). A cerebellar nucleocortical projection in the cat.
Anat. Rec. 184: 547.

TOLBERT,D.L., BANTLI, H. & J.R. BLOEDEL (1977). The intracerebellar nucleocortical projection in a primate.
Exp. Brain Res. 30: 425-434.

TOLBERT, D.L., BANTLI, H. & J.R. BLOEDEL (1978). Organisational features of the cat and monkey cerebellar nucleo-cortical projection.
J. Comp. Neurol. 182: 39-56.

TOLBERT, D.L. & W.M. PANNETON (1983). Transient cerebrocerebellar projections in kittens: postnatal development and topography.
J. Comp. Neurol. 221: 216-228.

TORVIK, A. (1956). Transneuronal changes in the inferior olive and pontine nuclei in kittens.
J. Neuropath. Exp. Neurol. 15: 119-145.

VOOGD, J. (1969). The importance of fibre connections in the comparative anatomy of the mammalian cerebellum.
In "Neurobiology of Cerebellar Evolution and Development." R. Llinas (Ed.), American Medical Association, Chicago.

VOOGD,J., BIGARE, F., GERRITS, N.M. & E MARANI (1981). Structure and fibre connections of the cerebellum.
Prog. Clin. Biol. Res. 59A: 259-268.

WADDINGTON, G. & P. BANKS (1981). The development of pre- and postsynaptic componants of the noradrenergic system in the rat cerebellum.
J. Neurochem. 37: 576-581.

WALBERG, F. (1980). Olivocerebellocortical projection in the cat as determined with the method of retrograde axonal transport of horseradish peroxidase I. Topographical pattern.
In "The Inferior Olivary Nucleus: Anatomy and Physiology" J. Courville, de Montigny and Y. Lamarre (Eds.), Raven Press, New York.

WALKER, A.E. & E.H. BOTTERELL (1937). The syndrome of the superior cerebellar peduncle in the monkey.
Brain 60: 329-353.

WALL, P.D. (1977). The presence of ineffective synapses and the circumstances which unmask them.
Phil Trans. R. Soc. Lond. B 278: 361-372.

WATSON, C.R.R. & R.C. SWITZER (1978). Trigeminal projections to cerebellar tactile areas in the rat - origin mainly from nucleus interpolaris and nucleus principalis.
Neurosci. Letts. 10: 77-82.

WETTS, R. & K. HERRUP (1983). Direct correlation between Purkinje and granule cell number in the cerebella of lurcher chimeras and wild - type mice.
Dev. Brain Res. 10: 41-47.

WOODWARD, D.J., BICKETT, D. & R. CHANDA (1975). Purkinje cell dendritic alterations after transient developmental injury of the external granule layer.
Brain Res. 97: 195-214.

WOODWARD, D.J., HOFFER, B.J. & L.W. LAPHAM (1969). Postnatal development of electrical and enzyme histochemical activity in Purkinje cells.
Exp. Neurol. 23: 120-139.

WOODWARD,D.J., HOFFER, B.J., SIGGINS, G.R. & F.E. BLOOM (1971). The ontogenetic development of synaptic junctions, synapse activation and responsivness to neurotransmitter substances in rat cerebellar Purkinje cells.
Brain Res. 34: 73-97.

WOODWARD, D.J., SEIGER, A., OLSON, L. & B.J. HOFFER (1977). Intrinsic and extrinsic determination of dendritic development as revealed by Golgi studies of cerebellar and hippocampal transplants in oculo. Exp. Neurol. 57: 984-998.

YAMAMOTO, T., ISHIKAWA, M. & C. TANAKA (1977). Catecholaminergic terminals in the developing and adult rat cerebellum. Brain Res. 132: 355-361.

YEH, H.H., LIN, C.-S. & D.J. WOODWARD (1982). Mossy fibre and Purkinje cell axon collateral arborisation patterns in normal and X-irradiated rat cerebellum: a light microscopic study using horseradish peroxidase fibre filling techniques. Dev. Brain Res. 2: 169-175.

ZEMAN, W. & J.R.M. INNES (Eds.) (1963). "Craigie's Neuroanatomy of the rat." Academic Press, London, New York.

ZIMMER, J. (1973a). Extended commissural and ipsilateral projections in postnatally de - entorhinated hippocampus and fascia dentata demonstrated in rats by sivler impregnation. Brain Res. 64: 293-311.

ZIMMER, J. (1973b). Changes in Timm sulphide silver staining pattern of the rat hippocampus and fascia dentata following early postnatal deafferentation. Brain Res. 64: 313-326.

ZIMMER, J. (1974). Proximity as a factor in the regulation of aberrent axonal growth in the postnatally deafferented fascia detata. Brain Res. 72: 137-142.