SOME OBSERVATIONS ON THE POSTNATAL DEVELOPMENT OF THE RAT CEREBELLUM FOLLOWING UNILATERAL PEDUNCLOTOMY

By

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For Gregory and Jeremy,
with love.

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SUMMARY

The structure of the cerebellum, its cortex and the cells contained therein have been described. Also, the distribution of both afferent and efferent fibres has been summarised from the literature. The cerebellar development and experiments which have been performed upon it have also been briefly outlined.

The technique for surgical pedunclotomy is described and its effect on cerebellar and Purkinje cell development were found. The effect of pedunclotomy on cerebellar growth and the Purkinje cell numbers is differential, a lesion on postnatal day 7 causing the most severe effect. Also, the Purkinje cell size was reduced following pedunclotomy after the age of 3 days. However the cortical histology was otherwise qualitatively normal.

The response of the noradrenergic afferents to the cerebellum to pedunclotomy was observed. Qualitatively the density of fibres was equivalent to normal after 4 days, which is in keeping with previous experiments, but there was no hyperinnervation 35 days post pedunclotomy a result not in agreement with the studies using 6-hydroxydopamine.

The olivocerebellar projection 35 days after pedunclotomy on days 3 and 7 shows a reinnervation of the deafferented hemicerebellum. However, this response was not seen after pedunclotomy on day 10. The topography of

this aberrant path is organised roughly into sagittal bands although not as discretely as that seen in the normal animal. In neonatal rats there was some evidence that this path may already exist in those animals pedunclotomised on days 3 and 7. Also, an additional ipsilateral projection was found following acute pedunclotomy, which entered the cerebellum through the ipsilateral inferior cerebellar peduncle.

The results have been discussed in relation to other experiments on cerebellar development and with studies in the literature on plasticity within the central nervous system.

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LIST OF ABBREVIATIONS

BPN	basal pontine nucleus
DAO	dorsal accessory olive
dmcc	dorsomedial cell column
dPO	dorsal lamella of the principal olive
E13	embryonic age i.e. thirteenth day in utero
LRN	lateral reticular nucleus
MAO	medial accessory olive
NRTP	nucleus reticularis tegmenti pontis
PO	principal olive
PRN	paramedian reticular nucleus
vPO	ventral lamella of the principal olive
6-OHDA	6-hydroxydopamine

GENERAL INTRODUCTION

CHAPTER ONE

THE STRUCTURE AND DEVELOPMENT OF THE RAT CEREBELLUM

The rat cerebellum is a relatively discrete part of the central nervous system whose structure and connections have been thoroughly investigated (e.g. Eccles et al., 1967; Palay and Chan-Palay, 1974). In addition, its development is quite rapid (approximately 40 days) and occurs principally postnatally. Therefore, it is a suitable model for studying the factors which are important in neuronal development and what capacity the immature central nervous system has for responding to injury. Therefore an attempt has been made to try and assess the relative autonomy of cerebellar development or its dependence on its afferents.

CEREBELLAR MORPHOLOGY

The rat cerebellum is part of the hindbrain and is situated posterior to the cerebral hemispheres and dorsal to the pons and medulla where it forms the roof of the fourth ventricle. It is connected to the brainstem by three pairs of peduncles, superior, middle and inferior, which carry its afferent and efferent fibres. The cerebellum can be divided into a fairly broad midline vermis and two hemispheres. The division between these areas is marked by the paramedian fissure, a shallow depression on the dorsal surface which deepens inferiorly. The

cortex is folded into roughly parallel transverse folia and the intervening fissures divide the cerebellum into lobules. The division of the cerebellum into lobules either anatomically or according to the type of afferent input (e.g. spinal or cerebral) has produced several different terminologies (Dow, 1942), therefore the definition and nomenclature of Larsell (1952) has been used and is seen in Fig. 1.1.

The cerebellum is made up of a central medulla of fibres and an outer cortex. The white matter contains the deep nuclei, of which there are four in each hemisphere; the medially placed fastigial nucleus, the nucleus interpositus anterior and posterior and the lateral nucleus. A thin lamella of white matter passes into each folium containing the afferents and efferents of the cortex. The cortex is a trilaminar sheet, which if the folia were flattened, would not make a continuous layer (Braitenberg and Atwood, 1958). The area of this sheet in the rat is 270 mm^2 (Armstrong and Schild, 1970). The cortex is divided into a superficial plexiform molecular layer, a monolayer of Purkinje cells and an inner granular layer. It has the same structure throughout the cerebellum. The molecular layer contains the Purkinje cell dendritic tree, which lies perpendicular to the parallel fibre axons of granule cells, an arrangement that produces a lattice-like structure not found in other cortices (Braitenberg and Atwood, 1958). It also contains two types of neurons, basket and stellate cells, terminal parts of climbing and monoamine afferents and the processes of Bergmann glial

Figure 1.1

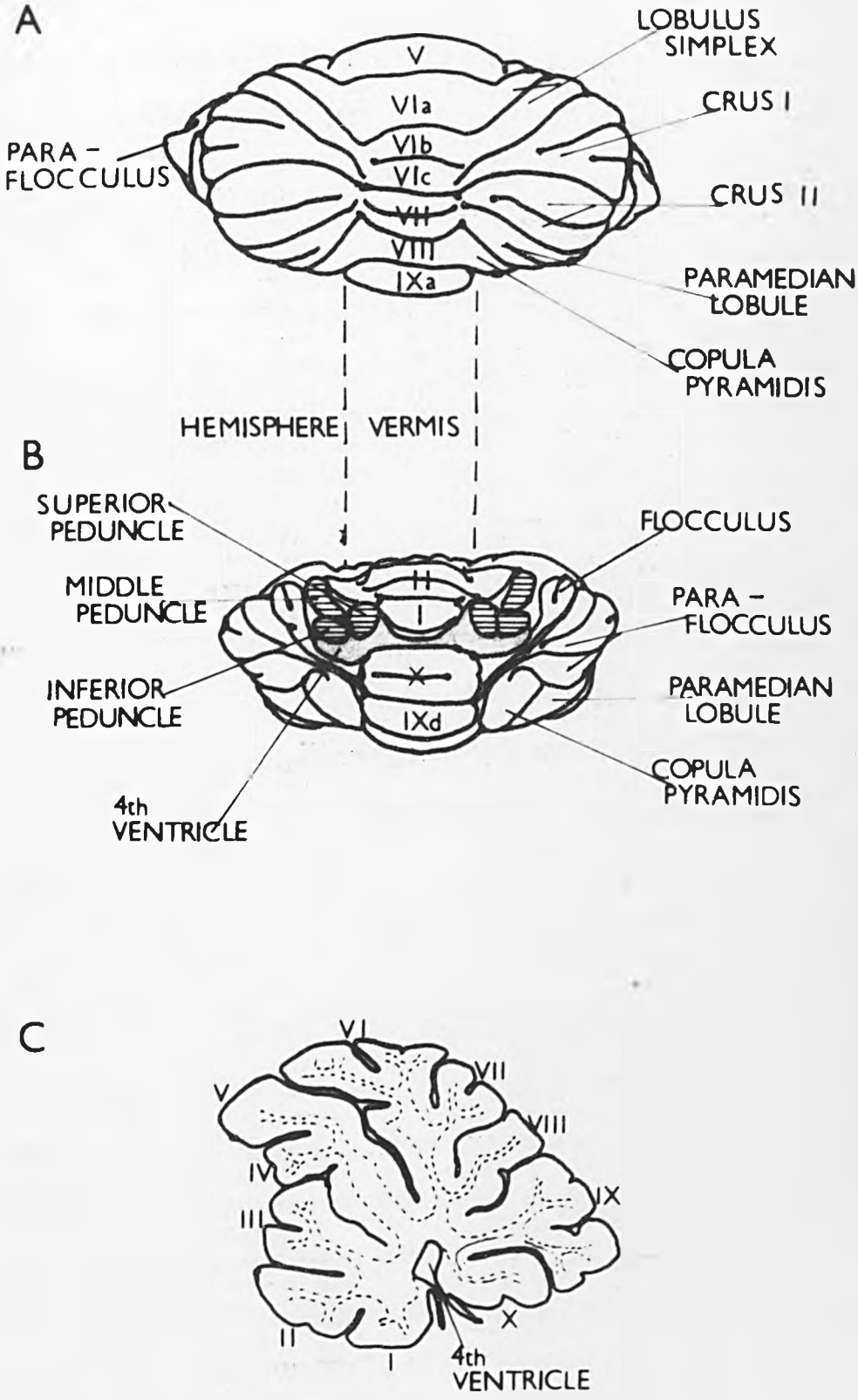
Cerebellar morphology redrawn from Larsell (1954)

Vermal Lobules I-V and their lateral extensions form the anterior lobe which has no true hemisphere. Lobules VI-X and the hemisphere form the posterior lobe. The flocculus is attached to the nodulus (lobule X) by a thin stalk and forms the flocculo-nodular lobe.

A. Dorsal view

B. Ventral view

C. Midsagittal section


A
LOBULUS SIMPLEX
V
VIa
CRUS I
PARA – FLOCCULUS
VIb
VIc
CRUS II
VII
VIII
IXa
PARAMEDIAN LOBULE
COPULA PYRAMIDIS
HEMISPHERE
VERMIS
B
SUPERIOR PEDUNCLE
FLOCCULUS
MIDDLE PEDUNCLE
II
I
PARA – FLOCCULUS
X
INFERIOR PEDUNCLE
IXd
PARAMEDIAN LOBULE
COPULA PYRAMIDIS
4th VENTRICLE
C
VI
VII
V
VIII
IV
IX
III
X
II
I
4th VENTRICLE

cells. The granular layer consists predominantly of small granule cells and their dendrites, but also contains Golgi and Lugaro neurons and the specialised 'rosette' terminals of mossy fibre afferents.

Purkinje Cells

Purkinje cells were first described by Purkinje in 1837 (Palay and Chan-Palay, 1974) from handcut slices of cerebellum. They are large pear-shaped neurons 21 µm by 25 µm in the rat (Palay and Chan-Palay, 1974) although they are larger in higher mammals. From its apex a large highly arborised dendritic tree ascends through the molecular layer, and the axon projects into the granular layer from the base of the cell. The Purkinje cells lie with no apparent pattern in a monolayer and are central in cortical circuitry (Braitenberg and Atwood, 1958 ; Armstrong and Schild, 1970; Palay and Chan-Palay, 1974). The perikarya are closely packed, approximately 1200/mm^2 (Armstrong and Schild, 1970), and are often separated only by a thin glial sheet (Palay and Chan-Palay, 1974). Each has a large nucleus, which only stains lightly due to the thinly dispersed chromatin, and a prominent usually centrally placed nucleolus. The cytoplasm has a whorl-like appearance due to the circumferential arrangement of organelles about the nucleus (Palay and Chan-Palay, 1974). Some cells appear less basophilic because of enlarged cisternae of the endoplasmic reticulum and probably indicate a different metabolic state (Scherini et al., 1981).

The dendritic tree arises from one to four trunks and ascends towards the pia mater. It is broad in the sagittal plane i.e. perpendicular to the axis of the folium (De Camilli et al., 1984), and is approximately 300-400 µm wide (Palay and Chan-Palay, 1974). However, it is not entirely flat and is 15-20 µm deep coronally (Palay and Chan-Palay, 1974) and appears in this plane as straight segments passing vertically through the molecular layer (De Camilli et al., 1984). Within the tree there are spaces for parallel fibres, basket and stellate cells and small blood vessels (Fox and Barnard, 1957). The primary dendritic trunks arising from cells on the sides of the folia lie more vertically than those arising from cells at the apex or deep in the sulci which are more horizontally angled. The primary dendritic trunks divide into secondary dendrites which spread laterally, sometimes running parallel to the surface. From these arise the tertiary dendrites, which divide many times with multiple-branch points often giving three to five branchlets of different calibres to fill the whole molecular layer with a rich arbour (Palay and Chan-Palay, 1974). The small dendrites usually subdivide immediately into terminal branches while the larger calibre fibres tend to pass on toward the pia mater. The small terminal dendrites are only 1-2 µm thick and are covered with small spines, which gives them their name of "spiny branchlets" (Fox and Barnard, 1957). These spiny branchlets occupy on average 92% of the whole tree and have

1.74 spines/μm (Palay and Chan-Palay, 1974). This gives an estimate for the rat of 17,354 spines per tree, which is twice the number that Smolyaninov (1971) found but is less than the numbers obtained for either the cat (Palkovits et al., 1971c) or monkey (Fox et al., 1964). The spines on the terminal dendrites have a long thin stem and a round head and arise from all over the dendrite. This is different from the type of thorns found on the large primary and secondary trunks which have a short stem and larger heads and are grouped together leaving most of the dendritic surface smooth. This second type of thorn receives climbing fibre afferents (Palay and Chan-Palay, 1974).

The Purkinje cell axon arises from a small cone at the basal pole of the cell. Its thin initial segment runs for 40-50 μm before becoming myelinated (Palay and Chan-Palay, 1974) where its diameter increases and then remains constant for the rest of its course (De Camilli et al., 1984). In the cat the axon is 2-5 μm in diameter and thus can be distinguished from afferent fibres (Voogd et al., 1981). It has a tortuous course through the granular layer (Chan-Palay, 1971) where it develops varicosities that mark the points from which collaterals arise (O'Leary et al., 1968; Chan-Palay, 1971). The route through the granular layer depends on the position within the folium of the cell of origin (O'Leary et al., 1968; Palay and Chan-Palay, 1974). Axons from apical Purkinje cells descend vertically to the

white matter, while those arising on the shoulder of a folium travel through the granular layer at an angle. Cells situated at the side or base of a folium project their axons through the granular layer so they have to turn through a right angle to enter the white matter. Within the central white matter axons from adjacent folia retain the two dimensional geometry of their dendritic trees (De Camilli et al.,1984).

Collaterals leave the Purkinje cell axon at an acute angle and ascend back towards the Purkinje cell layer (Chan-Palay, 1971). They are thin thread-like fibres (De Camilli et al., 1984) possessing 0.4-0.5 µm beads (Palay and Chan-Palay, 1974) and give off branches which end in many varicose terminals. The collaterals and their branches are given off in the plane perpendicular to the folium (Palay and Chan-Palay, 1974; De Camilli et al., 1984) and their terminal field forms a triangle which is not quite flat coronally and has its apex to the white matter and base at the Purkinje cell layer (O'Leary et al., 1968; Palay and Chan-Palay, 1974). This triangular field is symmetrical about the parent axon at the apex of the folium but is small and irregular in the attenuated cortex of the sulci (O'Leary et al., 1968; Palay and Chan-Palay, 1974). The collaterals which originate distally deep in the granular layer form a plexus within this layer and synapse on Golgi cells (O'Leary et al., 1968; Chan-Palay, 1971). However, those

collaterals branching from the parent axon in the middle and upper thirds of the granular layer ascend towards the Purkinje cell layer. They turn horizontally 10 µm below the Purkinje cell layer and follow a tortuous course for 150-200 µm in the sagittal plane (O'Leary et al., 1968; Palay and Chan-Palay, 1974; De Camilli et al., 1984). These fibres bifurcate several times and give short terminal branches to form the dense infraganglionic plexus, where they synapse on Purkinje cell somata and Lugaro cells (O'Leary et al., 1968; Chan-Palay, 1971; De Camilli et al., 1984). From this plexus some tertiary branches ascend into the molecular layer to form the supraganglionic plexus. This plexus is only sparse in the adult rat and its fibres terminate on the Purkinje cell dendrites and basket cell somata (Chan-Palay, 1971; De Camilli et al., 1984).

Basket and Stellate Cells

There are two types of neurons found in the molecular layer, the basket and stellate cells. Generally the basket cells lie in the lower third of the layer and the stellate cells in the upper two thirds. Basket cells are pyramidal or ovoid in shape, 10 µm long (O'Leary et al., 1968; Palay and Chan-Palay, 1974) and lie with their long axes parallel to the Purkinje cell layer in the sagittal plane (Braitenberg and Atwood, 1958). The perikarya are almost filled by the nucleus and possess scanty cytoplasm which contains little Nissl substance (Ariëns Kappers et al., 1936; Palay and Chan-Palay, 1974). Three or

four dendrites arise from the upper surface of the soma forming a fan-like appearance in the sagittal plane (Ariëns Kappers et al., 1936; Palay and Chan-Palay, 1974). They are moderately straight, thick fibres which have only a few spines and not many branches (Chan-Palay and Palay, 1970). The majority of these dendrites ascend, even right up to the pia mater, although there are horizontal fibres which usually also terminate as ascending branches (Ramon y Cajal, 1911; Ariëns Kappers et al., 1936; Chan-Palay and Palay, 1970). In addition a few thin, short basket cell dendrites descend towards the granular layer (Ramon y Cajal, 1911; Ariëns Kappers et al., 1936; O'Leary et al., 1968; Palay and Chan-Palay, 1974). The dendritic field is orientated sagittally and therefore interdigitates with the Purkinje cell dendrites, climbing fibres, Bergmann glial processes, other basket cell axons and parallel fibres. Despite this, basket cells receive a relatively small parallel fibre input (Palay and Chan-Palay, 1974). The basket cell axon can arise from either the soma or one of the major dendrites (Palay and Chan-Palay, 1974). It runs in the sagittal plane horizontally among the Purkinje cell primary dendrites (Ariëns Kappers et al., 1936; Palay and Chan-Palay, 1974) contributing to the supra-ganglionic plexus (O'Leary et al., 1968). The initial segment is thin and straight but at the first bifurcation the axon widens, after which it winds its way through the Purkinje cell dendrites, giving ascending, transverse and

descending collaterals (Ariëns Kappers et al., 1936; Chan-Palay and Palay, 1970). The fine ascending collaterals pass into the middle third of the molecular layer and entwine with the Purkinje cell tertiary dendrites. The transverse collaterals broaden the basket cell sagittal field by one Purkinje cell each side in the rat (Palay and Chan-Palay, 1974), although it is wider in the cat (Palkovits et al., 1971c), and many join the plexuses around the Purkinje cell perikarya. The basket cell axon gives off many thick descending collaterals which branch profusely around the Purkinje cell somata giving short twigs that terminate as bulbs on the Purkinje cell surface (Ariëns Kappers et al., 1936; Palay and Chan-Palay, 1974) and on Lugaro cells (O'Leary et al., 1968). Each axon may contribute several collaterals to one 'basket' and each 'basket' receives branches from 3-7 different axons (Palay and Chan-Palay, 1974). Some fibres from the perisomatic basket descend further and form a plexus around the initial unmyelinated segment of the Purkinje cell axon (Ariëns Kappers et al., 1963; O'Leary et al., 1968; Palay and Chan-Palay, 1974). The basket cell contribution to these pericellular baskets decreases as the cells of origin lie more superficial in the molecular layer.

The stellate cells can be divided into two classes, those situated most superficially in the molecular layer and the larger cells placed more deeply. Both types are orientated sagittally across the folium (Braitenberg and

Atwood, 1958) and receive parallel fibre afferents (Palay and Chan-Palay, 1974). The small superficial cells are fusiform, 5-10 µm long, and possess only scanty cytoplasm around the nucleus (O'Leary et al., 1968; Palay and Chan-Palay, 1974). The few dendrites have an irregular calibre and there are only a small number of spines or varicosities along their contorted path. They either run horizontally or ascend to the pia mater where some twigs terminate and others cascade back towards the cell body (O'Leary et al., 1968; Palay and Chan-Palay, 1974). The dendrites lie in the sagittal plane, only 15-20 µm wide coronally, and interleave with the Purkinje cell dendritic tree. The axon arises from the soma which, after a thin initial segment, has a meandering course giving off a simple arbour of thin crooked varicose collaterals (Palay and Chan-Palay, 1974). Their field is not quite flat, 40 µm coronally, and terminates on only a few Purkinje cells. The larger more deeply placed stellate cells have triangular somata 20 µm long (O'Leary et al., 1968) and have a large number of contorted highly branched dendrites which radiate into the molecular layer. The axon is long, 450 µm parasagittally, and remains at the level of the soma. From the first third it gives off thin varicose collaterals which either ascend and form a scanty arbour or descend and branch many times, occasionally contributing to the basket cell nests. The distal two thirds of the axon gives off very few collaterals (Palay and Chan-Palay, 1974).

Granule Cells

The granular layer consists predominantly of granule cells. They lie clustered together in tight groups or rows (Gray, 1961) and Smolyaninov (1971) estimated that there are $3.2 \times 10^6/mm^3$ which averages at a ratio of 250 granule cells for each Purkinje cell. However Bedi et al. (1980) calculate this ratio as 335:1. In cats and higher mammals their size and packing density appears to vary in different regions of the cortex; there are fewer larger granule cells in phylogenetically old areas than in phylogenetically newer regions (Lange, 1982). Granule cells are small round neurons only 5-6 μm in diameter (Palay and Chan-Palay, 1974) and they have very little cytoplasm around the nucleus, in which the chromatin is highly condensed (Gray, 1961; Palay and Chan-Palay, 1974). Each cell has 3-4 short sinuous dendrites which radiate from the perikaryon and divide into a claw-like terminal (Ariëns Kappers et al., 1936). These terminal claws surround the mossy fibre rosette (Gray, 1961) and up to 6 granule cells can terminate around a single rosette (Palay and Chan-Palay, 1974). The axon arises from the cell body or, more often, from a thick dendritic stem (Ariëns Kappers et al., 1936; Palay and Chan-Palay, 1974) and ascends vertically towards the molecular layer. In the upper third of the granular layer the axons from adjacent neurons join together into bundles to traverse the Purkinje cell layer with accompanying Bergmann glial cell

processes (Palay and Chan-Palay, 1974). The varicose ascending axon is 0.1-0.3 µm thick and once in the molecular layer develops protuberances which probably represent synaptic contacts with Golgi and Purkinje cell dendrites. In the molecular layer the axon bifurcates in either a Y- or T-shape and sends two slightly twisted processes in opposite directions. These 'parallel-fibres' are 0.1-0.2 µm thick and often the two sides will ascend to different levels within the molecular layer before they turn parallel to the pia mater along the axis of the folium (Ariëns Kappers et al., 1936; Palay and Chan-Palay, 1974). The two branches of the parallel fibre are not always the same length, especially if the parent granule cell is situated laterally in a folium (Palay and Chan-Palay, 1974; Schild, 1980). Although Smolyaninov (1971) estimated that parallel fibres are 0.9 mm long in the rat there is a large range of lengths, 0.8-3.0 mm, with the majority between 1.3-2.3 mm for the longer superficial fibres and 1.0-1.5 mm for the shorter deep fibres (Schild, 1980). Parallel fibres generally seem to be thicker in the lower part of the molecular layer (Fox and Barnard, 1957; Palkovits et al., 1971c; Smolyaninov, 1971) although this does not seem as apparent in the rat as in higher mammals (Palay and Chan-Palay, 1974). This is probably because the cells of origin of the deep lying parallel fibres tend to lie deep in the granular layer and have thicker, myelinated axons.

Because the parallel fibres run along the axis of the folium they pass perpendicularly through the dendritic trees of Purkinje, stellate, basket and Golgi cells with which they synapse via axonal varicosities (Fox and Barnard, 1957; Palay and Chan-Palay, 1974). It has been estimated that in the rat each parallel fibre synapses on 30 Purkinje cells (Smolyaninov, 1971). Approximately 40% of the parallel fibre is occupied by synaptic varicosities, which are 1.7 µm apart although over 50% are separated by less than 1 µm. This indicates that each parallel fibre has on average six synaptic varicosities for each Purkinje cell dendritic tree it traverses and each varicosity synapses with several dendritic spines. Large varicosities are more frequently found on the parallel fibres in the lower molecular layer and it is possible that these large varicosities synapse with basket cell dendrites which predominate in the deeper part of the layer (Fox and Barnard, 1957; Palay and Chan-Palay, 1974).

Golgi and Lugaro Cells

The two other types of neuron in the granular layer are Golgi and Lugaro cells. Golgi cells were first described by Golgi in 1874. He described two types of cells, one of which is now called the Lugaro cell. However the Golgi cells can also be divided into two groups of large and small neurons. Both these types appear to be differentially distributed in the cerebellum, being

more numerous in the vermis than the hemisphere (Lange, 1982). The large Golgi neurons lie mainly in the upper half of the granular layer. They are large cells, almost as big as Purkinje cells, being 10.7-23.8 µm long and 9.4-17.9 µm wide with a diameter of 9-16 µm (Palay and Chan-Palay, 1974). Eccentrically placed within the soma is a large circular or ovoid nucleus, which has dispersed chromatin and a round non-central nucleolus. The cytoplasm contains coarse clumps of Nissl substance (Palay and Chan-Palay, 1974). Two to four thick dendrites arise from the superficial surface of the cell, they bifurcate near to the soma often sending horizontal branches in the infraganglionic plexus (O'Leary et al., 1968) before they turn up towards the molecular layer (Palay and Chan-Palay, 1974). In the molecular layer the main dendrites give rise to relatively few secondary and tertiary branches that produce a fairly sparse three-dimensionally widespread arborisation which ascends even up to the pia mater and often overlaps with the trees of adjacent cells (O'Leary et al., 1968; Palay and Chan-Palay, 1974; Lange, 1982). The dendrites are 1.0-1.5 µm thick and are generally smooth and straight, although the thinner terminal branches become slightly varicose and have a few perpendicular spines (Palay and Chan-Palay, 1974). Due to the three-dimensional nature of the dendrite arborisation it overlaps with stellate, basket and Purkinje cell dendrites as well as parallel fibres.

From the deep surface of the soma or a major dendrite up to three axons can arise (Palay and Chan-Palay, 1974) of which one is always more branched than the others; however O'Leary et al. (1968) have not found evidence for more than one axon on each Golgi cell. Initially the axon is straight but it rapidly branches dichotomously, each branch being perpendicular to the stem (O'Leary et al., 1968; Palay and Chan-Palay, 1974), to form a dense plexus. The distal branches are thinner and varicose and often end in small terminal bulbs. The density of the plexus is not constant throughout being denser around the mossy fibre rosettes and having gaps where the fibres have to pass round granule cells. The plexus can either fill the whole depth of the granular layer or just a small part of it (Palay and Chan-Palay, 1974) and generally the plexuses arising from Golgi cells lying at the apex of the folium are less complex (O'Leary et al., 1968). The small Golgi neuron lies mainly in the lower part of the granular layer. These small neurons, 6-11 μm in diameter, are often grouped in twos or threes and can be distinguished from the granule cells by a pale ellipsoidal nucleus which has a prominent eccentric nucleolus and is surrounded by very little cytoplasm (Palay and Chan-Palay, 1974). The dendrites radiate from the soma and have irregular contours. They ascend towards the molecular layer although many remain within the granular layer (O'Leary et al., 1968; Palay and Chan-Palay, 1974). The

axons have the same structure as those of the large Golgi cells except that they form either two plexuses joined by a single branch or a long thin plexus along the granular layer-white matter junction (Palay and Chan-Palay, 1974). The Golgi cells receive input from the parallel fibres, basket and stellate cells, Purkinje cell recurrent collaterals and mossy and climbing fibres. The mossy fibres form large axo-somatic synapses which have been described as 'chestnut-like' (Chan-Palay and Palay, 1971b; Palay and Chan-Palay, 1974). The axons terminate on granule cell dendrites.

The Lugaro cells were first described as a type of Golgi neuron. They are fusiform shape, 9-10 x 25-30 μm, laying parallel to the folial axis in the granular layer just below the Purkinje cell layer. They are horizontal cells, although some lie almost vertical (O'Leary et al., 1968; Palay and Chan-Palay, 1974), containing an ellipsoidal nucleus surrounded by little cytoplasm in which there is scanty Nissl substance. One dendrite normally arises from each end of the cell and runs horizontally in a fairly straight arc along the Purkinje cell layer for 300-600 μm in the sagittal plane (O'Leary et al., 1968; Palay and Chan-Palay, 1974). Branches arise distally and run in approximately the same horizontal plane although some do ascend into the lower molecular layer (O'Leary et al., 1968; Palay and Chan-Palay, 1974). Both the main trunks and the branches have twisted

appendages and spines, which receive input from parallel fibres, Purkinje cell axon collaterals in the infra-ganglionic plexus (Palay and Chan-Palay, 1974) and possibly basket cell axons (O'Leary et al., 1968). The axon takes origin from either the soma or the base of a dendrite and travels obliquely over the Purkinje cell somata to ramify in the supraganglionic plexus (Palay and Chan-Palay, 1974). It has tenuous varicose ascending branches, which terminate on basket cell somata (Fox, 1959; O'Leary et al., 1968), and thick smooth descending branches which are through the granular layer to the white matter (Palay and Chan-Palay, 1974).

Glial Cells

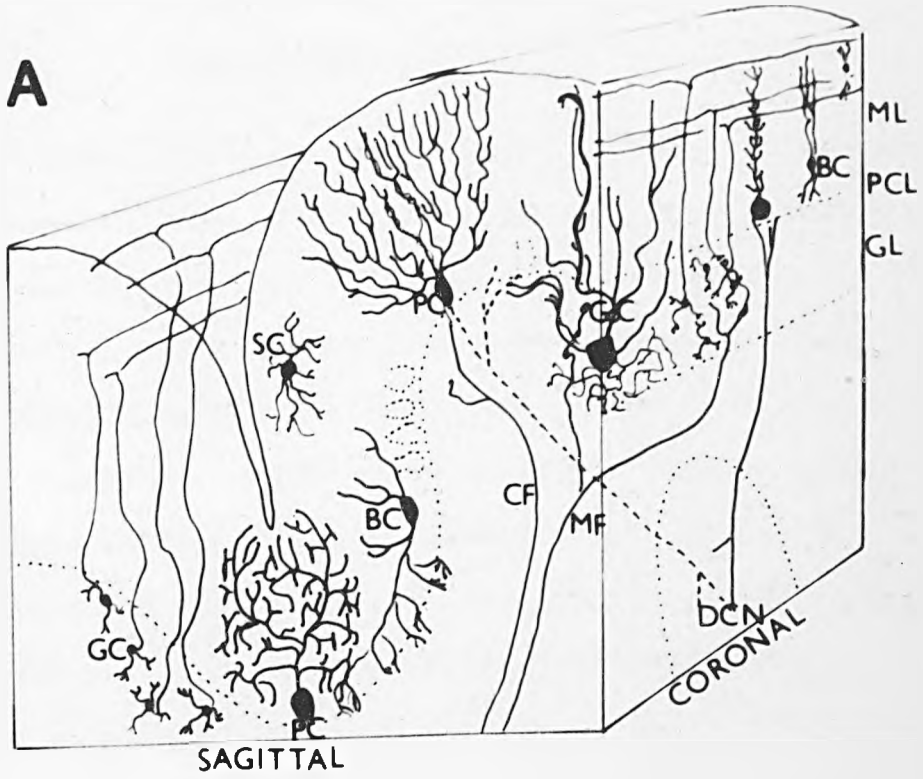
The cerebellar cortex also contains oligodendrocyte and astrocyte glial cells although microglia are not normally found. The oligodendrocyte is found mainly in the granular layer around the Purkinje cell axon and collaterals. The astrocytes can be divided into smooth and velate cells which are found mainly in the granular layer and the Golgi epithelial or Bergmann glial cells. These are situated in the Purkinje cell layer and project their varicose processes vertically through the molecular layer to the pia mater (Palay and Chan-Palay, 1974).

Figure 1.2

A. A diagrammatic representation of the cerebellar cortical organisation in sagittal and coronal planes (from Palay and Chan-Palay, 1974).

B. The organisation of the extracerebellar afferents to the cortex (from Chan-Palay, 1975).

SC = stellate cell
BC = basket cell
GC = granule cell
GoC = Golgi neuron
PC = Purkinje cell
ML = molecular layer
PCL = Purkinje cell layer
GL = granular layer
CF = climbing fibre
MF = mossy fibre
LC = noradrenergic afferents
5HT = serotoninergic afferents
DCN = deep cerebellar nuclei


A
ML
PCL
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BC
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SC
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MF
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GC
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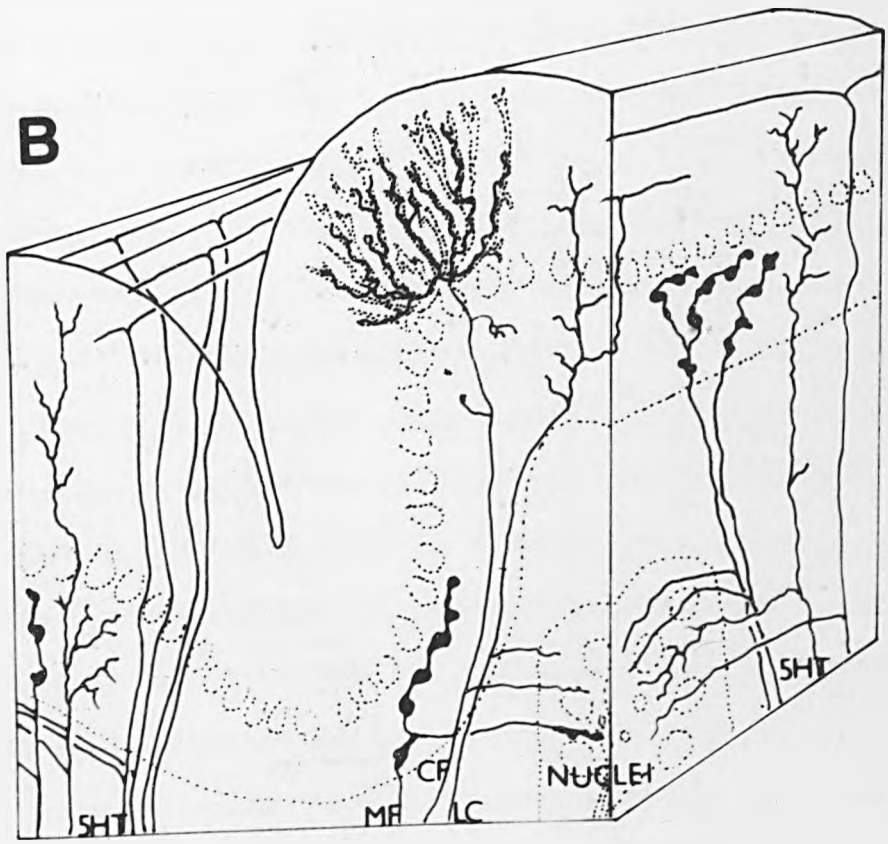
DCN
CORONAL
SAGITTAL


B
SHT
CF
NUCLEI
MF
LC
SHT

CEREBELLAR AFFERENTS

The cerebellar cortex has three types of extracerebellar afferents, the climbing, mossy and monoaminergic fibres. Climbing fibres arise from the contralateral inferior olive, mossy fibres from many brainstem nuclei and the spinal cord and the monoamine fibres from either the locus coeruleus or the raphe nuclei. Both climbing and monoamine fibres terminate directly onto Purkinje cells while mossy fibres act via the granule cells. In addition the cortex also receives an intracerebellar projection arising in the deep nuclei.

CLIMBING FIBRES

Climbing fibres were first observed in the cerebellar cortex by Ramon y Cajal (1888), who described all their important characteristics. He noted that the fibres emerge from the central white matter and follow a tortuous course through the granular layer to the Purkinje cell layer where they divide, in the same plane as the Purkinje cell dendrites, into varicose branches. These branches arborise about the Purkinje cell dendritic tree and give off thin tendrils which run parallel to their parent stems. Because of this mode of termination, Ramon y Cajal called these fibres 'climbing arborisations'. He clarified these observations by studying climbing fibre development in neonates and concluded that only one climbing fibre synapsed on each Purkinje cell.

Little was added to this description until Scheibel and Scheibel's (1954) Golgi study of climbing fibre intracortical branching and possible connections. However, it was not until the ultrastructure of the fibre was recognised (Larramendi and Victor, 1967) that the details of its terminals and synaptic connections could be studied.

The climbing fibre can be recognised in the white matter of a folium as a myelinated fibre of 2-3 µm diameter which follows a relatively straight course. It gives off collaterals, which pass into the granular layer, as does the main fibre, often by bending through approximately a right angle (Palay and Chan-Palay, 1974). It ascends obliquely through the granular layer following a tortuous course, presumably passing around groups of granule cells (Palay and Chan-Palay, 1974). Within this layer the climbing fibre gives off two types of collaterals (Chan-Palay and Palay, 1971a). The first is a long thin varicose fibre, which runs either close to the main stem or perpendicularly away from it. These collaterals synapse primarily on the major dendrites of Golgi and granule cells but also on the Golgi cell soma. The second type of collateral is a short stout fibre of the same diameter as the main stem (2 µm) which spreads into an efflorescence similar to that of a mossy fibre glomerulus, although with a more basophilic core. This second type of collateral also synapses with granule cell

dendrites and Golgi cell somata. These collaterals do not remain in the same plane as the terminals, therefore the climbing fibre divergence within the granular layer is not easily seen (Scheibel and Scheibel, 1954; Palay and Chan-Palay, 1974).

At the Purkinje cell layer the climbing fibre loses its myelin sheath and splits into a terminal arborisation (Chan-Palay and Palay, 1970; Palay and Chan-Palay, 1974). Fine tendril collaterals are given off which descend into the granular layer (Szentagothai and Rajkovits, 1959) and ramify in the infraganglionic plexus (Scheibel and Scheibel, 1954; Palay and Chan-Palay, 1974). These probably then synapse onto 5 to 10 adjacent Purkinje cell somata, also stellate and basket cells (Scheibel and Scheibel, 1954). Through these collaterals the climbing fibre terminal arborisation is not confined to the plane of the Purkinje cell dendritic tree as Ramon y Cajal (1888) first thought. The climbing fibre divides with the Purkinje cell primary dendrites and follows a similar, although not exactly comparable, arborisation, which is mainly confined to the lower two-thirds of the molecular layer (Chan-Palay and Palay, 1970). As Ramon y Cajal (1888) first noted the branches give off fine beaded collaterals, which run parallel to the parent stem (Scheibel and Scheibel, 1954; Chan-Palay and Palay, 1970). However, these collaterals do not necessarily synapse onto Purkinje cell dendrites, but can cross to basket cell and

stellate cell somata and granule cell ascending axons (Palay and Chan-Palay, 1974).

With the electron microscope climbing fibres are seen to be filled with microtubules and have synaptic boutons packed with round vesicles. They synapse onto large thorns on Purkinje cell primary dendrites and not directly onto the dendritic shaft (Larramendi and Victor, 1967; Chan-Palay and Palay, 1970).

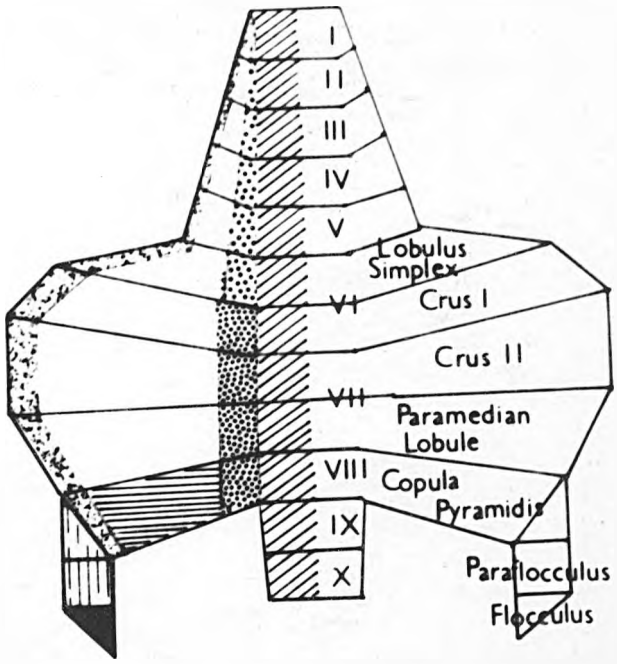
The Olivo-cerebellar Projection

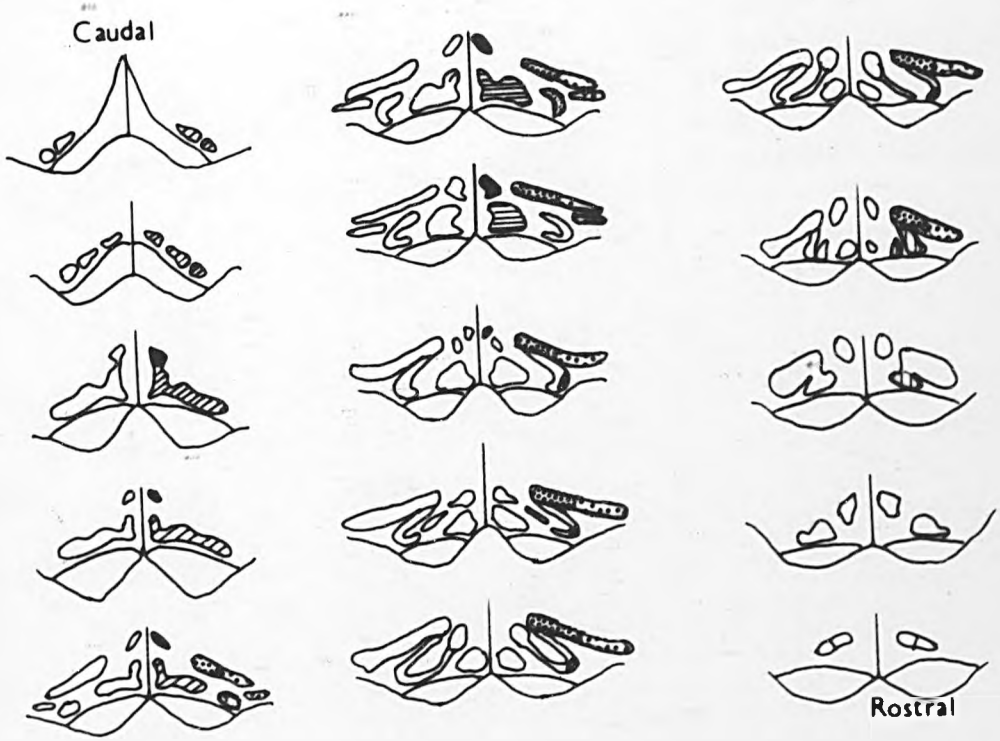
A connection between the inferior olives and the cerebellum was observed in man (Holmes and Stewart, 1908) after finding discrete degeneration of the contralateral inferior olive in patients with localised cerebellar disease, and they proposed that the olivocerebellar projection is topographically organised. Olivocerebellar fibres were found to terminate as climbing fibres only in 1959 (Szentagothai and Rajkovits) because previous studies (Carrea et al., 1947) had elicited degeneration in the granular layer alone following lesions to the inferior olive. Climbing fibre degeneration has since been shown following inferior olivary lesions (Grant, 1970; Desclin, 1974) but the rapidity with which the degenerating fibres disappear (48 hours) makes this technically difficult (Desclin, 1974). Climbing fibre termination of the olivocerebellar projection has been confirmed physiologically (Eccles et al., 1964) and autoradiographically

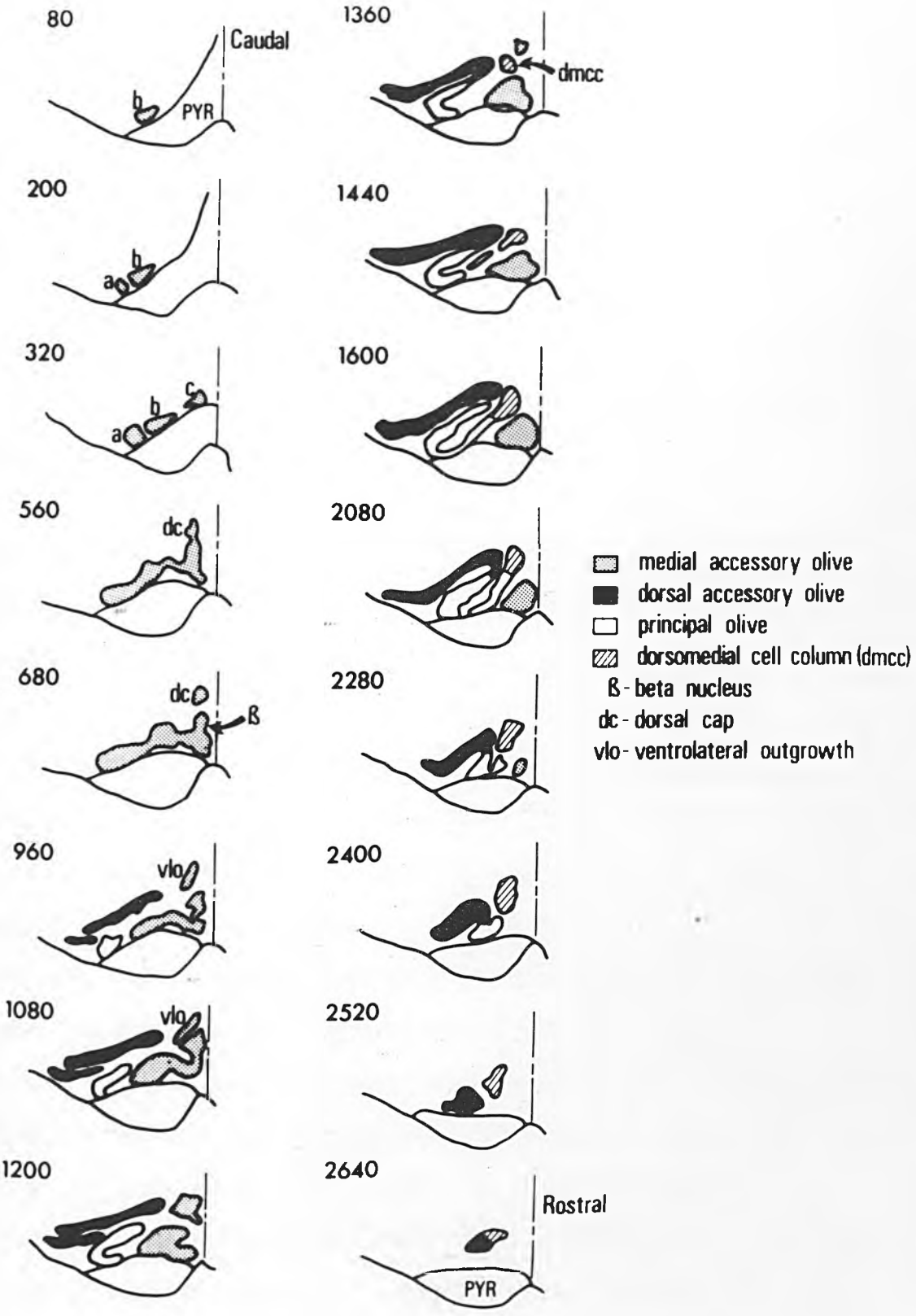
Figure 1.3

Olivocerebellar Projection

A diagram to summarise the major topographical regions in the olivocerebellar projection of the rat from the right inferior olive to the left hemicerebellum. The unshaded left inferior olive is not included as part of the projection to the hemisphere cortex but shows the inferior olivary subdivisions in accordance with those described by Gwyn et al. (1977) as seen overleaf. The diagrammatic flattened cortex is from Campbell and Armstrong (1983 a & b) and the nomenclature and vermal lobule numbering, in brackets for the posterior lobe, follows that of Larsell (1952).


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


Caudal
Rostral


80
Caudal
b
PYR
200
b
a
320
c
b
a
560
dc
680
dc
ß
960
vlo
1080
vlo
1200
1360
dmcc
1440
1600
2080
2280
2400
2520
2640
Rostral
PYR
medial accessory olive
dorsal accessory olive
principal olive
dorsomedial cell column (dmcc)
ß - beta nucleus
dc - dorsal cap
vlo - ventrolateral outgrowth

(Murphy et al., 1973; Courville et al., 1974; Courville, 1975; Courville and Faraco-Cantin, 1978; Campbell and Armstrong, 1983a).

The topography of the olivocerebellar projection was first studied in detail in cats by Brodal (1940) who observed retrograde changes in the contralateral inferior olivary neurons following small cerebellar lesions. He concluded that different olivary subdivisions projected to different lobules of the cerebellum. Using anterograde degeneration following inferior olivary lesions Voogd (1969) concluded that the olivocerebellar fibres were organised in parasagittal strips which ignore the inter-lobule fissures. Parasagittal branching of the olivo-cerebellar projection was readily confirmed physio-logically (Faber and Murphy, 1969; Armstrong et al., 1971, 1973c, 1974) and autoradiographically (Courville et al., 1974; Courville, 1975; Chan-Palay et al., 1978; Campbell and Armstrong, 1983a). Autoradiography of the cerebellar cortex following injection of a radiolabelled aminoacid into the contralateral inferior olive, shows the molecular layer banded into alternately labelled and unlabelled strips. Other workers using a combination of anterograde degeneration and autoradiography (Groenwegen and Voogd, 1977: Groenwegen et al., 1979) or wheat germ agglutinin conjugated to horseradish peroxidase (Beyerl et al., 1982) obtained similar results. Physiological experiments also revealed climbing fibre branching (Armstrong et al.,

1973 a & b), a result which correlates well with the disparity in the number of inferior olivary neurons and Purkinje cells (rat - Armstrong and Schild, 1970; Schild, 1970: cat - Escobar et al., 1968; Palkovits et al., 1971a: man - Braitenberg and Atwood, 1958; Escobar et al., 1968) and has since been confirmed anatomically (Brodal et al., 1980).

The precision of the olivocerebellar topography has only been established using retrograde labelling of the inferior olive following microinjections of horseradish peroxidase into the cerebellar cortex (for a review see Brodal and Kawamura, 1980). The majority of this work has been undertaken in cats and it is only comparatively recently that the rat olivocerebellar projection has been investigated in detail using either autoradiography (Chan-Palay et al., 1978; Armstrong et al., 1982; Campbell and Armstrong, 1983 a & b) or retrograde transport of horseradish peroxidase (Brown, 1980; Eisenman, 1981a; Hess, 1982b; Eisenman, 1984; Furber and Watson, 1983).

It is generally agreed that in the rat the vermis receives climbing fibres from the caudal medial accessory olive (MAO) (Brown, 1980: Eisenman, 1981a; Campbell and Armstrong, 1983b; Eisenman, 1984; Furber and Watson, 1983). In addition lobules I-III (in the nomenclature of Larsell, 1952) receive a projection from the caudolateral dorsal accessory olive (DAO) (Furber and Watson, 1983),

and lobules IV and V from the midrostral DAO and subnuclei 'a' and 'c' of the MAO (Furber and Watson, 1983). However, more laterally the paravermal zone of lobules I-IV receives a projection from the lateral DAO (Campbell and Armstrong, 1983b). Lobule VI is a terminal area for subnuclei 'b' and 'c' of the caudal MAO as well as the caudal two-thirds of the medial DAO (Furber and Watson, 1983). Subnuclei 'b', 'c' and 'β' of the midrostral MAO project to lobule VII (Hess, 1982b; Furber and Watson, 1983) while the subnuclei 'b' and 'c' of the caudal MAO and the dorsomedial cell column (dmcc) project to lobule VIII (Furber and Watson, 1983). Multiple injections of horseradish peroxidase into lobules V-VIII indicated a projection from subnuclei 'b' and 'c' of the caudal MAO only, while the paravermal region of lobules VI-VIII receive climbing fibres from the medial DAO (Campbell and Armstrong, 1983b).

A more detailed study of the olivary projection to lobules VIII and IX using retrograde transport of horseradish peroxidase (Eisenman, 1981a, 1984) reveals three parasagittal zones in each lobule. In lobule VIII the medial zone receives a projection from the lateral (possibly subnucleus 'a') caudal MAO, the intermediate zone from nucleus β of the MAO and the lateral zone from the lateral (perhaps subnucleus 'a') rostral MAO. The medial region of lobule IX receives afferents from subnuclei 'c' and 'β' of the MAO, the intermediate area from

the dmcc and the lateral area from the ventral lamella of the principal olive (vPO). Interestingly a retrograde double-label study of the intermediate zone of lobule VIII and the medial zone of lobule IX (both receiving afferents from nucleus β of the MAO) reveals that while cells projecting to either region are scattered throughout the nucleus, very few actually innervate both areas (Eisenman and Goracci, 1983).

However, the topography of the olivocerebellar projection to the cerebellar hemispheres is less clear. The lobulus simplex receives a projection from the medial part of the principal olive (PO) and the dmcc (Furber and Watson, 1983) while its extreme lateral zone receives afferents from the caudal PO (Campbell and Armstrong, 1983b). Climbing fibres to crus I arise in the lateral parts of both lamellae and the lateral bend of the PO and the dmcc (Furber and Watson, 1983), while those to the more medial part of the lobule arise in the rostral two thirds of the PO, the DAO and the dmcc (Brown, 1980) and to its lateral edge are from the caudal PO (Campbell and Armstrong, 1983b). Using retrograde transport of horseradish peroxidase Brown (1980) also finds a projection to crus I from subnuclei 'b' and 'c' of the MAO, but her injection site encroached onto vermal lobule VII, which is known to have a projection from the MAO (Furber and Watson, 1983). Crus II receives climbing fibres from the medial part of the PO and the dmcc (Furber and Watson, 1983), its more

medial part from the lateral PO, rostral DAO and the dmcc (Brown, 1980) and its extreme lateral edge from the caudal PO (Campbell and Armstrong, 1983b). As with her investigation into crus I, Brown (1980) also finds a projection from the MAO but the medially placed injection may have spread into lobule VII, which does receive fibres from the MAO. The paramedian lobule is innervated by either the whole (Furber and Watson, 1983) or just the dorsal lamella (Brown, 1980) of the PO. Its medial region receives fibres from the medial DAO and the dmcc (Brown, 1980), and its lateral region from either the caudal PO (Campbell and Armstrong, 1983b) or the midrostral dPO (Brown, 1980). The medial part of the copula pyramidis receives fibres from the lateral DAO (Eisenman, 1981a) but this may overlap with the paravermal region of lobule VIII, which receives fibres from the medial DAO (Campbell and Armstrong, 1983b). Fibres to the intermediate copula pyramidis arise in the midrostral MAO, and those to the lateral zone come from the caudal tip of the PO (Eisenman, 1981a). The paraflocculus receives its climbing fibre afferents from the rostral PO (Furber and Watson, 1983) and the flocculus from the lateral bend of the rostral PO and the dorsal cap of the MAO (Blanks et al., 1983).

These results would suggest that the cerebellar hemispheres can be divided into three longitudinal zones which receive climbing fibres from different divisions of the contralateral inferior olive. Afferents to the medial

paravermal zone arise mainly in the DAO, those to the intermediate zone project from the PO and possibly the dmcc, while a thin lateral strip receives fibres from the caudal PO. Also this parasagittal banding does not appear to continue into the paraflocculus and flocculus.

The olivocerebellar projection in the rat is very similar to that of the cat, although there are a few important differences. The most detailed description of the cat's olivocerebellar projection is by Brodal and his coworkers (for review see Brodal and Kawamura, 1980) using the retrograde transport of horseradish peroxidase, whose results compare most favourably with the physiological map produced by Armstrong et al. (1974). Therefore the topographical projection described by Brodal will be compared with that of the rat.

The olivocerebellar projection to the vermis is very consistent in both rats and cats arising primarily in the caudal MAO. In lobules I-IV of the rat Furber and Watson (1983) found an additional projection from the DAO, a projection considered to go to the paravermis by Campbell and Armstrong (1983b) and be confined to the lateral part of the vermis in the cat (Brodal and Walberg, 1977a). Similarly, Furber and Watson (1983) found a projection to lobule VII in rats from the midrostral as well as the caudal MAO. In the cat the midrostral MAO projects to the hemisphere of the anterior lobe (Brodal and Walberg, 1977a) which might indicate a genuine species difference

or a spread of tracer from the injection site. The olivary projection to lobules VII and the medial and intermediate zones of IX are the same with the addition of a nucleus β projection to lobule VIII of the rat (Eisenman, 1981a). However, the lateral zone of lobule IX has a different olivary projection in the rat, from vPO, and the cat from the lateral rostral MAO. In the cat the olivary projection to the cerebellar hemispheres is organised in longitudinal zones rather than to the different lobules. The medial region of the hemisphere, including both crura and the lobulus simplex, receives afferents from the medial DAO (Kotchabhakdi et al., 1978b) and this may correlate with the "paravermal cortex of the posterior lobe" of rats (Campbell and Armstrong, 1983b). The more lateral areas of the crura and lobulus simplex have a reverse projection in the rat and cat. In the cat the lobulus simplex and crus I have the same projection from the medial part of both PO lamellae, while the lateral lamellae and the lateral bend of the PO project to crus II (Walberg, 1980). But in the rat the lobulus simplex and crus II have the same olivary projection from the medial part of the principal olive and it is crus I which receives its projection from the lateral bend of the PO (Furber and Watson, 1983). The paramedian lobule is quite difficult to compare between the rat and the cat because in the rat it lies transversely in the posterolateral hemisphere while in the cat it is

just lateral and parallel to the posterior vermis. Perhaps because of this there is a difference in its olivary projection between the two species, in the rat its afferents arise in the PO (Furber and Watson, 1983), possibly just the dorsal lamella (Brown, 1980), but in the cat it also receives a projection from the midrostral MAO (Brodal and Walberg, 1977b). The olivary projection to the paraflocculus of the rat arises in the rostral PO (Furber and Watson, 1983), while in the cat the cells of origin lie in the lateral PO and the rostromedial MAO (Walberg, 1980).

Although it is now generally accepted that the contralateral inferior olive is probably the sole source of cerebellar climbing fibres, there has been some controversy whether it is the exclusive origin of these afferents and whether there is also a projection to the ipsilateral cerebellum. After unilateral lesions to the inferior olive O'Leary et al. (1970) found degenerating fibres in both the granular and molecular layers of the cerebellum and, under the electron microscope, many normal climbing fibre terminals. Therefore they concluded that some climbing fibres arose in other brainstem nuclei. A similar result was obtained autoradiographically (Murphy et al., 1973) where unilateral injection of radioisotope into the inferior olive labelled both mossy and climbing fibres in the contralateral cerebellum and showed a small ipsilateral pathway. They also found that injections

into the medial reticular nucleus produced the greatest amount of climbing fibre labelling, a result which has not been confirmed. An extra-olivary source of climbing fibres was also proposed by Courville (Courville et al., 1974; Courville, 1975) because injections of radio-isotope into the whole inferior olive only produced strips of labelling in the cerebellar cortex, a result which was reiterated by Chan-Palay et al. (1978). Physiological experiments also gave evidence for an extra-olivary source of climbing fibres (Batini et al., 1976) because Purkinje cells would respond with a typical climbing fibre induced complex spike potential after pontine stimulation. Batini et al. (1976) also injected horseradish peroxidase into the cerebellar vermis, cut the ipsilateral inferior cerebellar peduncle and still found labelling in the contralateral inferior olive, via a proposed climbing fibre path through the middle or superior cerebellar peduncle. However, Courville changed his view (Courville and Faraco-Cantin, 1978) and concluded that the unlabelled strips in the cerebellar cortex were innervated by unlabelled regions of the inferior olive. In addition they noticed that fibres from the rostral inferior olive pass rostrally through the brainstem and over the brachium conjunctivum before turning caudomedially to enter the contralateral inferior cerebellar peduncle. Since they found no labelling within either the middle or superior cerebellar peduncles this loop of olivocerebellar fibres

could explain the physiological results of Batini et al. (1976). A more recent autoradiographic study (Campbell and Armstrong, 1983a) has shown that extensive olivary labelling produces climbing fibre labelling throughout the cerebellum and that any unlabelled strips can be accounted for by incomplete olivary labelling. They also postulate that the alternated strips of labelled cortex found by Chan-Palay et al. (1978), even after total labelling of both inferior olives, might be caused by differential biochemical metabolism of methionine in the cerebellum, a possibility since the metabolism of 5-nucleotidase varies in different sagittal zones of the cortex (Scott, 1964). It is also possible that the high specific activity of the radiolabel used (sulphur 35) could excite silver halide crystals of the nuclear emulsion at some distance from the neuronal source of emission, therefore giving the impression of total inferior olivary labelling, when in fact some neurons did not take up and transport sufficient tracer to produce visibly labelled terminals in the cerebellum.

The question of a small ipsilateral olivocerebellar projection has generally been raised by workers using anterograde tracing techniques, either autoradiography (Murphy et al., 1973; Chan-Palay et al., 1978) or horseradish peroxidase conjugated to wheat germ agluttinin (Beyerl et al., 1982). The results from the latter technique may possibly be caused by the uptake of

horseradish peroxidase-wheat germ agglutinin by fibres from the contralateral inferior olive passing through the injection site. An ipsilateral projection found by autoradiography is most probably caused by spread of the labelled aminoacid from the injection site to either the contralateral inferior olive or the laterally adjacent lateral reticular nucleus, which projects to the cerebellum bilaterally (Chan-Palay et al., 1978). Injection sites totally confined to the inferior olive have not revealed an ipsilateral projection (Courville et al., 1974; Courville, 1975; Courville and Faraco-Cantin, 1978; Armstrong et al., 1982; Campbell and Armstrong, 1983 a & b). Retrograde tracing techniques have not revealed an ipsilateral olivo-cerebellar projection with one exception (Brown, 1980) when horseradish peroxidase was injected into the cerebellar vermis and both inferior olives were labelled. This result is probably due to spread of the tracer across the midline or a possible bilateral olivary innervation for the most medial vermis.

MOSSY FIBRES

Ramon y Cajal (1888) first reported mossy fibres in the cerebellar granular layer. He noticed that they formed the majority of the fibres within the layer and possessed nodules at frequent intervals along their length. He called them 'mossy fibres' because he thought that the nodules, which consisted of short varicose branches,

looked like clumps of moss.

In the white matter of the cerebellum the mossy fibre is thick, myelinated (Palay and Chan-Palay, 1974) and filled with neurofibrils (Craigie, 1926). The fibre branches to send collaterals to different folia (Smolyaninov, 1971) and within each folium it divides many times sending 20-30 collaterals into the granular layer, where they branch several times more to form a loose arbour (Palay and Chan-Palay, 1974). These myelinated branches within the granular layer follow a tortuous course to their terminations, often running parallel to the pia mater, and give off myelinated preterminal branches (Yeh et al., 1982). There appear to be two types of fibres, one thin of 0.4 µm diameter (Palay and Chan-Palay, 1974) and the other thicker with a diameter of 1-1.5 µm (Palay and Chan-Palay, 1974; Yeh et al., 1982). Also, Yeh et al. (1982) found a few much thicker fibres with a diameter of 4 µm. The mossy fibre branchings in the granular layer tend to be orientated in the sagittal plane, either at particular levels or throughout the whole depth of the layer (Smolyaninov, 1971). A fibre and its collaterals form a band in the cortex 400-700 µm sagittally by 100-250 µm coronally (Braitenberg and Atwood, 1958; Scheibel, 1977).

The mossy arborisations are discrete processes occurring along the fibre, at its bifurcation points and

at its terminals (Ramon y Cajal, 1888; Palay and Chan-Palay, 1974; Scheibel, 1977; Yeh et al., 1982) and can be found on both thick and thin fibres (Palay and Chan-Palay, 1974). These specialisations are spaced approximately every 20-80 µm along the fibre (Palay and Chan-Palay, 1974) although Braitenberg and Atwood (1958) found a differential spacing in the sagittal (280 µm) and coronal (50 µm) planes. In the mossy region, the fibre loses its myelin sheath and distends into a spindle shape 10-15 µm long (Braitenberg and Atwood, 1958), which is filled with curled neurofibrils (Craigie, 1926) and has short processes projecting in all directions (Palay and Chan-Palay; 1974: Yeh et al., 1982). Three types of mossy endings have been described; (1) a simple fusiform swelling possessing few short processes, (2) a large central expansion with twisted filiform appendages, and (3) a complex flower-like 'rosette' possessing curved tubular projections. All three types can be found with equal frequency (Palay and Chan-Palay, 1974). However, the simple fusiform type tends to occur more often on the thinner fibres (Yeh et al., 1982), the large expansions along the course of the fibre and the complex flower-like rosettes are usually seen at the terminals, mainly in the outer third of the granular layer (Palay and Chan-Palay, 1974). However, Brodal and Drabløs (1963) described two types of mossy fibre ending which are differentially distributed; one is the classically described 'rosette'

found throughout the cerebellar cortex and the other is more nodular with many short processes occurring on extensively branched fibres. The 'nodular' type of ending is found predominantly in the nodule, flocculus and paraflocculus and less frequently in other areas of the vermis, therefore it was concluded that these must be the terminals of primary vestibular afferents (Brodal and Drabløs, 1963).

The mossy fibre rosettes, both along the fibre and at its terminals, synapse onto granule cell dendrites (Gray, 1961), a contact first proposed by Ramon y Cajal (1911) since in Golgi preparations granule cell dendrites converge onto discrete spots in the granular layer. He also saw the axons of Golgi cells contacting granule cell dendrites at these points, which he called glomeruli because of their similarity to the glomeruli of the olfactory bulb. These neuron connections have been confirmed using the electron microscope, under which the mossy rosette is seen to be filled with mitochondria, neurofilaments and smooth endoplasmic reticulum. Also it synapses onto granule cell dendrites (Gray, 1961) and Golgi cell somata (Chan-Palay and Palay, 1971b). Golgi cell axons also synapse onto granule cell dendrites at the glomeruli (Gray, 1961).

Origin of Mossy Fibres

Unlike the climbing fibres, mossy fibre afferents have a wide origin from the spinal cord and brainstem nuclei

(for review see Bloedel, 1973). Connections from the spinal cord and brainstem to the cerebellum were observed early this century but there was great disparity in the results (for a review see Ariëns Kappers et al., 1936). However, Ariëns Kappers et al. (1936) concluded that there was a regional distribution of these afferents within the cerebellum. Experimental evidence for cerebellar afferents arising in different nuclei was not obtained until later. Mossy fibre afferents arise from primary vestibular afferents (Brodal and Hoivik, 1964), the vestibular nuclei (Dow, 1936), the spinal cord (Grant, 1962a), the external cuneate nucleus (Grant, 1962b), the reticular formation (Brodal, 1953), the pontine nuclei (Brodal and Jansen, 1946), the perihypoglossal nuclei (Brodal, 1952) and the trigeminal nerve nuclei (Pearson, 1949). The mossy fibre termination of brainstem afferents has been demonstrated with anterograde degeneration (Cupedo, 1965) and autoradiography (Murphy et al., 1973).

The topography of cerebellar afferents from the brainstem nuclei was first studied in the cat by Brodal and his colleagues using retrograde degeneration techniques (see review by Larsell and Jansen, 1972), and further detail has been added by the retrograde labelling of brainstem nuclei after horseradish peroxidase injections into the cerebellar cortex (for review see Gould, 1980). In a similar manner to climbing fibres, mossy fibre afferents have a sagittal organisation. This has

been demonstrated for spinal afferents both physiologically (cat, Oscarsson, 1976) and anatomically (ferret, Voogd, 1969) and in the cerebellar projections from the lateral reticular nucleus of the rat (Chan-Palay et al., 1978) and the cat (Kunzle, 1975b), results which compare favourably with the anatomical distribution of mossy fibres within the granular layer (Braitenberg and Atwood, 1958; Scheibel, 1977). The topography of mossy fibre afferents in the rat has been investigated only recently, predominantly using retrograde tracing of either horseradish peroxidase (e.g. Burne et al., 1978 a & b; Eisenman, 1976, 1980, 1981b, 1982; Mihailoff et al., 1981) or fluorescent dyes (Mihailoff, 1983; Payne, 1983b). Since the interspecies topographic differences are slight (Gould, 1980) only the details of the rat afferent topography will be described.

The Basal Pontocerebellar Projection

The cerebellar projection from the basal pontine nucleus is bilateral with a contralateral predominance (Burne et al., 1978 a & b; Eisenman, 1976, 1980, 1981b; Eisenman and Noback, 1980; Azizi et al., 1981; Mihailoff et al., 1981; Mihailoff, 1983; Payne, 1983b). The degree of homolaterality is greater from the caudal regions of the nucleus (Mihailoff et al., 1981) which generally project to the more medial aspects of the hemicerebellum (Eisenman and Noback, 1980; Azizi et al., 1981).

Lobule VI receives fibres from the midrostral ventromedial and ventrolateral regions of the nucleus and lobule VII from the rostral half of the medial, lateral and dorsolateral areas (Azizi et al., 1981). However, Eisenman (1976) found only the dorsolateral region of the nucleus projecting to lobules VI and VII. Afferents to lobule VIII arise in the dorsomedial and ventrolateral parts of the caudal tip of the pontine grey (Azizi et al., 1981; Eisenman, 1981b), while those to the paravermis adjacent to lobule VIII are from the central area of the caudal ventral grey (Eisenman, 1981b). There is some controversy over the origin of afferents to lobule IX; Eisenman and Noback (1980) find a projection from the caudal half of the pons, ventral peduncular cells projecting to sublobule 'a' and medial and ventrolateral cells projecting to sublobules 'b' and 'c'. However, Azizi et al. (1981) find the projection to sublobules 'a' and 'b' from the medial and ventral peduncular cells at the midrostral level, although they had afferents to sublobule 'c' arising in the caudal pons. These two results are quite difficult to compare because each uses a different system for describing the rostrocaudal position of their brainstem sections. It would appear that the two results are not totally incompatible because Eisenman and Noback (1980) do show labelled cells at the rostral end of the caudal half of the pons i.e. at the midrostral position, and Azizi et al. (1981) have some labelling in the caudal part of the nucleus.

Projections to the cerebellar hemisphere from the basal pontine nucleus appear to divide into two groups, the medial hemisphere receiving afferents from the more caudal parts of the pontine grey and the lateral hemisphere from more rostrally lying areas. The medial half of the lobulus simplex receives fibres from the caudal central ventral grey (Burne et al., 1978a) or the mid-rostral dorso- and ventromedial areas with a few fibres from the lateral- and dorsal-peduncular region (Mihailoff et al., 1981). Afferents to the lateral part of this lobule arise rostrally in the central ventral region (Burne et al., 1978a; Mihailoff et al., 1981). Injections into the lobulus simplex which encroached onto crus I, also revealed a projection to these areas from the rostral central ventral pons (Payne, 1983b). Crus I receives afferents from the medial, ventral and ventrolateral rim of the central ventral pons, and from neurons lying in the dorsal-peduncular region; cells projecting to the medial part of crus I lying midrostrally while those projecting to the lateral areas are placed further rostrally (Burne et al., 1978a; Mihailoff et al., 1981). Fibres projecting to the lateral part of crus I arise in the rostral centromedial, ventrolateral and ventral-peduncular areas of the pons (Burne et al., 1978a; Mihailoff et al., 1981). The paramedian lobule receives afferents from the central and ventral-peduncular pons throughout its rostrocaudal extent (Mihailoff et al.,

1981). However small injections into the medial and lateral parts of this lobule indicate a more precise projection from the medial and lateral ventral grey, from the caudal pons to the medial half and the rostral pons to the lateral half (Burne et al., 1978a; Mihailoff et al., 1981). The medial copula pyramidis receives fibres from the caudal ventromedial and ventrolateral pons and the midrostral ventral-peduncular area, and the lateral copula pyramidis receives a projection from the midrostral dorsomedial and ventrolateral grey and the dorsal-peduncular area (Eisenman, 1981b). Afferents projecting to the paraflocculus arise from the rostral dorsomedial and dorsolateral areas of the pontine grey (Burne et al., 1978 a & b), with those neurons projecting to the ventral paraflocculus lying further out on the extreme edges of these regions (Eisenman, 1980).

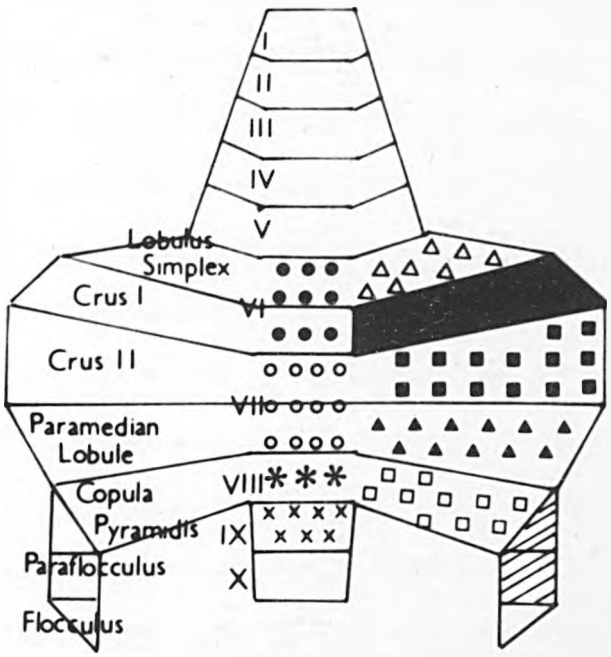
Since each region of the cerebellar cortex receives afferents from more than one area of the basal pontine nucleus, and the delineation between regions of the nucleus projecting to different cerebellar areas is relatively indistinct, the degree of pontocerebellar collateralisation between cortical lobules has been studied with double-label retrograde fluorescent tracers. Mihailoff (1983) found that there is far more intra- than inter-hemispheric branching. Intrahemispheric collaterals branch mainly between the lobulus simplex and crus II, and crus I and the paraflocculus, fewer have

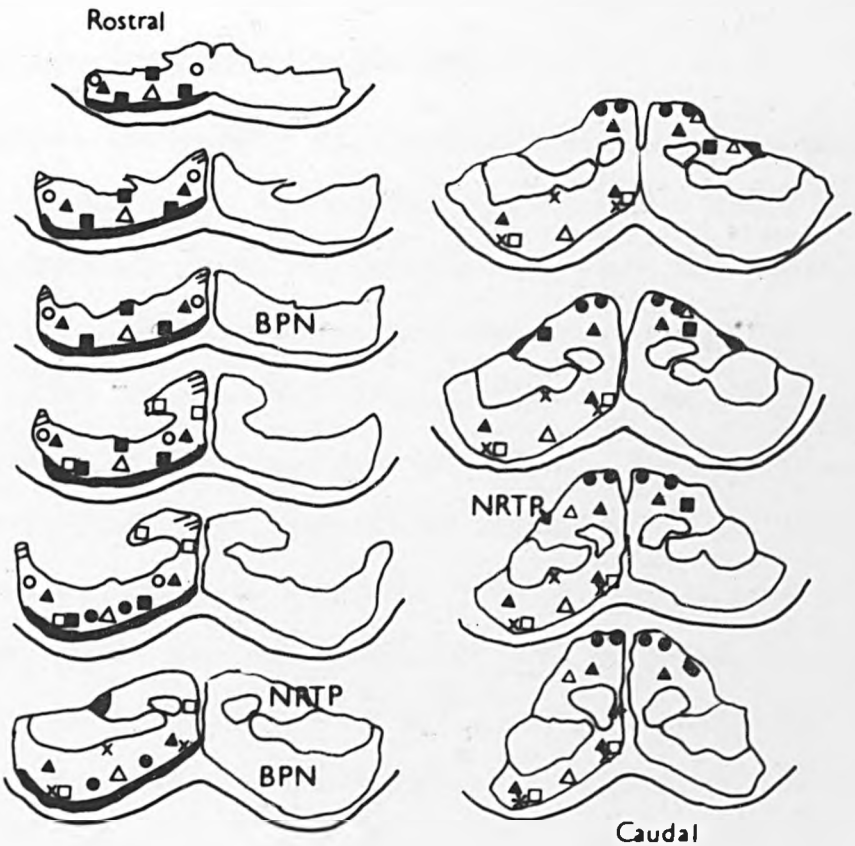
Figure 1.4

Basal and Tegmental Reticular Pontine Nuclear Projection to the Cerebellum

This diagram summarises the projection to the cerebellar posterior lobe from the basal pontine nucleus (BPN) and the nucleus reticularis tegmenti pontis (NRTP). The projection from the basal pontine nucleus, shown here as totally crossed, is bilateral with a contralateral predominance. The ipsilateral projection arises from symmetrically placed neuronal groups and has been left out to clarify the picture. It is interesting to note that there are some regions which contain closely adjacent groups which project to different cerebellar areas and these probably represent neurons possessing divergent collaterals.

The projection from the nucleus reticularis tegmenti pontis is also bilateral but the cells of origin from both nuclei have been demonstrated here because the projection does not appear to be symmetrical on both sides.


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


Rostral
BPN
NRTP
BPN
NRTP
Caudal

also been found dividing between the lobulus simplex and paramedian lobule, crus I and paramedian lobule and the anterior and posterior sublobules of crus II. These fibres always arise from the contralateral basal pontine nucleus. Interhemispheric branching is found to a lesser extent and is predominantly between homotopic lobules excepting the paraflocculus. Collaterals to heterotopic lobules are only frequently found between the paramedian lobule and either anterior or posterior sublobules of crus II, although they are present between the lobulus simplex and crus II, and crus I and the paramedian lobule. Collaterals between other heterotopic lobules have not been found.

Reticulocerebellar Projection

Detailed topography of the reticulocerebellar projection is relatively incomplete and although there is greater knowledge about the projection from the paramedian reticular nucleus in the cat and monkey (Somana and Walberg, 1978) than in the rat, studies on the rat appear no less complete than in other mammals. The majority of the reticulocerebellar projection arises in three nuclei; the nucleus reticularis tegmenti pontis (NRTP), the lateral reticular nucleus (LRN) and the paramedian reticular nucleus (PRN) (for review see Llinas and Simpson, 1981), but afferents to the cerebellar cortex have also been found arising in the nucleus reticularis giganto- and parvi-cellularis (Kimoto et al., 1978). Projections from

the NRTP are bilateral usually with a contralateral predominance, from the LRN are primarily ipsilateral with a small contralateral component, and there is a small bilateral projection from the PRN.

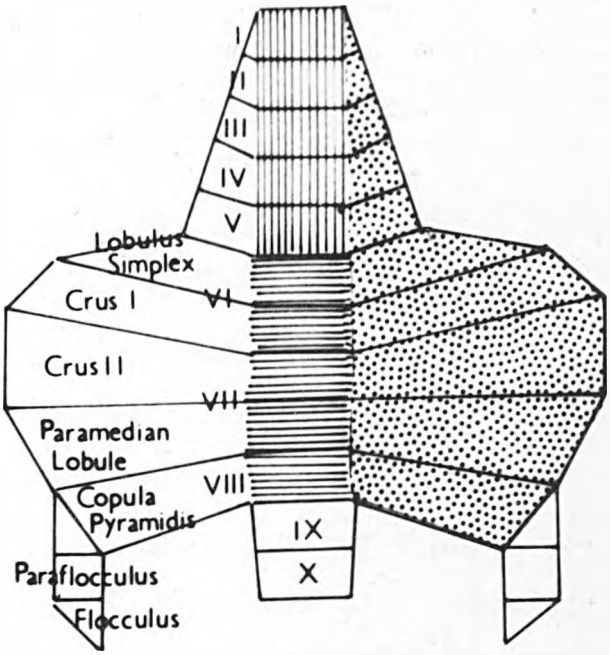
Vermal lobules II and III receive afferents from the border between the parvo- and magno-cellular parts of the ventromedial LRN (Hrycyshyn et al., 1982) and lobules IV and V from the ventromedial and dorsolateral magnocellular regions of the LRN throughout its rostrocaudal extent (Hrycyshyn et al., 1982). The PRN also projects to these lobules (Faull, 1977). The projection to lobules VI and VII from the LRN is small and arises in the rostral two-thirds of the medial magnocellular part (Hrycyshyn et al., 1982), and although cells throughout the NRTP project to lobule VI only neurons in the caudal medial area project to lobule VII (Azizi et al., 1981). Lobule VIII receives a large input from both parvo- and magno-cellular areas throughout the LRN (Hrycyshyn et al., 1981), although Eisenman (1982) finds a projection from the ventral areas only, and it appears that there is no connection from the NRTP (Azizi et al., 1981). Lobules VI-VIII also receive fibres from the PRN (Faull, 1977). Lobule IX does not receive fibres from either the LRN (Hrycyshyn et al., 1982) or the NRTP (Azizi et al., 1981). Although the studies of Faull (1977) are not sufficiently detailed to state that there is a projection from the PRN to lobule IX (he injects HRP into lobules III-IX), it is quite possible that such a connection does exist since it

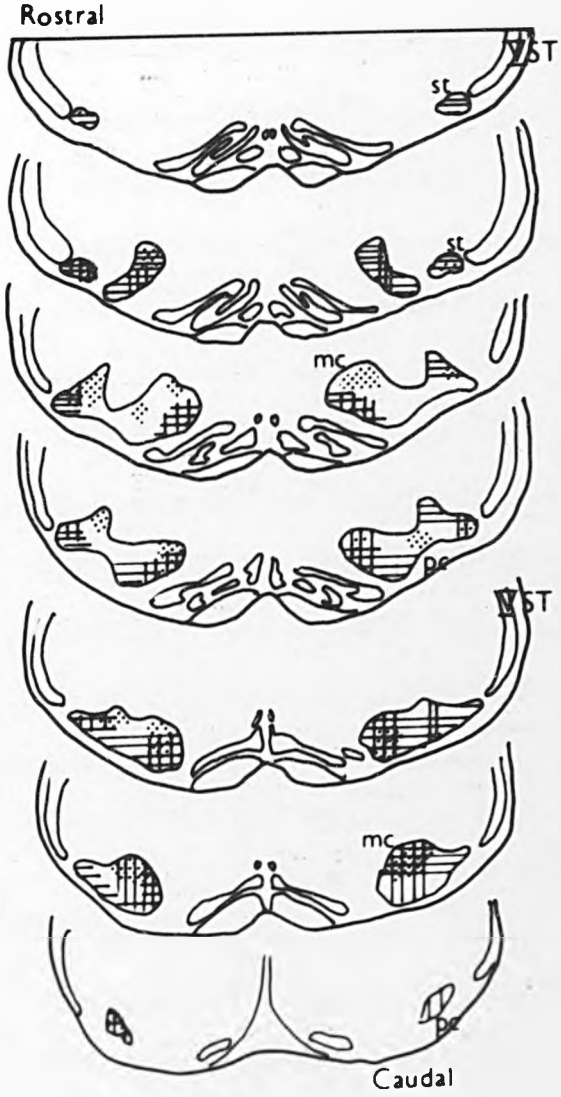
Figure 1.5

The Cerebellar Projection from the
Lateral Reticular Nucleus
(from Hrycyshyn et al., 1982)

This diagram illustrates both the bilaterality and broad poorly defined topography of the cerebellar projection from the lateral reticular nucleus.

V ST = spinal nucleus of the trigeminal nerve
mc = magnocellular part of the nucleus
pc = parvicellular region of the nucleus
st = subtrigeminal division of the nucleus


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


Rostral
VST
sc
sc
mc
pc
VST
mc
pc
Caudal

has been found in both the cat and the monkey (Somana and Walberg, 1978).

There is a small projection from the caudal two-thirds of the dorsal magnocellular region and the subtrigeminal division of the LRN to the lobulus simplex (Hrycyshyn et al., 1982), also afferents arising in the caudal half of the central NRTP (Mihailoff et al., 1981). However, lobulus simplex and crus I receive fibres originating throughout the LRN and NRTP and a few afferents from the PRN (Payne, 1983b). The detail of the lateral reticular projection to the ansoparamedian lobules has not been established, but these afferents arise in the rostral two-thirds of the dorsal magnocellular region (Hrycyshyn et al., 1982). In addition crus I possesses an input from throughout the NRTP including its dorsomedial parvicellular area, but there is only a small projection from the caudal half of the central region to crus II (Mihailoff et al., 1981). The input to the paramedian lobule from the NRTP takes origin from neurons lying in the central area throughout its rostrocaudal extent (Mihailoff et al., 1981). The copula pyramidis is projected upon by cells in the ventral parvo-, magnocellular and subtrigeminal areas of the LRN (Eisenman, 1982).

Other Precerebellar Nuclei

Several other nuclei send fibres to the cerebellum but the details of their projections have not been closely

investigated in the rat. The superior and medial vestibular nuclei project to lobules II-III and VIII-IX (Mehler, 1977). The cochlear nuclei project bilaterally to lobules VI-VIIIa and send fewer fibres to the crura and paramedian lobule (Huang et al., 1982). Spinal cord afferents project to the vermis and paravermis from the ipsilateral Clarke's nucleus, the contralateral border cells of the spinal grey matter and the bilateral central cervical nuclei (Snyder et al., 1978). Similarly the external cuneate nucleus projects to the vermis (lobules III-IX) (Faull, 1977), however a small projection has also been found to the ipsilateral lobulus simplex and crus I (Payne, 1983b). Afferents from the perihypoglossal nuclei terminate bilaterally in the vermis (Faull, 1977) and the lobulus simplex and crus I (Payne, 1983b). This projection differs from that in the cat in which there is no projection to the cerebellar hemispheres (Kotchabhakdi et al., 1978a). There is some disparity over the origin of cerebellar mossy fibres from the trigeminal nerve nuclei. The mesencephalic nucleus is found to project to lobule X (Cupedo, 1965) and the ventral vermis and hemisphere (Pearson, 1949). Watson and Switzer (1978) have shown a projection from the interpolaris and principal nuclei to both the vermis and the hemisphere, while Payne (1983b) finds a projection to the lobulus simplex and crus I only from the spinal nucleus. However, Faull (1977) has found a projection to the vermis from the

mesencephalic, principal and spinal trigeminal nuclei. Of the other cranial nerve nuclei, connections to the cerebellum have been found from the abducens nucleus to lobulus simplex and crus I (Payne, 1983b), the facial nerve nucleus and nucleus ambiguus to unspecified areas of the hemisphere (Kimoto et al., 1978) and the hypoglossal nucleus to the vermis (Faull, 1977) but not to the lobulus simplex and crus I (Payne, 1983b).

Correlations Between Mossy Fibre Origin and Morphology

Since the mossy fibres take origin from a large number of nuclei and there is more than one type of mossy fibre terminal the question has been raised whether rosette morphology reflects the mossy fibre's origin. Brodal and Drabløs (1963) described two types of mossy fibre rosettes, of which one was distributed almost exclusively in the 'vestibulocerebellum' and which they deduced was the terminal of primary vestibular afferents. Also Hamori et al. (1981) found two types of rosettes each with a different size distribution of synaptic vesicles. Studies of terminal degeneration following mossy fibre lesion reveal that after cervical cord hemisection predominantly simple type rosettes degenerate (Chan-Palay, 1973) and that in the cat mossy fibre rosettes from the pontine nucleus degenerate quicker than those from the spinal cord (Angaut and Sotelo, 1975). Whether these results indicate that the mossy fibre's origin is reflected in its terminal structure has not been settled.

MONOAMINE FIBRES

The monoaminergic afferents to the cerebellar cortex were discovered by Fuxe (1965), who observed a plexus of fluorescent fibres in the granular layer after treating the tissue according to the Falck-Hillarp method for biogenic amines.

In the central white matter the fibres have a typical thin varicosed appearance of preterminal monoaminergic fibres found elsewhere in the central nervous system. From here the fibres enter the granular layer where they form an intricate plexus (Hökfelt and Fuxe, 1969; Chan-Palay, 1977; Yamamoto et al., 1977; Moore and Bloom, 1979). The fibres are fine with intermittent varicosities of 0.4-2 μm diameter (Hökfelt and Fuxe, 1969; Schulman, 1983), these are usually round but a smooth elongated type of varicosity has been observed in the upper granular layer and in the fibres just underlying the pia mater (Yamamoto et al., 1977). Branches from the plexus within the granular layer ascend to the Purkinje cell layer, along which some collaterals run, and pass into the molecular layer. In the molecular layer the fibres give off further collaterals, which are not orientated in any particular plane (Hökfelt and Fuxe, 1969; Chan-Palay, 1977). The majority of these collaterals are found in the lower part of the layer but some ascend vertically towards the surface where they bifurcate and run just below and parallel to the pia

mater (Bloom et al., 1971; Chan-Palay, 1977; Yamamoto et al., 1977; Moore and Bloom,1979).

The monoaminergic afferents synapse on the primary dendrites, and possibly the somata, of Purkinje cells, (Bloom et al., 1971; Segal et al., 1973; Pickel et al., 1974b) and have an inhibitory action (Hoffer et al., 1971 & 1973). It has also been proposed that these fibres do not all terminate in conventional synapses and may have a modulatory function (Schulman, 1983).

The majority of monoaminergic afferents are noradrenergic although Hökfelt and Fuxe (1969) did observe some serotoninergic fibres. With fluorescence techniques the serotoninergic fibres appear very similar to those containing noradrenaline although a large proportion of them terminate as parallel fibres in the molecular layer. The large "mossy" like terminals found in the granular layer (Chan-Palay, 1977) are only observed after intraventricular infusion of tritiated-serotonin and are probably not serotoninergic terminals (Schulman, 1983). It has also been postulated that very few or none of these fibres terminate in conventional synapses. A third type of monoaminergic fibre containing dopamine has also been proposed as an afferent system to the cerebellum, but there does not appear to be any direct evidence of these fibres (Schulman, 1983).

Noradrenergic afferent fibres have been demonstrated in all regions of the cerebellar cortex although in fewer

numbers in the flocculonodular lobe (Hökfelt and Fuxe, 1969). The major source of these fibres is the locus coeruleus and it was first thought that the fibres arose in the dorsolateral region of the nucleus (Olson and Fuxe, 1971) and entered the cerebellum through the inferior peduncle (Hökfelt and Fuxe, 1969). More recent studies have revealed that these afferents arise in all regions of the locus coeruleus, pass through the superior peduncle (Segal et al., 1973; Pickel et al., 1974b) and terminate bilaterally with a slight (approximately 25%) ipsilateral prodominance (Chan-Palay, 1977; Kimoto et al., 1978; Mason and Fibiger, 1979; Pasquier et al., 1980; Nagai et al., 1981; Room et al., 1981; Lindvall and Bjorklund, 1983). However, Kobayashi et al. (1974) suggest that the projection to the anterior lobe is only ipsilateral. In addition, smaller bilateral projections to the cerebellum have also been found arising in the subcoeruleus area (Chan-Palay, 1977; Kimoto et al., 1978; Pasquier et al., 1980) nuclei A5 and A7 (Pasquier et al., 1980) and possibly A4 (Nagai et al., 1981).

Serotoninergic afferents have only been found in the anterior lobe (Hökfelt and Fuxe, 1969) and the posterior vermis (Shinnar et al., 1973). These fibres arise in the raphe nuclei dorsalis, superior centralis, magnus, pontis, obscurus and pallidus (Shinnar et al., 1973).

NUCLEOCORTICAL FIBRES

There has been much controversy whether or not there is a projection from the deep nuclei to the cerebellar cortex because anterograde degeneration techniques inevitably involve fibres of passage (Carrea *et al.*, 1947). More recently, using axonal transport techniques, these fibres have been found in the cat (Gould and Graybiel, 1976; Tolbert *et al.*, 1976; Dietrichs and Walberg, 1979) and the monkey (Chan-Palay, 1977; Tolbert *et al.*, 1977) and the projection is topographically organised (Tolbert *et al.*, 1978). These fibres are also present in the rat (Hess, 1982a) and are topographically organised (Payne, 1983a). The vermis receives fibres from the fastigial nucleus, the lobulus simplex from the medial nucleus interpositus and the dorsolateral protuberance of the fastigial nucleus. Fibres to crus I arise in the lateral nucleus interpositus and the medial lateral nucleus (Payne, 1983a). These fibres terminate as mossy fibres (Hamori *et al.*, 1981) and are collaterals of the deep nuclear cerebellofugal fibres (Tolbert, 1982; Payne, 1983a).

CEREBELLAR EFFERENTS

The Purkinje cells provide the sole outflow from the cerebellar cortex (Palay and Chan-Palay, 1974) which terminates mainly in the deep nuclei, however some fibres continue to the vestibular and some other brainstem nuclei

(Palay and Chan-Palay, 1974; Haines et al., 1982; De Camilli et al., 1984). The path is ipsilateral (Armstrong and Schild, 1978 a & b) and the Purkinje cell axons arborise in the deep nuclei and have a fairly large terminal field (Chan-Palay, 1977; Palay and Chan-Palay, 1982; p.370) terminating on both dendrites and somata (Chan-Palay, 1977; De Camilli et al., 1984). The corticonuclear projection has a basic organisation of three longitudinal zones: the vermis projects to the fastigial nucleus, the intermediate cortex to the interpositus nucleus and the lateral cortex to the lateral nucleus (Goodman et al., 1963). However there is further topographic organisation within these zones. Within the vermal projection to the fastigial nucleus there is a rostrocaudal, dorsoventral and mediolateral specificity (Armstrong and Schild, 1978a; Haines et al., 1982). In the hemisphere Armstrong and Schild (1978b) found a general mediolateral specificity of this projection although Goodman et al. (1963) concluded that there were only intermediate and lateral zones projecting to the interpositus and lateral nuclei respectively. However the medial portion of the hemisphere projects extensively to the dorsolateral protuberance of the fastigial nucleus (Armstrong and Schild, 1978b). In addition, the corticonuclear projection also appears to have a functional localisation, since areas which receive a similar input i.e. the lateral anterior lobe, paramedian

lobule and copula pyramidis, all project to the same part of the deep nuclei, the medial anterior interpositus nucleus (Armstrong and Schild, 1978b).

With the exception of those Purkinje cells which project to the vestibular nuclei, the cerebellar efferents arise in the deep nuclei and travel through the superior and inferior peduncles. There is a major projection, particularly from the lateral nucleus, to the contralateral red nucleus and ventral thalamus. Also cerebellar efferents terminate throughout the brainstem and provide feedback loops to the afferent precerebellar nuclei (Chan-Palay, 1977).

CEREBELLAR DEVELOPMENT

The cerebellum develops from the cerebellar plate, a bilateral eminence on the dorsum of the mesencephalon. It is first seen laterally on the thirteenth day of gestation (E13) and it grows medially to join the opposite side by E16 (Altman and Bayer, 1978a). The rostral part of the cerebellar plate does not differentiate into cerebellum and within it can be seen medially developing locus coeruleus neurons and laterally the presumptive vestibular neurons (Altman, 1982). On day E14 the dorsocaudal region starts to differentiate to produce neurons of the deep cerebellar nuclei (Altman and Bayer, 1978a) which are followed on E15 by a second zone, of Purkinje cells, that lie between the deep nuclear cells and the

neuroepithelium (Das and Nornes, 1972; Altman and Bayer, 1978a). Where the caudoventral edge of the cerebellar plate joins the developing choroid plexus, the germinal trigone develops on E17. Its superior prong spreads rostrally over the deep nuclear zone and reaches the rostral pole by E22 (Addison, 1911; Altman, 1982) and with it develops a cleft which separates the dorsal surface of the cerebellar primordium from the overlying mesencephalon (Altman and Bayer, 1978a). Simultaneous with the start of the external granular layer development from the germinal trigone, the Purkinje cells migrate through the zone of deep nuclear cells to a thin band of fibres just below the external granular layer (Altman and Bayer, 1978a). Altman (1982) proposes that the Purkinje cell migration and the thin fibrous layer are important for the midline junction of the two cerebellar halves. By E19 the external granular layer is 2-5 cells thick (Addison, 1911) and the third cerebellar macroneuron, the Golgi cell, has begun to differentiate from the horizontal prong of the germinal trigone (Das and Nornes, 1972; Altman and Bayer, 1978a).

After birth the cerebellum grows rapidly (Altman, 1969; Heinsen, 1977; Papadopoulos et al., 1981) which produces folding of the cortex. The primary and secondary fissures are produced early by massive local proliferation (Larsell, 1952). Fissures first develop medially and grow laterally but those fissures of the

posterior lobe hemisphere which do not reach the vermis develop *in situ*. The posterolateral fissure which separates the flocculonodular lobe develops relatively late (Korneliussen, 1968 a & c). Within the cerebellum there is a rostroventral to dorsocaudal gradient of development with the rostral areas being more advanced (Larsell, 1952; Altman, 1969; Korneliussen, 1969). Similarly there is a mediolateral gradient with the hemispheres lagging behind the vermis and the paraflocculus developing last (Addison, 1911; Altman, 1969). Also the developing cortex appears to be divided into four longitudinal zones, which are related to the deep nuclei and are separated by raphes in the white matter and presumably are the first signs of the sagittal banding found in adults (Korneliussen, 1968 a & c, 1969). This very rapid growth continues for 20 days then slows to 35 days after which there is a small amount of white matter growth especially at puberty (Altman, 1969; Heinsen, 1977; Papadopoulos *et al.*, 1981).

At birth the external granular layer has 6-8 rows of cells, 5 x 7.5 μm (Addison, 1911). These cells proliferate very rapidly because, despite the increase in cortical area, the external granular layer deepens to 8-12 rows by 5 days post partum (Altman, 1969; Haas and Werner, 1973). At this age it can be divided into two regions, the outer proliferative layer, 4-5 cells deep, and an inner premigratory zone of bipolar cells (Addison,

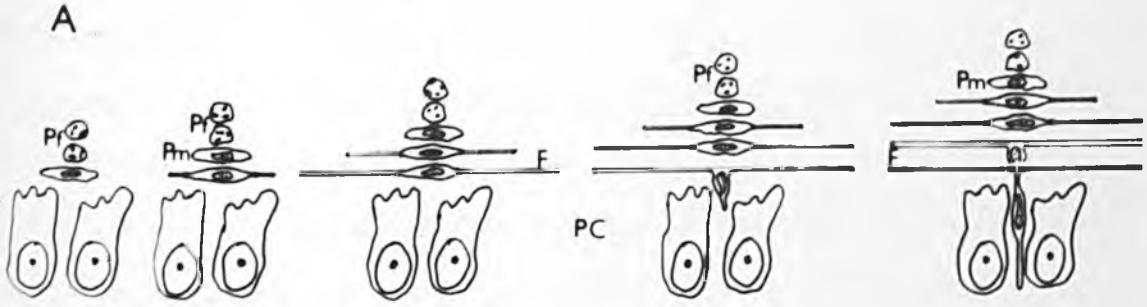
Figure 1.6

External Granular and Molecular Layer
Development from Altman 1972a

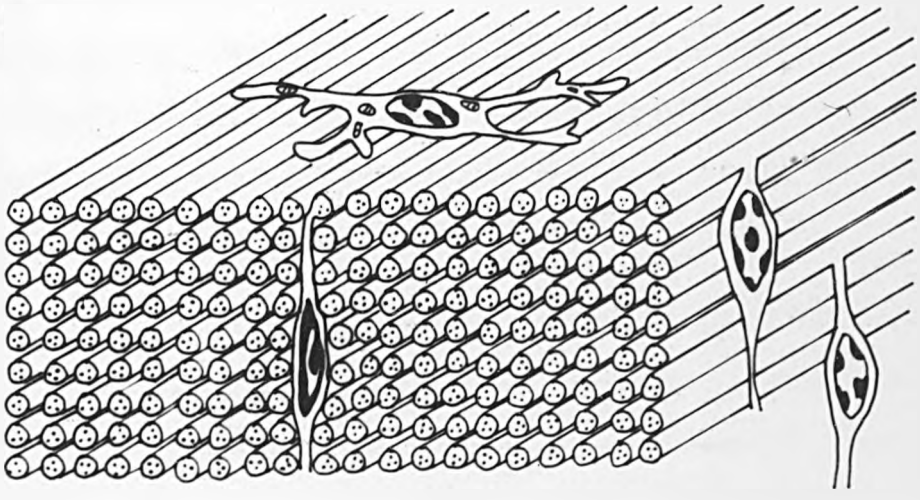
Figure 1.6A. The cells of the proliferative zone (Pf) in the external granular layer divide and increase the depth of the layer. The deeper lying cells become the bipolar cells of the premigratory zone (Pm) and produce parallel fibres (F) prior to descending through the Purkinje cell layer (PC) to the internal granular layer. The more latterly developed parallel fibres are progressively stacked upon those which are produced earlier.

Figure 1.6B. When basket and stellate cells differentiate the bipolar cells develop processes perpendicular to the direction of the parallel fibres and therefore become trapped in the developing molecular layer.

A



B



1911; Altman, 1972a). This remains constant until the 9th day after which the external granular layer decreases, the proliferative zone disappearing first, until day 13 when the premigratory zone is only 1-2 cells deep (Altman, 1969). The external granular layer completely disappears by 20 days post partum (Addison, 1911). The bipolar cells of the premigratory zone are 4-5 µm by 9-10 µm and grow processes from each end which run parallel to the developing fissures (Addison, 1911). When the 'parallel fibres' have grown the cell descends vertically along Bergmann glial cell processes (Rakic, 1971; Altman, 1975) through the developing molecular and Purkinje cell layers to the inner granular layer and as more parallel fibres are laid down the molecular layer deepens (Altman, 1972a). Some bipolar cells develop perpendicular to the direction of the parallel fibres and these cells develop into basket and stellate cells. The basket cells differentiate at 6-7 days and the stellate cells on days 9-10 post partum (Altman, 1972a).

The granule cells are produced continually from days 7 to 15 post partum, with the peak production occurring on days 10 and 11 (Addison, 1911; Altman, 1972c). Granule cell development also has a ventrorostral-dorsocaudal gradient within which the cells in the sulci are more advanced than those on the folial apices (Altman, 1969). The time of maximum granule cell migration to the inner granular layer occurs between days 9 and 17 (Addison, 1911)

and the density of cells within this layer rapidly increases (Heinsen, 1978). In the granular layer the granule cells do not further differentiate until the 15th day post partum when the dendrites develop and form claws around the immature mossy fibre rosettes (Altman and Das, 1970; Altman, 1972c). This development of the neuropil inevitably reduces the granule cell density, as does the reduction in the size of the somata which occurs at this time (Heinsen, 1978).

Also developing within the granular layer are the Golgi and Lugaro cells. The Golgi cell develops prenatally from the germinal trigone (Altman, 1972c; Haas and Werner, 1973; Altman and Bayer, 1978a) and by days 5-7 its dendrites have grown into the molecular layer and the axon within the granular layer where it first contacts granule cells on day 12. It has the adult appearance by day 15 although its reaction for acetylcholinesterase continues to increase until day 30 (Altman and Das, 1970; Altman, 1972c). Lugaro cells which are formed on day E21 (Das and Nornes, 1972) develop in the granular layer between days 8 and 13, although they are difficult to distinguish before day 15 (Addison, 1911; Altman, 1972c). Differentiating glial cells are also found in the granular layer (Altman, 1969; Basco *et al.*, 1977).

At birth the Purkinje cells lie in a band 6-12 cells deep below the external granular layer. They are 7 x 12 µm in size and have little cytoplasm around a polymorphous

Figure 1.7

Purkinje Cell Development from
Altman 1972b

Purkinje cell development illustrating the growth of its dendrites into the molecular layer as more parallel fibres are layed down with increasing age (days 3-21 are shown). Up to day 7 the Purkinje cell is contacted by climbing fibres on perisomatic processes. The early developed parallel fibres form synapses first with basket cells then the Purkinje cell dendritic spines laying low in the molecular layer and the basket cells develop axo-somatic synapses displacing the climbing fibres to the dendrites. As more parallel fibres are layed down and the molecular layer deepens, synaptogenesis moves up through the molecular layer towards the external granular layer. This is followed by the development of the glial sheath which covers the Purkinje cell and its dendrites and terminates synaptogenesis. This development is complete by 30 days post partum.

 = cells of the proliferative zone

 = bipolar cells of the premigratory zone

 = climbing fibre and synapse

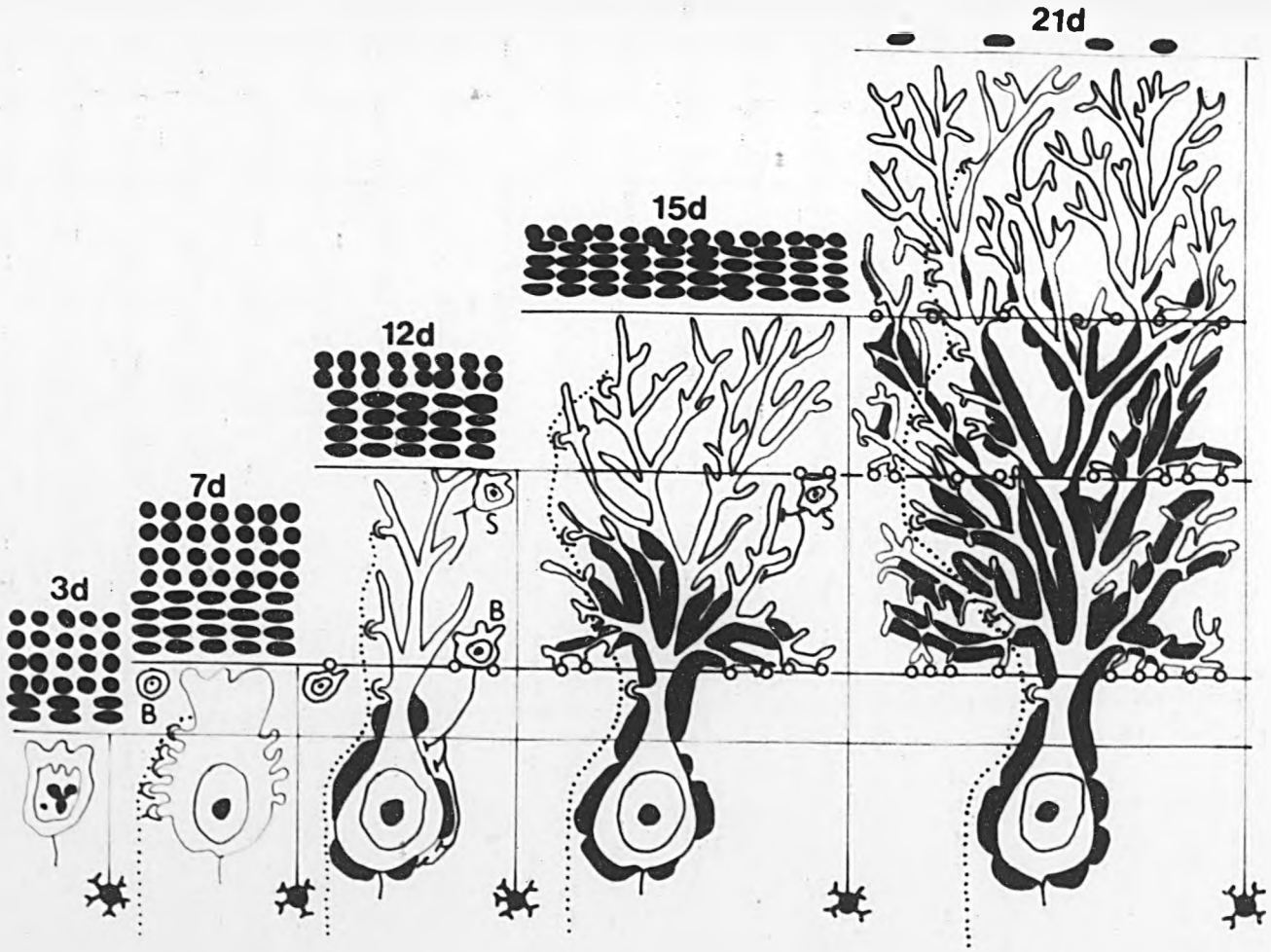
 = parallel fibre and synapse

B = basket cell

S = stellate cell

 = glial sheath

 = granule cell


21d
15d
12d
7d
3d
S
B
B

nucleus which often contains more than one nucleolus (Addison, 1911; Altman, 1972b). The cells lying superficially are more advanced than the deeper ones (Altman, 1972b) and are already electrically active (Woodward et al., 1969). The cells develop more cytoplasm and by 3-4 days are 8 x 14 µm and are almost in a monolayer (Addison, 1911; Altman, 1972b). Although the cells do not possess synaptic contacts (Altman, 1972b) they do respond to future transmitters e.g. glutamate and noradrenaline (Woodward et al., 1971) indicating a presynaptic specific sensitivity. By the 5th day the apical cytoplasm has considerably increased and perisomatic processes have developed and are in contact with presumed climbing fibres (Addison, 1911; Altman, 1972b; Berry and Bradley, 1976a) which induce electrical activity typical of the climbing fibre stimulation by day 7 (Woodward et al., 1969). Between days 5 and 10 post partum the perisomatic processes are also briefly contacted by mossy fibres (Altman and Das, 1970) and the apical cytoplasm continues to grow. By days 11-12 the Purkinje cells have grown to 12 x 18 µm and the cytoplasm contains Nissl substance (Addison, 1911). Most cells have developed a primary dendrite and their perisomatic processes are being replaced by basket cell axon contacts (Altman, 1972b). Also the Purkinje cell axon has grown down into the white matter (Addison, 1911). By 15 days post partum the dendrites have branched with great

complexity (Addison, 1911) and have formed the full width of the dendritic tree, although the height of the tree is still small (Berry and Bradley, 1976a). Also the lower branches receive parallel fibre contacts and the climbing fibres which have ascended from the perisomatic processes (Altman, 1972b). The Purkinje cell dendritic tree continues to grow in height up to day 30 (Berry and Bradley, 1976a) when its synaptogenesis and glial sheath are complete, despite a mature appearance by day 21 (Altman, 1972b).

CEREBELLAR AFFERENT DEVELOPMENT

MONOAMINE FIBRES

Locus coeruleus neurons differentiate at days E10-13 and the raphe nuclei on days E11-15 (Olson and Seiger, 1972; Lauder and Bloom, 1974) and they have started producing monoamines by days E12-14 (Seiger and Olson, 1973; Lauder and Bloom, 1975). Noradrenergic fibres reach the cerebellum prenatally and although early studies did not find a high concentration of noradrenaline (Loizou, 1969; Loizou and Salt, 1970) more recent techniques reveal that at birth the terminals are in the same concentration as there is in the adult (Yamamoto et al., 1977; Waddington and Banks, 1981) and they are already functioning despite being morphologically immature (Loizou, 1972). The concentration of terminals increases around the Purkinje cell layer (Yamamoto et al., 1977) to a maximum at 7 days

and then decreases again to reach the adult level by day 15 (Waddington and Banks, 1981). Noradrenergic receptors have been found biochemically at 4 days post partum and their number increases with Purkinje cell development to 15 days.

CLIMBING FIBRES

The cells of the inferior olive develop from the rhombic lip on days E13-14 (Ellenberger et al., 1969; Altman and Bayer, 1978b) and migrate along the course of the future inferior cerebellar peduncle to the ventral brainstem. The DAO, dorsal MAO and dPO are formed first, then the vPO and neurons for the ventral MAO last (Ellenberger et al., 1969; Altman and Bayer, 1978b; Marchand and Poirier, 1982). By day 2 post partum the climbing fibres have reached the cerebellar white matter lying in conventional sagittal bands (Sotelo et al., 1984) and although Purkinje cells will respond to climbing fibres at day 3 (Crepel, 1971; Mariani and Changeux, 1981) it seems unlikely this is via a conventional synapse. By the 5th day post partum the climbing fibres form nests at the infraganglionic plexus and the Purkinje cell layer (Ramon y Cajal, 1960; O'Leary et al., 1971; Altman, 1972b; Dupont et al., 1981; Sotelo et al., 1984). Each nest receives axons from several climbing fibres (Ramon y Cajal, 1960) and each Purkinje cell is innervated by more than one climbing fibre (Crepel, 1971;

Crepel et al., 1976; Mariani and Changeux, 1981; Crepel, 1982). Between days 7 and 12 the climbing fibres develop granular layer collaterals and ascend towards the Purkinje cell primary dendrites, so dispersing the infraganglionic plexus nests (O'Leary et al., 1971). Also during this development the multiple innervation of the Purkinje cells is changed to the adult pattern of only one climbing fibre innervating a Purkinje cell (Crepel et al., 1976; Mariani and Changeux, 1981). It would appear that this regression of climbing fibre contacts only slightly refines the longitudinal zonal topography of the adult olivocerebellar projection (Dupont et al., 1981; Sotelo et al., 1984).

MOSSY FIBRES

The brainstem nuclei sending mossy fibres to the cerebellum differentiate over several days, E13-19 (Altman and Bayer, 1978b), depending on the nuclear group. The fibres grow into the white matter by postnatal day 3 (Hamori and Somogyi, 1983b) and progress into the granular layer by day 7 (Altman, 1972c). The fibres ramify quite quickly (Ramon y Cajal, 1960) and form rosettes up to day 15 (Hamori and Somogyi, 1983b) although they do not mature synaptically until the fourth postnatal week (Altman and Das, 1970; Altman, 1972c; Hamori and Somogyi, 1983b).

EXPERIMENTS ON CEREBELLAR DEVELOPMENT

The organogenesis of the cerebellum involves the simultaneous growth of different cell and fibre systems which presumably interact. Great efforts have been made to try and establish the relative importance of genetic and epigenetic factors during its development and since the Purkinje cell is the total cortical outflow, its development in an abnormal environment has been studied. The majority of experiments alter the parallel fibre input to the Purkinje cells by reducing the number of granule cells through various techniques; genetic mutation, X-irradiation, drugs and malnutrition. Also the extra-cerebellar afferents can be removed either individually or all together.

GRANULOPRIVAL CEREBELLUM

Experiments producing granuloprival cerebella can be divided into those causing total degranulation and those which just reduce the granule cell population. Within these two groups the results are little affected by the method used.

Agranular cerebella are found in weaver and staggerer mutant mice (Sotelo, 1981), and neonates treated with panleukopenia virus (Herndon *et al.*, 1971 a & b) or X-irradiated from birth to 13 days post partum (Altman and Anderson, 1972). In all cases the cerebella are much reduced in size and the Purkinje cells are randomly scattered throughout the cortex (Herndon *et al.*, 1971 a & b;

Altman and Anderson, 1972; Bradley and Berry, 1978a; Sotelo, 1981). The Purkinje cells are often multipolar (Herndon et al., 1971 a & b; Altman and Anderson, 1972; Bradley and Berry, 1978a; Sotelo, 1981) and the dendritic tree is much reduced in size (Bradley and Berry, 1978a). Although small tertiary branchlets are rarely found (Bradley and Berry, 1978a) the primary and secondary dendrites are covered with spines which are often enveloped by glia (Herndon et al., 1971b; Altman and Anderson, 1972; Bradley and Berry, 1978a; Sotelo, 1981) and can be extremely large (Berry and Bradley, 1976b; Sotelo, 1982). Mossy fibres synapse onto the Purkinje cells (Altman and Anderson, 1972; Sotelo, 1981) and the neonatal multiple climbing fibre innervation is retained by 39-53% of Purkinje cells (Crepel and Mariani, 1976; Delhaye-Bouchaud et al., 1978; Crepel et al., 1980; Crepel et al., 1981; Sotelo, 1981)

Partial degranulation can be induced by malnutrition (Dobbing et al., 1971; Bedi et al., 1980; Chen and Hillman, 1980; Hillman and Chen, 1981a; Borges and Lewis, 1983; Dvergsten et al., 1983), chemical injury to the external granular layer with cyclophosphamide (Nathanson et al., 1969), cycasin (Hirano et al., 1972) or methyl-azoxymethanol (Woodward et al., 1975; Bradley and Berry, 1978b; Lovell and Jones, 1980b), or X-irradiation (Bradley and Berry, 1976a; Crepel et al., 1980; Altman, 1982). The effect of these different methods depends on the

severity of degranulation. This, in turn, depends on the time of injury to the external granular layer, which has a great capacity for regeneration. Extensive degranulation produces results very similar to those found in the agranular cerebellum (Woodward et al., 1975; Lovell and Jones, 1980b). In the less damaged cortex the Purkinje cells lie in a monolayer and some are multipolar (Woodward et al., 1975; Bradley and Berry, 1976a; Crepel et al., 1980). The dendritic tree is smaller than normal (Nathanson et al., 1969; Bradley and Berry, 1976a, 1978b; McConnell and Berry, 1978a; Chen and Hillman, 1980; Crepel et al., 1980) with few tertiary branchlets and is often "weeping-willow" shaped (Bradley and Berry, 1976a, 1978b; Crepel et al., 1980). The dendrites are covered with spines some of which only contact glia (Hirano et al., 1972; Chen and Hillman, 1980; Lovell and Jones, 1980b) and others which receive multiple parallel fibre synapses (Lovell and Jones, 1980b; Hillman and Chen, 1981b). The Purkinje cells also receive mossy fibre innervation (Hamori, 1969; Woodward et al., 1975; Yeh et al., 1982) and 12% of them retain multiple climbing fibre input (Hamori, 1969; Crepel et al., 1980). After the external granular layer regenerates the parallel fibres are laid down in the sagittal plane and the Purkinje cell dendrites are correspondingly reorientated (Herndon and Oster-Granite, 1975; Woodward et al., 1975; Altman, 1982). Also, ectopic clumps of granule cells are found in the

molecular layer and the depth to which they have migrated depends on how early regeneration of the external granular layer occurs (Hamori, 1969; Nathanson et al., 1969; Altman, 1982).

CLIMBING FIBRE ABLATION

The climbing fibre afferents to the cerebellum can be removing by lesion of the inferior olive (Kawaguchi et al., 1975; Bradley and Berry, 1976a) or the inferior peduncle (Sotelo and Arsenio-Nunes, 1976; Angaut et al., 1982; Alvarado-Mallart et al., 1983). The cerebellar histology is not grossly affected and the molecular layer is the normal depth (Sotelo and Arsenio-Nunes, 1976). The Purkinje cell dendritic tree is smaller than usual (Bradley and Berry, 1976a), although there is some disparity whether the spiny dendrites are relatively normal (Angaut et al., 1982) or ascend straight through the molecular layer with very little branching (Kawaguchi et al., 1975; Sotelo and Arsenio-Nunes, 1976). However, spines are consistently found on the primary dendritic trunks normally contacted by climbing fibres (Sotelo and Arsenio-Nunes, 1976; Angaut et al., 1982). There is also evidence that climbing fibres from the intact inferior olive can reinnervate Purkinje cells lying medially in the deafferented cortex (Angaut et al., 1982; Alvarado-Mallart et al., 1983).

MOSSY FIBRE REDUCTION

Because mossy fibres have such an extensive origin it is impossible to remove them totally. However pontine and

vestibular mossy fibres have been removed (Hamori, 1969; Fritzsch, 1981) which delays granule cell production resulting in fewer granule cells (Hamori, 1969) but does not appear to have any other effect.

NORADRENERGIC AFFERENTS

There have been several attempts to assess the role of noradrenaline in postnatal cerebellar development but the method used to damage noradrenergic fibres, the neurotoxin 6-hydroxydopamine (6OHDA), allows them to regrow and hyperinnervate the cerebellum (Bendeich et al., 1978; Schmidt and Bhatnagar, 1980; Schmidt et al., 1980; Sievers et al., 1980; Schmidt et al., 1981; Harston et al., 1982; Kostrzewa et al., 1982). This ability to regenerate fibres to the cerebellum is lost if the toxin is given after day 12 post partum (Schmidt et al., 1980, 1981). Administration of 6OHDA causes a reduction in cerebellar size, with fewer granule cells and smaller Purkinje cell dendritic trees due to the decreased number of parallel fibres (Sievers et al., 1981; Lovell, 1982). However, the same results are found even if the uptake of 6OHDA by the noradrenergic fibres, and resultant damage to the nerves, is prevented (Sievers et al., 1981). This has led Berry et al. (1980 a & b) to conclude that noradrenaline has no effect on the postnatal cerebellar development. However, any effect that alterations to the noradrenergic afferents may have on cerebellar development

could be disguised by the non-specific effects of 6OHDA on the pia mater, external granular layer and glia (Sievers et al., 1980). More recently Dr. Balazs (Palay and Chan-Palay, 1982, p. 49) has commented that this work needs repeating with the relevant blocking-agents to prevent the non-specific effects of 6OHDA.

CEREBELLAR DEAFFERENTATION

Cerebellar development with no external afferents has been studied using transplants of the cerebellar anlage onto adult tissue (Woodward et al., 1977; Alvarado-Mallart and Sotelo, 1982) or in vitro culture (Calvet et al., 1976; Privat and Drian, 1976; Bland and Seil, 1982). The three cortical layers develop relatively normally although the Purkinje cell dendritic tree is small with few branches, however these do develop normal spines (Calvet et al., 1976; Privat and Drian, 1976; Woodward et al., 1977; Blank and Seil, 1982; Alvarado-Mallart and Sotelo, 1982). If the transplant is treated with methylazoxymethanol, to destroy the granule cells, the cortex develops with the same disordered multipolar Purkinje cells as are found in the non-transplanted agranular cortex (Calvet et al., 1976; Privat and Drian, 1976).

OTHER MUTANT MICE

The 'reeler' mutant mouse provides a model for studying the Purkinje cell development in different

environments. The immature Purkinje cells fail to migrate prenatally and lie in the deep nuclei, white matter, granular layer or a few in the Purkinje cell layer. In the three former sites the Purkinje cells all develop dendrites with spines but they receive both mossy and climbing fibre input in the absence of parallel fibres (Sotelo, 1981).

Two other types of mutant mice reveal the dependence of granule cell development upon the Purkinje cells. In 'lurcher' mice the Purkinje cells die while the granule cells are differentiating and migrating. However the granule cells degenerate when the parallel fibres normally form synapses on the Purkinje cells because they have lost their major postsynaptic target (Wetts and Herrup, 1983). Manipulation of lurcher-wild type chimeras shows that the number of granule cells remaining is directly proportional to the number of surviving Purkinje cells (Wetts and Herrup, 1983). Also it appears that the size of the granule cell population is dependent on the length of time the Purkinje cells are present at a "critical stage" in granule cell development (Wetts and Herrup, 1983). This is endorsed in the 'nervous' mutant mouse in which the Purkinje cells die late in ontogeny after the cerebellar cortical circuitry has been established. Even 14 months after the Purkinje cell death approximately

50% of the parallel fibres and granule cells have survived (Sotelo, 1981).

The different types of experiments on cerebellar organogenesis have caused disagreement about the autonomy of Purkinje cell development; whether it requires interneurons to direct its polarity (Altman, 1982) or whether it will develop anyway towards maximum input (Woodward et al., 1977; Sotelo, 1982) and only requires the parallel fibres to orientate the dendritic tree (Herndon and Oster-Granite, 1975). But each technique has its advantages and disadvantages. In genetic mutations the locus of the mutation and its effect on phenotypic expression is unknown (Herndon and Oster-Granite, 1975; Stent, 1980). Irradiation has a delayed direct effect on Purkinje cells and most drugs have systemic actions which may influence cerebellar development (Herndon and Oster-Granite, 1975). Malnutrition, inevitably, affects the whole central nervous system (Dvergsten et al., 1983). With the exception of transplants, the removal of external afferents has only been selective, while moving immature tissue to a foreign environment may directly affect its subsequent development.

Surgical transection of all the neonatal cerebellar peduncles will provide an opportunity to try and assess the effect of an alteration in the extracerebellar

afferents upon postnatal cortical development, without some of the complications described above. The effect of total unilateral pedunclotomy, which removed most of the afferents to an hemicerebellum, on cerebellar growth was observed by the change in weight of the vermis and the deafferented and intact hemispheres. The number of Purkinje cells in the hemisphere ipsilateral to the pedunclotomy was counted in order to try and establish whether the final Purkinje cell number is dependent on the cortical volume or vice versa. In the hemisphere ipsilateral to the pedunclotomy the Purkinje cell diameters were measured to see whether Purkinje cell growth is reduced in the absence of extracerebellar afferents.

In order to try and interpret the effects of pedunclotomy on cerebellar development the afferents which pass directly to the Purkinje cells were also studied. The response of the noradrenergic fibres to neonatal injury was observed without the side-effects of 6OHDA. In addition, the developing olivocerebellar projection and its alteration after transection was investigated.

CHAPTER TWO

TECHNIQUE FOR CEREBELLAR PEDUNCLOTOMY

Animals Used

The animals used throughout these experiments were inbred Wistar rats of either sex which were housed in conditions of standardised heating and lighting. For neonatal age the day of birth was considered to be Day 0 and all neonates were weaned between 22 and 25 days of age. Litter size was standardised to ten pups at the time of operation unless it was unusually large, in which case the extra animals were removed earlier. Litters of less than eight pups were not used.

Neonatal pups aged 3 to 10 days also those aged 1, 15, 22 and 30 days underwent unilateral cerebellar pedunclotomy and were kept for 35 days postoperatively. When the weight data results from these groups indicated at which operative age the cerebellum was most vulnerable to deafferentation further operations were performed on pups aged 5, 7, 10 and 22 days to study the time-course of the cerebellar response to pedunclotomy. Survival times during these time-course experiments were 5, 10, 15, 20, 25 and 30 days.

Anaesthesia

Neonatal rats up to 15 days of age were anaesthetised in an ether jar for 1-2 minutes until the required depth

of anaesthesia was obtained - no hindlimb withdrawal when the foot was pinched. If necessary the neonate can easily be returned to the anaesthetic jar during the operation.

This is a good anaesthetic procedure because it is quick and despite temporary anoxia in the ether jar the young neonates recover rapidly which greatly reduces the time they are separated from their mothers. A minimum separation time is very important because the neonates suffer from lack of food and also become extremely cold. Another advantage of ether anaesthesia is that it has a long term analgesic effect which obviates the need for supplementary analgesics.

Rats aged 22 and 30 days did not react well to ether anaesthesia. Unlike the young neonates these animals do not tolerate high levels of anoxia and the division between the anaesthesia being too light, causing the animal to be distressed during the operation, and the animal dying was too fine for routine work. These animals were anaesthetised using ketamine hydrochloride containing 0·06mg/ml atropine (Ketalar, Parke, Davis & Co.) at 60 mg/kg administered intraperitoneally followed after 5 minutes with pentobarbitone sodium (Sagatal, May and Baker) at 20 mg/kg. This second anaesthetic was freshly diluted 1:3 with physiological saline to decrease the rate of uptake.

Figure 2.1

The position of the animal during the pedunclotomy, with its head perpendicular to its body.

(Photograph courtesy of Dr. Bower)

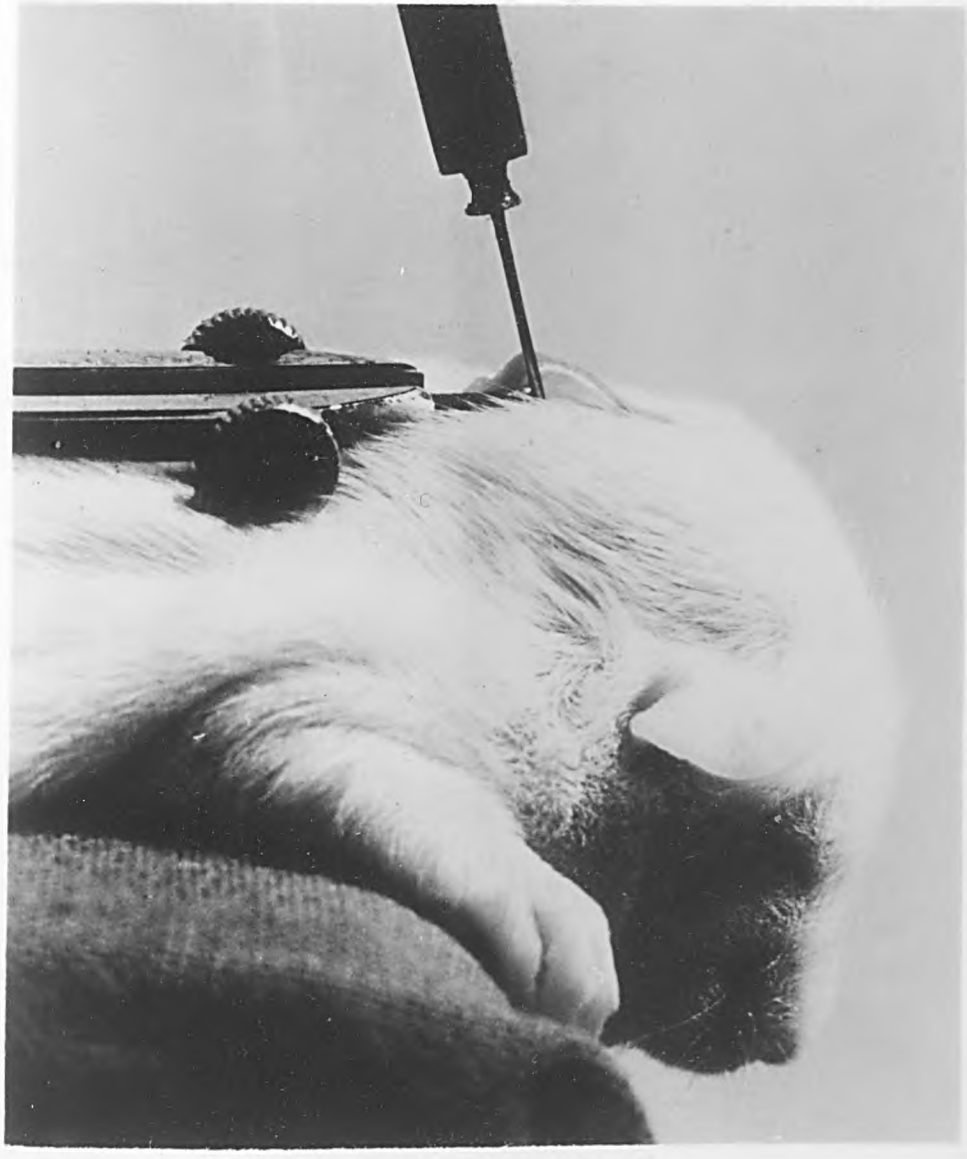
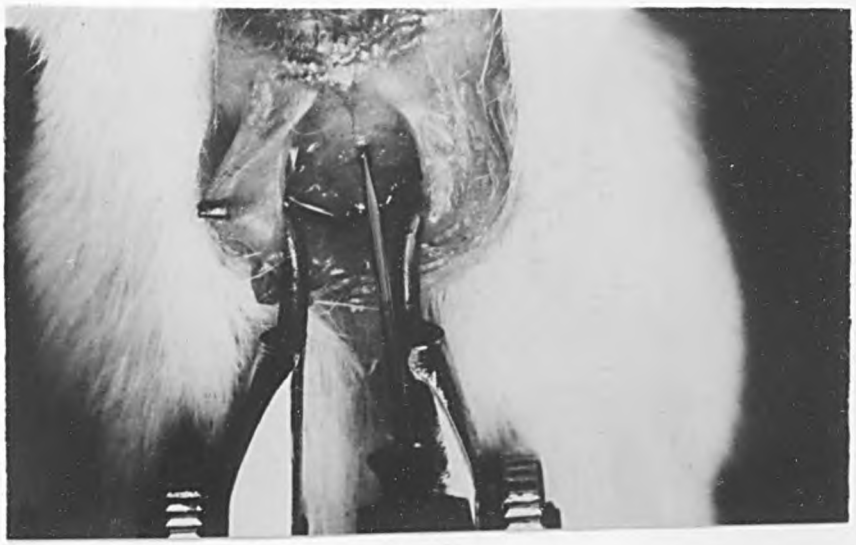
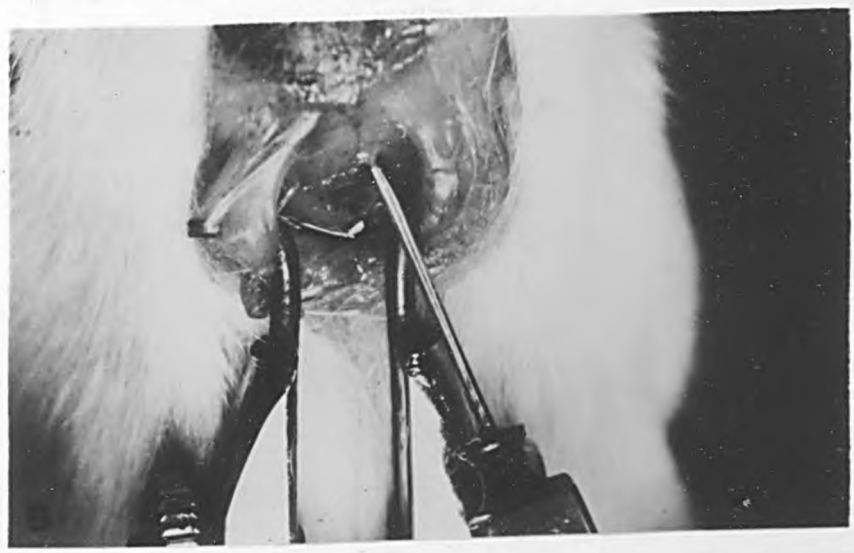


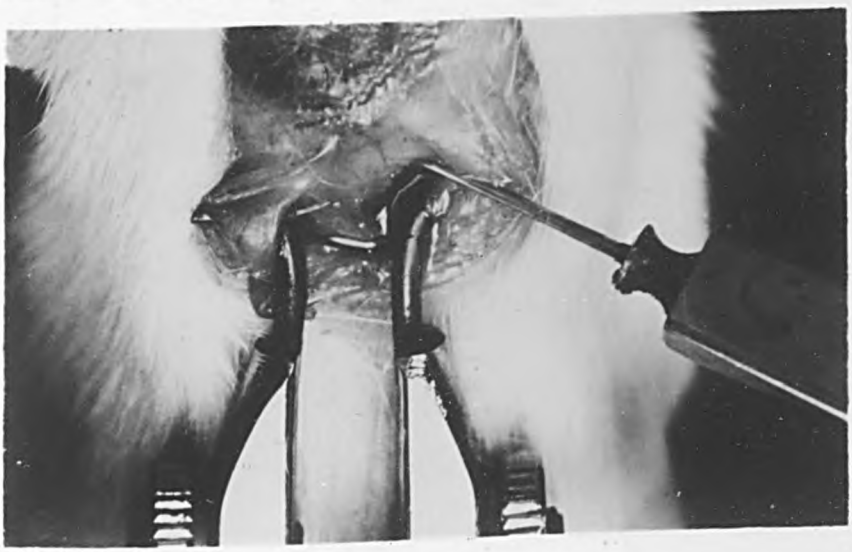
Figure 2.2

These photographs illustrate the rotation of the capsulotomy knife necessary to transect the left cerebellar peduncles.

(Photographs courtesy of Dr. Bower)







Operation

In aseptic conditions the anaesthetised animal is laid on a box so that its head can hang down at 90° to its body (Fig. 2.1). A 1cm midline incision is made running caudally from the occipital crest. The skin and longitudinal muscles of the neck are reflected laterally to expose the posterior atlanto-occipital membrane. A Williamson-Noble capsulotomy knife (John Weiss, London) is inserted through the membrane parallel to the brainstem and into the fourth ventricle. With the blade facing to the left the knife is rotated about an axis perpendicular to the brainstem until it touches the left side of the skull (Fig. 2.2). Taking care not to push hard against the bone, which damages important blood vessels to the cerebellum, the left peduncles are cut by sweeping the knife backwards in an arc towards the foramen magnum and withdrawn. The wound is sprayed with a broad spectrum antibiotic (Polybactrin, Calmic Medical Division, Wellcome Foundation), and the skin is sutured with silk and sealed with a plastic dressing (Nobecutane, Astra Pharmaceuticals Ltd., Watford, U.K.). Since the longitudinal muscles of the neck were only separated and not cut, it was found unnecessary to suture them.

This technique is a modification from that of Bower and Waddington (1981) to cut the peduncles unilaterally instead of bilaterally. Also Bower and Waddington's initial technique needed a separate procedure to cut the

superior cerebellar peduncle. A test series of animals showed that the present modification also cut the superior peduncle while decreasing the incidence of damage to the ventral surface of the cerebellum and the transverse sinus which lies just rostral to the peduncle.

Because some animal ages were the same in different groups (e.g. a 15 day pup will be in either the 5 or 10 day operative group surviving for 10 or 5 days respectively) in most litters some pups were kept as sham-operated littermate controls. These are referred to as controls throughout the project. The sham-operation involved inserting the capsulotomy knife into the fourth ventricle but not moving it laterally.

Since this operative technique is quite quick, about 5 minutes per animal the animals did not require any extra heating during the surgical procedure.

Postoperative Care

Both experimental and control pups were kept in a lined box warmed under a lamp until they had completely recovered from the anaesthetic. After at least 30 minutes the whole litter was returned to its mother, a procedure which minimized the incidence of maternal savaging. Those animals aged 22 and 30 days at the operation, which had been anaesthetised with ketamine hydrochloride and pentabarbitone sodium, took several hours to recover postoperatively. It was noticed that these animals had polyuria therefore when they were

sufficiently awake they had to be encouraged to drink water to prevent them dying from dehydration.

Discussion of the Technique

This operative technique has the major advantage of being relatively atraumatic to the neonates. Previous techniques used to cut the cerebellar peduncles in monkeys (Ferraro and Barrera, 1936; Walker and Botterell, 1937) and rats (Pickel et al., 1973) involve removing the suboccipital bone or thermocoagulation (Sotelo and Arsenio-Nunes, 1976), which is too traumatic for neonates and cannot be used. However the present technique has a great disadvantage in that being intracranial and therefore unseen the extent of any lesion cannot be assessed at the time of the operation. At post mortem careful examination of the brains was essential to ensure that only those correctly lesioned were retained. Animals with the following lesions were all discarded from the experiment:-

(i) Necrosis of the left cerebellar hemisphere, caused by severence of the vasculature, was usually complete. This occurred extremely quickly and was found even in short term survival groups (5 days).

(ii) Damage to the dorsum of the brainstem usually presented immediately post operation as signs of vestibular damage and these animals were destroyed. Those which did not so present usually had lesions

on the dorsal column nuclei.

(iii) Necrosis of the caudal occipital cortex and the midbrain tectum was caused by inserting the knife too deeply through the foramen magnum. This occurred mainly in the younger age groups where the whole brain is very small at the time of operation and the margin for operative error is minimal.

THE EFFECT OF PEDUNCLOTOMY

ON

CEREBELLAR DEVELOPMENT

CHAPTER THREE

COLLECTION OF WEIGHT DATA AND TISSUE PROCESSING

METHODS

KILLING AND HISTOLOGICAL FIXATION

After the required survival time the animals were killed with an overdose of anaesthetic ether and weighed. The brains were removed from the cranium and stored in 10% buffered formalin (Appendix I) at 4°C for two days after which the tissue was sufficiently fixed to allow handling and collection of weight data.

Neural tissue is usually fixed by intracardiac perfusion but the numbers of animals involved in this experiment (10 pups per litter) made it an impractical method. Since the pieces of tissue were small and the rate of infiltration is likely to be consistent for a given tissue type, fixation by infiltration was tried. Therefore an initial study was made to compare the quality of histological preservation by perfusion and infiltration fixation. While the former was better, the preservation by infiltration was perfectly adequate for the present study. Indeed neither the cerebellar weight nor the Purkinje cell diameters were different between perfused and infiltration-fixed brains, see Appendix V.

BRAIN DISSECTION FOR WEIGHT DATA

After an initial fixation for two days the brains were blotted dry and weighed. In order to standardise the

procedure as much as possible, the olfactory bulbs, if they had not been left within the cranium, were removed and the brainstem was cut at the obex. The cerebellum was removed, weighed and trisected by two parasagittal cuts along the superior cerebellar veins to remove the hemispheres from the vermis, and all these components were also weighed. The lateral edges of both hemispheres and the right cut surface of the vermis were labelled with drawing ink. This is important for correct orientation during blocking out. The cerebellar components and the brainstems were then further fixed in fresh 10% buffered formalin for at least one week.

STATISTICS

Since biological weight follows a normal distribution the comparison of the weight data from those groups surviving 35 days post operation could be analysed using a students t-test. Because the 'n' values in each group were small ($n \simeq 6$) and 5 components were weighed in each animal the confidence limits of $p \leq 0.01$ were chosen to minimise the risk of any differences between the two groups being due to chance. However in the time-course studies the growth curves from each operative age were compared with the control curves by a weighted t-test. In this method the mean value for each point on the experimental group curve is compared with its relevant control by a t-test but the confidence limits depend on the sum of all the observations for each point on both

the experimental and control curves. The confidence limit can be calculated from the formula $1-(0.95)^{1/n}$ where n is the total number of observations made on the experimental and control curves. If any point on the experimental curve is significantly different from control it indicates not only that the two curves are different but also where the difference occurs.

CRITERIA FOR INCLUDING MATERIAL IN THE EXPERIMENT

To try and ensure that only cerebella which had undergone a total unilateral pedunclotomy were studied several criteria had to be fulfilled before a brain was included in the data. Initially the only criterion was that the left hemisphere did not weigh less than 10% of the total cerebellar weight. Because a control hemisphere weighs approximately 30% of the cerebellum, it was considered that the few cerebella with left hemispheres smaller than 10% of the total weight must have undergone some vascular deprivation.

Inspection of some of these cerebella revealed evidence of incorrect lesions, either the peduncles were only partially cut or the ventral surface of the cerebellum removed by the knife. Therefore the cerebella were carefully examined on post mortem to check there was no necrotic tissue, that the peduncles had been completely removed and that there was a depression on the ventral surface of the cerebellum ipsilateral to the

lesion where, on the contralateral side, the peduncles enter the cerebellum.

With these new criteria some groups had to be repeated. The second results for animals 5, 6 and 7 days at operation were quite different from the first. Therefore a third set of operations was performed to ascertain the reproducibility of the pedunclotomy, hence the large n value in these groups.

A summary of the criteria used is:-

1) The left hemisphere must weigh more than 10% of the cerebellum.
2) There must be no obvious evidence of lesions to the dorsal brainstem, tectum or occipital cortex.
3) All three peduncles must be absent.
4) On histological examination the contralateral inferior olive and the ipsilateral lateral reticular nucleus must be degenerate. This criterion is also used by Angaut et al. (1982) for including material in their study. Also the contralateral basal pontine nucleus must be reduced in size.

TISSUE PROCESSING AND SECTIONING

The cerebellar hemispheres and vermis were dehydrated in alcohols and blocked out into JB4 (Polysciences Inc, USA), a plastic resin made from two stock solutions 'A' and 'B'. The procedure was as follows:-

a) Dehydration

The tissue can be dehydrated straight from the buffered formalin, it does not require washing. Dehydration proceeds into alcohols:-

70%	1 hr
80%	1 hr
90%	1 hr
absolute	1 hr
absolute	1 hr

b) Embedding

To make the embedding mixture 0.9g catalyst is added to 100 ml solution A with stirring until it has completely dissolved, about 15 minutes at room temperature.

The tissue is embedded in freshly made catalysed solution A overnight at room temperature.

c) Blocking Out

Tissue is blocked out in a 1:40 mixture of solution B and catalysed solution A. Each piece is carefully orientated with the ink label uppermost and the mixture is sealed with a metal chuck since air inhibits the polymerisation. The blocks are left to polymerise for 48 hours at 4°C.

This is a modification of the recommended procedure which involved embedding for 2 hours and blocking out in a 1:25 mixture of solutions B and A and polymerising for 75 minutes at room temperature. The modification

was necessary because with the recommended procedure the centre of each block was usually inadequately impregnated causing tissue destruction during sectioning. The most likely reason for this was that the mixture of solutions A and B started to polymerise before it had fully infiltrated the tissue, therefore an increased polymerisation time was preferable.

Parasagittal sections were cut on an LKB 'Historange' microtome with glass Ralph knives made with an LKB Bromma 2078 Histo-Knifemaker. Sections 2 µm thick were taken every 200 µm, expanded on distilled water and mounted on clean glass slides by heating to 80°C and then stained with 1% toluidine blue (Appendix II).

JB4 was used in preference to paraffin wax because it allows semithin sections to be cut. Also the embedding medium is not subsequently removed in strong organic solvents which can distort the sections. The advantage of JB4 over araldite is that it does not require heating for polymerisation.

One or more brainstems were picked at random from each experimental group and the histology of the basal pontine and lateral reticular nuclei and the inferior olives was analysed as an additional check that the cerebellar peduncles had been transected. The number of brainstems studied in each group can be found in Table 3.1. Since the brainstems were chosen randomly a few

TABLE 3.1

This table shows the number of brainstems randomly chosen in each experimental group for the examination of degenerative changes in the basal pontine, lateral reticular and inferior olivary nuclei.

Age at Operation (days)	Number of Brainstems Examined						
	Post Operative Survival Time (days)						
	5	10	15	20	25	30	35
3							2
4							2
5	3	2	2	2	4	3	2
6							2
7	2	2	2	3	2	4	2
8							2
9							2
10	2	2	4	3	2	2	2
15							1
22	1	1	3	2	3	2	2
30							2

Figure 3.0

A low power micrograph (x 20) of a coronal section through the cerebellum and the brainstem of an 11 day neonate (7-4) illustrating the total left pedunclotomy (the right of the field of view is the left side of the brain). This section is taken from an autoradiograph and the silver labelling in the left inferior cerebellar peduncle proximal to the lesion is clearly seen. Whereas the more lightly labelled right inferior peduncle lies more dorsally into the deep white medulla of the cerebellum.

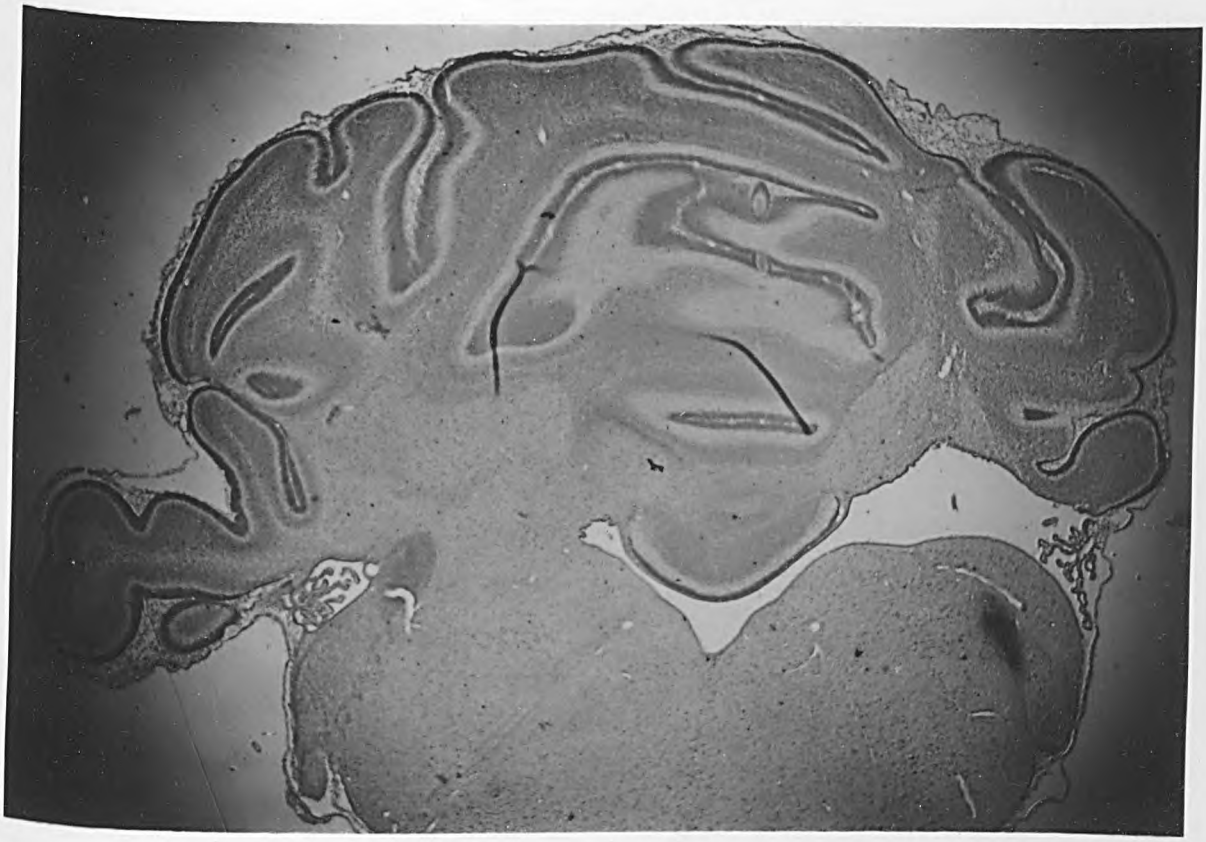


Figure 3.1

Micrographs A-D show the great reduction or abolition of the right basal pontine nucleus (BPB), depending on the rostrocaudal position of the section, following a left cerebellar pedunclotomy. Also, the reduced right nucleus reticularis tegmenti pontis (NRTP) can be seen in micrographs B and C.

The left side of the micrographs is the right side of the brainstem.

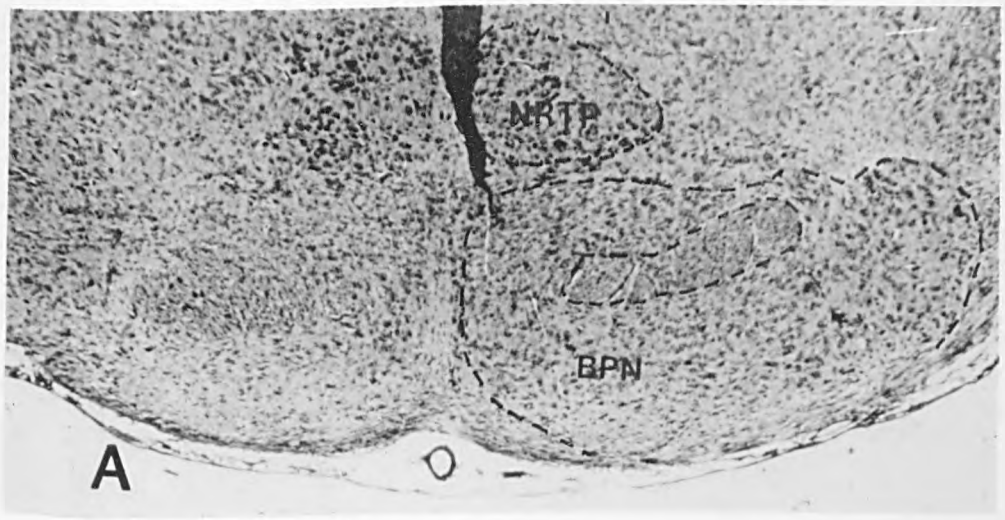
A = 10-5 (i.e. aged 10 days at operation and surviving 5 days)

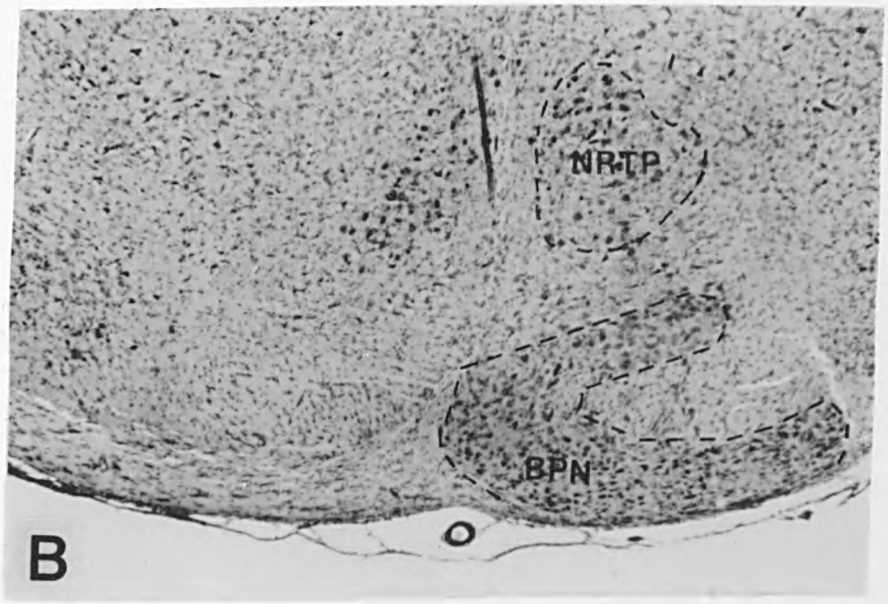
B = 7-20

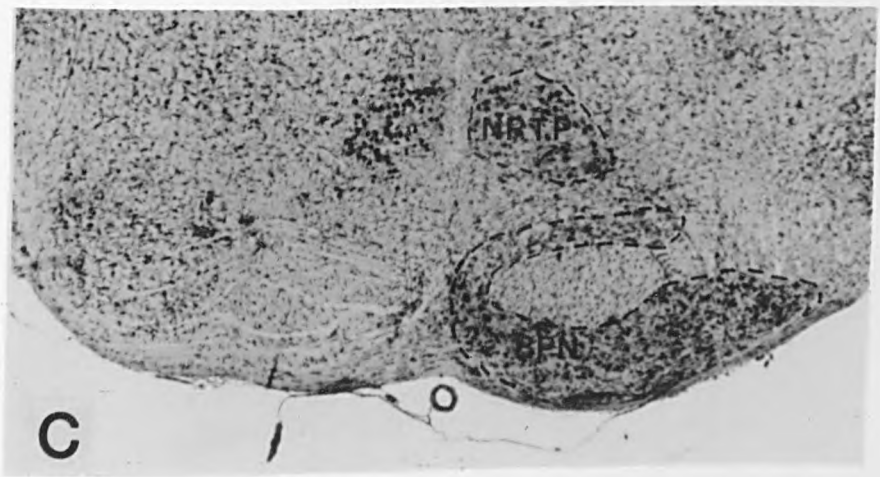
C = 3-35

D = 8-35

Magnification = x 36


NRTP
BPN
A


NRTP
BPN
B


NRTP
BPN
C

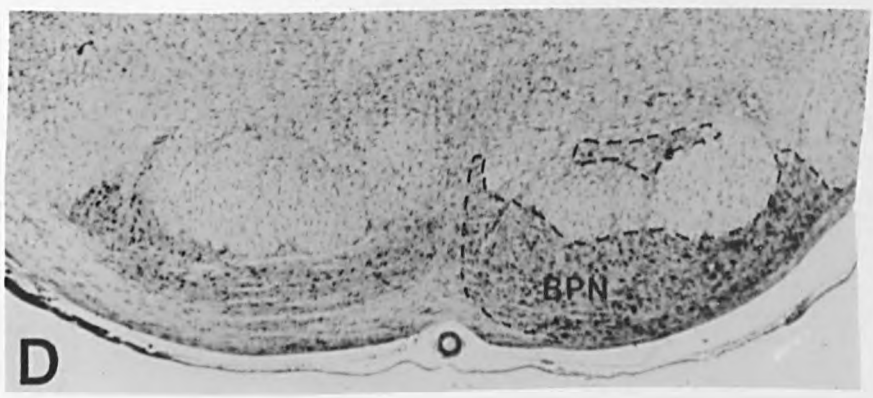

BPN
D

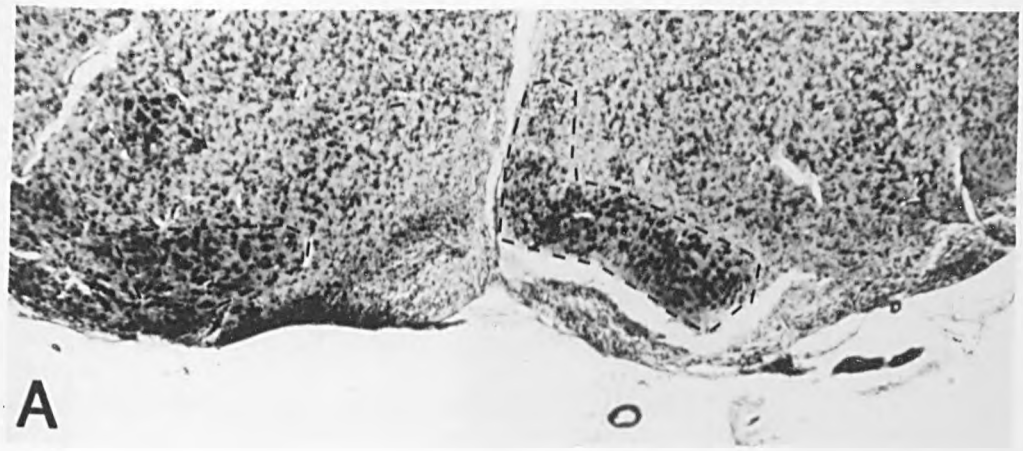
Figure 3.2

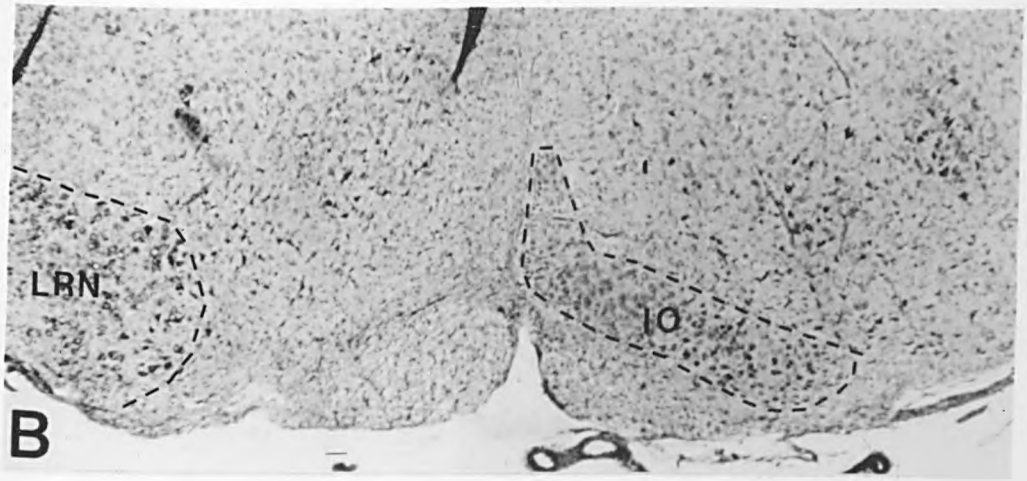
Figures A-D show the degeneration of the right inferior olive and the left lateral reticular nucleus following left cerebellar pedunclotomy. The site of the degenerated inferior olive is often marked by small darkly stained glial cells (A & B). The different size of the left inferior olivary nucleus is a reflection of the rostrocaudal level of the section.

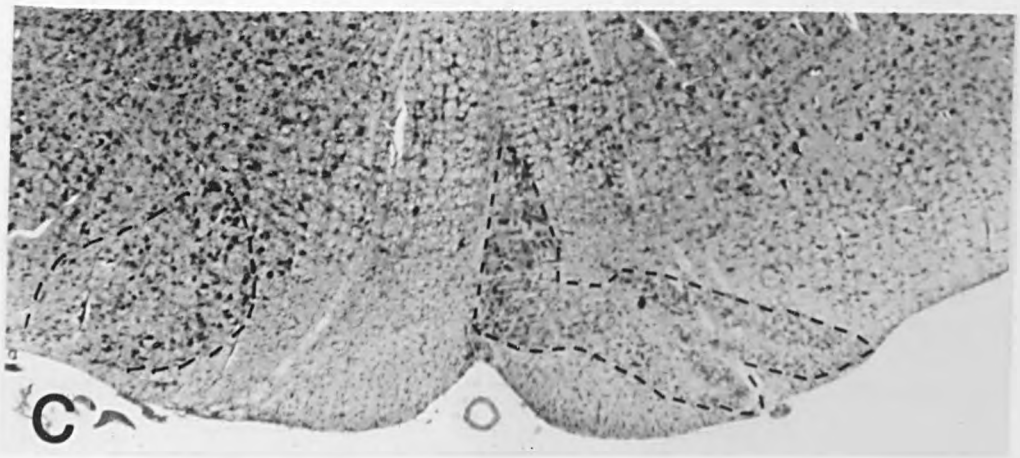
The left side of the micrographs is the right side of the brainstem.

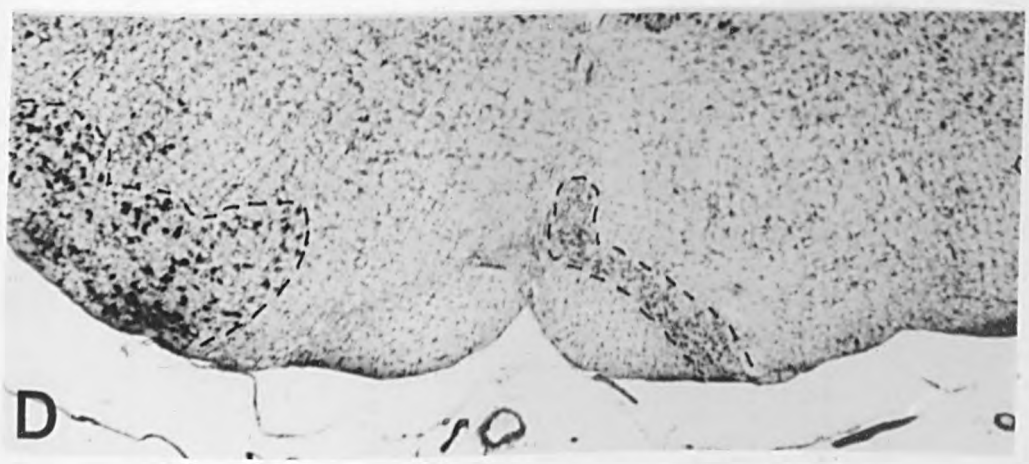
A = 5-5 x 48
B = 7-10 x 48
C = 5-35 x 36
D = 9-35 x 48

IO = left inferior olive
LRN = right lateral reticular nucleus


A


LRN
IO
B


C


D

samples were considered to be a sufficient representation of the whole group. The brainstems for histological examination were dehydrated, cleared and embedded using a Shandon Southern 2L processor and blocked out in paraffin wax. Sections 10 µm thick were taken every 200 µm. mounted on clean glass slides using glycerine albumin and stained with 1% cresyl fast violet (Appendix IV).

RESULTS

BRAINSTEM HISTOLOGY

The degenerative changes in the brainstem nuclei observed were the same in all animals irrespective of the age at the operation or the length of the survival time. Therefore retrograde cell degeneration in neonatal rats was extremely rapid and occurred in less than 5 days.

The right basal pontine nucleus was considerably reduced and contained many small dark microglial cells. The degenerative changes did not occur uniformly throughout the nucleus, however in all groups there was consistent cell loss in the ventromedial, ventral and ventrolateral regions such that the cerebral peduncle was adjacent to the ventral surface of the brainstem (Fig. 3.1). Occasionally the more dorsally placed cells were also affected. Also there appeared to be a slight reduction in the size of the right nucleus reticularis tegmenti pontis.

Figure 3.3

This series of photographs A-H (magnification x 1.7) illustrates the rapid growth of the cerebellum from the primordial structure seen at postnatal day 1 (A, arrowed) to the fully developed organ at 30 days post partum (H).

A = 1 day
B = 3 days
C = 5 days
D = 7 days
E = 10 days
F = 15 days
G = 22 days
H = 30 days

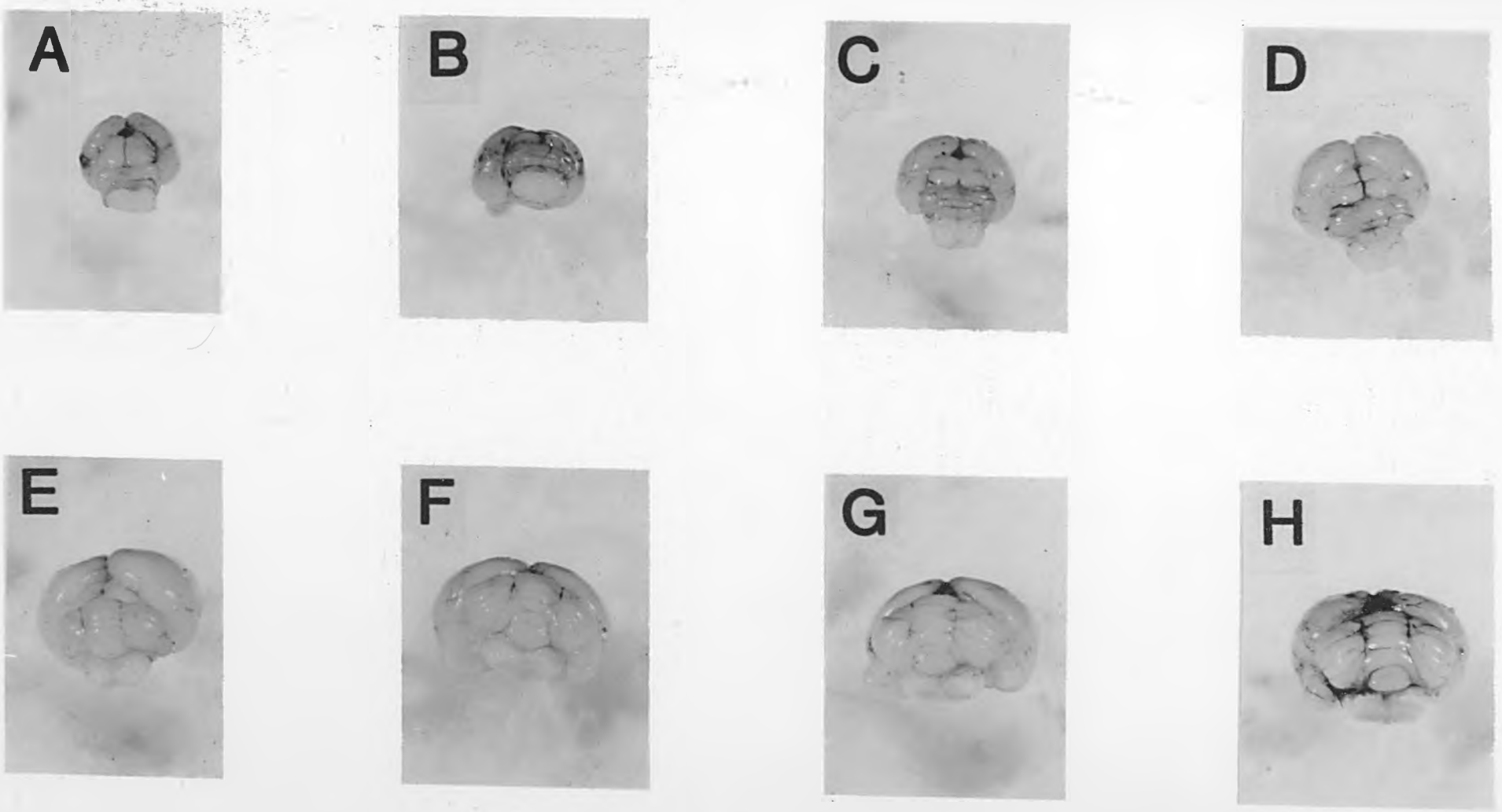
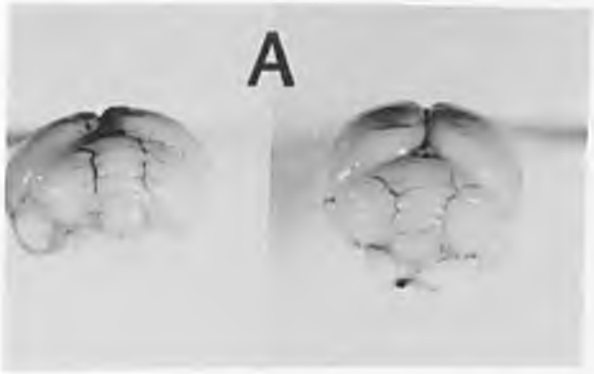

A
B
C
D
E
F
G
H

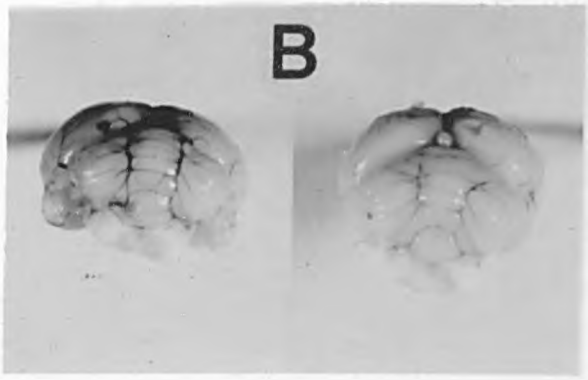
Figure 3.4

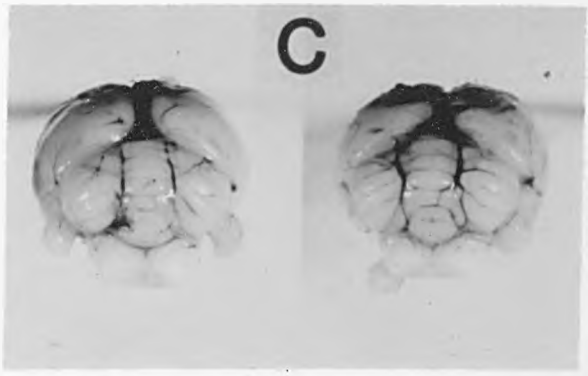
Cerebellar Growth after Pedunclotomy
on Day 22

The morphology of cerebella 5(A), 10(B), 25(C) and 35(D) days after left unilateral pedunclotomy is quite normal when compared with the control cerebella seen on the right in each pair. With increasing post operative survival time the pedunclotomised left hemisphere decreases in size and after 35 days a distinct gap can be seen between the ventral surface of the cerebellum and the brainstem (D, arrow).

(x 1.7)


A


B


C

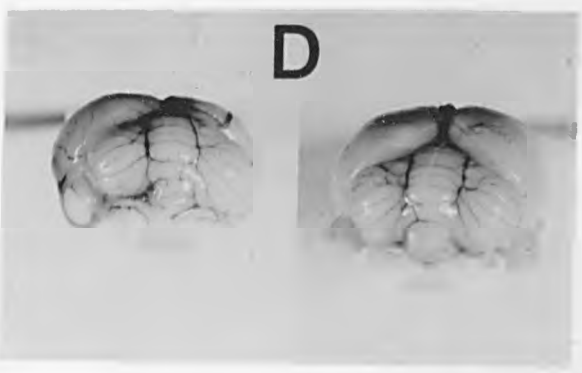

D

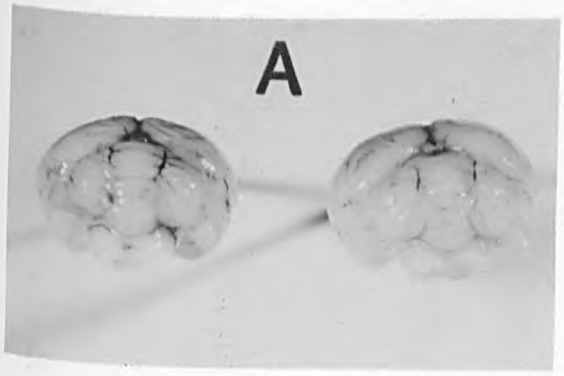
Figure 3.5

Cerebellar Growth after Pedunclotomy
on Day 10

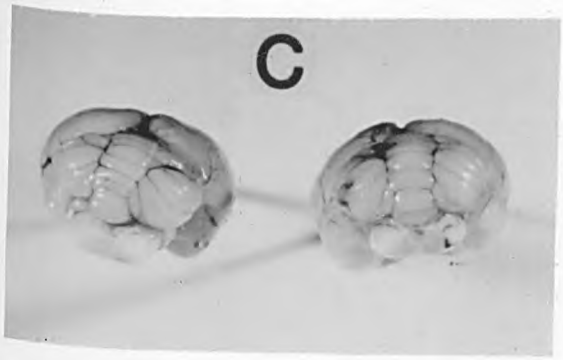
Figures A to G illustrate the development of the cerebellum at increasing intervals after pedunclotomy aged 10 days (on the left of each pair) compared with a normal cerebellum (on the right). In the experimental brains the left cerebellar hemisphere is smaller and has a less mature appearance than normal. In D the sublobulation of crus II is absent in the experimental brain (arrows).

A = 5 day survival
B = 10 day survival
C = 15 day survival
D = 20 day survival
E = 25 day survival
F = 30 day survival
G = 35 day survival

(x 1.7)

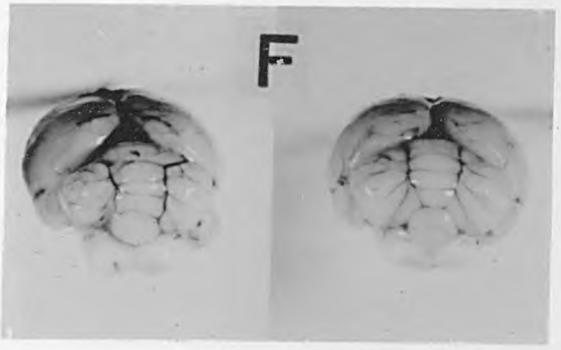

A


B


C


D


E


F

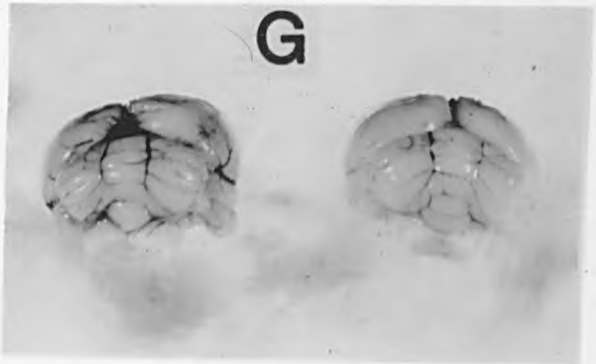

G

Figure 3.6

Cerebellar Growth after Pedunclotomy
on Day 7

Following pedunclotomy on day 7 the left cerebellar hemisphere is smaller than in the control brain (the right of each pair) and after most survival times there is a gap between it and the dorsal surface of the brainstem.

A = 5 day survival
B = 10 day survival
C = 15 day survival
D = 20 day survival
E = 25 day survival
F = 30 day survival
G = 35 day survival

(x 1.7)

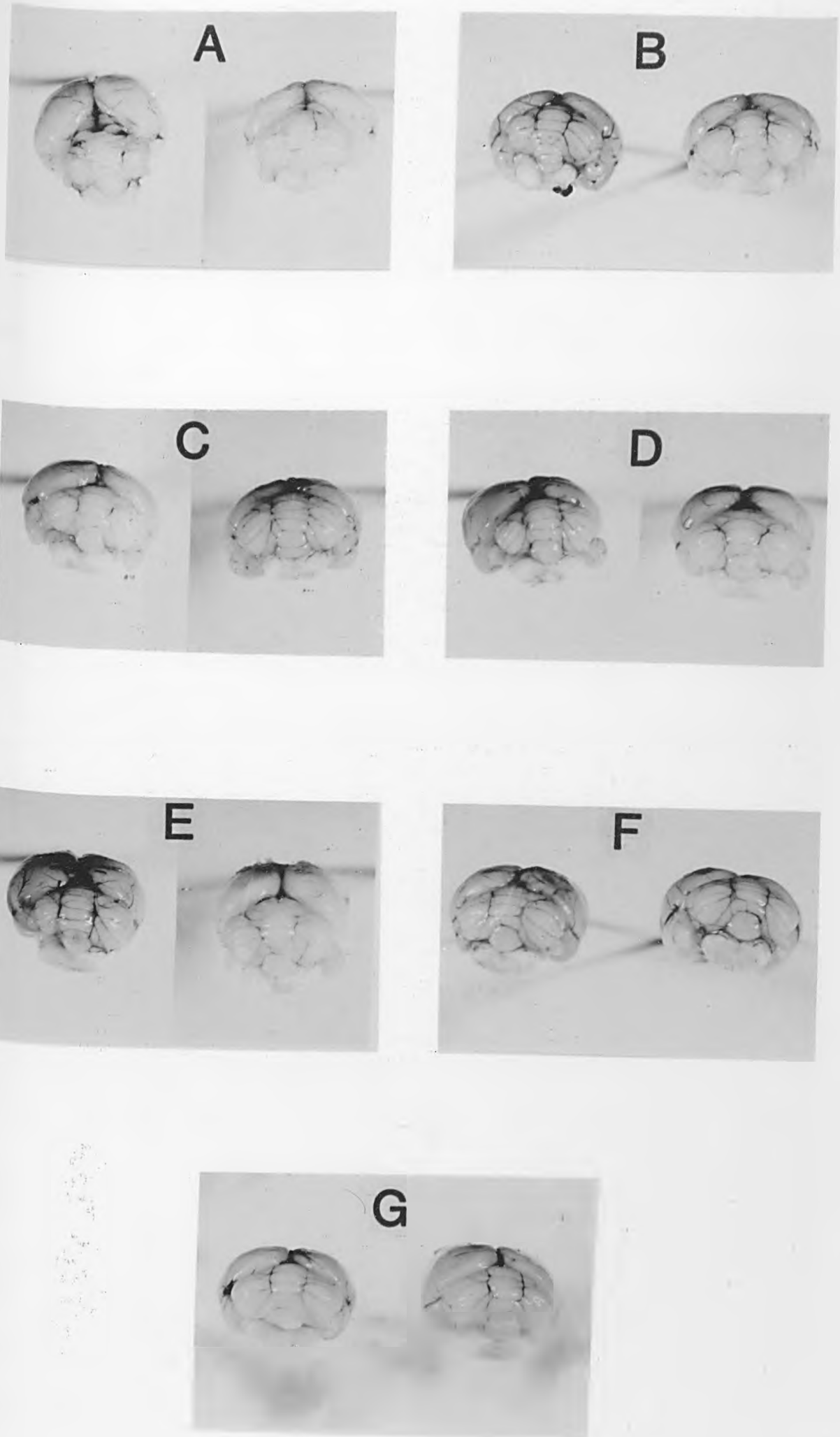

A
B
C
D
E
F
G

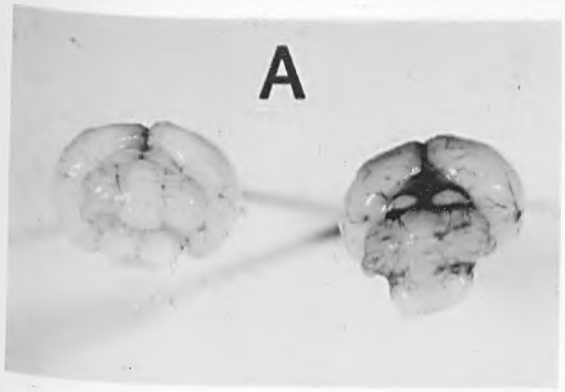
Figure 3.7

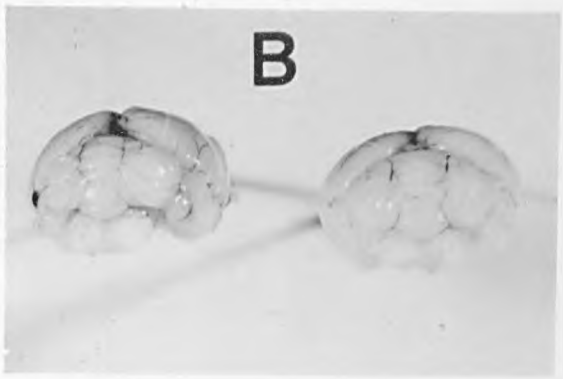
Cerebellar Growth after Pedunclotomy
on Day 5

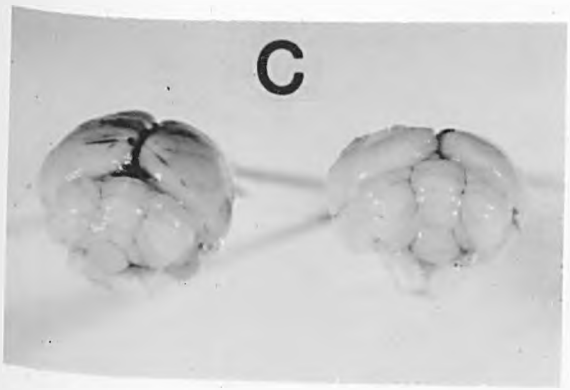
After pedunclotomy at 5 days of age the morphology of the experimental cerebella (on the left of each pair) develops relatively normally despite the reduced size of the left hemisphere. The decrease in ventrolateral growth and the frequent loss of the lateral lobule is clearly seen in the 30 and 35 day survivals (F and G, arrowed).

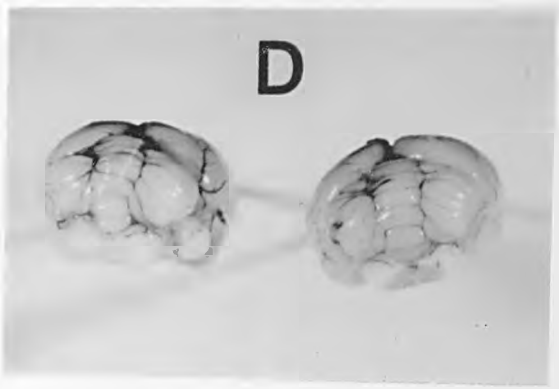
A = 5 day survival
B = 10 day survival
C = 15 day survival
D = 20 day survival
E = 25 day survival
F = 30 day survival
G = 35 day survival

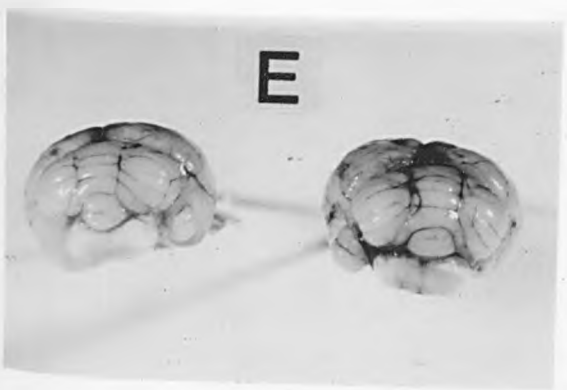
(x 1.7)

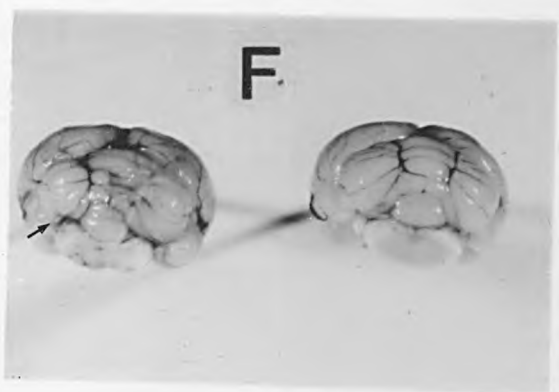

A

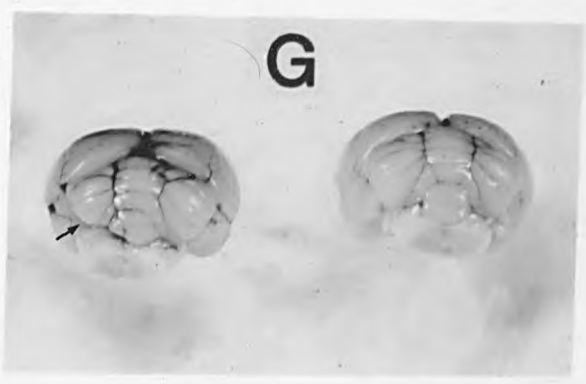

B


C


D


E


F


G

The left lateral reticular nucleus was found to have completely disappeared. However, although the right inferior olive also degenerated its position was quite often marked by a band of small glial cells (Fig. 3.2).

MORPHOLOGICAL CHANGES

The forebrains of the animals in the experimental groups did not look different from controls. In all cases the left cerebellar hemisphere in pedunclotomised rats looked smaller than in controls although the difference was only slight for those animals aged 22 days at operation. In the experimental animals the left cerebellar hemisphere did not extend as far ventrolateral as normal and it was not closely adjacent to the dorsum of the brainstem (e.g. Figs. 3.4 and 3.6). The general appearance of the pedunclotomised left hemispheres was that they were less mature than normal. For the animals aged 7 days at operation which survived 5 days the left hemisphere and vermis more closely resembled the cerebella from control animals aged 7 days (compare Fig. 3.6A and Fig. 3.3D). Also in the group which underwent pedunclotomy at 10 days the deafferented left hemisphere from animals surviving 25 days (i.e. aged 35 days) was almost identical to a normal cerebellum for the 5 day survival group (i.e. aged 15 days) (Fig. 3.5 E & A).

In animals aged 22 days at operation the cerebella appeared remarkably normal for all post operative survival

times. Although the left hemisphere looked slightly smaller than the controls the fissures and folia were not altered, and it was not closely adjacent to the dorsum of the brainstem. However, in the group which underwent pedunclotomy at 10 days post partum, the fissures of the left hemisphere were not as clearly marked as those either in the contralateral hemisphere or in controls. Also the additional minor sulci found within lobules, which Larsell (1952) described, were not visible (Fig. 3.5) especially in those animals which survived 5, 10, 20 and 30 days post operatively. One animal in the group which survived 30 days after pedunclotomy had an unusual left cerebellar hemisphere (Fig. 3.5F). However it was difficult to ensure whether this appearance was due to abnormal fissures or just a reorganisation of the pial blood vessels. The latter seems more probable.

Cerebellar pedunclotomy on animals aged 7 days produced a much smaller left hemisphere even after 5 days survival and this appearance became more pronounced with the longer survival times (Fig. 3.6). Despite this the folia and fissures appear quite normal. In the vermis of an animal surviving 10 days post operatively (Fig. 3.6B) lobule IX was deviated towards the right possibly because of a differential growth gradient between right and left hemispheres. Also five days after pedunclotomy the vermis was broader than in the control (Fig. 3.6A).

The cerebella of animals which underwent pedunclotomy aged 5 days had the same appearance of a smaller left hemisphere with normal-looking folia and fissures (Fig. 3.7). The cerebella from the 30 and 35 day survival groups illustrate particularly well the reduced lateral extension of the pedunclotomised hemispheres (Fig. 3.7 F & G).

THE EFFECT OF PEDUNCLOTOMY ON GROWTH

The operation did not have any effect on body weight and in no group was the mean body weight of the experimental animals significantly less than control. Therefore any weight or neuronal loss in the experimental animals was not due to malnutrition. Tables of the mean body weight for each group can be found in Appendix VI.

The first experiment involved performing unilateral cerebellar pedunclotomy on animals aged between 1 and 30 days and allowing them to survive 35 days post operation. The results are in Table 3.2.

Only in those animals which underwent cerebellar pedunclotomy between 7 and 10 days of age and on day 22 was the whole brain weight smaller than in controls. The operated brains were 5-12% smaller and this was only statistically significant ($p < 0.001$) for the 7-10 day operative ages. The experimental cerebella were smaller than controls for each age group. This reduction was only significant for animals aged 7-10 days ($p < 0.001$) or

22 days ($p < 0.01$) at operation when they weighed 20-25% less than controls. Similarly the pedunclotomised left hemispheres were smaller than normal in all age groups and, with the exception of the 3 and 4 day operative groups, this was highly significant ($p < 0.001$). The amount by which the left hemispheres were smaller than the control after pedunclotomy varied from 12% in the 3 day operative age group to a maximum of 48% in animals aged 7 days at operation. The deficit between experimental and control left hemispheres was also greater than 40% for groups which underwent pedunclotomy at 8, 9, 10 or 22 days. The relative vulnerability of the left hemisphere to pedunclotomy at different ages is illustrated in Fig. 3.8A. In the experimental groups, with the exceptions of those aged 3, 5 and 6 days at operation, the vermes were also smaller than normal although this was only significant ($p < 0.001$) after pedunclotomy at 7, 8 or 10 days of age when the deficit was between 13% and 18%. The 23% difference between the experimental and control vermes in the 22 day age group was also just significant ($p < 0.01$). Unilateral cerebellar pedunclotomy had very little effect on the right hemisphere. In the experimental groups the right hemispheres were not consistently either smaller or larger than the controls and none of the differences were significant. The relative vulnerability of the vermis and right hemisphere to pedunclotomy at different ages is shown in Figs. 3.8 B & C.

TABLE 3.2

The mean weight data for animals undergoing cerebellar pedunclotomy at different ages and surviving 35 days post operatively.

The bracketed fractions in the left column indicate the 'n' values in operated and control ($^{o}/_{c}$) groups.

† = $p < 0.01$

* = $p < 0.001$

Age at Operation (Days)	Weight (mg)									
	Brain		Cerebellum		L. Hemisphere		Vermis		R. Hemisphere	
	Op	C	Op	C	Op	C	OP	C	Op	C
1($^{7}/_{6}$)	1797.7 ±65.2	1761.4 ±72.8	237.9 ±20.3	250.2 ±16.4	52.3 * ± 6.7	69.8 ± 6.4	98.8 ± 7.5	101.8 ± 9.9	79.5 ± 7.1	77.2 ± 7.6
3($^{5}/_{10}$)	1795.1 ±82.6	1716.9 ±60.2	252.3 ± 9.9	252.5 ± 6.0	59.7 ± 7.8	68.5 ± 8.0	111.4 ± 5.4	101.3 ± 7.0	79.4 ± 5.3	76.7 ± 4.0
4($^{7}/_{6}$)	1806.1 ±69.0	1841.9 ±54.9	233.3 ±23.2	261.2 ± 8.8	55.1 † ±12.2	75.3 ± 3.9	105.1 ±10.0	114.1 ± 5.2	73.6 ± 6.7	74.9 ± 5.7
5($^{6}/_{8}$)	1799.3 ±70.2	1555.4 ±70.7	225.2 ±18.9	241.6 ±17.5	48.1 * ± 6.7	69.4 ± 7.1	99.7 ± 6.5	95.3 ± 4.3	73.1 ±11.1	75.5 ± 8.9
6($^{4}/_{13}$)	1942.6 ±68.8	1599.6 ±74.3	248.2 ±18.1	249.5 ±27.0	51.5 * ± 8.4	74.0 ± 9.5	107.8 ± 6.4	95.9 ± 7.2	84.2 ± 6.6	81.5 ± 9.4
7($^{12}/_{14}$)	1416.1 * ±90.5	1613.3 ±88.0	188.3 * ±19.9	250.4 ±14.2	36.9 * ±10.4	71.2 ± 6.3	83.7 * ± 9.6	100.9 ±10.1	73.0 ± 9.9	79.3 ± 8.6
8($^{17}/_{22}$)	1432.5 * ±93.3	1578.8 ±65.1	199.2 * ±18.4	248.8 ±19.9	40.6 * ±11.7	72.0 ±10.7	84.1 * ± 9.4	96.9 ± 9.1	75.5 ± 5.5	78.5 ± 8.2
9($^{10}/_{14}$)	1449.8 * ±85.3	1648.7 ±81.5	207.0 * ±23.9	257.1 ±18.0	45.4 * ± 9.8	78.3 ± 8.9	88.2 ±11.1	99.6 ±10.1	72.9 ± 8.9	80.1 ± 9.4
10($^{11}/_{13}$)	1524.8 * ±70.3	1638.9 ±55.7	206.2 * ±20.9	256.4 ±13.0	46.1 * ±13.4	76.1 ± 6.9	79.9 * ± 7.0	98.2 ± 6.7	74.6 ± 7.8	78.5 ± 9.7
15($^{4}/_{10}$)	1817.3 ±96.7	1779.7 ±71.2	244.8 ±11.7	270.8 ±11.2	63.1 * ± 6.2	80.2 ± 4.5	98.1 ± 3.8	102.6 ± 7.3	79.8 ± 3.9	82.4 ± 3.6
22($^{5}/_{5}$)	1775.3 ±34.6	1866.9 ±114.9	225.1 † ±15.6	277.4 ±22.2	44.5 * ± 7.7	79.7 ± 6.7	88.5 † ± 5.9	114.7 ±11.5	81.9 ± 4.9	81.5 ± 7.8
30($^{9}/_{5}$)	2001.0 ±91.9	2037.5 ±86.8	288.1 ±27.5	310.7 ±10.9	68.4 * ± 2.7	85.5 ± 4.5	121.8 ± 7.8	130.3 ± 7.3	87.0 ± 5.0	89.2 ± 5.7

FIGURE 3.8

Figures 3.8A to 3.8C are histograms illustrating the effect of cerebellar pedunclotomy performed on animals of different ages and surviving 35 days post operatively.

The values shown are found in Table 3.2 and represent the mean ± standard deviation.

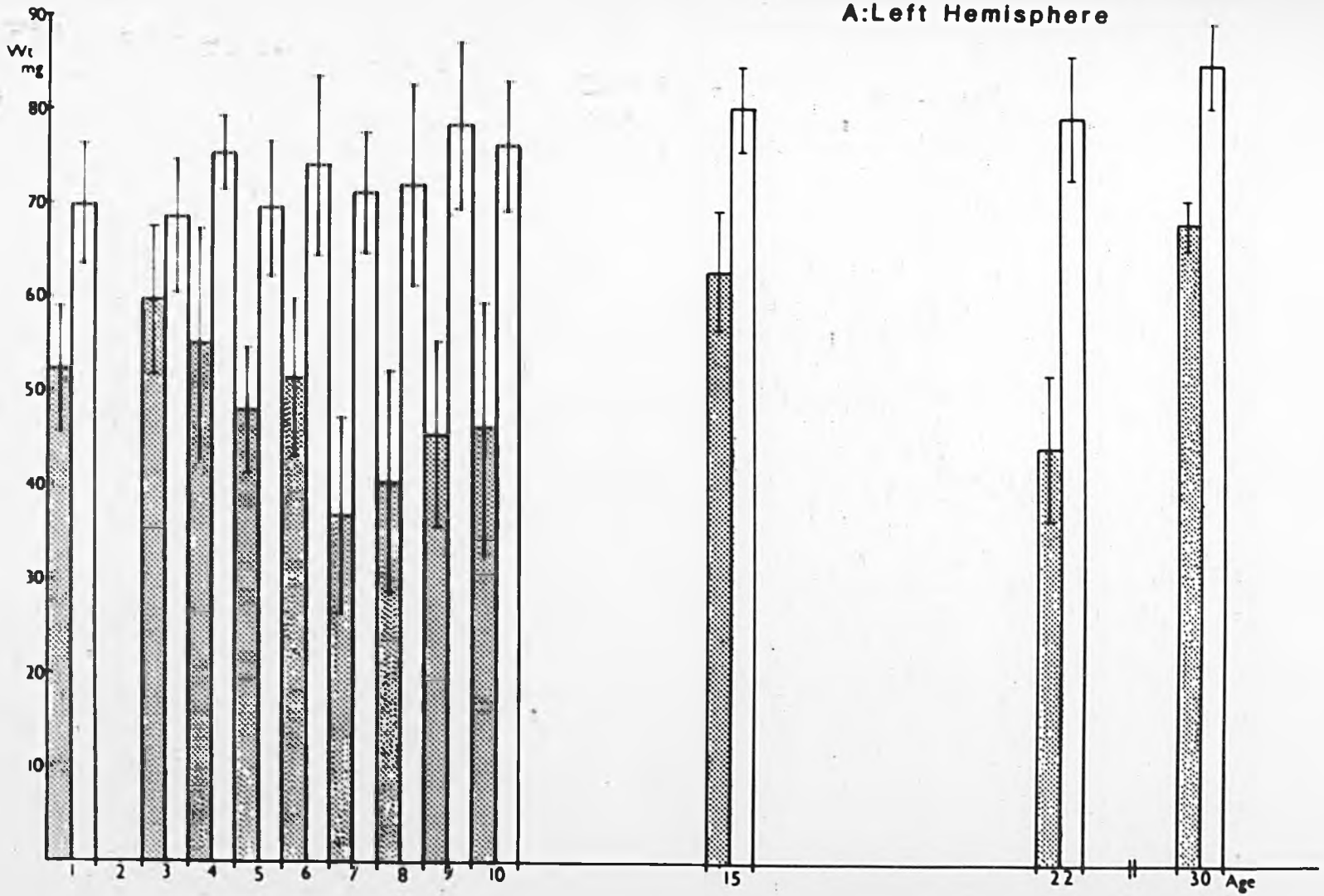
3.8A = the effect on the left hemisphere

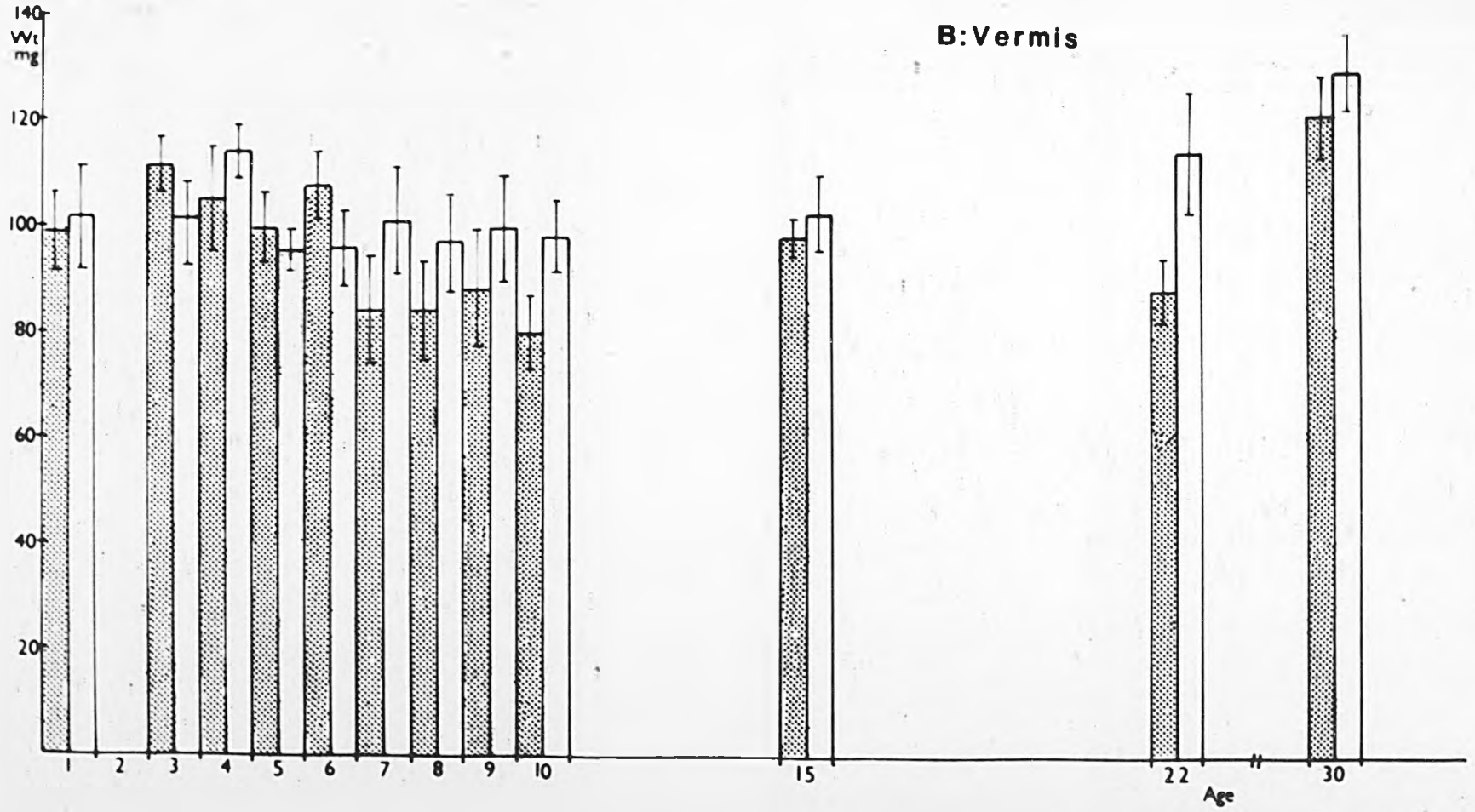
3.8B = the effect on the vermis

3.8C = the effect on the right hemisphere.

▒ = operated

□ = control


A:Left Hemisphere
Wt
mg
90
80
70
60
50
40
30
20
10
1
2
3
4
5
6
7
8
9
10
15
22
30
Age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Wt
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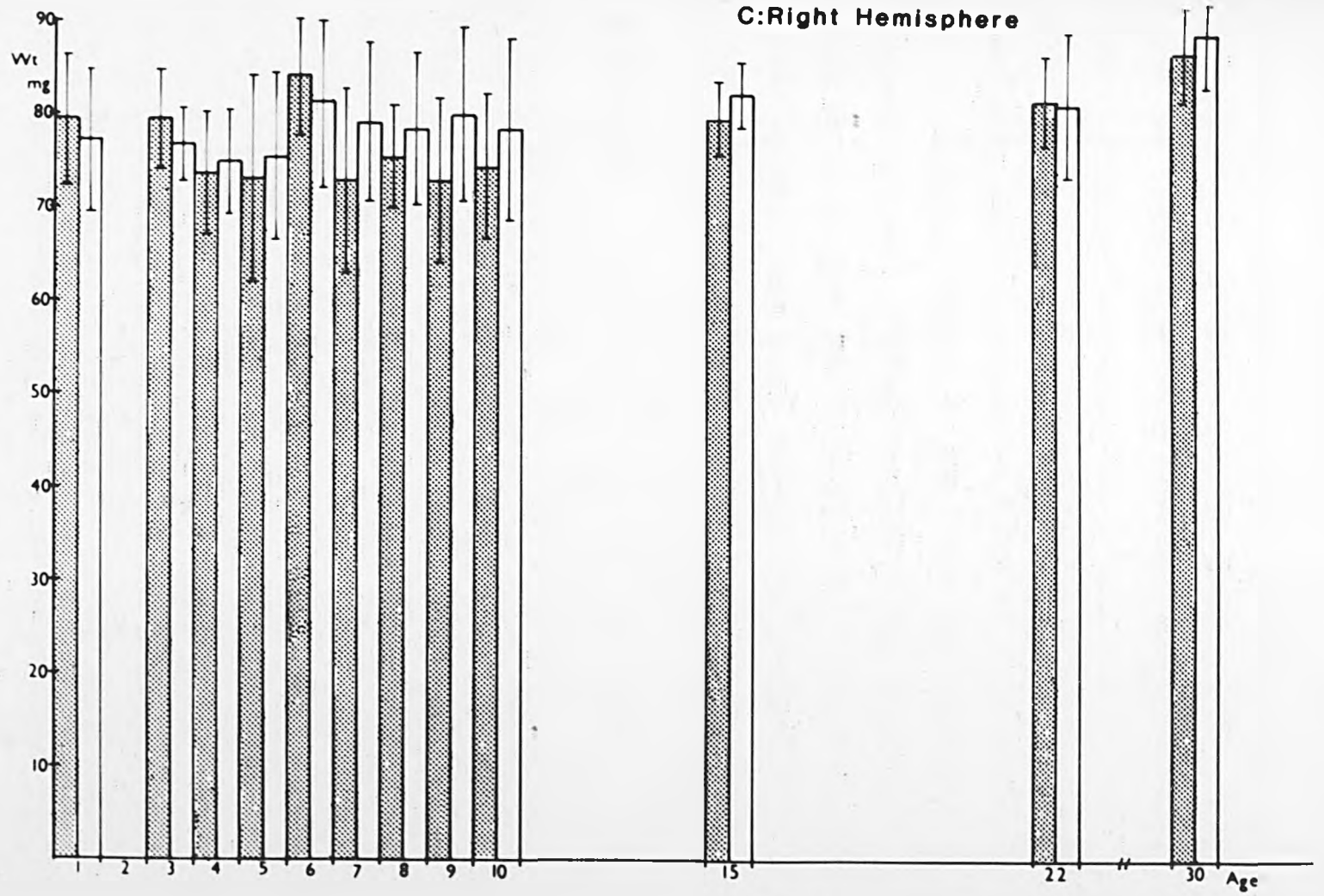
Age


C:Right Hemisphere
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Age

The results from this first experiment indicate that the degree of the effect of pedunclotomy depends on the age at which it was performed and that animals aged 7 days at operation were most severely affected (Fig. 3.8A). Therefore the second experiment involved animals aged 7 days, which were highly vulnerable, also 5 and 10 days, which both responded in a similar manner, and 22 days, at which age only the synaptogenetic stage of development has not finished. Animals of the above ages underwent pedunclotomy and were allowed to survive for intervals of 5 days up to 35 days post operatively. The brain and cerebellar weights from these animals are in Table 3.3. Using the weighted t-test in which the number of observations made for each growth curve is taken into account, the experimental values were only significantly different from the control if $p < 0.001$. Therefore any comparisons which are described as significant are so at the above confidence limit.

5 Day Pedunclotomy Time Course

The weight data values for this experimental group are shown in Table 3.3A. With the exception of the 35 day survival the mean weight of the whole brain was less in the experimental animals than the controls, a difference which was not statistically significant. For all post operative survival times the cerebella in the pedunclotomised groups were smaller than in the controls. These values were

significantly different after 5, 10, 15 and 30 days survival.

After each survival time the experimental left cerebellar hemisphere was always smaller than the control hemisphere. The pedunclotomised hemispheres weighed between 31% and 36% less except for the 5 and 20 day survival groups which were 50% below the control value. These differences between experimental and control groups were significant except for the 25 day survival point. Although the pedunclotomised left hemisphere was only half its normal weight after 5 days post operative survival it had still grown considerably weighing 70% more than at the operation. Nevertheless, the rate of growth was much less than in the control group which increased its weight by 260% between 5 and 10 days of age. But in the next five days up to 15 days of age the experimental group grew faster than the control animals. Thereafter in both groups the rate of growth was much less. After 35 days survival the vermis of the pedunclotomised animals was larger than in the controls. However, after the other survival times the experimental values were slightly below normal, up to 6% less apart from the 30 and 5 day survival times which were respectively 13% and 18% smaller. These differences were only significant for those animals surviving 5 days post operatively, which indicates that the growth of the vermis following unilateral pedunclotomy is very little different from normal. The right hemispheres of experimental animals were similar to controls,

the maximum deficit of 18% was found in the 25 day survival group, but none of these differences were significant.

7 Day Pedunclotomy Time Course

The mean weight data for the brain and cerebellar growth in animals pedunclotomised aged 7 days is shown in Table 3.3B. In the experimental animals the brains were smaller than in the controls for each survival time. The weight deficit was not significant except for the 10 and 35 day survival groups in which it was 11%. The cerebella of pedunclotomised animals were 23-30% smaller than normal which was a significant difference except for the group surviving 15 days.

For each survival time group the left cerebellar hemisphere was 48-53% and significantly below the control weight. The pedunclotomised hemisphere 5 days after the operation had only increased its weight by 20% compared with 140% in the controls. But between 5 and 10 days survival both groups grew at a similar rate, increasing their weight by approximately 50%. For longer survival times both experimental and control groups appear to have a similar growth pattern. With the exception of those animals surviving 15 days, the vermes in the experimental groups were significantly different from the controls, being between 17% and 30% smaller. But there were no significant differences between the right hemispheres of

experimental and control animals at any post operative survival time.

10 Day Pedunclotomy Time Course

The data for this time course are shown in Table 3.3C. Apart from those animals which survived 30 days post operatively, the brain weights of the pedunclotomised animals were 3-9% less than in the controls. These differences were only significant for those animals in the 20 and 35 day survival groups. The cerebella of experimental animals were 12-20% smaller than normal and the deficit was only not significant after 10 or 30 days survival.

Within the cerebellum, the experimental left hemisphere weighed 31-48% less than the controls, which was a significant difference for those animals surviving 5, 10, 15 and 35 days post operatively. After 5 days post operative survival the pedunclotomised hemisphere had lost weight although between 5 and 10 days survival it gained more weight than did the controls and then both the experimental and control groups had similar growth rates. For each survival time, the vermis in animals which had undergone pedunclotomy was smaller than in the controls. This was significant after 10, 25, 30 and 35 days survival when the deficit was between 10% and 19%. With the exception of the 10 day survival group, in which the experimental hemisphere was 12% smaller than in the controls, there was less than 5% difference between the operated and control

right hemispheres, and none of these differences was significant.

22 Day Pedunclotomy Time Course

Table 3.3D shows the mean data values for this time course experiment. In those animals which had undergone pedunclotomy, the mean brain weight varied between 5% less and 11% greater than the control value. But only the 15 day survival group, which was 7% smaller than normal, was significant. The cerebella of pedunclotomised rats were smaller than in the control animals, but the 8-20% differences were only significant for those animals which had survived 15, 25 or 30 days post operatively.

Apart from the 5 and 30 day survival groups, the left cerebellar hemispheres in the experimental animals were significantly smaller than normal. Between 10 and 20 days post operation this deficit was 27% and it increased to 38% and 44% by 25 and 35 days respectively. The vermal weights in the pedunclotomised animals were also below the control values at each survival time. However only in the 15 day survivals, which were 16% less, and the 25 and 30 day survivals, which were 22% less, were the differences significant. After left unilateral pedunclotomy the right hemispheres were almost identical to the controls, the greatest deficit in weight was 10%, but none of the differences were significant.

TABLE 3.3

Tables 3.3A - 3.3D show the mean weight data from animals which underwent cerebellar pedunclotomy at 5, 7, 10 and 22 days post partum and survived for variable times up to 35 days post operatively. The values in the row for '0' survival time are the weights from animals of the relevant age of operation. The bracketed numbers in each left column indicate the 'n' values for operated and control ($^{o}/_{c}$) groups.

* = $p < 0.001$

3.3A: 5 DAY PEDUNCLOTOMY TIME COURSE

Post Operative Survival Time (Days)	Weight (mg) ± S.D.									
	Brain		Cerebellum		L. Hemisphere		Vermis		R. Hemisphere	
	Op	C	Op	C	Op	C	Op	C	Op	C
0 (6)		551.2 ±37.6		35.3 ±3.3		9.4 ±1.4		14.6 ±1.8		10.4 ±0.6
5 ($^{8}/_{8}$)	1028.3 ±46.0	1080.3 ±33.1	81.4* ±6.8	105.5 ±5.6	16.1* ±2.7	34.2 ±5.9	34.6* ±3.6	42.2 ±2.5	27.6 ±2.8	28.8 ±3.9
10 ($^{6}/_{8}$)	1342.4 ±29.9	1390.7 ±40.7	147.4* ±6.8	169.9 ±5.4	33.4* ±3.3	48.1 ±4.8	64.1 ±3.0	67.8 ±3.7	47.0 ±4.2	49.8 ±2.8
15 ($^{4}/_{8}$)	1458.9 ±67.2	1511.3 ±29.1	176.5* ±9.6	203.3 ±8.6	39.6* ±2.6	61.5 ±4.9	76.6 ±3.7	81.1 ±2.1	60.5 ±5.2	60.8 ±4.3
20 ($^{4}/_{7}$)	1618.9 ±104.5	1722.1 ±35.0	192.6 ±25.6	237.4 ±12.5	39.5* ±10.7	74.1 ±8.5	87.6 ±11.5	91.1 ±3.8	63.8 ±4.9	78.1 ±6.4
25 ($^{5}/_{5}$)	1669.7 ±92.2	1718.8 ±30.0	212.5 ±19.5	239.2 ±13.9	45.7 ±6.6	68.7 ±8.6	92.4 ±7.0	95.4 ±8.3	68.4 ±8.3	73.1 ±3.2
30 ($^{6}/_{6}$)	1550.5 ±177.3	1669.2 ±43.0	205.5* ±11.3	246.9 ±8.8	44.7* ±5.3	70.3 ±2.4	87.2 ±11.4	99.9 ± 4.7	64.6 ±5.8	72.7 ±4.4
35 ($^{6}/_{8}$)	1799.3 ±70.2	1555.4 ±70.7	225.2 ±18.9	241.6 ±17.5	48.1* ±6.7	69.4 ±7.1	99.7 ±6.5	95.3 ±4.3	73.1 ±11.1	75.5 ±8.9

3.3B: 7 DAY PEDUNCLOTOMY TIME COURSE

Post Operative Survival Time (Days)	Weight (mg) ± S.D.									
	Brain		Cerebellum		L. Hemisphere		Vermis		R. Hemisphere	
	Op	C	Op	C	Op	C	Op	C	Op	C
0 (6)		896.9 ±35.5		70.5 ±6.0		17.4 ±2.6		29.7 ±2.6		20.3 ±1.9
5 ($^{6}/_{5}$)	1207.2 ±62.0	1331.5 ±48.3	108.8* ±9.9	150.7 ±9.7	20.9* ±1.7	41.9 ±3.1	45.9* ±6.2	62.4 ±3.9	40.3 ±4.5	43.1 ±3.6
10 ($^{5}/_{4}$)	1305.6* ±53.4	1470.9 ±21.0	145.3* ±7.9	208.7 ±2.5	32.0* ±4.7	61.1 ±1.1	56.8* ±5.1	83.1 ±2.9	55.6 ±6.2	61.9 ±2.9
15 ($^{5}/_{6}$)	1407.0 ±80.3	1475.4 ±69.5	159.4 ±19.2	208.3 ±18.2	30.1* ±5.9	61.4 ±4.9	66.1 ±5.2	84.3 ±10.2	57.8 ±7.9	62.6 ±5.6
20 ($^{5}/_{5}$)	1537.3 ±76.6	1613.3 ±25.2	181.4* ±8.4	238.4 ±5.0	32.6* ±6.0	65.9 ±2.7	78.0* ±3.6	96.0 ±3.1	67.6 ±6.4	71.6 ±2.8
25 ($^{6}/_{9}$)	1579.8 ±29.3	1639.9 ±81.0	185.2* ±6.2	239.1 ±16.2	31.6* ±4.5	67.2 ±6.8	76.7* ±3.8	97.2 ±6.3	68.7 ±5.9	68.6 ±5.3
30 ($^{5}/_{4}$)	1597.1 ±60.9	1640.3 ±40.6	187.9* ±14.5	249.5 ±3.2	34.2* ±7.1	68.7 ±4.8	79.0* ±5.3	104.9 ±7.3	71.3 ±4.8	73.3 ±1.2
35 ($^{12}/_{14}$)	1416.1* ±90.5	1613.3 ±88.0	188.3* ±19.9	250.4 ±14.2	36.9* ±10.4	71.2 ±6.3	83.7* ±9.6	100.9 ±10.1	73.0 ±9.9	79.3 ±8.6

3.3C: 10 DAY PEDUNCLOTOMY TIME COURSE

Post Operative Survival Time (Days)	Weight (mg) ± S.D.									
	Brain		Cerebellum		L. Hemisphere		Vermis		R. Hemisphere	
	Op	C	Op	C	Op	C	Op	C	Op	C
0 (4)		1223.4 ±21.4		116.4 ±11.3		30.9 ±4.1		48.2 ±1.9		36.4 ±4.7
5 ($^{4}/_{8}$)	1313.1 ±33.5	1390.7 ±40.7	137.6* ±6.7	169.9 ±5.4	24.8* ±1.6	48.1 ±4.8	61.8 ±7.9	67.8 ±3.7	47.7 ±6.4	49.8 ±2.8
10 ($^{6}/_{4}$)	1421.9 ±22.9	1502.2 ±45.5	164.1 ±8.8	199.1 ±12.9	33.9* ±6.4	56.6 ±5.5	70.6* ±4.8	84.9 ±3.9	54.9 ±3.5	52.1 ±6.9
15 ($^{5}/_{7}$)	1574.3 ±82.5	1722.1 ±35.0	196.2* ±13.9	237.4 ±12.5	47.2* ±5.4	74.1 ±8.5	83.7 ±8.1	91.1 ±3.8	73.9 ±9.7	78.1 ±6.4
20 ($^{6}/_{5}$)	1556.9* ±44.7	1718.8 ±30.0	198.9* ±10.2	239.2 ±13.9	46.1 ±7.9	68.7 ±8.6	86.6 ±4.1	95.4 ±8.3	64.4 ±3.3	73.1 ±3.2
25 ($^{6}/_{6}$)	1614.1 ±54.7	1669.2 ±43.0	209.4* ±15.5	246.9 ±8.8	48.4 ±11.9	70.3 ±2.4	87.2* ±4.4	99.9 ±4.7	71.8 ±5.2	72.7 ±4.4
30 ($^{4}/_{8}$)	1578.2 ±85.7	1555.4 ±70.7	214.2 ±21.7	241.6 ±17.5	45.8 ±9.3	69.4 ±7.1	86.2* ±1.4	95.3 ±4.3	75.1 ±9.7	75.5 ±8.9
35 ($^{11}/_{13}$)	1524.8* ±70.3	1638.9 ±55.7	206.2* ±20.9	256.4 ±13.0	46.1* ±13.4	76.1 ±6.9	79.9* ±7.0	98.2 ±6.7	74.6 ±7.8	78.5 ±9.7

3.3D: 22 DAY PEDUNCLOTOMY TIME COURSE

Post Operative Survival Time (Days)	Weight (mg) ± S.D.									
	Brain		Cerebellum		L. Hemisphere		Vermis		R. Hemisphere	
	Op	C	Op	C	Op	C	Op	C	Op	C
0 (5)		1475.4 ±69.5		196.3 ±14.7		58.8 ±3.3		78.3 ±8.1		58.5 ±3.3
5 ($^{3}/_{5}$)	1531.9 ±85.2	1613.3 ±25.2	212.1 ±7.8	238.4 ±5.0	54.8 ±5.9	65.9 ±2.7	83.7 ±5.0	96.0 ±3.1	69.9 ±2.5	71.6 ±2.8
10 ($^{3}/_{9}$)	1615.9 ±43.4	1639.9 ±81.0	210.7 ±16.2	239.1 ±16.2	49.2* ±5.8	67.2 ±6.8	86.6 ±4.7	97.2 ±6.3	68.6 ±5.9	68.6 ±5.3
15 ($^{5}/_{6}$)	1730.2* ±35.6	1856.8 ±51.3	222.5* ±12.7	271.0 ±7.6	54.1* ±8.5	74.9 ±4.5	94.2* ±5.9	112.6 ±6.4	71.7 ±4.3	79.3 ±2.8
20 ($^{5}/_{14}$)	1793.9 ±83.7	1613.3 ±88.0	231.5 ±14.0	250.4 ±14.2	52.5* ±4.6	71.2 ±6.3	98.1 ±10.2	100.9 ±10.1	75.8 ±4.7	79.3 ±8.6
25 ($^{4}/_{5}$)	1801.7 ±70.7	1787.6 ±28.5	225.2* ±17.4	280.8 ±6.4	48.0* ±8.1	77.9 ±7.2	91.7 ±8.6	117.8 ±2.7	77.8 ±4.3	81.7 ±3.8
30 ($^{2}/_{6}$)	1775.1 ±25.8	1873.7 ±70.1	239.1* ±1.9	274.3 ±9.5	66.4 ±2.5	76.9 ±2.9	92.0* ±1.0	117.2 ±5.6	75.4 ±0.9	77.3 ±3.6
35 ($^{5}/_{5}$)	1775.3 ±34.6	1866.9 ±114.9	225.1 ±15.6	277.4 ±22.2	44.5* ±7.7	79.7 ±6.7	88.5 ±5.9	114.7 ±11.5	81.9 ±4.9	81.5 ±7.8

FIGURE 3.9

Figs. 3.9A to 3.9C show the growth curves of parts of the cerebellum after pedunclotomy at 5, 7, 10 or 22 days post partum. The data for these graphs can be found in Tables 3.3A-D. The standard deviations have been omitted to improve the clarity of the diagrams.

3.9A = Left hemisphere growth curves

3.9B = Vermis growth curves

3.9C = Right hemisphere growth curves

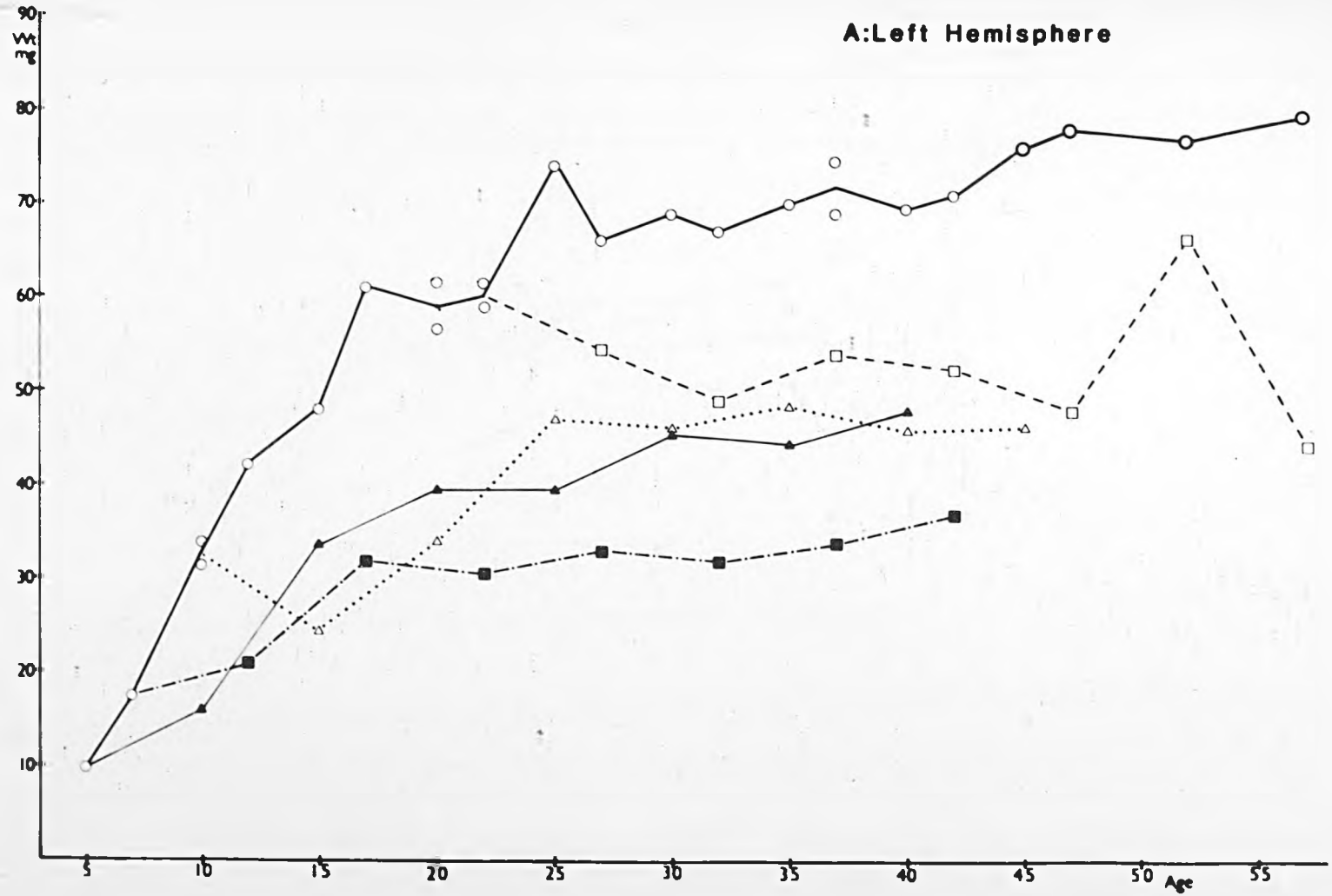
—○— = control group

—▲— = 5 day pedunclotomy group

—·■·— = 7 day pedunclotomy group

···△··· = 10 day pedunclotomy group

– –□– – = 22 day pedunclotomy group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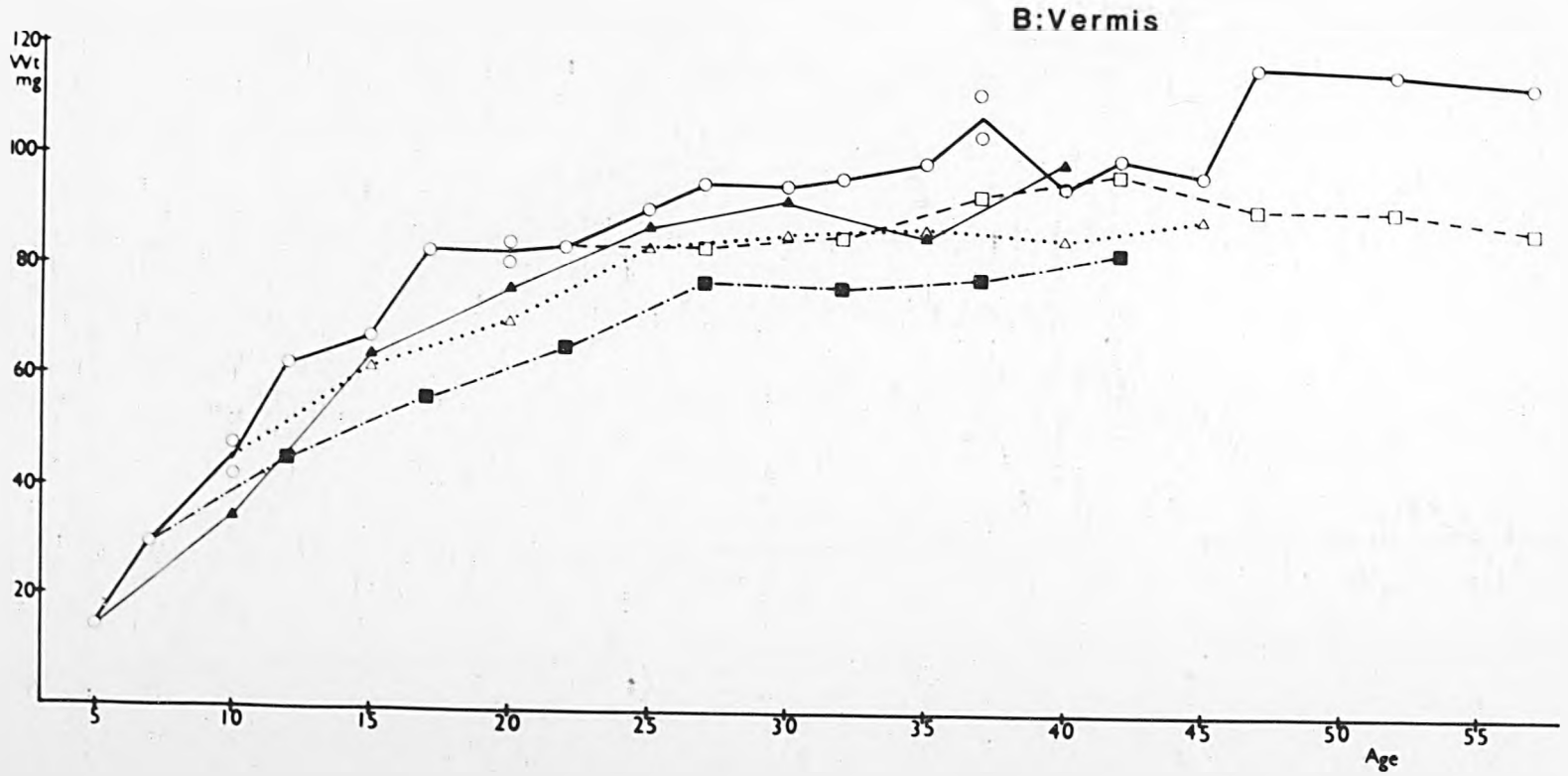
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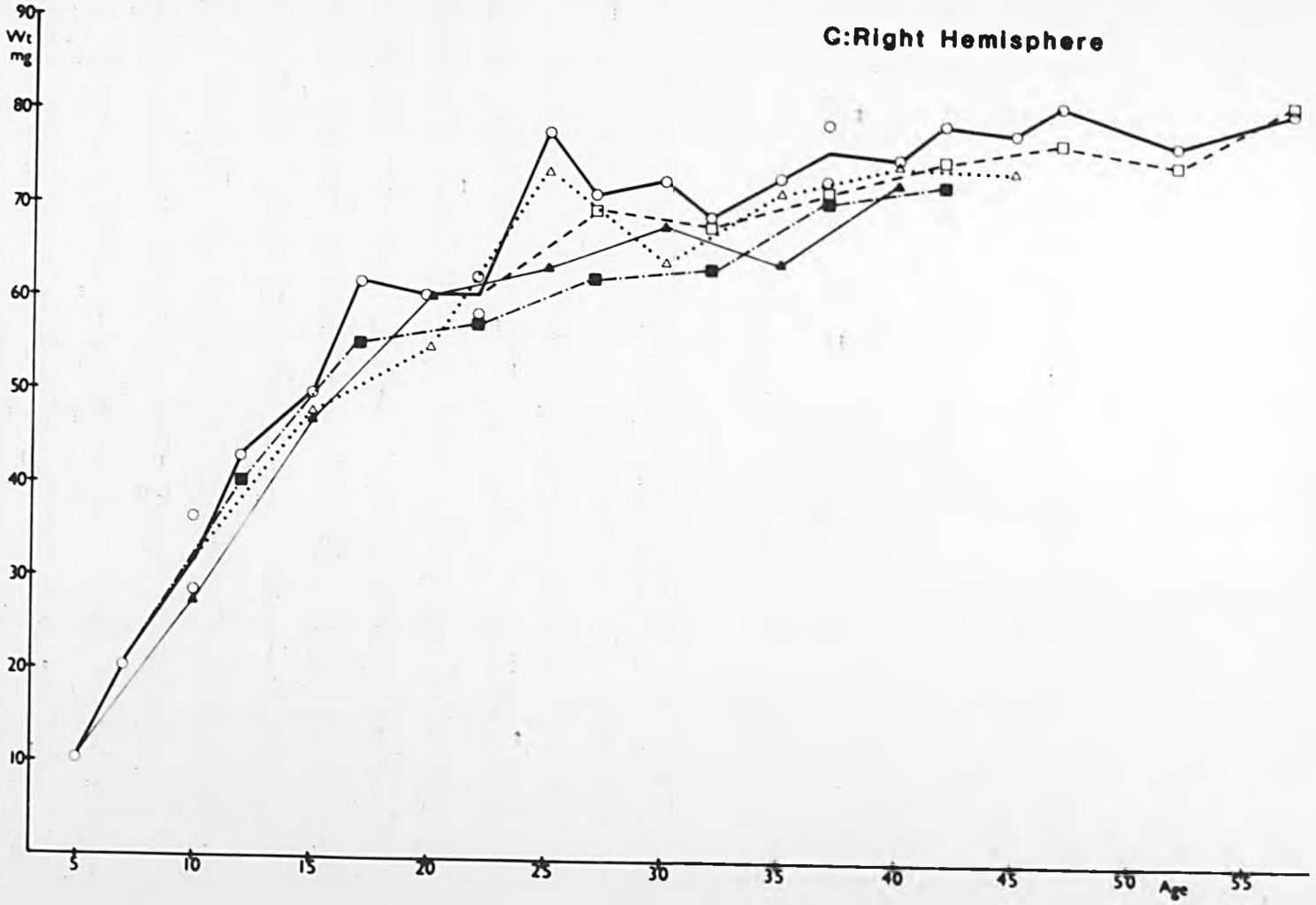
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CHAPTER FOUR

CEREBELLAR HISTOLOGY AND PURKINJE CELL DATA

INTRODUCTION

In the experimental groups the weights of the left hemispheres were smaller than in controls and since the histology looked qualitatively normal it seemed likely that overall there would be fewer Purkinje cells in the deafferented cerebellar hemisphere. Several authors have quantified Purkinje cells in man (Braitenberg and Atwood, 1958), cat (Palkovits *et al.*, 1971a) and in rats (Inukai, 1928; Armstrong and Schild, 1970; Hillman and Chen, 1981a). Each method involved finding the area of the Purkinje cell monolayer and the density of the Purkinje cells within it. The area of the monolayer was consistently found by multiplying the sum of the measured sagittal length in individual sections by the intersection distance. However the method of measuring Purkinje cell density varied; either direct areal density measured in tangential sections (Braitenberg and Atwood, 1958; Armstrong and Schild, 1970; Palkovits *et al.*, 1971a); by finding the sagittal linear density and applying stereological corrections (Hillman and Chen, 1981a) or just by counting the linear density in sections every 45 µm across the cerebellum (Inukai, 1928). However the method used here uses a formula devised to measure synaptic area on neurons (Mayhew,

1978) in which the density per unit area is dependent on the measured linear density and the mean diameter.

METHODS

Purkinje cell data for the left cerebellar hemisphere were collected on a Summagraphics Bit Pad One connected to a Commodore CMB 4043 computer using a Wild microscope with a side arm attachment. At low magnification the length of the interface between the granular and molecular layers was measured in each section and the total length multiplied by the intersection distance to give the area of the Purkinje cell sheet. At a higher magnification all Purkinje cell profiles were counted, since no single cell will appear in two sections, and the diameters were obtained from the circumference of cells containing nucleoli.

The density of Purkinje cells per unit area of monolayer was obtained by dividing the measured linear density by the mean Purkinje cell diameter (see Mayhew, 1978). The product of this density and the area gives the total number of Purkinje cells. The Purkinje cell numbers and diameter were measured on only two slides for each left cerebellar hemisphere. These were generally 800 µm and 2000 µm from the first section.

DISCUSSION OF THE METHOD

To verify this method, sections from adult cerebella (3.3×10^5 cells) were quantified and the results were compared and found

to agree with those available in the literature (Inukai, 1928; Armstrong and Schild, 1970; Hillman and Chen, 1981a). Also since the procedure is lengthy and to enable the maximum number of experimental groups to be quantified it was essential to find the minimum number of slides necessary to give consistent results. Initially sections were taken every 50 µm and the molecular/granular layer interface measured at high power, the difference between the results thus obtained and those from sections every 200 µm measured at low magnification was less than 2%. Also the measured linear density of Purkinje cells was remarkably constant in each section, with minimal variation from medial to lateral parts of the cerebellum. The standard deviation of this density within each individual hemisphere was less than 0.5%. Therefore calculating a mean Purkinje cell density and number using values from only two measured slides consistently obtained results less than 4% different than if every slide had been measured. These differences were considered to be within biological variation between animals as well as operator error in collecting the data.

The Purkinje cell density was not calculated by measuring the areal density in sections tangential to the Purkinje cell monolayer because if sagittal sections are cut the areas of Purkinje cell monolayer seen tangentially are very small and tend to occur more frequently laterally and at the basal corners of the

folia. Since the linear Purkinje cell density varies with position in the folium (Braitenberg and Atwood, 1958; Armstrong and Schild, 1970) the overall density obtained will depend on where the tangential areas lie. Also if some tissue was sectioned tangential to the Purkinje cell layer the total monolayer area cannot be derived. Therefore the mean Purkinje cell number would have to be calculated using Purkinje cell layer area and density from different animals. Despite proposals by Palkovits et al. (1971a), Smolyaninov (1971) and Altman and Winfree (1977) that Purkinje cells lie in a geometrical array and Eccles et al. (1967) finding different densities in the sagittal and coronal planes other studies have been quite unable to find any regularity in the Purkinje cell spatial arrangement (Braitenberg and Atwood, 1958; Armstrong and Schild, 1970; Palay and Chan-Palay, 1974). The use of sagittal sections was also supported because both Braitenberg and Atwood (1958) and Armstrong and Schild (1970) found the mean Purkinje cell density was the same in sagittal, coronal and horizontal planes.

The quantitative methods of both Inukai (1928) and Hillman and Chen (1981a) involved sagittal sections. Inukai (1928) did not use stereological corrections since he was working before they were devised. However, Hillman and Chen (1981a) used the correction factor of Abercrombie (1946) probably because they were using 50 µm sections. Stereological corrections were not considered

necessary in this study because underestimation of numbers due to the Holme's effect does not occur unless the section thickness is greater than 20% of the mean diameter being measured (Elias et al., 1971). Also Abercrombie's correction is not essential unless the diameter of the object being counted is less than ten times the section thickness (Mayhew, 1979).

When the linear Purkinje cell density was measured all Purkinje cell profiles were counted, not just those containing nucleoli because this has been found to give a more reliable estimate of neuronal numbers (Mayhew and Momoh, 1973).

Purkinje cell numbers and diameters were measured in animals aged 3, 5, 7, 10 or 22 days at operation which survived 35 days post operatively. Also, animals pedunclotomised at 5 days of age which survived for 5, 15 or 25 days were analysed to assess whether the Purkinje cell numbers and diameters were related to the left cerebellar hemispheric growth curve. Neonates operated on at day 7 which survived 5 or 15 days were also studied to see the relation between cerebellar development and Purkinje cell numbers. The 25 day survival time was left out because the left hemisphere growth curve flattened by day 17 and it was considered that the 15 and 35 day survival times were sufficient. Further analysis was made on animals aged 10 days at operation

which had survived for 10 days post operatively because these animals were a direct comparison to those pedunclotomised on day 5 which had survived 15 days. Also in the group receiving pedunclotomy aged 10 days the growth curve flattened after a 10 day survival.

STATISTICS

The mean Purkinje cell numbers in comparable experimental and control groups were analysed using a Students t-test because Von Euler's central limiting theorem states that the distribution of very large numbers is likely to approach normality. Within any group of animals either experimental or control a mean Purkinje cell diameter and standard deviation was found for each animal. It is known that mean values do have a normal distribution therefore the mean of these means (i.e. the mean of any given group) can be subject to a t-test. However, this is only valid if the diameter distribution is the same for both experimental and control groups. This can be tested by analysing the distribution of the standard deviations for each group. The distribution of standard deviations is not normal because they cannot be negative, while the range of a normal distribution lies between plus and minus infinity. Therefore, the standard deviations must be put through a variance stabilising transformation in order that the standard deviation distribution will approach normality. The

logarithms of the standard deviations for each group were found and a mean and standard deviation was calculated. These new values were analysed with a t-test. Logarithms are used because the logarithms of small numbers are negative therefore this extends the lower end of the standard deviation distribution further out below zero which makes it similar to a normal distribution.

RESULTS

CEREBELLAR HISTOLOGY

Adult Cerebella

The left cerebellar hemispheres from animals aged 3, 5, 7 and 10 days at pedunclotomy which survived 35 days post operatively all had a similar appearance. The total area of a section was smaller than in controls but the pattern of foliation was relatively normal and individual lobules could be recognised. However, each lobule looked shorter dorsoventrally and to have less minor sublobules and cortical folding than in the control animals (Fig. 4.1). In addition, the cerebella in the experimental groups had the apparently normal organisation of 3 cortical layers and a central medulla of white matter (Fig. 4.1).

The molecular layer contained many parallel fibres with scattered basket and stellate cells as were found in the normal cortex. Also parts of Purkinje cell

Figure 4.1

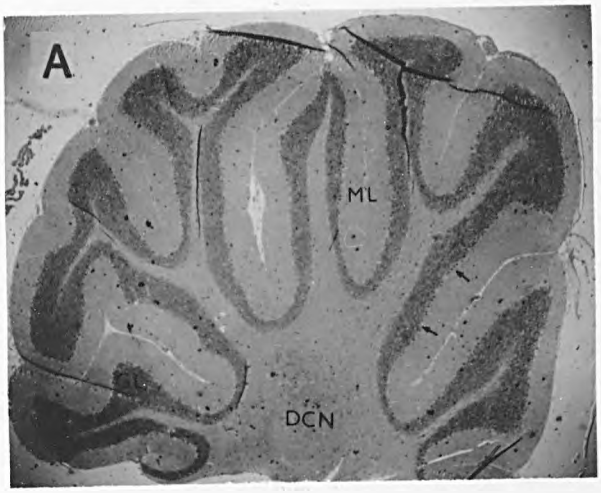
A. A sagittal section through the left hemisphere of a normal 38 day old cerebellum, approximately 1 mm from the medial edge of the hemisphere showing the pattern of lobulation and the three cortical layers:- molecular layer (ML), granular layer (GL) and the Purkinje cell monolayer (arrowed). Also neurons of the deep nuclei (DCN) can be seen (x 15).

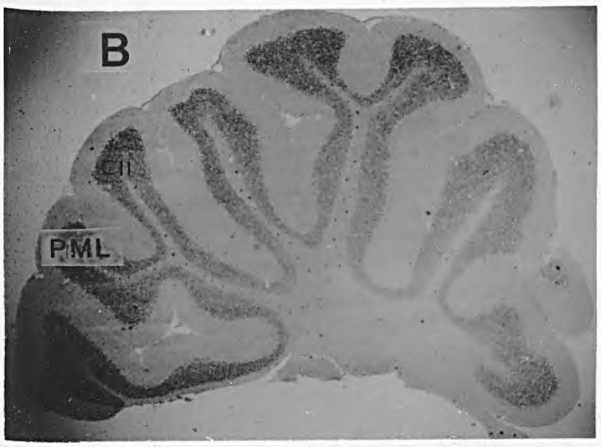
B. A section of the left hemisphere from an animal surviving 35 days after pedunclotomy on day 3 and it is in the same mediolateral plane as A. The dorso-ventral extent of the lobules is smaller than in the control cerebellum and both crus II (CII) and the paramedian lobule (PML) are considerably reduced (x 15).

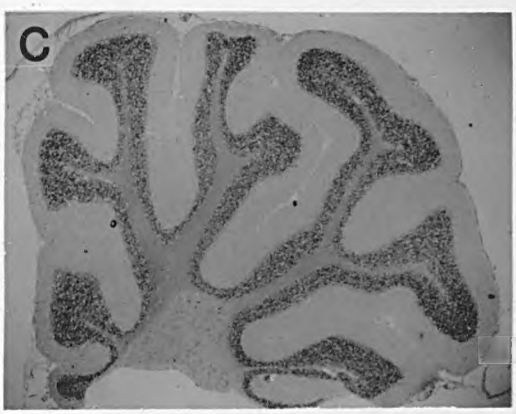
C. The left hemisphere of an animal aged 5 days at pedunclotomy which survived 35 days and is also 1 mm from the medial edge of the hemisphere. The size of the section is much smaller than the control seen in A but the cortex and pattern of lobulation looks normal (x 15).

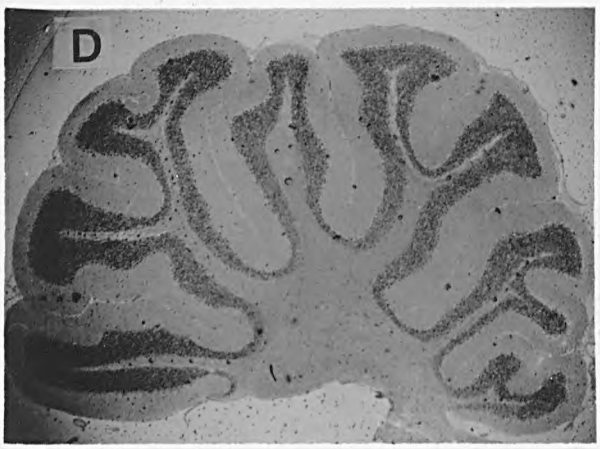
D. A section from the left hemisphere of an animal 35 days after pedunclotomy on day 10. Apart from a reduced dorsoventral height the cortex and lobules are quite normal in appearance (x 15).

E. Thirty-five days after pedunclotomy on day 7 the dorsoventral height of the lobules is considerably reduced although the cortical layers look normal (x 15).


A
ML
DCN


B
CII
PML


C


D

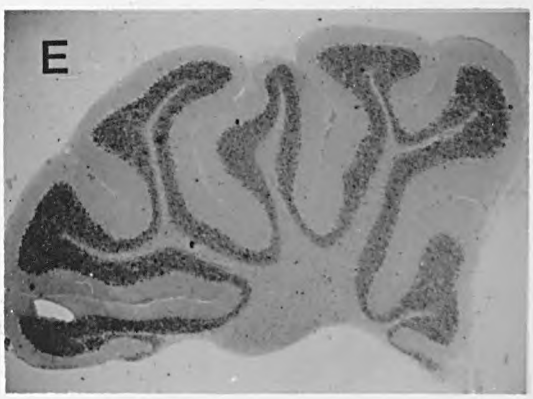
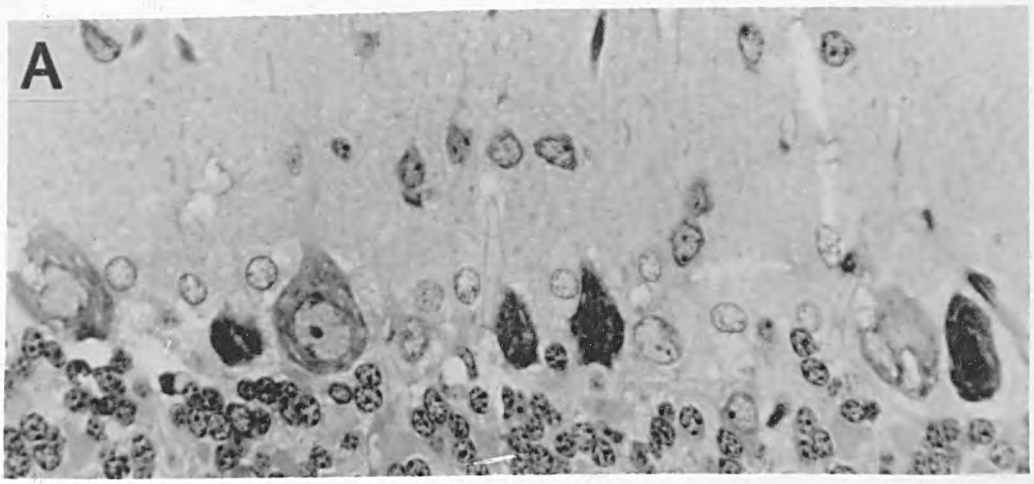
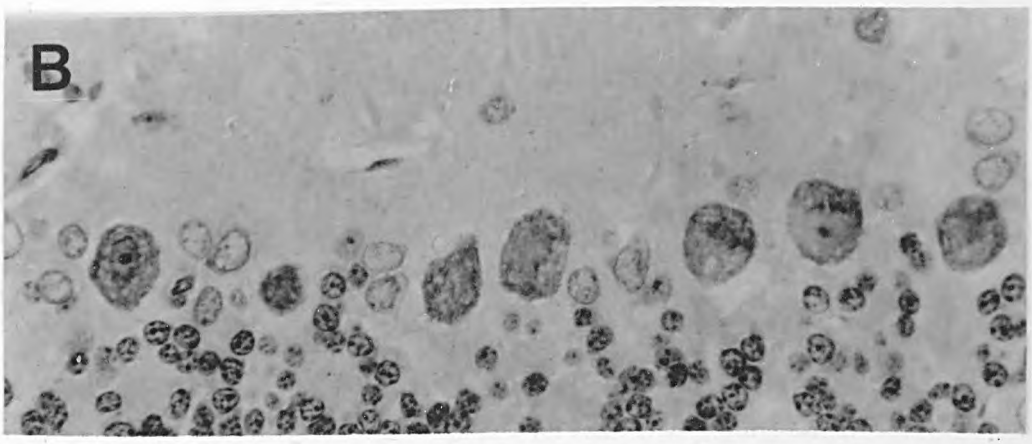

E

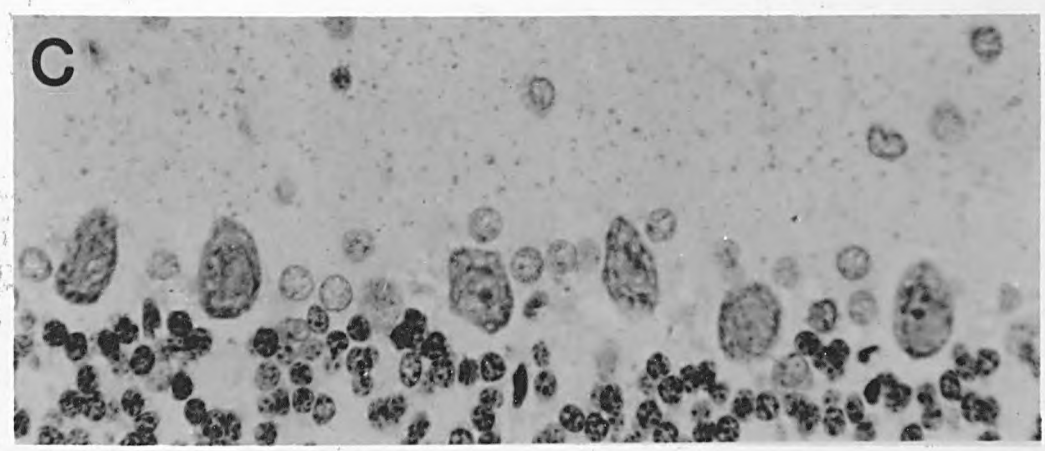
Figure 4.2

Figure A shows the Purkinje cell layer of crus I from a 42 day control left hemisphere. Figures B-D show the normal appearance of the Purkinje cell layer, also in crus I, from animals aged 5(B), 7(C) and 10(D) days at operation which have survived 35 days (x 620).

These can be compared with figures 4.6 and 4.9 which show the abnormal Purkinje cell layer in those animals which survived 5 days post operatively.


A


B


C

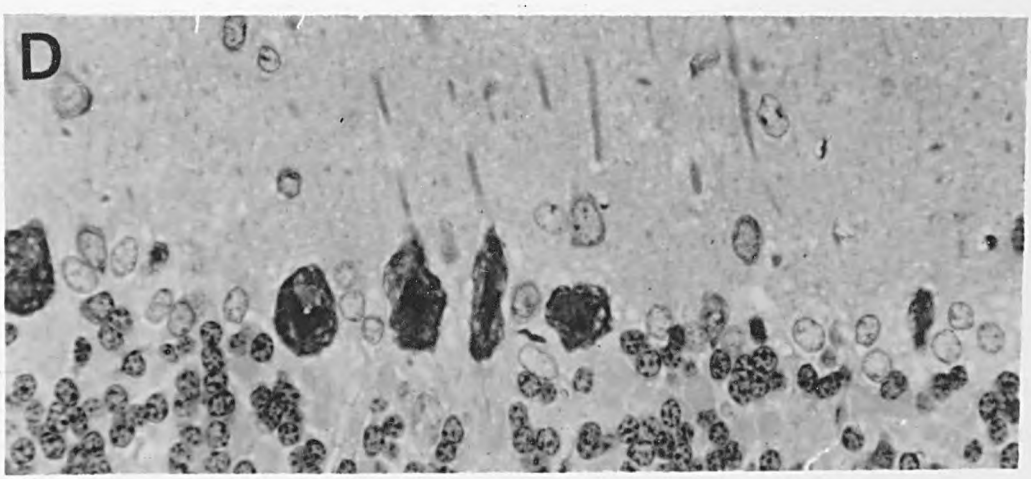

D

Figure 4.3

Photograph A shows the granular layer of crus II from a 42 day cerebellum, in which the clumps of granule cells are separated by mossy fibre glomerular 'islands'.
In figures B-D the granular layer of experimental left cerebellar hemispheres, the granule cells are more tightly packed than they are in the control cortex.

B = 3-35 operated paramedian lobule
C = 7-35 operated crus II
D = 10-35 operated crus II

(x 620)

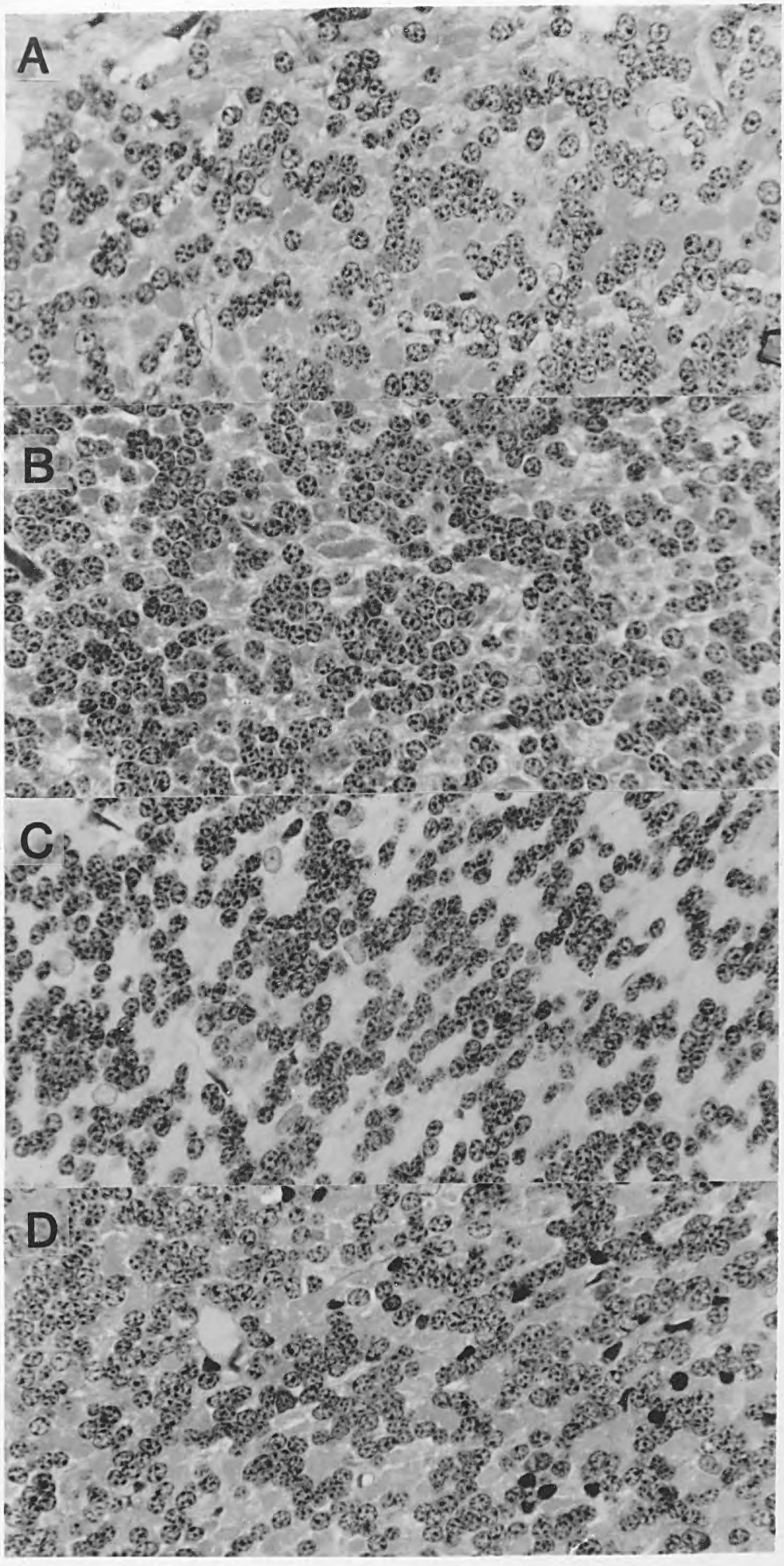
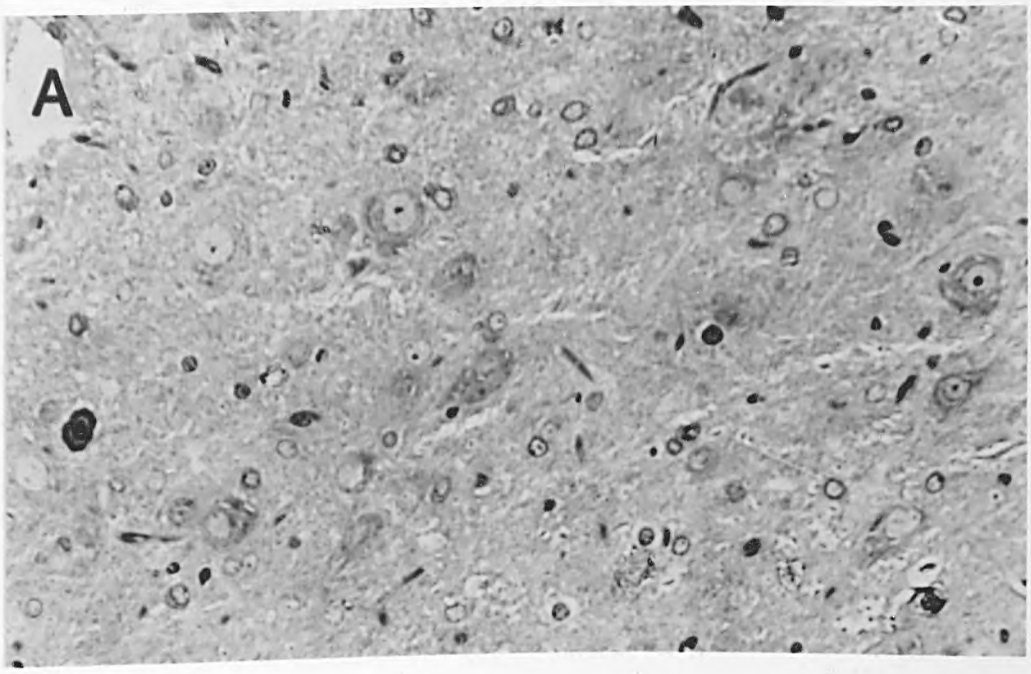
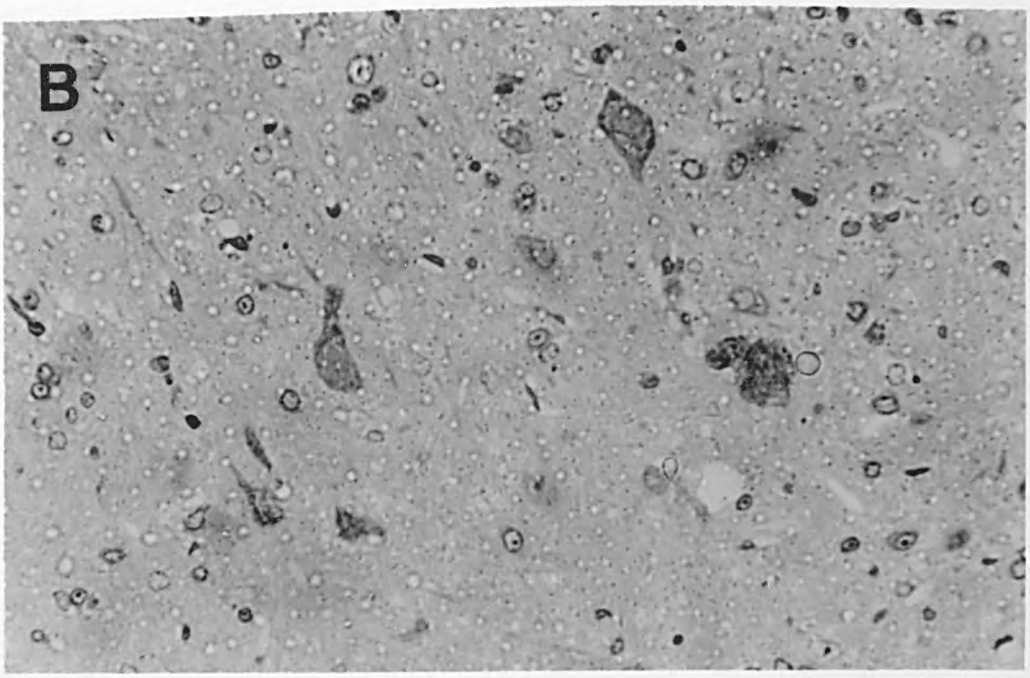
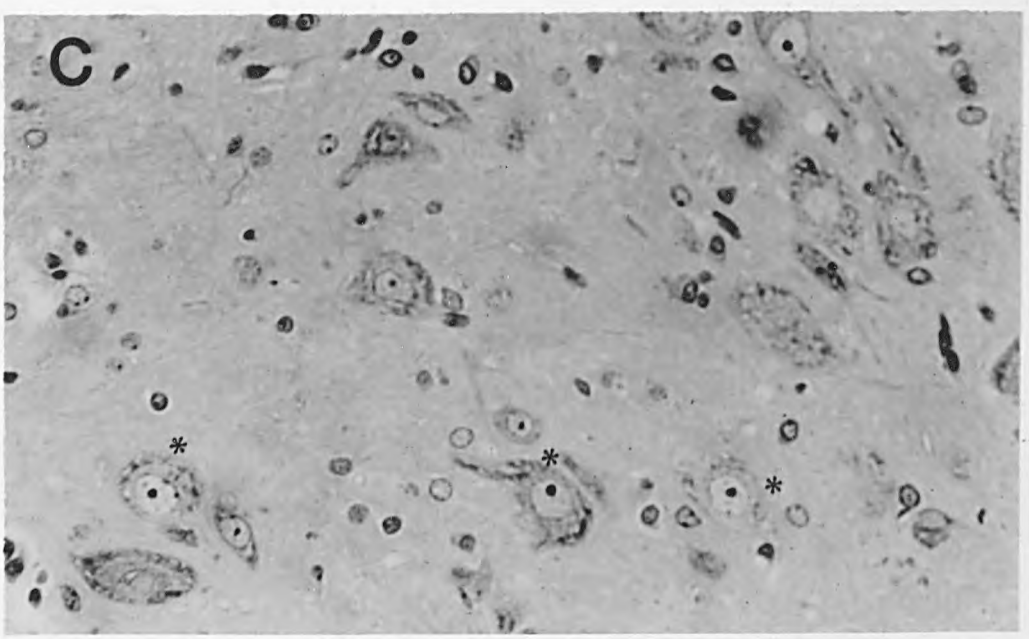

A
B
C
D

Figure 4.4

Photograph A shows the normal deep cerebellar nuclei of a 38 day rat. Thirty-five days after pedunclotomy on day 3 (B) there are considerably fewer neurons in the deep nuclei. In photograph C (5-35) some of the deep nuclear neurons exhibit chromatolysis (asterisked). (x 380)


A


B


C
*
*
*

dendrites were visible ascending through the layer and were the same in both experimental and control groups. The Purkinje cells lay in a monolayer and were spaced in the same manner as the controls, with fewer cells in the depths of the sulci than on the apices of the folia. Also their morphology appeared normal with whorl-like cytoplasm around a large pale-stained central nucleus containing a prominent nucleolus (Fig. 4.2). The granular layer contained many small granule cells but in the experimental groups these looked more closely packed with fewer gaps for ascending or descending fibres or mossy fibre glomeruli (Fig. 4.3). Perhaps because of this the Golgi cells seemed more prominent. The central white matter also had a normal appearance in the pedunclotomised hemispheres, however there may have been fewer large neurons in the central deep nuclei (Fig. 4.4).

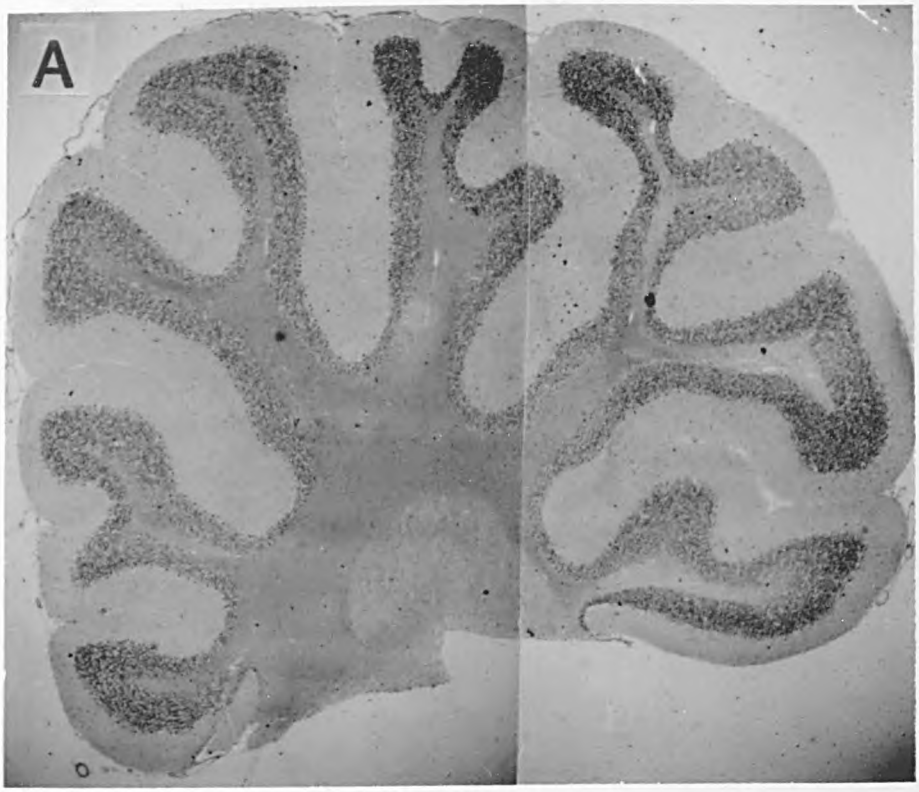
The histology of the left hemispheres from those animals which underwent pedunclotomy on day 22 and survived 35 days, was different from the other experimental groups. The pattern of lobules was much more like the controls, however there were gaps between some of the folia which were not normally seen. In the cortex there were the usual 3 layers; however both the molecular and granular layers were thinner than in the control hemisphere and there were considerably fewer granule cells. These were widely spaced and were often singly placed rather than in clumps (Fig. 4.5). However the white

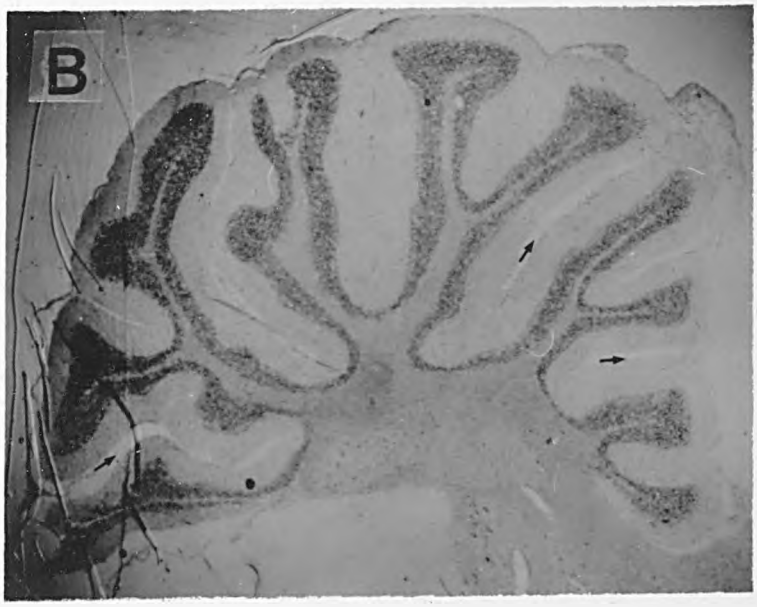
Figure 4.5

Animals Pedunclotomised Aged 22 Days

A and B (x 22) show the considerable difference in the size of the left hemisphere 35 days after pedunclotomy (B) compared with the control (A). In the experimental hemisphere (B) both the molecular and granular layers are thinner and there are gaps between the folia (arrowed). But the pattern of foliation is no less complex than normal.

C and D (x 227) illustrate the reduction in the depth of the molecular layer in dorsal crus I 35 days after the pedunclotomy (D) when compared with the same region of the control hemisphere (C).


A


B

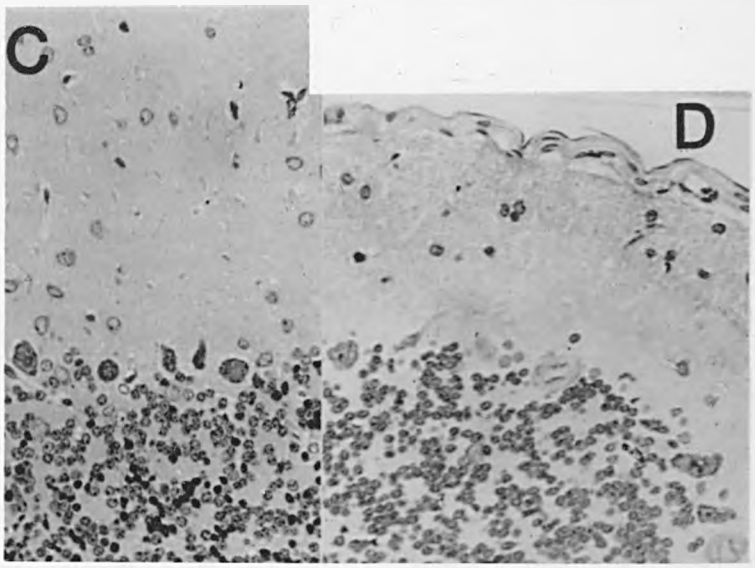

C
D

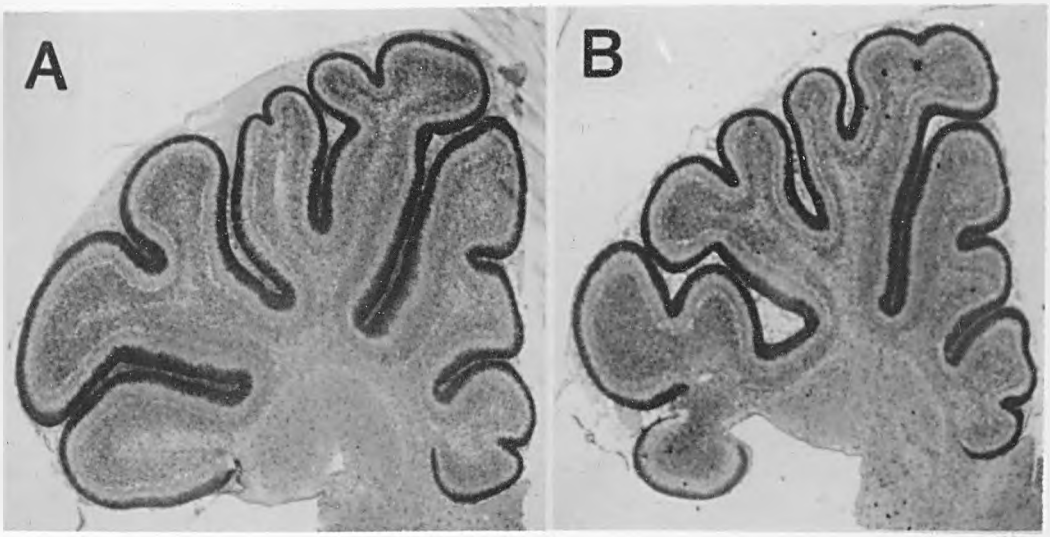
Figure 4.6

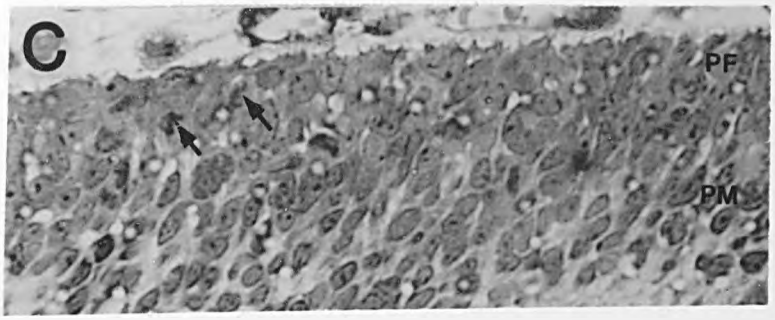
Left Cerebellar Hemisphere from Neonatal Rats
Pedunclotomised Aged 5 Days and
Surviving 5 Days

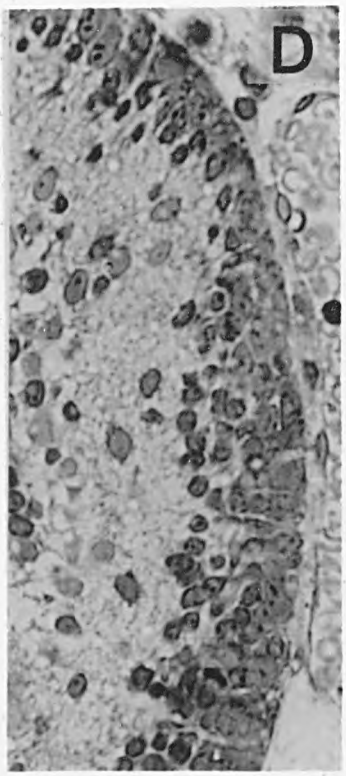
Photograph A shows a sagittal section through a normal left hemisphere 0.6 mm lateral to the medial edge of the hemisphere. After pedunclotomy (B) the hemisphere is smaller but retains the same histological layers as the control cerebellum although the folia are not as well developed. (x 22)

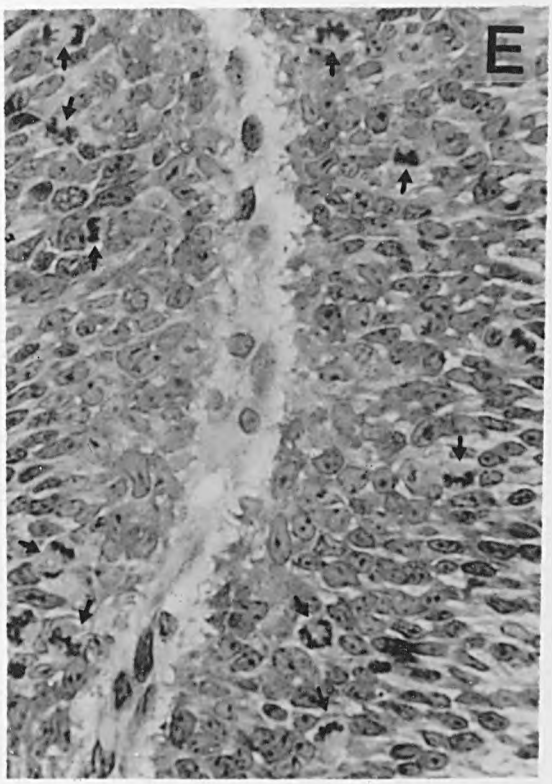
Figure C shows the normal external granular layer of crus II containing proliferative (PF) and premigratory (PM) zones. Also a few mitotic figures are visible (arrowheads). After pedunclotomy the external granular layer becomes uneven, which can be seen in crus I (D) and there are more mitotic figures, shown at the junction of lobulus simplex and crus I (E). (x 620)

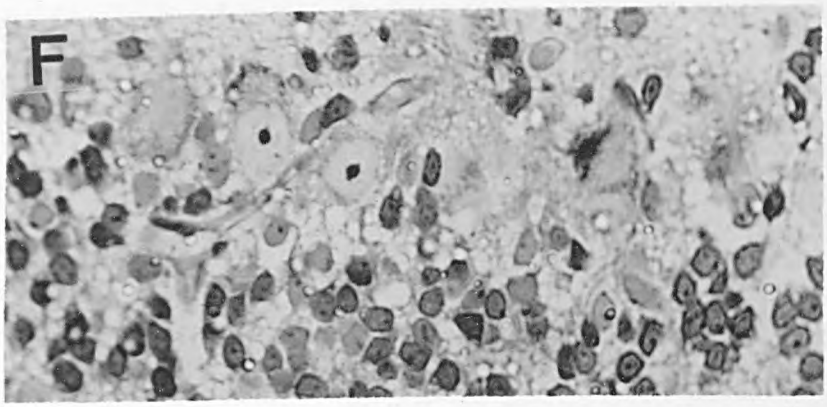
In the control cerebellum the Purkinje cell layer is a monolayer by day 10 (F) while after pedunclotomy the neurons are unevenly spaced. This can be seen in the lobulus simplex with either a Purkinje cell bilayer (H) or a large gap between adjacent cells (G). (x 620)

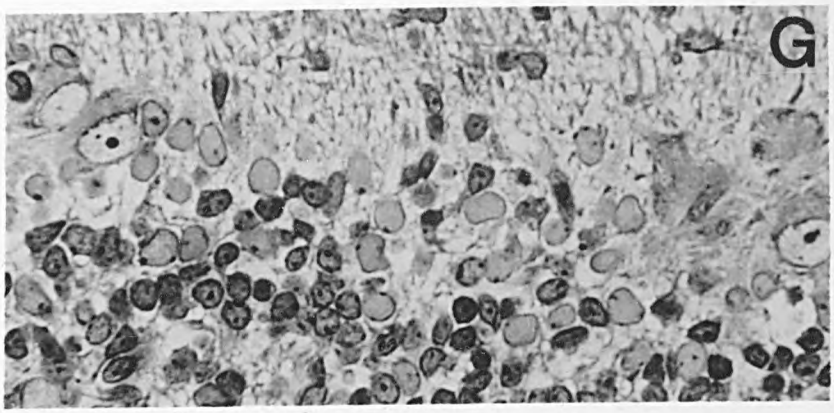

A
B

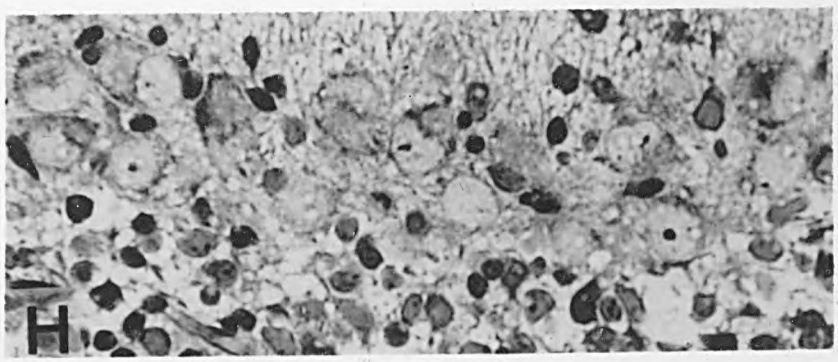

C
PF
PM


D


E


F


G


H

medulla and the neurons of the deep nuclei had the same appearance in both the experimental and control groups.

Groups Pedunclotomised at 5 and 10 Days of Age

The cerebellar histology of neonates which underwent pedunclotomy aged 5 days was observed after 5, 15 and 25 days survival. In the normal 10 day neonate the cerebellar folia are quite recognisable, however they were much less clear in those animals pedunclotomised at 5 days which had survived 5 days. The cortex contained four layers:- the external granular, molecular, Purkinje cell and internal granular layers. In the control animal aged 10 days the external granular layer was in two zones, the outer proliferative layer of 4-5 cells thick and an inner premigratory zone of approximately 4 rows of bipolar cells. Normally this layer was of fairly constant thickness, however it was much less even in the experimental group being generally thicker on the sides of the folia and thinner at the apices. In both experimental and control neonates aged 10 days mitotic figures could be found in the external granular layer, however they were more numerous in the pedunclotomised rats (Fig. 4.6). In both normal and experimental animals aged 10 days the molecular layer was thin and contained basket cells and descending granule cells. The Purkinje cells have basically formed a monolayer by day 10 and they were closely packed so that

Figure 4.7

A. The superficial part of the molecular layer and the scarce monolayer of the remaining external granular layer in a normal 20 day rat. (x 380)

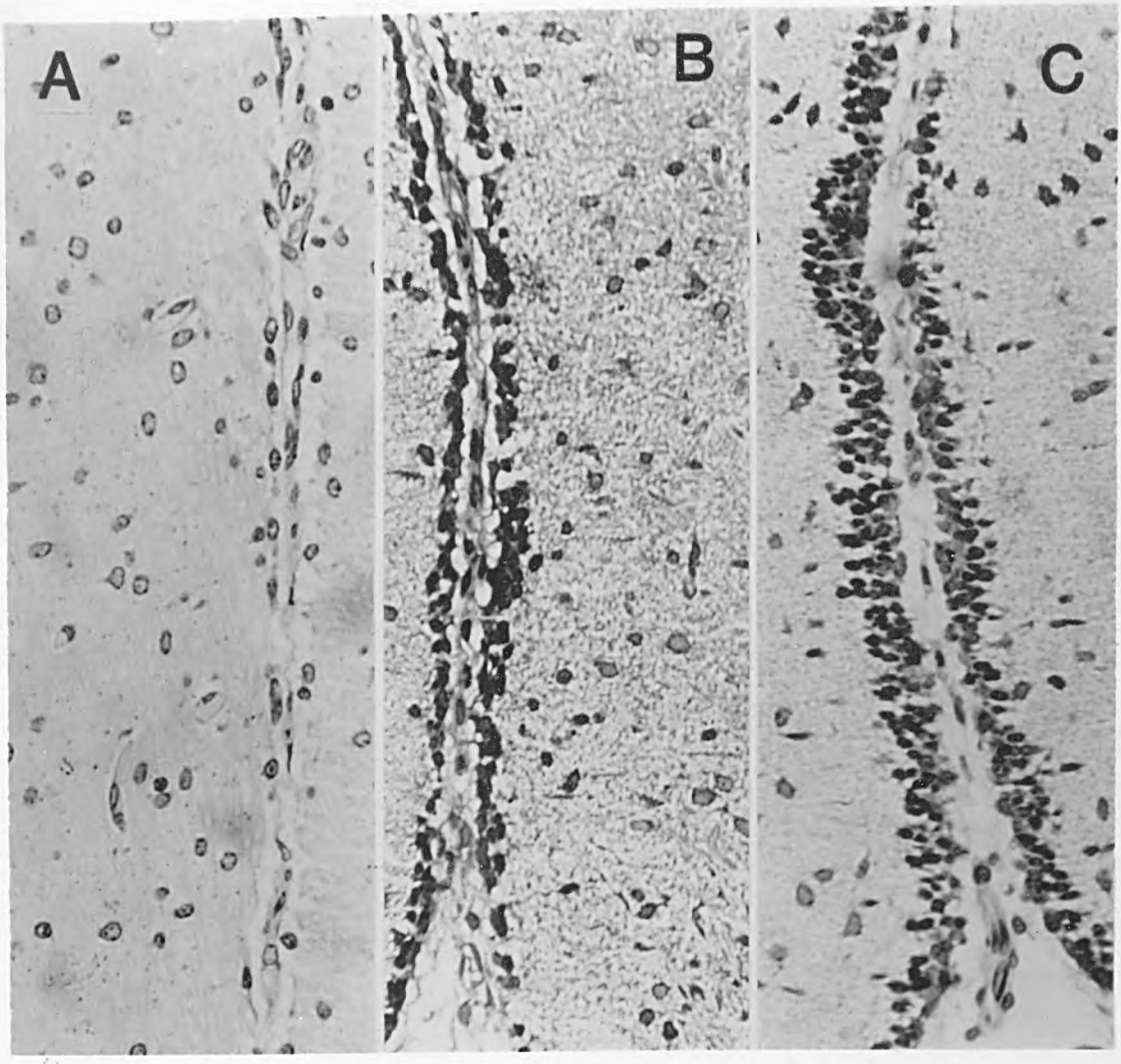
B. The remaining external granular layer in crus I of the left hemisphere from a neonate pedunclotomised aged 5 days and surviving 15 days is still a complete monolayer. (x 380)

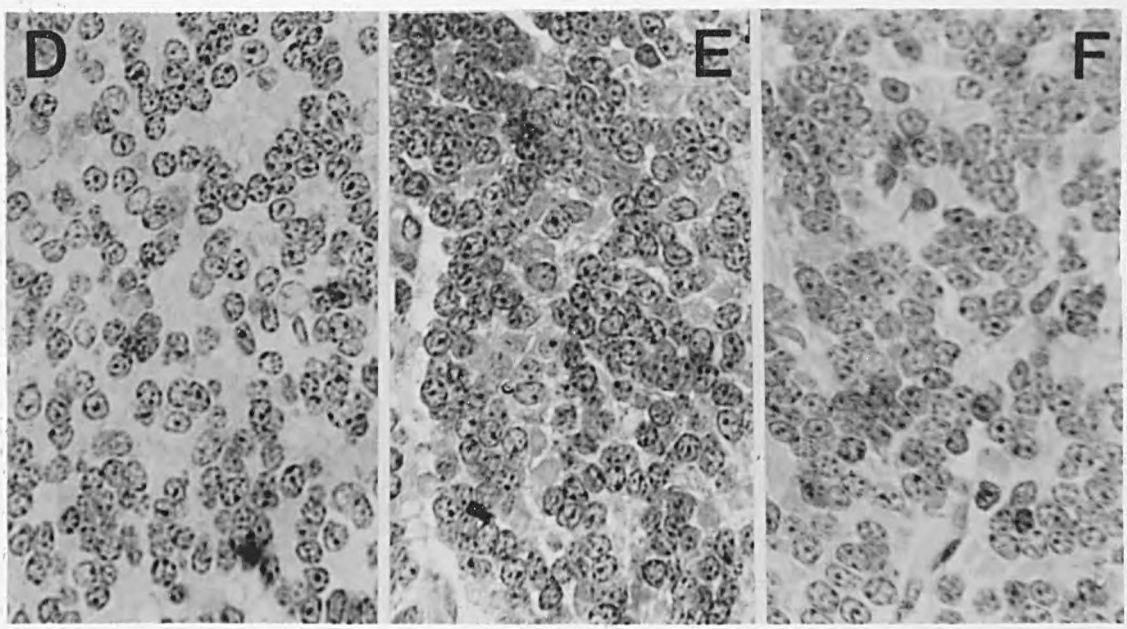
C. In the paramedian lobule 10 days after pedunclotomy on day 10, the external granular layer is still as much as 4 cells thick. (x 380)

D. The granular layer of crus I in the 20 day old cerebellum. (x 620)

E. After pedunclotomy on day 5 and 15 days survival the granule cells in the lobulus simplex are more densely packed leaving few spaces for the developing mossy fibre rosettes. (x 620)

F. The granular layer in the lobulus simplex 10 days after pedunclotomy on day 10 is also more densely packed with granule cells. (x 620)


A
B
C


D
E
F

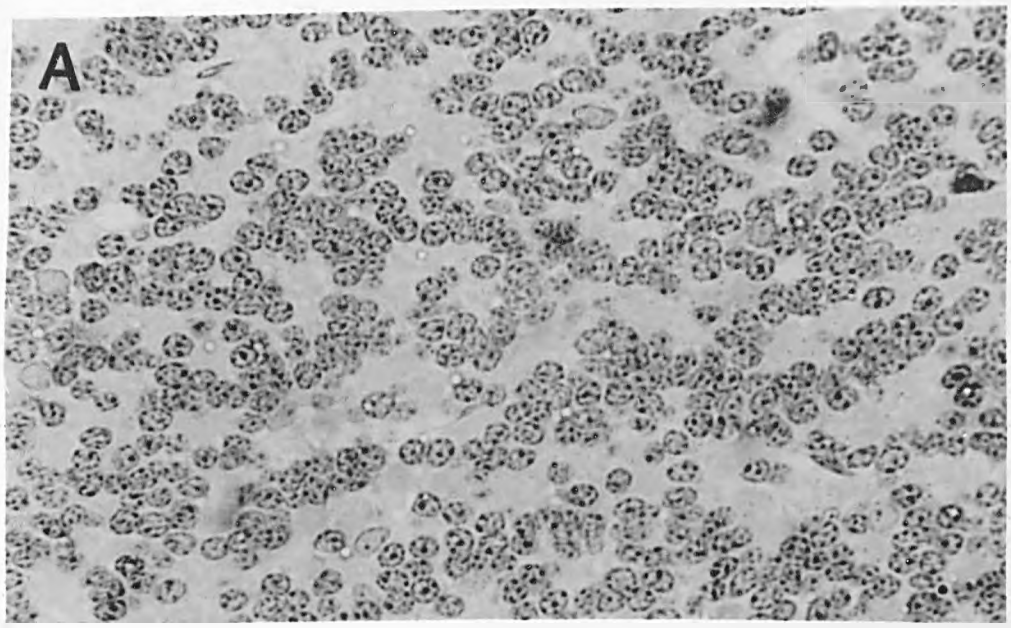
adjacent cells often touch. Also most cells had a large apical cone of cytoplasm projecting towards the molecular layer. In the experimental animals the cells were less regularly placed being not always in a monolayer and in some places there were gaps in the layer with no cells. In addition the Purkinje cell polarity was less marked in the experimental animals and some cells possessed prominent basal cytoplasm (Fig. 4.6). In both control and experimental groups the internal granular layer contained thinly packed granule cells and small immature Golgi cells. The white matter was tenuous and contained many glial cells and looked the same in both normal and pedunclotomised cerebella.

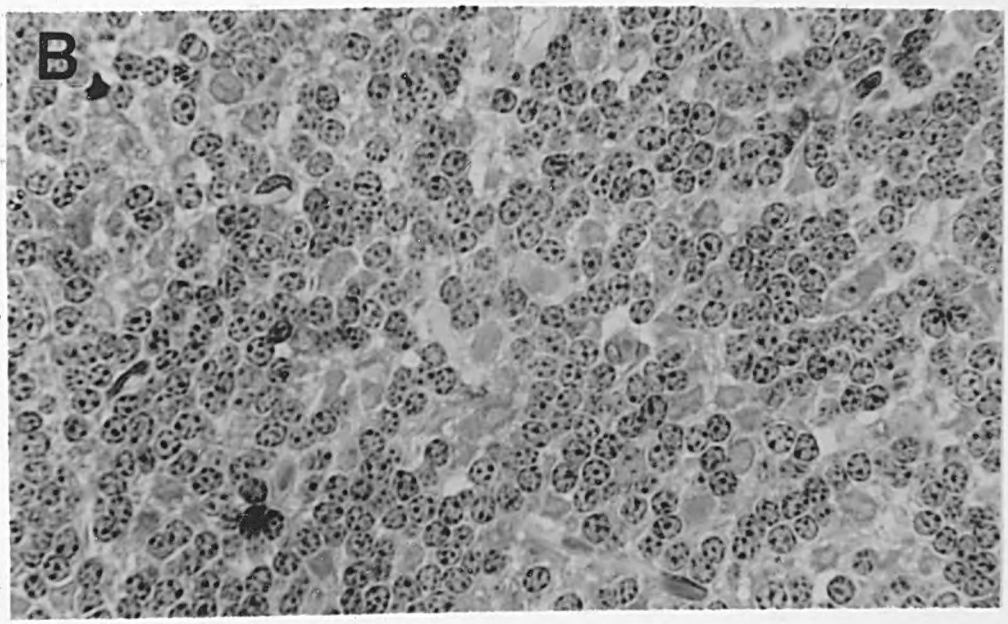
By 20 days post partum the cerebellum had developed an almost adult appearance and the different folia were recognisable in both control and experimental animals which had undergone pedunclotomy on day 5 and survived 15 days or on day 10 and survived 10 days. In the control animals the external granular layer was not a complete layer, however in the paraflocculus it was still 2-3 cells thick. In both experimental groups it was thicker. In those rats which underwent pedunclotomy aged 5 days, the external granular layer was a complete monolayer in the anterior lobe and 2-4 cell layers thick in the posterior lobe, while in those animals pedunclotomised on day 10, it was 2-3 cell layers thick in most lobules (Fig. 4.7). The molecular

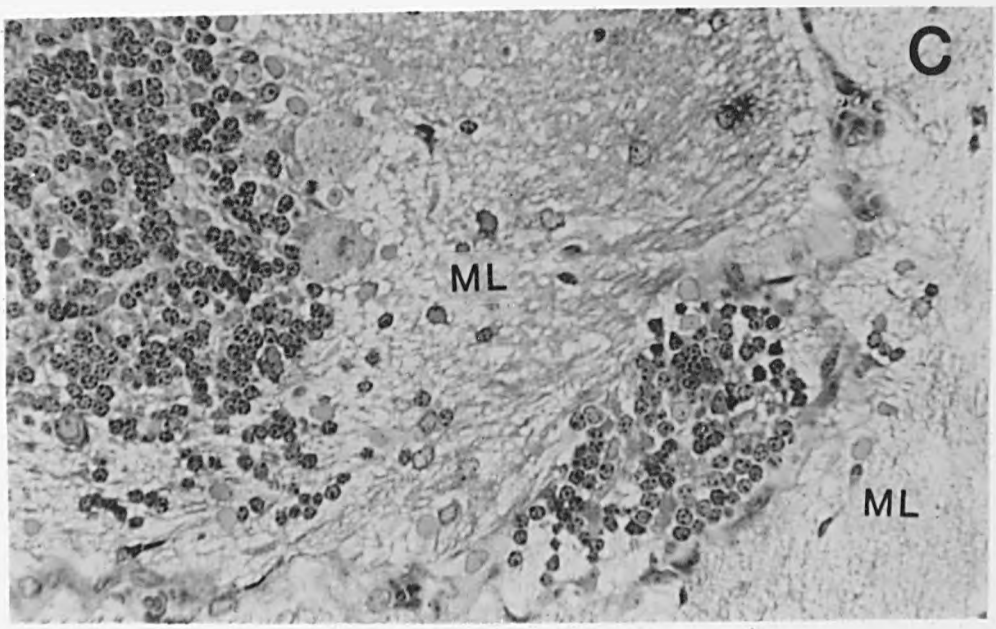
Figure 4.8

Photograph A shows the granular layer in crus II from a 30 day cerebellum (x 620) and B shows a similar region of the granular layer on day 30 but following pedunclotomy on day 5 (x 620). In the experimental cerebellum the islands of mossy fibre rosettes are much less evident.

Micrograph C shows a group of ectopic granule cells in the left hemisphere paramedian lobule from a rat pedunclotomised aged 5 days which survived 25 days. The ectopic island can be seen between two adjacent molecular layers (ML). (x 380)


A


B


C
ML
ML

layer of both experimental groups was the same depth as in the control and also contained basket, stellate and descending granule cells. The Purkinje cells lay in a monolayer in both experimental and control animals aged 20 days and they have an adult cytological appearance. But in those rats which underwent pedunclotomy on day 5 the Purkinje cells were less evenly spaced than in either the control or 10 day pedunclotomised groups. In experimental and control groups the internal granular layer contained many small granule cells with scattered Golgi cells. In both experimental groups the granule cells appeared more closely grouped than in controls and the mossy fibre glomeruli were much less evident (Fig. 4.7). The central white medulla was identical in experimental and control animals and neurons of the deep nuclei were found in the more medially placed sections.

After 30 days post partum the cerebellum was almost completely developed and both the normal and experimental rats pedunclotomised on day 5 had the adult pattern of lobules. The external granular layer had disappeared in both groups and the molecular layer contained basket and stellate neurons but there were no descending granule cells. The Purkinje cell monolayer was not different in the experimental group. In the granular layer the granule cells were grouped together between spaces for the mossy fibre glomeruli and this appearance was also found in the experimental group with

Figure 4.9

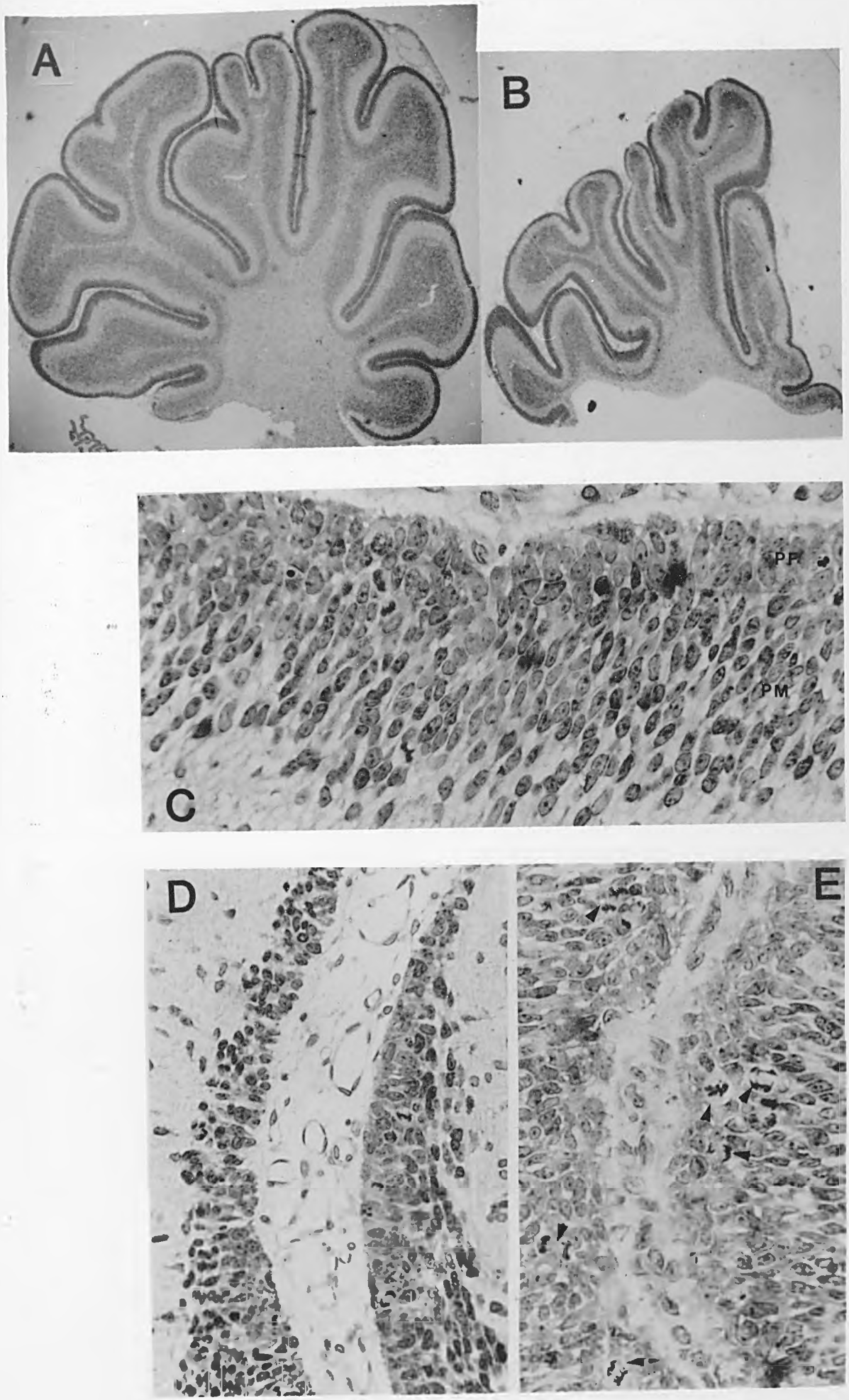
Cerebellar Histology from Neonates
Pedunclotomised Aged 7 Days
Surviving 5 Days

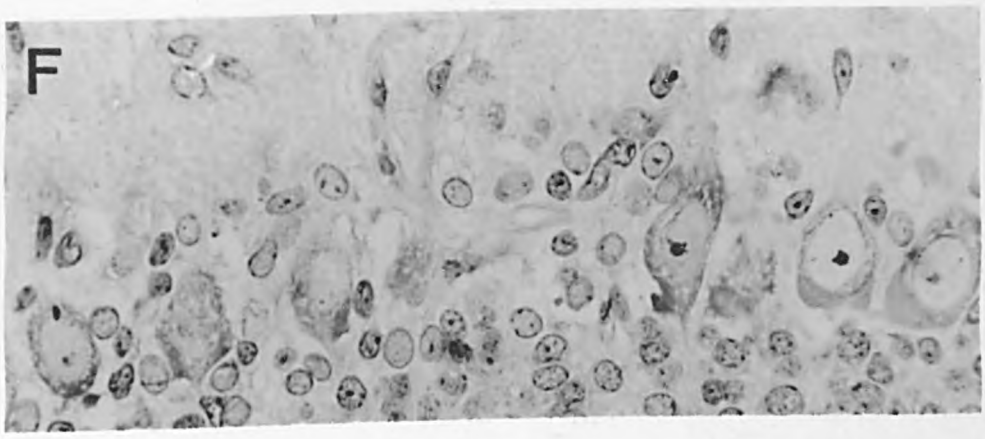
Following pedunclotomy on day 7, the left cerebellar hemisphere is considerably reduced in size and the folia are less well developed (B) than in the control cerebellum (A). (x 22)

Photograph C (x 620) shows the external granular layer of lobulus simplex in a 12 day cerebellum containing proliferative (PF) and premigratory (PM) zones. After pedunclotomy the external granular layer becomes uneven as can be seen in the lobulus simplex (D) (x 380) and contains more mitotic figures (arrowed) as is shown in the crus I (x 620).

By day 12 the Purkinje cells lie in a monolayer which can be seen in crus II (F), but after pedunclotomy the interneuronal spacing was uneven, for example in the paramedian lobule (G) in which only two Purkinje cells are present and lobulus simplex (H), also the somatic polarity was less pronounced (H). (x 620)

After pedunclotomy there are fewer neurons in the deep nuclei (J) when compared with the control (I). Also none of their nuclei contain the usual prominant nucleolus. (x 380)


A
B
PP
PM
C
D
E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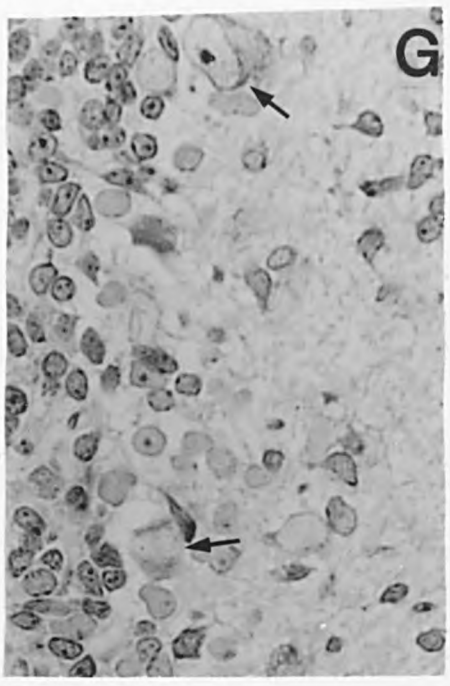
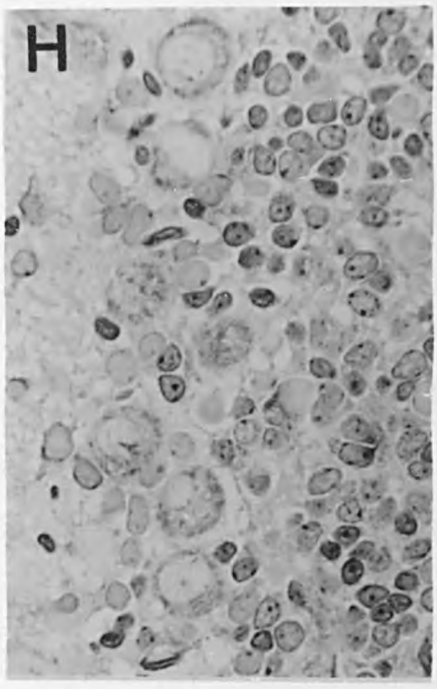
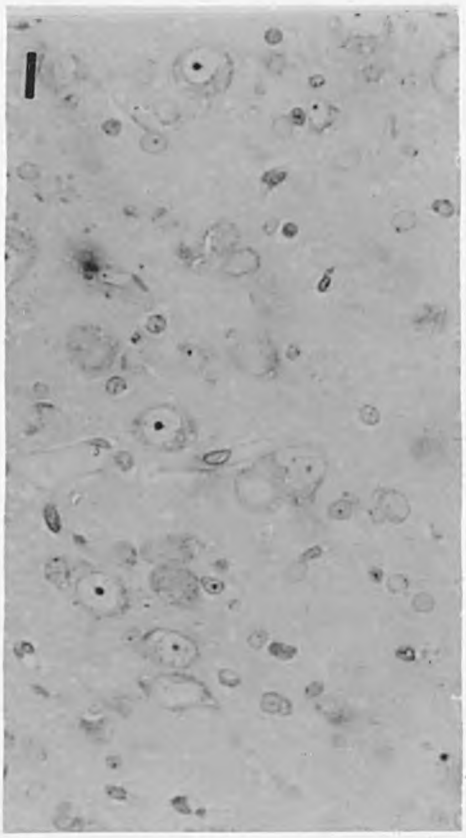
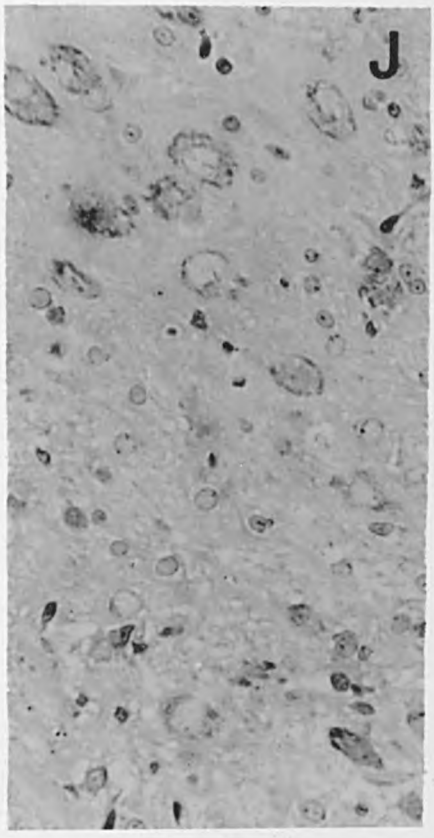

G


H


I


J

the exception that the glomerular gaps were less evident. The central white medulla and deep nuclear neurons appeared the same in both experimental and control groups. However, occasionally in the pedunclotomised cerebella some ectopic granule cells were found in the molecular layer lying in the depths of the sulci (Fig. 4.8).

7 Day Pedunclotomy Group

Animals which had undergone pedunclotomy on day 7 and survived 5 or 15 days post operatively were studied. The normal 12 day neonatal cerebellum had the same general structure as at day 10 but the external granular layer was thicker with the proliferative zone containing 5-6 cell layers and the internal granular layer contained more granule cells. In the experimental animals after a 5 day post operative survival the pattern of folia was less clear and individual lobules were not so easily recognised (Fig. 4.9). The external granular layer was unevenly thick in the experimental cortex and consisted of up to 8 layers in the outer proliferative zone and had more mitotic figures (Fig. 4.9). The molecular layer contained basket and descending granule cells in both control and pedunclotomised groups but the layer looked slightly thinner in the operated animals. After pedunclotomy the Purkinje cells lay predominently in a monolayer but many cells did not possess the apical-basal polarity of the normal cerebella. In the internal

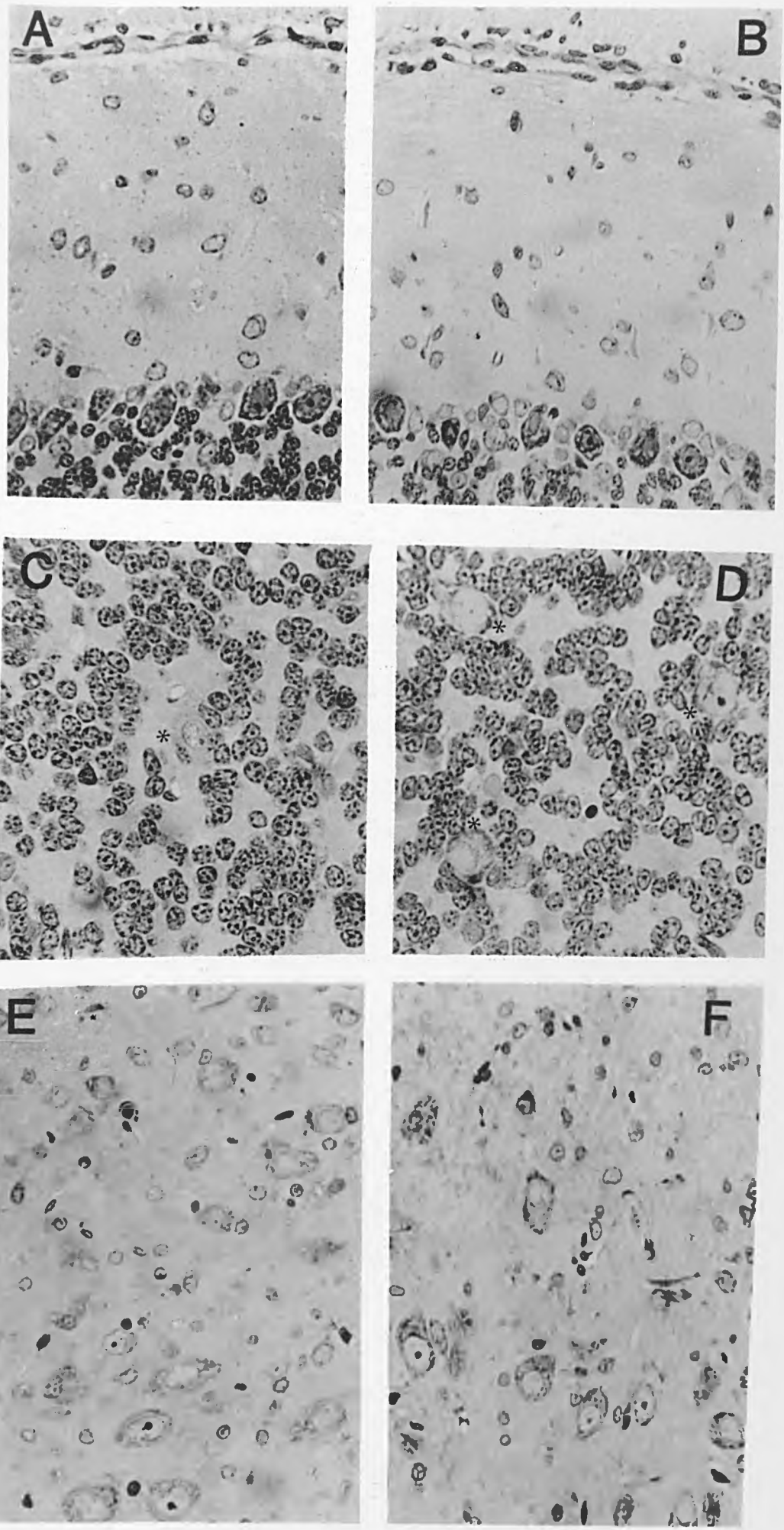
Figure 4.10

Cerebellar Histology Fifteen Days
After Pedunclotomy on Day 7

By day 22 the external granular layer has almost disappeared (A), but following pedunclotomy a persistent monolayer is retained (B). These micrographs from crus I also show that the molecular and Purkinje cell layers are the same in both control and experimental cerebella. (x 380)

The granular layer in the experimental animal (D) has a similar appearance to that of the control (C) with normal-looking glomerular 'islands'. However, Golgi neurons (asterisked) are more prominant in the pedunclotomised hemisphere. (x 620)

In the deep cerebellar nuclei there are slightly fewer neurons after pedunclotomy (F) than normal (E). (x 380)


A
B
C
D
E
F

granular layer of the experimental group the granule cells were grouped together more closely than normal. Neither experimental nor control left hemispheres had much central white matter, but there were fewer neurons in the deep nuclei of the pedunclotomised group.

By day 22 the cerebellum looked similar to that of day 20, however the external granular layer had decreased to a scarce monolayer in all lobules. But in the experimental animals the external granular layer was still a monolayer and even 2 cells thick deep in the sulci. In both experimental and control groups the molecular layer looked similar, it contained basket and stellate cells and a few still descending granule cells, and the Purkinje cell monolayer appeared the same (Fig. 4.10). In the internal granular layer of both groups the granule cells were clustered together and there were glomerular spaces even in the experimental rats. The central white medulla did not look different between the two groups except there were fewer deep nuclear cells in the pedunclotomised hemisphere.

PURKINJE CELL NUMBERS

The number of Purkinje cells was counted in only the left cerebellar hemispheres of experimental and control groups. The groups which were quantified were those animals aged 3 and 22 days at operation which had survived 35 days. Also animals of the 5 day operative age group

TABLE 4.1

The mean number of Purkinje cells in the left cerebellar hemisphere from animals which underwent pedunclotomy aged 3, 5, 7, 10 or 22 days and survived up to 35 days post operatively.

† = $p < 0.01$

* = $p < 0.001$

Age at Operation (Days)		Purkinje Cell Numbers per Left Cerebellar Hemisphere														
		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	72,885	10,865
	C													6	78,161	6,697
5	Op	6	68,696†	13,356				4	69,136†	12,933	6	59,406†	16,028	6	69,652	16,319
	C	6	94,059	9,977				4	97,391	8,895	5	87,326	5,621	6	81,313	6,899
7	Op	6	66,445†	21,809				5	62,004*	9,923				6	50,350*	2,991
	C	5	104,420	12,086				4	104,066	7,761				6	77,983	5,663
10	Op				6	75,078	15,580							6	79,498	8,983
	C				4	97,391	8,895							5	93,257	7,246
22	Op													3	83,376	18,436
	C													5	89,086	8,567

which survived 5, 15, 25 and 35 days post operatively and those of the 7 day group which had survived 5, 15 and 35 days. In addition animals which underwent pedunclotomy at 10 days post partum and survived 10 and 35 days post operatively were also analysed. The results are shown in Table 4.1.

Even in control groups there was quite large variation in the Purkinje cell numbers. They varied between 78,161 and 104,420 with standard deviations of between 6.5% and 10.5%. Apart from the 5 and 15 day survivals in the 7 day operative age group the Purkinje cell numbers did not appear to be age related. There were fewer Purkinje cells in the experimental groups irrespective of the age at operation or the survival time. However, the inter-animal variation was increased and the standard deviations were generally near 20%.

Both the experimental groups which underwent pedunclotomy at 3 and 22 days of age had 7% less Purkinje cells than controls, however these were not significant reductions. Animals aged 10 days at operation which survived 10 and 35 days had 23% and 15% reductions in the number of Purkinje cells but these were not statistically significant.

Animals which were aged 5 days when the pedunclotomy was performed all had a similar number of Purkinje cells except those animals which survived 25 days post

operatively. However the groups which survived 5 or 15 days post operatively had significantly fewer Purkinje cells than controls ($p < 0.01$), on average 30% less, while the lower number of Purkinje cells in the group surviving 35 days, 14%, was not statistically significant. In those animals which survived 25 days there were also 30% fewer Purkinje cells in the experimental group ($p < 0.01$) probably because the number in control animals was lower than for the other survival times.

The 7 day operative age group had on average 40% less Purkinje cells than controls which is highly significant ($p < 0.001$). However, within the group there was also a large difference between animals which survived 5 days and those which survived 35 days. In the control groups this difference was significant ($p < 0.01$) while in the experimental groups it was not.

PURKINJE CELL DIAMETERS

The Purkinje cell diameters were measured in the same groups as those in which the Purkinje cell numbers were counted and they were measured on only those Purkinje cell profiles containing nucleoli. The mean Purkinje cell diameters for each group are shown in Table 4.2.

The average adult Purkinje cell diameter is 20 μm

(Palay and Chan-Palay, 1974) and this value was obtained in all control groups which survived 35 days post operatively, with the exception of the controls from the 5 day operative group, which had a mean diameter of only 18.8 μm. The adult size of diameter was also obtained in normal young rats by day 20 i.e. the 5 and 7 day groups which survived 15 days. However, the Purkinje cell diameter was smaller in younger neonatal rats. The mean diameter for neonates aged 10 days was 16.7 μm and this had increased to 18 μm by day 12.

In the experimental groups the average Purkinje cell diameter in those animals aged 3 days at operation and surviving 35 days was not different from control. In the 5 day operative age group those animals which survived 5, 15 or 25 days had smaller Purkinje cells than normal, however the 35 day survival group had larger Purkinje cells than the controls. In the groups which survived 15 or 25 days the mean Purkinje cell diameters were 4%, but not significantly, smaller than the controls while the 5 day survival group had average diameters 7% smaller than normal ($p < 0.01$).

All animals which underwent pedunclotomy aged 7 days had smaller Purkinje cells than the controls. After 15 days the mean diameter was only 5% smaller than normal, however the 5 and 35 day survival groups had mean Purkinje cell diameters respectively 10% and 15% less, which were significantly different ($p < 0.001$).

The average Purkinje cell diameters of the 10 day operative age group with 10 day survival were only 2% smaller than the controls which was not statistically significant. However, in the group which survived 35 days the mean diameter was 12% below the control value ($p < 0.01$). After pedunclotomy at 22 days of age and 35 day post operative survival the mean Purkinje cell diameter was 11% smaller than in the controls ($p < 0.001$).

The type of diameter distribution for each group was the same for experimental and control animals (see Appendix VIII. In the normal adult rat the range of Purkinje cell diameters was 13-30 µm with the majority lying between 17 µm and 22 µm and this distribution was found in rats as young as 20 days. In younger neonates the range of Purkinje cell diameters lay further towards the left. In a 12 day rat the range was between 11 µm and 29 µm with most cells measuring 16-20 µm. At 10 days of age the diameter range was 10-24 µm and most cells measured 15-18 µm. In the neonatal rats the distribution of Purkinje cell diameters was the same shape as in the adult, however the shift to the left was significantly different ($p < 0.01$).

In the experimental group which underwent pedunclotomy on day 3 and survived 35 days the diameter distribution was the same as the control. However, the animals pedunclotomised aged 5, 7, 10 or 22 days which

survived 35 days all had a similar Purkinje cell diameter distribution with a range of 11-28 μm and the majority of diameters being between 15 μm and 21 μm. In a similar manner to the controls this diameter distribution was also found in animals aged 20 days or more irrespective of the age at which pedunclotomy was performed. (Compare animals aged 5 days at operation surviving 15 or 25 days, 7 days at operation surviving 15 days and 10 days at operation surviving 10 days.) The Purkinje cell diameter distribution of animals aged 7 days at operation which survived 5 days was also moved to the left, the range being 10-27 μm and the majority of diameters lying between 14 μm and 18 μm. However, in animals aged 5 days at operation which survived 5 days post operatively, the range of the Purkinje cell diameter distribution was the same as in controls, 10-23 μm, but the majority of cells were smaller, 13-17 μm. Despite this the shape of the diameter distribution of this experimental group was not significantly different from either the 10 day or adult control.

TABLE 4.2

The mean Purkinje cell diameter from animals which underwent pedunclotomy at 3, 5, 7, 10 or 22 days of age and survived up to 35 days post operatively.

† = $p < 0.01$

* = $p < 0.001$

Age at Operation (Days)		Mean Purkinje Cell Diameter (μm)														
		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	19.3	0.47
	C													6	19.5	0.5
5	Op	6	15.5†	0.5				5	19.2	0.75	6	20.5	1.26	6	19.3	0.94
	C	6	16.7	0.75				4	20.0	0.71	5	21.2	0.75	6	18.8	1.07
7	Op	6	16.3*	0.47				5	19.0	0.89				6	17.5*	0.76
	C	5	18.0	0.0				4	20.0	0.71				6	20.5	1.12
10	Op				6	19.7	0.47							6	17.5†	0.96
	C				4	20.0	0.71							5	19.8	0.75
22	Op													3	18.3*	0.47
	C													5	20.6	0.49

Figure 4.11

Purkinje Cell Diameter Size/Frequency Distribution

The following graphs illustrate the Purkinje cell diameter distribution in experimental and control animals for each age group. The numbers of cells for each size are expressed as a percentage of the population because the 'n' values for the operated and control groups were rarely the same. The raw data for these graphs can be found in Appendix VIIIA. The numbers in the top right-hand corner of each graph indicate the operative age and the survival time e.g. 5-5 shows animals pedunclotomised aged 5 days which have survived 5 days.

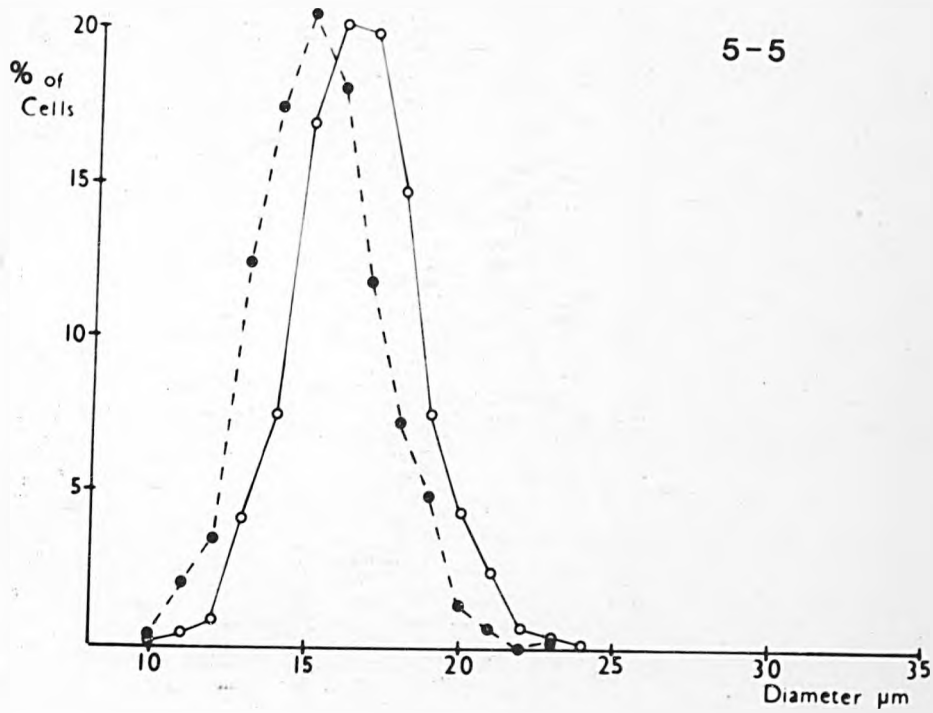
The diameter distribution for the 5-15 and 10-10 groups have been put onto the same graph so that any difference in the effect of pedunclotomy at the two ages can be directly assessed.

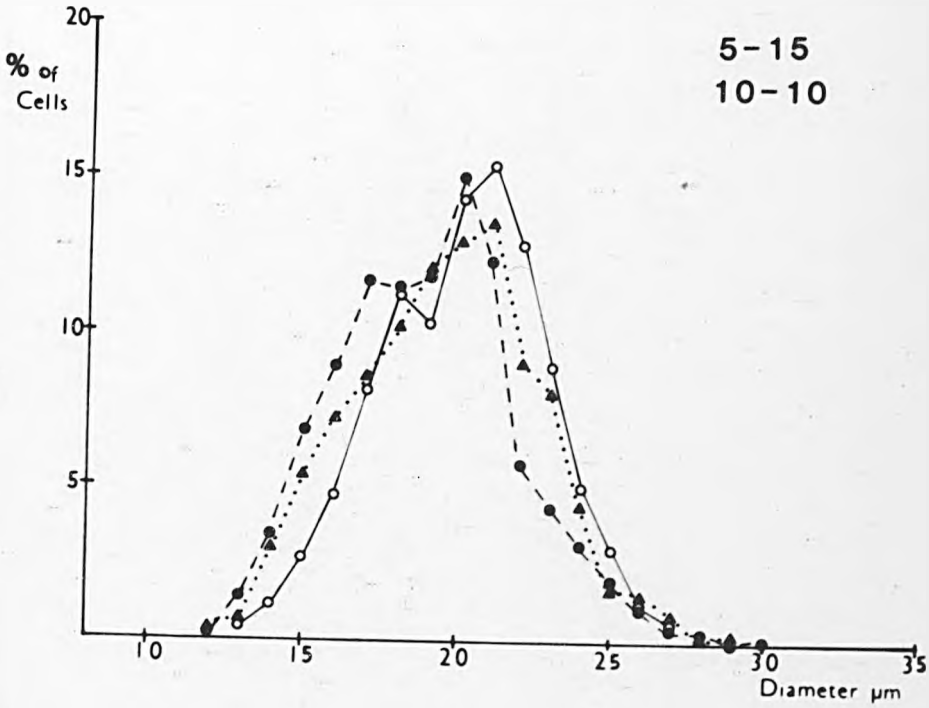
—o— = control

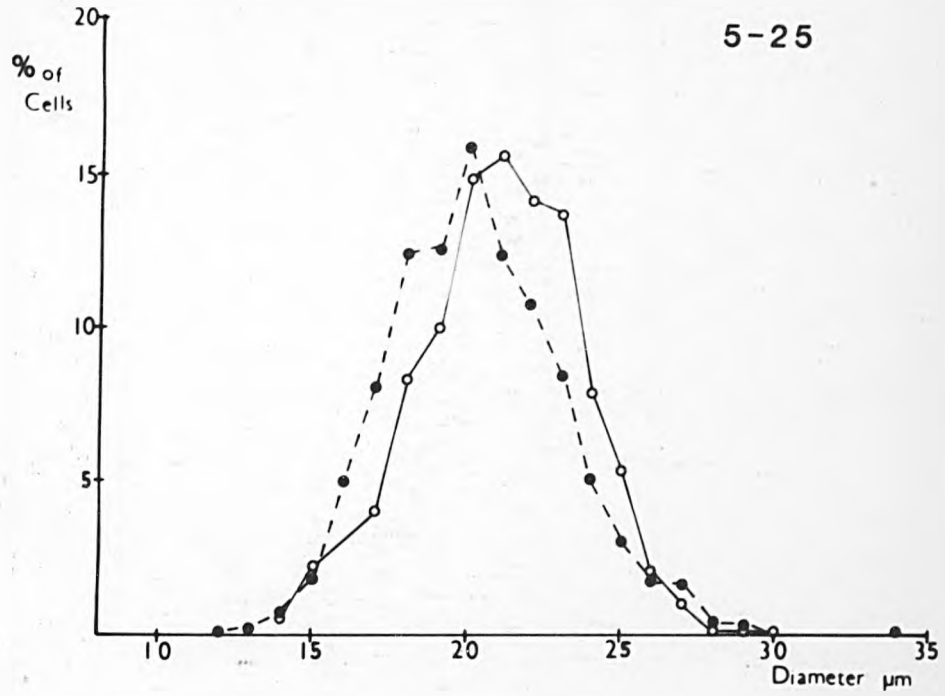
—•— = experimental

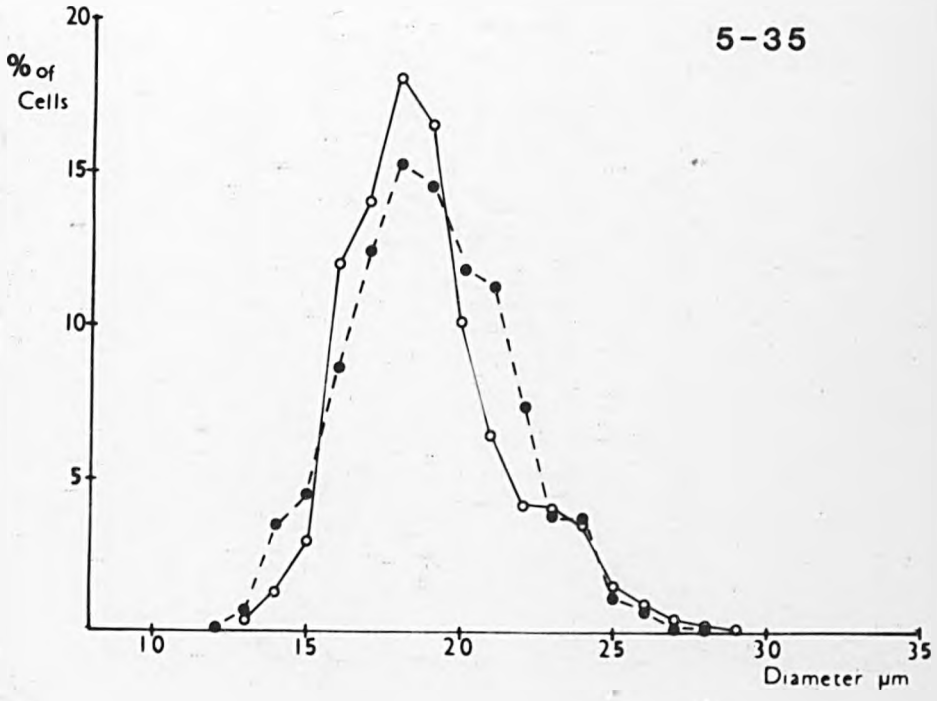
··▲·· = 10-10 operated.

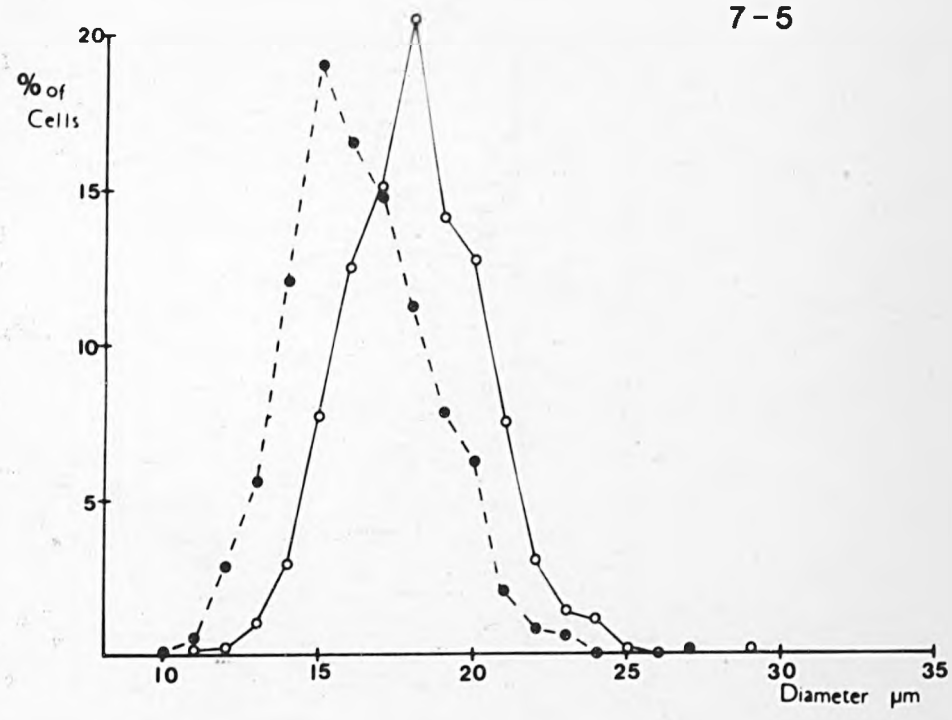
It is interesting to note that the 5-5 and 7-5 groups have narrower distributions than in the older animals, however the shapes of the two types of curve are not significantly different. Also, with the exception of the 5-35 and 3-35 groups, the distribution of diameter size in the experimental animals lies to the left of the controls.

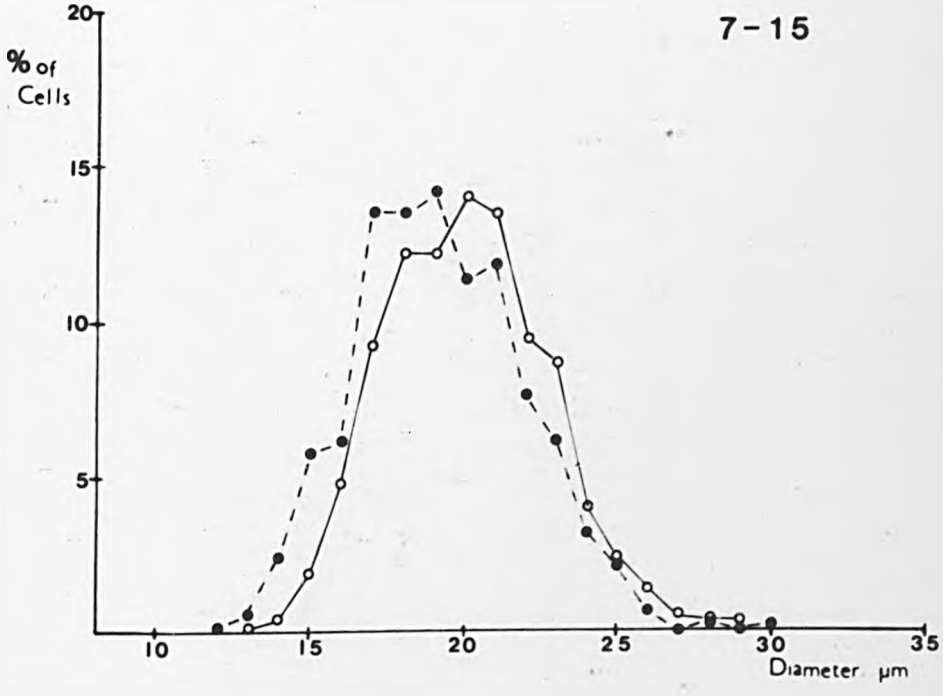

5-5
% of Cells
20
15
10
5
10
15
20
25
30
35
Diameter µm

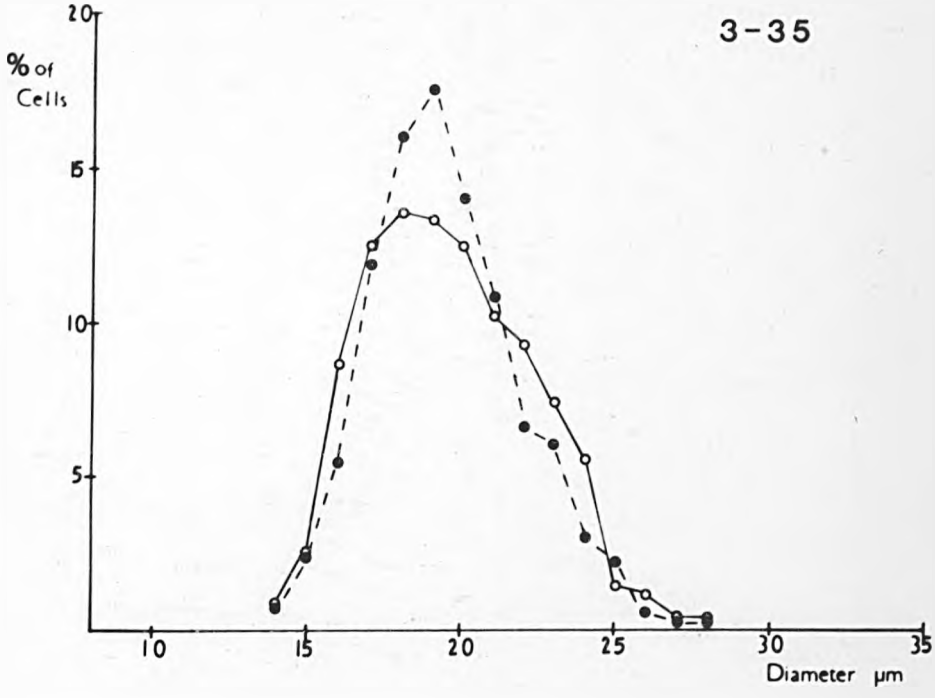

5-15
10-10
% of Cells
20
15
10
5
10
15
20
25
30
35
Diameter µm

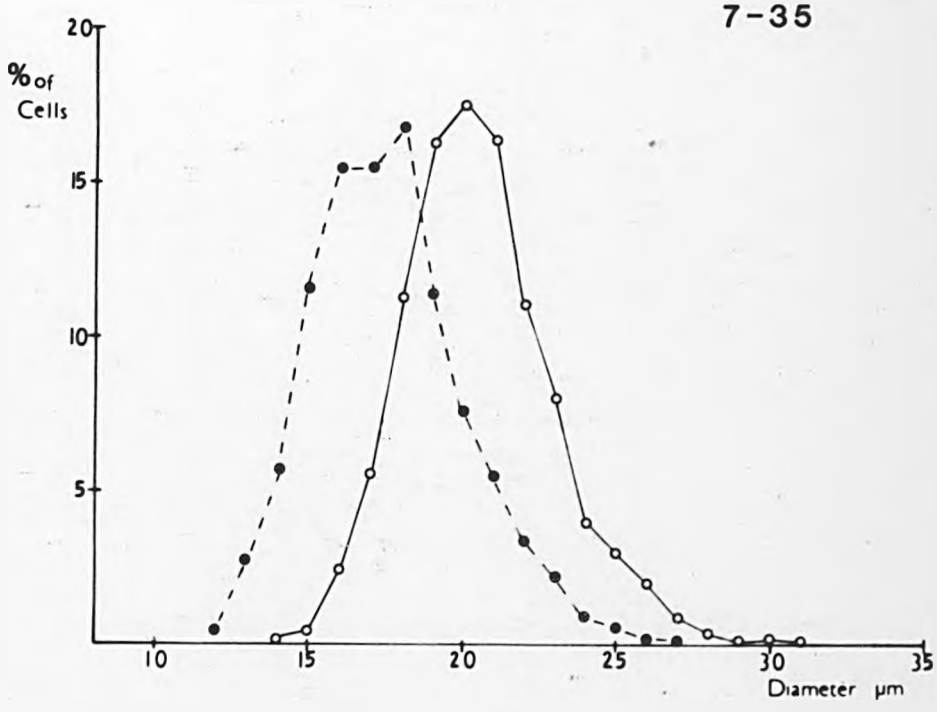

5-25
% of Cells
20
15
10
5
10
15
20
25
30
35
Diameter µm

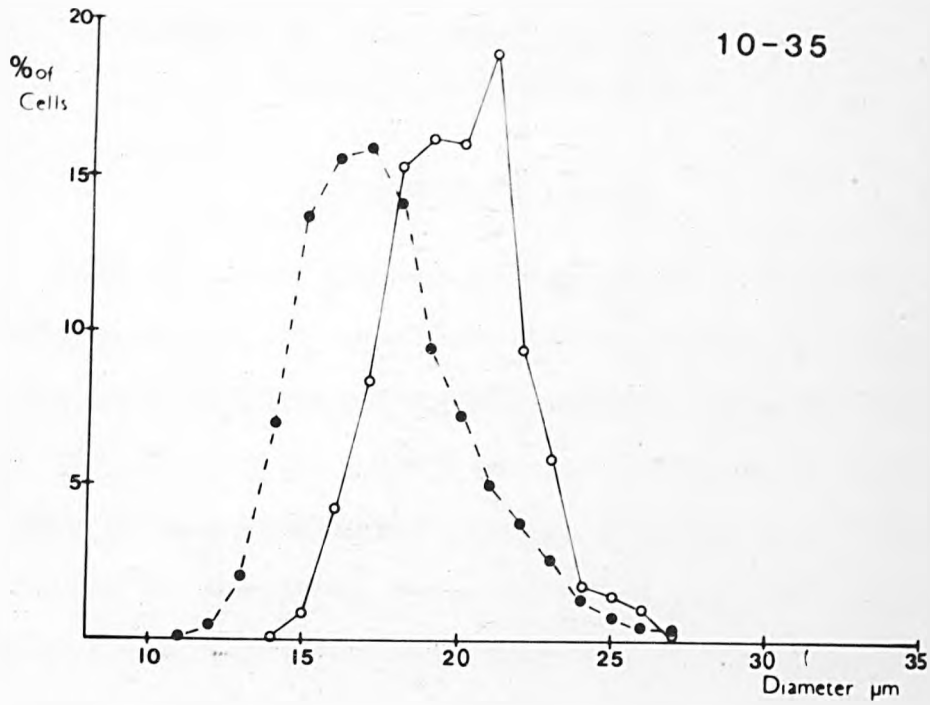

5-35
% of Cells
20
15
10
5
10
15
20
25
30
35
Diameter µm

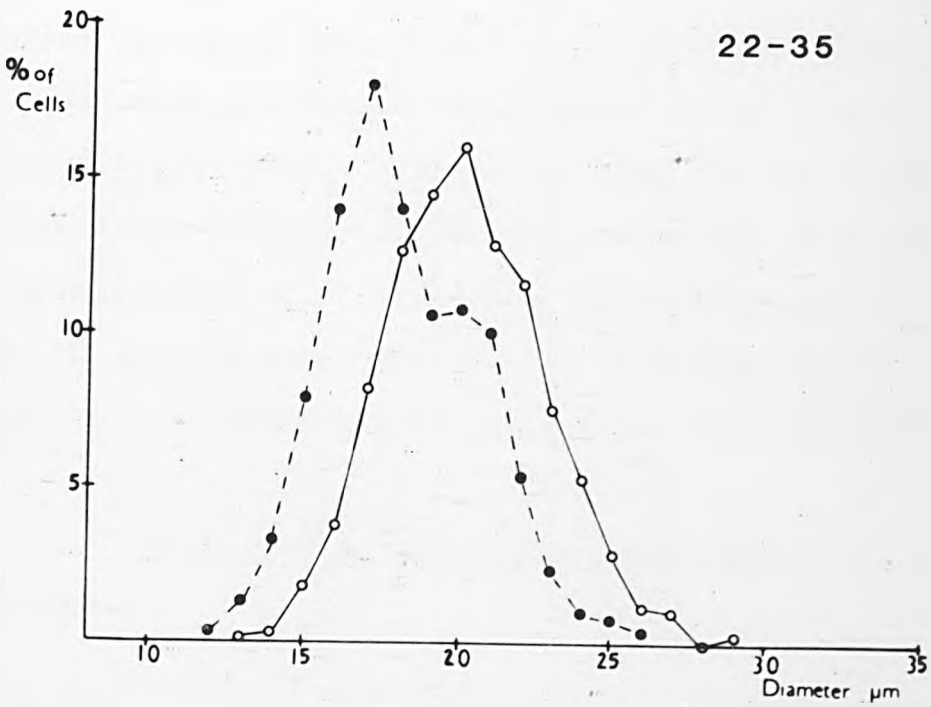

7-5
% of Cells
20
15
10
5
10
15
20
25
30
35
Diameter µm


7-15
% of Cells
20
15
10
5
10
15
20
25
30
35
Diameter µm


3-35
% of Cells
20
15
10
5
10
15
20
25
30
35
Diameter µm


7-35
% of Cells
20
15
10
5
10
15
20
25
30
35
Diameter µm


10-35
% of Cells
20
15
10
5
10
15
20
25
30
35
Diameter µm


22-35
% of Cells
20
15
10
5
10
15
20
25
30
35
Diameter µm

CHAPTER FIVE

DISCUSSION ON THE EFFECT OF PEDUNCLOTOMY ON CEREBELLAR DEVELOPMENT

BRAINSTEM HISTOLOGY

A study of the brainstem histology is a further extremely accurate criterion for pedunclotomy in addition to noting that the peduncles were missing, because in some animals the site of the peduncles was occupied by arachnoid mater and, on the cerebellar ventral surface, scar tissue. Degeneration of the right inferior olive and left lateral reticular nucleus demonstrates that the inferior peduncle has been cut (Angaut et al., 1982), and transection of the middle peduncle is revealed by the massive reduction of the basal pontine nucleus, which projects bilaterally to the cerebellum (Burne et al., 1978 a & b; Eisenman, 1976, 1980, 1981b; Eisenman and Nobak, 1980; Azizi et al., 1981; Mihailoff et al., 1981; Mihailoff, 1983; Payne, 1983b). The degenerative changes of the brainstems are the same for all survival times which indicates the developing neurons respond to axotomy more rapidly than do mature neurons which disappear after approximately eight days (Brodal, 1981).

CEREBELLAR MORPHOLOGY AND HISTOLOGY

MORPHOLOGY

The morphological appearance of the experimental cerebella was remarkably normal despite the reduced size of the

left hemisphere. This decrease in left hemisphere size is consistent with the small cerebella in staggerer and weaver mutant mice (Jacobson, 1978) and the reduced foliation following treatment with methylazoxymethanol (Woodward et al., 1975). But this is different from the abnormal pattern of foliation seen after deafferentation using intracisternal 6-OHDA (Berry et al., 1980b). Also, the cerebella of the experimental animals appeared less mature than in control animals of equivalent age. This would imply that the operation induces a delay in the cerebellar development, a phenomenon also seen after partial destruction and regeneration of the external granular layer (Nathanson et al., 1969; Woodward et al., 1975; Altman, 1982).

HISTOLOGY

The normality of the cerebellar histology in experimental animals shows that pedunclotomy produces a different experimental model for studying development from either mutant mice (Sotelo, 1982) and extensive degranulation (Herndon et al., 1971 a & b; Altman and Anderson, 1972) in which the Purkinje cells are highly disorganised, or mild degranulation (Woodward et al., 1975; Bradley and Berry, 1976a & 1978b; Crepel et al., 1980; Altman, 1982) where the Purkinje cell dendrites often descend towards the granular layer. The disorganisation in the above experimental models is possibly caused because the Purkinje cells are seeking to maximise their input by retaining multiple

climbing fibre synapses (Hamori, 1969; Crepel et al., 1980) and forming connections with mossy fibres (Hamori, 1969; Woodward et al., 1975; Yeh et al., 1982) both of which are drastically reduced or removed after pedunclotomy. However, a relatively normal cortical histology is found after malnutrition (Bedi et al., 1980; Chen and Hillman, 1980; Hillman and Chen, 1981a; Borges and Lewis, 1983; Dvergsten et al., 1982) or climbing fibre ablation (Kawaguchi et al., 1975; Bradley and Berry, 1976a; Sotelo and Arsenio-Nunes, 1976; Angaut et al., 1982; Alvarado-Mallart et al., 1983). The interfolial spaces described in animals pedunclotomised on day 22 and surviving 35 days, have also been found after perinatal malnutrition and is probably due to reduced granule cell production (Hillman and Chen, 1981a). Although the granule cells were not quantified in these experiments, it is likely that there are fewer of them because of the massive decrease in left hemisphere volume despite the possible slight increase in granule cell density.

Ectopic granule cells were not often found after pedunclotomy, but in those animals they were the result was similar to the ectopias found throughout the molecular layer after temporary injury to the external granular layer and subsequent regeneration which delays granule cell migration (Nathanson et al., 1969; Altman, 1982). Another demonstration that the cerebellar development is either delayed or slowed following pedunclotomy is the Purkinje cell morphology.

After pedunclotomy at either day 5 or 7 the Purkinje cells are not evenly distributed into a monolayer by day 10 although in control animals they are by day 5 (Addison, 1911), added to this the cytological appearance of the deafferented Purkinje cells is less advanced than normal. This result is exaggerated in cerebellar development *in vitro* where the Purkinje cells are unevenly dispersed as a monolayer and retain their perisomatic processes as late as 20 days post partum (Privat and Drian, 1976).

Those animals which survived for short periods post operatively (e.g. 5, 10 or 15 days) showed that the pedunclotomy induced changes in the external granular layer; an increase of mitotic figures and uneven thickness of the layer. The apparent increase in the number of mitotic figures might indicate an increased length of metaphase in the external granular layer neuroepithelium, which in turn would decrease the number of granule cells. This is a different result from that seen after ethanol poisoning (Borges and Lewis, 1983) but it seems to be in agreement with the decreased proliferation induced by zinc deficiency (Dvergsten *et al.*, 1983). Similarly, pedunclotomy causes an uneven thickness of the external granular layer, which is quite unlike its hyperplasic response to chemical injury (Woodward *et al.*, 1975; Borges and Lewis, 1983). It is unfortunate that the external granular layer has not been studied in malnourished rats for comparison since in cultures the pia mater is damaged and the external

granular layer cells migrate out from the tissue and consequently greatly reduce granule cell production. A further change in the external granular layer following pedunclotomy is that it remains longer than is usual, a response which has also been seen in zinc deficient animals (Dvergsten et al., 1983).

The presence in the pedunclotomised hemicerebella of deep nuclear neurons which exhibit chromatolysis show that their axons have been transected (with the superior peduncle) and that the perikaryon can be maintained by its nucleocortical collateral, a finding also demonstrated in the mammillary body (Fry and Cowan, 1972). Deep nuclear cells also develop in transplants of the cerebellar anlage and are maintained by mossy fibre connections with the cortex (Woodward et al., 1977; Alvarado-Mallart and Sotelo, 1982). But the development of the deep nuclei is clearly disturbed even though they are formed prenatally (Korneliussen, 1968c) because in both sagittal and coronal sections it is difficult to distinguish the normal nuclear divisions (Korneliussen, 1968b) in the left hemicerebellum. Whether this is caused by the loss of extracerebellar afferents or their proximity to the lesion is not possible to assess.

CEREBELLAR GROWTH

Measuring the weight of the cerebellum and its three components gives a good indication of its growth and how it is affected by unilateral pedunclotomy.

CHRONIC EXPERIMENTS

As has been said above the small left hemicerebellum in pedunclotomised animals is consistent with the other experimental methods for studying cerebellar development (Woodward et al., 1975; Jacobson, 1978; McConnell and Berry, 1978a; Bedi et al., 1980; Dvergsten et al., 1983). It is likely that this reduced size is caused by the pedunclotomy itself because malnutrition would both lower the body weights and affect the whole cerebellum, thus reducing the growth of the right cerebellar hemisphere as well. A study of the blood supply to the pedunclotomised cerebella has shown that there is no vascular insufficiency (Bower and Sherrard, unpublished observations) especially since the cerebellum receives a dual blood supply along the peduncles flowing centrifugally and from the pia mater flowing centripetally (Koppel et al., 1982).

It is interesting that from day 3 the cerebellum becomes increasingly vulnerable to the pedunclotomy until day 7 after which the effect is reversed. Also, between days 5 and 10 (excluding day 6) the histogram (Fig. 3.8A) is almost symmetrical about day 7. This might suggest that cerebella pedunclotomised on days 5 or 10 respond to deafferentation in the same manner, however this seems improbable considering the different stages of development at these two ages. There is also the question of why the cerebellum is so vulnerable to pedunclotomy on day 7, whether it is because of extreme susceptibility of the development taking place

or that there is some definitive change in the afferents at that age. It seems likely that the high value obtained from animals which underwent pedunclotomy aged 15 days is erroneous, perhaps because the 'n' value was only 4 and all the animals were male. Unlike Hillman and Chen (1981a), in this study the weights of brains from adult males, either experimental or control, were greater than those of females in equivalent groups. The large difference between the weights of the left cerebellar hemisphere after pedunclotomy at 22 and 30 days indicates the vulnerability of a system deafferented pre-synaptogenesis (Torvik, 1956; Kelly and Cowan, 1972) and the relative stability that the formation of synapses endows (Sotelo, 1981).

TIME COURSE EXPERIMENTS

The results from the four time course experiments undertaken confirm the findings of the chronic experiments. As might be expected, left cerebellar pedunclotomy has very little effect on the right hemisphere and a larger although still quite small effect on the vermis. This is probably because the remaining afferents of the right hemicerebellum sprout extra terminals to fill the adjacent vacated synaptic sites, as has been shown in other areas of the central nervous system (Lynch et al., 1972, 1973a & b; Zimmer, 1973 a & b; Hickey, 1975; Raisman, 1977; Angaut et al., 1982).

The cerebellar pedunclotomy has a devastating effect on the growth of the left hemisphere for each operative age.

Despite developing to the same terminal weights, cerebella pedunclotomised at 5 or 10 days do grow at different rates. The shape of the growth curve after pedunclotomy on day 5 is more like the control curve than it is in the 10 day experimental group. This might suggest that the development in the former group is relatively more normal, which would imply that the younger less mature cerebellum is more able to adapt to alterations in its environment. This loss of capacity to plasticise with increasing age has already been observed in the cerebellum (e.g. Nathanson et al., 1969; Schmidt et al., 1980; Altman, 1982; Sievers and Klemm, 1982). Also, this result may indicate that although the cortical histology appears the same for both groups 35 days post operatively its circuitry may be quite different. Following pedunclotomy on day 10 the substantial weight loss after 5 days would either represent a massive loss of cells and neurites or a technical error at this point. The latter explanation would seem more likely because pedunclotomy on days 5 and 7 produces a rapid loss of Purkinje cells without a concomitant loss in hemispheric weight.

Unilateral pedunclotomy on day 7 affects the whole cerebellum, even the right hemisphere is more consistently reduced than are the other experimental groups. The drastic curtailment of the left hemisphere's growth shows how important the climbing fibres or the normal density of mossy and noradrenergic afferents are to cortical development

especially since vascular deprivation has been excluded (see above). By day 17 all the cerebellar neurons have been formed, therefore the low hemispheric weight achieved by this age would imply a smaller granule cell population and the slight growth occurring after this age suggests that the normal neurite and glial proliferation is much reduced.

By day 22 all the cerebellar neurons are formed and have migrated to their final positions. Between 22 and 30 days the neurons form their connections and the newly developed synapses become sealed in a glial sheath. Denervation on day 22 will primarily affect the granule cells because the majority of their input is of extra-cerebellar origin. Neuronal degeneration following de-afferentation is most extensive at the time normal synaptogenesis would occur (Kelly and Cowan, 1972) and this probably explains the steady post operative decline of the left hemisphere weight. The weight of the left hemisphere in animals which survived 30 days post operatively is unexpectedly high. It is a mean of only two values and is taken from animals heavier than normal for their age.

Cerebellar Development on Postnatal Day 7

The extreme vulnerability of the 7 day old cerebellum to deafferentation must be caused by either a particular developmental event occurring at this time or some

unexpected response by the remaining afferents. The latter point will be discussed in Chapter 8, therefore, in order to try and understand why the cerebellum responds in the manner it does, the stage of cerebellar development taking place around day 7 will be summarised.

Postnatal day 7 marks the start of rapid differentiation of the external granular layer neuroepithelium into granule cells (Addison, 1911; Altman, 1972c) and is the second of the two days on which basket cells are formed (Altman, 1972a). In comparison, on day 5 the neuroblasts are proliferating rapidly (Altman, 1969; Haas and Werner, 1973) and by day 10 granule cell differentiation is well established and migration has begun (Addison, 1911). The Golgi cells have developed their dendrites by day 5 (Altman and Das, 1970) and are probably quite well established by day 7. Despite electrophysiological evidence that Purkinje cells respond to climbing fibre stimulation, the great majority of these fibres do not form synapses upon the perisomatic processes until day 7 (Woodward et al., 1969; O'Leary et al., 1971) while by day 10 the climbing fibres are ascending towards the primary dendrite and being replaced on the perikaryon by basket cell axons (Altman, 1972b). Also by day 7 the mossy fibres have reached the internal granular layer (Altman, 1972c), in which they rapidly ramify (Ramon y Cajal, 1960), and the noradrenergic fibre plexus is at its maximum density prior to subsequent rapid reduction

(Waddington and Banks, 1981).

The above description shows that cerebellar pedunclotomy performed on neonatal rats aged 7 days occurs simultaneously with at least four important events in cerebellar development:- 1) it will greatly reduce the noradrenergic input to the external granular layer, 2) just as it is changing from proliferation to differentiation, and noradrenaline is known to be important for neural development (Lawrence and Burden, 1973). 3) It will prevent a major percentage of the mossy fibre afferents from reaching the cerebellar cortex and 4) it will remove the climbing fibre afferents to the Purkinje cells just as these connections have been formed and before they can be replaced by basket cell axons. Therefore the lesion is likely to affect both granule and basket cell production and the Purkinje cells. The results obtained in this experiment are compatible with those of malnutrition studies, in which there are fewer granule cells and the cerebellum is smaller than normal (McConnell and Berry, 1978a; Bedi et al., 1980; Dvergsten et al., 1983) because reduced mossy fibre input is known to delay the production of granule cells (Hamori, 1969) and early climbing fibre ablation reduces the size of the Purkinje cell dendritic tree (Bradley and Berry, 1976a).

PURKINJE CELL NUMBERS

CONTROL ANIMALS

The method used to calculate the Purkinje cell numbers has been discussed in Chapter 4. When the mean Purkinje cell numbers obtained are compared with those described in the literature, $4.2\text{-}5.5 \times 10^5$ (Inukai, 1928), $3.2\text{-}3.6 \times 10^5$ (Armstrong and Schild, 1970) and $2.7\text{-}2.8 \times 10^5$ (Hillman and Chen, 1981a) they are found to be in agreement.

Even in control animals the number of Purkinje cells is quite variable. Including the values for younger animals there is a range of 26,000 cells among the control groups, a large interanimal variation which was also noted by Armstrong and Schild (1970). The slightly larger figure in the younger animals may indicate a loss of Purkinje cells during development. This type of neuronal death has been shown within the developing nervous system (e.g. Cowan, 1978).

PEDUNCLOTOMISED ANIMALS

The individual animal's response to pedunclotomy is demonstrated in the Purkinje cell numbers as well as the weight data. Despite each neonate undergoing the same lesion the standard deviations of the number of Purkinje cells in experimental groups are approximately 20% of the mean population. The 8% standard deviation seen in control animals shows that this is a differential response to the pedunclotomy rather than the handling, anaesthetic,

skin lesion and suturing. After pedunclotomy on day 5 a large variation in the average number of Purkinje cells is found in each survival time group, whereas in those animals which were pedunclotomised aged 7 days the variability of Purkinje cell numbers decreased with longer survival times. This might suggest that despite interanimal variation pedunclotomy on day 7 ultimately has the same effect on all cerebella.

The slight and statistically non-significant reduction in the number of Purkinje cells after pedunclotomy on day 3 is consistent with the small difference in left hemisphere weight between this group and the control value. However, in the rats pedunclotomised at 22 days there is no loss of Purkinje cells despite a large loss of left hemisphere weight because the linear Purkinje cell density is increased in the pedunclotomised cerebella. This would imply that in this group the weight loss is primarily due to granule cell and concomitant parallel fibre (i.e. molecular layer) degeneration. In addition, it is interesting that the equality of left hemisphere weights between the animals surviving 35 days after pedunclotomy aged either 5 or 10 days is also reflected in the Purkinje cell numbers. A difference of 10,000 neurons, considering the variation between normal animals, would not appear to be a significant finding. This similarity between neonates pedunclotomised aged 5 or 10 days is also found in left hemisphere weight and Purkinje cell numbers of animals aged 20 days.

5 Day Pedunclotomy Time Course

Neonates pedunclotomised aged 5 days all have a similar number of Purkinje cells excepting those which survived 25 days which had fewer cells. It seems probable considering the normal variation of Purkinje cell numbers and the high standard deviations in these experimental groups, that the lower value found in the 25 day survival group is not significant. Despite all having approximately the same number of Purkinje cells, the values in the experimental cerebella of animals surviving 5 or 15 days are significantly lower than in controls, but because of a probable normal loss of Purkinje cells the difference at 35 days is not significant. However, it also shows that in the same manner as the brainstem a large number of neurons die and disappear extremely quickly, i.e. 5 days.

7 Day Pedunclotomy Time Course

After pedunclotomy on day 7 the loss of Purkinje cells is statistically significant for each survival time studied. In order to ensure that the significance of this loss in those animals surviving 5 and 15 days is not due to the slightly higher control values, the Purkinje cell numbers were also compared with the results from control neonates aged 10 and 20 days respectively. The Purkinje cell loss after 5 days was just still significant ($p < 0.02$) while after 15 days survival it remained highly significant ($p < 0.001$).

Unlike the neonates pedunclotomised aged 5 days there continues to be substantial Purkinje cell loss even after the initial extensive cell death of the first 5 days survival. This continued loss could be caused directly by deafferentation of the cell at a crucial stage or the reduced hemisphere volume in response to pedunclotomy. In calculating the Purkinje cell number their linear density was found. In the 7, as well as 5 and 10 day, experimental groups surviving 35 days, the normal adult density of 20 cells/mm was retained. In young neonatal cerebella the Purkinje cell linear density is considerably higher (approximately 45 cells/mm) but it steadily decreases to the adult level by postnatal day 30. This might imply that the Purkinje cell loss in the 7 day experimental group is a result of its small left hemisphere and that there is an optimal packing density greater than which the neurons will not tolerate. However, in the 22 day experimental group despite a reduced hemispheric volume the Purkinje cell number is unchanged and the cells do survive in a more densely packed environment.

CONCLUSION

The Purkinje cell data indicate that in the normal developing cerebellum there is some Purkinje cell death, which is consistent with the development of other systems (see reviews Cowan, 1978; Lund, 1978), and that there is considerable interanimal variation in normal adult animals.

In most of the experiments on cerebellar development the number of Purkinje cells has not been determined and apart from those mutant mice in which the Purkinje cells specifically die (Sotelo, 1981; Wetts and Herrup, 1983) the Purkinje cell numbers are unaffected (Berry et al., 1980b; Chen and Hillman, 1980; Hillman and Chen, 1981a), which is a totally different result from this experiment. But, transneuronal degeneration of immature neurons following deafferentation has been described (Kelly and Cowan, 1972) and it takes place extremely quickly (Torvik, 1956).

PURKINJE CELL DIAMETERS

At birth Purkinje cells are 7 x 12 µm (Addison, 1911; Altman, 1972b) and they grow to 12 x 18 µm by 11-12 days (Addison, 1911). In this experiment the Purkinje cell diameters at day 10 were 16.7 µm and they increase to 18 µm by day 12 and have reached the adult size by day 20. During this rapid growth the variation of Purkinje cell size increases, possibly because of the individual neurons environment or the dorsorostral-ventrocaudal growth gradient within the cerebellum (Larsell, 1952; Altman, 1969; Korneliussen, 1969).

The mean Purkinje cell diameter is not affected by pedunclotomy on day 3, a result which is consistent with both the left hemisphere weight and the number of Purkinje cells. But after pedunclotomy on day 5 the Purkinje cells

are significantly smaller ($p < 0.01$) in those animals surviving 5 days post operatively but after longer survival times they are not different from the control values. This would imply that after an initial post operative delay the Purkinje cell soma can develop apparently normally. An initial post operative delay in Purkinje cell growth is also seen if the pedunclotomy is performed on day 7 and in a similar manner the Purkinje cell appears to 'catch up' and reach its normal size by day 22. This response may also occur in the 10 day pedunclotomy group although there is no 5 day survival to prove it. However, by 35 days post operation on days 7 or 10, the Purkinje cells have shrunk and are significantly smaller ($p < 0.001$ and $p < 0.01$ respectively) than those of control cerebella. These results suggest that during the normal period of synaptogenesis (21-30 days post partum) the Purkinje cells shrink because they do not receive their normal complement of synaptic input, as is demonstrated in the 22 day pedunclotomy group. Since the distributions of Purkinje cell diameters are the same as their age-matched controls, the pedunclotomy probably affects all the Purkinje cells in the same manner, either in decreasing their growth or causing them to shrink after synaptogenesis.

Although the smaller Purkinje cells are a different response from the malnutrition studies of Bedi et al. (1980) they are probably consistent with the smaller dendritic trees found after partial deafferentation

(Bradley and Berry, 1976 a & b; Sotelo and Arsenio-Nunes, 1976; Woodward et al., 1977), total deafferentation (Privat and Drian, 1976; Woodward et al., 1977; Alvarado-Mallart and Sotelo, 1982; Blank and Seil, 1982), malnutrition (McConnell and Berry, 1978 a & b; Hillman and Chen, 1981a) and the smaller neuronal size found after ganglionic (Black et al., 1972) and nuclear deafferentation (Garey and Blakemore, 1977).

CEREBELLAR DEVELOPMENTAL RESPONSE TO PEDUNCLOTOMY

The normal cortical morphology and the reduction in Purkinje cell numbers indicates that pedunclotomy is a totally different experimental model from the mutant mice or agranular cerebella. The results are more similar to light degranulation, which the reduced cortical volume would suggest, especially if it is induced by malnutrition rather than drugs or X-irradiation which cause misorientated Purkinje cell dendrites. But malnutrition studies do not find a reduced Purkinje cell population.

As might be expected, the histology is more like that seen in cerebellar transplants and tissue culture, except that in those models only small areas of cerebellar cortex develop, probably because of the trauma of the operation and the loss of the pial vasculature. However, the normal histology and small Purkinje cell dendritic tree (possibly reflected in this experiment by the reduced somatic diameter) are comparable in the two experiments.

Both the weight data and the Purkinje cell numbers show that the stage of cerebellar development at the time of the pedunclotomy is crucial to the subsequent effect. At 3 days post partum pedunclotomy will remove some of the developing noradrenergic fibres and the few further developed climbing fibres and the results indicate that the cerebellum is still sufficiently immature to adapt to this. By day 7 the effect of pedunclotomy is critical, most probably because four developmental stages change on this day (see above) and the cerebellum is further developed and less capable of adaptation. The lesser severity of pedunclotomy at later ages is possibly a reflection of the greater stability of a more developed system which contains some functional connections. This hypothesis is supported in those animals pedunclotomised aged 10 and 22 days surviving 35 days, in which the cerebellar weight is less than normal but the Purkinje cells are maintained even though they are of smaller size.