UCSF
UC San Francisco Electronic Theses and Dissertations

Title
Neuronal specificity and the nervous system of the Reeler mouse

Permalink
https://escholarship.org/uc/item/3r62v0f4

Author
Steindler, Dennis A.

Publication Date
1978

Peer reviewed|Thesis/dissertation

eScholarship.org Powered by the California Digital Library
University of California

Abstract

There are unconventional patterns of cytoarchitecture in the forebrain and hindbrain cortical structures of reeler mice. In the afoliate and undersized reeler cerebellum, most cortical neurons are maloriented and do not attain their appropriate position within radially organized laminae. The laminar disarray is characterized by most granule cells being either intermingled with, or lying external to, populations of Purkinje cells. The cerebellar distribution of the dorsal and ventral spinocerebellar tracts (DSCT and VSCT) was compared in normal and reeler mice using anterograde axonal degeneration techniques to assess the extent of cerebellar afferent organization in the mutant. After low thoracic spinal cord hemisections in normal mice, degenerating DSCT and VSCT fibers were found to accumulate in a cerebellar commissure that is at the level of or just above the deep cerebellar nuclei. These fibers radially ascend and distribute within midline regions of particular vermian lobules of the anterior and posterior lobes. Following similar lesions in reeler mice, it was found that DSCT and VSCT axons in reeler descend to populations of granule cells that are primarily below the level of the deep cerebellar nuclei. This phenomenon is presumed to be due to an attempt by these afferents to locate displaced populations of postsynaptic target cells. The topographical (mediolateral and rostrocaudal) distribution of reeler DSCT and VSCT axons also conforms

to a pa
distribu
rostroca
cortical
lobuliza
of DSCT
a miniat
and segr
cerebell
Finally,
qualitat
This for
collatera
granule
from stu
that cere
aberrant
afferents
reeler ce
and appro
of an aut
developme
similar p
notion as
developmer

to a pattern that is dictated by regional variations in the distributions of target neurons. A decrease in the overall rostrocaudal and mediolateral distributions of cerebellar cortical neurons in reeler, as a result of the failure in lobulization, produces a compressed topographical distribution of DSCT and VSCT fibers. This topographical distribution is a miniature DSCT and VSCT representation in that laterality and segregated anterior and posterior spinal recipient cerebellar zones are similarly delineated in both phenotypes. Finally, the deep cerebellar nuclei in reeler appear to be, qualitatively, increased in size relative to the normal. This form of hypertrophy might reflect an increased afferent collateralization that results from reduced numbers of target granule cells in the reeler cerebellar cortex. Thus, results from studies of connectivity in the reeler cerebellum suggest that cerebellar cortical neurons in the mutant that reside in aberrant positions may make connections with their appropriate afferents. Long projection afferents within the malformed reeler cerebrum also display modified axonal trajectories and appropriate topographic distributions. This is suggestive of an autonomisity in the expression of cytoarchitecture and development of patterns of interneuronal connectivity. A similar phenomenon in the mutant cerebellum reinforces this notion as well as implies that there are parallel factors for development of both the forebrain and hindbrain cortices.

Introduction

Examination of long projection afferent systems within restricted abnormal cortical loci in the genetic mutant reeler mouse provides a measure of the specificity involved in synaptogenesis by isolating potentially independent factors which influence or direct neuronal position, orientation and connectivity. Studies on the nervous system of reeler allow an assessment of the deleterious effects of a single gene mutation on the development of functional cortical circuitry in both the cerebrum and cerebellum. Because reeler possesses cytoarchitectural abnormalities in both cortical structures, it is possible to identify common developmental factors which influence circuitry in both structures.

The present study is based upon the premise that an examination of the rostrocaudal, mediolateral and radial components of afferent trajectories and distributions in the mutant cortices allows an assignment of the potentially separable phenotypic phenomena of cell position and the topographic specificity of axonal connections.

The nervous system of the reeler (rl) mutant mouse is unique among the seven[1] well known neurological mutations in

---

[1] (leaner (see Sidman et. al., 1965, 1968a,b); lurcher (see, for example, Swisher and Wilson, 1977); nervous (see, for example, Landis, 1973); Purkinje cell degeneration (see Mullen et. al., 1976); staggerer (see for example, Sidman, et. al., 1962); weaver (see, for example, Rakic and

Introduction

Examination of long-projecting afferent systems within restricted abnormal cortical loci in the [illegible] reeler mouse provides a measure of the specificity involved in synaptogenesis by isolating potentially independent factors which influence or direct neuronal position, orientation and connectivity. Studies on the nervous system of reeler allow an assessment of the deleterious effects of a single gene mutation on the development of functional cortical circuitry in both the cerebrum and cerebellum. Because reeler expresses cytoarchitectural abnormalities in both cortical structures, it is possible to identify common developmental factors which influence circuitry in both structures.

The present study is based upon the premise that an examination of the rostrocaudal, mediolateral and radial components of afferent trajectories and distributions in the mutant cortices allows an assignment of the potentially separable phenotypic phenomena of cell position and the topographic specificity of axonal connections.

The nervous system of the reeler (rl) mutant mouse is [illegible] among the seven[1] well known neurological mutations [illegible]

---

[1] (Lemmer (see Sidman et. al., 1965, 1965a,b); Lurcher (see, for example, Zwisher and Wilson, 1977); nervous (see, for example, Landis, 1973); Purkinje cell degeneration (see Mullen et. al., 1976); staggerer (see for example, Sidman, et. al., 1962); weaver (see, for example, [illegible]

mice in that it possesses anomalous development of both cerebral and cerebellar cortical structures. The rl autosomal recessive mutation in mice (Falconer, 1951), in linkage group XVII and chromosome 5 (Falconer, 1952; Lane, 1967; Committee on Standard Genetic Nomenclature for Mice, 1972), is believed to affect neuronal migration and subsequent cell class-specific aggregation during neurogenesis. This results in aberrant patterns of cell lamination and orientation in the cerebral and cerebellar cortices (Hamburgh, 1960; Meier and Hoag, 1962; Hamburgh, 1963; Sidman, et. al., 1965; Sidman, 1968a,b; Sidman, 1972; Caviness and Sidman, 1972; Caviness and Sidman, 1973a,b; Caviness, 1976a,b).

One way of assessing organization in the reeler nervous system is to examine the distributions of particular sub-cortical afferent systems within the cytoarchitecturally abnormal cortices. A previous examination of patterns of connectivity in the mutant telencephalon and diencephalon (Steindler and Colwell, 1976; Welt and Steindler, 1977; Steindler, 1977a) has shown that the specificity of neuronal connections in the forebrain may be independent of those factors which direct cell position and orientation during development. The present study is primarily concerned with afferent distributions within the malformed reeler cerebellum (for a preliminary report, see Steindler, 1975). This

---

Sidman, 1973a,b; Sotelo, 1975)

represents
afferent r
similar ph
Caviness,
Stanfield,
1976; Cavi
1977a; Wel
contribute
may underl
circuitry

Studies on

Many
system have
organizatio
standard ne
perikarya a
distinctive
absence of
layer. Ha
neuronal ty
present wit
not organiz

Sidman
"cell-birth
young neuro
manative ve

represents one of the first attempts made to relate possible afferent reorganization within the mutant hindbrain to similar phenomena known to occur in the forebrain (Devor, Caviness, and Derer, 1975; Frost and Caviness, 1974; Stanfield, Caviness and Cowan, 1975; Steindler and Colwell, 1976; Caviness and Yorke, 1976; Caviness, 1976a,b; Steindler, 1977a; Welt and Steindler, 1977). Such an endeavor will contribute to our understanding of parallel factors which may underlie the development and maturation of neuronal circuitry in both the cerebrum and cerebellum.

Studies on the Neuronal Organization of the Reeler Forebrain

Many previous investigations of the reeler nervous system have been concerned primarily with deciphering the organization of the mutant forebrain. Hamburgh (1963), using standard neurohistological methods for examining neuronal perikarya and their processes, suggested that the most distinctive feature of the mutant cerebral cortex was the absence of a well-defined molecular (external plexiform) layer. Hamburgh also recognized that all appropriate neuronal types (e.g. fusiform, pyramidal and granular) were present within the reeler cortex, but he believed they were not organized into cell class-specific laminae.

Sidman (1968a,b), in doing a tritiated thymidine "cell-birthday" study, showed that during neurogenesis, young neurons arose at appropriate times along the germanative ventricular and subventricular zones of the reeler

cerebrum,
migration
were exte
and Sidma
studies o
have demo
migratory
the last
Thus the
be referr
migrate p
neurons e
Sidman (19
in reeler,
migrate p
randomly
attributed
neuronal
in those
the so-cal

Resul
organizati
1972), ret
in reeler
trary to p
1) The cyt
show rando

cerebrum, and that they also displayed appropriate rates of migration into the cortical plate. (These observations were extensively elaborated upon in the paper by Caviness and Sidman, 1973b.) Tritiated thymidine autoradiography studies of normal mouse cerebrum (Angevine and Sidman, 1961) have demonstrated that the earliest differentiated post-migratory neurons occupy the deepest cortical layers, and the last postmitotic neurons reside more superficially. Thus the laminar deposition of migrating young neurons can be referred to as "inside-out". Later forming young neurons migrate past temporally defined class-specific differentiating neurons en route to more superficial cortical regions. Sidman (1968b) originally believed that migratory neurons in reeler, upon entering the cortical plate, "...fail to migrate past their predecessors and instead, mingle randomly among them..." Such a phenomenon was hypothetically attributed to an improper cell to cell communication during neuronal migration in the mutant nervous system, especially in those laminated cortical structures which develop by the so-called "inside-out" pattern (Sidman, 1968a,b).

Results from further investigation into the neuronal organization of forebrain olfactory (Caviness and Sidman, 1972), retrohippocampal, hippocampal, and related structures in reeler (Caviness and Sidman, 1973a) suggested that contrary to prior beliefs (Hamburgh, 1963; Sidman, 1968a,b): 1) The cytoarchitecture of the mutant forebrain did not show random organization. Rather, with regard to the

cerebrum, and that they also disclosed appropriate rates of migration in the cortical plate. (These observations were extensively elaborated upon in the paper by Davinee and Sidman, 1973.) Initiated thymidine autoradiography studies of normal mouse cerebrum (Angevine and Sidman, 1961) have demonstrated that the earliest differentiated post-migratory neurons occupy the deepest cortical layers, and the last postmitotic neurons reside more superficially.

Thus the laminar deposition of migrating young neurons can be referred to as "inside-out". Later forming young neurons migrate past temporally defined class-specific differentiating neurons en route to more superficial cortical regions.

Sidman (1961) originally believed that migratory neurons in reeler, upon entering the cortical plate, "...fail to migrate past their predecessors and instead, migrate randomly about them..." Such a phenomenon was hypothetically attributed to an improper cell to cell communication during neuronal migration in the mutant nervous system, especially in those laminated cortical structures which develop by the so-called "inside-out" pattern (Sidman, 1968a,b).

Results from further investigation into the neuronal organization of forebrain olfactory (Davinees and Sidman, 1972), retrohippocampal, hippocampal, and related structures in reeler (Davinees and Sidman, 1973a) suggested that contrary to prior beliefs (Rugbarth, 1973; Sidman, 1968a,b): 1) the cytoarchitecture of the mutant forebrain did not show random organization. Rather, with regard to the

parasubiculum, presubiculum, subiculum and anterior hippocampal cortex, the relative positions of deep polymorphic and superficial phramidal cells appeared to be reversed in reeler relative to the normal (Caviness and Sidman, 1973a). 2) An improper "inside-out" histogenesic pattern was not the immediate source of the reeler cortical malformation. The cytoarchitecture appeared normal in the olfactory bulbs of reeler (Caviness and Sidman, 1972). The hippocampus, a structure which also follows an "inside-out" pattern for development has also been shown to display atypical, but nonetheless recognizable patterns of cellular lamination in reeler (Hamburgh, 1963; Sidman, 1968a,b; Caviness and Sidman, 1973a; Caviness, 1973; Stanfield, Caviness and Cowan, 1975; Stanfield, 1977). Recent findings (Stanfield, 1977) have also confirmed the idea (see Caviness, 1973) that young neurons destined to form pyramidal and granule cells of the mutant hippocampus and dentate gyrus arise at appropriate times and places, and that their duration of proliferation is similar to that of normal mouse.

The inversion of cortical layers appears to be the most prevalent theme of neuronal organization in the forebrain of reeler. The laminar disarray has been attributed to defective intracortical migration patterns which invert the relative positions of neurons occupying the upper and lower cortical layers (Caviness and Sidman, 1973a,b). In the normal mouse, a cell-sparse zone (external-plexiform or

molecul
neurons
boundar
(see, f
the mos
populat
boundar
matter.
positio
cells a
that re
laminat
granule
shown t
sensory
Van der
mice wa
Cavines
1977. I
examinat
through
not reve
"upside-

Des
posterio
subfield
area in

molecular layer) forms the outermost lamina I, and cortical neurons of layer VI contribute to a well-delineated inner boundary at the junction of the gray and white matters (see, for example, Welt and Steindler, 1977). In contrast, the most superficial cortical zone in reeler mice is densely populated with small neurons, and there is an uneven ventral boundary in which some neurons penetrate into the white matter. Caviness (1976a,b) has concluded that the relative positions of superficial pyramidal cells and deep polymorphic cells are reversed in reeler. It should therefore follow that reeler possesses some form of cell class-specific lamination within other cortical layers (for example, granule cells of layer IV). For instance, it was recently shown that the topographical organization of the somatosensory (SmI) layer IV barrel field (see Woolsey and Van der Loos, 1970; Welker and Woolsey, 1974) of reeler mice was similar to that of the normal (Welker, 1976; Caviness, 1976a,b; Welt and Steindler, 1977; Steindler, 1977. For contrasting findings, see Cragg, 1975). However, examination of Nissl stained coronal and sagittal sections through the SmI (as well as other regions) of reeler does not reveal a clearly laminated cortex which is merely "upside-down".

Despite an appropriate medio-lateral and anterior-posterior representation of the postero-medial barrel subfield (PMBSF) in reeler (Welt and Steindler, 1977), an area in normal animals known to contain the largest barrels

[illegible]

[illegible] Lyon, [illegible] 1 [illegible] 1 [illegible] 1

that are functionally related to individual vibrissae of the contralateral face (Van der Loos and Woolsey, 1973; Welker, 1976), there are irregular features also demonstrated by this region. Using the techniques and criteria laid down by Welker and Woolsey (1974) for assessing normal development of a barrel field, it has been shown that the arrangements of neuronal perikarya within reeler barrels in tangential sections are abnormal (Welt and Steindler, 1977). Results from this study suggest that: 1) It is the radial dimension of barrel topology that suffers the most in reeler. That is, within the dorso-ventral (pia--white matter) axis of layer IV in reeler, the cellular aggregates that comprise barrels usually appear more distorted and less distinct. 2) "Some of the disorganization seen in barrels of the reeler mutant may involve inappropriate arrangements of granule cells." 3) "There may be an aberrant migration of pyramidal neurons into the barrel field." 4) "Defects in the cellular organization of barrels could also involve a deficit in total numbers of neurons and glia or an alteration in the packing density of these elements." 5) "There may be structural changes in the elaboration of dendritic and/or axonal processes..." indicating abnormal neuronal development in conjunction with the laminar disarray in reeler. Also, many cortical neurons in reeler deviate from a radially (vertical) columnar pattern of orientation to the extreme of complete inversion (also see Caviness, 1976a,b). Thus, incomplete and sometimes improper aggregation of cellular elements

constit

that "

sequen

(aggreg

numerou

Studies
of the

Th

cerebru

on cell

charact

cyto- a

attribu

are par

are top

and phy

hypothe

reeler

1975a f

mouse c

can be

arise a

cells:

tions o

system

2) Are

cortex

normal (

constituting unique arrangements of barrels in reeler suggest that "...developmental events in addition to, or as a consequence of those factors which misdirect cell migration (aggregation), and orientation produce a cortex with numerous defects" (Welt and Steindler, 1977).

Studies on the Afferent Organization and Connectivity of the Reeler Forebrain

The degree of organization within the abnormal reeler cerebrum cannot be assessed solely from observations made on cell and fiber-stained material. Besides assessing the characteristics of neuronal organization from data on cyto- and myeloarchitecture, on must also examine other attributes of organization such as connectivity. There are particular patterns of neocortical connectivity that are topologically representative of certain anatomically and physiologically defined cortical regions. Based on the hypothesis that the architectonic map of the neocortex of reeler is identical to that of the normal (see Caviness, 1975a for a reappraisal of Krieg's parcellation of normal mouse cerebral cortex; and Caviness, 1976a), three questions can be posed regarding the patterns of connectivity that arise amidst radically malpositioned and disoriented cortical cells: 1) What are the trajectories and terminal distributions of afferents arising from other areas of the nervous system within the cytoarchitecturally abnormal cortices? 2) Are the efferent projections that arise from the abnormal cortex organized in a similar fashion to those seen in the normal (Steindler, 1976)? 3) What is the pattern of

innervati
(e.g. cal
Devo
there are
cortices
tation.
to trace
reeler (
cortex;
tions wi
trajecto
olfactor
LOT) is
limb of
course i
Sidman,
bulb eff
both nor
fields -
olfactor
anterior
amygdal
Krieg.
the top
laminar
6) The
a lamin

innervation between two abnormal cortical structures (e.g. callosal cortico-cortical connections)?

Devor et. al. (1975) showed that, for the most part, there are appropriate fiber projections within the olfactory cortices of reeler despite cellular malposition and disorientation. They used axonal degeneration techniques in order to trace two characteristic central olfactory connections in reeler (projections from the olfactory bulb to the olfactory cortex; and ipsilateral cortico-cortical association projections within the piriform cortex). They found that: 1) The trajectory of the efferent fibers from the main and accessory olfactory bulbs (referred to as the lateral olfactory tract, LOT) is normal in the reeler mouse. 2) Fibers of the anterior limb of the anterior commissure, however, follow an aberrant course in the olfactory peduncle (also see Caviness and Sidman, 1972). 3) The distribution of main olfactory bulb efferents are ipsilateral in normal and reeler, and both normal and reeler mice display the following LOT terminal fields - anterior olfactory nucleus, piriform cortex, olfactory tubercle, rostral portion of the hippocampus, anterior amygdaloid area, rostral portion of the cortical amygdaloid nucleus and lateral entorhinal area, area 28b of Krieg. 4) There are some fine quantitative differences in the topology of some of these projections in reeler. 5) The laminar distribution of LOT axons of reeler mice is normal. 6) The axons and terminals of association projections occupy a lamina just beneath the terminals of the LOT in reeler as

in norm
pyramid
deep ra
have ap
LOT. T
cellula
reeler.
on norm
project
reeler
reeler
1) Are
modifie
In othe
of reel
that is
get cel
systems
the ree
say, th
derived
example
specifi
afferen
onto vi
project

in normal. 7) Many of the malpositioned and disoriented pyramidal cells of the piriform cortex, whose somata occupy deep rather than superficial positions in reeler, do not have apical dendrites that ascend far enough to reach the LOT. Therefore, there may be aberrant patterns of intercellular connectivity in at least the piriform cortex of reeler. To date though, there is no ultrastructural data on normal or abnormal synaptic relationships involving long projection afferents or local circuits anywhere in the reeler cerebrum.

Subsequent studies of patterns of connectivity in the reeler forebrain have been directed toward two major issues: 1) Are there particular fiber projections that exhibit modified axonal trajectories that are appropriately organized? In other words, are the trajectories and terminal distributions of reeler cortical afferent systems reorganized in a manner that is reminiscent of afferents seeking out displaced target cells? 2) Are the projections of particular afferent systems (beside olfactory projections already described) in the reeler cerebrum topographically organized? That is to say, the architectonic map of the neocortex of reeler, as derived from studies using cell and fiber stains (see for example, Caviness, 1976a), should contain topographic specificity in the regional distributions of particular afferent systems (e.g. lateral geniculate nucleus projects onto visual cortical areas, the thalamic ventrobasal complex projects upon somatosensory cortical areas, etc.).

It
by exam
tivity
neurona
of the
particu
mutant
phenoty
of axon
also ta

Re
suggest
factors
determi
studies
Cavines
Yorke,
1976; W
It has
techniq
Hayes,
labeling
specific
extremel
populati
thalamoc
topograp

It soon became obvious to several investigators that by examining these issues, one is able to isolate connectivity from cytoarchitecture as a result of displacement of neuronal elements within the reeler cortices. An examination of the topographical (tangential) and radial components of particular afferent trajectories and distributions in the mutant would allow an assessment of potentially separable phenotypic phenomena of cell position and the specificity of axonal connections. It follows that such phenomena may also take place during development in normal brains.

Results from the study of Devor, et. al. (1975) suggested that in the reeler olfactory system, developmental factors which determine axonal trajectories and those which determine neuronal position are independent. Several studies in other forebrain systems of reeler (see Frost and Caviness, 1974; Steindler and Colwell, 1976; Caviness and Yorke, 1976; Caviness, 1976a,b; Caviness, Frost, and Hayes, 1976; Welt and Steindler, 1977) tend to support this notion. It has been shown, from studies using anterograde degeneration techniques (Frost and Caviness, 1974; Caviness, Frost and Hayes, 1976) and combined anterograde-retrograde axonal labeling techniques (Steindler and Colwell, 1976), that specific thalamocortical projections in reeler will follow extremely aberrant trajectories in order to locate particular populations of displaced target cells. Further, these thalamocortical projections circumscribe characteristic topographical regions within the mutant cerebrum that are

homolo
nucleu
visual
Steindl
1976a)
regions
1976; C
1977; S

Wi
malposi
was sho
specific
fashion
cortica
normal
of neur
Colwell
an inves
offered
amidst d
neurons
were sho
that cel
their po
to proje

Wit
forebrai

homologous to the normal (for example, the lateral geniculate nucleus projects onto what appears to be area 17 of the visual cortex (see Drager, 1975; Frost and Caviness, 1974; Steindler and Colwell, 1976; Caviness, 1975b; Caviness, 1976a) and the ventrobasal complex projects to somatosensory I regions containing the "barrel field" (Steindler and Colwell, 1976; Caviness, Frost and Hayes, 1976; Welt and Steindler, 1977; Steindler, 1977).

With regard to efferent projections that arise from malpositioned and disoriented cortical cells in reeler, it was shown that particular cortical regions project back upon specific thalamic relay nuclei in a precisely reciprocal fashion (Steindler and Colwell, 1976). Reciprocal thalamo-cortical-corticothalamic (TC-CT) pathways, as seen in both normal and abnormal brains, may be a fundamental characteristic of neuronal organization and development (see Colwell, 1975; Colwell and Merzenich, 1975; Colwell, 1976). For this reason, an investigation of highly reciprocal TC-CT projections offered a precise parameter for the assessment of connectivity amidst distorted laminar arrangements and disoriented cortical neurons in the mutant forebrain. Because these projections were shown to be maintained in reeler, it can be inferred that cells of a particular cortical locus, regardless of their position or orientation, are genetically determined to project in a reciprocal fashion back to the thalamus.

With regard to diencephalic structures of the reeler forebrain, there is an extreme paucity of information

regardin
example,
(Welt and
stained
complex i
aggregate
In additi
structure
vibrissae
reciproca
cortex (S
The corti
lattices
relay neu
thalamoco
results s
normal in
these str
patterns
the corti
normality
of findin
neurons i
it would
some degr

Othe
(callosal

regarding cyto- or myeloarchitectural organization of, for example, the thalamus. However, it was recently shown (Welt and Steindler, 1977; Steindler, 1977) that in cell stained material, a division of the thalamic ventrobasal complex in reeler contains appropriate examples of cellular aggregates referred to as "barreloids" (Van der Loos, 1976). In addition to their normal appearance in reeler, these structures, which are believed to be related to individual vibrissae of the contralateral face, display appropriate reciprocal connections with the primary somatosensory cortex (SmI) (Welt and Steindler, 1977; Steindler, 1977). The corticothalamic axons from SmI project to primarily the lattices of the thalamic barreloids, and thalamocortical relay neurons within the lattices give rise to the strongest thalamocortical projections back onto SmI. Even though these results suggest that subcortical brainstem structures are normal in reeler, further information is needed. Because these structures normally do not possess the recognizable patterns of cellular lamination and orientation as seen in the cortices, an assessment of their cytoarchitectural normality may be currently unattainable. However, in light of findings on aberrant axonal trajectories from thalamic neurons in attempting to reach displaced cortical neurons, it would be surprising if thalamic somata did not display some degree of malposition or disorientation as well.

Other cortical efferent systems such as cortico-cortical (callosal) projections have also been recently examined in

[illegible] cytoarchitectural [illegible] [illegible], however, it was [illegible] (Welt and Steindler, 1977; Steindler, 1977) that in cell stained material, a division of the thalamic ventrobasal complex in reeler contains appropriate examples of cellular [illegible] referred to as "barreloids" (Van der Loos, 1[illegible]). In addition to their normal appearance in reeler, these structures, which are believed to be related to individual vibrissae of the contralateral face, display appropriate reciprocal connections with the primary somatosensory cortex (SmI) (Welt and Steindler, 1977; Steindler, 1977). The corticothalamic axons from SmI project to primarily the lattices of the thalamic barreloids, and thalamocortical relay neurons within the lattices give rise to the strongest thalamocortical projections back onto SmI. Even though these results suggest that subcortical brainstem structures are normal in reeler, further information is needed. Because these structures normally do not possess the recognizable patterns of cellular lamination and orientation as seen in the cortices, an assessment of their cytoarchitectural normality may be currently unattainable. However, in light of findings on aberrant axonal trajectories from thalamic neurons in attempting to reach displaced cortical neurons, it would be surprising if thalamic somata did not display some degree of malposition or disorientation as well.

Other cortical efferent systems such as cortico-cortical (callosal) projections have also been recently examined in

reeler (Steindler and Colwell, 1976; Caviness and Yorke, 1976). The pattern of innervation between the abnormal cerebral hemispheres, as in the normal, is homotypically (topographically) organized. Combined horseradish peroxidase (HRP)-tritiated amino acid (TAA) injections in the reeler hemispheres revealed HRP neurons and autoradiographic (ARG) grains within the contralateral homotypic cortex, and these projections displayed a different radial distribution relative to the normal (Steindler and Colwell, 1976). In reeler, the greatest concentration of HRP and ARG labeling was in the deep strata of the contralateral homotypic cortex, while in the normal the greatest concentration was located more superficially (Steindler and Colwell, 1976). These results suggest that there is a maintenance of reciprocal interhemispheric projections, mediated through the appropriate cortical interneurons, in reeler as in the normal. The origins, trajectories and terminations of these projections are radially modified in a manner that is retrospective of laminar inversion. The distributions of thalamocortical, corticothalamic and cortico-cortical axons appear to be determined by the relative positions of their target cells (Caviness and Yorke, 1976; Devor, et. al., 1975; Caviness, Frost and Hayes, 1976; Caviness, 1976a; Steindler and Colwell, 1976).

Finally, with respect to afferent organization within the reeler hippocampus: 1) Major afferent systems arrive at appropriate times during development of the hippocampus

and dentat

cortex (by

and commiss

graphic mod

afferent la

gyrus displ

placed popu

1975; Stanf

All of

order to es

postsynapti

long projec

specificity

Studies on

Cerebe

mice have b

Sidman et.

Mullen, et.

1973a,b; Sc

reeler cere

as extensiv

part. Besi

also produc

tion and ma

sized cereb

(Hamburgh,

Rakic and S

and dentate gyrus. 2) Projections from the entorhinal cortex (by way of the pertorant path) and associational and commissural systems are arranged in appropriate topographic modes within these structures. 3) The patterns of afferent lamination within the hippocampus and dentate gyrus display radial aberrancies in association with displaced populations of hippocampal cells (Stanfield, et. al., 1975; Stanfield, 1977).

All of the forementioned results suggest that in order to establish appropriate connections with displaced postsynaptic populations of neurons in the reeler forebrain, long projection afferent axons manifest both topographic specificity and modified radial distributions of axons.

Studies on the Neuronal Organization of the Reeler Hindbrain

Cerebellar malformations due to genetic mutations in mice have been examined in several studies (for example, see Sidman et. al., 1965; Swisher and Wilson, 1977; Landis, 1973; Mullen, et. al, 1975; Sidman, et. al, 1962; Rakic and Sidman, 1973a,b; Sotelo, 1975). A review of the literature on the reeler cerebellum has revealed that this area has not been as extensively examined as its forebrain neocortical counterpart. Besides affecting the cerebrum, the reeler mutation also produces defects in the cerebellum where lack of foliation and malpositioned cortical neurons result in an undersized cerebellum in the adult, which appears embryonic (Hamburgh, 1963; Sidman, et. al, 1965; Sidman, 1968a,b, 1972; Rakic and Sidman, 1972; Steindler, 1975; Rakic, 1976). Some

studie
Hamburg
reduced
reeler
activit
tissue)
observe
ephrine
of reel
is abou
might r
anatomi
within
of the
choline

Th
surface
also di
cytoarc
bellum
reeler
cell la
cell-st
feature
1968a,b,
Rakic,
and orie

studies (Sidman, et. al., 1965; Sidman, 1968a,b, 1972; Hamburgh, 1963) suggest that granule cell numbers are reduced and that the concentration of cholinesterase in reeler mice is two to three times normal (cholinesterase activity expressed in units per milligram wet weight of tissue) (Hamburgh, 1963). Landis et. al. (1975) also observed a twofold increase in the concentration of norepinephrine and number of fluorescent aminergic fibers per unit of reeler mutant cerebellar cortical area. However, there is about a 50% reduction in cerebellar size in reeler that might result in compressed topographic representations. An anatomical increase in density of neurons and afferents within any given section through the rostral-caudal extent of the mutant cerebellum would support the increases in cholinesterase and norepinephrine.

The radial aspect of cerebellar morphology (pial surface to white matter, or dorso-ventral axis) in reeler is also difficult to assess. There is an aberrant pattern of cytoarchitecture and cell orientation in the reeler cerebellum that obscures the characteristic three layers of the reeler cerebellar cortex: molecular, Purkinje and granule cell laminae. In electron microscopic and light microscopic cell-stained material, the following cytoarchitectonic features of the reeler cerebellum were recognized (Sidman, 1968a,b, 1972; Hamburgh, 1963; Rakic and Sidman, 1972; Rakic, 1976): 1) The degree of aberrancy in cell position and orientation is different within different regions.

2) There
layer be
molecular
many area
within po
Purkinje
granule c
radial or
in the mu
and deep
be morpho
Sidman et.
and Sidman
substantia
occupy the
cerebellum
6) The cer
extends to
display abr
these cells
the externa
Bergmann gl
within the
obliquely.
coat the ext
coming in co
Bergmann gli

2) There is, for the most part, a persistence of a molecular layer beneath the pial surface. 3) The segregated nature of molecular layer basket and stellate neurons is lost. 4) In many areas, disoriented Purkinje cells reside beneath or within populations of granule cells. In some areas, Purkinje cells occupy normal external positions relative to granule cells, and their somata and dendrites display normal radial orientations. Purkinje somas are found everywhere in the mutant cerebellum except within the molecular layer and deep nuclei. 5) The deep cerebellar nuclei, thought to be morphologically normal in reeler (see Hamburgh, 1963; Sidman et. al., 1965; for contrasting results, see Caviness and Sidman, 1972, where these authors describe, without substantiation, the lateral nucleus as being abnormal), occupy the greater part of the radial extent in the reeler cerebellum as a result of cerebellar cortical dysgenesis. 6) The cerebellar white matter, in some instances, almost extends to the pial surface. 7) Bergman glial cells also display abnormalities in position and orientation. Fibers of these cells ascend radially in the molecular layer and coat the external surface in normal animals. In reeler, some Bergmann glia are inverted. They are aften malpositioned within the molecular layer and possess fibers that course obliquely. Bergmann glial end-feet in reeler often do not coat the external surface, resulting in neuronal elements coming in contact with the pial surface. The fibers of Bergmann glia are known to be related with, and act as a

guidance
external
In reeler
orientat
maintain
rather th
molecular

In t
prelude t
the devel
(see Caja
Miale and
The develo
variations
may be alr
the commen
discussion
Nooreddin,
studies hav
three-layer
prior to em
postmitotic
cells) begi
fourth ventr
and Sidman,
(possibly Be
around E12 -

guidance for, migratory young neurons that descend from the external granule cell layer (see, for example, Rakic, 1971a). In reeler, in spite of the abnormalities in position and orientation of Bergmann cells and fibers, granule cells maintain migratory routes along attainable Bergmann fibers rather than attempting an unassisted descent through the molecular layer.

In the normal mouse, particular neuronal migrations prelude the final histogenetic phenomena and contribute to the development of gross and microscopic cerebellar morphology (see Cajal, 1929; Bonnevie and Brodal, 1946; Uzman, 1960; Miale and Sidman, 1961; Fujita, et. al., 1966; Fujita, 1969). The development of fissures and lobules may result from regional variations in cerebellar corticogenesis, and these variations may be already expressed within the germinal zones prior to the commencement of migration (to be elaborated upon in the discussion, also see Korneliussen, 1968a,b; Haddara and Nooreddin, 1966). Tritiated thymidine autoradiography studies have shown that the development of a conventional three-layered cerebellar cortex begins, in the normal mouse, prior to embryonic day 11 - 13 (E11 - E13) when young postmitotic neurons (presumptive deep nuclear and Purkinje cells) begin to migrate from the ventricular zone of the fourth ventricle into the cerebellar cortical plate (Miale and Sidman, 1961). Golgi II neurons and some neuroglia (possibly Bergmann glial cells) undergo their final mitosis around E12 - E15 and migrate from the ventricular zone also

around thi
mitotic ac
E17. Ther
small neur
and outwar
ment and d
does not o
cells form

Purkin
differentia
cortex arou
zone, calle
around E13
margin of t
(Miale and
EGL neurons
posterolate

During
cover the c
istic ventr
(although t
characteris
Cajal (1929
of daughter
and positio
stages in G
neurons from

around this time. According to Miale and Sidman (1961), mitotic activity in this ventricular zone ceases around E17. Therefore, Purkinje cells, Golgi cells and large and small neurons of the deep nuclei undergo their final mitoses and outward migration prior to birth. Characteristic allignment and differentiation of these populations of neurons does not occur until after birth (for example, Purkinje cells form a single lamina around postnatal day 10 (P10)).

Purkinje, Golgi and deep nuclear cells begin their differentiation within the normal developing cerebellar cortex around the time another germinal zone appears. This zone, called the external granular layer (EGL) arises at around E13 in the ventricular zone at the lateral caudal margin of the roof of the fourth ventricle (the rhombic lips) (Miale and Sidman, 1961). By E15, a blanket of these young EGL neurons completely covers the cerebellar plate in a posterolateral to anteromedial direction.

During the period E13 - E15, the EGL cells that now cover the cerebellum begin another mitotic cycle. Characteristic ventricular zone divisions do not apply to the EGL (although this layer does probably exhibit "elevator"movement characteristic of "matrix" cells, see Fujita, 1969), and Cajal (1929) categorized the stages of division and migration of daughter cells from the EGL according to changes in shape and position of these cells as they appeared in successive stages in Golgi impregnated material. Postmitotic young neurons from the EGL assume a horizontal bipolar shape just

below a s
tritiated
Sidman, 19
phase of m
cells beco
nuclear mi
adult posi
Rakic and
some neuro
mitosis and
and other
and past s
position in
mentioned b
and Sidman,
radial guid
adult posit
this migrat
(e.g. granu
laying down
Sidman, 196
in the EGL
during the

With r
tritiated t
Purkinje ce
and spatial

below a superficial zone in the EGL where, according to tritiated thymidine autoradiographic studies (Miale and Sidman, 1961), mitotic activity is still high. The next phase of migration is characterized by the horizontal bipolar cells becoming vertically bipolar as a result of vertical nuclear migration of granule cells en route to their presumed adult position in deeper layers (Cajal, 1929; Rakic, 1971a; Rakic and Sidman, 1970). Basket cells, stellate cells, and some neuroglia remain within the molecular layer following mitosis and a short migration from the EGL. Granule cells, and other neuroglia, must migrate through the molecular layer and past somata of Purkinje cells to reach their adult position in the presumptive internal granule cell layer. As mentioned before, it is believed (see, for example, Rakic and Sidman, 1970; Rakic, 1971a) that these cells migrate along radial guidelines of Bergmann glial fibers to reach their adult positions. (It has been suggested that it is during this migration that there is cell to cell communication (e.g. granule cell - Purkinje cell) that results in the laying down of synaptogenic relationships (see Miale and Sidman, 1961; Sidman, 1968a,b)). Mitotic activity ceases in the EGL around P15, and the EGL completely disappears during the third postnatal week (Miale and Sidman, 1961).

With regard to development of the reeler cerebellum, tritiated thymidine autoradiography studies have shown that Purkinje cells and granule cells display appropriate temporal and spatial origins (Sidman, 1968a,b). In reeler, Purkinje

cells aris
align prop
Most granu
postnatall
Purkinje s
degree of
but granule
orientatio
cell death
the mutant
(see Sidman
there is no
EGL in reel
which influ
cells from
have gone a
cytoarchite

Sidman
manifested
communicatio
In other wo
cortices may
young cortic
cell-surface
nition - see
evidence fro
mutant corti

cells arise on E11 - E12 but fail to migrate, aggregate and align properly within the outer part of the cerebellar cortex. Most granule cells in both reeler and normal animals originate postnatally, but in the mutant most fail to descend past Purkinje somata (Sidman, 1968a,b). This results in some degree of granule cell aggregation, in the form of ectopia, but granule cells in reeler also display aberrancies in orientation. As stated earlier, there may be some atypical cell death in the EGL that reflects the first development of the mutant phenotype. Unlike the weaver cerebellum though (see Sidman, 1968a,b; Rakic and Sidman, 1973a,b; Sotelo, 1975) there is no strong evidence for significant atrophy of the EGL in reeler. Therefore, in reeler, the developmental factors which influence patterns of migration and alignment of young cells from the EGL, and/or ventricular zone, must somehow have gone astray producing a nonfoliated, undersized and cytoarchitecturally disorganized cerebellum.

Sidman (1968a,b) suggests that the reeler mutation is manifested at the level of cell to cell interactions or communication during neuronal migration in the cortices. In other words, the histogenetic malformation in the mutant cortices may be present intrinsically within populations of young cortical neurons (for example, factors coating the cell-surface leading to differential cellular adhesion/recognition - see Discussion). Such a notion is supported by evidence from tissue culture experiments where embryonic mutant cortices, supposedly free of subcortical influences,

reaggregat
seen in vi
experiment
interactio
of cortica
young neur
be involve
afferent-ta
subsequent
1968a, in
mouse cere
within the
migratory P
that cerebe
controlling
layer durin
this from f
aberrant mos
rat cerebell
within the e
been a simil
the mutant c

Studies on t
Reeler Hindb

An archi
produced, and
the extensive

reaggregate normally and display the cytoarchitectural deficits seen in vivo (DeLong and Sidman, 1970; unfortunately, these experiments were never successfully repeated). If cellular interaction is necessary, are the possible aberrant interactions of cortical cells in reeler solely restricted to the somata of young neurons within the cortices, or may presumptive neurites be involved? It is also possible that there are abnormal afferent-target cell communications during migration and subsequent neuronal alignment within the cortices (Sidman, 1968a, in looking at pyridine-silver preparations of E14 normal mouse cerebella, described subcortical afferents present within the cerebellar plate and often in the direct path of migratory Purkinje cells. Also, Altman (1973) has suggested that cerebellar mossy fibers may be involved in eliciting or controlling granule cell migrations from the external granular layer during development of the cerebellar cortex. He postulated this from findings on cerebellar granule cell ectopia due to aberrant mossy fiber distribution in x-irradiated neonatal rat cerebella. Such a phenomenon has not been investigated within the ectopic granule cell layer of reeler, nor has there been a similar assessment of potential afferent influences in the mutant cerebrum.

Studies on the Afferent Organization and Connectivity of the Reeler Hindbrain

An architectonic map of the reeler cerebellum has not been produced, and such an effort would be difficult in light of the extensive mixing of neuronal elements. The questions

then arise: 1) Can one assess the extent of cerebellar organization and development in reeler by examining the topographical and radial distributions of particular cerebellar afferents? (Such an examination has enhanced our understanding of organization in the mutant forebrain - see previous sections). 2) Might common developmental factors influence cell position and orientation in the mutant cerebellum and cerebrum? 3) Is the mutant cerebellum cytoarchitecturally inverted in a similar fashion to the cerebral inversion?

An electron microscopic study of synaptic arrangements and cellular morphology in the reeler cerebellar cortex (Rakic and Sidman, 1972; Rakic, 1976) has revealed that cellular morphology and synaptic relationships are not grossly affected. Long projection afferents (climbing fibers from the inferior olivary nuclei; and mossy fibers from several possible brainstem nuclei or the spinal cord) were recognized within the reeler cerebellum, and they engaged in appropriate synaptic relationships (climbing fibers contacting Purkinje, basket and stellate dendrites, and Purkinje soma; mossy fiber terminals contacting granule cell dendrites in "simplified glomeruli").

Rakic and Sidman (1972) have deduced that synaptic generating mechanisms may be present in the reeler cortices in spite of cellular malpositioning. To date though, there are only two studies that have examined the topographical and radial distributions of specific afferent projections within the shrunken and malformed cerebellum (Steindler, 1975;

Steindler,
such an exa
the mutant
of particul
malformed r

The pr
of observat
convey prop
recipient z
preliminary
mossy fiber
lamina VII
brodal and
Sherrington
1969), and
1977) in se
focussed on
lower trunk
of origin o
(dorsal spi
and Grant,
tract proje
normal anim

The pr
degeneratio
cerebellar
reeler cere

Steindler, 1977b). Based on the aforementioned premise that such an examination will shed light on the organization of the mutant hindbrain, I have undertaken a systematic evaluation of particular brainstem and spinal projections within the malformed reeler cerebellum.

The present study represents a detailed anatomical report of observations made on spinocerebellar projections that convey proprioceptive information to a restricted spinal recipient zone (SRZ) in normal and reeler cerebella (for preliminary observations, see Steindler, 1975). Spinocerebellar mossy fibers are known to arise from Clarke's column (within lamina VII of Rexed (1954)) (see, for example, Grant, 1962; Brodal and Grant, 1962), spinal border cells (Cooper and Sherrington, 1940; Hubbard and Oscarsson, 1962; Ha and Liu, 1969), and other spinal gray regions (see, for example, Snyder, 1977) in several mammalian species. My attention will be focussed on the cerebellar representation of the hindlimb and lower trunk in the normal and reeler cerebellum. The neurons of origin of these representations reside in Clarke's column (dorsal spinocerebellar tract projections, see Larsell, 1953; and Grant, 1962) and spinal border areas (ventral spinocerebellar tract projections, see Grant, 1962; and Ha and Liu, 1969) in normal animals.

The present study was undertaken, using primarily axonal degeneration techniques, to reveal one of two possible spinocerebellar distributions that were anticipated to exist in the reeler cerebellum: 1) Spinocerebellar mossy fibers may

distribute without any recognizable order or topography.
2) There may be an order to the distribution of this specific afferent system in the mutant cerebellum as follows:
a) spinocerebellar mossy fibers may reorganize and synapse within non-spinocerebellar sites within the cerebellum (as determined by comparisons of the afferent distribution in normal mouse cerebella) as a result of reduced numbers of displaced target cells (granule cells normally receive the strongest mossy fiber input - see, for review, Palay and Chan-Palay, 1974) as well as the overall reduction of cerebellar size in reeler, b) spinocerebellar mossy fibers may follow some specific reeler blue-print for the development of afferent-target cell relationships, and hence show some organization distinct from the pattern of a normal distribution,
c) spinocerebellar mossy fibers may distribute densely within topographically appropriate areas of the abnormal cerebellum with an overall topology that is similar to the normal. (This assumes that the mutant cerebellum is basically a miniature cerebellum, and that within a reeler blue-print for cerebellar development, there resides a fundamental mechanism for the laying down of presumptive topographical boundaries.)

Results from these experiments will contribute to our understanding of afferent organization and the maintenance of appropriate patterns of connectivity amidst malpositioned and disoriented hindbrain cortical neurons. This will be accomplished by attempting to parcel the reeler cerebellum

based on topographical projections of particular afferent systems, and examining trajectories and radial distributions of these axons and terminals in order to deduce whether or not specific cerebellar afferents seek out particular displaced populations of reeler cerebellar cortical cells.

Materials

Descripti

Hist

normal an

microscop

differenc

tectonics

basis for

tions wit

A se

3 heteroz

age from

The mice

C57BL/6J

Sidman, 1

Bar Harbo

possible,

inbred str

phenotypic

tonic anom

(Caviness,

affected

and mental

(though so

viorally a

a gene dos

Materials and Methods

Descriptive Anatomical Studies on Normal and Reeler Hindbrain

Histological sections of brain and spinal cord from normal and reeler mutant mice were compared using the light microscope, in order to assess the apparent cytoarchitectural differences present within the two genotypes. The cytoarchitectonics of the normal and mutant cerebellum provided a basis for subsequent studies dealing with afferent distributions within each.

A series of twelve mice, 3 normal (homozygous wild-type), 3 heterozygous, and 6 homozygous reeler mutants varying in age from 16 to 30 postnatal days were used in this study. The mice were from the C57BL/6J-*rl* congenic strain and C57BL/6J x C3H/HeJ F1 hybrid strain (see Caviness, So and Sidman, 1972) originally obtained from The Jackson Laboratory, Bar Harbor, Maine. Hybrid reeler mice where used, whenever possible, because of their increased vitality compared to inbred strains (Caviness, So and Sidman, 1972). Despite phenotypic differences in the two strains, the cytoarchitectonic anomalies appear to be identically expressed in both (Caviness, So and Sidman, 1972). Behaviorally, homozygous affected reeler mice exhibit motor dysfunctions, passiveness and mental deficiencies beginning around postnatal day 14 (though sometimes earlier). Heterozygous mice appear behaviorally and histologically normal; therefore, there is not a gene dosage effect in reeler. Affected animals (in all

experiment
well as th
for exampl
zygous rec
incidence

The a
with eithe
phosphate
(see Valve
brains and
months, an
tion of ne
Golgi meth
in gelatin
sagittal pl
cresyl viol
perform a G

In add
mutant brai
1 µm using
methylene b
(1960).

Sectio
cytoarchite
depicted in
Sidman, et.

experiments) were recognized by their abnormal phenotype as well as through the aid of a genetic marker hammertoe (see, for example, Sidman, et. al., 1965) which results in homozygous reeler being homozygous hammertoe with about 12% incidence of crossover.

The animals were perfused through the left ventricle with either 10% formol-saline, 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2), or an osmium-dichromate fixitive (see Valverde, 1970), under ether or metafane anesthesia. The brains and spinal cords were removed, postfixed for up to two months, and either immediately block stained for the examination of neurons and their processes using the original Rapid Golgi method (see, for example, Valverde, 1970), or embedded in gelatin-albumin and serially sectioned in frontal and sagittal planes with subsequent staining of perikarya using cresyl violet. Some brains were not fixed in order to perform a Golgi-Cox method (Ramón-Moliner, 1970).

In addition, slices of cerebellar tissue from normal and mutant brains were embedded in epon-alraldite, sectioned at 1 µm using a Reichert Om U2 ultramicrotome and stained with methylene blue according to the method of Richardson, et. al. (1960).

Sections were examined using bright-field microscopy, and cytoarchitectonic parcellations were compared with those depicted in the atlas of the mouse brain and spinal cord by Sidman, et. al. (1971).

Anatomic
in the C

In
butions
cerebell
spinal c
mutant h
age from
in our la
stock pre

The
anesthesi
tenth cos
tissue we
processes
vertebrae
using a sm
crushing c
forceps.

Follo
other unre
days were
or 4% para
removed and
transferred
and serially
plane allows

Anatomical Studies on the Distribution of Spinal Afferents in the Cerebella of Normal and Reeler Mutant Mice

In order to define the trajectories and terminal distributions of spinocerebellar mossy fibers in normal and reeler cerebella, the cerebellar cortices, underlying brainstems and spinal cords from 3 normal, 3 heterozygous, and 4 homozygous mutant hybrid reeler mice were studied. The mice ranged in age from 16-22 postnatal days, and were bred and maintained in our laboratory from known heterozygotes of the B6C3H hybrid stock previously described.

The animals were anesthetized by ether or metafane anesthesia, and dorsal skin incisions were made caudal to the tenth costal-vertebral joint. Overlying muscle and connective tissue were reflected above several vertebrae, and the spinous processes and entire dorsal surface of the twelfth thoracic vertebrae (for the cases documented in the present study) using a small hemostat and forceps. A hemisection was made, crushing one-half of the cord with a number 5 watchmakers forceps.

Following survivals of five days (found to be optimal from other unreported studies where survival times of one to seven days were tried) and cardiac perfusion with 10% formol-saline or 4% paraformaldehyde, the brains and spinal cords were removed and postfixed for at least one week before being transferred to 15%, then 30% sucrose-phosphate buffer, frozen, and serially sectioned at 30 µm in the frontal plane (this plane allows one to accurately assess both the radial and

medio-la

One in t

other se

or Fink-

basicall

procedur

fiber pr

cortex (

Sec

projectin

tion in t

to some e

blood ves

struction

radial di

in the no

<u>An Experi</u>
<u>Using Com</u>

One

ongoing in

relationsh

the brains

Injec

peroxidase

various ce

gous reeler

days. 90 μ

medio-lateral distributions of cerebellar afferent fibers). One in three sections was stained with cresyl violet, and all other sections were stained by either the Nauta-Gygax (1954) or Fink-Heimer (1967) methods (a Fink-Heimer variant that basically combines both of the Fink-Heimer I and Fink-Heimer II procedures that has proved to be successful in localizing mossy fiber preterminal and terminal degeneration in the rat cerebella cortex (Steindler, unpublished observations)).

Sections were drawn using a Bausch and Lomb overhead-projecting microscope, and the total extent of axonal degeneration in the cerebella (as well as brainstems and spinal cords to some extent) were plotted from the light microscope using blood vessels and other landmarks. Frontal and surface reconstructions were made in order to assess the topography and radial distribution of spinocerebellar mossy fiber projections in the normal and reeler cerebellum

An Experimental Study of Cerebellar Afferents and Efferents Using Combined Anterograde-Retrograde Axonal Labeling

One case that is also reported in this study is from an ongoing investigation of reciprocal afferent and efferent relationships between the cerebellum and certain nuclei of the brainstem (also, see Steindler, 1977b).

Injections of 0.05 - 0.2 µl combined horseradish peroxidase (HRP) - tritiated amino acid (TAA) were made in various cerebellar regions of three normal and four homozygous reeler mice, varying in age from 22 to 30 postnatal days. 90 µm frontal sections through the normal and reeler

mid- and hindbrain were processed for HRP alone (Graham and Karnovsky, 1966; LaVail, Winston and Tish, 1973) and adjacent 30 µm sections were processed for either autoradiography (ARG) alone (Cowan, et. al., 1972) or for combined HRP-ARG (Colwell, 1975) using Kodak NTB-2 nuclear track emulsion. Autoradiographic and combined HRP-ARG sections were exposed in the dark for up to three months, then developed in D-19 and examined by bright- and bark-field microscopy (for further details, see Colwell, 1975; Steindler and Colwell, 1976; Steindler, 1977b).

Results

Cytoarchitecture of the Normal Cerebellum

Investigations on the cytoarchitectonics and patterns of connectivity within the reeler cerebellum have shown: 1) Distorted laminar arrangements and disoriented cortical neurons obscure the characteristic radial organization of the cerebellar cortex. 2) For the most part, granule cells can be considered as occupying ectopic positions in relation to mal-positioned and disoriented Purkinje cells. 3) The improper radial development of the cerebellar cortex results in altered and compressed rostrocaudal and mediolateral representations of cortical neurons. 4) Cerebellar afferents such as spino-cerebellar mossy fibers radially distribute within primarily ventral regions of the afoliate reeler cortex (as opposed to dorsal regions in the normal) in a manner that may indicate seeking-out of particular displaced populations of target cells. 5) The dorsal and ventral spinocerebellar tracts (DSCT and VSCT) display modified axonal trajectories. In accordance with rostrocaudal and mediolateral regional alterations in cytoarchitecture, DSCT and VSCT projections give rise to a reorganized topographic spinocerebellar representation that can be mapped within presumptive vermian (paleocerebellar) areas that appear to be homologous to the normal. 7) The deep cerebellar nuclei in reeler, which may be larger than normal, display appropriate afferent and efferent associations with specific brainstem nuclei (for example, the inferior olivary complex).

These results, which approximate previous findings in the reeler forebrain cortical structures, will now be presented in detail.

The normal mouse cerebellum, in cell-stained sections, reveals the same characteristic geometric relationships of cerebellar cortical neurons and their afferents as seen in other mammalian species (see Cajal, 1952; for review, see Palay and Chan-Palay, 1974). Figures 1a, 2a, and 11 show the uniform dorso-ventral (radial) arrangement of three class-specific cellular layers in the normal mouse cerebellar cortex. The layer immediately beneath the pial surface, the molecular layer, contains Purkinje cell dendrites, granule cell axons (parallel fibers), cell bodies and neurites of basket and stellate cells, and neuroglia (for example, Bergmann glial fibers). The cell bodies of Purkinje cells form a single row beneath the molecular layer. The most distinctive feature of the cerebellar cortex is the thick granule cell layer which consists of densely-packed granule cells, Golgi II cells, and various neuroglia. This continuous lamina is extremely convoluted throughout the dorso-ventral, medio-lateral, and rostral-caudal extent of the cerebellum. These convolutions give rise to regional variations in the form of folia and lobuli. These variations are also expressed in the thickness and overall conformity of the layer below the granule cell layer - the white matter. The cerebellar white matter contains afferents en foute to the cortical alyers (mossy fibers which contact primarily the claw-like dendrites of granule cells

within glomerular structures (see, for example, Eccles, Itto and Szentagothai, 1967) and climbing fibers which are synaptically related with Purkinje cells, their dendrites, and cells and processes in the molecular layer (see for review, Palay and Chan-Palay, 1974)) as well as efferents from Purkinje cells. Most Purkinje cells' axons terminate within the deep cerebellar nuclei (dentate (lateralis), interpositus and fastigial (medialis) nuclei) which are a mass of gray matter situated within the cerebellar commissures deep to the convoluted cortex.

A homogeneous distribution of cerebellar cortical neurons in the normal cerebellum makes it difficult to recognize the existence of cytoarchitectonic fields (see, for example, Braitenberg and Atwood, 1958). Hence, most of the attempts at functional parcellization of the mammalian cerebellum have focussed on: 1) Phylogenetic examination and gross dissection of surface landmarks, fissures, and folia to assemble topologically a three dimentional model of cerebellum made up of lobes and lobules[1] (see Bolk, 1906; Larsell, 1952; Voogd, 1975); 2) Interpreting behaviors that result from partial or complete cerebellectomy in humans with cerebellar disease (see, for example, Dow and Moruzzi, 1958), or from animals

---

1 (See Results section - "The Distribution of Spinocerebellar Afferents Within the Cerebellum of the Normal Mouse.")

with hereditary or infectious disease; 3) Physiological mapping of localized somatotopic representations (see, for example, Snider and Stowell, 1944; Snider and Eldred, 1952; Shambes, et. al., 1976); and 4) The somatotopic organization of cerebellar afferent systems that arise from particular subcortical nuclei. Particular afferent projections might also contribute to regional differences seen in myeloarchitecture[1] (see, for example, Voogd, 1969; Voogd, et. al., 1969; Voogd, 1975).

Cytoarchitecture of the Reeler Cerebellum

Extensive cytoarchitectural anomalies are present in the reeler cerebellum, and they are regularly reproduced from animal to animal. Upon first examination, the most striking histological feature of the mutant cerebellar cortex is the lack of foliation that dramatically reduces surface area (see Fig. 1b). A discernible subpial molecular layer encircles most of the smooth cortical sheet (see Figures 1b, 3, 12, 13a, 14). The molecular layer contains scattered clusters of displaced granule cells as well as varied numbers of basket and stellate cells. The three divisions of the deep cerebellar

---

1 (See Results section - "The Distribution of Spinocerebellar Afferents Within the Cerebellum of the Normal Mouse.")

nuclei are cytoarchitecturally distinct in reeler, but often appear to be qualitatively greater in size (see Fig. 1b). There is not a well-delineated boundary at the junction of gray and white matter of the cortex, and displaced populations of granule and Purkinje cells are often found in the white matter. The configuration of the white matter does, however, adhere to the regional variations that result from changes in thickness of the cortical sheet (Fig. 1b). The white matter does not wrinkle forming "medullary sheets" to accomodate for cortical surface expansions as seen in the normal (see Haddana and Nooreddin, 1966), and there may be a greater concentration of axons per unit of white matter in reeler as well. There are three geometric representations of aberrantly positioned and oriented granule and Purkinje cells in reeler that contribute to the severe laminar disarray (see Fig. 2b): 1) Some granule cells may lie beneath a row of Purkinje cells as in the normal (see Fig. 13a); 2) Populations of granule cells occupy positions above Purkinje cells (also see Fig. 13a); and 3) Granule cells anomalously intermingle with Purkinje cells. It appears that most granule cells in reeler are ectopic in relation to populations of Purkinje cells. The relative <u>dorsoventral</u> (radial) arrangement of Purkinje and granule cells may be reversed, yet extreme heterogeneous distributions of neuronal elements within various cerebellar regions of reeler complicates this issue. Most likely, factors which misdirect cellular migration, aggregation, and orientation produce a cerebellar cortex with numerous

laminar (radial) defects (see Introduction: Studies on Neuronal Organization of the Reeler Forebrain).

As stated earlier, the homogeneity in distribution of cerebellar cortical neurons in the normal cerebellum is not conducive for the assignment of cytoarchitectonic fields (for cytoarchitectonic parcellations of the normal cerebrum, based on variations in cellular lamination, see Brodmann, 1909; Krieg, 1946a,b; Caviness, 1976a,b). In addition, extremely heterogeneous distributions of cerebellar cortical neurons in reeler obscures the characteristic landmarks necessary for both direct comparison with the normal and construction of an architectonic map. Thus, it becomes necessary to focus on the mediolateral and rostrocaudal distributions of cortical neurons in order to provide another basis for assessing afferent organization in the normal and mutant cerebellum.

In cell-stained sections from embryonic Cetacea and rat cerebellum, Korneliussen (1968a,b; 1969) has elaborately described mediolateral variations in the onset and rate of cerebellar cortical differentiation. Mediolateral variations in the thickness, concentration, position and continuity of neurons in the embryonic cerebellar cortical anlage are representative of longitudinal zones. These zones consist of four rostrocaudal segments on each side of the midline (i.e. medial, medial intermediate, lateral intermediate, and lateral zones). They are well-defined as a result of "abrupt changes and interruptions within the cortical anlage, as well as by raphe-like

differentiations within the underlying medullary substance..." (Korneliussen, 1968a). Korneliussen also believed that these zones were associated with the medial, anterior interpositus, posterior interpositus, and lateral cerebellar nuclei, respectively.

Thus, by comparing regional variations in the thickness, concentration, and position of the ectopic granule cell layer (EGL) in reeler, with regional variations expressed during corticogenesis in the normal, one would be able to pin-point the exact time the cerebellar defect is expressed (For example, compare Fig. 3, section b, with Fig. 1c of Korneliussen, 1968a). The proposed presence of longitudinal zones in the reeler cerebellum strongly suggests a maintenance of recognizable topographical organization. Figure 1b shows the <u>rostrocaudal</u> deviations in thickness and continuity of the EGL that contribute to diversification in the position and degree of mixing of neuronal elements. These regional variations in the reeler EGL correspond topographically to lobular formations seen in the granule cell layer of the normal (compare Figures 1a and 1b). Furthermore, frontal sections through the mutant cerebellum (Fig. 3) reveal that these rostrocaudal deviations appear as variations in the <u>mediolateral</u> distributions of the EGL. These mediolateral variations in the reeler cerebellar cortex are strikingly similar to longitudinal cortical zones present during normal corticogenesis (see Korneliussen, 1968a,b; 1969). For example, an interruption in the EGL can be seen in sections

a - e of Fig. 3. This interruption occurs in what appears to be the medial (and perhaps, medial/intermediate) zone of Korneliussen. The interruption results from populations of granular neurons migrating from the EGL to form sheets (section a, b of Fig. 3; Fig. 14) and cylindrical condensations (sections c - f of Fig. 3; Fig. 12) in the midline. Evidence will be presented in support of the notion that these midline cellular condensations of the medial zone in reeler represent presumptive paleocerebellar(vermian) regions.

In conclusion, data on the organization of hindbrain cytoarchitectonics are too ambiguous to reliably parcel the reeler cerebellum. The reeler cerebellum consists of a roof plate that contains white matter, deep cerebellar nuclei, and cellular condensations in the midline (presumptive vermis) (see Figures 1b, 3). The reeler cerebella cortex, which for the most part superposes the roof plate, is mainly comprised of a relatively thin sheet of ectopic granular neurons (EGL) that have undergone a partial migration from the external granule cell layer but failed to convolute and form lobules. Many Purkinje cells lie beneath or amidst this layer, and their aberrant radial relationships with granule cells contribute to almost unrecognizable patterns of lamination. An abnormal radial development of the reeler cerebellar cortex also contributes to reduced rostrocaudal cortical expanse and alterations in the mediolateral representations of cortical neurons. (According to Haddara and Nooreddin, 1966, regional variations in the dorsoventral growth of the external granule cell layer

of the normal mouse contribute to lobulization and an increase in the rostrocaudal cortical expanse. The direction of folding is also apparently determined by a tendency for the cortex to spread rostrocaudally at a rate of more than four times that from medial to lateral (Haddara and Nooreddin, 1966). Nonetheless, the rostrocaudal and mediolateral variations in concentration, position, and continuity of cerebellar cortical neurons suggest that presumptive topographical regions and boundaries (in the form of immature longitudinal cortical zones) do exist in the reeler cerebellum. However, as a result of reduced rostrocaudal and mediolateral cortical expanse in reeler, cerebellar topography appears to be greatly compressed relative to the normal.

The Distribution of Spinocerebellar Afferents Within the Cerebellum of the Normal Mouse

These results will focus on two tracts that occupy two distinct areas of the spinal white matter, arise from particular populations of neurons in the spinal gray matter, and convey proprioceptive (and perhaps exteroceptive) impulses to the paleocerebellum (see Oscarsson, 1973). The two tracts are named the dorsal and ventral spinocerebellar tracts (DSCT and VSCT). There are at least two other systems that perform a similar function, the cuneocerebellar projections arising from the external cuneate nucleus of the caudal medulla and projecting neck, forelimb and upper trunk information onto the paleocerebellar spinal recipient zone (SRZ) (Grant, 1962; Voogd, et. al., 1969), and Oscarsson's (1973) rostral spinocerebellar

tract (also see Snyder, 1977). Attention will be focussed on the cerebellar representation of hindlimb and lower trunk (via DSCT and VSCT projections).

The DSCT and VSCT projections are organized in a segmental fashion based on the topographical representation of their afferents from dorsal root ganglia (see Grant and Rexed, 1958). Therefore by predominately placing lesions at lower spinal segments in this study, it has been possible to isolate particular body segments such as hindlimb and lower trunk that project to the cerebellum. Anterograde axonal degeneration studies are based upon the demonstration of distal portions of interrupted axons, hence axons arising from DSCT and VSCT neurons rostral to the lesion were unaffected and would appear normal in sections treated with the silver salts. The topological pattern of somatotopic DSCT and VSCT projections is the paradigm for assessment of afferent organization within the normal and dysgenesic reeler mouse cerebellum.

A. <u>The Spinal Course of the Normal Mouse DSCT and VSCT</u>

The spinal course of the mouse DSCT and VSCT is similar to that described in other mammalian species (Anderson, 1943; Vachanada, 1959; Grant, 1962; Brodal and Grant, 1962; Voogd, et. al., 1969). After T12 - T13 hemisections in normal and heterozygous reeler mice, and subsequent application of Nauta and Gygax (1954) and Fink and Heimer (1967) reduced silver techniques, the following observations were made with regard to the spinal course of the DSCT and VSCT: 1) Rostral to the

lesion site, degenerating axons were seen in the ipsilateral spinal tract of Flechzig (1876) that lies in the dorsolateral funiculus. This tract, known to be the DSCT, is believed to arise from ipsilateral secondary neurons of Clarke's column (see, for example, Larsell, 1953; Grant, 1962). 2) Degenerating axons in this tract were traced rostrally to their point of entry into the cerebellum via the ipsilateral inferior cerebellar peduncle (ip in Figures 5, 7 and 8). 3) Degeneration was also observed in the ipsilateral and contralateral ventrolateral funiculus, in a region presumed to contain the VSCT. It is believed that these axons arise predominately from contralateral populations of spinal border cells (Cooper and Sherrington, 1940; Morin and Gardner, 1953; Hubbard and Oscarsson, 1962; Ha and Liu, 1968), hence, a bilateral representation of degenerating VSCT fibers, rostral to the lesion site, is suggestive of destruction of VSCT cells, VSCT fibers that have already crossed, and VSCT fibers that have not crossed at the site of the lesion. 4) Degenerating fibers within the ipsilateral and contralateral VSCT ascended through the pons, and superior to the fifth nerve nucleus they turned dorsad and curved corso-caudally into the superior cerebellar peduncle to enter into the cerebellum. 5) In some animals, a small amount of degeneration was also observed in the contralateral inferior cerebellar peduncle as well as the ipsilateral (see Figures 5, 7, 8). This most likely resulted from compression of the contralateral half of the cord or damage to the vasculature, while performing the hemisection. Even though unlikely, there might

also be a small crossed component of the DSCT and/or crossed projections from Clarke's column. In light of similar results in the cerebellar topographic distribution of the DSCT and VSCT in this study and that of Voogd, et. al. (1969) who claim the achievement of relatively pure hemisections, there is no great concern for this phenomenon.

B. The Cerebellar Distribution of the Normal Mouse DSCT and VSCT

Figures 5 and 6 represent reconstructions from frontal sections through the cerebellum and underlying medulla that demonstrate degenerating DSCT and VSCT fibers in a 22-day old normal mouse following interruption at T12 - T13 and show presumed projections of hindlimb and lower trunk regions within the cerebellum (case 9086 - this case is also represented in Figures 10 and 11. The results in this case adequately represent the major characteristics of spinocerebellar distribution displayed in the non-documented cases.) Degenerating axons which are depicted as dots or small lines, can be seen entering the cerebellum through the inferior and superior cerebellar peduncles. Degenerating DSCT and VSCT fibers appear to coalesce near the midline in a cerebellar commissure at the level of, or just above the deep cerebellar nuclei. This commissure is a mass of white matter formed by the uniting of the inferior, middle, and superior cerebellar peduncles, and runs rostrocaudal as far back as the most caudal extent of the deep nuclei. Also, many degenerating fibers were seen crossing to the contralateral side within the commissure at the level of the superior cere-

bellar peduncle, and most likely represent VSCT fibers that cross again upon entry into the cerebellum. (see Fig. 6D). The appearance of degenerating spinocerebellar mossy fibers and their terminal arbors within the white matter and granule cell layer was consistent with Brodal and Grant's (1962) findings in the cat (also see Figures 4, 10, 11). Degenerating axonal debris was observed in the interpositus and medial deep cerebellar nuclei, and Figure 5A - C shows degenerating fibers descending in a radial fashion to terminate within the lingula (li), and ascending (radially) into the centralis (ce) and culmen (cu) of the anterior lobe. Analagous sites of DSCT and VSCT termination have been described previously in rat, cat, and monkey (Anderson, 1943; Grant, 1962; Voogd, et. a., 1969; Oscarsson, 1965; Vachanada, 1959). Besides the dense projection present in the anterior lobe, there is also a projection present posteriorally to the pyramis lobule (Figure 6D - F). This projection has also been described by Grant (1962) who observed a spinocerebellar projection to other lobules of the posterior lobe as well (i.e. pyramis and paramedian. It should be noted that some cases in the present study, especially those with the longest survival times, also displayed some evidence for paramedian projections). As far as the radial component (pia to white matter) of these spinocerebellar projections within the lingula, centralis, culmen and pyramis lobules, it should be noted that in frontal sections (Figures 5 and 6) there are patches of radially oriented DSCT and VSCT fibers that arise from longitudinally and tangentially coursing axons

in the white matter to enter the granule cell layer as discontinuous columns that are radially organized (most easily recognized in Figure 6E - F).

Besides the radial (columnar) and topographical (rostrocaudal from the lingula and centralis lobules of the anterior lobe to the pyramis lobule of the posterior lobe) dimensions to the normal mouse spinocerebellar projections, there is also a mediolateral representation revealed within these frontal sections (Figures 5 and 6) that suggests the DSCT and VSCT project in a bilaterally symmetric fashion (also see, for example, Voogd, et. al., 1969). The ipsilateral innervation of a given cerebellar lobule is similar if not equal to the contralateral projection. This bilateral spinocerebellar projection may also be indicative of an extensive amount of mixing of spinal proprioceptive and exteroceptive efferent information from body regions within numerous and overlapping cerebellar regions, or may reveal a different type of cortical system within the cerebellum where there is no strong evidence for a callosal system (as there is in the cerebral hemispheres) that interconnects ipsilateral and contralateral homotypic body representations. Further, there appears to be an equivalent cerebellar midline with periphery represented on both sides; this division into body halves is consistent with anatomical and physiological findings of spinocerebellar distributions in cat, monkey, and other species (for example, see Boogd, et. a., 1969; Voogd, 1975; Vachanada, 1959; Oscarsson, 1973; Snider and Stowell, 1944; Oscarsson, 1965).

Also, with regard to the mediolateral distribution of DSCT and VSCT axons, the discontinuous columns of degeneration seen within the white matter and granule cell layer of particular lobules of the normal mouse anterior and posterior lobe correspond to "mediolaterally arranged maxima and minima in the termination of the spinocerebellar fibres..." (Voogd, et. al., 1969) seen in various other mammalian species (Voogd, et. al., 1969; Voogd, 1969). Voogd (1969), in looking at regional differences in cerebellar myeloarchitecture using various axonal staining techniques, has described discrete longitudinal compartments in the cerebellar white matter that display regional differences in concentration and position of afferent (and most likely efferent) cortical axons. This compartmentalization most likely accounts for the pattern of maxima and minima of spinocerebellar degeneration observed in consecutive frontal sections through the mouse cerebellum (Figures 5 and 6, particularly 6E and F). These "maxima and minima" contain axons that course in various planes, but terminal distributions appear to adhere to a predominately radial (dorso-ventral) pattern. Tracing these so-called columns of maxima and minima in a rostrocaudal direction suggests that they are representative of continuous topographical bands (see Voogd, 1969; Boogd, et. a., 1969). This banding might also illustrate a consistent mode of cerebellar afferent distribution (for similar findings on the topological organization of cuneocerebellar and olivocerebellar projections, see Voogd, et. al., 1969; Groenewegen and Voogd, 1977), organization (see Scott,

1964), function (for review, see Oscarsson, 1973), and development (see Korneliussen, 1968a,b; 1969).

The Distribution of Spinocerebellar Afferents Within the Cerebellum of the Reeler Mouse

A. The Spinal Course of the Reeler DSCT and VSCT

The spinal course of the DSCT and VSCT in abnormal reeler mice appears to be identical to that previously described in their normal littermates (see Section A of last section). The reeler cases depicted here (cases 9084 and 9076) are representative of DSCT and VSCT projections from below T12 in the mutant, and the spinal lesions in these cases were similar to the lesion in the case of the normal (case 9086) illustrated in Figures 5 - 6.

B. The Cerebellar Distribution of the Reeler DSCT and VSCT

Figures 7 and 8 represent reconstructions from frontal sections through the cerebella and underlying medullas showing degenerating DSCT and VSCT fibers in two 22-day old reeler mice (cases 9084 and 9076) following spinal interruption at T12 - T13. (It should be noted here that no degeneration was observed in silver-stained sections of control reeler mice that did not receive spinal lesions. Hence, there is no evidence for spontaneous degeneration of axonal systems within the mutant cerebellum.) Because of the placement of the lesion at this low thoracic level, these projections are also presumed to be hindlimb and lower trunk representations. (Compare the reconstructions of Fink-Heimer stained sections in Fig. 7 with adjacent Nissl-stained sections shown in Fig. 3. Also,

see micrographs of histological sections from cases 9084 and 9076 in Figures 12, 13, and 14.) Degenerating DSCT and VSCT axons appear to coalesce near the midline in a cerebellar commissure in reeler as in the normal, and also as seen in the normal, this commissure is at the level of or just above the deep cerebellar nuclei (see, for example, Fig. 7C). Also within this commissure, degenerating axons of presumably the VSCT are seen crossing the midline near their level of entry in the superior cerebellar peduncle (see Figures 7A - B, 8A). Many DSCT and VSCT fibers, before reaching the commissure and distributing within cortical areas, are seen entering the interpositus and medial nuclei bilaterally, and there is some suggestion that the amount of axonal degeneration products seen in the deep nuclei is greater than that seen in normal animals following a similar lesion (for example, compare spinocerebellar projections to the interpositus and medial nuclei of reeler (Fig. 7) with those of the normal Figures 5 and 6)). This finding may relate to an increased size of the reeler deep cerebellar nuclei as seen in cell-stained sections (i.e. Fig. 1), and will be analyzed in more depth in the Discussion.

Upon leaving the cerebellar commissure, degenerating axons distribute to particular cerebellar regions in reeler in a manner that is different from the normal. Degenerating spinocerebellar fibers and terminals in consecutive frontal sections through the shrunken reeler cerebellum appear to be more densely concentrated at any one level than the projections

in the no
consisten
and Grant
often fou
a clear s
In a rost
fibers ca
fashion t
fourth ve
reeler se
the norma
represents
Also in a
degenerati
within mid
in sheets
(Figures 7
with those
that the d
condensati
represent
presumptiv
butions of
regions of
7C - 7D, 8
fiber dist
an area tha

in the normal. The appearance of argyrophilic debris was consistent with the findings in normal mouse and cat (Brodal and Grant, 1962), and these degenerating mossy fibers were often found terminating within glomerular regions defined by a clear space surrounded by granule cell nuclei (see Fig. 4b). In a rostral region of the reeler cerebellum, degenerating fibers can be seen descending from the commissure in a radial fashion to enter a population of granule cells just above the fourth ventricle (Figures 7A and 8A). Comparing the levels of reeler sections 7a and 7b with levels of section 5a and 5b in the normal, it appears that this degeneration in reeler represents an innervation of a presumptive lingule lobule. Also in a relatively rostral region of the reeler cerebellum, degenerating axons descend from the commissure to arborize within midline populations of granule cells that are arranged in sheets (Figures 7A, 8A, 14) and cylindrical condensations (Figures 7B, 7C, 12). Again, by comparison of these levels with those of the normal (Figures 5A and 5B), one can infer that the degenerating axons that closely follow granule cell condensations in the midline (Figures 12b, 14c) probably represent another spinocerebellar distribution within a presumptive centralis lobule in reeler. The radial distributions of degenerating DSCT and VSCT fibers in more caudal regions of this presumed anterior lobe in reeler (sections 7C - 7D, 8C - 8D) are suggestive of spinocerebellar mossy fiber distributions within populations of granule cells in an area that is analogous topographically to the culmen

lobul

80 - 8

I

cereb

granul

cerebe

in the

commis

the de

case f

innerva

and dev

Hence,

some de

compres

Be

midline

project

VSCT pr

in the

bellum (

patch of

projecti

tially a

cells of

The midli

likely re

lobule of the normal mouse (compare Figures 7C - 7D and 8C - 8D with Figures 5B - 5D).

It is of interest that these projections descend from the cerebellar commissure to enter populations of displaced granule cells that are at the level of or below the deep cerebellar nuclei in reeler, and spinocerebellar projections in the normal mouse anterior lobe ascend from the cerebellar commissure to innervate granule cells in lobules well above the deep cerebellar nuclei. This represents the strongest case for cerebellar inversion in reeler, but this pattern of innervation most likely results from a failure in lobulization and development of dorsoventral cortical expanse in reeler. Hence, paleocerebellar granule cells that have undergone some degree of radial migration tend to occupy dorsoventrally compressed regions in the midline (also see Fig. 3).

Besides the DSCT and VSCT projections to a presumed midline anterior lobe region in reeler, there is another projection present that appears to correspond to DSCT and VSCT projections to the pyramis lobule of the posterior lobe in the normal. Within posterior regions of the reeler cerebellum (Figures 7E, 13, and 8D - 8E), there is a condensed patch of degeneration in the midline, and there is lateral projection as well that consists of axons that course tangentially and radially to distribute amongst displaced granule cells of the ectopic granule cell layer (EGL) (see Fig. 13). The midline patch of degeneration (Figures 7E, 8D - 8E) most likely represents the midline portion of the pyramis lobule

in the no
(again, s
analogous
tion in t
possibili
cerebella
the mutan
normal th

With
DSCT and
a bilatera
ipsilatera
part simil
Further, t
midline wi
DSCT and v
degenerati
and minima
obscured i
There is,
organizatio
if one foll
cerebellar
section to
dorsoventra
in reeler, a
groups of th

in the normal (Figures 6E - 6F), and the lateral distribution (again, see Figures 7E, 8D - 8E) in reeler appears to be analogous topographically to the lateral pyramis distribution in the normal (Figures 6E - 6F). With regard to the possibility of cerebellar inversion in reeler, the spinocerebellar distributions in the lateral pyramis region of the mutant are predominately dorsolateral, where in the normal they are more ventrolateral.

With respect to the mediolateral representation of DSCT and VSCT fibers in reeler, these systems project in a bilaterally symmetric fashion. As in the normal, the ipsilateral innervation of a given region is for the most part similar to the contralateral (see Figures 7 and 8). Further, there is a consistent appearance of a cerebellar midline with representations of periphery on both sides (via DSCT and VSCT projections). The columns of spinocerebellar degeneration in the normal mouse cerebellum, termed maxima and minima (see Voogd, et. a., 1969), are for the most part obscured in frontal sections through the reeler cerebellum. There is, however, a subtle representation of columnar organization in the radial distributions of these fibers if one follows particular descending projections from the cerebellar commissure (see, for example, Fig. 7D) from section to section. Because of the altered mediolateral and dorsoventral representations of cerebellar cortical neurons in reeler, afferent systems in association with particular groups of these neurons display reorganization in the form

of compres
columns of
anterior l
deviations
This is al
DSCT and V
and latera
display cl
columnar a
trajectori
populations
by the extr
ascend obli
with groups

Thus,
bellum appe
architectur
position, c
in reeler r
fiber distr
of DSCT and
particular
placed popu
appear to b
Projections
cells (see P
expanse of t

of compressed mediolateral distributions. The vertical columns of descending DSCT and VSCT fibers in midline anterior lobe regions of reeler (Figures 7 and 8) display deviations in the form of modified axonal trajectories. This is also true in the posterior lobe where degenerating DSCT and VSCT fibers are densely concentrated within medial and lateral regions (see Figures 7E, 8D - 8E) that do not display clear maxima and minima. However, the distorted columnar arrangements are suggestive of axons with modified trajectories presumed to be trying to reach displaced populations of cortical cells. This is further exemplified by the extreme case of spinocerebellar mossy fibers that ascend obliquely into the molecular layer to be associated with groups of displaced granule cells (see Fig. 14).

Thus, the afferent organization in the reeler cerebellum appears to conform to the aberrant patterns of cytoarchitectural organization. Regional variations in the position, concentration, and continuity of cortical neurons in reeler result in reorganization of particular afferent fiber distributions. There is an order to the distribution of DSCT and VSCT projections in the mutant that suggests particular mossy fibers presumably seek out particular displaced populations of target cells that occupy areas which appear to be analogous topographically to the normal animal. Projections to particular midline condensations of granule cells (see Figures 3 and 14) suggests that the rostrocaudal expanse of these condensations is topographically equivalent

to th
bellu
diate
Korne
is co
that
revea
can b
hindl
culme
in th
physi
Snide
repre
mossy
bellar

I
rostro
reeler
are co
cortic
affere
Cavine
the fo
in reel
zone ma
and pos

to the normal paleocerebellum. However, the reeler paleocerebellum actually resembles the medial and medial intermediate zones of the embryonic cerebellum described by Korneliussen (1968a,b; 1969). The reeler paleocerebellum is comprised of densely concentrated neurons and afferents that display regional topographic specificity. This is revealed in the surface reconstructions of Figure 9 where it can be seen that there is a double representation of the hindlimb and lower trunk, one in the anterior lobe centralis, culmen, and lingula, and another in the posterior lobe pyramis in the normal mouse cerebellum (this also conforms to the physiologically-defined cerebellar somatatopy described by Snider and Edlred, 1952). In reeler, there also is a double representation of hindlimb and lower trunk spinocerebellar mossy fibers within particular anterior and posterior cerebellar regions (see Fig. 9).

Therefore, the distinctive dorsoventral (radial), rostrocaudal, and mediolateral DSCT and VSCT distributions in reeler suggest that: 1) The radial distributions of afferents are considerably modified as a result of laminar disarray of cortical neurons. Thus, the raidal distribution of cerebellar afferents must be determined by their target cells (also, see Caviness, Frost and Hayes, 1976, for similar hypotheses in the forebrain). 2) DSCT and VSCT projections from below T12 in reeler uniformly project to a distinct spinal recipient zone made up of granule cell populations in midline anterior and posterior zones. There are also projections to more

lateral
viewed a
normal r
projecti
recipier
reeler c
the norr
boundari
distribu
5 - 14).

Cerebell
Mutant M

Par
of the m
of topog
of parti
1977b).
injectio
several
gradely
In partic
oralis ar
principal
pheric lo
mouse. T
also foun
these regi

lateral regions of the posterior zone in reeler that are viewed as analogous to lateral pyramis projections of the normal mouse. There also appears to be no DSCT and VSCT projections to regions outside of the paleocerebellar spinal recipient zone (e.g. hemispheric areas) in reeler. 3) The reeler cerebellum is topologically miniature (about one-half the normal size) but nonetheless containing appropriate boundaries and topographic specificity in the regional distributions of particular afferent systems (see Figures 5 - 14).

Cerebello-Olivary and Olivo-Cerebellar Projections in Reeler Mutant Mice

Particular brainstem nuclei project onto distinct regions of the mutant cerebellum in a manner that supports the notion of topographic specificity in the regional distributions of particular afferent systems. In a previous report (Steindler, 1977b), it was shown that following horseradish peroxidase (HRP) injections into various regions of the mutant cerebellum, several brainstem nuclei displayed distributions of retrogradely labeled neurons similar to that seen in the normal. In particular, trigemino-cerebellar projections from the oralis and interpolaris divisions of the spinal trigeminal and principal sensory nucleus were found to project upon hemispheric lobules (for example, Crura I and II) in the normal mouse. Trigemino-cerebellar projections in the mutant were also found in lateral cerebellar regions, indicating that these regions can be considered as cerebellar hemispheres.

Fol
tiated a
into va
in norma
nucleus
and rec
nuclei (
appear t
and they
olivary
sory nuc
in diffe
(see Mar
et. al.,
into por
well as
mutant m
connecte
and medi
appears
The ante
nuclei a
accessory
1976).

Figu
injection
and interp

Following injections of combined L-(^{3}H) leucine (tritiated amino acid, TAA) and horseradish peroxidase (HRP) into various cerebellar cortical and deep nuclear regions in normal and reeler mutant mice, the inferior olivary nucleus was consistently found to be both topographically and reciprocally related with the cerebellum via the deep nuclei (see Fig. 15). Cerebello-olivary projections appear to arise from the dentate and interpositus nuclei, and they project upon all three divisions of the inferior olivary complex (principal nucleus, dorsal and medial accessory nuclei). These pathways have been extensively analyzed in different mammalian species by several investigators (see Martin and Henkel, 1975; Martin, et. al., 1976; Tolbert, et. al., 1976). After placing combined HRP-TAA injections into portions of the dentate and interpositus nuclei (as well as overlying cerebellar cortex) in normal and reeler mutant mice, these nuclei were found to be reciprocally connected with areas of the contralateral principal, dorsal and medial accessory olivary nuclei. The dentate nucleus appears to be primiarily related with the principal nucleus. The anterior interpositus and the posterior interpositus nuclei are related with the dorsal accessory and medial accessory nuclei, respectively (also see Tolbert, et. al., 1976).

Figure 15 shows one case in which two combined HRP-TAA injections were made in the left cerebellar cortex, dentate, and interpositus nuclei of a reeler mouse. Subsequent

applic
reveal
of aut
neuron
comple
repres
and Vo
latera
olivar
recipr
bellar
(see To
and Voo

Th
(e.g. c
(e.g. s
appear
appears
common
tures in
of affer
malposit
and conn

application of histochemical and autoradiographic techniques revealed compatability between the topographic distribution of autoradiographic grains and horseradish peroxidase-stained neurons in portions of the contralateral inferior olivary complex. Peroxidase-stained neurons in the inferior olive, representing the olivo-cerebellar projection (see Groenewegen and Voogd, 1977), appear to project back into those contralateral deep nuclear regions giving rise to the cerebello-olivary projection in a precisely reciprocal fashion. The reciprocal nature of these cerebello-olivary and olivo-cerebellar pathways has been implicated in other species as well (see Tolbert, et. al., 1976; Martin, et. al., 1976; Groenwegen and Voogd, 1977).

Thus, the efferent projections from the mutant cerebellum (e.g. cerebello-olivary) like the afferent projections (e.g. spinocerebellar, trigemino-cerebellar, olivo-cerebellar), appear to be appropriately organized. Therefore, there appears to be at least one developmental phenomenon that is common to both the cerebral and cerebellar cortical structures in reeler - the maintenance of appropriate patterns of afferent and efferent projections in spite of cellular malposition and disorientation suggests that cytoarchitecture and connectivity are individually regulated.

Discussi

The
of spin
cerebel
mechanis
of cellu
from Ca
of displ
one occa
with reg
as well
All thes
and can
surmount
"Presuma
but we d
that sur
priate t

Obs
of malor
and norma
and Sheib
axon is d
course is
surroundi
an experim

Discussion

The findings of appropriately reorganized distributions of spinocerebellar projections in the malformed reeler cerebellum suggest that highly specific synaptic generating mechanisms (Rakic and Sidman, 1972) may be present in spite of cellular malpositioning. Jacobson (1970) cites a quotation from Cajal (1929) with regard to axonal growth and connections of displaced and disoriented neurons - "In all neural organs one occasionally sees atypical and accidental arrangements with regard to the locations and relationships among neurons, as well as with regard to the path and orientation of axons... All these aberrations are produced during fetal development and can be explained by the obstacles which neurons must surmount during migration." Jacobson also states that... "Presumably, many of these neurons do not survive to maturity, but we do not have sufficient data to determine whether those that survive in an aberrant location make connections appropriate to their proper location or to their aberrant location.

Observations from descriptive silver impregnation studies of maloriented cortical neurons in normal chick (Cajal, 1928) and normal rabbit (Stensaas, 1967; Van der Loos, 1965; Globus and Sheibel, 1967) suggest that the initial growth of the axon is determined by the parent cell body, but its further course is influenced by extrinsic factors present in the surrounding neuropil (e.g. cells and their processes). In an experimental anatomical investigation by Stefanelli (1950),

rostroca
neurons
axons i
the app

Si
mammals
turally
light pr
neuronal
the mamm
organiza
for asse
position
of facto
connecti
also bee
et. al.,
Caviness,
Welt and
of both f
likely ex

In th
in the ree
topographi
cerebellar
DSCT and VS
radially, s

rostrocaudal inversion of presumptive amphibian Mauthner's neurons resulted in an initial misgrowth of the Mauthner axons in the wrong direction followed by a new growth in the appropriate direction within the neuraxis.

Similar phenomena may take place during histogenesis in mammals, and studies of connectivity in the cytoarchitecturally abnormal cortices of the adult reeler mouse shed light precisely on this issue of the relationship between neuronal polarity (and position) and axonal projection in the mammalian brain. The maintenance of regional topographic organization in the mutant cerebellum has been the paradigm for assessing the possibility that the acquisition of neuronal position and orientation during development is independent of factors which determine the specificity of neuronal connections. In view of the fact that such a hypothesis has also been set forth regarding the reeler forebrain (Devor, et. al., 1975; Steindler and Colwell, 1976; Caviness, 1976a,b; Caviness, Frost and Hayes, 1976; Caviness and Yorke, 1976; Welt and Steindler, 1977), parallel factors for development of both forebrain and hindbrain cortical structures most likely exist.

In the present study, regional topographic organization in the reeler cerebellum has been appraised at the level of topographical arrangements of the dorsal and ventral spinocerebellar (DSCT and VSCT) systems. It was found that the DSCT and VSCT projections are characteristically reorganized radially, suggesting that these afferents may seek out dis-

placed post-synaptic populations of neurons. DSCT and VSCT axons follow aberrant trajectories in order to locate particular populations of target cells. Similar findings were obtained in studies upon the mutant forebrain (Frost and Caviness, 1974; Steindler and Colwell, 1976). Finally, the numbers of axons derived from the DSCT and VSCT in reeler must be accommodated in a fashion according to limitations imposed by the aberrant cytoarchitecture, in that the mutant cerebellum appears to have twice the concentration of cells and degenerating axonal debris per given section through its rostrocaudal extent, and its surface area is about half as large as the normal. Once more, these findings will be discussed with regard to the radial and topographical components of cellular and afferent organization in the reeler cortices (see Caviness and Yorke, 1976a, who have also employed this form of analysis in the mutant cerebrum).

Radial Organization of the Reeler Cerebellum

A. Cytoarchitectonics

The radial cytoarchitectural organization of the cerebellum is a product of: 1) the particular arrangement of cortical neurons into cell class-specific laminae (e.g. molecular layer, Purkinje cell layer, granule cell layer), and; 2) neuronal polarity with respect to a dorsoventral axis between the pia and white matter (e.g. Purkinje cells with sagittally oriented dendritic arbors, and primary dendrites that are perpendicular to the pial surface; granule cells with axons that ascend perpendicular to the molecular layer

where they bifurcate and course longitudinally, parallel to the pial surface.) Both of these constituents are greatly altered in the reeler cerebellum. With regard to the laminar disarray which displays regional variations, most granule cells are either intermingled with, or external to, populations of their primary post-synaptic target neurons - Purkinje cells. In some areas, however, Purkinje cells lie external to granule cells, and they display appropriate patterns of dendritic elaboration and orientation within a molecular layer. Purkinje cells within these appropriate geometric positions display less abnormal neuronal development than those which are aberrantly positioned and disoriented (see Rakic and Sidman, 1972; Rakic, 1976).

The major defect in the reeler cerebellar cortex appears to be a result of the failure of appropriate cellular alignment and position taking place with respect to the radial (dorso-ventral) axis. Neocortical neurons in reeler generally do not attain their appropriate position within radially organized laminae, but there is to some degree, an achievement of class-specific aggregation (e.g. some cells are in appropriate positions, as just described; the ectopic granule cell layer displays a pattern of granule cell aggregation; clusters of granule cells are segregated into certain midline condensations, see Figures 12 and 14). Cellular orientation, like position, follows a radial pattern, and polarity of cerebellar cortical neurons and their neurites in reeler display marked aberrancies. In the normal animal, the geometric configura-

tions of dendritic and axonal elaboration are subject to restraints imposed by radial boundaries (e.g. pia and white matter), and previously formed neural matrix (e.g. other cells and processes). In reeler, many malpositioned and disoriented cerebellar cortical neurons display inappropriate dendritic morphologies (Rakic and Sidman, 1972; Rakic, 1976). For example, some Purkinje dendrites exhibit the "weeping willow" effect characteristic of the x-irradiated, semi-agranular, rat cerebellum (see Altman, 1973). All of these radial abnormalities in the reeler cerebellum may represent epiphenoma resulting from a basic histogenetic malformation that influences the radial design of the cortices. Even though all of the appropriate types of cerebellar cortical neurons are present in reeler (see Hamburgh, 1963; Sidman, 1968a,b; 1972; Rakic and Sidman, 1972; Rakic, 1976), the characteristic attributes of cell-class specific lamination and orientation have developmentally gone astray. The radial determination of neuronal position and orientation most likely occurs during migration from the germinal zone and subsequent alignment within the cortical plate. This leads to the development of cell class which might be independent of cell position (see Williams, et. al., 1975), and could represent a phenomenon that is already dictated at the site of cellular origin (e.g. the germinal zone). The development of cellular position, orientation and cell-class may arise during histogenesis from mechanisms that are <u>intrinsic</u> to the young cortical neurons (e.g. cell to cell communica-

tion during histogenesis); or 2) factors that are extrinsically present within the milieu of the cortical plate (e.g. afferent-target cell communication).

It is known that during histogenesis in both the cerebral and cerebellar cortices, young neurons migrate from their site of origin (e.g. the ventricular zone, or external granule cell layer) in a radial fashion into the cortical plate where they often pass the somata of previously formed neurons (e.g. the "inside-out" pattern of histogenesis already mentioned in the cerebrum, and granule cell migration from the external layer past somata of Purkinje cells that are beginning to align and differentiate (see Sidman, 1968a,b; 1972; Rakic and Sidman, 1970; Rakic, 1971a). The question is what developmental factors might preclude this radial development of the cortex? Sidman (1968a,b) suggests that during neuronal migration, cell to cell communication may result in the laying down of synaptogenic relationships as well as intrinsically conveying positional and orientation cues to populations of migratory/pre-aligning young neurons.

With regard to dorso-ventral cortical dysgenesis in reeler, DeLong and Sidman (1970) suggest that "...the reeler allele (and the wild-type in its turn) affects the majority of cortical neurons at the particular stage when they are to become aligned and oriented in vivo." There are at least three types of cellular interactions that may occur during normal radial histogenesis of both of the cortical structures which may go amiss in the mutant:

1) The ultimate position and orientation of migratory neurons may be affected by postmigratory neurons that have already developed axonal or dendritic coordinates, or postmigratory neurons that have not yet developed an axon or dendritic appendages and so are not yet aligned. Young neurons that migrate past the somata of previously formed neurons may express particular affinities toward certain neuronal populations. This could result in an interaction that influences the position and orientation of populations of migratory young neurons. These migratory young neurons may also use radial glial guidelines to assist them during their relatively lengthy trans-cortical excursion (see Rakic and Sidman, 1970; Rakic, 1972). In reeler, aberrant cell surface properties may be expressed at the level of postmigratory/aligned neuron -→ migratory neuron, postmigratory/prealigned neuron -→ migratory neuron, or migratory neuron -→ migratory neuron. In other words, the radial histogenetic malformations in the mutant cortices may be present intrinsically within populations of young cortical neurons and may be independent of extrinsic factors that arise from other areas of the nervous system.

2) The development of neuronal position and orientation may also be affected by developing local circuits within the cortices. Developing dendrites and axons of earlier formed cells may synaptogenically or non-synaptogenically (e.g. other biotrophic phenomenon) interact with migratory young neurons, and hence affect their eventual position and orientation.

3) It is also possible that factors extrinsic to the developing cortices, such as early arising subcortical projections (see Sidman, 1968a,b) may play a part in determining cell position and orientation. Different types of afferent trajectories (e.g. radial, tangential, longitudinal) could produce particular gradients within the developing cortices that contribute to the occurrence of cell class-specific lamination and orientation. Any afferent-migratory neuron interactions may be synaptogenic and like both of the previously mentioned interactions, they may be mediated via factors coating the cell surface (afferent-target cell communication will be discussed in more depth in following sections that deal with afferent organization).

Thus there may be mechanisms intrinsic to the cortical neurons such as cell to cell communication that give rise to particular migratory patterns in neocortical systems (see Sidman, 1968a,b). These patterns may produce the epiphenomenon of cell class-specific lamination and orientation. Cell surface properties underlying this cell to cell communication could produce radial gradients within developing cortices that determine particular recognition factors within populations of migratory/pre-aligning young neurons. DeLong and Sidman (1970) proposed that there are "...cell-to-cell binding factors assymetrically distributed along the length of the cells..." In one study of cell surface components relevant to neuronal migration and cell to cell communication, Hatten, Trenkner, and Sidman (1976) postulate

particular "carbohydrate-containing surface macromolecules" may be present differentially within different populations of migratory/pre-aligning neuroblasts in the normal mouse. From studies using lectins as probes for these particular macromolecules, these investigators found that "...Different regions of the E13 brain were found to agglutinate with different lectins, indicating probable differences in cell surface carbohydrates..." During neuronal migration, cellular recognition of previously formed neural matrix may be a result of differential cellular adhesions. The role of carbohydrate-binding proteins in intercellular adhesion has been well-documented in lower systems (see, for example, Rosen, et. al., 1973 and 1974), and it is possible that similar macromolecular components present on the cell surface of mammalian neuroblasts may mediate neuronal recognition leading up to appropriate migratory patterns and cell class-specific aggregation. To my knowledge, similar experiments to those done by Hatten, et. al. (1976) have not been conducted in reeler with regard to possible differences between normal and mutant cell surface carbohydrate properties.

The foregoing discussion has examined the numerous possibilities of common histogenetic factors and interactions that may have gone astray in both of the reeler cortices producing aberrant radial patterns of cellular position and orientation. The developmental phenomena leading up to the development of cell class, position, and polarity have been discussed in a highly speculative manner. Observations on

the abnormal radial patterns of cytoarchitecture in the cortices of reeler shed little light on the issue of the mechanisms behind normal development of characteristic attributes of cell-class. It suffices to say that displaced and disoriented cortical neurons in reeler most likely result from young neurons failing to surpass the obstacles that are encountered during migration and subsequent aggregation and alignment (see Jacobson, 1970). Any trophic factors that were described as cell surface mediated and involved in determining neuronal position and polarity are purely hypothetical. However, Jacobson (1970) believes that three conclusions can be drawn from previous observations (Cajal, 1928; Van der Loos, 1965; Stensaas, 1967; Globus and Sheibel, 1967; Stefanelli, 1950, 1951; Jacobson, 1964; Hibbard, 1965) on the possible factors that determine neuronal polarity:

"1) The neuron's polarity is intrinsically determined so that the axon and dendrites sprout in a predetermined manner, irrespective of the orientation of the neuron.

2) After the axon has grown a short distance in the wrong direction it reorientates itself with respect to its substratum. Rarely do the extrinsic factors override the intrinsic factors, and the axon sprouts in the correct direction regardless of the orientation of the neuron.

3) The dendrites do not reorientate but conform to the orientation of the cell body. In such a case, in the absence of normal axodendritic connections, the dendrites remain stunted and the dendritic tree becomes malformed..."

Results from experimentation in reeler tend to support these hypotheses that are derived from observations on normally present or experimentally induced displaced and disoriented neurons in amphibian and mammalian cortices. Studies to be discussed on the afferent organization and patterns of connectivity that arise amidst displaced and disoriented cortical neurons in reeler get at the other question posed by Jacobson (1970): Do neurons that survive in an aberrant location make connections that are appropriate to their proper location or their aberrant location? In other words, might an examination of the radial distribution of particular cortical afferent systems in reeler reflect reorganization in a manner that implicates afferents seeking out particular populations of displaced target cells?

B. Afferent Organization

In the previous section, the radial abnormalities in reeler cortical cytoarchitecture were discussed in relation to factors which may determine cellular position and orientation. With regard to the radial component of afferent distribution in the reeler cortices, one can again address the issue of the possible contributions afferent systems make in influencing neuronal position and orientation (e.g. afferent-target cell communication). On the other hand, an examination of the radial distributions of particular afferents also bears upon the issue of whether or not cerebellar afferent systems, like cerebral afferent systems in reeler, possess anomalous trajectories and distributions,

which it is presumed are due to an attempt to locate displaced populations of post-synaptic target cells.

The findings of altered radial distributions of DSCT and VSCT fibers in the reeler cerebellum do not directly address the issue of possible interactions of migratory/ pre-aligned cortical neurons with afferent systems that may be at fault in reeler, and subsequently may contribute to abnormalities in positioning and polarity of cells (see Caviness and Sidman, 1972). Early arising subcortical projections (see, for example, Sidman, 1968a,b; Morest, 1970; Hinds, 1972; Marin-Padilla, 1971; Caviness and Sidman, 1972) may, hypothetically, play a part in determining cell position and orientation in the cortices. For example, disruption of a thalamocortical column results in the development of a disorganized architectural organization in the rodent cerebrum. In looking at the afferent conveyance of information from the mystacial pad in rodents to specialized somatosensory thalamic barreloids (Van der Loos, 1976) and cerebral cortical barrels (Woolsey and Van der Loos, 1970; Welker and Woolsey, 1974; Welker, 1976), it has been shown that removal of a facial vibrissa at particular developmental stage results in an abnormal thalamocortical projection from a barreloid in the thalamic ventrobasal complex and leads to a malformed cortical barrel (Killackey, et. al., 1976). This suggests that a collection of somatotopically organized afferents may contribute to: 1) the definition of the arrangement of target cells; and 2) the sculpturing of

morphologically indifferent young cortical neurons. In reeler, because some neurons reach appropriate positions and acquire normal orientations within the mutant cortices, the question arises as to whether these "normal" cells are deviant from an abnormal intrinsic blue-print (e.g. cell to cell communication), or actually reflect the inconsistency of an abnormal extrinsic design of patterning.

The role of afferent-target cell communication during histogenesis of the normal and mutant cerebral and cerebellar cortices needs to be elucidated in order to determine if afferent fibers may influence cell position as well as differentiation. To date, many theories along this line are highly speculative and often nonsubstantiated. For instance, Caviness, Frost, and Hayes (1976) claim that somatosensory cortical neurons may reach their radial position in the cortex before their specific thalamocortical afferents arrive. However, a blue-print for the development of cyto-architecture may be carried in early arising afferent systems that are present, nonsynaptically, in the pre-migratory cortical plate (see, for example, Olson, et. al., 1973; and Molliver, et. a., 1973). Also, there is some evidence to date that suggests neurons may migrate toward particular afferent fibers (see, for example, Levi-Montaleini, 1964a,b; Altman, 1973; and Gaze, 1970). Suffice it to say that rearranged radial distributions of spinocerebellar axons in the cytoarchitecturally aberrant reeler cerebellum, which convey information from normal[1] distributions of peripheral

receptors such as muscle spindles (Werner and Steindler, unpublished observations), may have produced abnormal cytoarchitectonic patterns in reeler, or may reflect the inappropriate organization of another uninvestigated afferent system that initially induced the cytoarchitectural disarray. For the reasons stated in the following paragraph, it is probable that the modified radial distributions of DSCT and VSCT axons in reeler are a result of the afferent axon seeking out the target cell. The results of the experiments upon spinal afferents to the mutant cerebellum indicate that axons seek out particular displaced populations of granule cells. DSCT and VSCT axons primarily descend radially from a cerebellar commissure in presumed anterior lobe regions to intermingle with condensed and topologically aberrant populations of granule cells within midline areas. Similar patterns of reorganization in the radial distributions of the DSCT and VSCT were also recognized in particular posterior lobe regions. There is a distorted columnar arrangement to this radial

---

1 Altered organization of the periphery may modify organization in the CNS (see Killackey, et. a., 1976). However, the morphology and quantitative distribution of muscle spindles in reeler appear appropriate (Werner and Steindler, unpublished observations). Hence, any inherent abnormalities in the projections of the DSCT and VSCT in reeler would not appear to relate to peripheral irregularities.

distribution that is unlike the normal, and appears to result from limitations that are geometrically imposed by erratic positions of target cells. Particular distributions of DSCT and VSCT mossy fibers deviate from the usual radial pattern to obliquely approach displaced granule cells. In some instances, degenerating mossy fibers were also found in or near the molecular layer where they appeared to be related with ectopically positioned granule cells. Columns of degenerating axons may also be radially extended. This would be in agreement with similar findings in the mutant cerebrum that suggests a greater radial spread of target cells (see Caviness, Frost and Hayes, 1976).

It was most interesting to observe a reversal in the pattern of degenerating mossy axons that leave the cerebellar commissure in reeler. As already mentioned, in anterior lobe regions in reeler, columns of degenerating DSCT and VSCT mossy fibers descend to populations of cortical neurons at the level of or beneath the deep cerebellar nuclei. This is exactly opposite from the situation in the normal mouse anterior lobe where axons ascend to cortical lobules that display a dorsal expanse relative to the deep nuclei. In posterior lobe regions in reeler, DSCT and VSCT distributions are found within dorsolateral populations of ectopically positioned granule cells that appear to be analogous to ventrolateral pyramis projections in the normal. However, examining these relative distributions (Figures 6D - F; 7E; 8D - E) tends to suggest that granule cells of the ectopic

granule cell layer in reeler that are dorsolaterally positioned would have come to lie ventrolaterally if appropriate patterns of migration and lobulization had occurred. Nonetheless, these findings infer that, as in the reeler cerebrum (Steindler and Colwell, 1976; Caviness and Yorke, 1976), the relative distributions of spinocerebellar projections in the mutant cerebellum may be inverted. Most likely though, these aberrant radial distributions of DSCT and VSCT projections in reeler accommodate a basic anomalous radial development of cytoarchitecture.

Thus, all lines of evidence on the radial distributions of DSCT and VSCT axons in reeler tend to strongly point toward the phenomenon of DSCT and VSCT axons seeking out radially displaced populations of target granule cells. There have been contrasting findings of similar phenomena in the reeler forebrain, although a majority of axonal systems examined tend to concur with this plan for establishing connectivity (see, Steindler and Colwell, 1976 and Caviness and Yorke, 1976, for discussions of thalamocortical, corticothalamic and corticocortical connections in reeler). However, as reported by Devor, et. al. (1975), particular populations of maloriented pyramidal cells of the piriform cortex in reeler may not receive appropriate distributions of lateral olfactory tract or perforant path axons that fail to reorganize and seek out these cells. Hence, the degree of seeking out by particular axonal systems may be somehow limited according to undefined factors, and certain afferent systems may display more success

in trying to reach target cells (see, for review, Sperry, 1965).

Topographic Organization of the Reeler Cerebellum

A. Cytoarchitectonics

The topographic organization of cytoarchitecture in reeler was assessed with respect to the occurrence of regional variations in rostrocaudal and mediolateral distributions of cortical neurons (see Results). It was found that regional variations in the thickness, concentration, position and continuity of the ectopic granule cell layer in reeler compare with longitudinal (mediolateral) and rostrocaudal zones present in the embryonic cerebellum (see Korneliussen, 1968a,b; 1969). Hence, the mutant cerebellum has developed a compressed topographical representation of the body with altered distribution of cerebellar cortical neurons. Nonetheless, the topographical zones of cortical neurons that exist in the adult reeler cerebellum appear to contain presumptive topographical regions and boundaries (e.g. cellular condensations in a medial zone that represent vermian regions; and lateral zones that correspond topographically to hemispheric lobules). The histogenetic phenomenon that has gone astray in the reeler cerebellum resulting in a failure in lobulization, produces a reduced rostrocaudal and mediolateral expanse. This gives rise to an overall cerebellar topography that is greatly compressed relative to the normal. There are similar variabilities in the packing density of cortical neurons in the reeler cerebrum that may also reflect altered topographical arrangements in the mutant forebrain (see, for example, Welt and

Steindler, 1977).

B. Afferent Organization

1. The Normal Mouse

The topographic distribution of spinocerebellar mossy fibers were the paradigm for assessment of the afferent conveyance of cerebellar somatotopy because of their segmentally organized projections arising from spinal cord neurons that are known to be functionally related to body segments (see Grant and Rexed, 1958; Oscarsson, 1973). The organization of these pathways within the normal mouse cerebellum was studied as to possible geometric modes of body representation via topological patterns of a particular afferent system. This organization also provided a basis for analysis of organization within the mutant cerebellum. Within the cerebellum of the mouse there are two components of spinocerebellar afferent organization that implicate certain characteristics of cerebellar afferent conveyance of somatotopy. First, the vertical (radial) distribution already discussed that is organized into columns (or rostrocaudal bands, see Voogd, et. al., 1969). Second, the topographical (e.g. rostrocaudal) arrangements of spinocerebellar projections that seem to corroborate physiological findings (e.g. see Snider and Stowell, 1944) of double representations of the body within anterior and posterior spinal recipient zones.

The aforementioned columnar arrangement of spinocerebellar afferents that is present in both the anterior and posterior spinal recipient zone (SRZ) may reveal a new inter-

pretation of the way in which the cerebellum receives and processes afferent information. For example, in looking at the comparative distribution of spinocerebellar pathways in various mammals Voogd, et. al. (1969; Voogd, 1969) described longitudinal zones of afferents running rostro-caudally within the cerebellum. These may correspond to the columns of spinocerebellar afferents in mouse that I observed in consecutive frontal sections. The rostrocaudal continuity of these columns reflects a topographical banding.

Voogd's description of afferent zones within the cerebellar white matter and granule cell layer, and Korneliussen's (1968a,b; 1969) observations on longitudinal zones in the embryonic cerebellum, suggest that this topographical banding may be a fundamental form of organization within the mammalian cerebellum.

Figures 5 and 6 somewhat reveal this topographical banding in the normal mouse. Discontinuous columns of spinocerebellar mossy fibers ascend from the white matter into the granule cell layer within lobules of the anterior and posterior lobes. Because of the cerebellar convolutions, these discontinuous columns must be traced serially in a rostral-caudal fashion in order to appreciate their band-like nature. It is possible that the three-dimensional organization of particular afferent systems is "slab-like". Even though the columnar arrangement of afferents within the radial vector is partially obscurred as a result of foliation, cerebellar afferents may be organized within functional/

morphological slabs, not unlike afferent systems within the cerebral cortex. The ascending thalamocortical fibers within somatosensory cortical areas, for example, form radially oriented functional columns contributing to topographical bands within SI (primary somatosensory) cerebral cortex (Hand and Van Winkle, 1977).

With regard to other topographical features of the DSCT and VSCT projections in the normal mouse, the double representations present in the anterior and posterior SRZ can be assessed in the following way. First of all, the SRZ of the cerebellum is far more extensive than revealed by the frontal reconstructions presented and discussed in this paper (this case represents only one of a series of 12 experiments that consistently displayed the SRZ of the mouse). Spinocerebellar projections to lingula, centralis, culmen, and pyramidis only reflect the extent and position of the spinal lesion. The presented case involved a lesion at T12 - T13 which interrupted spinocerebellar fibers conveying proprioceptive and exteroceptive impulses from secondary neurons in Clarke's column and spinal border cells from below this level. Below this level, ascending axons are functionally and morphologically related to the hindlimbs and lower trunk. Hence the double representation of spinocerebellar afferents (one representation in the anterior lobe - lobulus centralis, culmen and lingula; and another representation in the posterior lobe - pyramis and possibly paramedian) morphologically represents the hindlimb, lower trunk, and tail within the

SRZ of the mouse cerebellum. These findings corroborate the predictions of Larsell (1952) and Bolk (1906) who believed the caudopelvis and tail musculature in certain species are mapped within these lobules.

As mentioned earlier, these investigators based their assumptions of somatotopy on the regional variations that are present in the anatomy of the cerebellum through evolution. As Larsell (1952) states:

> "The lobule immediately adjacent to the lingula...is large in the rat, spider monkey (labelled L. centralis in plate 1, Chang and Ruch, '49) and many other species of mammals. In many species of birds the corresponding folium II is subfoliated into IIa and IIb, a suggestion of such subdivision also being present in the rat and spider monkey, as well as in many other mammals. The human cerebellum, however, shows great variability of the portion of lobule II that corresponds to IIa in species which show this sublobule. According to Elftman ('32) variability also is one of the primary characteristics of the pelvic musculature of man."

The spinocerebellar representation of the caudopelvis of the mouse is reflected by the rather extensive projection observed in the lobulus centralis.

> "Degenerating fibers, in the experiment of Chang and Ruch, were also found in the folia of the lobulus centralis adjacent to the lingula, indicating that this portion of the central lobule also is related to tail function, as I have long suspected on comparative anatomical grounds."

Based on the premise that the somatotopic distribution of afferents from spinal cord reveals the synaptic conveyance of somatotopy from one CNS region to another, the morphological depiction of the orderly body representation within these regions can be inferred (see Fig. 9). The neurophysiological

correlate of a double body representation, from evoked potential studies (see Snider and Eldred, 1952) on tactile and proprioceptive impulses within the monkey cerebellum are corroborated by the morphological representation inferred from Fig. 9 (compare Fig. 9 with Fig. 16 of Voogd, 1975).

From certain comparative physiological studies of the representations of particular body regions within the cerebral cortex, such as the mapping of the receptive fields of paw regions in the somatosensory cortex of racoons (Welker and Seidenstein, 1959) or the physiological and anatomical descriptions of the representations of the mystacial vibrissae as "barrels" in the somatosensory cerebral cortex of rodents (Woolsey and Van der Loos, 1970; Welker, 1976), it appears that areas of the body that dominate in certain important behaviors of various animals reveal a characteristically enlarged representation. A mouse certainly relies heavily on the activities of its tail, and it appears that the elaborate evolution of the lobulus centralis in mice with an extensive spinocerebellar representation of this caudopelvic area may be a dominant feature of the rodent cerebellum.

Of course, the representation of the tail (caudopelvis) contributes to only a portion of the described spinocerebellar projection. In agreement with the physiological findings of hindlimb and lower trunk representations within the anterior lobe and pyramidis-paramedianus of the posterior lobe of cat and monkey, the projection of spinocerebellar fibers from T12 and below in the normal mouse conform to an overall topography

that is consistent with these representations.

Finally, the double representation of the cerebellar cortex may be related to a double representation within the deep cerebellar nuclei. In the present study, degenerating mossy fibers were observed in the anterior and posterior interpositus, and medial nuclei. Courville and Diakiw (1976) has suggested that the anterior representation may be related to output of the anterior portion of the interpositus nucleus, and the posterior lobe to the posterior portion of the interpositus complex. If this were the case, this could represent a way in which cerebellar output might interact with segregated thalamic areas (e.g. VL - VA) that are known to project to both representations (Motor I and Motor II) within the cerebral cortex. The afferent conveyance of somatotopic representations leading to double representations within various CNS regions can then be serially traced. The cerebellum might then represent the first major component to produce a double representation (e.g. anterior and posterior SRZ) through the mixing of particular ipsilateral, contralateral, and bilateral segmental projections of the spinocerebellar tracts, and subsequently forwarding these somatotopic representations to certain prosencephalic regions.

2. <u>The Reeler Mouse</u>

In reeler, the radial and mediolateral distributions of DSCT and VSCT axons are altered in a manner that obscures the characteristic mediolateral maxima and minima of afferents that are clustered into columns. As already mentioned, al-

terations in the radial and mediolateral distributions of cerebellar cortical neurons in reeler results in modified DSCT and VSCT distributions, presumably in search of displaced populations of target neurons. The rearranged radial topology of these populations into unique cellular condensations (e.g. sheets and cylindrical configurations, Figures 12 and 14) disrupts the columnar distribution. However, tracing the vertical distributions of degenerating DSCT and VSCT axons throughout the rostrocaudal extent of the mutant cerebellum reveals the presence of some form of columnar organization within what appears to be a medial (vermian) zone that is present during corticogenesis in the normal (Korneliussen, 1968a,b; 1969). Thus, this aberrant form of radial organization of particular afferent systems in the reeler cerebellum may actually reflect the beginning of development of columnar organization as it appears during histogenesis in the normal cerebellum. Nonetheless, the altered radial distributions of afferents in reeler still conforms to a pattern for the establishment of appropriate neuronal connectivity by way of afferents seeking out and conforming to distinct topological representations of particular target neurons.

The topographical distribution of DSCT and VSCT axons conveying hindlimb and lower trunk information to the reeler SRZ is a miniature version of the normal (see Fig. 9). There are projections to anterior and posterior SRZ regions that appear to be homologous to the normal. These projections

are compressed and may be twice as dense in any given section through the rostrocaudal extent of the mutant cerebellum. There is a well-defined boundary between DSCT and VSCT projections to anterior and posterior regions that is manifested by these particular afferent systems in the reeler cerebellum. Thus, the topographical somatotopic relationships between the spinal cord and the cerebellum appears to be appropriate in the mutant (for similar findings on maintenance of topography in the reeler cerebrum, see Steindler and Colwell, 1976). The afferent conveyance of somatotopy in the reeler cerebellum is manifested in spite of cytoarchitectural abnormalities. Therefore, the afferent conveyance of somatotopy is not necessarily dependent upon, nor reflected by cytoarchitecture, and the malposition of neurons in the reeler mutant is an independent phenomenon from the topographic specificity of axonal connections (see Steindler, 1977b).

Finally, with regard to the topographic organization of the deep cerebellar nuclei in reeler, DSCT and VSCT projections were found within the same divisions of these nuclei as in the normal (e.g. interpositus and medial nuclei). However, these projections appeared greater in density than in the normal. This was not due to a compression of axons into nuclei of reduced size, as the deep nuclei are actually larger than they are in the normal. Although the absolute number of cells with the deep nuclei of normal and mutant animals was not measured, one can speculate that there was an increased mossy

fiber collateralization within the reeler deep nuclei as a result of reduced numbers of cortical target neurons available for synaptic contact. This phenomenon might also lead to less cell death and an hypertrophied appearance of the reeler deep cerebellar nuclei.

Concluding Remarks

It is necessary that we understand the mechanisms which underlie the development and maturation of functional cortical circuitry in the human brain. The acquisition of properly functioning cortical circuits is dependent upon precise developmental phenomena that determine neuronal position, orientation, differentiation and connectivity. Human CNS disturbances (for example, lissencephaly-pachygyria, cerebral palsy, Parkinson's disease, mental retardation) often result in disrupted neural circuits that produce abnormal motor, sensory, emotional, or intellectual behaviors. An understanding of the primary defect and development of the components of cortical circuitry (e.g. neuronal position, orientation, differentiation and connectivity) in neurologically mutant mice may lead to a better understanding of the mechanisms of the development of neuronal circuitry in the brains of other mammals, including that of man.

Findings from electrophysiological experiments (Drager, 1975; Bliss and Chung, 1974) indicate that there is for the most part, normal function within the reeler mutant cortices (expressed as somatotopic representations, appropriate receptive field properties and latencies in time of response of particular neurons to particular stimuli). Despite basic qualitative findings of appropriate patterns of neuronal connectivity within the cytoarchitecturally abnormal cortices, the reeler mouse possesses abnormal phenotypic behaviors

(e.g. motor dysfunctions, passiveness, possible mental retardation). On the one hand, these behaviors may be an expression of quantitative or qualitative defects in neuronal circuitry that are histologically, ultrastructurally, and electrophysiologically obscurred because of the current state of the art. Ultrastructural studies (Rakic and Sidman, 1972; Rakic, 1976) of the mutant cerebellum have not revealed a significant presence of anomalous synaptic relationships. This might be difficult to assess in light of the hypothesis suggesting presynaptic elements dictate the morphology of the postsynaptic structure (see Mugnaini, 1969). On the other hand, there may be an aberrant blue-print for neuronal connectivity that does not favor the conventional spatial-temporal conveyance and processing of neural information. In other words, aberrant trajectories of afferent systems that are in search of displaced populations of target neurons in reeler, contribute to uniquely different morphological/functional patterns of neuronal connectivity.

With regard to the radial and topographical arrangements of cortical cells and their afferents in reeler, there are at least two histogenetic factors that seem common to both the mutant cerebellum and cerebrum: 1) The distinct topographic organization within particular cortical regions is maintained. The topographic representation of the periphery must be intrinsically present within, or somehow conveyed to, populations of young neurons that spatially occupy prospective topographical positions within their germinal zone. 2) Radial patterns

of cytoarchitecture are greatly disturbed, and this often results in characteristic reorganization of afferent distributions manifested by modified axonal trajectories. This suggests that the radial development of the cortex is determined within the cortical plate, either during migration or subsequent alignment, and is subject to intrinsic, as well as extrinsic restraints (e.g. cell to cell, or afferent-target cell communication). 3) The specificity of neuronal connections may be independent of those factors which determine cell position and orientation. Displaced cortical neurons with aberrant polarities and malformed dendritic appendages appear to be genetically programmed to attempt to engage in particular synaptic relationships with particular populations of afferents. 4) There is a maintenance of appropriate patterns of reciprocal connectivity between the cerebrum and the thalamus (Steindler and Colwell, 1976) and the cerebellar cortex and deep cerebellar nuclei with certain precerebellar nuclei (e.g. inferior olive).

The autosomal recessive mutation in mice, reeler, is similar in phenotype to the human malformation lissencephaly-pachygyria (Williams, et. al., 1975). This malformation also results in distorted laminar and columnar arrangements in the cerebral cortex, and observations on the development of cytoarchitecture in lissencephaly-pachgyria indicate that "...cell class is an inherent property of the postmitotic neuron and a property independent of cell position..." (Williams, et. al., 1975). With regard to an attribute of

cell class such as connectivity, examination of afferent organization in reeler tends to suggest that there is a genetic specificity of synaptic generating mechanisms (Rakic and Sidman, 1972) that is separate from the genetic determination of cell position and orientation. Of course, as pointed out by Changeux and Danchin (1976), there are not enough genes to code for individual synaptic relationships, and hence there must be specific connections that are genetically specified between classes of cells.

The data presented in this study extend the forebrain notion of maintenance of appropriate patterns of interneuronal connectivity in spite of aberrant cellular polarity and malposition to the hindbrain cortical structures in reeler. The reeler cerebellum, like its forebrain cortical counterpart, has also attained some degree of organization in spite of severe cytoarchitectural anomalies. The reeler gene appears to affect particular histogenesic factors that are common to both the development of cerebral and cerebellar cortical cytoarchitecture, yet spares the laying down of patterns of interneuronal connectivity. Appropriate patterns of cerebellar connectivity, revealed by modified axonal trajectories and maintained topographical distributions of spinocerebellar, olivo-cerebellar, cerebello-olivary, and trigemino-cerebellar pathways in reeler, suggest that in both forebrain and hindbrain cortical structures - the topographic specificity of axonal connections is manifested irregardless of neuronal position and polarity.

Acknowledgements

This thesis would not have evolved without the advice, assistance, criticism, encouragement and love that was so timelessly given by: Dave Akers, Allan Basbaum, Carol Basbaum, Patricia Calarco, Steven Colwell, Karen Emold, Howard Fields, Mary Fisher, Eldon Giesert, Jennifer LaVail, Matt LaVail, Mary Liu, Patrick Mantyh, William Mehler, Mike Merzenich, Mimi Love Morgan, Terry Perkins, Diane Ralston, Peter Ralston, Sal Rapisardi, Steven Rosen, Peter Sharp, Russel Snyder, Helen Krawchuk, Janice J.U. Steindler, Jerome Steindler, Joshua Nicholai Steindler, Natalie Steindler, Sue Tieman, Patricia Ward, Carol Welt, and Joan Werner. I would especially like to thank Peter Ralston for sharing his intuition with me, as well as giving me the opportunity to study how the brain works. Janice J.U. Steindler showed me that divine patience and understanding does exist, even amidst thesis-induced schizophrenia. This work was supported by Grant NS - 11614 from the NIH.

References

Altman, J. 1973 Experimental reorganization of the cerebellar cortex III. Regeneration of the external granular layer and granule cell ectopia. J. Comp. Neurol. 149: 153-180.

Anderson, R.F. 1943 Cerebellar distribution of the dorsal and ventral spinocerebellar tracts in the white rat. J. Comp. Neurol. 97: 415-423.

Bliss, T.V.P. and Chung, S.H. 1974 An electrophysiological study of the hippocampus of the "reeler" mutant mouse. Nature 252: 153-155.

Bolk, L. 1906 Das cerebellum der Saugetiere. Haarlem, De Erven F. Bohn; Jena. Gustav Fischer.

Bonnevie, K. and A. Brodal. 1946 Hereditary hydrocephalus in the house mouse. IV. The development of the cerebellar anomalies during foetal life with notes on the normal development of the mouse cerebellum. Skrifter Norske Videnskaps-Akad. Oslo, I. Mat.-Naturv. Kl. No. 4: 1-60.

Braitenberg, V. and R.P. Atwood. 1958 Morphological observations on the cerebellar cortex. J. Comp. Neurol. 109: 1-33.

Brodal, A. and G. Grant. 1962 Morphology and temporal course of degeneration in cerebellar mossy fibers following transection of spinocerebellar tracts in the cat. Exp. Neurol. 5: 67-86.

Brodmann, K. 1909 Vergleichende Lokalisatrons-lehre der Grosshirnrinde in ihren Prizipien dargestellt auf Grund des Zellenbaues. J.A. Barth, Leipzig.

Cajal, S. Ramón y. 1928 Degeneration and Regeneration of the Nervous System, R.M. May (trans.) Hafner, New York, 1959.

Cajal, S. Ramón y. 1929 Studies on Vertebrate Neurogenesis. L. Guth (transl.) Springfield, Ill., Thomas, 1959.

Cajal, S. Ramón y. 1952 Histologie du systeme nerveus de l'homme et des vertebres, vols. I and II. Paris.

Caviness, V.S., Jr. 1973 Time of neuron origin in the hippocampus and dentate gyrus of normal and reeler mutant mice: an autoradiographic analysis. J. Comp. Neurol. 151: 113-120.

Caviness, V.S., Jr. 1975a Architectonic map of neocortex of the normal mouse. J. Comp. Neurol. 164: 247-264.

Caviness, V.S., Jr. 1975b Structure of visual cortex of the reeler mutant mouse: a Golgi analysis. Neurosci. Abs. 1: 101.

Caviness, V.S., Jr. 1976a Patterns of cell and fiber distribution in the neocortex of the reeler mutant mouse. J. Comp. Neurol. 170: 435-448.

Caviness, V.S., Jr. 1976b Reeler mutant mice and laminar distribution of afferents in the neocortex. Exp. Brain Res. Suppl. 1: 267-273.

Caviness, V.S., Jr. and R.L. Sidman. 1972 Olfactory structures of the forebrain in the reeler mutant mouse. J. Comp. Neurol. 145: 85-104.

Caviness, V.S., Jr. and R.L. Sidman. 1973a Retrohippocampal, hippocampal, and related structures of the forebrain in the reeler mutant mouse. J. Comp. Neurol. 147: 235-254.

Caviness, V.S., Jr. and R.L. Sidman. 1973b Time of origin of corresponding cell classes in the cerebral cortex of normal and reeler mutant mice: An autoradiographic analysis. J. Comp. Neurol. 148: 141-152.

Caviness, V.A., Jr., D.K. So, and R.L. Sidman. 1972 The hybrid reeler mouse. J. Hered. 63: 241-246.

Caviness, V.S., Jr. and C.H. Yorke, Jr. 1976 Interhemispheric neocortical connections of the corpus callosum in the reeler mutant mouse: A study based on anterograde and retrograde methods. J. Comp. Neurol. 170: 449-460.

Changeux, J.-P. and A. Danchin. 1976 Selective stabilisation of developing synapses as a mechanism for the specification of neuronal networks. Nature 264: 705-712.

Colwell, S.A. 1975 Thalamocortical-corticothalamic reciprocity: a combined anterograde-retrograde tracer technique. Brain Res. 92: 443-449.

Colwell, S.A. 1976 Combined anterograde-retrograde tracing of the connections of reeler mouse cortex (II): Thalamocortical-corticothalamic reciprocity. Anat. Rec. 184: 380.

Colwell, S.A. and M.M. Merzenich. 1975 Organization of thalamocortical and corticothalamic projections to and from physiologically defined loci within primary auditory cortex in the cat. Anat. Rec. 181: 336.

Committee on Standard Genetic Nomenclature for Mice. 1972 Standard karyotype of the mouse, Mus musculus. J. Hered. 63: 69-72.

Cooper, S. and C.S. Sherrington. 1940 Gower's tract and spinal border cells. Brain 63: 123-134.

Courville, J. and N. Diakiw. 1976 Cerebellar corticonuclear projections in the cat. The vermis of the anterior and posterior lobes. Brain Res. 110: 1-20.

Cowan, W.M., D.I. Gottlieb, A.E. Hendrickson, J.L. Price, and T.A. Woolsey. 1972 The autoradiographic demonstration of axonal connections in the central nervous system. Brain Res. 37: 21-51.

Cragg, B.G. 1975 Absence of barrels and disorganization of thalamic afferent distribution in the sensory cortex of reeler mice. Exp. Neurol. 49: 858-862.

DeLong, G.R. and R.L. Sidman. 1970 Alignment defect of re-aggregating cells in cultures of developing brains of reeler mutant mice. Develop. Biol. 22: 584-600.

Devor, M., V.S. Caviness, Jr., and P. Derer. 1975 A normally laminated afferent projection to an abnormally laminated cortex: some olfactory connections in the reeler mouse. J. Comp. Neurol. 164: 471-482.

Dow, R.S. and G. Moruzzi. 1958 The Physiology and Pathology of the Cerebellum. Univ. of Minn. Pr., Minneapolis.

Drager, U.S. 1975 Physiologic properties of cells in the primary visual cortex of the reeler mutant mouse. Neuroscience Abst. 1: 102.

Eccles, J.C., M. Ito, and J. Szentagothai. 1967 The Cerebellum as a Neuronal Machine. Springer Verlag, Berlin-Heidelberg-New York.

Falconer, D.S. 1951 Two new mutants "trembler" and "reeler" with neurological actions in the house mouse (Mus musculus). J. Genet. 50: 192-201.

Falconer, D.S. 1952 Location of "reeler" in linkage group III of the mouse. Heredity 6: 255-258.

Fink, R.P. and L. Heimer. 1967 Two methods for selective silver impregnation of degenerating axons and their synaptic endings in the central nervous system. Brain Res. 4: 369-374.

Flechsig, P. 1876 Die leitungsbahnen im Gehirn und Ruckenmark des Menschen. Leipzig: W. Engelmann.

Frost, D.O. and V.S. Caviness, Jr. 1974 Patterns of thalamic projections to the neocortex of normal and reeler mutant mice. In: Program and Abstracts of the Fourth Annual Meeting of the Society for Neuroscience. p. 217.

Fujita, S. 1969 Autoradiographic studies on the histogenesis of the cerebellar cortex. In: Neurobiology of Cerebellar Evolution and Development. (R. Llinas, Ed.) Chicago: AMA-ERF. Institute for Biomedical Research. p. 743-747.

Fujita, S., M. Shimada, and T. Nakamura. 1966 ^{3}H-Thymidine autoradiographic studies on the cell proliferation and differentiation in the external and internal granular layers of the mouse cerebellum. J. Comp. Neurol. 128: 191-208.

Gaze, R.M. The Formation of Nerve Connections. 1970. Academic Press. London-New York. p. 288.

Globus, A. and A.B. Sheibel. 1967 Pattern and field in cortical structure: the rabbit. J. Comp. Neurol. 131: 155-172.

Graham, R.C., Jr. and M.J. Karnovsky. 1966 The early stages of absorption of injected horseradish peroxidase in the proximal tubules of mouse kidney: Ultrastructural cytochemistry by a new technique. J. Histochem. cytochem. 14: 291-302.

Grant, G. 1962 Spinal course and somatotopically localized termination of the spinocerebellar tracts (An experimental study in the cat). Acta Physiologica Scan., vol. 56, Supplem. 193.

Grant, G. and B. Rexed. 1958 Dorsal spinal root afferents to Clarke's column. Brain 81: 567-576.

Groenewegen, H.J. and J. Voogd. 1977 The parasagittal zonation within the olivocerebellar projection. I. Climbing fiber distribution in the vermis of the cat cerebellum. J. Comp. Neurol. 174: 417-488.

Ha, H. and C.N. Liu. 1968 Cell origin of the ventral spinocerebellar tract. J. Comp. Neurol. 133: 185-207.

Haddara, M.A. and M.A. Nooreddin. 1966 A quantitative study on the postnatal development of the cerebellar vermis of the mouse. J. Comp. Neurol. 128: 245-254.

Hamburgh, M. 1960 Observations on the neuropathology of "Reeler", a neurological mutation in the mouse. Experientia, 16: 460.

Hamburgh, M. 1963 Analysis of the postnatal developmental effects of "reeler," a neurological mutation in mice. A study in developmental genetics. Develop. Biol. 8: 165-185.

Hand, P.J. and T. Van Winkle. 1977 The efferent connections of the feline nucleus cuneatus. J. Comp. Neurol. 171: 83-110.

Hibbard, E. 1965 Orientation and directed growth of Mauthner's cell axons from duplicated vestibular nerve roots. Exp. Neurol. 13: 289-301.

Hinds, J.W. 1972 Early neuron differentiation in the mouse olfactory bulb. J. Comp. Neurol. 146: 233-252.

Hubbard, J.I. and O. Oscarsson. 1962 Localization of cell bodies of the ventral spinocerebellar tract in lumbar segments of the cat. J. Comp. Neurol. 118: 199-204.

Jacobson, C.-O. 1964 Motor nuclei, cranial nerve roots, and fibre pattern in the medulla oblongata after reversal experiments on the neural plate of Axolotl. larvae. I. Bilateral operations. Zool. Bidrag. Uppsala 36: 73-160.

Jacobson, M. 1970 Developmental Neurobiology. Holt, Rinehart and Winston, Inc. p. 465.

Killackey, H.P., G. Belford, R. Ryugo, and D.K. Ryugo. 1976 Anomalous organization of thalamocortical projections consequent to vibrissae removal in the newborn rat and mouse. Brain Res. 104: 309-315.

Korneliussen, H.K. 1968a Comments on the cerebellum and its division. Brain Res. 8: 229-236.

Korneliussen, H.K. 1968b On the ontogenetic development of the cerebellum (nuclei, fissures, and cortex) of the Rat, with special reference to regional variations in corticogenesis. J. Hirnforsch. 10: 379-412.

Korneliussen, H.K. 1969 Cerebellar organization in light of cerebellar nuclear morphology and cerebellar corticogenesis. In: Neurobiology of Cerebellar Evolution and Development. (R. Llinas, Ed.) Chicago: AMA-ERF. Institute for Biomedical Research. p. 515-523.

Krieg, W.J.S. 1946a Connections of the cerebral cortex. I. The albino rat. A.Topography of the cortical areas. J. Comp. Neurol. 84: 221-275.

Krieg, W.J.S. 1946b Connections of the cerebral cortex. I. The albino rat. B. Structure of the cortical areas. J. Comp. Neurol. 84: 277-324.

Landis, S.C. 1973 Ultrastructural changes in the mitochondria of cerebellar Purkinje cells of nervous mutant mice. J. Cell Biol. 57: 782-797.

Landis, S.C., W.J. Shoemaker, M. Schlumpf, and F.E. Bloom. 1975 Catecholamines in mutant mouse cerebellum: fluorescence microscopic and chemical studies. Brain Res. 93: 253-266.

Lane, P.W. 1967 Linkage groups III and XVII in the mouse and the position of the light ear locus. J. Hered. 58: 21-24.

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

Larsell, O. 1953 The cerebellum of the cat and the monkey. J. Comp. Neurol. 99: 135-199.

LaVail, J.H., K.R. Winston, and A. Tish. 1973 A method based on retrograde intraaxonal transport of protein for identification of cell bodies of origin of axons terminating within the CNS. Brain Res. 58: 470-477.

Levi-Montalcini, R. 1964a Events in the developing nervous system. Progr. Brain Res. 4: 1-29.

Levi-Montalcini, R. 1964b Growth and differentiation in the nervous system. In: The Nature of Biological Diversity. (J.M. Allen, ed). McGraw-Hill, New York.

Marin-Padilla, M. 1971 Early prenatal ontogenesis of the cerebral cortex (neocortex) of the cat (Felis domestica). A Golgi study. I. The primordial neocortical organization. Z. Anat. Entwickl-geschichte 134: 117-145.

Martin, G.F. and C.K. Henkel. 1975 Cerebello-olivary fibers: An analysis of their origin, course, and distribution using horseradish peroxidase, autoradiographic and degeneration techniques. Neurosci. Abstr. 1: 208.

Martin, G.F., C.K. Henkel, and J.S. King. 1976 Cerebello-olivary fibers: their origin, course and distribution in the North American Opossum. Exp. Brain Res. 24: 219-236

Meier, H. and W.G. Hoag. 1962 The neuropathology of "reeler", a neuromuscular mutation in mice. J. Neuropathol. Exp. Neurol. 21: 649-654.

Miale, I.L. and R.L. Sidman. 1961 An autoradiographic analysis of histogenesis in the mouse cerebellum. Exp. Neurol. 4: 277-296.

Molliver, M.E., I. Kostovic, and H. Van der Loos. 1973 The development of synapses in cerebral cortex of the human fetus. Brain Res. 50: 403-407.

Morest, D.K. 1970 A study of neurogenesis in the forebrain of opossum pouch young. Z. Anat. Entwickl-geschichte 130: 265-305.

Morin, F. and E.D. Gardner. 1953 Spinal pathways for cerebellar pathways in the monkey (Macaca mulatta). Am. J. Physiol. 174: 155-161.

Mugnaini, E. 1969 Ultrastructural studies on the cerebellar histogenesis II. Maturation of nerve cell populations and establishment of synaptic connections in the cerebellar cortex of the chick. In: Neurobiology of cerebellar evolution and development. (R. Llinas, Ed.) Chicago: AMA-ERF. Institute for Biomedical Research. p. 749-782.

Mullen, R.J., E.M. Eicher, and R.L. Sidman. 1976 Purkinje cell degeneration, a new neurological mutation in the mouse. Proc. Natl. Acad. Sci. (Wash.) 73: 208-212.

Nauta, W.J.H. and P.A. Gygax. 1954 Silver impregnation of degenerating axons in the central nervous system. Stain Technol. 29: 91-93.

Oscarsson, O. 1965b Functional organization of the spino- and cuneocerebellar tracts. Physiol. Rev. 45: 495-522.

Oscarsson, O. 1973 Functional organization of spinocerebellar paths. In: Handbook of Sensory Physiology, Vol. II, Somatosensory System. Springer-Verlag, Berlin. p. 339-380.

Palay, S. and V. Chan-Palay. 1974 Cerebellar Cortex, Cytology and Organization. Springer-Verlag, Berlin.

Rakic, P. 1971a Neuron-glia relationship during granule cell migration in developing cerebellar cortex. A Golgi and electron microscopic study in Macacus rhesus. J. Comp. Neurol. 141: 283-312.

Rakic, P. 1972 Mode of cell migration to the superficial layers of fetal monkey neocortex. J. Comp. Neurol. 145: 61-84.

Rakic, P. 1976 Synaptic specificity in the cerebellar cortex: study of anomalous circuits induced by single gene mutations in mice. Cold Spring Harbor Symp. Quant. Biol. 40: 333-346.

Rakic, P. and R.L. Sidman. 1970 Histogenesis of cortical layers in human cerebellum, particularly the lamina dissecans. J. Comp. Neurol. 139: 473-500.

Rakic, P. and R.L. Sidman. 1972 Synaptic organization of displaced and disoriented cerebellar cortical neurons in reeler mutant mice. J. Neuropathol. Exp. Neur. 31: 192.

Rakic, P. and R.L. Sidman 1973a Weaver mutant mouse cerebellum: defective neuronal migration secondary to specific abnormality of Bergmann glia. Proc. Natl. Acad. Sci. (Wash.) 70: 240-244.

Rakic, P. and R.L. Sidman 1973b Sequence of developmental abnormalities leading to granule cell deficit in cerebellar cortex of weaver mutant mice. J. Comp. Neurol. 152: 103-132.

Ramon-Moliner, E. 1970 The Golgi-Cox Technique. In: Contemporary Research Methods in Neuroanatomy. (W.J.H. Nauta and S.O.E. Ebbesson, Eds.) Springer-Verlag. New York, Heidelberg, Berlin. p. 32-55.

Rexed, B. 1954 A cytoarchitectonic atlas of the spinal cord in the cat. J. Comp. Neurol. 100: 297-397.

Richardson, K.C., L. Jarett, and E.H. Finke. 1960 Embedding in epoxy resins for ultrathin sectioning in electron microscopy. Stain Tech. 35: 313-323.

Van der Loos, H. 1976 Barreloids in mouse somatosensory thalamus. Neuroscience Letters 2: 1-6.

Shambes, G.M., J.M. Gibson, and W. Welker. 1976 Somatotopic organization of rat cerebellar tactile cortex using micromapping methods. Neuroscience Abst., Vol. II, p. 117.

Sidman, R.L. 1968a Abnormal cell migrations in developing brains of mutant mice. In: Expanding Concepts in Mental Retardation. A Symposium from the Joseph P. Kennedy, Jr. Foundation. (G.Z. Jervis, Ed.) Charles C. Thomas, Springfield, Illinois. p. 40-49.

Sidman, R.L. 1968b Development of interneuronal connections in brains of mutant mice. In: Physiological and Biochemical Aspects of Nervous Integration. (F.D. Carlson, Ed.) Prentice Hall, New Jersey. p. 163-193.

Sidman, R.L. 1972 Cell interactions in developing Mammalian central nervous system. In: Cell Interactions, Proc. Third Lepetit Colloquium (L.G. Silvestri, Ed.) North-Holland, Amsterdam. p. 1-13.

Sidman, R.L., J.B. Angevine, and E.T. Pierce. 1971 Atlas of the Mouse Brain and Spinal Cord. Harvard University Press, Cambridge, Massachusetts. p. 261.

Sidman, R.L., M.C. Green and S.H. Appel. 1965 Catalog of the Neurological Mutants of the Mouse. Harvard University Press, Cambridge, Massachusetts.

Sidman, R.L., P.W. Lane and M.M. Dickie. 1962 Staggerer, a new mutation in the mouse cerebellum. Science 137: 610-612.

Snider, R. and E. Eldred 1952 Cerebro-cerebellar relationship in the monkey. J. Neurophysiol. 15: 27-40.

Snider, R.S. and A. Stowell. 1944 Receiving areas of the tactile, auditory and visual systems in the cerebellum. J. Neurophysiol. 7: 331-358.

Snyder, R.L. 1977 A comparative study of the neurons of origin of cerebellar afferents in the rat, cat, and squirrel monkey based on the horseradish peroxidase (HRP) retrograde tracer technique: The spinal afferents. Anat. Rec. 187: 719.

Sotelo, C. 1975 Anatomical physiological and biochemical studies of cerebellum from mutant mice. II. Morphological study of cerebellar cortical neurons and circuits in the weaver mouse. Brain Res. 94: 19-44.

Sperry, R.W. 1965 Embryogenesis of behavioral nerve nets. In: Organogenesis (R.L. Dettaan and H. Ursprung, Eds.) Holt, Rinehart and Winston, New York.

Stanfield, B. 1977 The morphogenesis of the hippocampus in the normal and reeler mutant mouse. Anat. Rec. 187: 721-722.

Stanfield, B.B., V.S. Caviness, Jr., and W.M. Cowan. 1975 The organization of the afferents to the dentate gyrus of the hippocampal formation in the reeler mutant mouse. Neuroscience Abst. I: 774.

Stefanelli, A. 1950 Studies on the development of Mauthner's cell. In: Genetic Neurology. (P. Weiss, Ed.) Univ. of Chicago Press, Chicago.

Stefanelli, A. 1951 The Mauthrerian apparatus in the Ichthyopsida; its nature and function and correlated problems of histogenesis. Quart. Rev. Biol. 26: 17-34.

Steindler, D.A. 1975 Distribution of spinal afferents in the cerebellum of the reeler mutant mouse. Neuroscience Abst. 1: 516.

Steindler, D.A. 1976 Combined anterograde-retrograde tracing of the connections of reeler mouse cortex (I): On neuronal specificity in the mutant nervous system. Anat. Rec. 184:540.

Steindler, D.A. 1977a Cortical barrels, thalamic barreloids, and trigemino-cerebellar projections in normal and reeler mutant mice. Anat. Rec. 187: 722-723.

Steindler, D.A. 1977b Trigemino-cerebellar projections in normal and reeler mutant mice. Neuroscience Letters (In press).

Steindler, D.A. and S.A. Colwell. 1976 Reeler mutant mouse: maintenance of appropriate and reciprocal connections in the cerebral cortex and thalamus. Brain Res. 113: 386-393.

Stensaas, L.J. 1967 The development of hippocampal and dorsolateral pallial regions of the cerebral hemisphere in fetal rabbits. II. Twenty millimeter stage, neuroblast morphology. J. Comp. Neurol. 129: 71-84.

Swisher, D.A. and D.B. Wilson. 1977 Cerebellar histogenesis in the Lurcher (Lc) mutant mouse. J. Comp. Neurol. 173: 205-217.

Tolbert, D.L., L.C. Massopust, M.G. Murphy and P.A. Young. 1976 The anatomical organization of the cerebello-olivary projection in the cat. J. Comp. Neurol. 170: 525-544.

Uzman, L. 1960 The histogenesis of the mouse cerebellum as studied by its tritiated thymidine uptake. J. Comp. Neurol. 114: 137-159.

Vachananda, B. 1959 The major spinal afferent systems and the cerebellar corticonuclear connections in macaca mulatta. J. Comp. Neurol. 112: 303-352.

Valverde, F. 1970 The Golgi method. A tool for comparative structural analyses. In: Contemporary Research Methods in Neuroanatomy. (W.J.H. Nauta and S.O.E. Ebbesson, Eds.) Springer-Verlag. New York, Heidelburg, Berlin. p. 12-31.

Van der Loos, H. 1965 The "improperly" oriented pyramidal cell in the cerebral cortex and its possible bearing on problems of growth and cell orientation. Bull. Johns Hopkins Hosp. 117: 228-250.

Van der Loos, H. 1976 Barreloids in mouse somatosensory thalamus. Neuroscience Letters 2: 1-6.

Van der Loos, H. and T.A. Woolsey. 1973 Somatosensory cortex: structural alterations following early injury to sense organs. Science 179: 393-398.

Voogd, J. 1969 The importance of fiber connections in the comparative anatomy of the mammalian cerebellum. In: Neurobiology of cerebellar evolution and development. (R. Llinas, Ed.) A.M.A. Chicago. p. 493-514.

Voogd, J., G. Broere, and J. Van Rossum. 1969 The mediolateral distribution of the spinocerebellar projection in the anterior lobe and the simple lobule in the cat and a comparison with some other afferent fibre systems. Psychiat. Neurol. Neurochir. 72: 137-151.

Voogd, J. 1975 Bolk's subdivision of the mammalian cerebellum (Growth centres and functional zones). Acta Morphol. Neerl.-Scand. 13: 35-54.

Welker, C. 1976 Receptive fields of barrels in the somatosensory neocortex of the rat. J. Comp. Neurol. 166: 173-190.

Welker, C. 1976 Cytoarchitectural organization of the somatosensory (SmI) cortical "barrel field" in reeler mutant mice. Anat. Rec. 184: 560-561.

Welker, C. and T.A. Woolsey. 1974 Structure of layer IV in the somatosensory neocortex of the rat: description and comparison with the mouse. J. Comp. Neurol. 158: 437-454.

Welker, W.I. and S. Seidenstein. 1959 Somatic sensory representation in the cerebral cortex of the racoon (Procyon lotor). J. Comp. Neurol. 111: 469-502.

Welt, C. and D.A. Steindler. 1977 Somatosensory cortical barrels and thalamic barreloids in reeler mutant mice. J. Neurosci. (In press).

Williams, R.S., R.J. Ferrante, and V.S. Caviness, Jr. 1975 Neocortical organization in human cerebral malformations: A Golgi study. Neuroscience Abst. 1: 776.

Woolsey, T.A. and H. Van der Loos. 1970 The structural organization of layer IV in the somatosensory region (SI) of the mouse cerebral cortex: the description of a cortical field composed of discrete cytoarchitectonic units. Brain Res. 17: 205-242.

Figure Legends

Figure 1:

30 um sagittal sections through the hindbrain of 22-day old normal wild-type (a) and homozygous reeler (b) littermates, Nissl-stain. Note dysgenesis of cerebellar cortex in reeler (asterisk in b) compared to normal (asterisk in a), but increased size of underlying deep cerebellar nuclei in reeler. ic = inferior colliculus; dorsal (D) is up, ventral (V) is down, rostral (R) is to the left, and caudal (C) is to the right.

Figure 2:

Semi-thin plastic sections of 22-day old normal (a) and reeler (b) cerebellar cortex stained with toluidine blue.

a. Cytoarchitecture of the cerebellar cortex of the mouse, as in other mammalian species, is characterized by the presence of a cell-sparse molecular layer (M) beneath the pial surface. A single row of Purkinje somata (asterisk and P) lie just beneath the molecular layer, and a granule cell layer (G) is below this layer. Scale = 20 um.

b. In reeler, aberrant patterns of cerebellar cyto-architecture are revealed by the presence of many granule cells occupying positions above Purkinje cells (EGL), as well as Purkinje cells being intermingled with granule cells (P & G). There is, however, some areas with a discernible molecular layer and some Purkinje somata

above granule cells (top asterisk). For the most part, the dorso - ventral pattern of cerebellar lamination in reeler is depicted in Fig. 2b: molecular layer; some Purkinje cells; ectopic granule cell layer; Purkinje and granule cells intermingled. Asterisks mark Purkinje cells. Scale = 20 um.

Figure 3:

30 um frontal sections through the cerebellum of a 22-day old reeler mouse (case 9084, also depicted in Figures 7, 12, 13). Nissl stain. Section a is most rostral, i is caudal. The cortex consists of a sheet of darkly-stained cells that covers the afoliate cerebellar surface, and condensations of cells in the midline. In a, the cortical sheet, containing populations of densely-packed granule cells, dips down into midline areas forming sheets (section b) and cylindrical condensations (sections c, d, e). Caudal to section e, there are also populations of granule cells that are within midline areas at the level of or below the deep cerebellar nuclei (sections f - h). Most caudally (sections h - i), the layer of darkly-stained cortical cells can be seen coating the entire cerebellar surface. A portion of section d is also shown in a in Figure 12, and h is partially depicted in a of Figure 13.

Figure 4:

a. Golgi-Cox preparation, cerebellar cortex of a 22-day old reeler mouse. A mossy fiber axon can be seen giving

rise to a presumed terminal and continuing on a short distance to end in another smaller terminal. Scale = 6 um.

b. Fink-Heimer preparation, cerebellar cortex of a 22-day old reeler mouse following spinal cord hemisection on day 17. The parent axon of a mossy fiber is argyrophilic and beaded, and appears to end as a swollen argyrophilic terminal within a clear space (glomerular region) surrounded by granule cell nuclei that are also stained. Other argyrophilic products, presumed degenerating axonal debris, are also present. Scale = 6 um.

Abbreviations for Figures 5 - 9:

ip - inferior cerebellar peduncle
M.V. - medial vestibular nucleus
L.V. - lateral vestibular nucleus
N.L. - nucleus lateralis
N.I. - nucleus interpositus
N.M. - nucleus medialis
IV - fourth ventricle
XII - twelfth nerve nucleus
L.C. - lateral cuneate nucleus
N.Pc. - nucleus parvocellularis compactus
loc. - nucleus locus coeruleus
dor. coc. - dorsal cochlear nucleus
li - lingula
ce - centralis
cu - culmen
si - simplex
cr1 - hemisphere crus 1
cr2 - hemisphere crus 2
no - nodule
de - declive
st. cu. - stratum granulosum culmen
uv - uvula
py - pyramis
pa - paramedian
l.a.u.v. - lamina alba uvula vermis

Figures 5 - 6:

Reconstructions from 30 um frontal sections through the cerebellum of a 22-day old normal mouse (case 9086, also depicted in Figures 9 - 11) stained by the Fink-Heimer method following spinal cord hemisection at T12 - 13 on day 17. Degenerating axonal debris is plotted as small black dots or lines within the peduncles, deep cerebellar nuclei, cerebellar commissure, and particular regions of the cerebellar spinal recipient zone. Section A is rostral, F is caudal. Numbers to the right of sections indicate the number of that section from a consecutive series of 30 um frontal sections. Scale = 1 mm.

Section A:

Degenerating fibers from the dorsal spinocerebellar tract (DSCT) enter the cerebellum by way of the inferior cerebellar peduncle (ip) and intermingle with degenerating fibers of the ventral spinocerebellar tract (VSCT) that have entered the cerebellum from the superior cerebellar peduncle. DSCT and VSCT fibers accumulate within a cerebellar commissure that is at the level of or just above the deep cerebellar nuclei (N.M., N.I., N.L.) (commissure can also be seen in sections B - D). There appears to be a small projection to the lingula (li), but the bulk of degenerating DSCT and VSCT fibers, at this level, ascend from the commissure and distribute bilaterally amongst populations of granule cells in the centralis lobule (ce) of the anterior lobe vermis. Degenerating axonal debris can also be seen in the nucleus medialis (N.M.) and nucleus

interpositus (N.I.) deep cerebellar nuclei.

Section B:

This section is similar to section A. The cerebellar commissure, which is just above the lingula, is seen to contain similar amounts of DSCT and VSCT degeneration on both sides of the midline. Degenerating axons within the centralis lobule appear as patches or "maxima" and "minima" (see Voogd, et. al., 1969; and text for elaboration).

Section C:

Degenerating DSCT and VSCT fibers radially ascend from the cerebellar commissure to reach populations of granule neurons in the stratum granulosum in the culmen lobule (st. cu.) of the anterior lobe.

Section D:

Degenerating fibers in the commissure (above the uvula, uv) at this level represent the "connector" (see Fig. 9) between anterior lobe and posterior lobe DSCT and VSCT projections. Fibers leaving the commissure at this level enter the pyramis lobule (py) of the posterior lobe. At this level, bilateral projections can be seen within lateral pyramis regions as well as in the nucleus medialis.

Section E:

Degenerating DSCT and VSCT projections are represented in almost a bilaterally symmetric fashion in midline and lateral regions of the pyramis lobule. The patches of degeneration, maxima and minima, (Voogd, et. a., 1969), when traced in consecutive sections (for example, sections E - F which are separa-

ted by approximately 300 um) appear to contribute to rostrocaudal bands (see Text and Voogd, et. al., 1969). A portion of section E is also illustrated in Fig. 10.

Section F:

Maxima and minima of spinocerebellar degeneration can be clearly seen in the pyramis lobule. Section is also illustrated in Fig. 11.

Figures 7 - 8:

Reconstructions from 30 um frontal sections through cerebella of 22-day old reeler mice (cases 9084 and 9076, also depicted in Figures 9, 12, 13 and 14) stained by the Fink-Heimer method following T12 - 13 hemisection on day 17. Again, section A is most rostral in both Figures.

Figure 7 (Case 9084):

Section A:

Degenerating fibers from the DSCT enter the cerebellum by way of the inferior cerebellar peduncle (ip) and intermingle with degenerating VSCT fibers that have entered via the superior cerebellar peduncle. As in the normal mouse, there is also a cerebellar commissure in the mutant that is just above the deep nuclei (N.I. in section A). Degenerating DSCT and VSCT fibers within the midline of this anterior region in the mutant cerebellum are distributed amongst granule cells below the level of the deep nuclei. This area is presumed to be analogous to the lingula and centralis lobules of the normal mouse anterior lobe (compare section 7A with

sections 6A - 6B). Degenerating axons can also be seen in the nucleus medialis deep cerebellar nuclei.

Section B:

Degenerating DSCT and VSCT axons descending from the commissure toward displaced populations of granule neurons in a presumptive centralis region, and a massive amount of degeneration (relative to the normal) can also be seen in the medial and interpositus deep cerebellar nuclei. As in the normal mouse cerebellum, DSCT and VSCT projections are almost equally distributed on both sides of the midline.

Section C:

The cerebellar commissure is quite prominent at this level, and again, degenerating DSCT and VSCT axons descend to neurons of the presumed centralis region. (Sections 7A - 7C are believed to correspond to sections 6A - 6B of the normal mouse anterior lobe.) This section is also illustrated in Fig. 12.

Section D:

Compare this section with section 6C from the normal mouse. A bilateral distribution of degenerating axons within a midline region appears to represent DSCT and VSCT innervation of a culmen lobule. Also, degenerating axons fan-out from the commissure to populations of ectopically positioned granule cells in lateral regions of the mutant cerebellum. This is presumed to represent the beginning of lateral pyramis innervation (compare with Fig. 6D of the normal). Degeneration can also be seen within the deep nuclei medialis and interpositus.

Section E:

Compare this section with sections 6E - 6F. A midline patch of degeneration is believed to represent a DSCT and VSCT posterior lobe innervation of midline pyramis neurons that are extremely condensed compared with the normal. Bilateral projections to lateral regions of the ectopic granule cell layer are believed to be analogous to lateral pyramis projections of the normal mouse (Figures 6D - 6F). As in all of the sections through the mutant cerebellum, the mediolateral distribution of DSCT and VSCT axons is seen to be altered relative to the normal, and hence the organization of afferents into "maxima" and "minima" is obscured. Section e is also illustrated in Fig. 13.

Figure 8 (Case 9076):

This case is rather similar to case 9084. Section A almost corresponds to section A of Fig. 7; section B with sections B - C of Fig 7; section C with sections C - D of Fig. 7; and section D - E with sections D - E of Fig. 7.

Figure 9:

These illustrations represent surface reconstructions of cases 9086 and 9084. Stippling on the surface of the normal and mutant cerebella indicates the mediolateral and rostrocaudal extent of degeneration plotted from frontal sections. These summary diagrams illustrate that:

1) The radial (dorsoventral) distributions of DSCT and VSCT axons are greatly altered in reeler (as seen in the frontal plane of these drawings). In the normal animal,

DSCT and VSCT projections predominately ascend from the commissure towards populations of granule cells which reside in cortical regions well above the deep cerebellar nuclei. In reeler (case 9084), these projections primarily descend toward populations of neurons at the level of or below the deep nuclei.

2) As a result of deviations in the radial distributions of cerebellar cortical neurons in reeler, the mediolateral distributions of DSCT and VSCT axons are also altered in an accomodating fashion. The mediolateral organization of the paleocerebellum in reeler appears to be greatly compressed.

3) Symmetrical DSCT and VSCT projections to both sides of the midline exist in the normal and mutant cerebellum.

4) The rostrocaudal distribution of DSCT and VSCT fibers is similar in both phenotypes, only greatly compressed ("miniature") in reeler (the mutant cerebellum is approximately one-half the size of the normal). There is a segregated DSCT and VSCT projection to anterior and posterior lobe regions in normal and reeler cerebella (centralis, culmen, and lingula of the anterior lobe; pyramis of the posterior lobe). An exaggerated "connector" is diagrammatically depicted here joining the anterior and posterior spinal recipient zones. This connector is made up of degeneration within midline areas of the white matter at the junction of the two zones.

Figure 10 (Case 9086, normal mouse cerebellum, previously described in Figures 5 - 6):

a) Section E of Fig. 6; the same blood vessel (b.v.) is noted in b. and c. Scale = 1 mm.

b) Nissl-stained section adjacent to section stained with Fink-Heimer method and drawn in section a. Granule cells (G) of the midline portion of the pyramis lobule (py) are bounded on either side by white matter (W). Scale = 70 um.

c) Fink-Heimer stained section. Normal axons (open arrow) and argyrophylic debris representing mossy fiber axonal and preterminal degeneration (closed arrow) can be seen in the white matter and granule cell layer. Scale = 3 um.

Figure 11 (Case 9086, normal mouse cerebellum, previously described in Figures 5 - 6):

a) Section F of Fig. 6; blood vessel also shown in b. Scale = 1 mm.

b) Adjacent Nissl-stained section. M is molecular layer; P is Purkinje cell layer; granule cell layer (G) and white matter (W). Lower granule cell layer is shown in C. Scale = 70 um.

c) Fink-Heimer stained section, photomicrograph from an area enclosed within box in a. Normal axons (open arrow) and degenerating mossy fiber axonal debris (closed arrows) within the white matter (W) and granule cell layer (G). Scale = 2.5 um.

Figure 12 (Case 9084, reeler mouse cerebellum, previously described in Figure 7):

a) Adjacent Nissl-stained section (area within outer box of insert drawing) showing large neurons (many of which are Purkinje cells) scattered amongst populations of smaller neurons (many of which are granule cells) in a midline regions of the presumed reeler anterior lobe. Cylindrical condensations of granule cells in the midline (partially enclosed in box) are assumed to be a portion of a presumptive centralis lobule. Scale in insert drawing = 1 mm; scale in a = 50 um.

b) Fink-Heimer stained section, photomicrograph from an area enclosed within box in a, inner box in insert drawing. Many degenerating mossy fibers (black dots) distributed amongst the granule cells that are arranged in a cylindrical fashion. The course of these DSCT and VSCT axons so much adheres to a pattern formed by the unique topology of the granule cells that one is tempted to describe this situation as mossy afferents seeking-out displaced populations of centralis granule neurons. Scale = 50 um.

Figure 13 (Case 9084):

a) Nissl-stained section, from the presumed lateral pyramis region of the reeler cerebellum. This section typifies the aberrant cytoarchitecture of the mutant cerebellum. Below the pial surface, there is a cell-sparse molecular layer (M). A few Purkinje cells (asterisks and P) occupy normal positions above granule cells, but most are either intermingled with (P & G) or

below what can be considered an ectopically positioned granule cell layer (EGL). Scale in insert drawing = 1 mm; scale in a = 40 um.

b) Fink-Heimer stained section, box in insert drawing. Dark-field photomicrograph. Degenerating DSCT and VSCT axons (bright dots) distribute amongst granule neurons in the Purkinje and granule cell layer (P & G) and ectopic granule cell layer. Scale = 40 um.

Figure 14 (Case 9076, reeler mouse cerebellum, previously described in Figures 7 - 8):

a) See Fig. 8. Area enclosed within square is shown in b, circle is shown in c. Scale = 1 mm.

b) Nissl-stained section showing large neurons (many of which are Purkinje cells) intermingled with granule cells that are arranged in sheets within a rostral (anterior lobe) region of the mutant cerebellum. A molecular layer (M), beneath the pial surface, is present at this level. Scale = 320 um.

c) Fink-Heimer stained section showing degenerating mossy fibers within the "sheets" of granule cells (open arrows). Preterminal degeneration can also be seen in the molecular layer (closed arrows) near granule cells that are ectopically positioned within this layer. Scale = 12 um.

Figure 15 - 30-day old reeler mouse with two combined horseradish peroxidase (HRP) - tritiated amino acid (TAA) injections that involved the lateral cerebellar hemispheric regions and

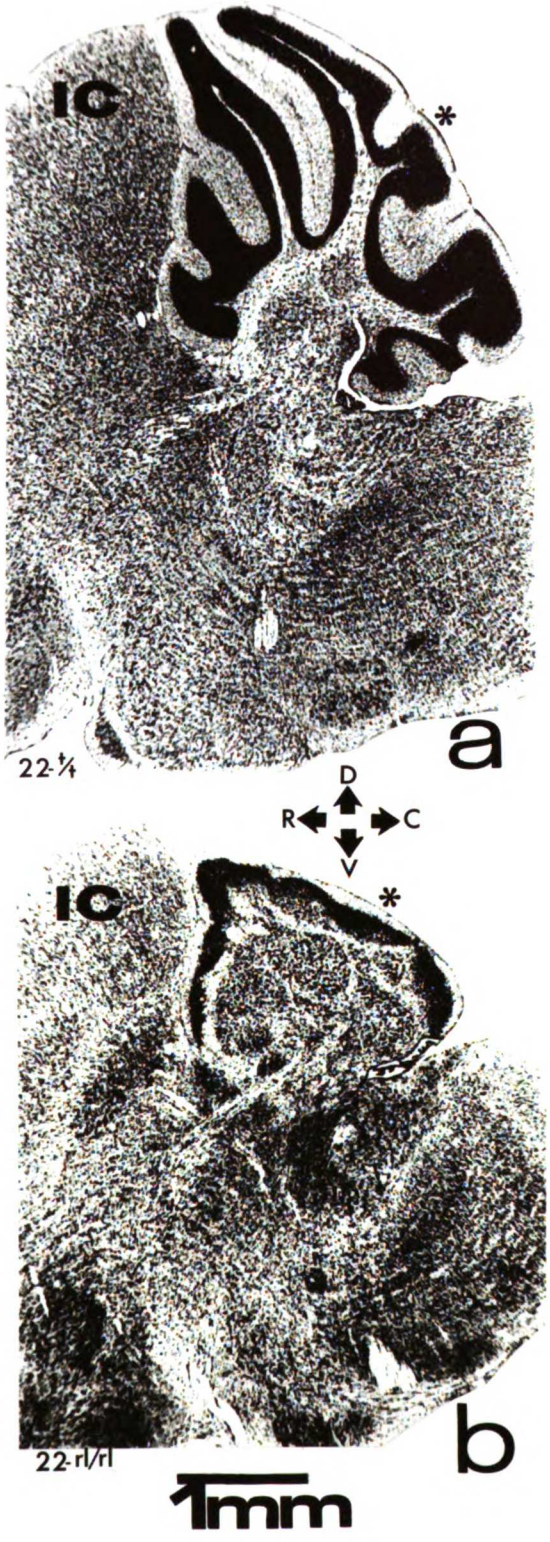
underlying lateral (N.L.) and interpositus (N.I.) deep cerebellar nuclei on the left side (also see Steindler, 1977b):

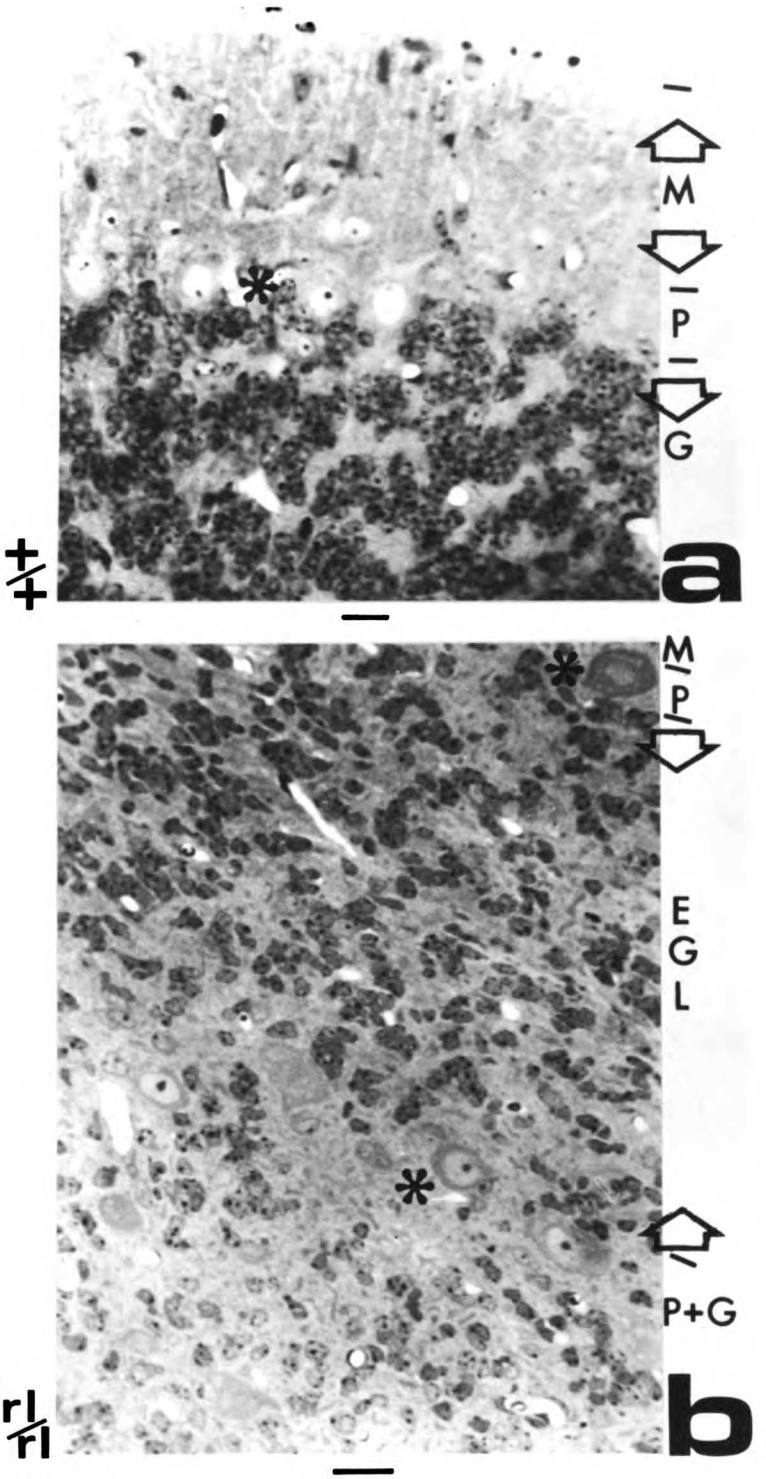
a) Bright-field photomicrograph showing the extent of peroxidase labeling within the injection sites in the mutant cerebellum from a section reacted solely for HRP. N.M. is nucleus medialis. Scale for both a and b = 500 um.

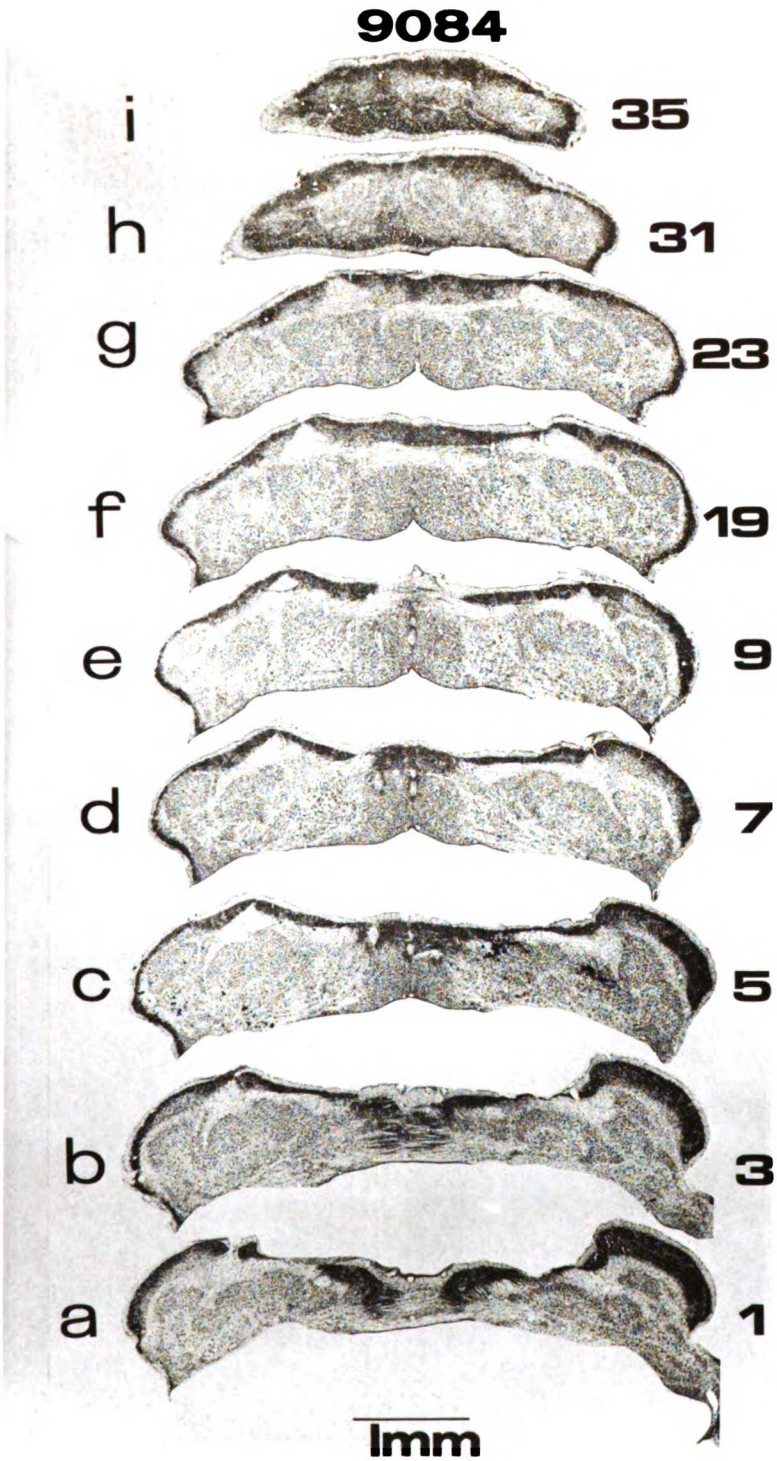
b) Dark-field photomicrograph of an adjacent section to a that was processed for autoradiography (ARG). This section shows the extent of the TAA labeling within the injection sites.

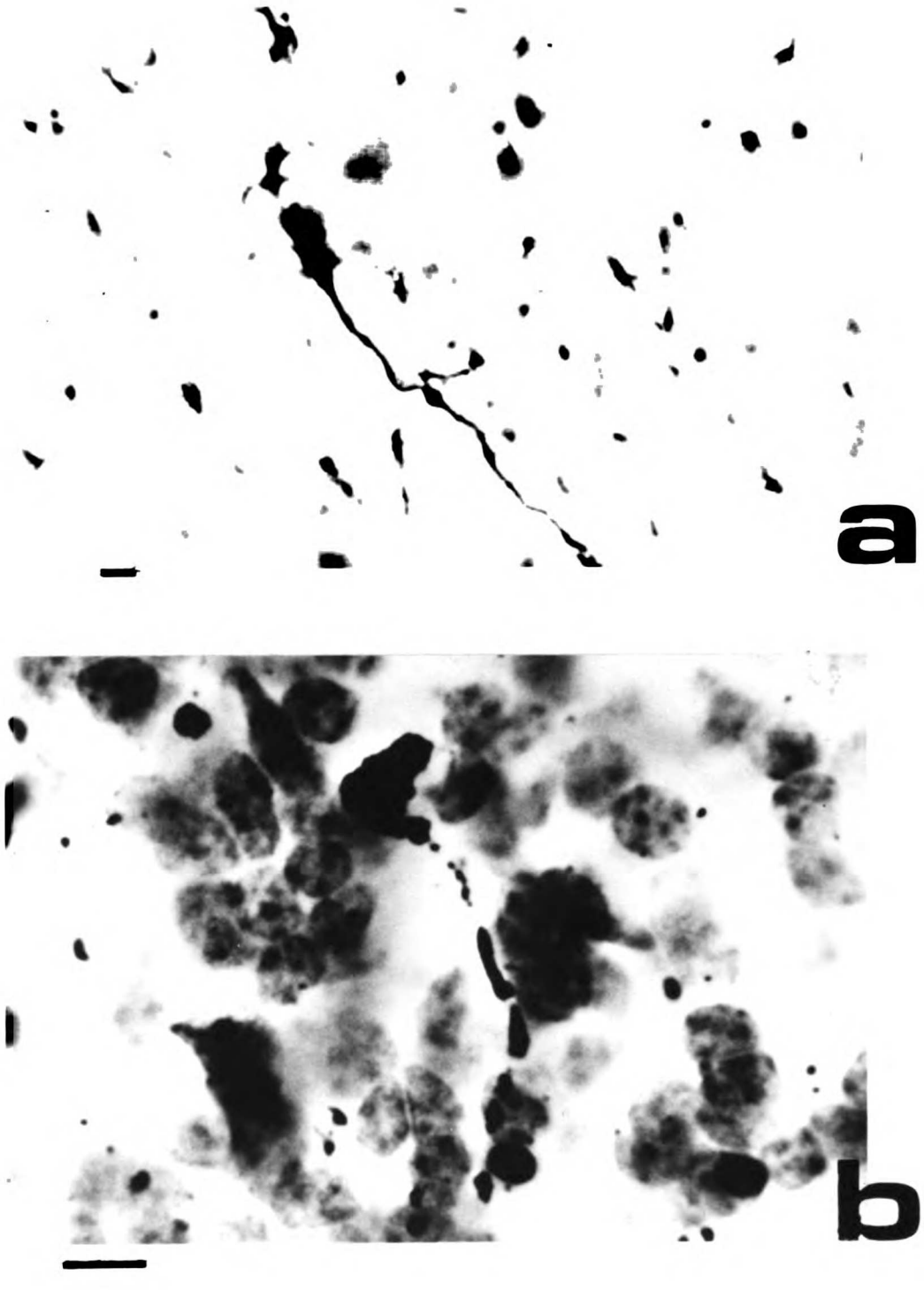
c) Drawing of a 30 um section from the brainstem of this animal. Abbreviations: int. v. = interpolaris division of the spinal trigeminal nucleus; IV = fourth ventricle; XII = twelfth nerve nucleus; n. Rol. = nucleus of Rolleri; io, mao, and dao = principal, medial accessory, and dorsal accessory divisions of the inferior olivary complex.

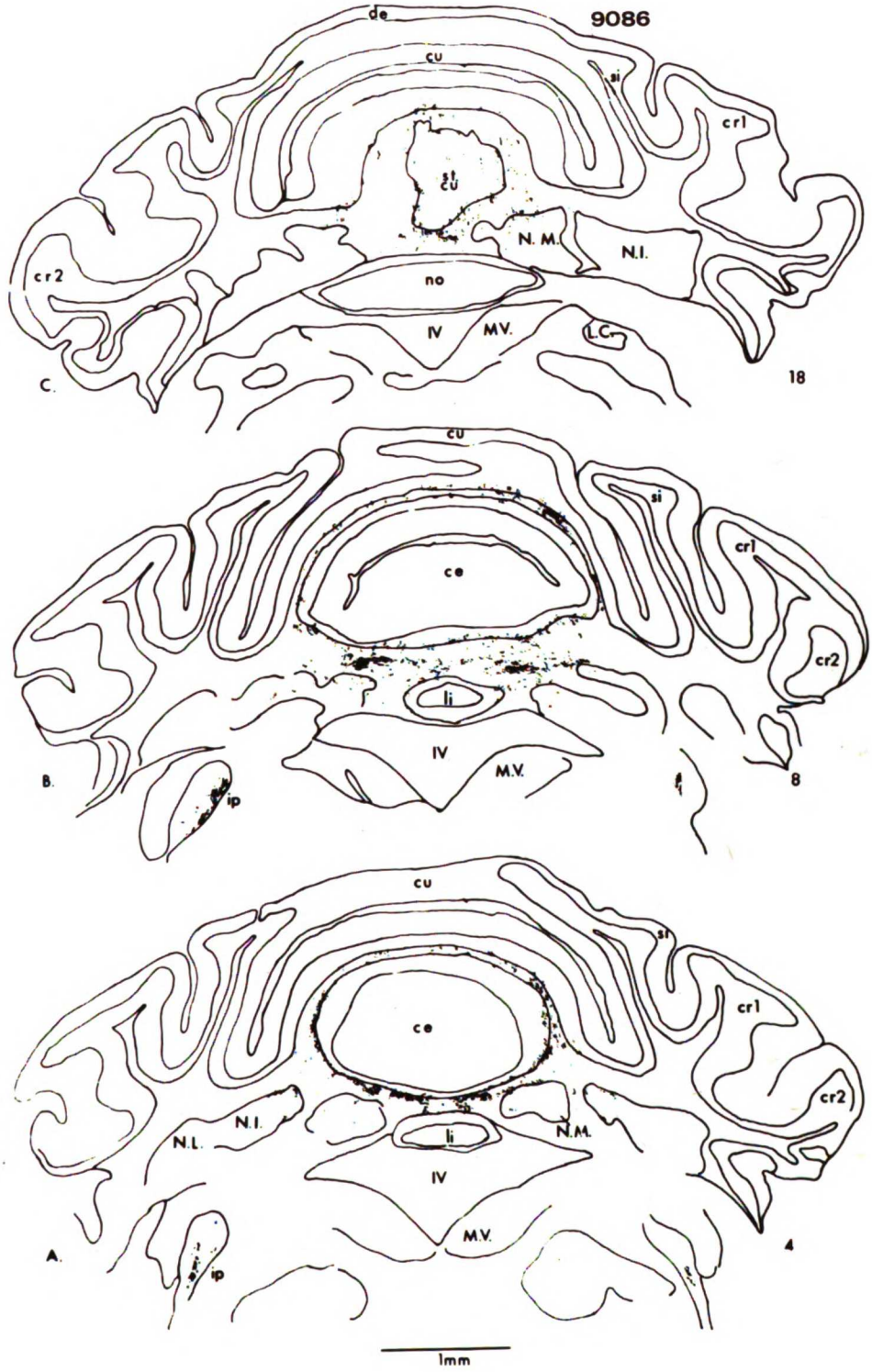
d) Dark-field photomicrograph (from box in C) of a portion of the dao showing ARG grains over cerebello-olivary axons and terminals, and retrogradely-labeled neurons (i.e. arrow) of the olivo-cerebellar projection. The cerebello-olivary and olivo-cerebellar projections are highly reciprocal in both normal and reeler mice. HRP-labeled neuron to the right of arrow in the dark-field photomicrograph is also shown in bright-field in the insert. Scale in d = 175 um; scale in insert = 12 um.

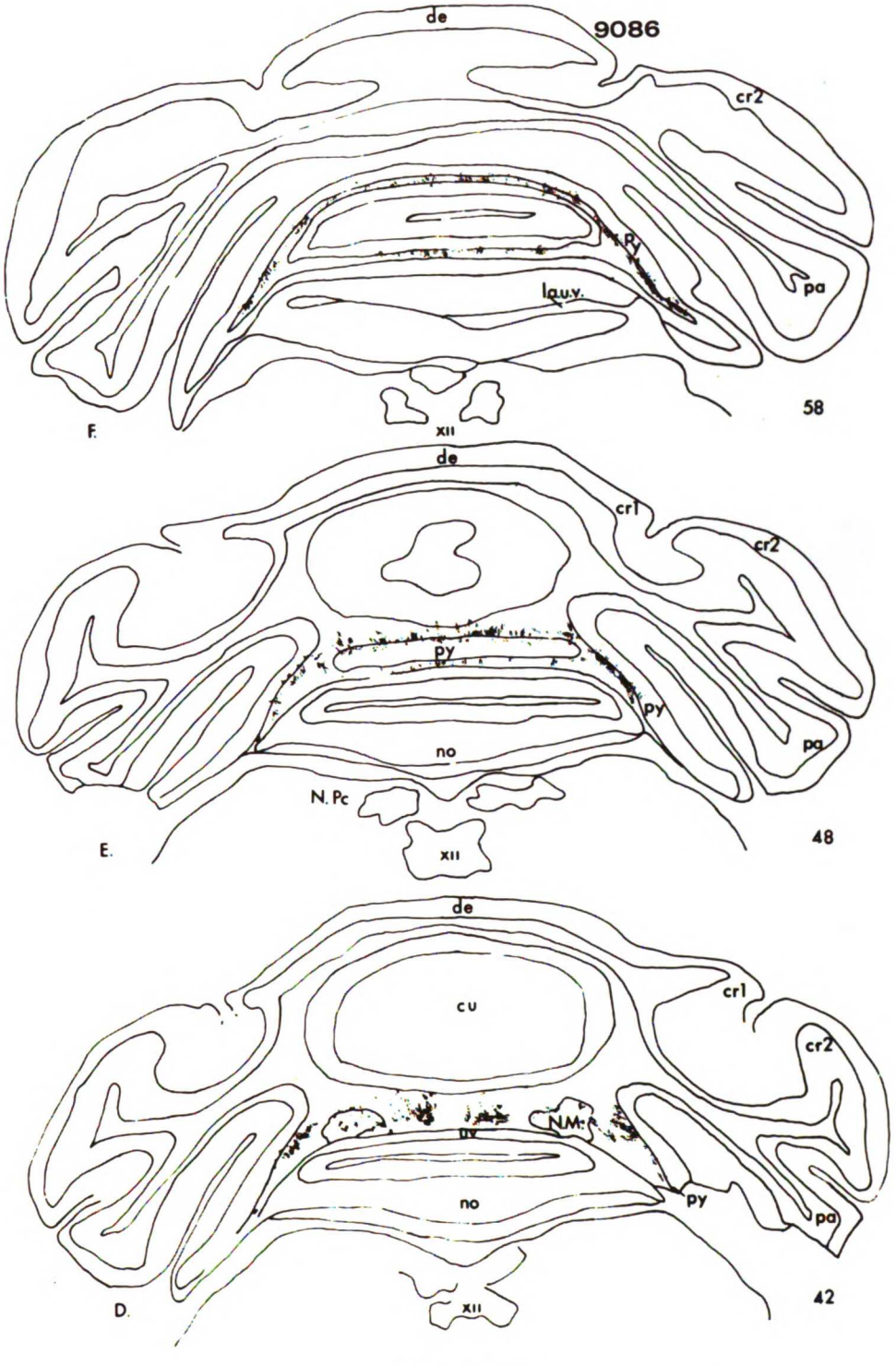

IC
*
22-¼
a
D
R
C
V
IC
*
22-rl/rl
b
1mm

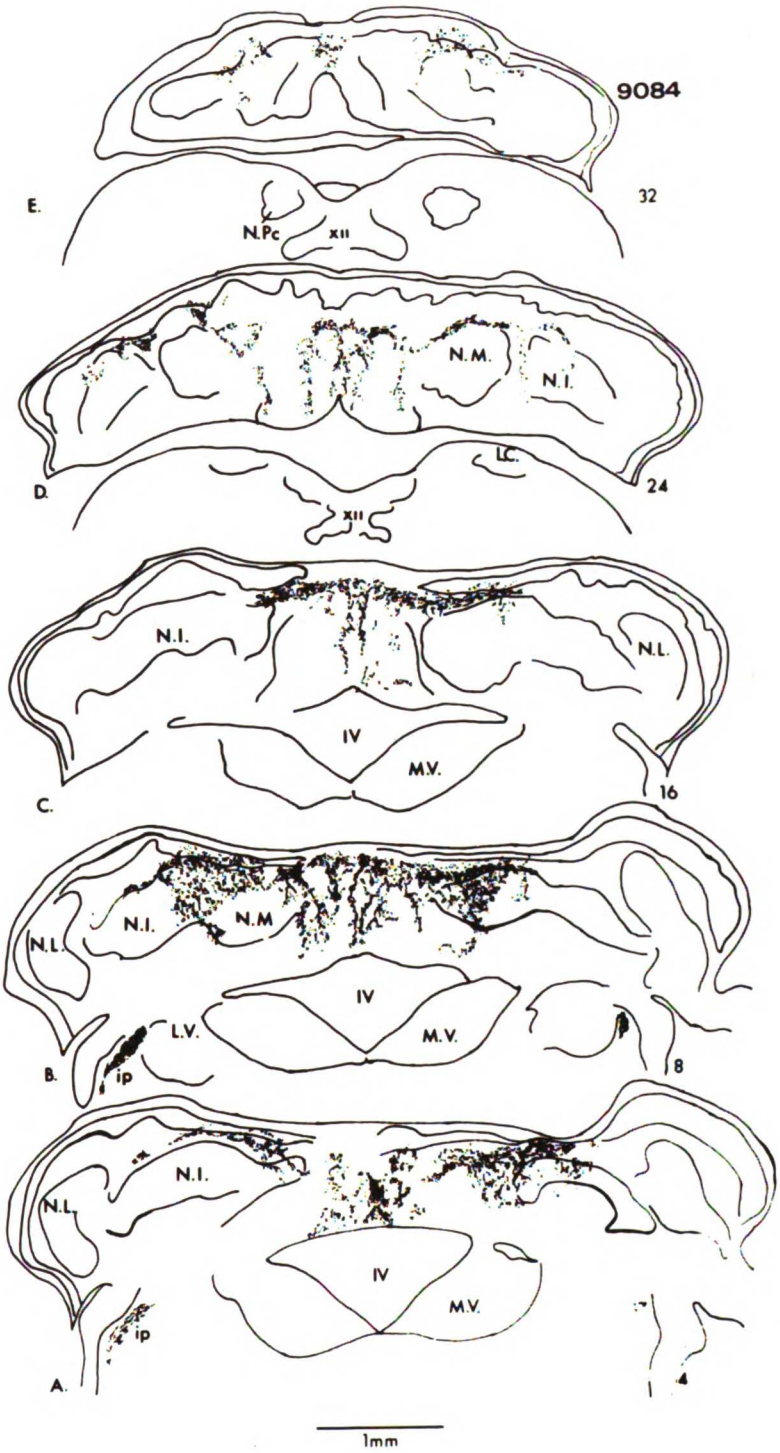

M
P
G
a
+/+
M
P
E
G
L
P+G
b
rl/rl

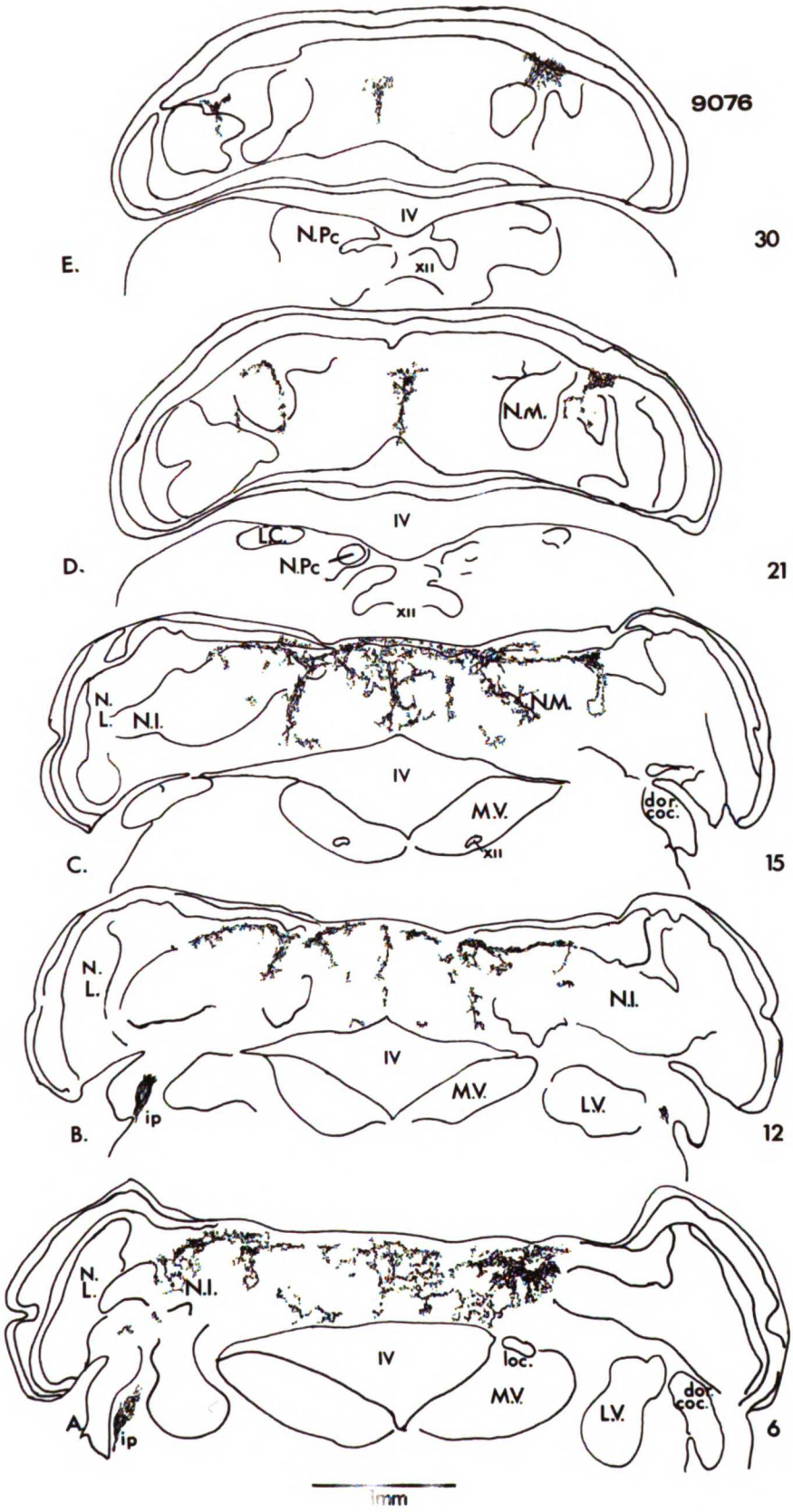

9084
i
35
h
31
g
23
f
19
e
9
d
7
c
5
b
3
a
1
1mm

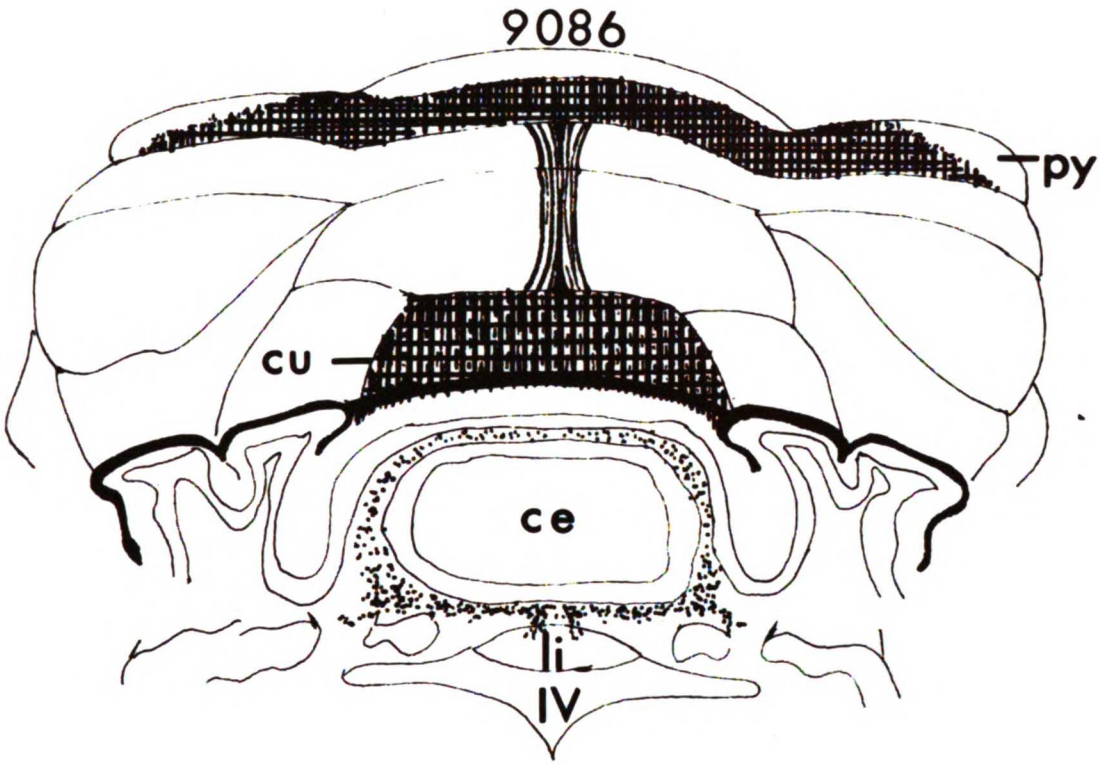

a
b

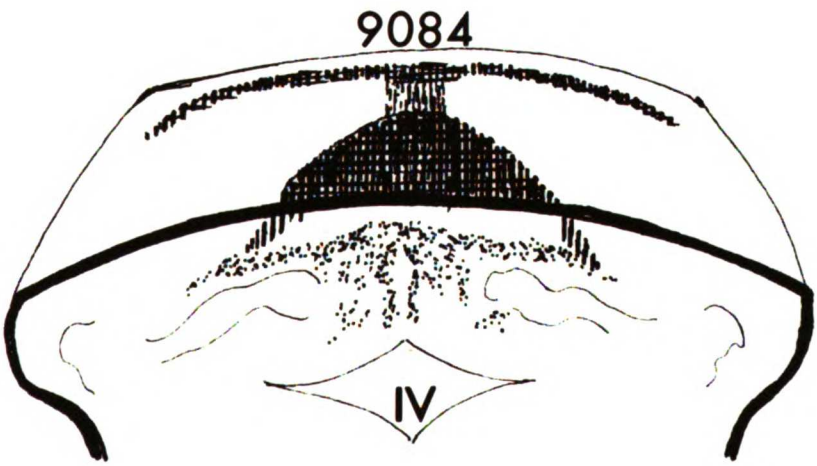

9086
de
cu
si
cr1
cr2
N. M.
N.I.
no
IV
M.V.
L.C.
C.
18
cu
si
cr1
cr2
ce
li
IV
M.V.
B.
ip
8
cu
si
cr1
cr2
ce
N.I.
N.L.
li
N.M.
IV
M.V.
A.
ip
4
1mm


de
9086
cr2
py
pa
lau.v.
58
F.
xii
de
cr1
cr2
py
py
pa
no
N. Pc
48
E.
xii
de
cu
cr1
cr2
N.M.
no
py
pa
42
D.
xii


9084
E.
32
N.Pc
XII
N.M.
N.I.
LC.
D.
24
XII
N.I.
N.L.
IV
M.V.
C.
16
N.I.
N.M
N.L.
IV
L.V.
M.V.
B.
ip
8
N.I.
N.L.
IV
M.V.
ip
A.
4
1mm


9076
E.
30
IV
N.Pc
XII
N.M.
D.
21
IV
LC.
N.Pc
XII
N.
L.
N.I.
N.M.
IV
M.V.
dor.
coc.
XII
C.
15
N.
L.
N.I.
IV
M.V.
L.V.
ip
B.
12
N.
L.
N.I.
IV
loc.
M.V.
L.V.
dor.
coc.
A
ip
6
1mm


9086
py
cu
ce
li
IV


9084
IV

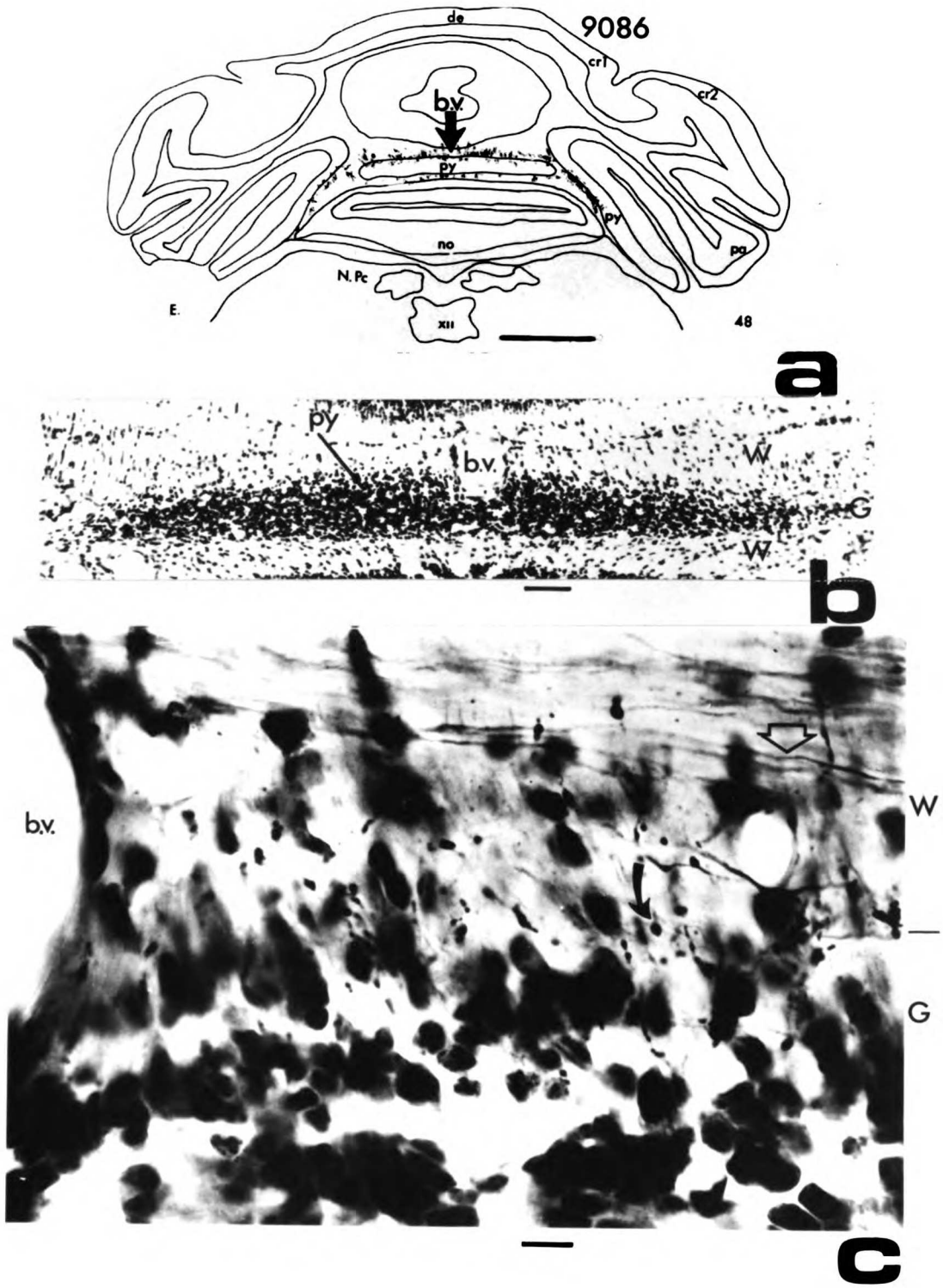
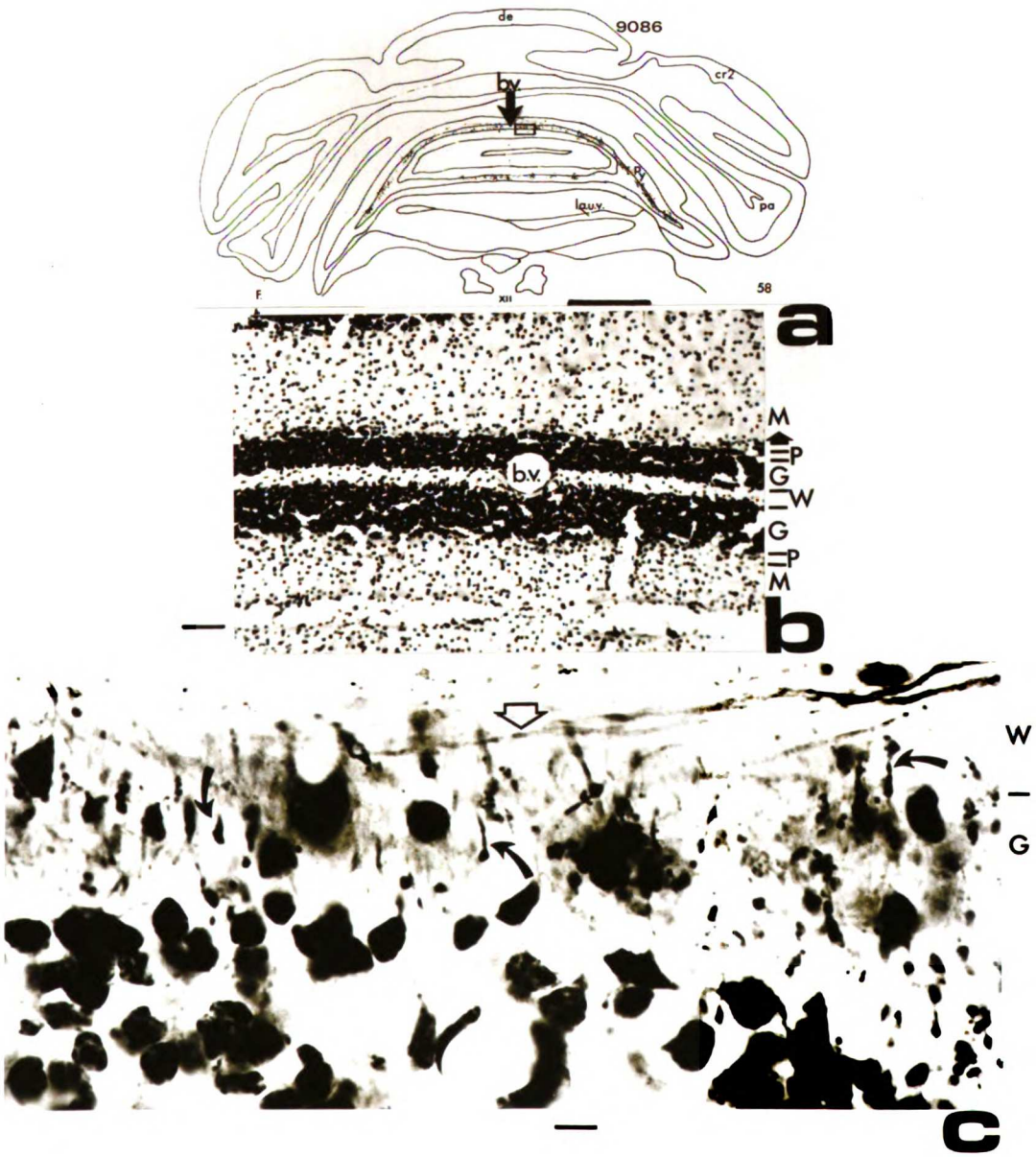
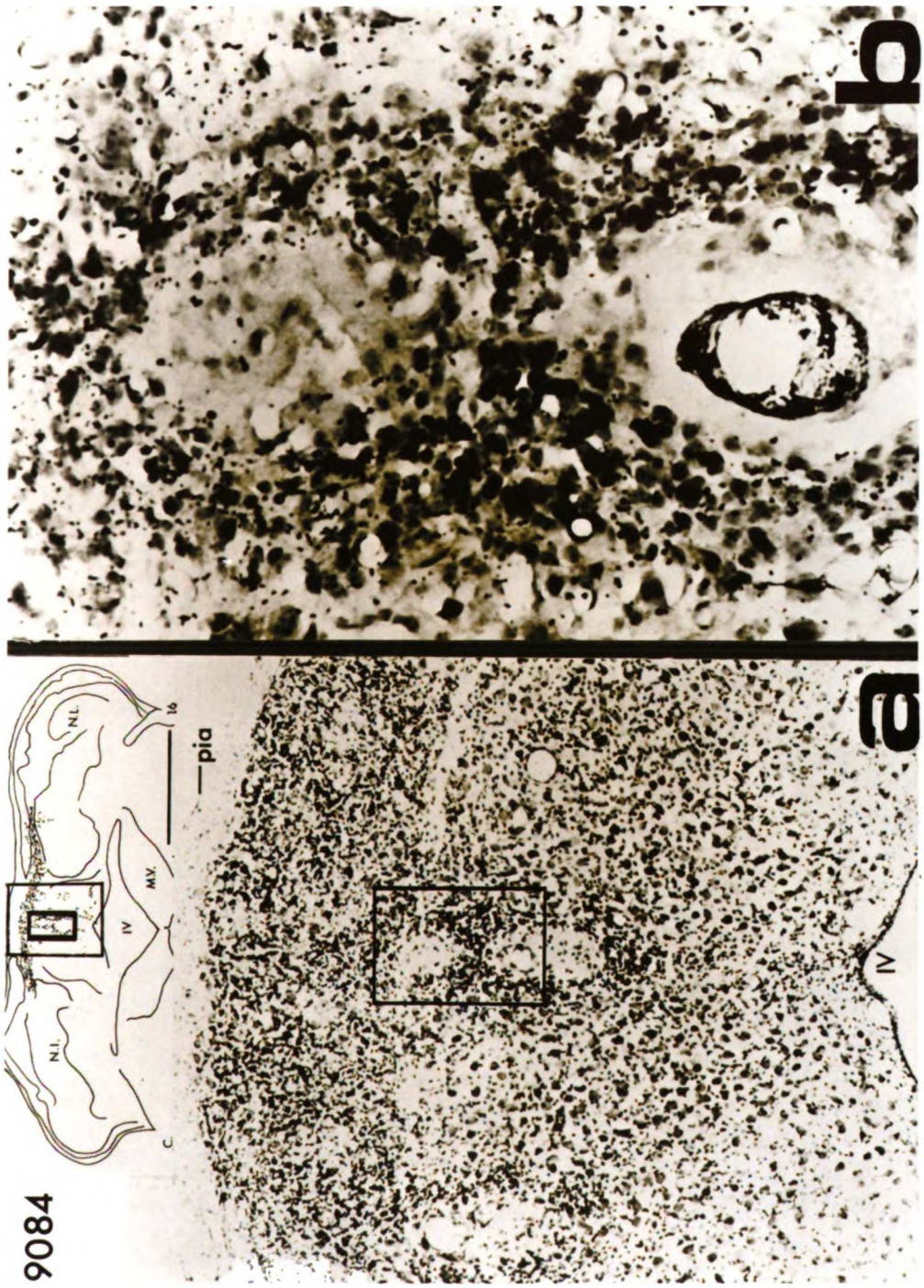
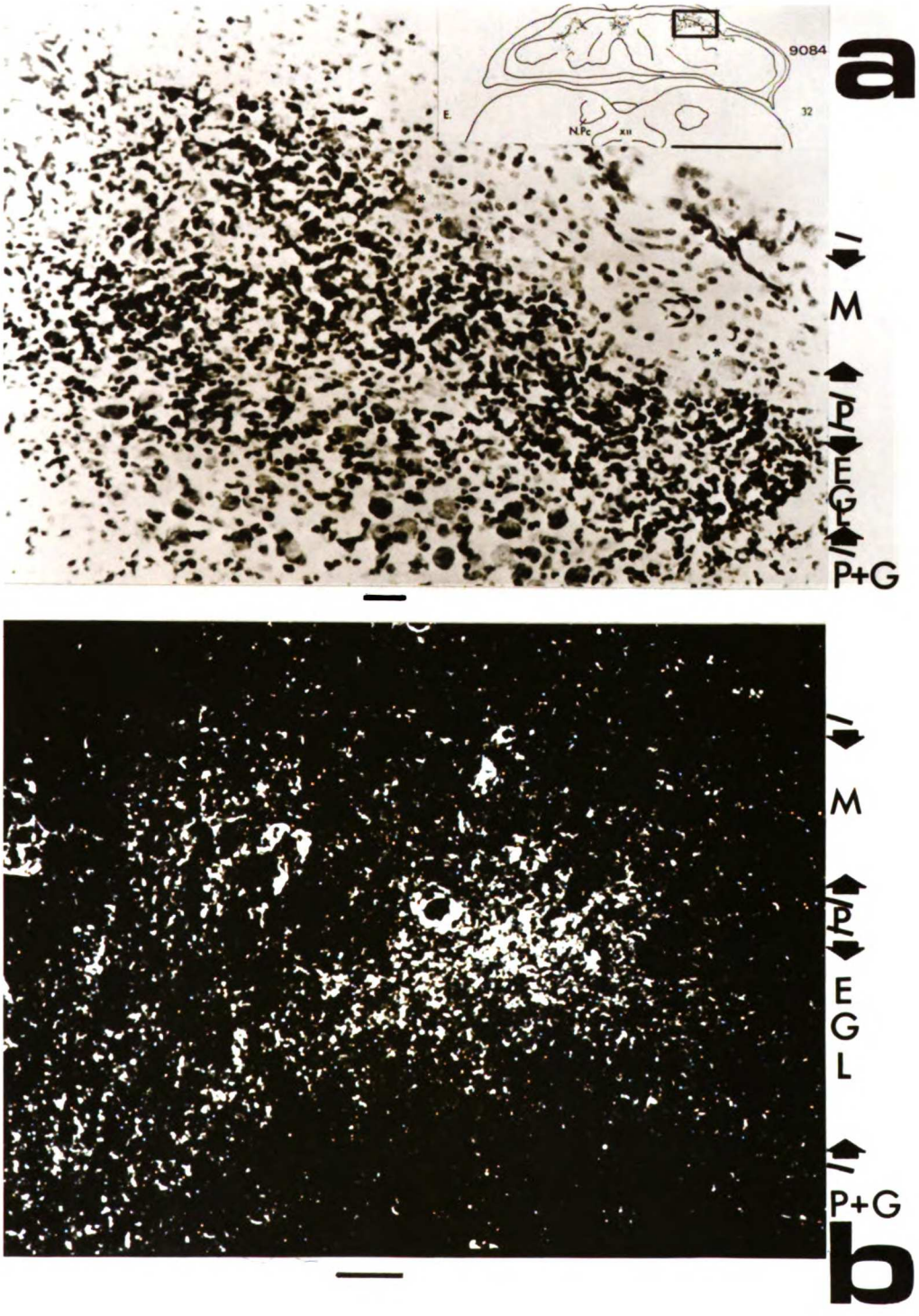
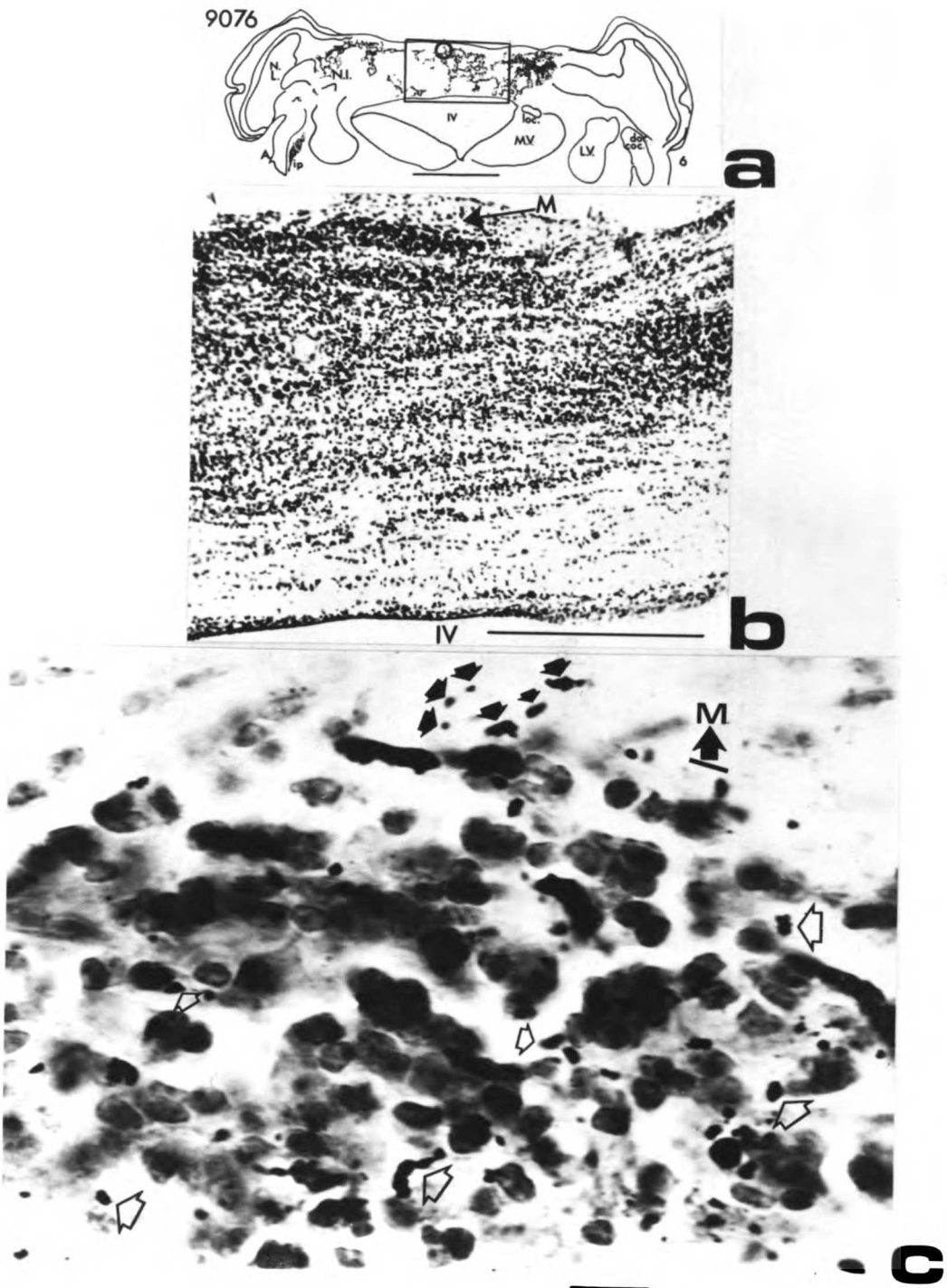

9086
de
cr1
cr2
b.v.
py
py
no
N. Pc
pa
E.
XII
48
a
py
b.v.
W
G
W
b
b.v.
W
G
c

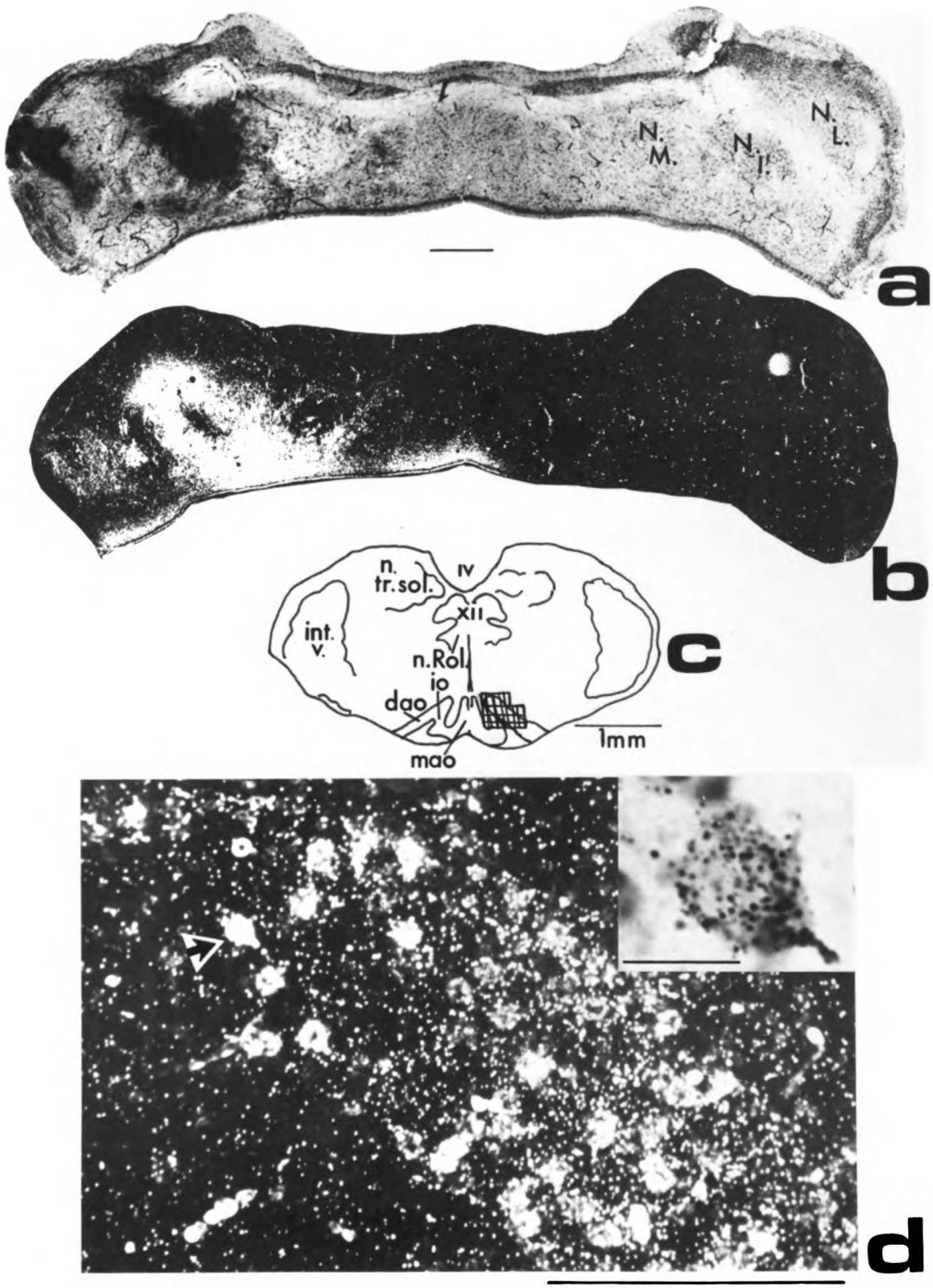
Figure 11




9084
N.L.
N.L.
C
IV
M.V.
Ia
pia
a
IV
b


9084
E.
N.Pc
XII
32
a
M
P
E
G
L
P+G
M
P
E
G
L
P+G
b


9076
N.L.
N.I.
IV
loc.
M.V.
L.V.
dor. coc.
A
ip
6
a
M
IV
b
M
c


N.
M.
N.
L.
a
b
n.
tr.sol.
IV
XII
int.
V.
n.Rol.
io
dao
mao
1mm
c
d

UC San Francisco LIBRARY
UNIVERSITY OF CALIFORNIA

FOR REFERENCE

NOT TO BE TAKEN FROM THE ROOM

CAT. NO. 23 012

PRINTED IN U.S.A.