A QUANTITATIVE CYTOARCHITECTONIC ANALYSIS OF THE INTERPEDUNCLAR COMPLEX OF THE ALBINO RAT

Thesis for the Degree of M. A.

MICHIGAN STATE UNIVERSITY

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1968

ABSTRACT

A QUANTITATIVE CYTOARCHITECTONIC ANALYSIS
OF THE INTERPEDUNCULAR COMPLEX
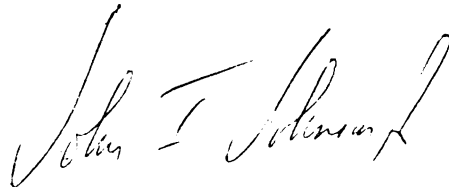OF THE ALBINO RAT

By

William Robert Ives

The interpeduncular complex (IPN), situated on the medio-
ventral surface of the midbrain, is universally present in all
vertebrates including man. Experiments on the functional signif-
icance of this structure, thus far unproductive, have generally
assumed cytoarchitectonic homogeneity although this is contra-
indicated by a number of qualitative studies. Because of the
lack of quantitative data on the structure of the IPN the pre-
sent study has undertaken an analysis of this structure using
measures of cell size and packing density as measures of sub-
nuclear differentiation. Cell size (area) and density were mea-
sured in 4 spatially separate cell groups; pars lateralis (PL),
pars medialis (PM),pars dorsalis parvocellularis (PDP), and pars
dorsalis magnocellularis (PDM). The IPN of 3 normal, Nissl-
stained, albino rats, sectioned in the horizontal, sagittal,
and coronal planes of section served as material. 60 cells
were randomly sampled from each of the 4 subnuclei for each
of the 3 planes of section and their areas determined by plan-
imetry. Density was measured within each subnucleus by count-
ing the numbers of cells contained in several sample volumes
of tissue. PDM had the largest cell size and the lowest den-
sity of any of the 4 subnuclei while PDP had the smallest cell
size and the highest density. PL and PM were intermediate in

both cell size and packing density and were not significantly different from one another. The differences were tested by a 2 way analysis of variance and Newman-Keuls tests at the .01 level of confidence. The absence of any quantiative differences between PL and PM casts doubt upon earlier distinctions based on qualitative studies of these 2 subnuclei. The distinction between PL and PM is a spatial one and not cytoarchitectural while PDM and PDP present both spatial and cytoarchitectural differences. These quantitative differences in cytoarchitecture may imply functional differentiation within the IPN which may help to explain the variety of behavioral deficits observed after gross lesions of this structure.

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A THESIS

ACKNOWLEDGEMENTS

The author wishes to express his gratitude to the following professors whose guidance during my education has been invaluable; Dr. G. I. Hatton who first aroused my interest in the nervous system, Dr. T. W. Jenkins who provided me with an invaluable introduction to neuroanatomy, Dr. J. I. Johnson who furthered my interest in the nervous system, and Dr. M. Balaban who introduced me to developmental neuroanatomy.

To my wife, whose efforts in reaching this stage in my education have been far greater than mine, I dedicate this thesis.

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ABBREVIATIONS USED IN PLATES 1-6

CIC.. commissure of the inferior colliculus

COL. SUP. .... superior colliculus

COR. PIN. .... pineal body

DBC.. decussation of the superior cerebellar peduncle

DEC. TEG. DORS. .... dorsal tegmental decussation

DEC. TEG. VENT. .... ventral tegmental decussation

FLM.. medial longitudinal fasciculus

GR. CENT. .... central gray

GR. PONT. .... pontine gray

LEM. MED. .... medial lemniscus

N. INTERPED. PARS DORSALIS
PARS MAGNO................................. interpeduncular nucleus pars dorsalis pars magnocellularis

N. INTERPED. PARS DORSALIS
PARS PARVO. .... interpeduncular nucleus pars dorsalis pars parvocellularis

N. INTERPED. PARS LATERALIS................ interpeduncular nucleus pars lateralis

N. INTERPED. PARS MED. .... interpeduncular nucleus pars medialis

N. LIN. .... linear nucleus

N. OCULO. .... oculomotor nucleus (III)

ABBREVIATIONS USED IN PLATES 1-6
(continued)

N. RUB. .... red nucleus

N. TROCH. .... trochlear nucleus (IV)

PED. CER. .... cerebral peduncle

PED. MAM. .... mammillary peduncle

RAD. MEY. .... radiations of Meynert

SUB. NIG. .... substantia nigra

TR. CORT. SP. .... corticospinal tract

TR. HAB. PED. .... habenulopeduncular tract

III... oculomotor nerve

INTRODUCTION

General Anatomy

Because of the juxtaposition of the interpeduncular nuc-
leu₋ (IPN) and the posterior perforated substance Forel (1877)
first termed this nucleus das Ganglion der lamina perforata
posterior. Gudden (1881) later termed this cell group das
Ganglion interpedunculare because of its position between the
cerebral peduncles in mammals. Subdivisions of the nucleus
were not described by these early investigators although
Cajal (1952) described a stratification of the nucleus into
two layers in the rabbit. The outer layer (i.e. dorsal) con-
sisted of large multipolar cells (peripheral zone) while the
inner (i.e. ventral) layer was described as consisting of
small to medium sized cells (plexiform layer). These early
descriptions for mammals were accepted until the work of Huber,
Crosby,Woodburne,Gillian,Brown, and Tamthai (1943) in which it
was noted that the IPN of a number of mammals (mink,rabbit,cat
dog,sheep,pig,rat, and armadillo) appeared to consist of two
spatially and cytoarchitectonically distinct subnuclei; a large
celled lateral subnucleus and a smaller celled medial subnuc-
leus. According to the latter authors the IPN reached its
greatest development in the rabbit where it consisted of three

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subnuclei; a compact dorsal portion, a large celled lateral portion, and a small celled medial portion.

Work on the submammalian IPN has been done by Herrick (1917) in the mudpuppy, Necturus, and by Jansen (1930) in the hagfish, Myxine. In the hagfish the nucleus consists of a rostrally located mesencephalic division which consists of large multipolar cells and a caudally located rhombencephalic division which contains large and small spindle shaped cells. A similiar subdivision of the nucleus into rostral and caudal components has been described for Necturus.

A schematic drawing of the generalized fiber connections of the vertebrate IPN is shown in Figure 1. The connections are general in the sense that all vertebrates minimally have them. The IPN receives a large contingent of fibers from the habenulae as the habenulopeduncular tract (HPT) which contains fibers from both the lateral and medial habenular nuclei (Kappers,Huber, and Crosby,1936). The IPN is also afferently connected with the mammillary bodies via the mammillopeduncular tract. The efferent outflow of the nucleus is directed to the dorsal tegmental nucleus of the central gray via the pedunculotegmental tract. The dorsal tegmental nucleus contributes its fibers to the dorsal longitudinal fasciculus which synapses with several visceral efferent cranial nerve nuclei (VII,IX,X,XI)(Zeman and Innes,1963).

Several general features of the vertebrate IPN need to be noted. That the nucleus is divided into at least two subnuclei in many vertebrates is quite evident. In Myxine and Necturus
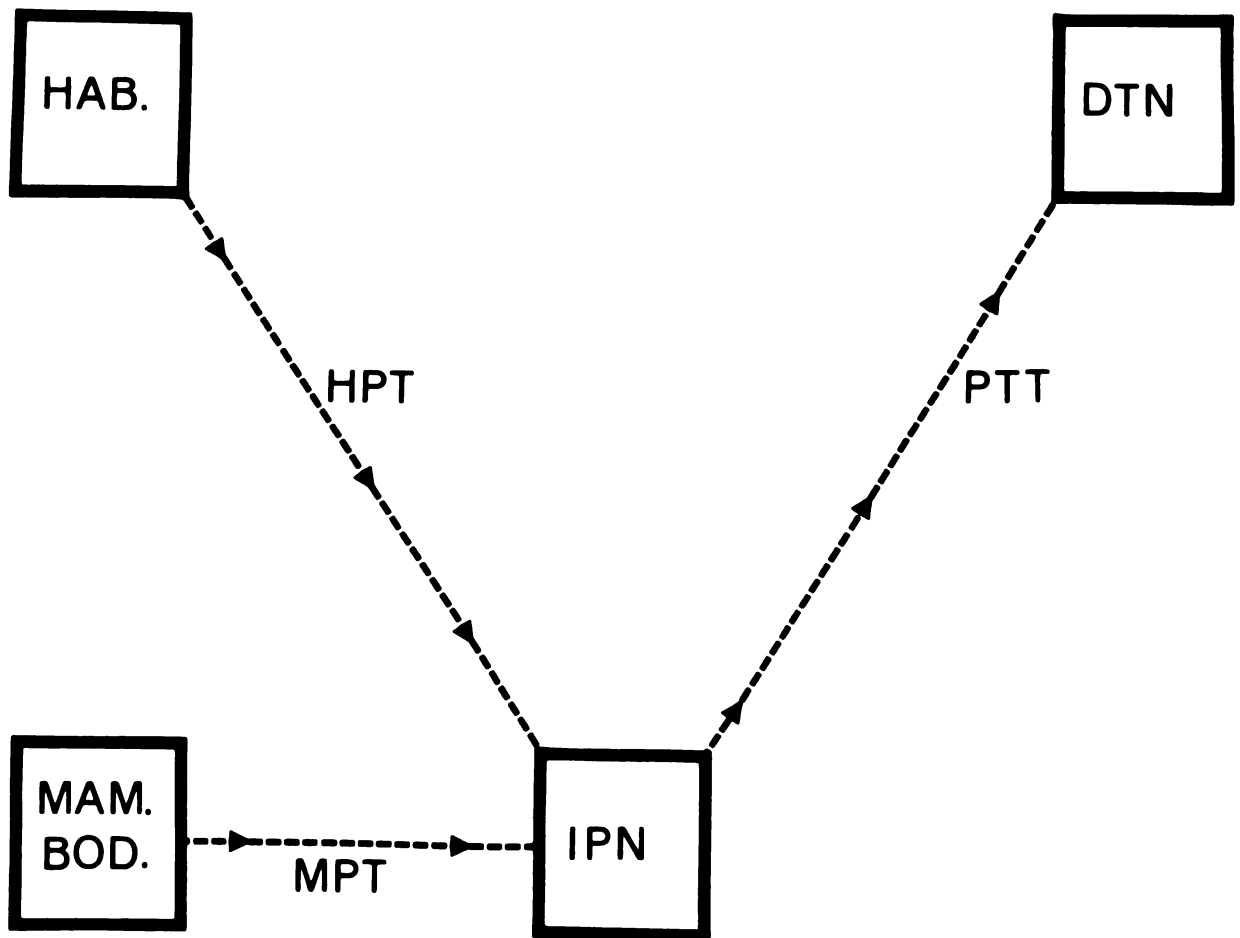
Figure 1. A schematic representation of the generalized
fiber connections of the vertebrate IPN. Arrows indicate
the direction of conduction of nerve impulses. HAB.= habenula,
DTN= dorsal tegmental nucleus, MAM. BOD.= mammillary body,
IPN= interpeduncular nucleus, HPT= habenulopeduncular tract,
PTT= pedunculotegmental tract, MPT= mammillopeduncular tract.

these divisions are rostral and caudal and as we ascend the phyletic scale they become medial and lateral. This shift in position of the subnuclei follows the shift in the position of the habenular subnuclei from a rostral-caudal subdivision to a medial-lateral one. The HPT also has been described as consisting of contributions from both the habenular subnuclei (Kappers,Huber, and Crosby,1936).

The general existence of a divided input (HPT) to a nucleus that also exhibits divisions indicates that there may be a certain specificity of projection upon the IPN. A given habenular subnucleus might project to a given IPN subnucleus. One might also hypothesize that there exists some specificity of output through the pedunculotegmental tract although there is no experimental evidence to corroborate either of these points.

The Problem

A recent study by Berman and Bowers (1967) has renewed interest in the cytoarchitectonics of the IPN. They described five subnuclei of the cat IPN which differ from one another on the basis of cell size, staining characteristics, and packing density. These subnuclei are the posterior, apical, central, paramedian, and intrafascicular (see Figure 2).

"The posterior nucleus comprises an outer division which forms a cellular cup surrounding the caudal part of the complex, and a bifurcated inner division separated from the outer by a cell sparse zone. The apical nucleus is embedded in the dorsal aspect of the outer division. The paramedian nuclei are paired columns of cells on either side of, but clearly separated from the inner division of the posterior nucleus. The central nucleus is an elongated structure rostral to, and partly fused with the inner
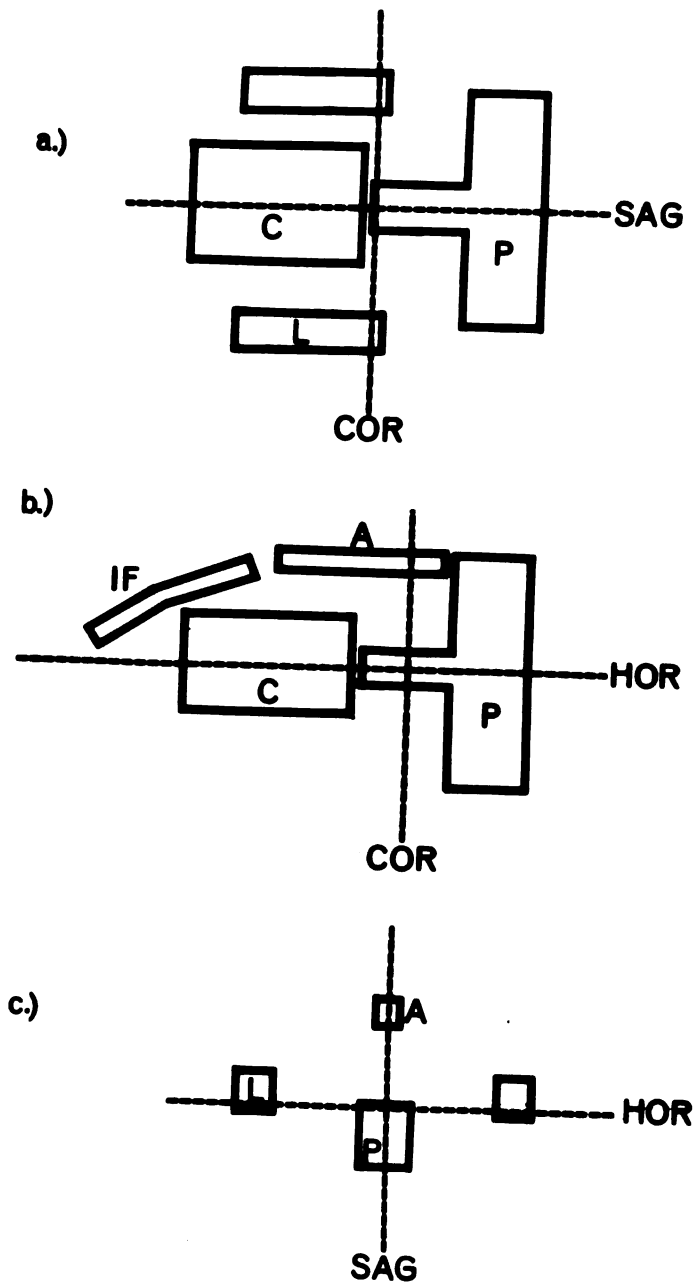
Figure 2. A schematic representation of the subnuclei of the cat IFN after Berman and Bowers (1967). In a, b, and c the nucleus is shown in the horizontal, sagittal, and coronal planes of section, respectively. A= apical, C= central, P= posterior, L=.paramedian, and IF= intrafascicular.

division of the posterior nucleus. At the rostral end of
the complex a fifth structure, the intrafascicular nucleus,
forms a cap over the central nucleus, but extends beyond
it as a narrow wedge in the median raphe between the two
habenulopeduncular tracts." Berman and Bowers, 1967, p.213.

These authors stated that it was not possible to tell whether
the intrafascicular nucleus was a part of the complex or a
part of the central linear nucleus with which it merged.

My own work in the rat (see plates and Figure 3) has
indicated the presence of four subnuclei which differ from
one another on the basis of location and/or cell size. These
are the pars dorsalis magnocellularis (PDM), pars dorsalis
parvocellularis (PDP), pars lateralis, and pars medialis. PDM
consists of large cells which lie in the dorsocaudal third
of the complex. PDP, located immediately rostral to PDM, con-
sists of small spherical cells with lightly staining nucleoli.
In sections through the red nucleus PDP turns ventral and
comes to occupy the medial portion of the nucleus as well. PL
consists of two well defined groups of cells which lie at the
lateral extremities of the nucleus. PM is located just ventral
to PDM and PDP and medial to PL. It is separated from all the
above subnuclei by areas of lower cell density.

The purpose of the present study was to quantitatively
assess the nature of the aforementioned IPN subnuclei through
measurement of cell size and packing density within the sub-
nuclei. The values obtained for each of these measures was
then compared with similiar measurements for the other subnuc-
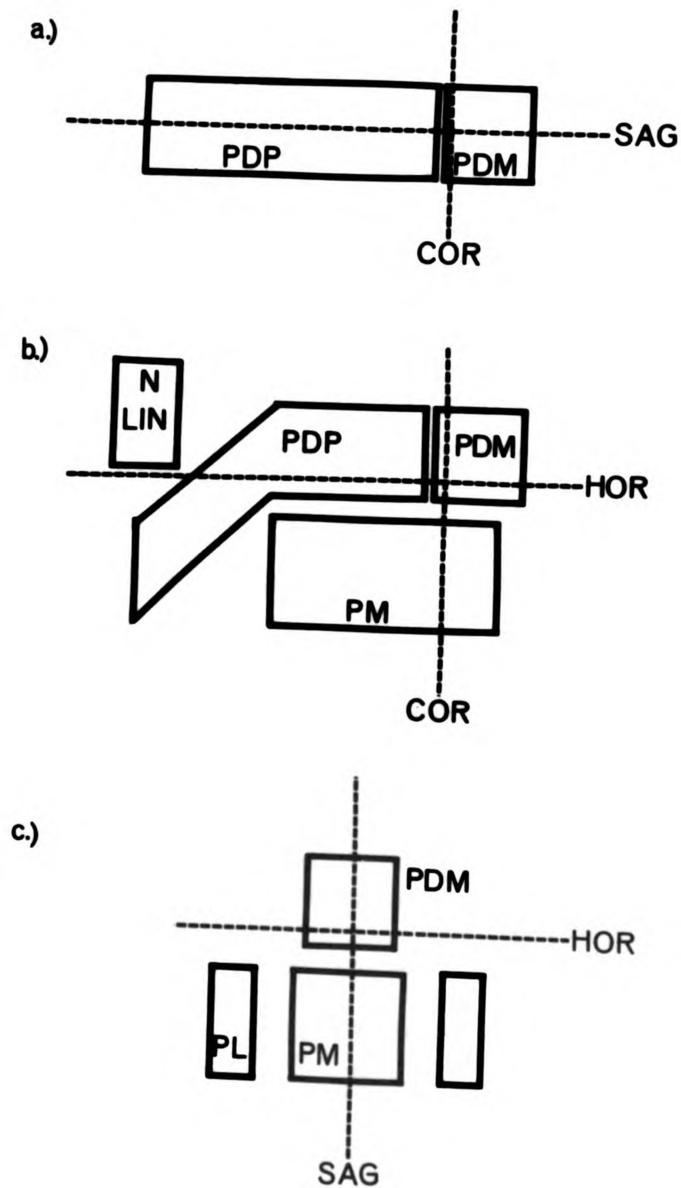lei through the use of appropriate statistical tests.

Figure 3. A schematic representation of the subnuclei of the
rat IPN. In a, b, and c the nucleus is shown in the hor-
izontal, sagittal, and coronal planes of section, respective-
ly. PL= pars lateralis, PM= pars medialis, PDP= pars dorsalis
parvocellularis, PDM= pars dorsalis magnocellularis, and
N LIN= linear nucleus.

METHOD

Specimens

Three albino rats were perfused intracardially with .87%
saline solution followed by a .87%-10% formalin mixture and
their brains removed and photographed. The brains were embedded
in celloidin and sectioned a 25 microns in three planes of sec-
tion, horizontal, coronal, and sagittal. Alternate sections
were stained with iron hematoxylin for fibers (Sanides
Haidenhain method) and thionin for cells bodies (Nissl method).
Refer to appendix A for the details of the histological pro-
cedure.

Cell Size Measurements

Cells of the four subnuclei were magnified 2800 times via
a camera lucida attachment on a Zeiss microscope. Ten cells
were randomly sampled from each subnucleus for six sections
through the nucleus such that a total of 60 cells were sam-
pled from each subnucleus for each plane of section. Only
cells whose nucleoli were visible were sampled. The areas of
the cells were determined by planimetry with a Keuffel and
Esser compensating polar planimeter. Three area measurements
were taken for each cell and the mean of these measurements
used as an estimate of the true area.

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Packing Density Measurements

Densities were measured by counting the number of cells contained in a grid placed over the nucleus. The grid was a Whipple-Hauser disc which was placed directly in the ocular of the microscope. Knowing the thickness of the section and the area of the grid, volume could be determined by multiplying thickness times area. Dividing the number of cells in a sample volume by the sample volume gives the density of cells within a given subnucleus.

Thickness of a given section was measured in the following manner. The slide was placed on the microscope stage and the fine focus dial adjusted until the first cell came into focus. At this point the number on the focus vernier was read. The fine focus knob was then turned until the thickness of the section had been focused through and the last cell was in focus. The vernier was then read again and one reading subtracted from the other. The resulting number was the thickness of the section in microns. Thickness was measured three times for a given section and the mean of the measurements used as an estimate of the true thickness of the section. In order to avoid errors due to differential thickness within a given section measurements were only conducted upon the IPN and on no other portion of the section.

RESULTS

Descriptive Anatomy

Plates 1-6 show the nuclear configuration of the IPN
and surrounding midbrain structures in a caudal to rostral
sequence. In caudal sections through the midbrain (Plates 1&2)
the IPN shows a division into three subnuclei, pars lateralis
(PL), pars medialis (PM), and pars dorsalis magnocellularis
(PDM). PL and PM are particularly well developed at this level
in terms of size and in fiber preparations fibers can be seen
issuing from the lateral borders of PM. The cells of PDM, while
being sharply segregated from the rest of the nucleus, appear
to trail off into the overlying nucleus centralis superior.

In middle and rostral sections through the midbrain
(Plates 3-6), PDM can be seen to be replaced by pars dorsalis
parvocellularis (PDP) on the dorsal aspect of the nucleus. In
the most rostral sections (Plates 5&6) PDP turns ventral to
replace PL and PM.

Cell Size

The results of the cell area measurements are presented
in Figures 4-8. On the abscissa of each figure is plotted
the value for cellular area in microns^2 X 10, while the ordinate
plots the frequency of occurrence of a given cell size. A
two-way analysis of variance revealed a significant difference
in mean cell areas between the three planes of section (F=33.33,

2/708 df, p< .01), a significant difference in mean cell areas between divisions (F=236.80, 3/708 df, p< .01) and a significant interaction (F=4.25, 6/708 df, p <.01). The results of this analysis are presented in tabular form in table 1. ω^2 (decimal fraction of the variance accounted for by the treatments) for planes was .04, for divisions .46, and for the interaction .01. Comparisons between all pairs of means by the method of Newman and Keuls (Table 2) revealed that the mean cell sizes within all subnuclei were significantly different from one another with five exceptions: PDPsag=PDPcor, PDPsag=PDPhor, PMcor=PLcor, PMsag=PLsag, PMhor= PLhor.

In order to ascertain whether or not the differences in cell size between planes (within a given subnucleus) could be attributed to shrinkage differences or differences in the actual orientation of the cells, ratios were computed. Three ratios for each plane were computed, PL/PM, PDM/PM, and PDP/PM. The values for these ratios are presented in table 3. It can easily be seen that the ratios remain essentially constant across planes which at least strengthens the argument for the cause of the differences being shrinkage. No statistical tests were conducted on these data.

Density

The results of the density measurements are presented in figures 9-11. The abscissa of each histogram plots the density as cells/micron^3 X 10^{-4} and the ordinate plots the frequency of occurrence of a given density. A two-way analysis

Table 1. Summary of the results of the analysis of variance conducted on the cell size data. $*$ = $p < .01$

SOURCE	SUM OF SQUARES	df	MEAN SQUARE	F	ω^2
Planes of section	282.69	2	141.34	33.33*	.04
Subnuclei	3,012.17	3	1,004.05	236.80*	.46
Interaction	120.07	6	20.01	4.71*	.01
Error	3,011.63	708	4.25	------	---
Total	6,426.56	719	--------	------	---

Table 2. Summary of the results of the Newman-Keuls tests conducted on the cell size data. Lines beneath two subnuclei indicate that they are not different from one another at the .01 level of confidence.
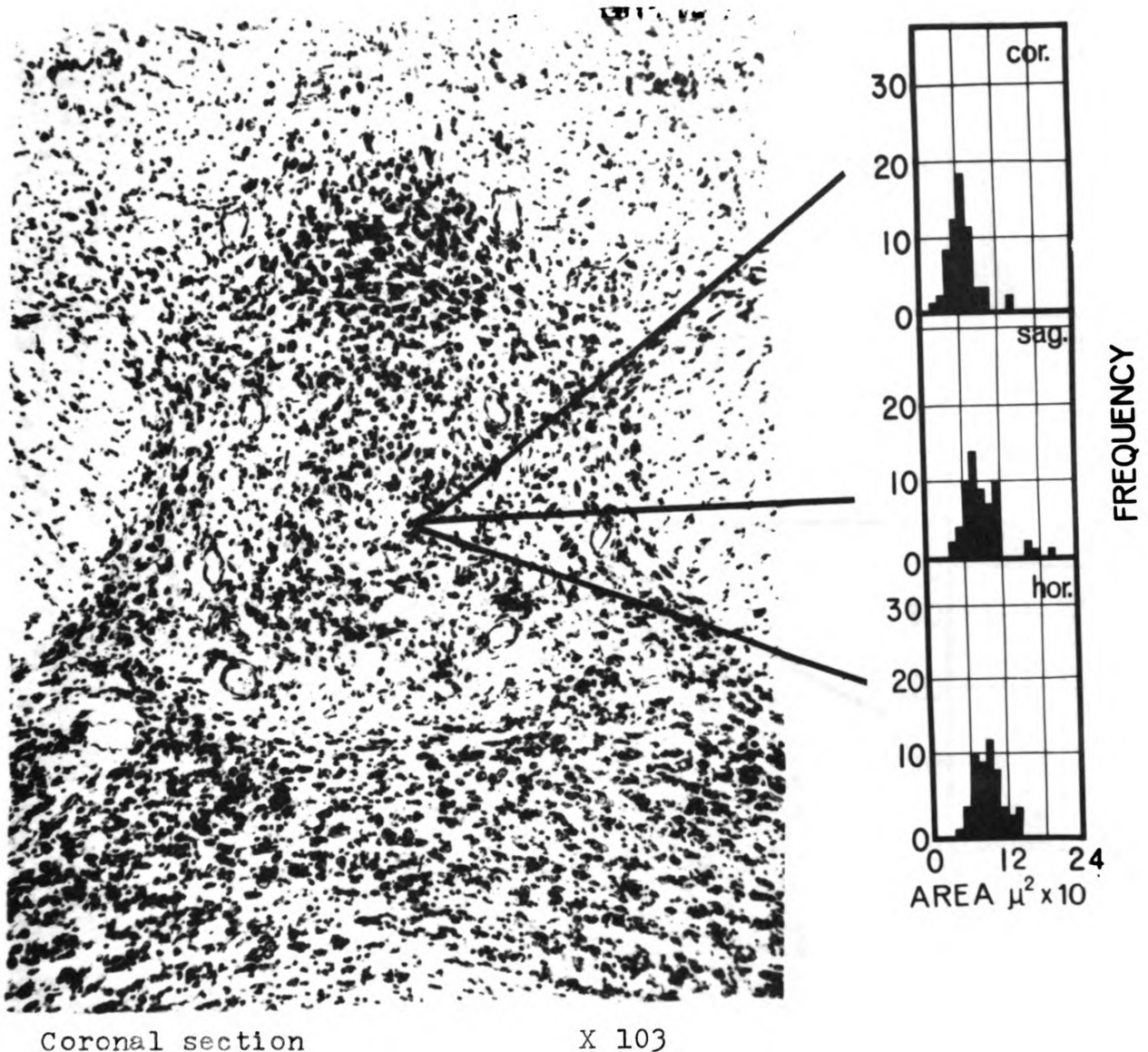
PDP_c PDP_s PDP_h PM_c PL_c PM_s PM_h PL_s PL_h PDM_c PDM_s PDM_h

\#

Table 3. Cell size ratios computed as the ratio between a given subnucleus and PM.

CORONAL

PL/PM=1.00

PDM/PM=1.63

PDP/PM=.53

SAGITTAL

PL/PM=1.09

PDM/PM=1.34

PDP/PM=.52

HORIZONTAL

PL/PM=1.05

PDM/PM=1.44

PDP/PM=.57

Coronal section   X 103

Figure 4. Results of cell area measurements for PM.
In this and the following figures the results are
based on a random sample of 60 cells from each of the
three planes of section.The means and standard devi-
ations for the respective planes are 69 microns^2 $\pm$ 20
for the coronal plane, 85 microns^2 $\pm$ 24 for the sag-
ittal plane, and 89 microns^2 $\pm$ 25 for the horizontal
plane. Comparisons of the means by the Newman-Keuls
method revealed $PM_c \neq PM_s$ and $PM_c \neq PM_h$ at the .01 level
of confidence. The section is approximately at the level
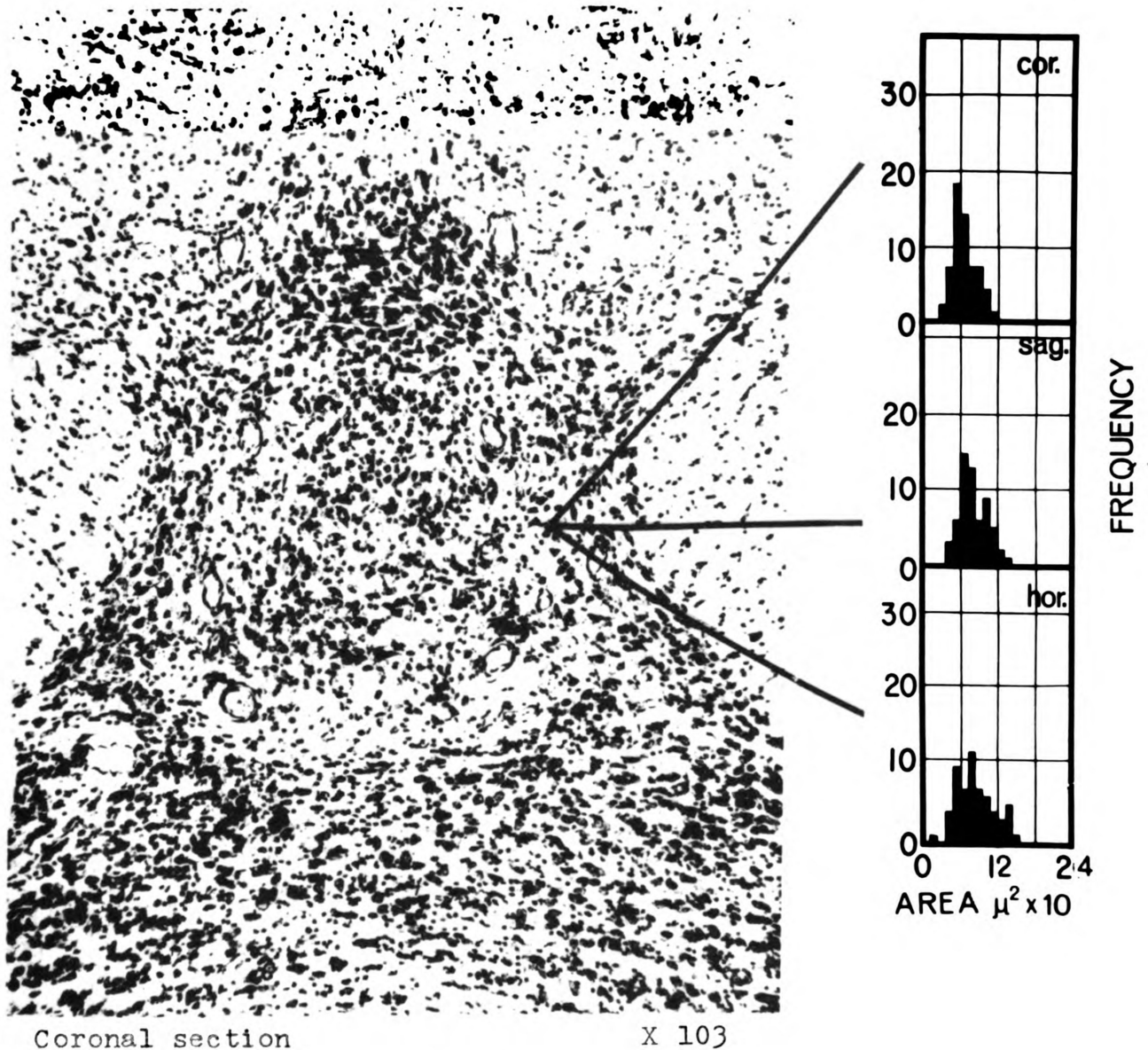of plate 2.

Coronal section   X 103

Figure 5. Results of cell area measurements for PL.
The means and standard deviations for the respective
planes of section are 69 microns^2 ± 22 for the coronal
plane, 93 microns^2 ± 29 for the sagittal plane, and 94
microns^2 ± 26 for the horizontal plane. Comparisons be-
tween the means by the Newman-Keuls method revealed
$PL_c \neq PL_h$, $PL_c \neq PL_s$, and $PL_s = PL_h$ at the .01 level of
confidence. The section is approximately at the level of
Plate 2.

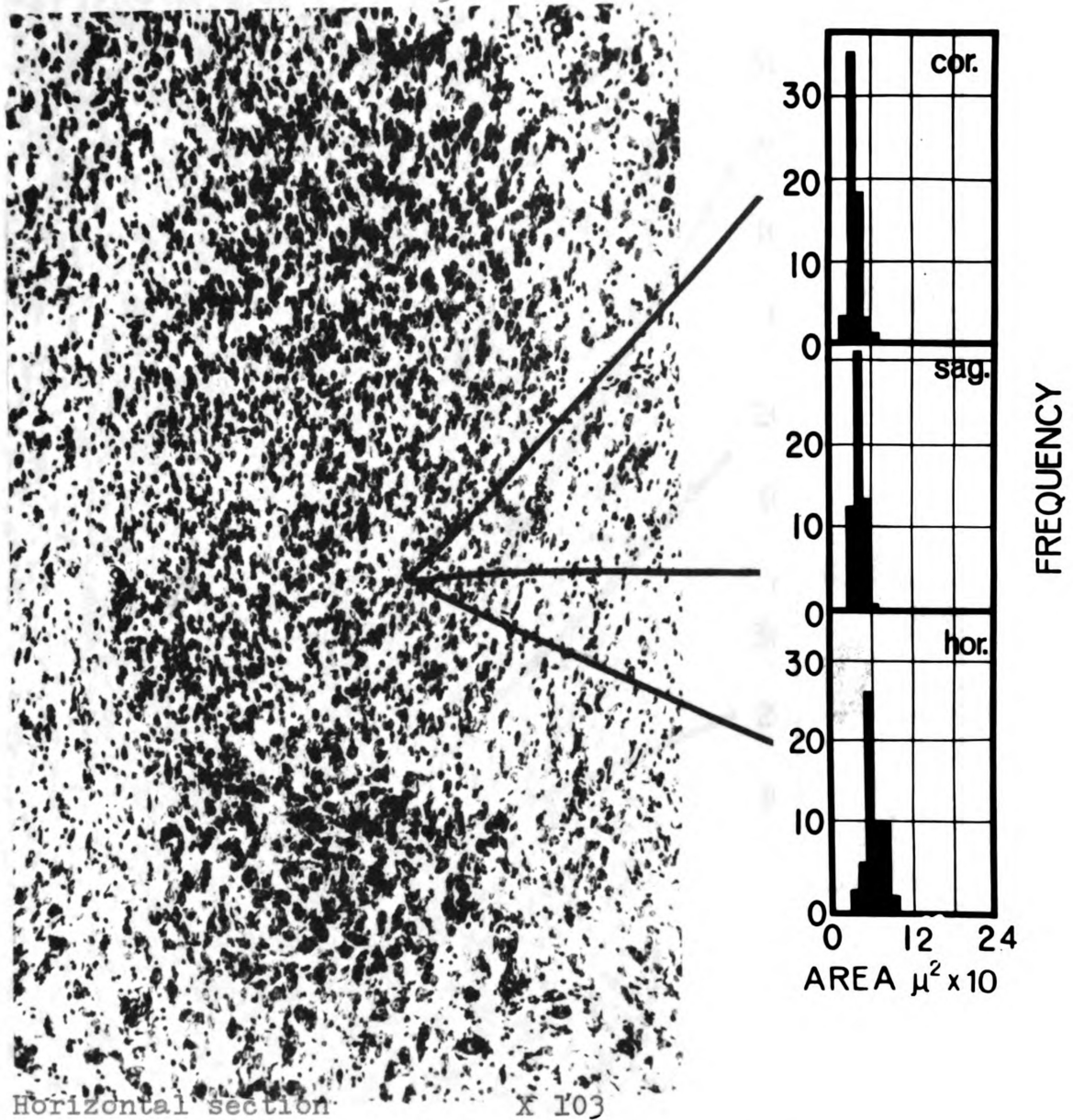Figure 6. Results of cell area measurements for PDP. The means and standard deviations for the respective planes of section are 36 microns^2 ± 8 for the coronal plane, 45 microns^2 ± 9 for the sagittal plane, and 51 microns^2 ± 14 for the horizontal plane. Comparisons between the means by the Newman-Keuls method revealed $PDP_c \neq PDP_h$ at the .01 level of confidence.
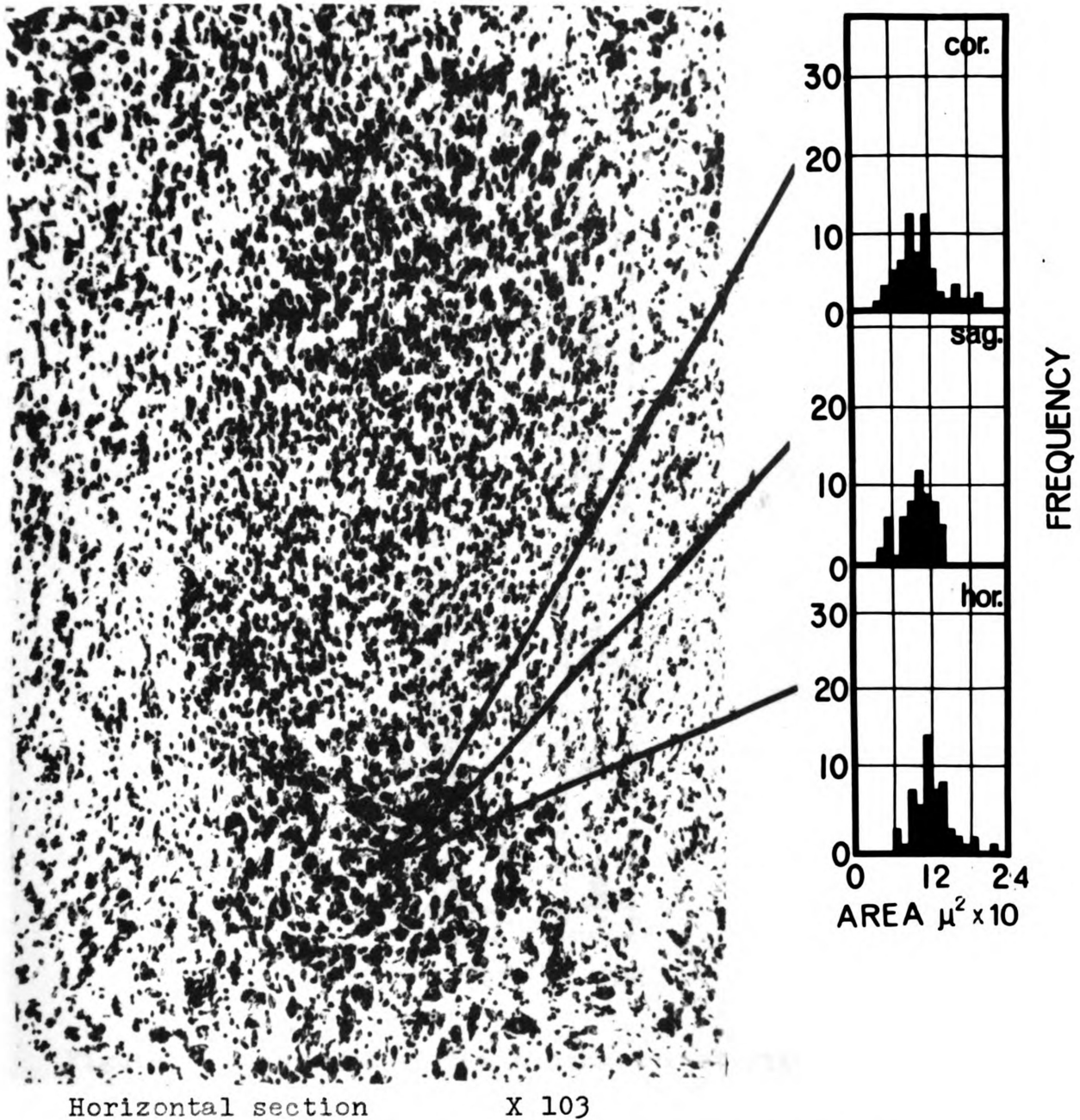
Horizontal section   X 103

Figure 7. Results of cell area measurements for PDM.
The means and standard deviations for the respective
planes are 112 microns^2 ± 36 for the coronal plane,
113 microns^2 ± 33 for the sagittal plane, and 129
microns^2 ± 36 for the horizontal plane. Comparisons
between the means by the Newman-Keuls method revealed
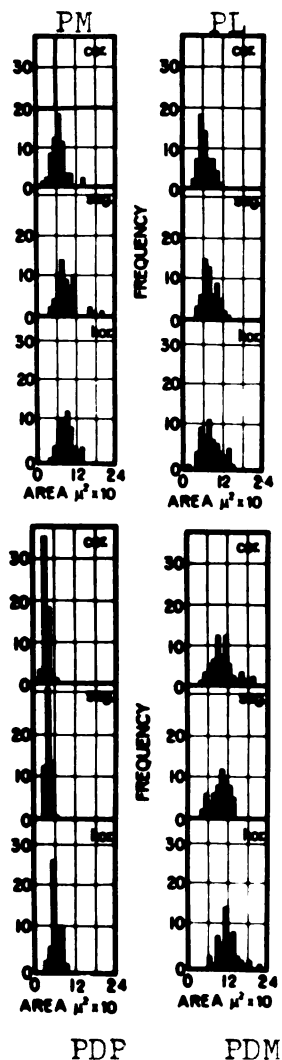$PDM_c \neq PDM_h$ at the .01 level of confidence.

Figure 8. Composite of the histograms of Figures 4-7 for purposes of comparison. Within planes comparsions of the means by the Newman-Keuls method revealed the following: Coronal plane: PL=PM. PDP and PDM were different from one another and PL and PM at the .01 level of confidence; Sagittal plane: PL=PM. PDP and PDM were different from one another and PL and PM at the .01 level of confidence; Horizontal plane: PL=PM. PDP and PDM were different from one another and PL and PM at the .01 level of confidence.

of variance (table 4) revealed a significant difference in
the mean cell densities between planes ($F=2.495$, 2/60 df,
$p < .05$) and a significant difference between subnuclei
($F=32.988$, 3/60 df, $p < .01$). There was no significant inter-
action.

All possible comparisons of the means by the method of
Newman and Keuls (Table 5) revealed PM=PL within each of the
three planes of section while PDP was significantly more dense
than PDM for the three planes of section.

As with cell size, ratios were computed to ascertain
what the differences in density between planes (within di-
visions) indicated. These ratios, computed as the ratio of
a given subnucleus to PM are shown in Table 6. The results
present a picture of equality across planes with two exceptions:
both PDM/PM and PDP/PM in the horizontal plane of section
are higher than the other corresponding ratios.

After the research was completed it was discovered that
there was an error in determining section thickness by the
method described previously. This error was due to the re-
fractive index of the mounting medium . The data was recal-
culated under the assumption that the sections were uniform-
ly cut at 25 microns. Although the mean densities changed in
all cases after the recalculation the ratios between the sub-
nuclei were not appreciably altered.

Table 4. Summary of the analysis of variance conducted on the density results.*= significant at .01 level of confidence.

SOURCE	SUM OF SQUARES	df	MEAN SQUARE	F	ω^2
Planes of section	14.81	· 2	7.40	2.49*	.01
Subnuclei	293.72	3	97.90	32.98*	.56
Interaction	16.49	6	2.74	.92	---
Error	178.09	60	2.96	-----	---
Total	503.11	71	-----	-----	---

Table 5. Summary of the results of the Newman-Keuls tests. A line beneath 2 subnuclei indicates that they are not significantly different at the .01 level of confidence.

PM_c PM_h PL_c PM_s PL_h PL_s PDM_c PDM_s PDM_h PDP_c PDP_s PDP_h

Table 6. Density ratios computed as the ratio between a given subnucleus and PM

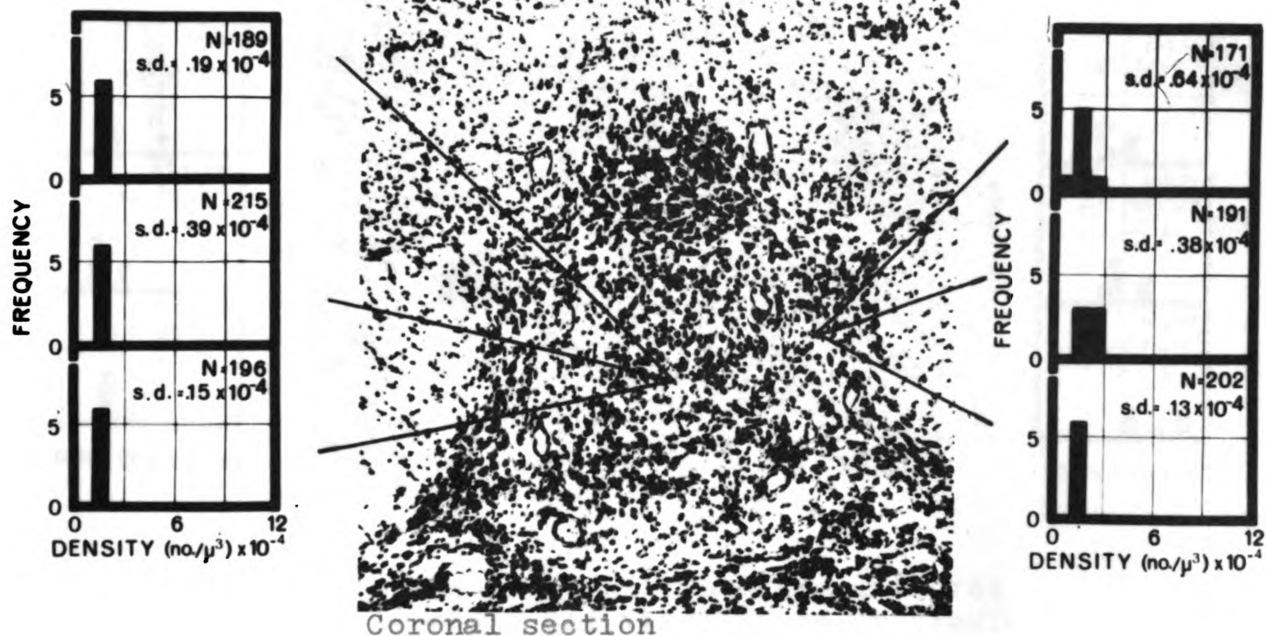| CORONAL	SAGITTAL	HORIZONTAL
PL/PM=1.11	PL/PM=1.07	PL/PM=1.06
PDM/PM=1.34	PDM/PM=1.24	PDM/PM=1.91
PDP/PM=3.70	PDP/PM=3.57	PDP/PM=5.26

Figure 9. Results of density measurements for PL and PM. In this figure and Figures 10 and 11 the abscissa represents the density as cells/micron^3 X 10^{-4} while the ordinate represents the frequency of occurrence of a given density. In the upper right hand corner of each histogram is the number of cells counted and the standard deviation of the sample. The coronal plane is represented by the upper histogram, the sagittal plane by the middle histogram, and the horizontal plane by the lower histogram. Mean densities for the respective planes were:

Coronal

PL   1.638 X 10^{-4} cells/micron^3
PM   1.469 X 10^{-4} "

Sagittal

PL   1.789 X 10^{-4} "
PM   1.663 X 10^{-4} "

Horizontal

PL   1.731 X 10^{-4} "
PM   1.622 X 10^{-4} "

Within planes comparsions of the means by the Newman-Keuls method revealed no significant differences between PL and PM.
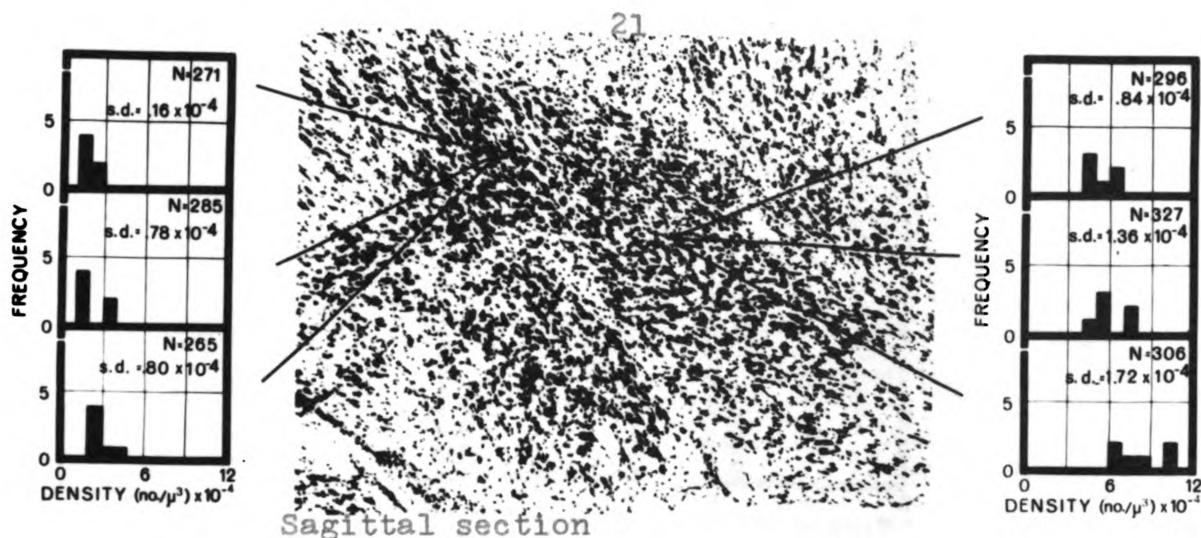

Figure 10. Results of density measurements for PDM and PDP. Ordinate and abscissa are the same as in Figure 9. In the upper right hand corner of each histogram is the number of cells counted and the standard deviation of the sample. The upper histogram represents the coronal plane, the middle histogram represents the sagittal plane, and the lower histogram represents the horizontal plane. The means for the respective planes were:

Coronal

PDP 5.938×10^{-4} cells/micron^3
PDM 1.979×10^{-4} "

Sagittal

PDP 5.935×10^{-4} "
PDM 2.072×10^{-4} "

Horizontal

PDP 8.171×10^{-4} "
PDM 3.148×10^{-4} "

Within planes comparsions of the means by the Newman-Keuls method revealed PDP significantly greater in density than PDM at the .01 level of confidence.
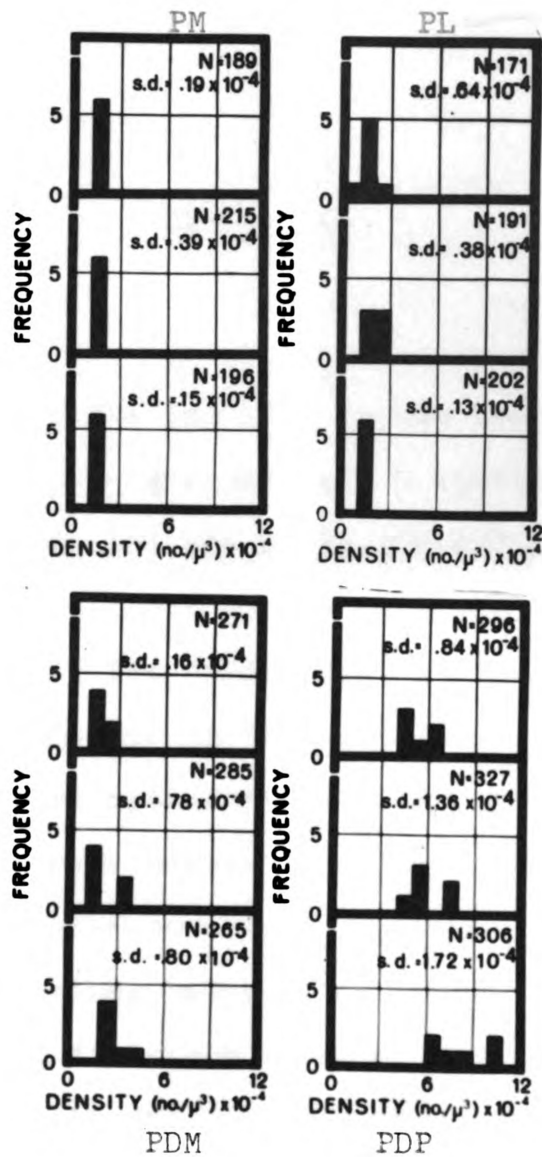
Figure 11. Composite of the histograms of Figures 9 and 10 for purposes of comparison. Refer to Table 5 for results of between planes comparisons of the mean cell densities.

DISCUSSION

An Overview

Generally, in the rat IPN, it has been found that the constituent cells are grouped into 4 spatially distinct subnuclei. These subnuclei are PL,PM,PDM,and PDP. While the latter two subnuclei exhibit marked differences in both cell size and packing density the former two are not significantly different from one another in terms of these measures. Because of the quantitative cytoarchitectonic similarity between PL and PM one might hypothesize that they are functionally similiar subnuclei which have been separated from one another by two intrinsic fiber bands. That quantitatively different cytoarchitectonic divisions exist within the IPN is assumed to indicate a functional differentiation of the nucleus.

Cell Size

The high value for ω^2 for subnuclei (.46) and its relatively low value for the interaction and planes effects indicates that a knowledge of the subnuclei is a more efficacious predictor of cell size than is a knowledge of planes of section or both subnuclei and planes of section. This measure may be interpreted as indicating that our uncertainty about the cell size is reduced by 46% if we know the division from which the cell was sampled. This is compared with a reduction

of uncertainty of only 4% if we know the plane in which the nucleus was sectioned. Given both planes of section and sub-nucleus the uncertainty in predicting cell size is only reduced by 1% over that given by the other two effects independently.

The virtual constancy of the ratios (Table 3) across planes (for a given ratio) indicates that differences which occurred between certain divisions across planes are probably due to shrinkage. Thus, for example, PLcor was significantly different from PLsag and PLhor, although the ratio, PL/PM, was essentially constant for the respective three planes. We can conclude that within animal comparisons (i.e. within a given plane of section) of mean cell size in different subnuclei can be done directly. This is opposed to between animal comparisons (between planes of section) which must take the form of ratios or some other similiar technique which effectively cancels out the variance due to histological procedures.

The general results of the Newman-Keuls test indicate that within a given plane of section PL and PM do not differ significantly in their mean cell size. This is to say that the samples of cells from these two divisions are not different in terms of cell size although their spatial location is different. The predictive value of this result is obvious. Given a sample of cells whose mean is similar to that of PL or PM it would be impossible to determine, on the basis of cell size, whether or not the cells had been sampled from PL or PM.

Given only cell size it is impossible to specify PL or PM. These subnuclei must be specified by their spatial location. This is not true of PDM and PDP as indeed the names are meant to imply. The latter two distributions of cell size are markedly different. The distribution of PDP has a relatively small variance and a mean cell size of approximately 40 microns^2 whereas the variance of the PDM cell size distribution is relatively greater and mean cell area is approximately 105 microns?

Density

As with the cell size data, the subnuclei factor of the analysis of variance demonstrated a high value for ω^2 (.56). In terms of the predictive value this indicates that a knowledge of the subnucleus (i.e. which subnucleus) reduces our uncertainty as to the packing density of the constituent cells by 56%. This is to be compared with the relatively low value for the planes factor (.01).

In general the ratios were constant with 2 exceptions; both PDM/PM and PDP/PM were much greater than expected. This indicates that PDP and PDM are more densely packed in the horizontal plane than in the other two planes of section with respective means of 8.171×10^{-4} and 3.148×10^{-4} cells/micron^3. Measurements of density in another rat brain sectioned in the horizontal plane were conducted in order to ascertain whether or not this finding was reliable. The means for this animal were PDM= 2.837×10^{-4} and PDP= 7.215×10^{-4} cells/ micron^3. The corresponding ratios were PDM/PM= 1.71 and PDP/PM=4.37. The close correspondence between the means and ratios for the

two animals sectioned in the **horizontal** plane indicate that
PDM **and** PDP are reliably more dense in the horizontal plane
as opposed to either the sagittal or coronal planes of sec-
tion.

An explanation of this paradoxical finding might be that
these dorsal subnuclei are laminated. The distance between the
laminae would be less than the average thickness of the sec-
tions since there were no areas of relatively low density
found interspersed with areas of relatively high density.

Synthesis

Huber,et.al. (1943) have described the rat IPN as con-
sisting of a large celled lateral subnucleus and a smaller
celled medial subnucleus. The results of the present study
place these latter findings in doubt since it was shown that
the lateral and medial subnuclei did not differ on the basis
of either cell size or packing density. That these results
were reliable across three animals obviates any explanation
of these results as due to insufficient sample size. Further-
more, the ratio, PL/PM, remained essentially constant across
animals which indicates that any observed differences between
PL and PM across animals were probably due to extraneous fac-
tors (most likely differential shrinkage).

Employing the quantitative techniques described in the
present paper it is possible to specify or define the com-
ponent subnuclei of the IPN in terms of three numbers; a cell
size term, a density term, and a coordinate or positional term.
In certain cases, as for example with PDP and PDM, it is

possible to adequately describe the given subnucleus using only the first two terms. With PL and PM, though, position must be specified if we are to distinguish between these two subnuclei.

The use of quantitative techniques is important because they provide for a greater degree of interobserver reliability than has generally been present in neuroanatomy. Given the appropriate measuring devices it would be expected that another observer would find the same results as presented in this paper. This does not assume that another observer would arrive at exactly the same numbers. What it does imply is that another observer would obtain the same results relative to the present ones.By computing ratios between the different subnuclei for both density and cell size, extraneous factors such as differing fixation and dehydration times are effectively cancelled out.

The raison d'etre of the nuclear subdivisions is not clear. It is my assumption that these differences in the cytoarchitectonics of the rat IPN indicate functional differences. The basis for this assumption is that one would expect cell size and packing density within the nucleus to be randomly distributed. When it is not, as with the IPN, functional differentiation is the first choice as an explanation of the findings. There are two lines of evidence which support this assumption; biochemical studies and studies of the fiber connections of the IPN.

Friede (1959) and Manocha and Bourne (1966) have

reported in the guinea pig and squirrel monkey, respectively, that there is differing activity of succinic dehydrogenase (SDA) and cytochrome oxidase (CO) within the IPN. In both species the ventral portion of the nucleus exhibits a high activity of these enzymes while the dorsal portion exhibits very low activity of SDA and CO. The differences in the activity of these enzymes is related to the degree of capillarization which in turn is related to the degree of metabolic activity (Sharrer,1945). The metabolic rate might be related to spontaneous activity within the nucleus which would predict a high spontaneous firing rate for cells in the ventral portion of the complex as opposed to those in the dorsal portion.

The fiber connections of the IPN have not been well studied in relation to the subnuclei described in the present paper. In the normal rat brains the author has observed that the fibers of the pedunculotegmental tract appear to issue from the lateral borders of PM. In the cat Smaha (1968) has provided some evidence that there are at least two efferent fiber systems of the IPN. One projects rostrally to the intralaminar system of the dorsal thalamus and the thalamic reticular nucleus. The caudally projecting fibers travel to the ventral tegmental nucleus of Gudden and the inferior olive. It is unfortunate that these fiber projections were not correlated with the nuclear morphology elucidated by Berman and Bowers (1967).

Afferently, the HPT appears to project to the lateral subnuclei but I have not ascertained whether it synapses there.

Cajal (1952) has described the endings of this fiber tract as showing extensive ramification throughout the nucleus so that such a conclusion might be premature until appropriate silver stains can be done. Massopust and Thompson (1962) have described efferent fibers in the HPT which terminate in the dorsolateral and dorsomedial thalamic nuclei and the lateral habenular nucleus, some traveling with the stria medullaris to terminate in the anterior hypothalamus. The existence of this pathway might explain why Smaha (1968) observed a rostrally projecting efferent fiber bundle arising from IPN.

Comparative Studies

In order to place the rat into the schema of the total order Rodentia, comparative studies have been conducted on several members of the suborders Caviamorpha and Sciuromorpha (paper in preparation). The rat has been taken to be the sole representative of the Myomorpha . Quantitative measurements of the cell size and packing density within the IPN subnuclei have been conducted on the guinea pig, Cavia, and the capybara, Hydrochoerus, two representatives of the Caviamorpha. Quantitative studies of the nuclear configuration of representatives of the Sciuromorpha have been conducted on the mountain beaver, Aplodontia. Qualitative studies have been conducted on the chinchilla, Chinchilla, a caviamorph, and the gray squirrel, Sciurus, the marmot, Marmota, the flying squirrel, Glaucomys, and the eastern pocket gopher, Geomys, which are members of Sciuromorpha.

Generally the IPN of all of the above mentioned rodents can be divided into the four subnuclei described for the rat with one exception. In the capybara, a discrete pars lateralis does not appear to exist. Rather, the large celled dorsal division appears to extend ventrally around the lateral extent of the nucleus. The small celled dorsal subnucleus has the same configuration in the other rodents studied as seen in the rat. In the rostral extent of its course it moves ventrally to occupy the whole extent of the nucleus in planes of section through the red nucleus. Pars medialis has essentially the same relative extent and position throughout all of the rodents studied.

An important qualitative observation concerns the relation between the large celled dorsal division and the nucleus centralis superior, a nucleus of the median raphe. In all of the rodents mentioned above it has been noted that this large celled division is intimately related to the nucleus centralis superior. Furthermore, the size of PDM seems to be correlated with the size of this latter nucleus. In rodents such as the guinea pig, where the nucleus centralis superior is small, PDM was found to be correspondingly reduced in size. In the mountain beaver, where nucleus centralis superior is large, PDM was correspondingly well developed.

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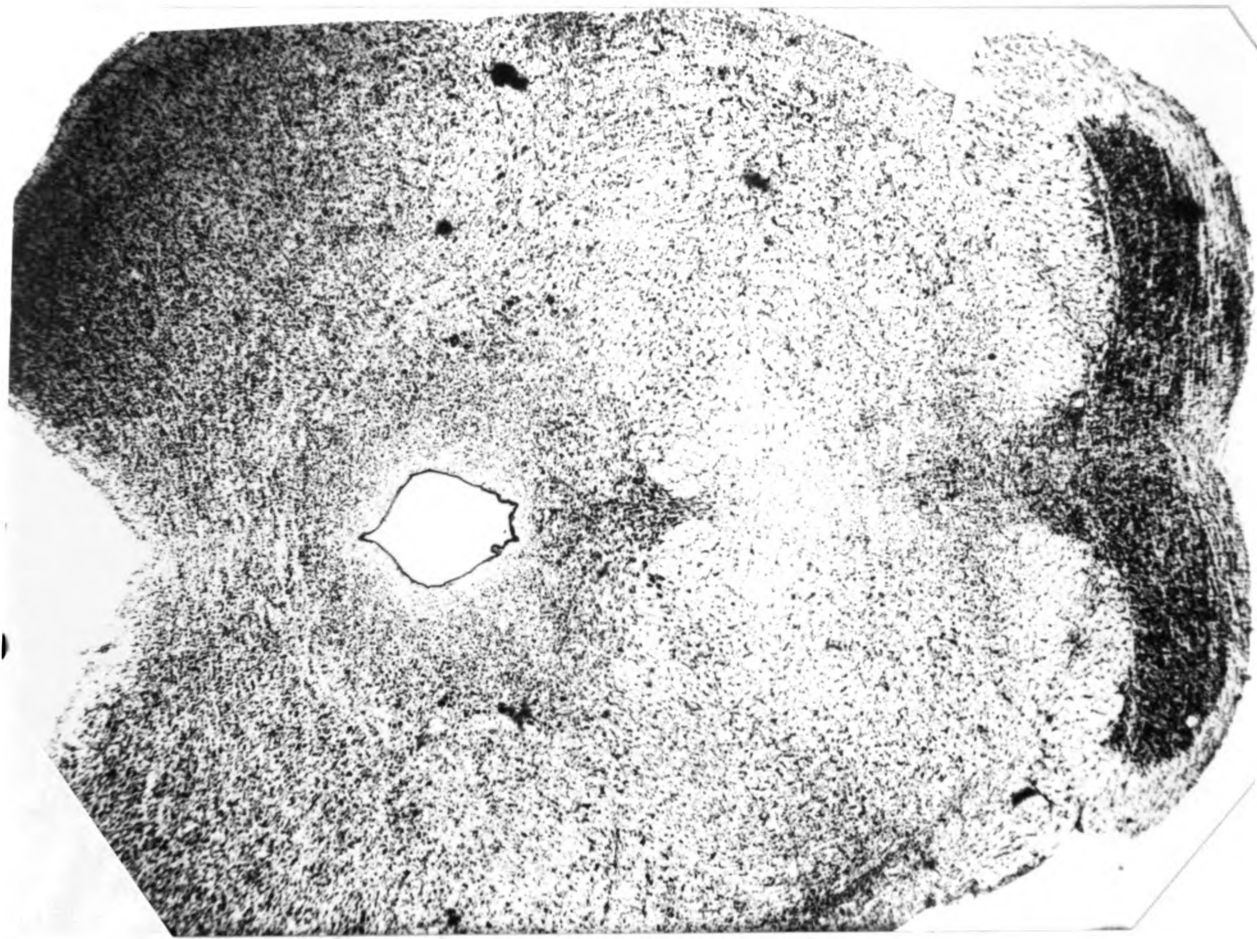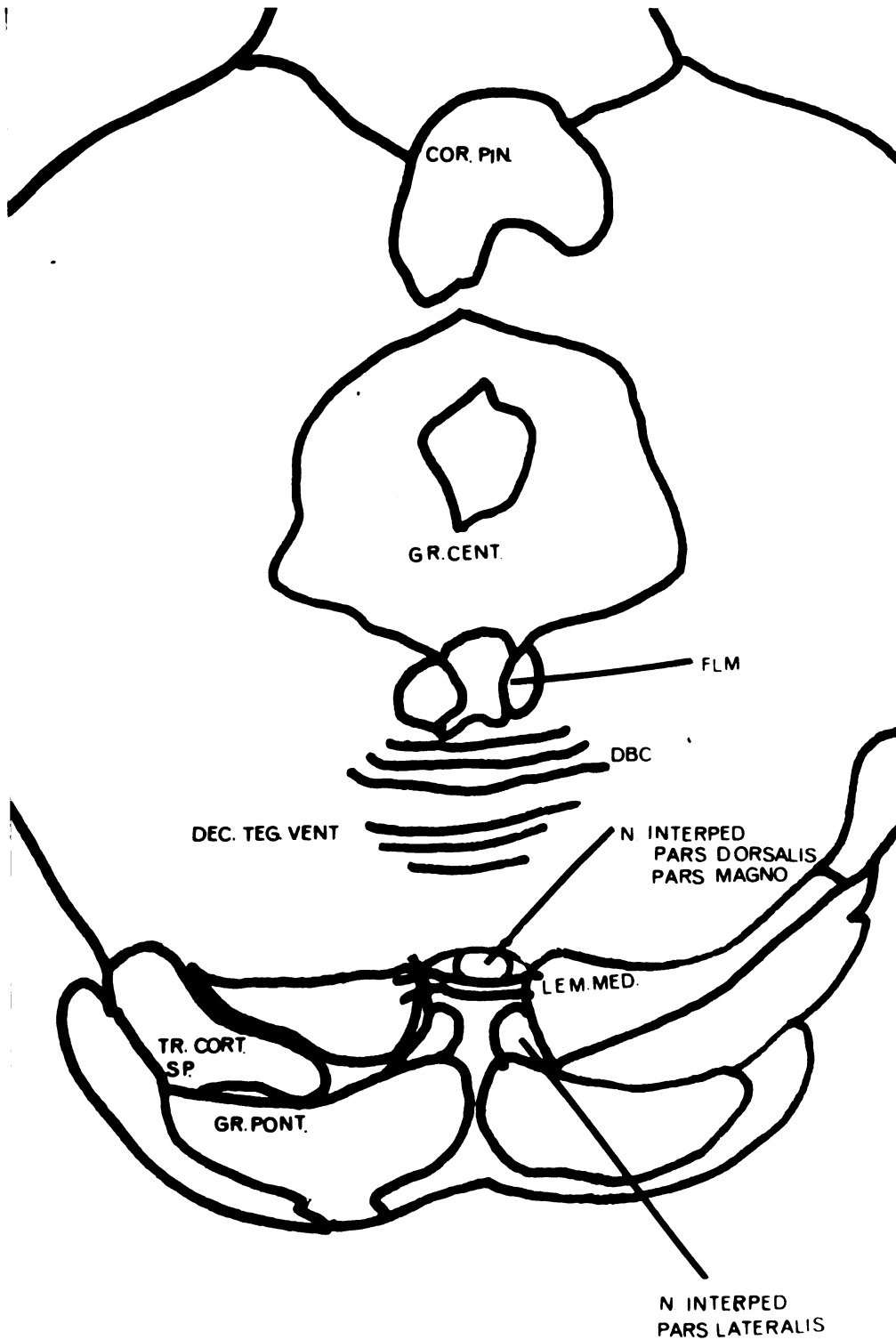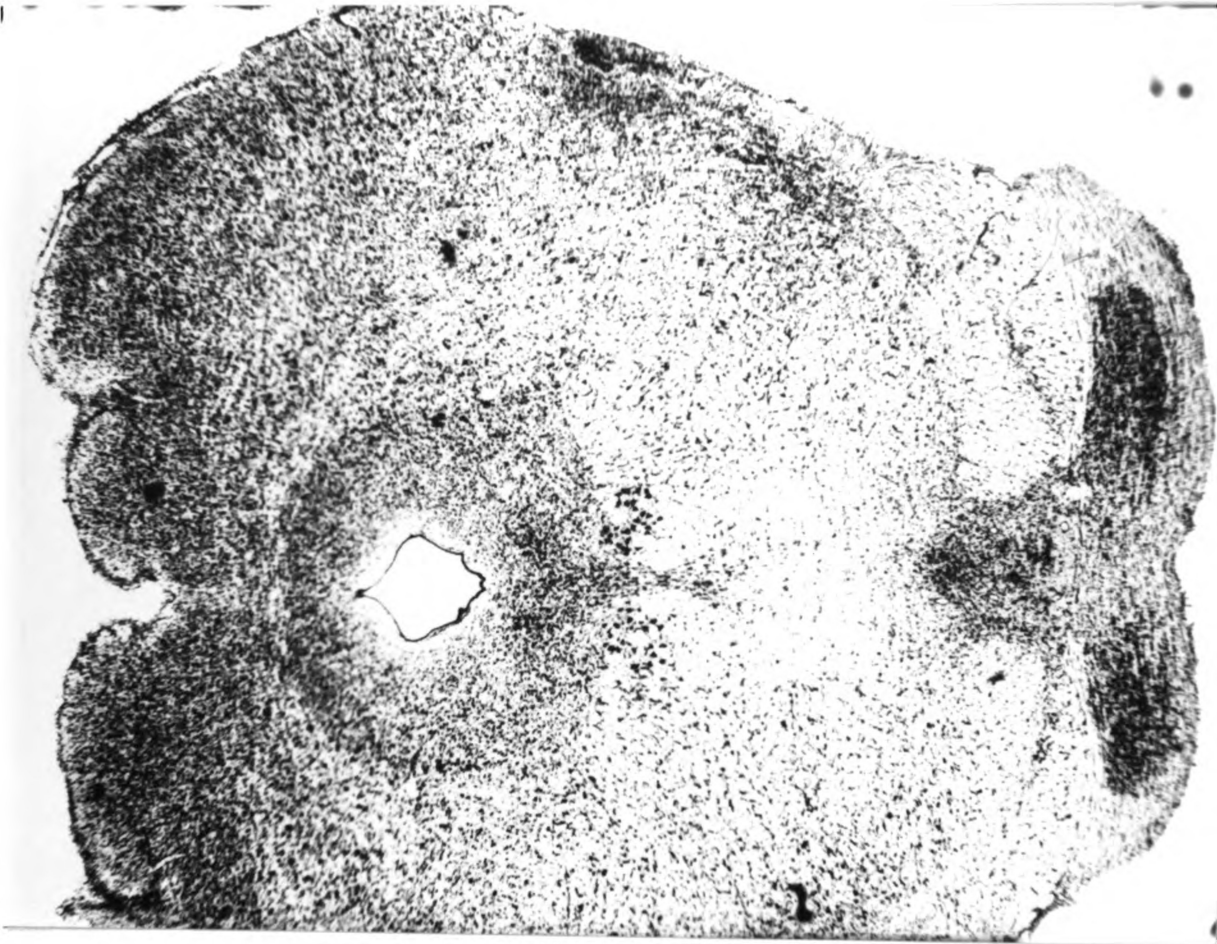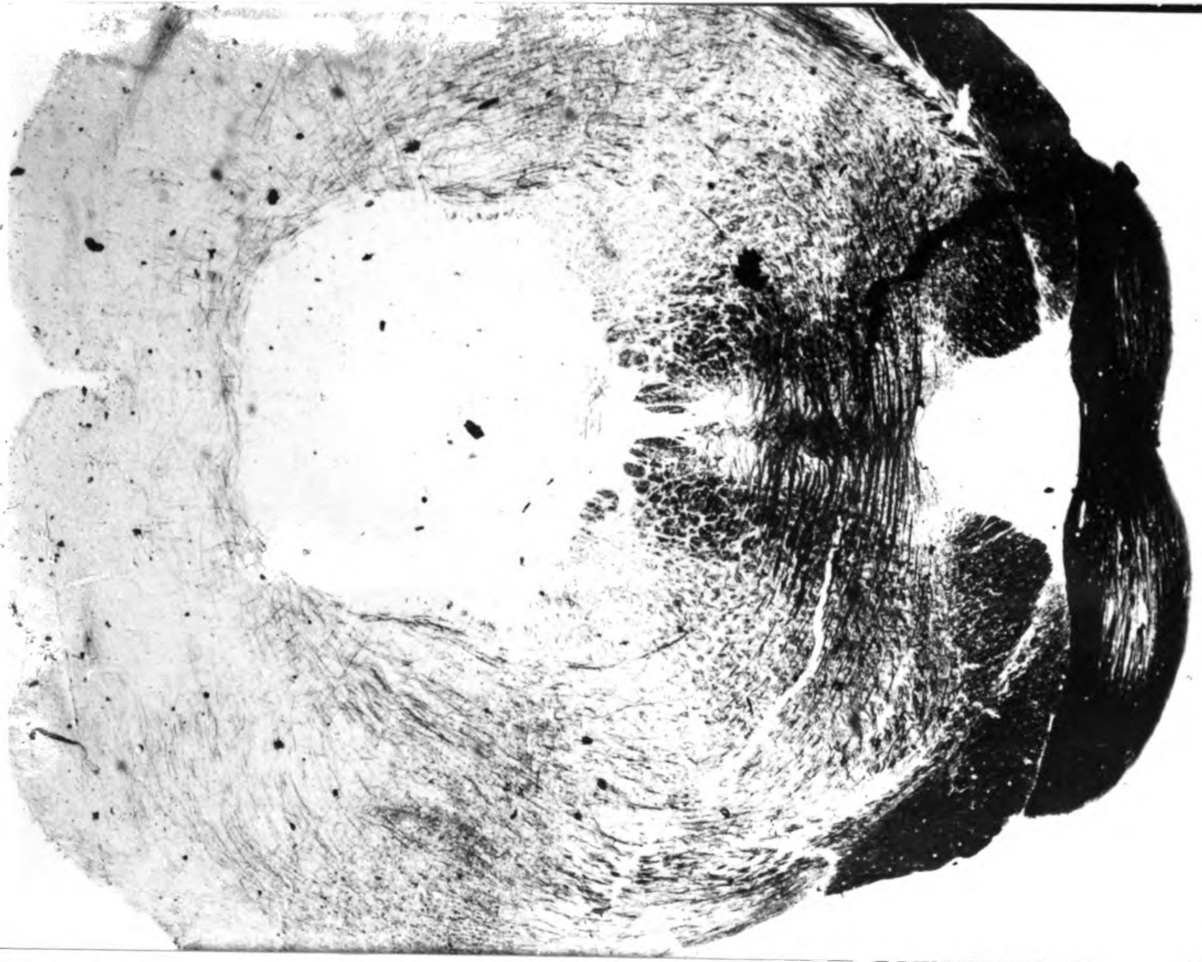
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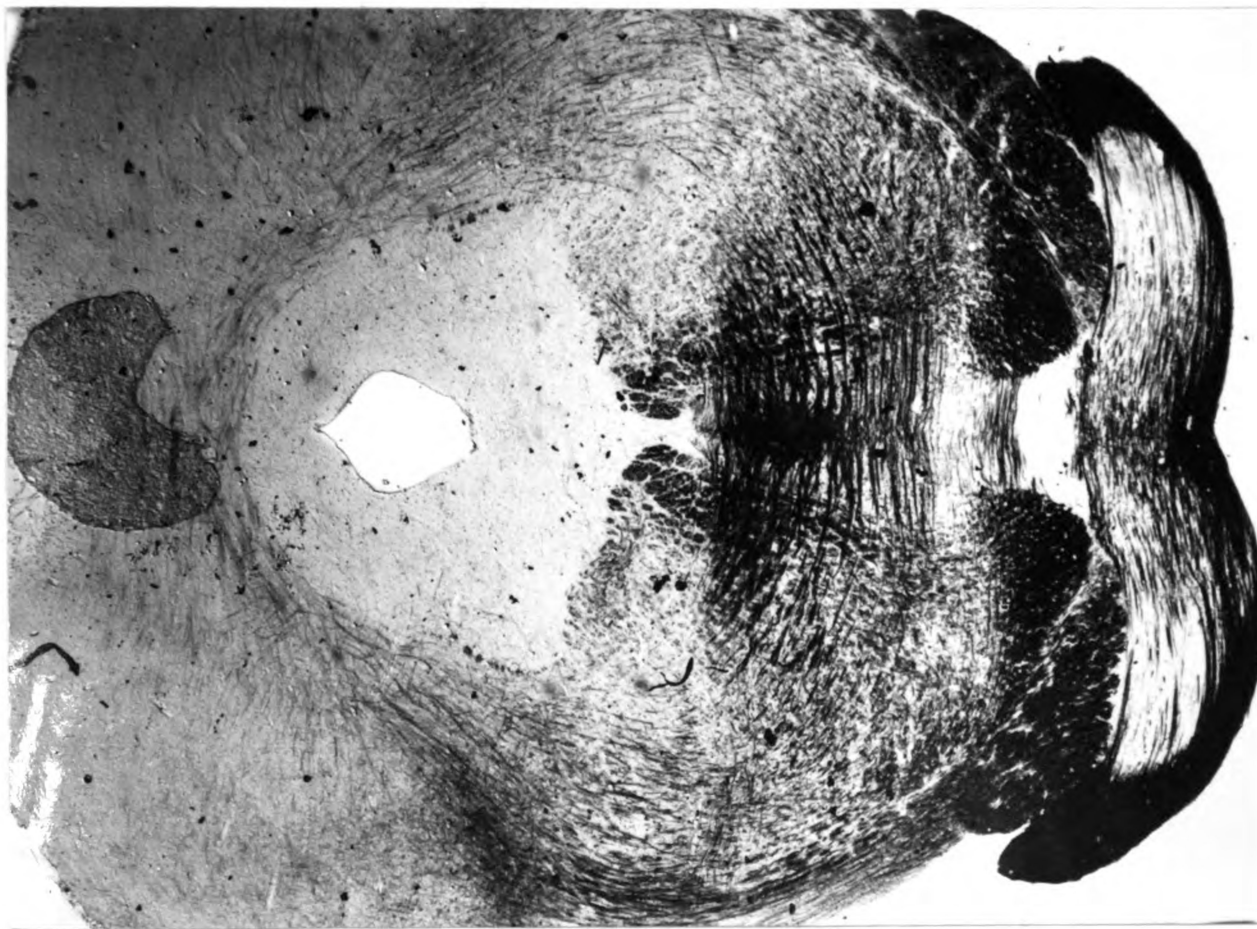
Haidenhain

Plate 1. Caudal section through the midbrain at the level
of the decussation of the superior cerebellar peduncle (DBC).
In this and the following plates the midbrain is magnified
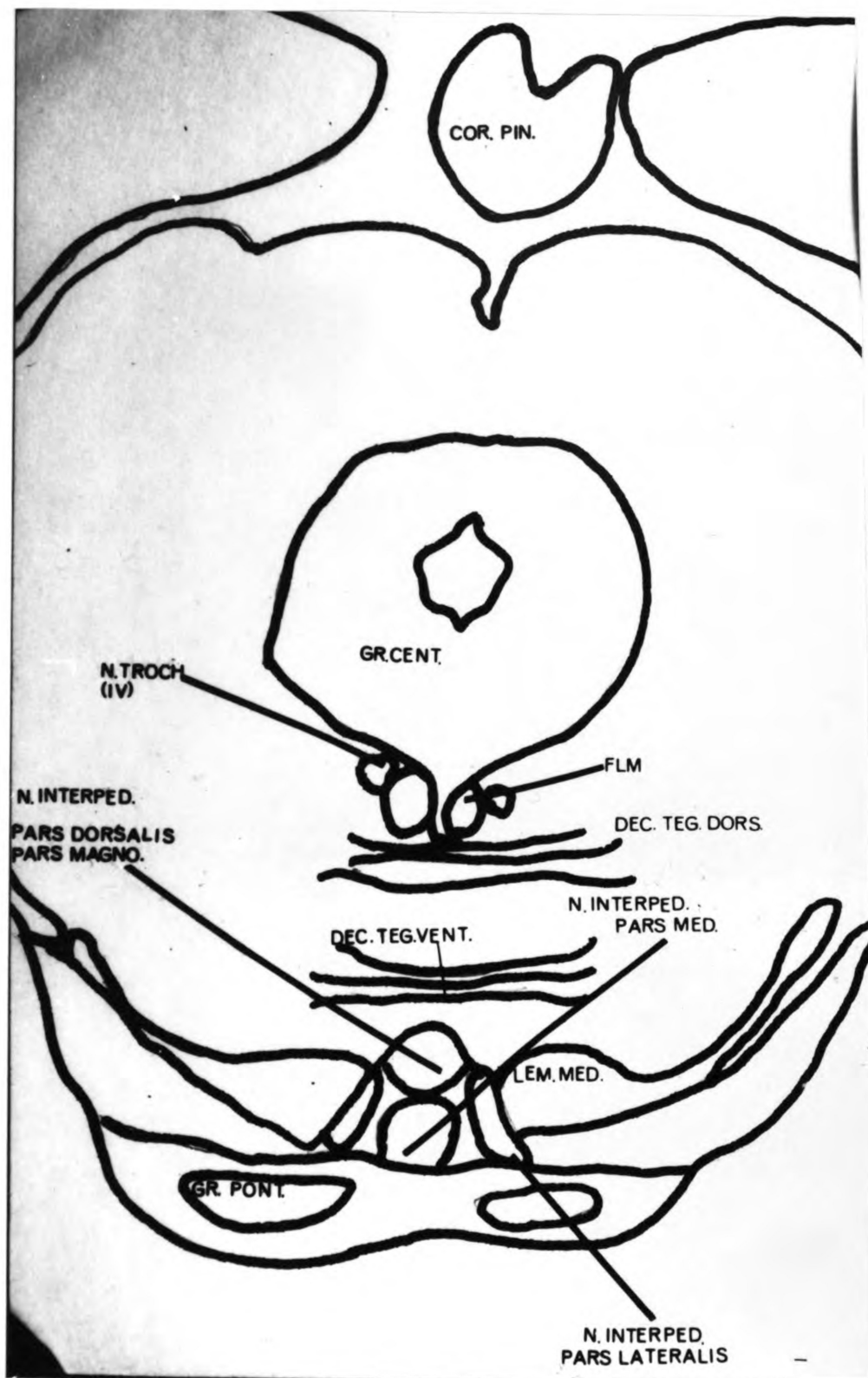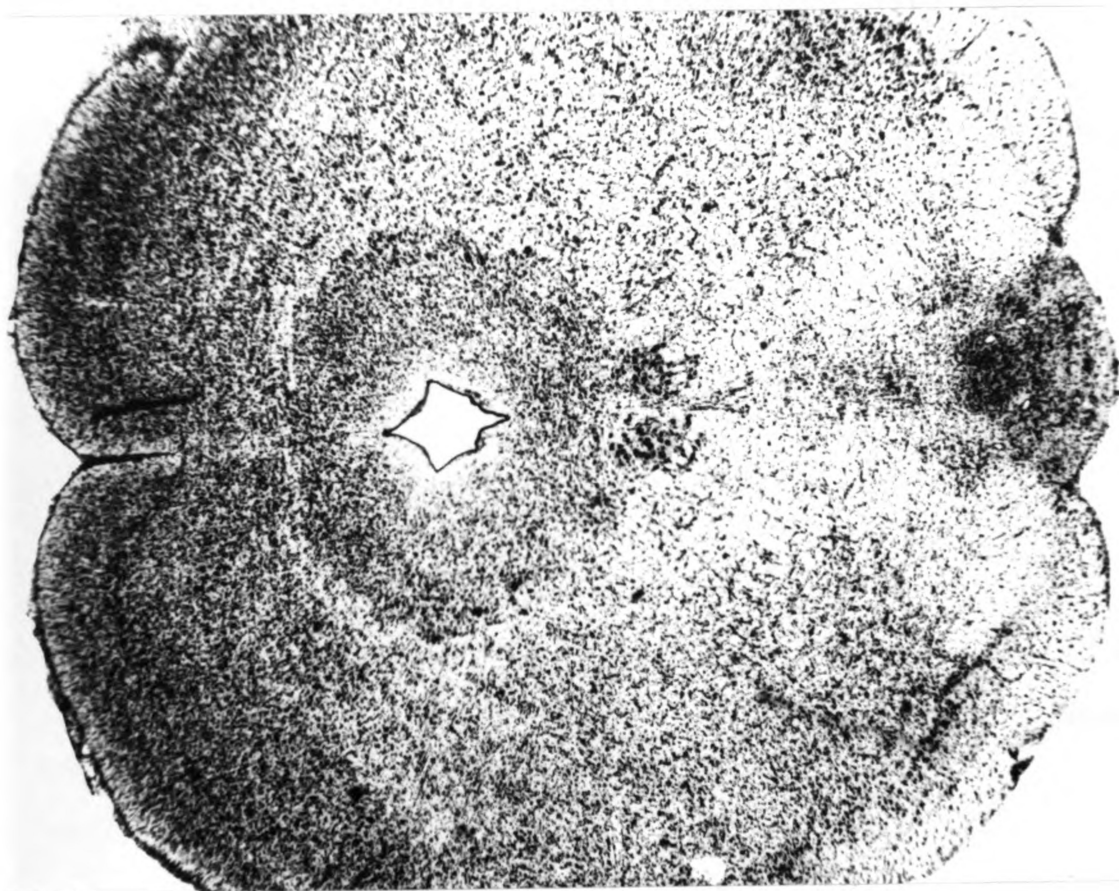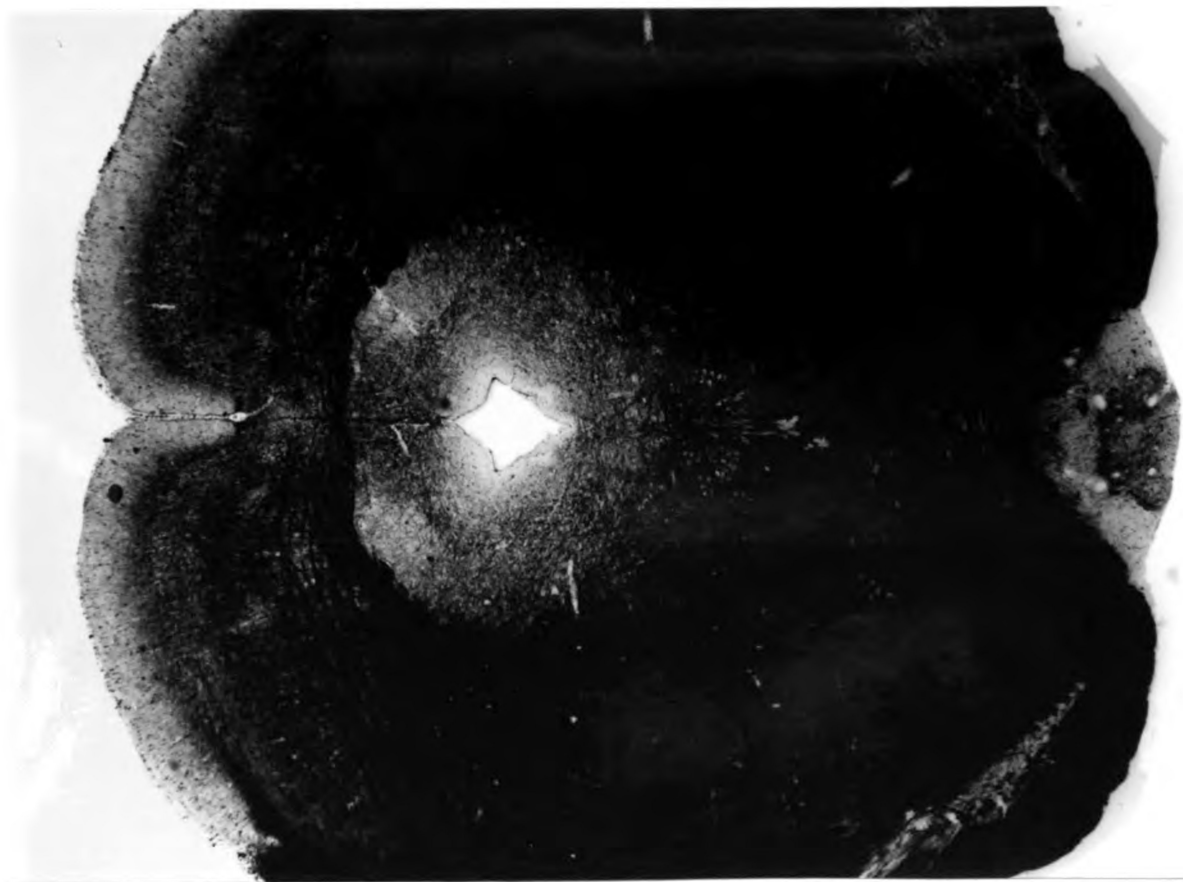30 times. For a further description refer to the text.

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Plate 2. Caudal section through the midbrain at the level of the trochlear nucleus (IV). For a description of the IPN at this level refer to the text.
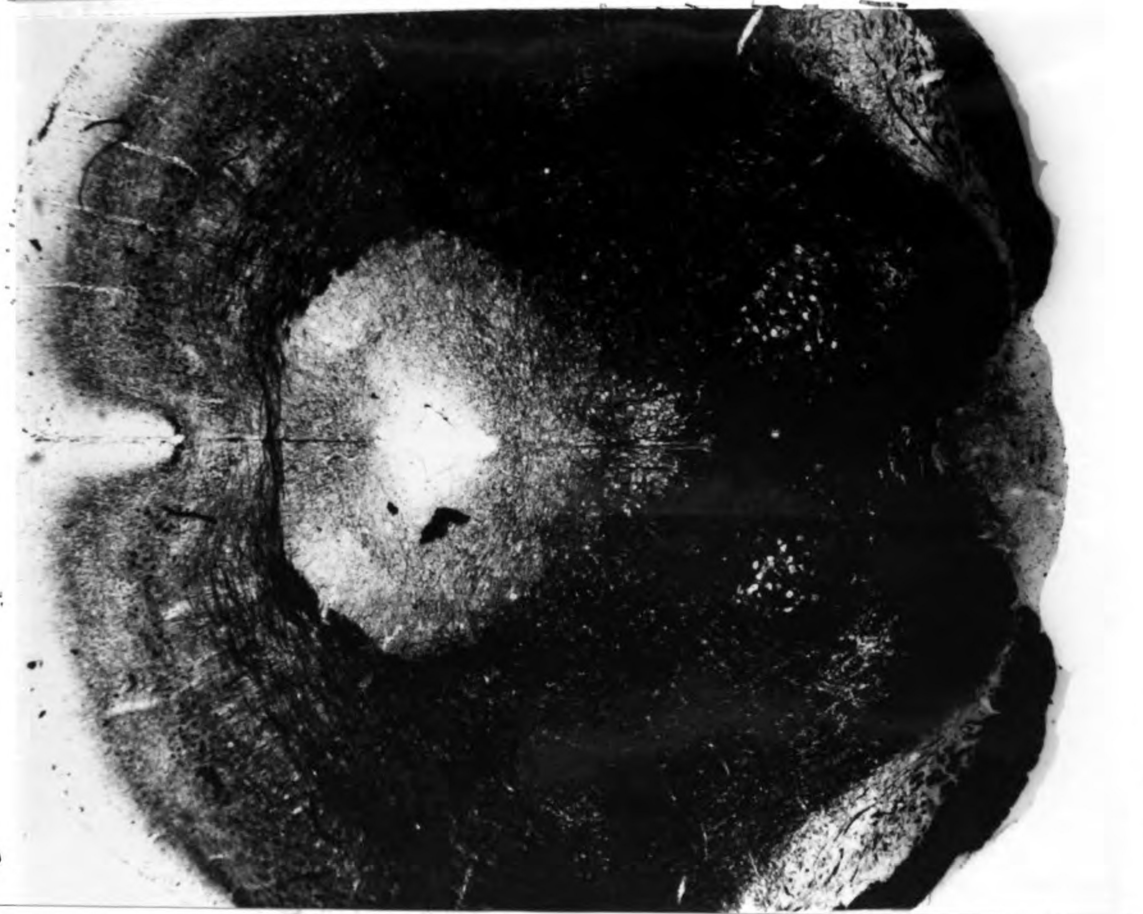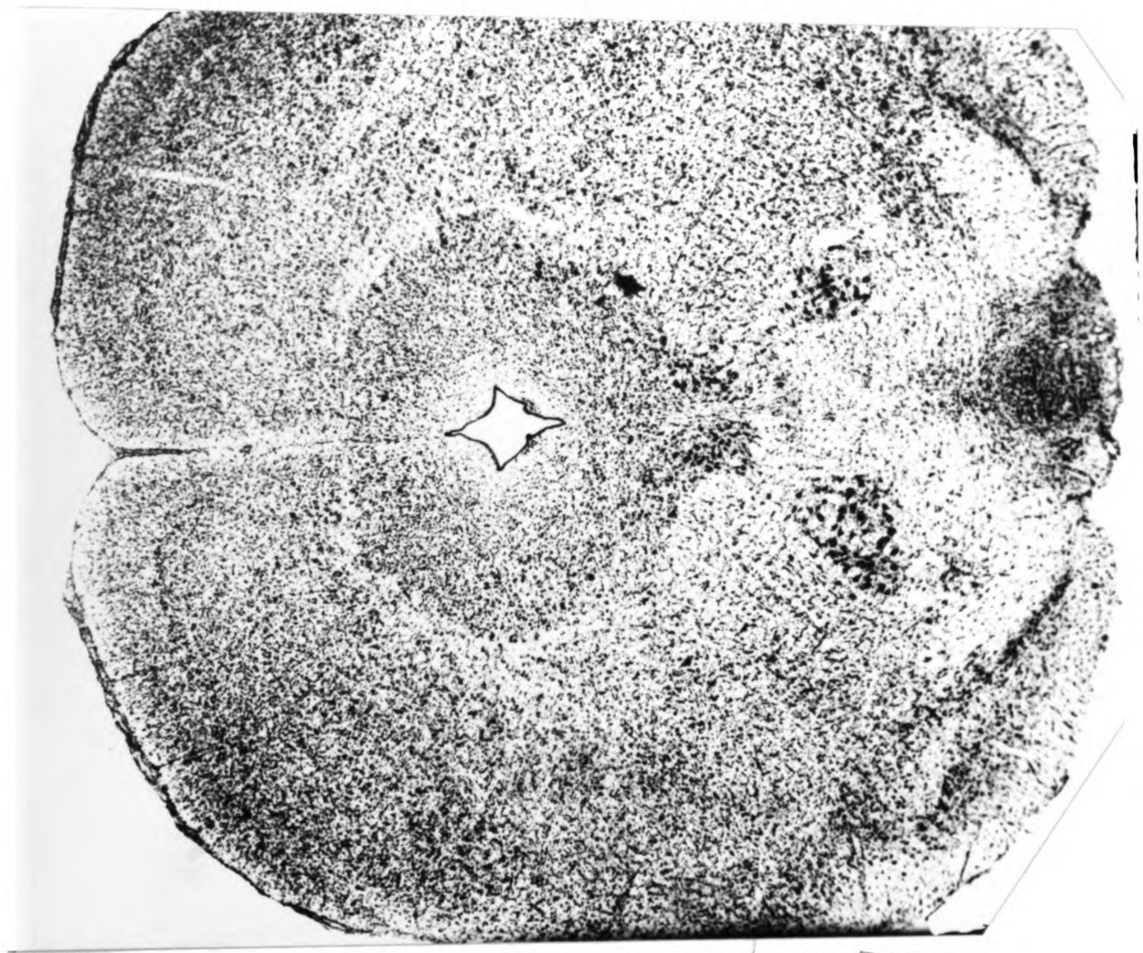
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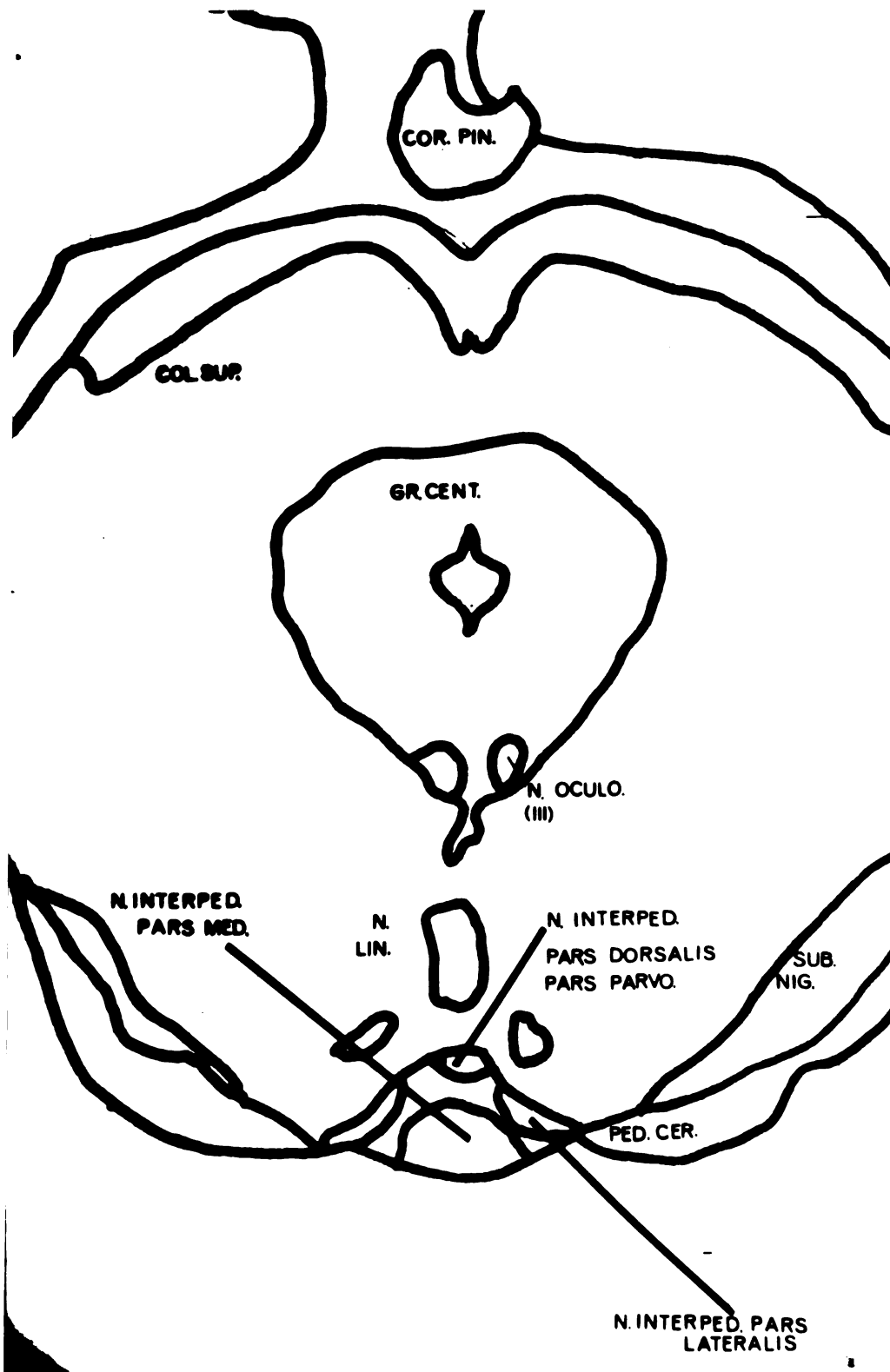
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Plate 3. Coronal section through the midbrain at the level of the caudal extent of the superior colliculus (COL. SUP.). For a description of the IPN at this level refer to the text.
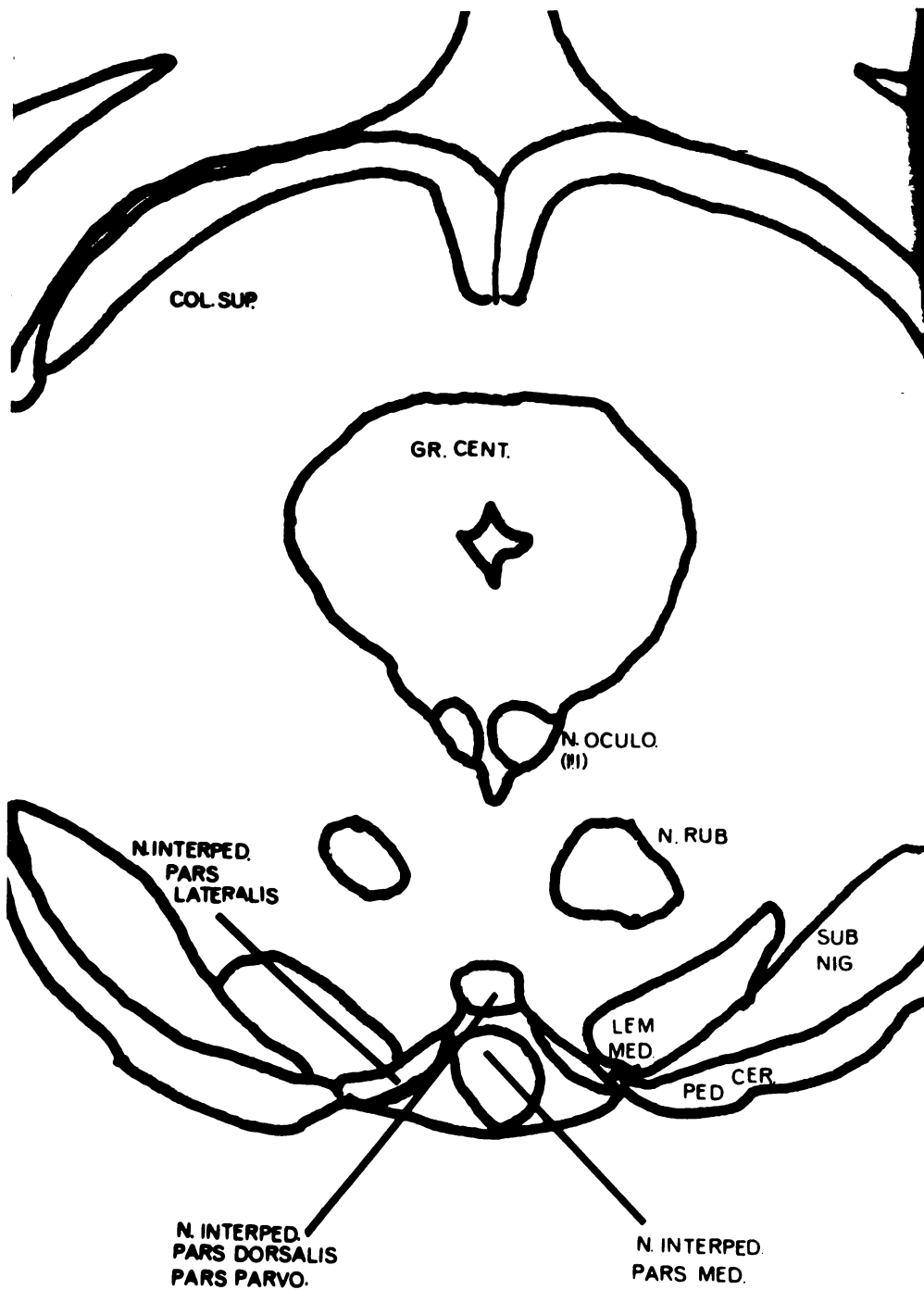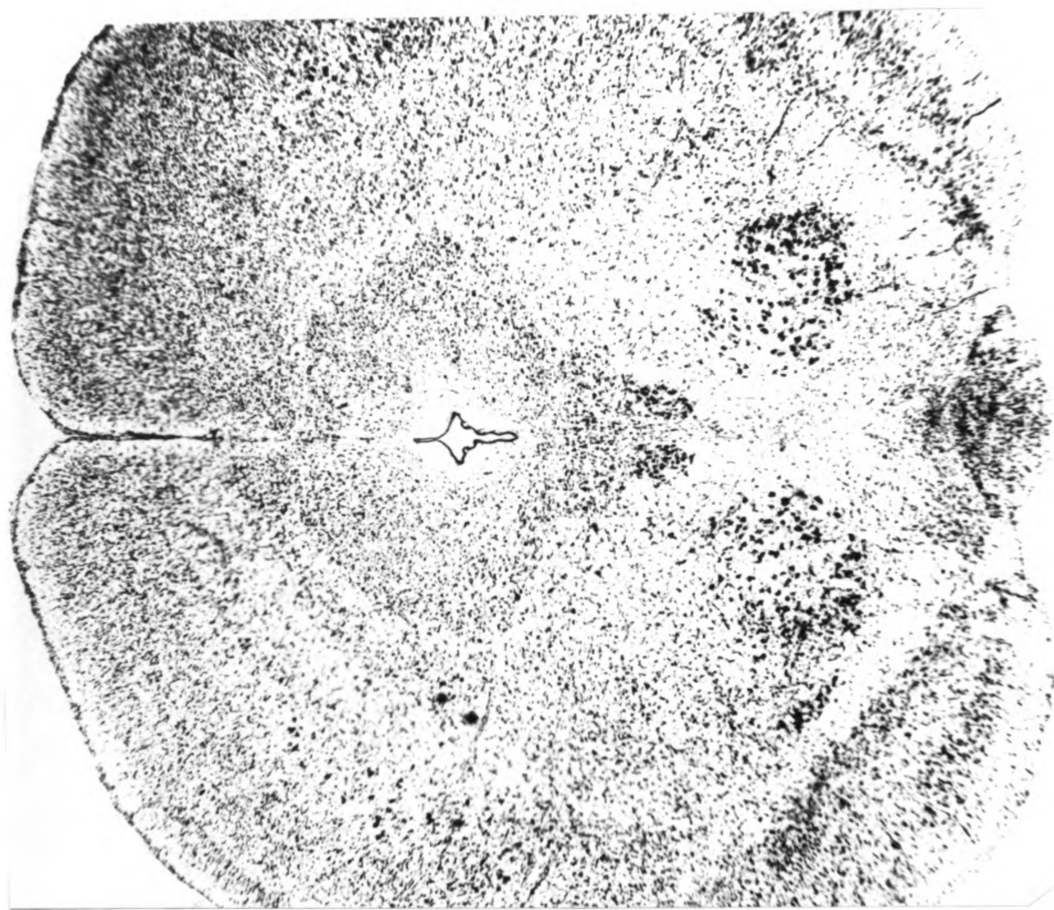
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COL. SUP.

GR. CENT.

N. OCULO.
(III)

N. INTERPED.
PARS
LATERALIS

N. RUB

SUB
NIG.

LEM
MED.

PED CER.

N. INTERPED.
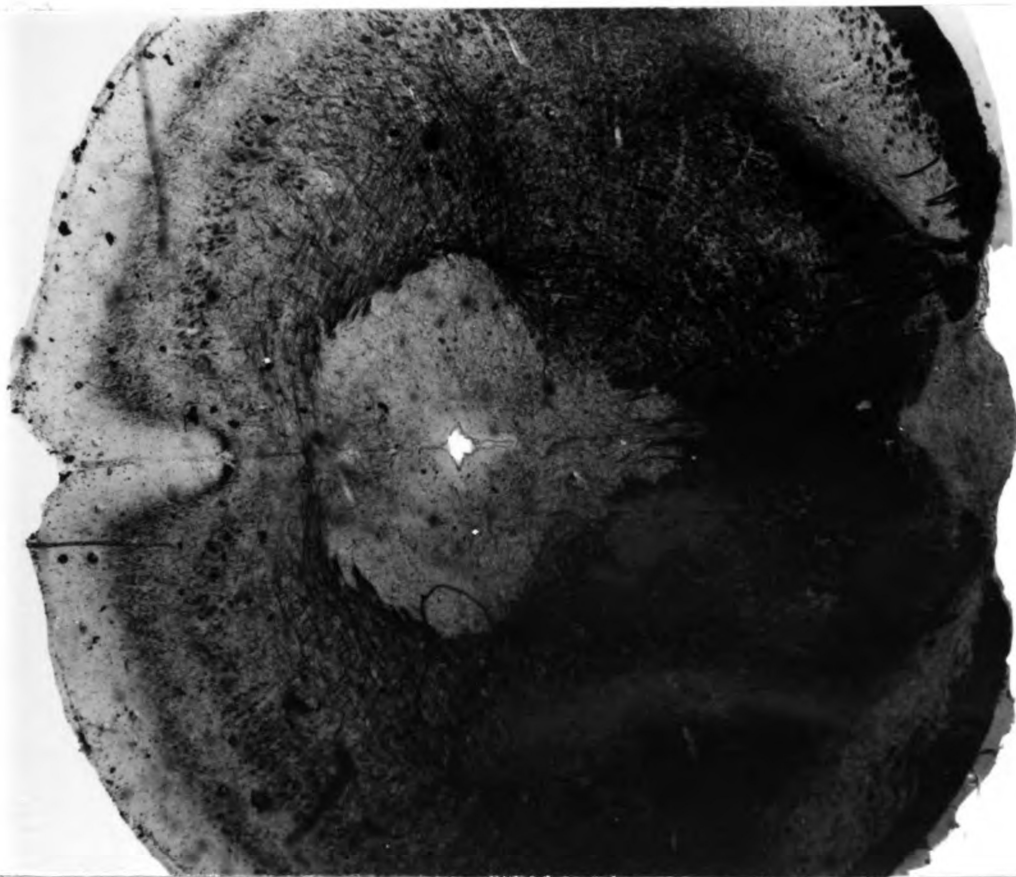PARS DORSALIS
PARS PARVO.

N. INTERPED.
PARS MED.

Plate 4. Coronal section through the midbrain at the level of
the oculomotor nucleus (III). For a description of the IPN at
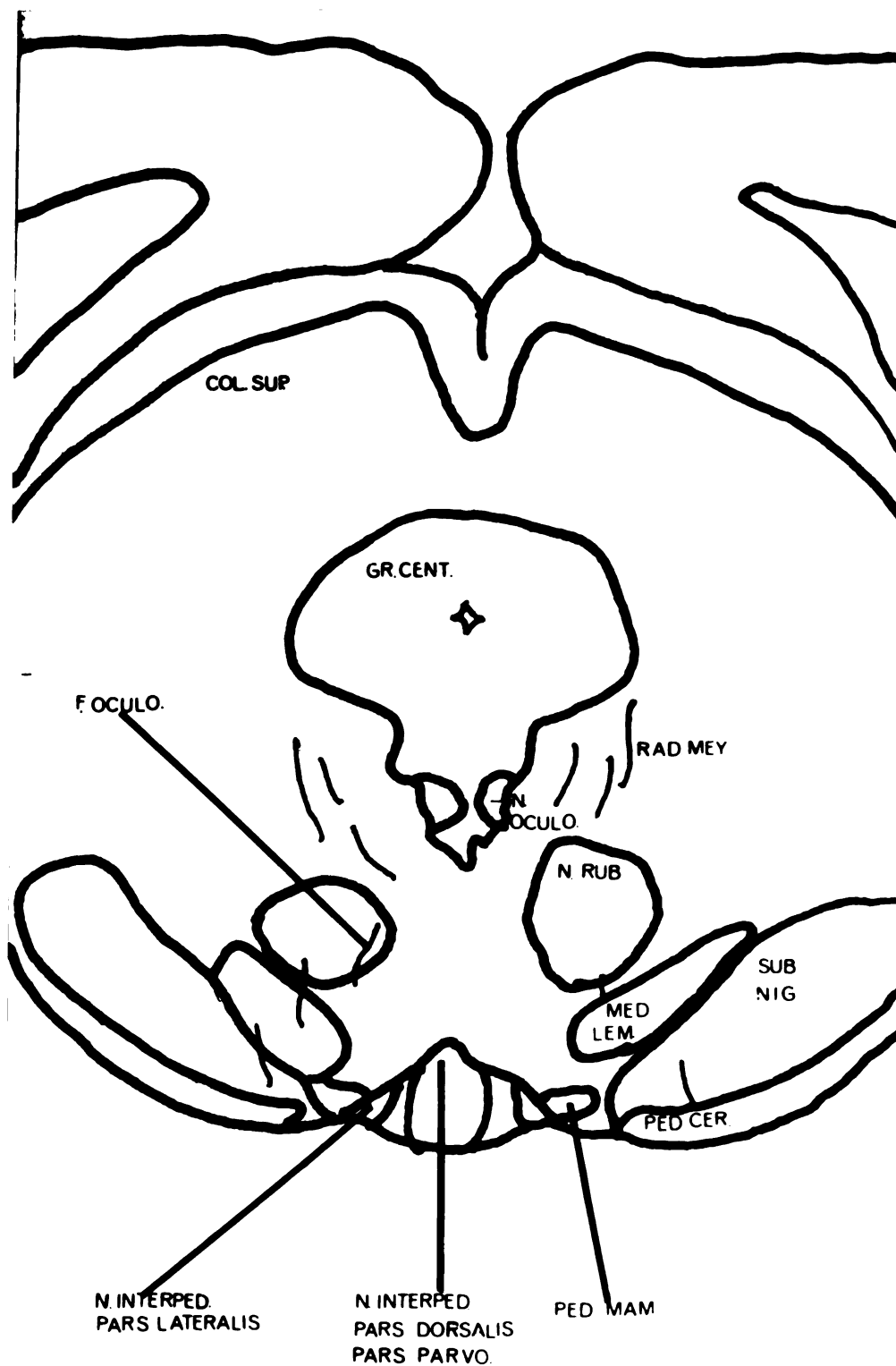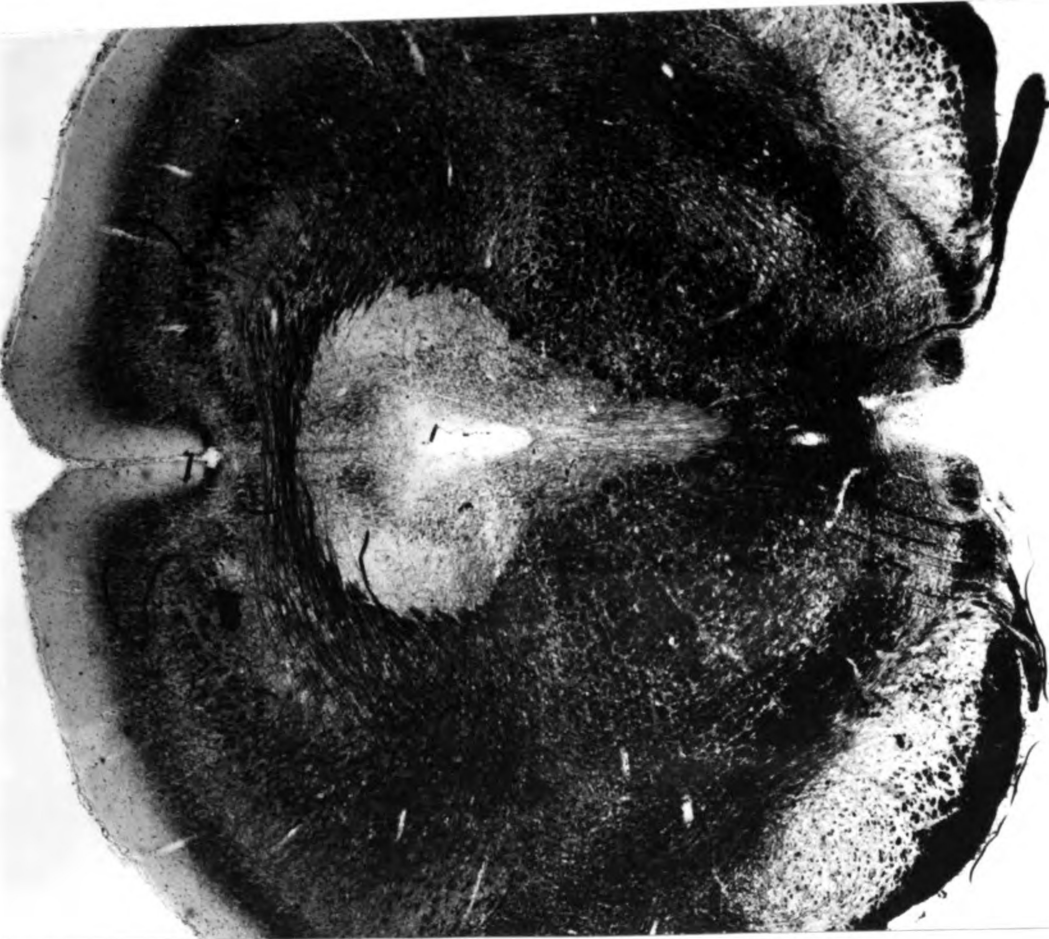this level refer to the text.

Thionin

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Plate 5. Coronal section through the midbrain at the level of the red nucleus (N. RUB.). For a description of the IPN at this level refer to the text.
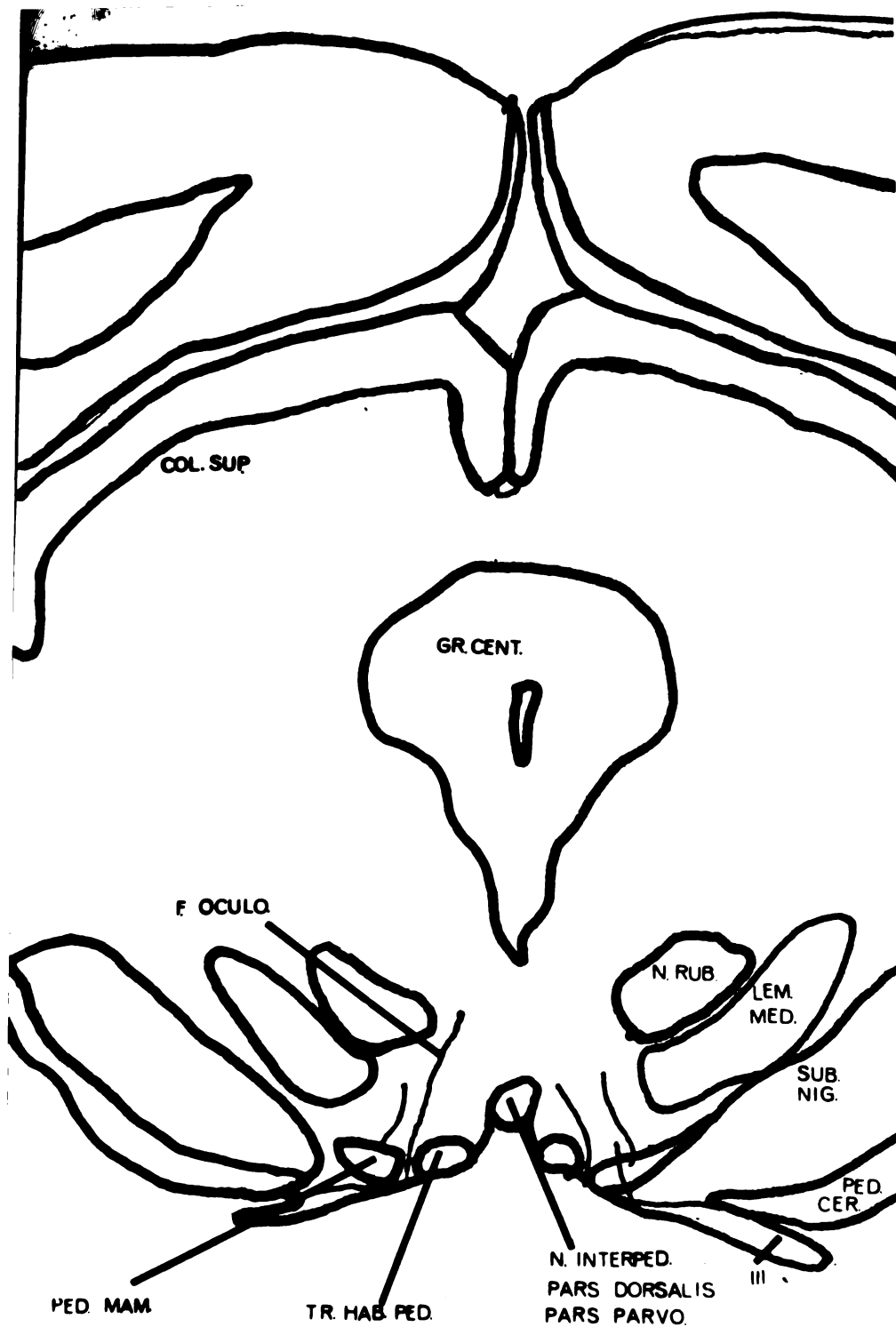
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Plate 6. Coronal section through the midbrain at the rostral
end of the IPN. For a description of the nucleus at this level
refer to the text.

APPENDIX A

THIONIN STAIN (NISSL METHOD)

1. Take sections off paper by floating each section into distilled water.

2. From the distilled water place the sections in the steaming stain (buffered to pH 4.0) in the oven for 15 min.(1% thionin sol.)

3. Transfer the sections to distilled water.

4. Transfer the sections to 80% ethyl alcohol and agitate to remove excess stain.

5. Place sections in aniline alcohol (50 cc. aniline, 450 cc. 95% ethyl alcohol). Change to fresh aniline as the color comes out.

6. When the sections are the desired color transfer to 95% alcohol and wash through 6 changes of 95% to remove the aniline

7. Clear sections in oil of cajeput rinse in at least 2 changes of xylene and mount in H.S.R. mounting medium or Permount.
****

SANIDES HAIDENHAIN MYELIN STAIN

Solutions: 1. 2.5% ferric ammonium sulfate
2. 10% hematoxylin in absolute ethyl alcohol allowed to sit for 2 weeks in the sunlight unstoppered.
3. Saturated lithium carbonate (store in refrigerator)

1. Place sections in distilled water for 1 hr. and change to fresh distilled water for a second hour.

2. Place 15-20 small sections or 5-7 large sections in a petri dish of solution 1 and allow to stand overnight.

3. Prepare staining solution of 50 cc. distilled water, 5 cc. solution 2, and 3-7 ml. of solution 3.

4. Pour the ferric ammonium sulfate solution off the sections and rinse in 2 changes of distilled water.

5. Pour the staining solution over the sections and let stand for 5 hours being sure to agitate the sections every hour.

6. Pour off stain, rinse in 3 changes of distilled water,and let sections stand in 80% alcohol until clear of reddish color.

SANIDES HAIDENHAIN MYELIN STAIN
(continued)

7. Pour on fresh 80% alcohol and let stand overnight.

8. Place sections in 95% alcohol for at least 24 hrs.

9. Dehydrate sections in one change of 95% alcohol and one change of absolute alcohol.

10. Drain and put through 2 changes on xylene and mount as described for the thionin stain.