A Mapping of the Distribution of Lactic Dehydrogenase in the Brain of the Rhesus Monkey[1]

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This article provides a quantitative mapping of the distribution of lactic dehydrogenase (LDH) activity in the nuclei and regions of the brain of the rhesus monkey. It represents one part of a program of systematic mappings of the distribution of various enzymes in the brain. Previous publications described the distribution of succinic dehydrogenase (SD) in guinea pig brain (Friede, '59 a, b; '60 a, b), four oxidative enzymes and capillarization in a cat brain brain stem atlas (Friede, '61 a), and DPN-diaphorase in human brain (Friede and Fleming, '62).

These studies were deliberately confined to enzymes involved in different phases of oxidative breakdown of glucose. A comparison of the data shows similar distribution of these enzymes in most of the regions of the brain; however, some exceptional nuclei showed definite differences in gross distribution. In addition, some finer differences in cytological distribution were found throughout the brain. Therefore, the present study emphasizes the comparison and the differences of patterns of LDH and SD with extensive reference made to the articles quoted above.

MATERIALS AND METHODS

Brains from healthy adult rhesus monkeys were blocked immediately after removal and fixed for one day in 10% neutral formalin at 5°. Sectioning and incubation procedures were as those described for fixed tissue in a recent publication (Friede, Fleming, and Knoller, '63a). Thirty-μ sections were incubated for one hour using the histochemical method for lactic dehydrogenase[2] (Allen and Slater, '61). Duplicate series of sections were always prepared, one for histochemical studies and the other for quantitative LDH measurements. The series for histochemical studies was incubated in medium containing Nitro-BT and the sections were mounted in glycerol gel. The other series was incubated in medium containing INT and quantitative measurements of the histochemical reaction were made (Friede and Fleming, '62).

Extensive collections of enzyme histochemical series for several other enzymes, both in monkey brain and other species, were available for comparison.

RESULTS

A. Distribution of LDH in the monkey brain

Measurements of LDH showing the gradations of this enzyme in nuclei and regions of the monkey brain are given in table 1. These measurements represent averages of measurements from three brains; they summarize a total of 2,014 measurements. For a better understanding of the cytological distribution of LDH that the measurements represent, the figures should be compared with the plates. For example, the plates demonstrate whether the enzyme was distributed homogenously in the neuropil or in the perikarya of a given region. In other regions, the measurements may represent only a fraction of the true enzyme activity in a given nucleus; for example, when there were either perforating fiber bundles or a markedly reticular arrangement of the neuropil.

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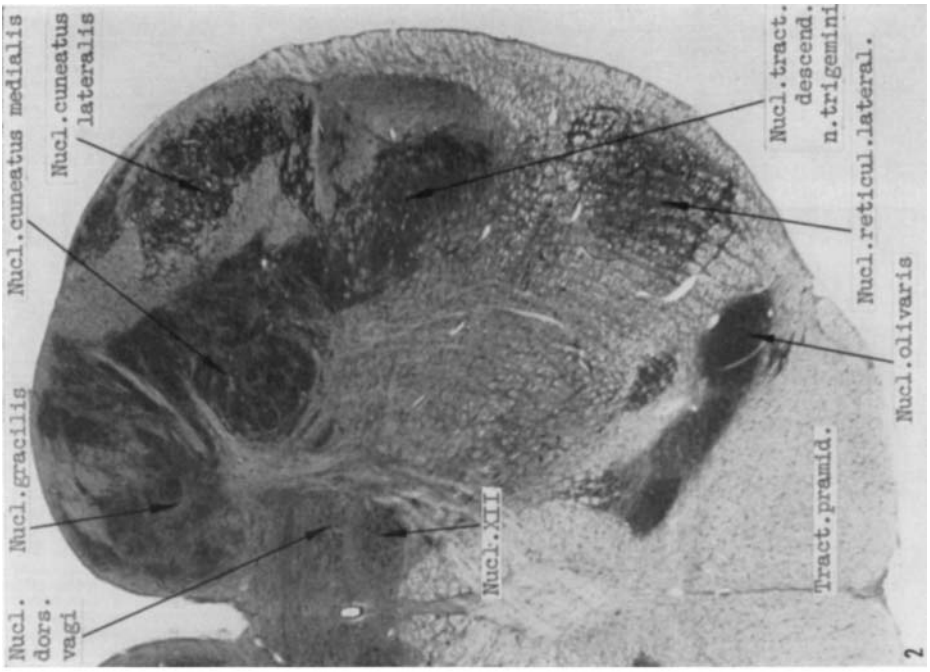
[2] The composition of the incubation media was as follows: 0.5 M NaDL lactate (Sigma), 3 ml; 0.01 M DPN, 3 ml; 0.1 M phosphate buffer, pH 7.5, 12 ml; 1 mg/ml Nitro BT, 7 ml; 0.03 M Potassium cyanide, 5 ml. pH adjusted to 7.5.

TABLE 1

Histochemical measurements of lactic dehydrogenase activity in the nuclei and regions of monkey brain

Medulla spinalis	
Columna ventralis	52 ± 3
Columna lateralis	54 ± 2
Lateral tracts	21 ± 5
Dorsal tracts	18 ± 1
Medulla oblongata	
N. cuneatus medialis	55 ± 6
N. cuneatus lateralis	58 ± 5
N. tractus desc. n. trigemini (general region)	44 ± 6
N. nervi hypoglossi	49 ± 5
N. tractus solitarii	35 ± 11
N. reticularis	38 ± 5
N. reticularis lateralis	52 ± 6
N. vestibularis medialis	55 ± 6
N. vestibularis lateralis	46 ± 5
N. prepositus hypoglossi	47 ± 7
Tractus pyramidalis	16 ± 1
Corpus restiforme	20 ± 2
N. nervi facialis	50 ± 4
N. reticularis gigantocellularis	41 ± 3
Nervus V	14 ± 2
Cerebellum	
Cortex (whole)	49 ± 11
N. fastigii	35 ± 9
N. dentatus	52 ± 7
Substantia alba	19 ± 3
Pons	
Nucl. reticularis	36 ± 3
N. nervi abducentis	62 ± 4
Griseum centrale	42 ± 7
N. coeruleus and griseum centrale	37 ± 4
N. nervi trigemini motorius	59 ± 6
N. parabrachialis	40 ± 2
N. parolivaris and n. olivaris superior	59 ± 4
N. reticularis Bechterew (retic. part)	44 ± 4
N. pontis (retic. part)	50 ± 6
Brachia pontis	17 ± 3
Brachium conjunctivum	22 ± 2
N. lemnisci lateralis	46 ± 2
Midbrain (colliculus inf.)	
Colliculus posterior (caudal portion)	74 ± 4
Griseum centrale (ventral portion)	43 ± 2
Midline n.	21 ± 1
N. mesencephalicus profundus	38 ± 4
Midbrain (colliculus sup.)	
Colliculus superior (laminae profundae)	43 ± 6
Regio S	36 ± 3
Griseum centrale (dorsolateral portion)	44 ± 4
Griseum centrale (ventromedial portion)	39 ± 3
Pretectal area	38 ± 5
N. nervi oculomotorii	64 ± 5
N. ruber	42 ± 4
N. niger	38 ± 5
Pedunculi cerebri	17 ± 2
N. interpeduncularis	42 ± 3
Diencephalon (thalamus)	
N. anterior (dorsalis)	48 ± 9
N. lateralis	39 ± 5
N. dorsomedialis	41 ± 5
Anterior midline nuclei	37 ± 3
Centre median	32 ± 3
Midline-nuclear group (deep transition into hypothalamus)	30 ± 3
Pulvinar	37 ± 4
N. geniculatus medialis	43 ± 4
N. geniculatus lateralis	41 ± 6
Capsula interna	18 ± 2
Tractus opticus	19 ± 3
N. reticularis thalami	39 ± 3
Diencephalon (subthal. centres)	
Tuber cinereum	44 ± 4
N. subthalamicus	51 ± 11
Zona incerta	35 ± 4
Peduncular zone of n. intercalatus	30 ± 4
Commissura anterior	21 ± 4
Corpus callosum	17 ± 2
Basal telencephalic centres	
N. caudatus (caput)	49 ± 8
Putamen	54 ± 6
Pallidum externum	33 ± 2
Pallidum internum	36 ± 4
Capsula interna	18 ± 2
Claustrum	35 ± 12
Amygdala	33 ± 5
Septal region	41 ± 7
Precentral motor cortex	
Laminae II-IV	44 ± 5
Frontal pole	
Laminae II-IV	40 ± 5
Substantia alba	20 ± 2
Parietal cortex	
Laminae II-IV	54 ± 3
Substantia alba	17 ± 3
Insular cortex (general)	43 ± 6
Occipital cortex	
Area 18, laminae II-IV	59 ± 9
Area 17, lamina IV	71 ± 6
Substantia alba	17 ± 2
Temporal cortex	
Laminae II-IV	39 ± 4
Substantia alba	21 ± 3
Deep temporal cortex	
Laminae II-IV	28 ± 3
Substantia alba	18 ± 2
Cornu ammonis (total)	45 ± 5
Subiculum	36 ± 2

The measurements indicate μg Formazan/0.131 mm^3 tissue 2h/36°C; they represent averages taken from a total of 2,014 measurements in the brains of three monkeys (Friede, Fleming and Knoller, '63b).



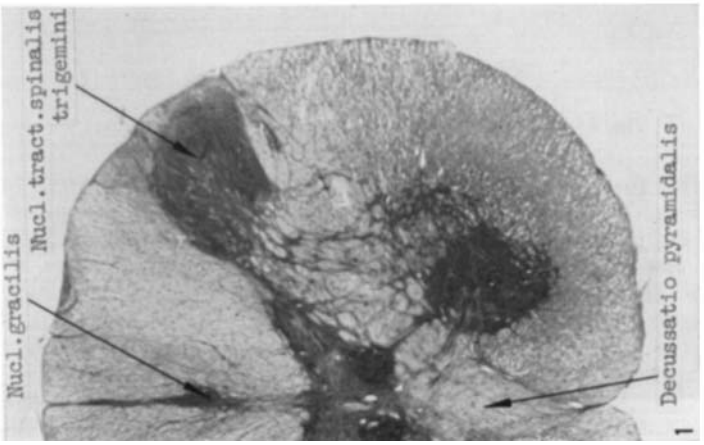


Fig. 1 Cervical cord.

Fig. 2 Medulla oblongata, caudal portion.

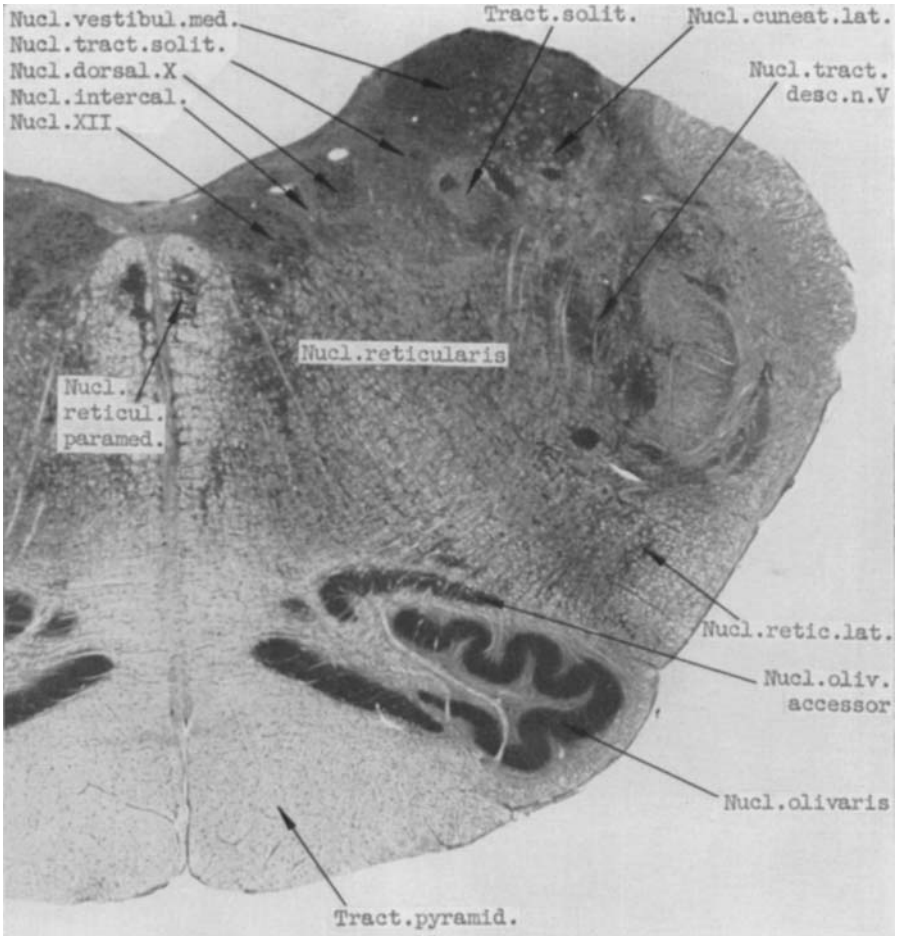


Fig. 3 Medulla oblongata, middle portion.

A description of the fine structure of the neuropil in individual nuclei and of the sharpness of the delineation of nuclei from each other was not included in this article because these features were extremely similar to those described in detail for DPN-diaphorase in the human brain (Friede and Fleming, '62).

If one compared the gradation of enzyme activity among the regions of the brain, the gradations of SD, DPN-diaphorase, and LDH among the nuclei of the medulla oblongata of various mammalian species were practically identical (figs. 1–6) except for the few particular nuclei listed below. Comparison of the measurements of the gradations of DPN-diaphorase in 41 nuclei of the human brain stem with LDH in the same nuclei of the monkeys showed a correlation coefficient of .80.

In the mesencephalon (figs. 7–9), there was more variation of enzyme patterns among species, while in the thalamus and telencephalon, marked differences were observed between guinea pig, monkey, (figs. 11, 12) and man. This was demonstrated by a comparison of microphotographs published in this article with such in previous publications (Friede, '59 a.b., '60 a.b.); it was substantiated by quantitative measurements of the histochemical reaction. The detailed results of this comparison will appear in a forthcoming publication.

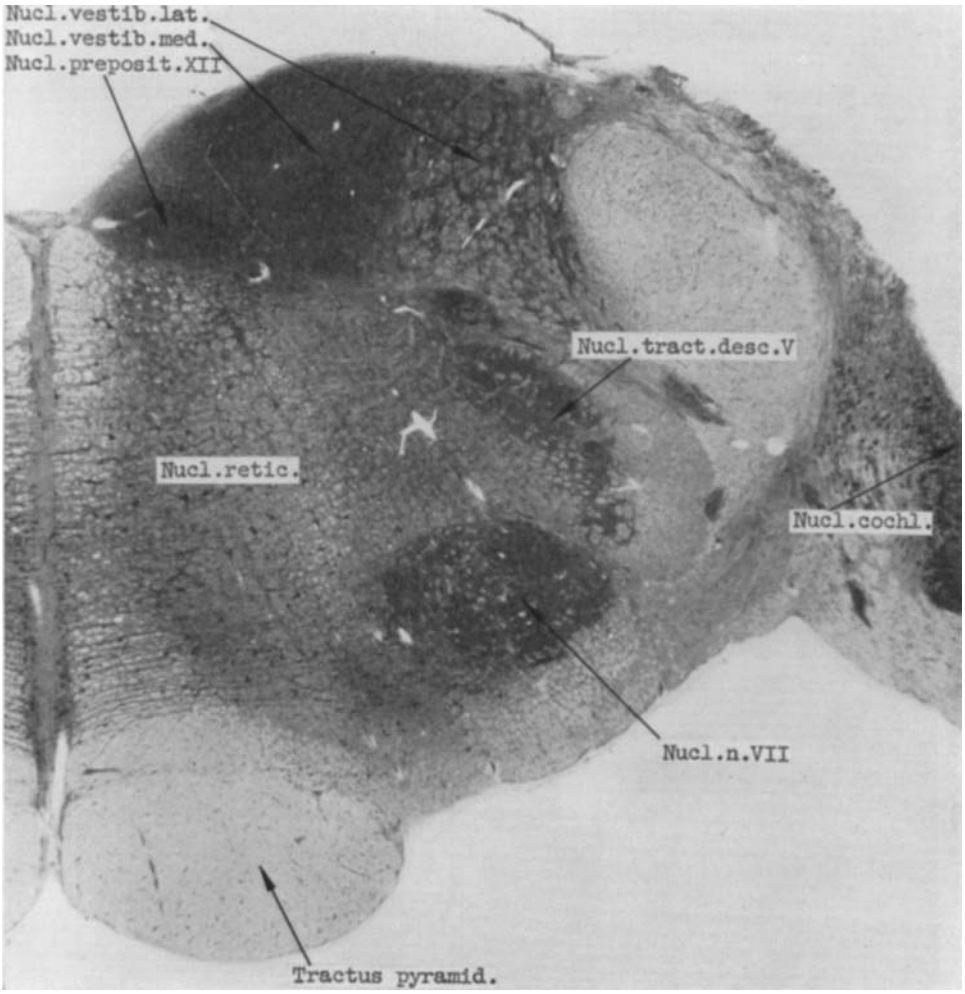


Fig. 4 Medulla oblongata, cranial portion.

The enzyme patterns in the areas of the cerebral cortex resembled those described in a histochemical mapping of the guinea pig cerebral cortex (Friede, '60 a). Sensory cortex (postcentral, auditory, and visual) showed strongest LDH. The visual cortex showed a striking lamination (fig. 15) similar to that of DPN-diaphorase in human area 17 (Friede and Fleming, '62). The strong LDH in the upper laminae terminated sharply at the borders of the area 17. Parietal, frontal (fig. 13), and temporal cortex (fig. 14), in declining order, showed weaker activity. Betz giant cells (fig. 13) were sharply outlined by strong LDH, while the cell patterns of the temporal cortex (fig. 14) were more diffuse.

B. Differences in the cytological distribution of SD and LDH

The cytological distribution of SD and LDH was similar; in some regions the neuropil contained much stronger activity than the perikarya, while in other regions the pattern was vice-versa (fig. 10). However, if one compared the distribution of SD and LDH in any given region, some difference of cytological distribution could be observed in that there was always more

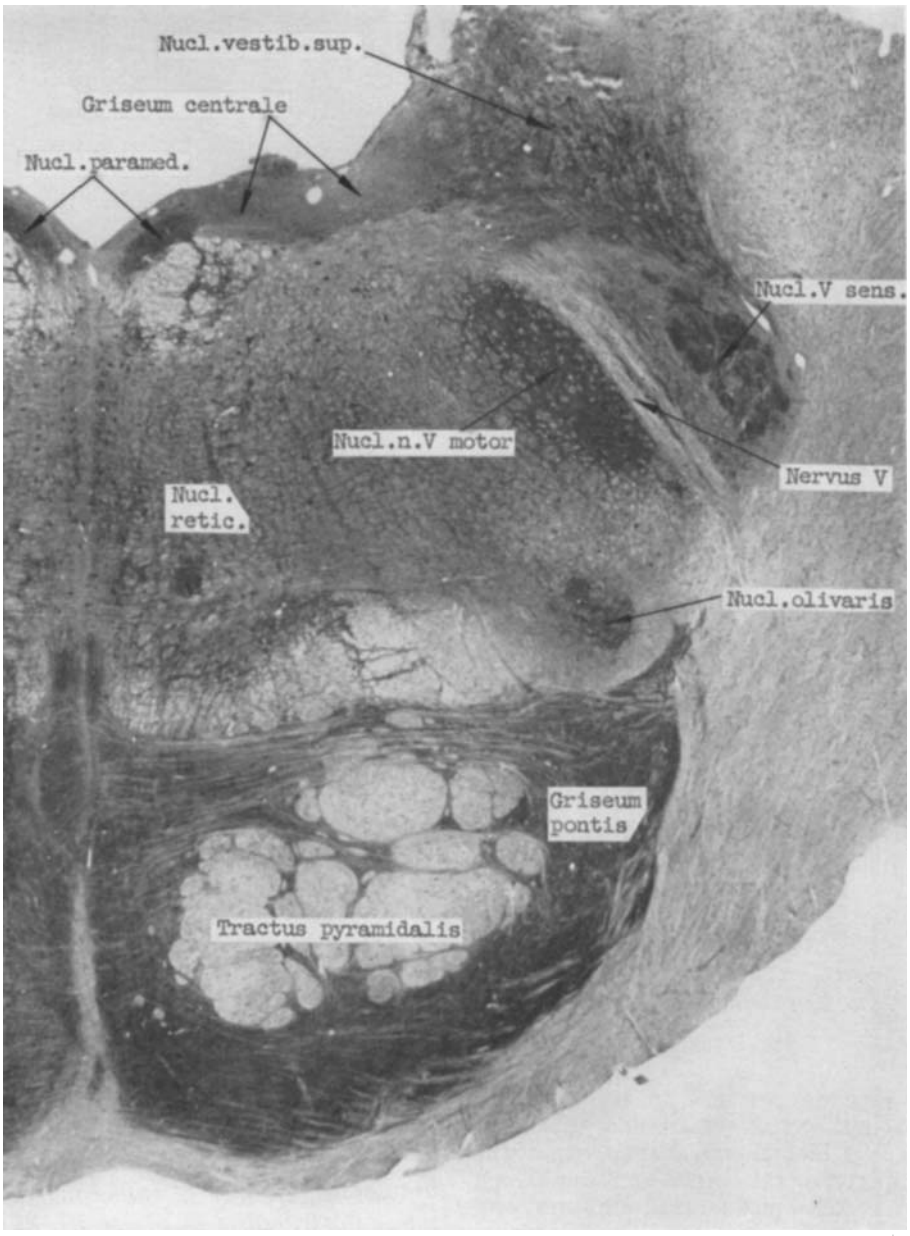


Fig. 5 Pons.

LDH than SD in the perikarya and more SD than LDH in the neuropil. A similar but less distinct difference in cytological pattern was observed when comparing SD and DPN-diaphorase (Friede, '61 a). A few characteristic regions, where this difference in cytological distribution of the enzymes was particularly evident, are described below.

The *second or third laminae of the cerebral neocortex* (figs. 13–15) had very little perikaryal SD, but the activity was strong in the neuropil where nerve cells were often discernible as "optically empty"

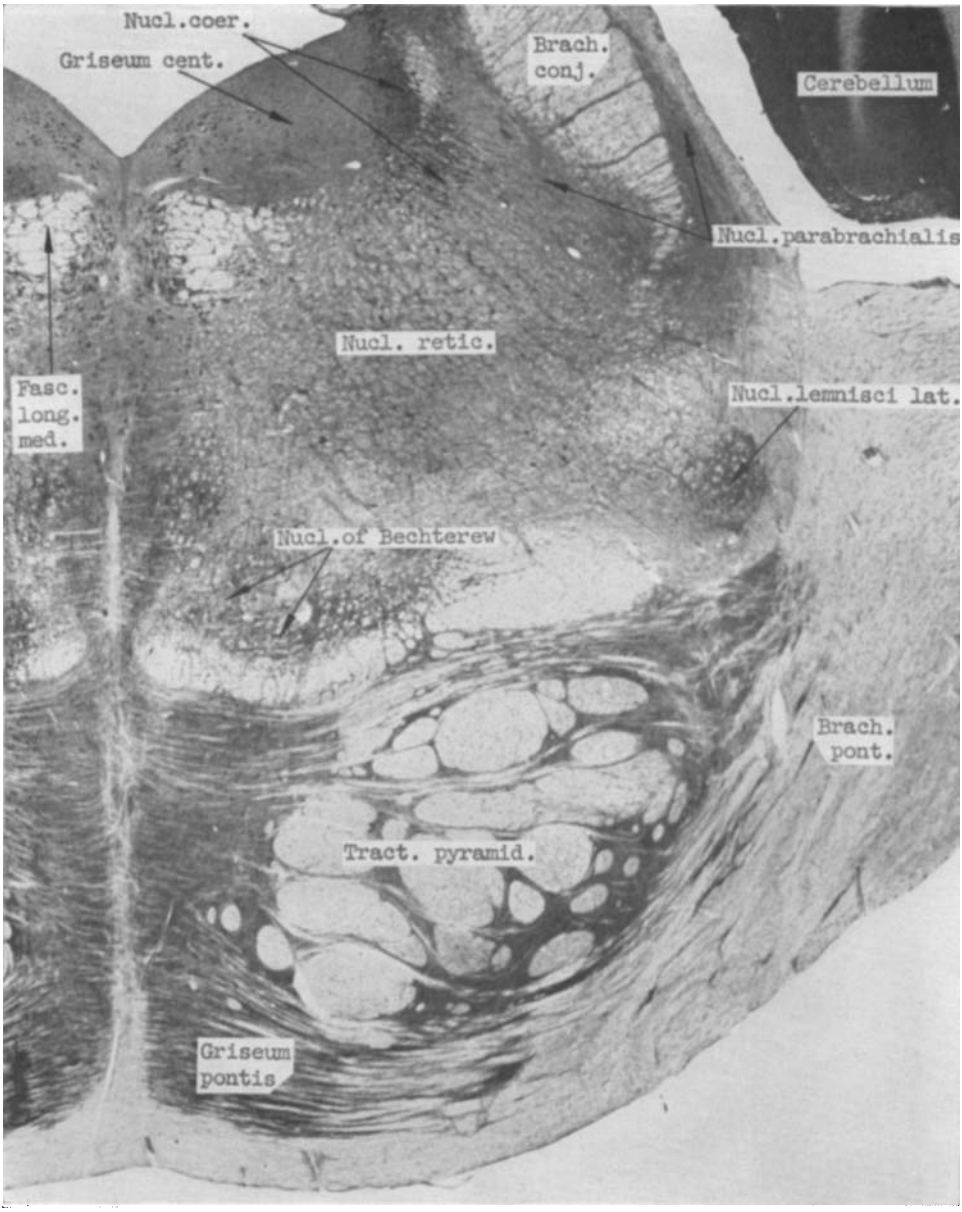


Fig. 6 Pons.

areas. On the other hand, LDH permitted one to recognize an abundance of nerve cells with slightly more activity in the perikarya than in the adjacent neuropil (fig. 14).

In the *fascia dentata,* the pyramidal cells showed no SD reaction; in the adjacent neuropil of the molecular layer, there was a strong reaction that distinctly increased in the distal portion of the layer. In contrast, there was marked LDH activity in the perikarya of the pyramidal cells (fig. 18) and the activity appeared to be equal throughout the molecular layer; sometimes there was more LDH in the proximal portion of the layer (fig. 16).

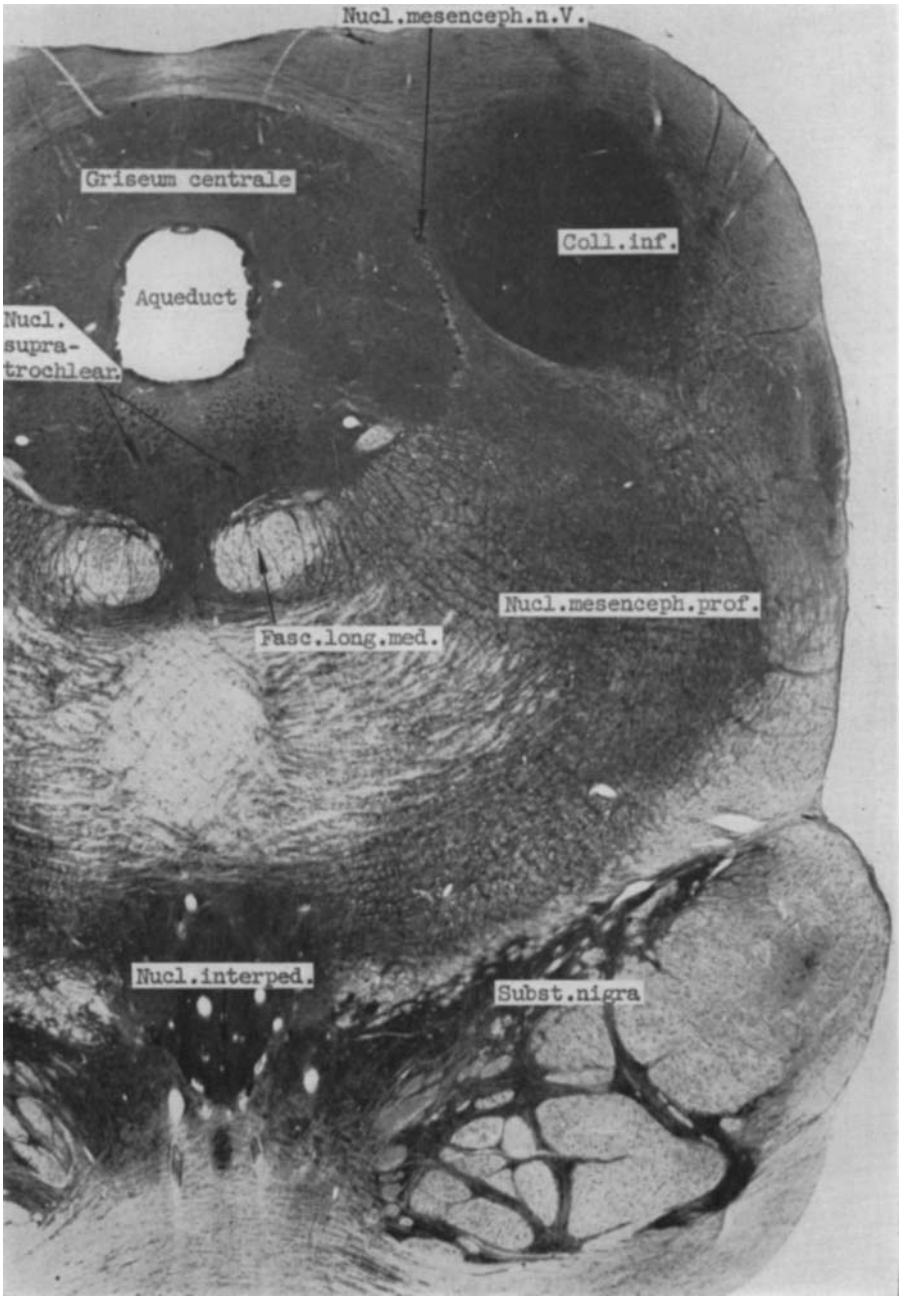


Fig. 7 Midbrain at the level of the inferior colliculus.

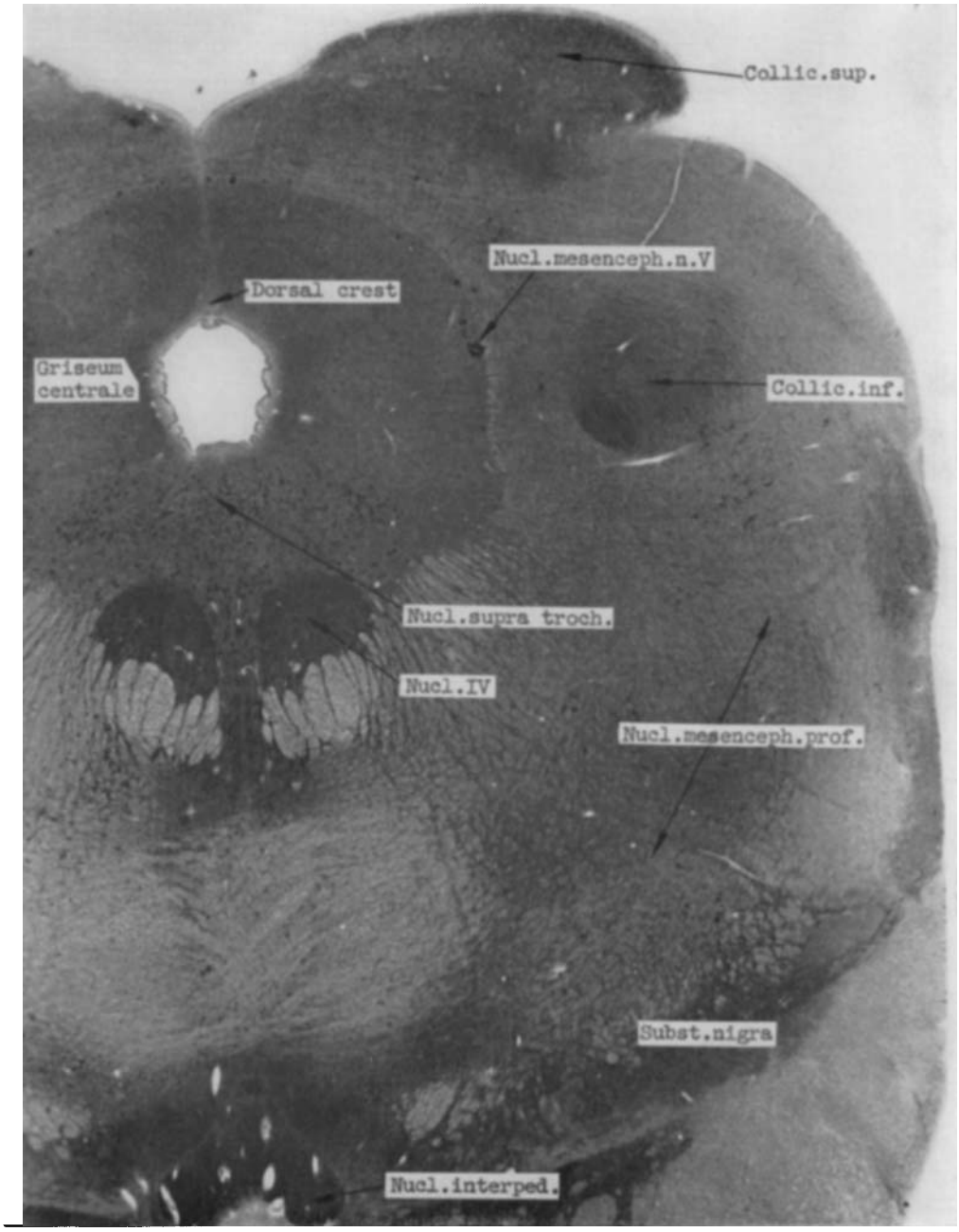


Fig. 8 Midbrain at intercollicular level.

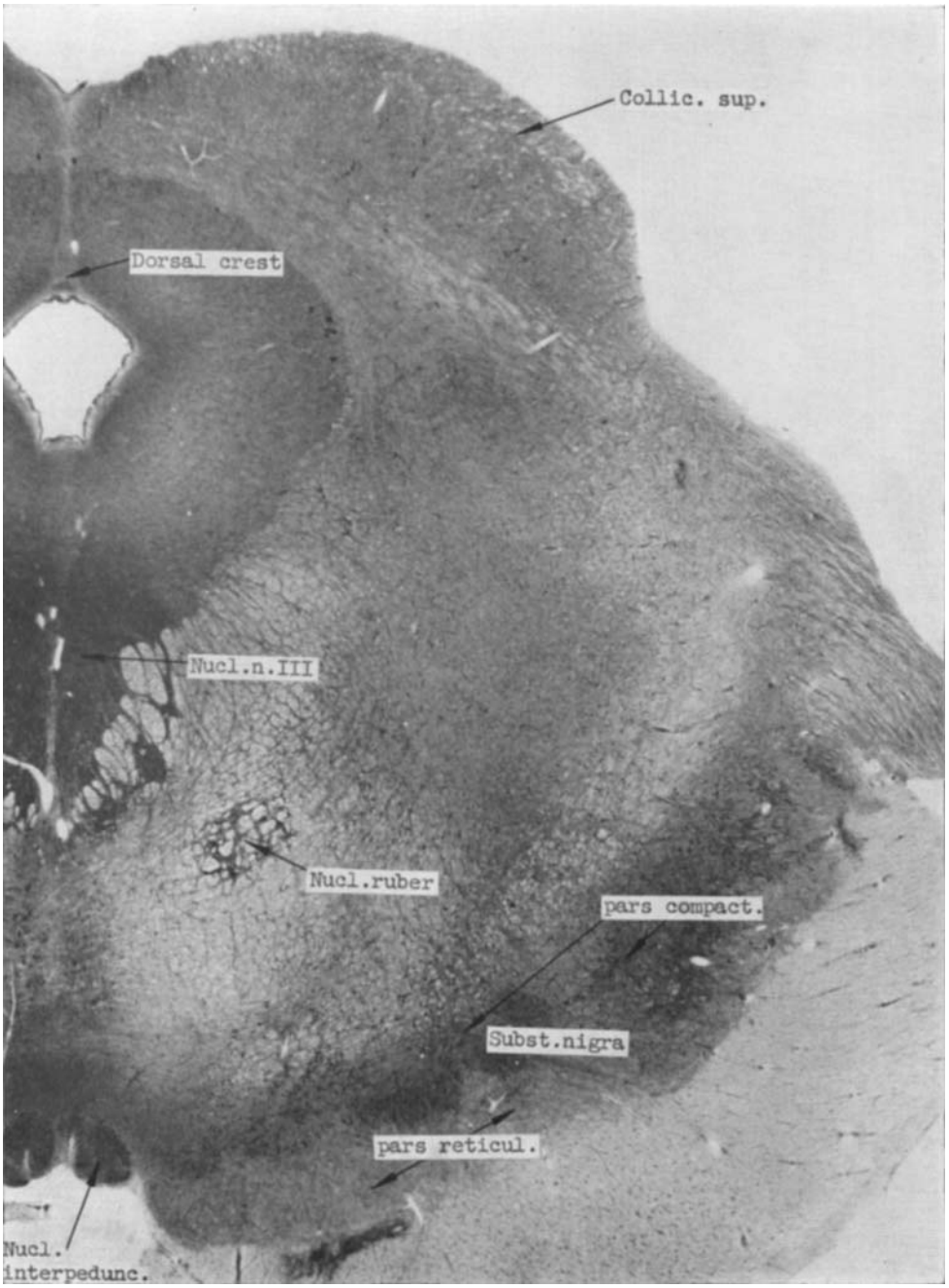


Fig. 9 Midbrain at the level of the upper colliculus.

Fig. 10 Basal telencephalic centers.

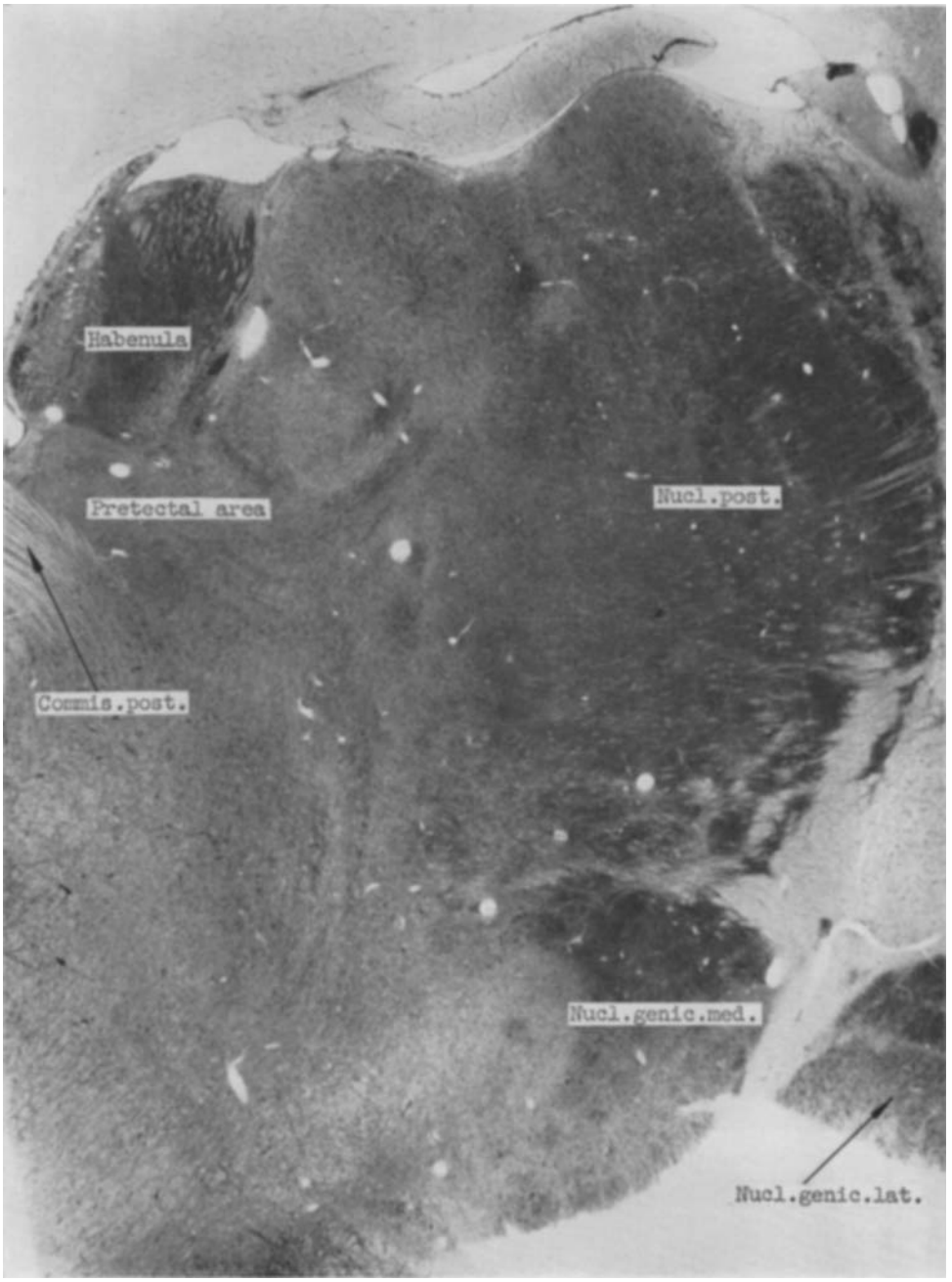


Fig. 11 Thalamus, posterior portion.

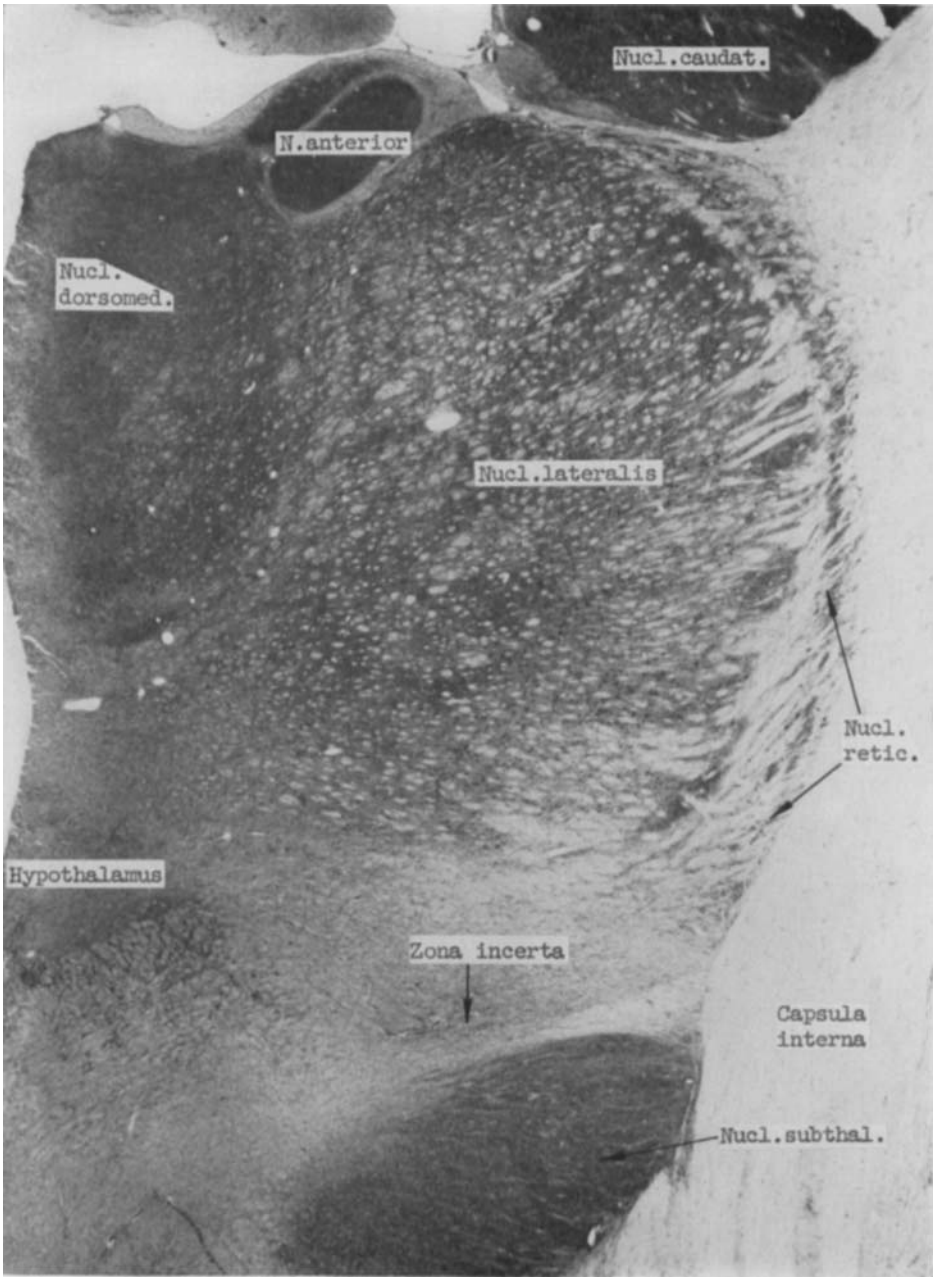


Fig. 12 Thalamus, anterior portion.

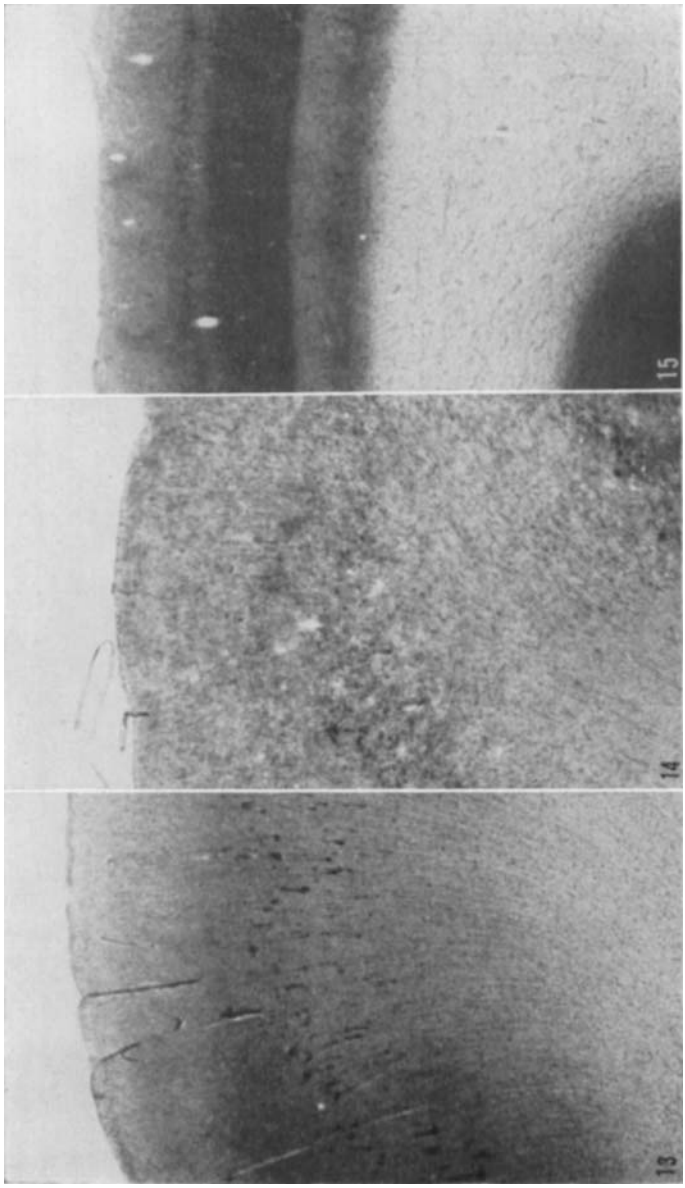


Fig. 13 Precentral motor cortex.

Fig. 14 Temporal cortex.

Fig. 15 Visual cortex (area 17).

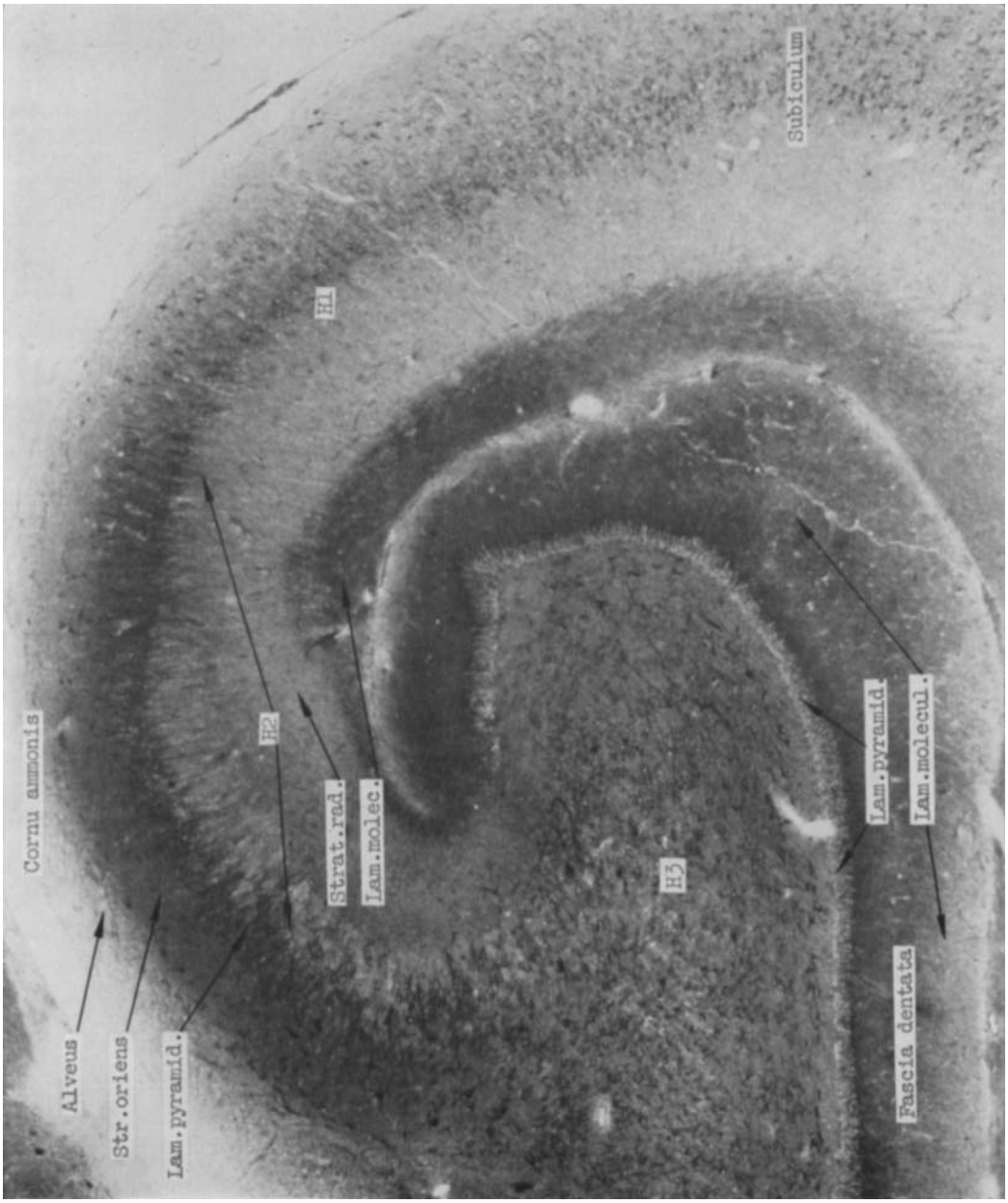


Fig. 16 Cornu ammonis and fascia dentata; survey.

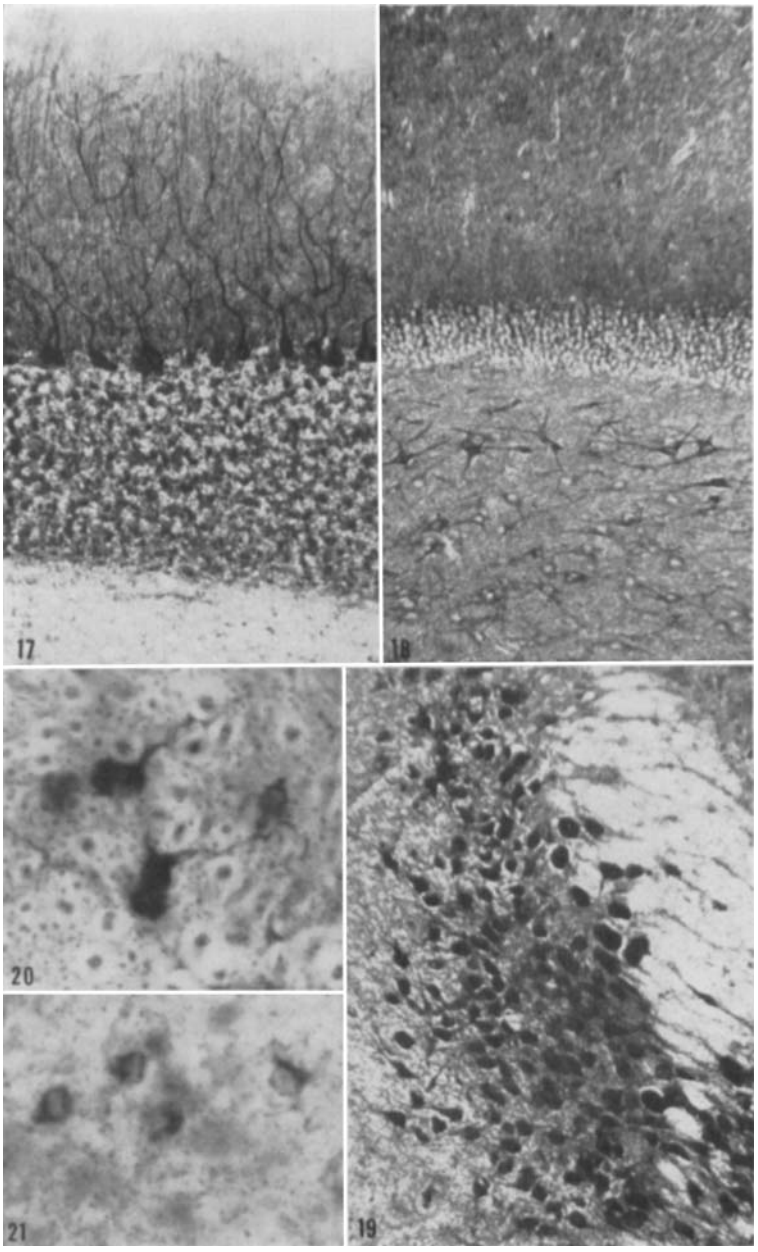


Fig. 17 Cerebellar cortex.

Fig. 18 Fascia dentata.

Fig. 19 Nucleus coeruleus.

Fig. 20 Oligodendroglia, type II, in white matter; note the reaction in axons and absence of a reaction in myelin sheaths.

Fig. 21 Oligondendroglia, type III or IV.

Many *thalamic nuclei*, as well as all *motor cranial nerve nuclei* of the medulla oblongata, showed very strong SD, both in the large nerve cells and in the neuropil, so that the distinction of the perikarya from the neuropil was difficult. With the LDH reaction, the perikarya and their long dendrites were sharply distinguished from the much weaker reaction in the neuropil (figs. 4, 5, 12).

In the *central gray matter*, perikarya were not distinguishable from the very homogenous distribution of SD in the neuropil. LDH often permitted one to recognize the perikarya of small nerve cells which had slightly more activity than the adjacent neuropil (figs. 7–9).

In the *cerebellar cortex*, the synaptic glomeruli and the Purkinje cell perikarya had equally strong SD activity. In the molecular layer, the proximal branches of Purkinje cell dendrites were difficult to distinguish against the background of strong activity homogenously distributed in the neuropil. The perikarya of the Purkinje cells had markedly stronger LDH reaction than the synaptic glomeruli (fig. 17); likewise, the dendrites had stronger activity than the neuropil of the molecular layer, so that the cells and dendritic ramifications were demonstrated with excellent contrast resembling silver impregnation. Also, fibers and perikarya of Bergmann glia cells and some glial perikarya in the molecular layer were distinguishable with LDH reaction, but were never seen with the SD reaction (fig. 17).

In the *colliculus superior*, the stratum griseum superficiale was characterized by extremely strong SD in the neuropil; the gradient between the molecular layer and the deeper cellular layers was much smaller for LDH (figs. 8, 9).

These regions represented particularly striking examples of a shift of enzyme pattern which was virtually ubiquitous throughout the central nervous system. This was not artifact produced by methodological differences: for example, the reaction for SD did not permit, at any staining intensity, the demonstration of Purkinje cell dendrites as they were seen with the LDH reaction. Also, the distribution of LDH was identical in formalin-fixed and in unfixed sections.

C. A system of nuclei with exceptionally strong LDH activity

The pattern difference observed when comparing SD and LDH distribution was excessively accentuated in some particular nuclei which were distinguished from all others by having very little SD activity throughout, but had extremely strong LDH activity in the perikarya of their nerve cells. These exceptional nuclei were the nucl. dorsalis vagi (figs. 2, 3), the nucl. coeruleus (figs. 6, 19), scattered cell groups near the raphe in the floor of the fourth ventricle at the level of the pons (figs. 5, 6), the pars compacta of the substantia nigra (fig. 9), the nucl. supratrochlearis (fig. 7), the nucl. paraventricularis and supraopticus in the hypothalamus, scattered cell groups in the dorsal and lateral aspects of the hypothalamus, a cell group at the ventral aspect of the pallidum with a small extension ventrally between putamen and pallidum, scattered cells in the ventral septal area, scattered cell groups dorsal and ventral of the anterior commissure, and the subcommissural organ.

Measurements of SD in some of these nuclei (Friede, '61a) showed lowest SD activity of all nuclei of the gray matter, ranging only slightly above white matter. In contrast, LDH was extremely strong in these nuclei.

For example, in the nucl. coeruleus, the nerve cells contained no, or extremely little, SD reaction and were discernible in sections only by the differences of the refractive index of their perikarya. On the other hand, the LDH reaction in these cells was excessive (fig. 19), and exceeded that in any other nucleus at this level of the brain stem. Similar observations were noted for all the other nuclei named above. In the substantia nigra, the difference between the pars compacta and pars reticularis was of interest. The pars compacta was barely distinguishable with the SD reaction; the cells of the pars reticularis showed a medium reaction. This pattern was inverse for LDH, which was quite strong in the cells of the pars compacta (fig. 9).

The area postrema also seemed to belong in this group of exceptional regions,

but the amount of activity in it apparently was subject to individual variations. The amygdala and part H_2 of the hippocampus (fig. 16) likewise had disproportionally more LDH than SD activity, but the difference was not as marked as in the other exceptional nuclei.

D. Distribution of LDH in white matter

The distribution of LDH in white matter of the monkey brain was quite similar to that of DPN-diaphorase in human brain (Friede, '61b). The white matter in comparison with gray matter had relatively stronger LDH than SD due to the much stronger LDH reaction in the oligodendroglia cells and, to some extent, in the capillaries and axons.

Of particular interest were regional differences of the number of oligodendroglia cells and the amount of LDH activity in them. Numerous oligodendroglia cells were observed in the white matter of the cerebral and cerebellar hemispheres and in many fibre tracts of the brain stem. These cells were identified as being oligodendroglia by counterstaining their nuclei in enzyme histochemical preparations with chrome alum-gallocyanin. Such cells showed no enzyme reaction in their nuclei and a small margin of cytoplasm with a moderate reaction for LDH (fig. 21). These cells were identified as being Hortega's oligodendroglia of types III and IV (Friede, '61b).

A different morphology of cells was observed in several tracts containing thick axons with strong LDH. In these tracts, most of the oligodendroglia cells were larger and had markedly stronger LDH than those in the white matter of the hemispheres (fig. 20). Representative for this pattern were the roots of motor cranial nerves, the fasciculus longitudinalis medialis, the pyramidal tract, portions of the capsula interna, and the portions of the frontal white matter and corpus callosum containing radiations or commissures, respectively, of the precentral cortex. In sections cut perpendicular to the course of the fibers, one could see the oligodendroglia processes attached to or encircling nerve fibers (fig. 20) or else glial perikarya intimately attached to the fibers, resembling Schwann cells. The latter was observed particularly frequently in the cranial nerve roots. By these features, the cells were identified as Hortega's oligodendroglia types I and II (Friede, '61b).

Enzyme histochemical preparations, therefore, supported the morphological distinction of types of oligodendroglia cells. These cells were observed in a characteristic distribution among fiber systems, in keeping with Hortega's ('28) original notes as to where these types of oligodendroglia cells can be found.

Throughout the white matter, astrocytes did not show activity of LDH; however, the molecular layer at the surface of the cerebral cortex showed numerous astrocytes with strong activity of LDH.

DISCUSSION

The mapping of LDH marks a new phase of our study of enzyme patterns in mammalian brain because it represents an enzyme involved in the glycolytic breakdown, as compared with SD which represents the citric acid cycle. Certain conclusions as to the relative significance of these two pathways of glucose breakdown can be drawn from the data. The vast majority of nuclei in the brain have amazingly similar gradations of LDH, SD, and capillarization; this indicates that these patterns do reflect general gradations of energy metabolism in the nuclei of the nervous system. The over-all differential gradation of these enzymes between perikarya and neuropil suggests the working hypothesis that perikarya depend relatively more on glycolytic metabolism than the dendrites in the neuropil do; dendrites, or neuropil, seem to depend relatively more on citric acid cycle.

Even more interesting are the nuclei characterized by marked differences of the distribution of LDH and SD. It certainly represents more than a coincidence that the capillarization of these nuclei showed an exceptional disproportion to their SD activity (Friede, '61a). Strong LDH activity in itself does not prove that this enzyme is really being utilized up to capacity. However, the very weak SD activity does prove that the nuclei cannot utilize the citric acid cycle to the same extent as other nuclei. If these nuclei utilize

glucose at all, it must be assumed that their metabolism is predominantly glycolytic. In addition, current investigations in the same nuclei showed exceptionally strong activity of two enzymes involved in glucose shunt metabolism; that is, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase.

All the data we have strongly suggests that this group of nuclei with particularly strong LDH is metabolically exceptional as it depends more on glucose shunt and glycolytic metabolism than other regions of the brain do. Observations of the resistance to anoxia of the supraoptic and paraventricular nucleus (Grenell and Kabat, '47) or on the long survival following circulatory arrest of the dorsal vagal nucleus (Heymans et al., '34, '38; Kalle, '33) are well in keeping with this interpretation. Our observations also suggest that oligodendroglia cells depend less on the citric acid cycle than nerve cells do.

This article represents an interim report of a continued effort to map enzyme patterns in the mammalian brain. The detailed mapping of one given enzyme, such as SD, would seem to be merely of academic interest. If such studies are continued along with parallel studies of other enzymes, similarities and dissimilarities of the patterns become evident and better foundations are obtained for the metabolic interpretation of enzyme patterns. Such information becomes available only through systematic, comparative mappings of most, or all, the nuclei of the brain.

SUMMARY

This article reports microphotographically the distribution of lactic dehydrogenase in the monkey brain. Extensive measurements of the histochemical reaction document the characteristic gradations among nuclei. The distribution of this enzyme closely resembles that of succinic dehydrogenase and DPN-diaphorase which had been mapped in preceding investigations. The patterns of these enzymes are almost identical in the medulla oblongata of various species, while differences among species increase in caudo-cranial order.

In contrast with these similarities of enzyme patterns, a number of differences could be observed in certain nuclei and also as to the cytological distribution of enzyme activity. Lactic dehydrogenase always was somewhat stronger in the cell bodies and somewhat weaker in the neuropil than succinic dehydrogenase. Likewise, lactic dehydrogenase was relatively stronger than succinic dehydrogenase in oligodendroglia cells, of which several types could be distinguished histochemically.

A system of histochemically exceptional nuclei was described; they were characterized by very little succinic dehydrogenase but extremely strong lactic dehydrogenase. These nuclei probably depend metabolically more on glycolytic and glucose shunt metabolism than on citric acid cycle.

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