Light Microscopic Immunocytochemical Localization of Pyruvate Dehydrogenase Complex in Rat Brain: Topographical Distribution and Relation to Cholinergic and Catecholaminergic Nuclei

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Pyruvate dehydrogenase complex (PDHC; EC 1.2.4.1, EC 2.3.1.12 and EC 1.6.4.3) includes 3 catalytically active mitochondrial enzymes involved in the formation of cellular energy through the tricarboxylic acid cycle and in the synthesis of ACh. We sought to determine whether immunocytochemically detected PDHC was enriched in neurons of the rat CNS, and, if so, whether the perikarya containing higher levels of PDHC immunoreactivity were differentially distributed with respect to their size or location within nuclear groups containing ACh, catecholamines or other unidentified transmitters. Under the labeling conditions used in this study, the peroxidase–antiperoxidase immunoreaction product for PDHC was detectable principally in neuronal perikarya. The intensity of immunoreactivity within perikarya was variable as judged visually and by cellular, computer-assisted densitometry. In the *forebrain*, the most intensely labeled perikarya were seen in the medial septal nuclei, the nuclei of the diagonal band, the nuclei basalis, the dorsal and ventral striatum, and the entorhinal cortex. *More caudally*, intense immunoreactivity was detected in perikarya in the supraoptic hypothalamic nuclei, reticular thalamic nuclei, lateral substantia nigra, most of the tegmental nuclei, lateral nuclei of the trapezoid body, raphe pontis and obscuris, and the caudal part of the lateral reticular nuclei. In addition, many of the motor nuclei of the cranial nerves, including the dorsal motor nuclei of the vagus and the hypoglossal nuclei, and the nucleus ambiguus contained perikarya with intense PDHC labeling. Densitometry revealed no differences in intensity of immunoreactivity in soma of varying sizes. However, the intensity of neuronal labeling for PDHC was significantly greater in several nuclear groups that were shown in adjacent sections to contain cholinergic, but not catecholaminergic, enzymes. In contrast, the primary olfactory

cortex, pyramidal cell layer of the regio inferior of hippocampus, and the Purkinje cell layer of the cerebellum were regions having perikarya with intense PDHC immunoreactivity but lacking both the synthetic and the degradative enzymes for ACh. These results provide the first morphological evidence that PDHC, a general metabolic enzyme complex, is enriched in selective perikarya that are heterogeneously distributed in brain and are especially abundant in many of the regions containing cholinergic neurons. The heterogeneity of PDHC immunoreactivity suggests that certain cholinergic as well as noncholinergic nuclei may be selectively vulnerable to mitochondrial diseases involving pyruvate utilization.

Pyruvate dehydrogenase complex (PDHC) [pyruvate dehydrogenase (PDH), EC 1.2.4.1; dihydrolipoyl transacetylase, EC 2.3.1.2; dihydrolipoyl dehydrogenase, EC 1.6.4.3; PDH_E kinase, EC 2.7.1.9; and PDH_E phosphatase, EC 3.1.3.43] is essential for the conversion of glucose into energy via the mitochondrial tricarboxylic acid cycle (Reed, 1981; Pratt et al., 1982; Teague et al., 1982; Sheu et al., 1983, 1984, 1985a; Stepp et al., 1983; Sheu and Kim, 1984). The cellular localization of PDHC in the CNS has not been investigated microscopically, largely due to the expectation that an enzyme complex with such general metabolic functions would be homogeneously distributed, with the possible exception of higher concentrations in large neurons having extensive terminal fields (Jones and Cowan, 1983). However, 2 lines of evidence suggest that PDHC might be enriched in certain central neurons irrespective of their size. First, PDHC has been shown to be involved in the synthesis of ACh (Tucek and Cheng, 1974; Gibson et al., 1975; Jope and Jenden, 1980; Perry et al., 1980; Sterri and Fonnum, 1980). Second, adrenergic neurons in the rostral ventrolateral medulla of the rat brain have been shown by quantitative electron microscopic analysis to have larger numbers of mitochondria than most neurons of comparable size, suggesting that these cells may also contain greater concentrations of associated mitochondrial enzymes such as PDHC (Milner et al., 1987). In this study, we used the peroxidase–antiperoxidase (PAP) immunocytochemical method to localize a rabbit antiserum to PDHC in aldehyde-fixed sections of rat brain in order to determine (1) whether immunoreactivity for PDHC was more readily detected in neuronal perikarya and, if so, (2) their distribution and (3) the relationship to neuronal size or location within nuclear groups containing either cholinergic or catecholaminergic perikarya. The cholinergic neurons were identified in adjacent sections by either immunocytochem-

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ical localization of CAT or the histochemical demonstration of AChE. The catecholaminergic neurons were identified by immunocytochemical localization of the synthesizing enzyme tyrosine hydroxylase (TH).

Materials and Methods

Antisera. Antiserum against a highly purified bovine kidney PDHC was produced in rabbits by previously described methods (Sheu and Kim, 1984; Sheu et al., 1985a). As described elsewhere, the antiserum cross-reacted with both rat and human PDHC (Sheu et al., 1985a) but did not discriminate between PDHC and phospho-PDHC (Sheu and Kim, 1984). The antiserum also reacted strongly with the thiamine pyrophosphate-dependent peptide (α E1) of the first catalytic enzyme (pyruvate dehydrogenase E1, EC 1.2.4.1) of PDHC; but weakly with the other peptide (β E1) of the same enzyme. Moreover, the antiserum cross-reacted with the second (E2; lipoate transacetylase EC 2.3.1.12) and third (E3; lipoamide dehydrogenase EC 1.6.4.3) catalytic enzymes of the complex. The E3 enzyme is also a component of the α -ketoglutarate dehydrogenase complex (KGDHC; Koike et al., 1974) and of the branched-chain keto-acid dehydrogenase complex, BCDHC (Pettit et al., 1978); thus, the antiserum may also recognize KGDHC and BCDHC.

The antiserum was further tested for specificity by an adsorption technique employing PDHC purified by previously described methods (Sheu et al., 1983; Sheu and Kim, 1984). For this, 50–200 μ g purified PDHC was added to 1 ml of the primary antiserum at the working dilution of 1:1000. After 2 hr of incubation at 37°C, the complexes formed were removed by centrifugation and the supernatant was substituted for the primary antiserum in the immunocytochemical labeling procedure on sections of tissue.

A monoclonal antibody to CAT produced from rat-mouse hybridomas according to methods described by Eckenstein et al. (1981) was obtained from Boehringer-Mannheim Biochemicals. Antiserum to TH was generously supplied by Dr. T. H. Joh (Department of Neurology, Cornell University Medical College) and was produced in rabbits by previously described methods (Joh and Ross, 1983). The antiserum has been tested for specificity by production of a single immunoprecipitate exclusively with the purified antigen, and by the absence of immunocytochemical labeling with the antiserum after adsorption with excess antigen (Joh and Ross, 1983).

Preparation of tissue. Twenty male Sprague-Dawley rats (180–220 gm) from Hilltop Lab Animals, Inc. were anesthetized with pentobarbital (50 mg/kg) and then perfused through the ascending aorta with 400 ml 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.6) for a 6 min period. At the termination of the perfusion, the brains were removed and cut into 4 mm coronal slices, which were placed for 30 min in the above fixative and then into 0.1 M phosphate buffer for temporary storage. Tissue that was to be frozen was placed additionally in 10% sucrose in 0.1 M phosphate buffer for 6 hr prior to sectioning.

Coronal sections (40 μ m) were cut from the entire brain either on a vibratome or a sliding microtome. A 1-in-6 series of the sections was processed through each of the following conditions: (1) PDHC immunocytochemistry, (2) CAT immunocytochemistry, (3) TH immunocytochemistry, (4) 2 AChE histochemical stains, and (5) thionin stain.

Immunocytochemistry. The antisera were localized in the tissue by a modification (Pickel, 1981; Pickel and Teitelman, 1986) of the PAP method of Sternberger (1979). Briefly, for the rabbit antisera, this procedure consisted of a sequential incubation of the sections with (1) a 1:1000 dilution of the PDHC antiserum or a 1:2000 dilution of TH antiserum for 18–24 hr; (2) a 1:50 dilution of goat anti-rabbit immunoglobulin (IgG) for 1 hr; and (3) a 1:100 dilution of rabbit PAP complex for 1 hr. The diluents were prepared with 1% goat serum in 0.1 M Tris-saline, pH 7.6. The PDHC antiserum also was diluted with and without 0.25% Triton X-100. All reactions were carried out at room temperature with continuous agitation. The peroxidase reaction product was demonstrated by incubation with 3,3'-diaminobenzidine and hydrogen peroxide. The sections then were mounted on glass slides coated with 1% gelatin, dehydrated, cleared in xylene, and mounted with a coverslip using histoclad.

For the rat monoclonal antibody the procedure was the same as above except that the sections were incubated sequentially with (1) a 1:40 dilution of the CAT antibody; (2) a 1:50 dilution of rabbit anti-rat IgG; and (3) a 1:100 dilution of rat PAP. Additionally, the diluent and washes were prepared in 1% rabbit serum in 0.1 M Tris-saline (pH 7.6) and the incubation of the primary antiserum was carried out at 4°C.

AChE histochemistry. A series of sections collected in 0.1 M phosphate buffer were mounted on gelatin-coated glass slides and stained for AChE according to the procedure of Hedreen et al. (1985). Subsequently, the slides were rinsed in water, dehydrated, cleared, and coverslipped. A second series of sections collected in 0.1 M sodium acetate buffer was processed according to a modification (Milner et al., 1983) of the Koelle (1954) copper thiocholine method.

Data analysis. Coronal sections throughout the entire brain were examined using Nomarski and bright-field optics on a Nikon Microphot microscope. Regional maps showing the distribution of perikarya with immunoreactivity for PDHC and CAT were drawn using a camera lucida attachment to a Leitz microscope. The boundaries of nuclei and fiber tracts, which were difficult to distinguish in the immunocytochemically stained sections, were delineated using both adjacent Nissl- and AChE-stained sections. The nomenclature for the nuclei and fiber tracts is that of Paxinos and Watson (1982).

All cells with immunoreactivity for PDHC significantly greater than the surrounding neuropil were plotted on the maps. The intensity of immunoreactivity for PDHC in each brain area was determined both visually and quantitatively using a semiautomated image analysis system (IAS; Eycom II and a Microvax computer) (Iadecola et al., 1983). For the latter, neurons from each anatomical area sampled were visualized using bright-field optics on a Leitz microscope attached to the IAS. Background optical density (O.D.) values generated by fluctuations in the light transmission to and electronically within the TV monitor were subtracted from the O.D. in tissue. The O.D. was standardized for every section by relating it to a Kodak gray scale. The average O.D. values of cytoplasm (i.e., neuronal area minus nuclear area) and corresponding cytoplasmic area were determined for each perikaryon. Regions that contained perikaryal labeling for PDHC were further correlated with the presence or absence of immunoreactivity for CAT and TH and staining for AChE.

Results

Labeling conditions

The most intense immunocytochemical labeling for PDHC was observed in unfrozen vibratome sections labeled in the presence of Triton X-100. This immunoreactivity was abolished by preadsorbing 1 ml of the antiserum with 100 μ g PDHC. Frozen sections, with or without the detergent, exhibited more diffuse and significantly reduced intensities of labeling for PDHC.

In vibratome sections labeled in the presence of Triton, PDHC immunoreactivity was primarily localized in neuronal perikarya and proximal, presumably dendritic, processes. However, more dense varicose processes having the characteristics of axons and axon terminals were detected in certain brain areas (e.g., the suprachiasmatic nucleus).

Quantitative analysis of PDHC immunoreactivity in relation to cell size

Low levels of PDHC immunoreactivity (O.D. = 0.030–0.040), above that seen in control sections, were present throughout the neuropil. However, selected neuronal perikarya having more intense immunoreactivities (O.D. = 0.060–0.170) were recognized only in certain nuclei (Fig. 1, Table 1). Linear regression analysis of the densities of immunoreactivity seen in the cytoplasm of 196 perikarya located throughout 10 regions revealed (1) that the density of immunoreactivity was not related to the area of cytoplasm and (2) the increasing densities were continuous over the low–high ranges (Fig. 1A). The same results were seen in individual nuclei such as the nucleus basalis, which contained almost all medium-sized cells (Fig. 1B).

Regional distribution of PDHC-labeled perikarya

Perikarya showing substantial (>0.060 O.D.) densities of immunoreactivity for PDHC were heterogeneously distributed within the various regions of the brain. The labeled cells in each

A. ALL REGIONS **B. NUCLEUS BASALIS OF MEYNER**

Figure 1. Linear regression analysis correlating cytoplasmic area to optical density (O.D.) for PDHC-immunoreactive perikarya from selected brain regions. *A*, All regions sampled were nucleus (n.) ambiguus, n. basalis, cerebellar cortex, layer VI of cortex, vagus, hypoglossal n., locus coeruleus, lateral reticular n., mesencephalic and motor n. of trigeminal nerve, and reticular thalamic n. *B*, Only neurons from nucleus basalis were sampled. Each dot represents 1 neuron. r = linear regression correlation coefficient.

region were arbitrarily divided into 4 groups of varying intensities (see Table 1) for descriptive purposes. Perikarya size was divided into 3 categories: small, 8–12 μm ; medium, 12–20 μm ; and large, >20 μm .

Telencephalon. The cerebral cortex contained a large number of PDHC-immunoreactive neurons (Fig. 2, *A–E*) throughout the rostrocaudal extent of layer VI and in layers II and V of the temporal cortex (Fig. 2, *D, E*). In layer VI, the perikarya were medium-sized and fusiform, with their axes oriented parallel to the corpus callosum. The cells in layers II and V were rounder and smaller than those in layer VI. A few medium-sized PDHC-immunoreactive neurons also were found in the claustrum and endopiriform nuclei (Fig. 2, *A, B*). The primary olfactory cortex contained a number of medium-sized, round, and multipolar labeled neurons (Fig. 2, *A–C*). A few large, fusiform, and multipolar PDHC-immunoreactive neurons were scattered throughout the olfactory tubercle (Fig. 2, *A, B*). The caudate putamen, particularly the dorsal and lateral quadrants (Fig. 2, *A–C*), contained several isolated perikarya with immunoreactivity for PDHC. The labeling in the striatum was seen in large cells. Medium-sized labeled neurons also were observed in the globus pallidus, especially in the caudal half (Fig. 2*B*).

A large number of PDHC-immunoreactive neurons were distributed throughout the rostral–caudal extent of the septal complex. The cells were small in the medial septal nucleus and medium-sized in the nucleus of the diagonal band (Figs. 2, *A, B*; 3*A*). Moreover, the cell bodies were often elongated on the plane of the fibers in both nuclei. Numerous medium-sized, fusiform, or multipolar labeled neurons were observed in the nucleus basalis and ventral pallidum (Fig. 2, *A, B*). The nucleus

accumbens, septohypothalamic nucleus, lateral part of the bed nuclei of stria terminalis and of the dorsal fornix had a few, small PDHC-immunoreactive neurons (Fig. 2, *A, B*).

Perikarya labeled for PDHC were found throughout the hippocampal formation (Fig. 2, *C–E*). There was no apparent rostrocaudal gradient. However, the labeled cells were distributed differentially in each of the subfields. In regio superior, medium-sized neurons containing PDHC labeling were found near the alveus and scattered throughout stratum oriens and the pyramidal cell layer (Fig. 4*A*) and in the border between stratum radiatum and stratum lacunosum-moleculare. In regio inferior, only a few labeled neurons were found in the alveus and stratum radiatum; a large number of the pyramidal cells, however, were immunoreactive (Fig. 4*B*). The hilus of the dentate gyrus also contained a few large neurons with labeling for medium-sized PDHC (Fig. 4*C*). In the subicular complex, the majority of the PDHC-immunoreactive perikarya were found near the alveus and scattered throughout the cell layer of the subiculum proper. Few labeled perikarya were found in the pre-, para-, or post-subiculum. Many of the perikarya in layer II of the entorhinal cortex contained PDHC immunoreactivity (Fig. 4*D*). Moreover, a few labeled cells were found in layers III, IV, and VI. In the amygdaloid complex, medium-sized PDHC-immunoreactive neurons were found throughout the basolateral nucleus. A few labeled cells were also seen in the lateral aspect of the basomedial nucleus of the amygdala (Fig. 2*C*).

Diencephalon. The medial preoptic areas and dorsal and lateral hypothalamus contained a few medium-sized PDHC-immunoreactive perikarya. Several neurons having the same features were also observed in the paraventricular hypothalamic,

Table 1. Comparison of intensity and distribution of PDHC immunoreactive neurons to the distribution of CAT-labeled and AChE-positive cells

	Intensity of PDHC-immuno- reactive neurons	Presence of CAT- immuno- reactive neurons	Presence of AChE- positive cells
Telencephalon			
Cerebral cortex (layer VI)	++(0.109 ± 0.020)	-(+ ^c)	+ ²
Primary olfactory cortex	++	—	—
Clastrum	++	—	—
Endopiriform nucleus	++	—	+
Olfactory tubercle	+++	+ ^{a,b}	+ ^{3,4}
Caudate putamen	+++	+ ^{a,b}	+ ^{3,4}
Globus pallidus	+++	+ ^{a,b,c}	+ ^{3,4}
Ventral pallidum	+++	+ ^{a,b,c}	+ ^{3,4}
Septal complex			
Medial septal n.	+++	+ ^{a,b,c}	+ ^{1,3-5}
N. of diagonal band	+++	+ ^{a,b,c}	+ ^{1,3-5}
Septohypothalamic n.	+++	+ ^{a,b}	+ ³
Accumbens n.	+++	+ ^{a,b}	+ ²⁻⁴
Bed n. stria terminalis	++	+ ^{a,b}	+ ¹⁻³
N. basalis	+++ (0.153 ± 0.040)	+ ^{a,b,c}	+ ¹⁻⁴
Hippocampal formation			
Regio superior (stratum oriens)	++	—	+ ^{2,5}
Regio inferior (pyramidal cell layer)	++	—	—
Dentate gyrus (hilus)	++	—	+ ^{2,5}
Subicular complex	++	—	+ ^{2,5}
Entorhinal cortex (layer II)	+++	—	+ ^{2,5}
Basolateral amygdaloid n.	++	—	+ ^{2,3}
Diencephalon			
Dorsal hypothalamic area	+	—	+ ^{1,4}
Lateral hypothalamic area	++	—	+ ^{1,2,4}
Medial preoptic area	+	—	+ ³
Paraventricular n.	++	—	+ ^{1,2}
Supraoptic hypothalamic n.	+++	—	+ ²
Supramammillary n.	++	—	+ ^{1,2}
Lateral mammillary n.	++	—	+ ²
Arcuate n.	+	—	+ ^{1,2}
Reticular thalamic n.	++++ (0.161 ± 0.029)	—	+ ²
Anteromedial thalamic n.	+	—	+ ²
Anterodorsal thalamic n.	++	—	+ ²
Paratenial thalamic n.	+	—	+ ²
Medial habenula	++	+ ^c	+ ²
Ventroposterior thalamic n. med	++	—	—
Lateroposterior thalamic n.	+	—	—
Dorsolateral geniculate n.	+	—	+ ²
Medial geniculate n.	+	—	—
Mesencephalon			
Central gray	+	—	+ ²
Substantia nigra			
Reticulata	++	—	+ ²
Compacta	++	—	+ ^{1,2}
Lateral	+++	—	+ ²
Ventral tegmental area	++	—	+ ²
Red nucleus	+++	—	+ ²
Superior colliculus	+	—	+ ²
Inferior colliculus	++	—	+ ²
N. mesencephalic tract trigeminal nerve	++++ (0.160 ± 0.02)	—	+ ¹
Dorsal raphe n.	++	—	+ ¹

Table 1. Continued

	Intensity of PDHC-immunoreactive neurons	Presence of CAT-immunoreactive neurons	Presence of AChE-positive cells
Oculomotor n. (III)	++	+ ^{a-c}	+ ^{1,2,4}
Trochlear n. (IV)	+++	+ ^{a-c}	+ ^{1,4}
Pons			
Laterodorsal tegmental n.	+++	+ ^{a,b}	+ ^{1,2,4}
Dorsal tegmental n.	++	—	+ ^{1,2}
Ventral tegmental n.	+++	—	+ ^{1,2}
Reticulotegmental n. pons	++	—	+ ²
Pedunculopontine tegmental n.	+++	+ ^{a,b}	+ ^{1,2,4}
Locus coeruleus	+++ (0.092 ± 0.013)	—	+ ^{1,2,4}
Lateral parabrachial n.	++	+	+ ²
Raphe pontis n.	+++	—	+
Medial n. trapezoid body	++	—	+ ²
Lateral n. trapezoid body	+++	+	+
Lateral superior olive	++	—	+ ^{1,2}
Medial superior olive	+	—	—
Motor trigeminal n.	+++	+ ^{a,b}	+ ^{1,2,4}
Principal sensory trigeminal n.	++	—	+
Lateral vestibular n.	+++	—	+ ^{1,2,4}
Medial vestibular n.	++	—	+
Parvocellular reticular n.	++	+ ^{a,b}	+ ^{1,2,4}
Ventral cochlear n.	++	—	+
Facial n. (VII)	+++	+ ^{a-c}	+ ^{1,2,4}
Cerebellar cortex—Purkinje cell layer	++ (0.117 ± 0.013)	—	—
Cerebellar nuclei			
Fastigial n.	++	—	+ ²
Dentate n.	++	—	+ ²
Interpositus n.	+	—	+ ²
Medulla oblongata			
Paratrigeminal n.	++	—	+
N. spinal tr. trigeminal nerve (ventral)	++	—	+ ²
Raphe obscurus	+++	+	+ ¹
Dorsal motor n. of vagus (X)	+++ (0.133 ± 0.014)	+ ^{a-c}	+ ^{1,2,4}
Hypoglossal n. (XII)	+++ (0.124 ± 0.017)	+ ^{a-c}	+ ^{1,2,4}
Ambiguus n.	+++ (0.155 ± 0.017)	+ ^{a-c}	+ ^{1,2,4}
Inferior olive (above pyramidal tract)	++	+	+ ^{1,2}
Cuneate n.	+	—	+ ²
Lateral reticular n. subtrigeminal	+++	—	+ ⁴
Rostral ventrolateral medulla (medial)	++	+ ^b	+ ^{1,2,4}
Caudal ventrolateral medulla	+++	—	+ ^{1,2,4}
Prepositus hypoglossi	++	+ ^{a,b}	+ ^{1,2}

Intensity of PDHC-immunoreactivity: +, light (O.D. = 0.060–0.089); ++, medium (O.D. = 0.090–0.119); +++, dark (O.D. = 0.120–0.159); +++++, very dark (O.D. ≥ 0.160).

Background O.D. = 0.030–0.040.

Average O.D. measurements for cytoplasmic areas of at least 20 neurons are indicated in parentheses for selected nuclei.

Presence of CAT-immunoreactive neurons and AChE-positive cells indicated by plus sign.

^a CAT-immunoreactive neurons previously reported by Armstrong et al. (1983).

^b CAT-immunoreactive neurons previously reported by Satoh et al. (1983).

^c CAT-immunoreactive neurons previously reported by Houser et al. (1983).

¹ AChE-positive cells previously reported by Jacobowitz and Palkovits (1974) and Palkovits and Jacobowitz (1974).

² AChE-positive cells previously reported by Parent and Butcher (1976) and Butcher and Woolf (1984).

³ AChE-positive cells previously reported by Bigl et al. (1982).

⁴ AChE-positive cells previously reported by Satoh et al. (1983).

⁵ AChE-positive cells previously reported by Milner et al. (1983).

Figure 2. Camera lucida drawings of coronal sections arranged from rostral (*A*) to caudal (*B*) through the rat brain show the distribution of PDHC-immunoreactive neurons (*left*) and CAT-immunoreactive neurons (*right*). Note the similarities in the patterns of distribution. Each section is separated by a distance of approximately 1 mm and each symbol indicates one cell. Scale bars, 1 mm.

supramammillary, and lateral mammillary nuclei. In addition, the supraoptic hypothalamic nuclei contained a substantial number of large labeled perikarya.

Only a few thalamic nuclei contained PDHC-immunoreactive

perikarya. The reticular thalamic nucleus was the most striking since numerous large, multipolar, and fusiform labeled neurons (Figs. 2*C*, 5*A*) were observed throughout the entire nucleus. A few small PDHC-immunoreactive perikarya were found inter-

C

D

scattered among the medial aspect of the habenula and the paraventricular, anterodorsal, anteromedial, ventroposterior, and reuniens thalamic nuclei (Fig. 2C). The dorsal, ventral, and medial divisions of the medial geniculate all contained a few small labeled neurons, the majority of which were located in

the rostral half (Fig. 2D). A few small labeled cells also were found in the dorsal lateral geniculate and the dorsal anterior pretectal area, as well as dorsal to the posterior commissure.

Mesencephalon. Small PDHC-immunoreactive neurons were situated primarily in the dorsal and lateral aspects of the central

G

H

gray (Fig. 2D). The substantia nigra contained a few medium-sized, round, multipolar immunoreactive neurons which were topographically distributed within each region (Fig. 2D). These were located throughout the pars lateralis but predominantly in the caudal two thirds in the ventral part of the pars reticulata and in the caudal third of the pars compacta. A few labeled perikarya also were detected in the ventral tegmental area (Fig. 2D). The red, principal oculomotor (III) and trochlear (IV) nuclei contained a number of large, labeled neurons throughout their entire rostrocaudal extent.

A substantial number of the extremely large, round neurons in the nucleus of the mesencephalic tract of the trigeminal nerve were immunoreactive for PDHC (Fig. 2F). A few medium-sized neurons in the superficial and intermediate gray layers of the

superior colliculus, inferior colliculus, and dorsal raphe also were labeled (Fig. 2E).

Pons. Most of the tegmental nuclei contained PDHC-immunoreactive perikarya. The majority were located in the lateral dorsal nucleus, while only a few were observed in the dorsal, ventral, and reticulo- and pedunculopontine nuclei (Fig. 2, E, F). A few small round or fusiform immunoreactive neurons were interspersed in the locus coeruleus and lateral and medial parabrachial and Kölliker-Fuse nuclei. The raphe pontis nucleus contained a large number of medium-sized labeled cells oriented parallel to the fiber bundles (Fig. 2F). Numerous small and large PDHC-immunoreactive perikarya were observed throughout the lateral and medial trapezoid nuclei, respectively (Fig. 2F). Only a few immunoreactive cells were found in the medial and caudal

Figure 3. *A*, Bright-field photomicrograph of a coronal section through a midrostrocaudal level of the septal region (corresponding to Fig. 2*A*) shows the distribution of PDHC-immunoreactive neurons. *B*, Photomicrograph of a semiajacent section showing the distribution of CAT immunoreactive neurons. A similar distribution pattern of the PDHC and CAT immunoreactive neurons is found in the diagonal band: Both types of immunoreactive cell bodies are often elongated in the plane of fibers in the ventral part (arrows) and avoid similar midregion (dashed line). Note that the differences in the section size is due to tissue shrinkage following the Triton treatment (i.e., tissue immunolabeled for PDHC). Scale bar, 100 μ m.

half of the lateral superior olivary nuclei. A substantial number of large, round, multipolar neurons in the motor trigeminal nuclei were immunoreactive, while only a few small labeled neurons were observed in the principal sensory trigeminal nucleus (Fig. 2*F*). Both small and large multipolar immunoreactive neurons were found interdispersed throughout the caudal, oral, and parvicellular reticular nuclei. A few labeled perikarya were found medially in the medial vestibular nucleus, while the lateral vestibular nucleus contained several immunoreactive cell bodies, especially in the ventral half (Fig. 2*G*). Many medium-sized immunoreactive neurons were seen in the ventral cochlear nucleus. All of the subdivisions of the facial nucleus contained numerous large, multipolar PDHC-immunoreactive neurons.

A substantial number of Purkinje cells in the cerebellar cortex were immunoreactive for PDHC (Fig. 2*F*). All of the deep cerebellar nuclei contained immunoreactive perikarya. Large, round, multipolar labeled neurons were located throughout the dentate nucleus. A few large immunoreactive neurons were also observed in the dorsal aspect of the interpositus nucleus while a few small labeled neurons were seen in the medial aspect of the fastigial nucleus.

Medulla oblongata. The nucleus ambiguus contained a substantial number of medium-sized PDHC-immunoreactive perikarya in its entire rostrocaudal extent (Fig. 2, *G, H*); however, the labeled cells were especially abundant in the rostral third (Fig. 6*A*). The dorsal motor nucleus of the vagus and the hypoglossal nucleus had a large number of labeled neurons (Figs. 2, *G, H*; 6*C*; 7*A*). Although the immunoreactive perikarya were distributed throughout the rostrocaudal extent of these nuclei, the majority of cells in these nuclei were concentrated in their caudal portions near the level of the area postrema (Fig. 2*H*). A few immunoreactive cell bodies also were observed in the paratrigeminal nucleus and in the rostroventral aspect of the nucleus of the spinal tract of the trigeminal nerve. The subtrigeminal region of the lateral reticular nucleus also contained a large number of medium-sized immunoreactive perikarya. Only a few neurons in the rostral ventrolateral medulla were immunoreactive for PDHC. The majority of these were medial to the lateral reticular nucleus but lateral to the inferior olive. In contrast, many more neurons in the caudal ventrolateral medulla, including some in the lateral reticular nucleus, contained PDHC immunoreactivity (Fig. 2*H*). A substantial number of labeled cell bodies, primarily small, were found throughout the rostrocaudal extent of the raphe obscurus (Fig. 2*G*). A few small labeled neurons also were observed in the rostromedial part of the cuneate nucleus (Fig. 2*H*). The prepositus hypoglossi, paramedian reticular, Roller (not shown), and inferior olivary nuclei contained a few randomly distributed immunoreactive cell bodies (Fig. 2, *G, H*).

Relationship between the distribution of PDHC- and CAT-immunoreactive neurons

Immunocytochemical labeling for CAT was found in unfrozen vibratome sections labeled in the absence of Triton X-100. The results of our analysis of the distribution of CAT in rat brain were in essential agreement with previous reports of Armstrong et al. (1983), Satoh et al. (1983), and Houser et al. (1983) (see Table 1). Thus, the localization will not be described except to note specific exceptions and to compare with the localization of PDHC. In contrast to an earlier report (Houser et al., 1983), CAT-labeled perikarya were not detected in the cerebral cortex

but were seen in the lateral trapezoid nucleus (Fig. 2*F*), medial and lateral parabrachial nuclei, and Kölliker-Fuse nucleus, and the midrostrocaudal part of the raphe obscurus and inferior olive (above the pyramidal tract) (Fig. 2*G*).

When the distribution of PDHC-immunoreactive neurons was compared to that of CAT-labeled cells, the cytological features and distribution of perikarya labeled with the 2 antigens were similar in a large number of regions (Fig. 2). For example, PDHC-immunoreactive neurons and CAT-labeled cells in the septal nuclei both had elongated perikarya and were located within the fiber bundles of medial septal and diagonal band nuclei, both were distributed throughout the rostrocaudal extent of the nuclei, and both were arranged in the same topographic pattern (compare Fig. 3, *A* and *B*). Additional similarities could be observed in the nucleus ambiguus (Fig. 6, *A* and *B*), the hypoglossal nucleus (Fig. 6, *C* and *D*), and the dorsal motor nucleus of the vagus (Fig. 7, *A* and *B*). In all cases, the rostrocaudal extent and pattern of distribution were nearly identical. The correspondence between neurons containing PDHC and CAT immunoreactivity was especially evident in regions that exhibited intense PDHC immunoreactivity (Fig. 2 and Table 1). There were, however, notable exceptions. These included neurons in the hippocampal formation and entorhinal cortex, the neocortex, supraoptic hypothalamic nuclei, reticular thalamic nuclei, red nuclei, lateral parts of the substantia nigra, mesencephalic nuclei of the trigeminal nerve, ventral tegmental nuclei, the raphe pontis, the lateral vestibular nuclei, the subtrigeminal lateral reticular nuclei, and the cerebellar Purkinje cells.

Relationship between PDHC-immunoreactive neurons and AChE-positive cells

The distribution of perikarya identified by the AChE staining was similar to that previously described (Jacobowitz and Palkovits, 1974; Palkovits and Jacobowitz, 1974; Parent and Butcher, 1976; Bigl et al., 1982; Satoh et al., 1983; Butcher and Woolf, 1984). Furthermore, the distribution resembled that of CAT-immunoreactive neurons. Thus, only the few notable differences are described. Table 1 lists the regions in which AChE-positive cells were found and correlates them to the distribution of both CAT- and PDHC-immunoreactive cells. AChE-positive cells were seen in certain areas not previously reported. These areas included the endopiriform nucleus, the lateral trapezoid body, raphe pontis, and the paratrigeminal nucleus (Table 1).

Most regions that contained PDHC-labeled perikarya also contained AChE-positive cells (Table 1). This is illustrated for the lateral reticular thalamic nucleus, nucleus basalis, and globus pallidus in Figure 5. Regions that contained PDHC-immunoreactive neurons but did not exhibit detectable AChE activity included the primary olfactory cortex, pyramidal cell layer of regio inferior of the hippocampal formation, ventro- and latero-posterior thalamic nuclei, medial geniculate, superficial gray layer of the superior colliculus, medial superior olive, and the Purkinje cell layer of the cerebellar cortex.

Relationship between PDHC-immunoreactive neurons and catecholaminergic neurons

The distribution of catecholaminergic cell bodies using immunocytochemical localization of TH was identical to that described previously by fluorescence histochemistry (Dahlström and Fuxe, 1964) and thus will not be presented here. For the majority of areas, the regional distributions of TH-containing neurons and of PDHC-immunoreactive neurons did not over-

Figure 4. Bright-field photomicrographs of a coronal section in the rostral third through the hippocampal formation. *A*, In regio superior, PDHC-immunoreactive neurons are found in the alveus (*Alv*) and scattered throughout stratum oriens (*SO*) and the pyramidal cell layer (*PL*). *B*, In regio inferior, a large number of pyramidal cell neurons (*PL*) contain PDHC immunoreactivity. Additionally, a few PDHC-labeled neurons are found in stratum oriens. *C*, In the dentate gyrus, many hilar (*H*) neurons contain PDHC immunoreactivity. Note the absence of labeling in the granule cell layer (*GL*). *D*, Layers II and III of the entorhinal cortex contain many intensely PDHC-labeled neurons. Scale bars, 50 μm.

Figure 5. Bright-field photomicrographs of a coronal section through the rostral thalamus. *A*, At this level, PDHC-immunoreactive neurons are most intense in the reticular thalamus nucleus (*Rt*), the nucleus basalis (*B*), and the globus pallidum (*GP*). *B*, Semiadjacent section to *A* that has been stained for the demonstration of AChE. Note the correspondence between the distribution of AChE-stained neurons and processes to that of the PDHC-immunoreactive neurons, especially in the reticular thalamic nucleus. Scale bar, 100 μ m.

Figure 6. Bright-field photomicrographs of a coronal section through the medulla oblongata. PDHC-immunoreactive neurons (*A*) and CAT-immunoreactive neurons and processes (*B*) are shown in the rostral part of the nucleus ambiguus. PDHC-immunoreactive neurons (*C*) and CAT-immunoreactive neurons and processes (*D*) in the hypoglossal nucleus. Note the correspondence between both the cellular morphology and distribution of PDHC- and CAT-immunoreactive neurons in both these nuclei. Scale bars, 50 μ m.

Figure 7. Bright-field photomicrographs of coronal sections through the solitary tract nucleus (NTS) and dorsal motor nucleus of vagus (X) show the distribution of PDHC-immunoreactive neurons (A), CAT-labeled cells (B), and TH-immunoreactive neurons and processes (C). PDHC-immunoreactive neurons have a similar distribution to CAT-labeled cells in the dorsal motor nucleus but are not found in the regions of the NTS that contain TH-labeled neurons. Scale bars, 50 μ m.

lap. An example is shown in Figure 7, in which low to nondetectable levels of PDHC immunoreactivity were seen in perikarya in the solitary tract nucleus (Fig. 7A), a region containing many TH-immunoreactive neurons (Fig. 7C). There were, however, 3 notable exceptions in which a few PDHC-labeled cells were found in topographic areas occupied by catecholaminergic neurons: (1) the lateral parts of the substantia nigra, pars compacta; (2) the locus coeruleus; and (3) the A1 region in the caudal ventrolateral medulla.

Discussion

The present study has shown that, under a specific set of conditions, PDHC immunoreactivity is present throughout the neuropil but is enriched in selective neuronal perikarya. The intensity of labeling in these perikarya was found to be independent of the cell size but closely related (1) to the brain region and, in some cases, (2) to the distribution of cholinergic enzymes.

Labeling conditions

The immunocytochemical localization of PDHC to neuronal perikarya was most evident in unfrozen vibratome sections labeled in the presence of Triton X-100. Presumably, permeabilization of aldehyde-fixed membranes was necessary to expose the otherwise cryptic antigenic sites within mitochondria. The requirement for the use of detergent presents technical limitations both with respect to future ultrastructural studies of the localization of PDHC and dual-labeling studies with soluble enzymes such as CAT. One solution to this problem under current investigation is combined immunocytochemical localization of the more soluble enzyme (e.g., CAT) prior to embedding the tissue in plastic, followed by postembedding immunogold labeling for the mitochondrial enzyme (Knecht et al., 1986). The thin sectioning exposes antigenic sites otherwise inaccessible without use of detergent.

Varying levels of PDHC in neuronal perikarya

The present detection of varying intensities of the PAP reaction product for PDHC in perikarya of different sizes suggests that these cells have different metabolic requirements for PDHC. Similar high levels of mitochondrial enzymes involved in glucose metabolism have been shown previously within neuronal perikarya histochemically identified by staining for reduced nicotinamide adenine dinucleotide phosphate (NADPH)-diaphorase in the dorsal nucleus of the vagus, hypothalamic neurosecretory nuclei and the locus coeruleus (Thomas and Pearce, 1961; Friede et al., 1963). While neurons in the dorsal motor nucleus contain ACh (Armstrong et al., 1983) and those in the locus coeruleus contain catecholamines (Hökfelt et al., 1974), the differential distribution of PDHC and NADPH-diaphorase suggests that factors other than the transmitter are also important in determining the detected levels of certain mitochondrial enzymes. The dissociations between intensity of immunoreactivity for PDHC and cell size, transmitter, or number of mitochondria are further supported by the low intensity of labeling within the region of the rostral ventrolateral medulla, where large neurons enriched in mitochondria have been shown to contain phenylethanolamine *N*-methyltransferase (Milner et al., 1987). Other factors relevant to the intensity of PDHC in perikarya may include either the terminal ramifications in target regions, the amount of afferent input (Jones and Cowan, 1983), or even methodological factors related to the subcellular distribution of the enzyme complex.

Relation of PDHC-immunoreactive neurons to cholinergic nuclei

The present detection of the highest intensity of labeling for PDHC in neuronal nuclei with CAT immunoreactivity supports biochemical evidence that PDHC activity is enhanced in cholinergic neurons (Tucek and Cheng, 1974; Gibson et al., 1975; Jope and Jenden, 1980; Sterri and Fonnum, 1980). Since CAT catalyzes the conversion of choline and acetyl-coenzyme A to ACh, it is generally recognized as an unambiguous indicator for cholinergic neurons (see Wainer et al., 1984, for review). Alternatively, the detection of intense PDHC-labeled perikarya in areas containing AChE, but not CAT, may indicate that the cells receiving cholinergic innervation also have high levels of the enzyme. AChE is the degradative enzyme for ACh and thus may be localized on the cytoplasmic membranes of perikarya receiving cholinergic innervation (Koelle, 1954; Silver, 1974; Wainer et al., 1984).

Several previous studies have analyzed the distribution of AChE-positive cell bodies in the rat brain (Jacobowitz and Palkovits, 1974; Palkovits and Jacobowitz, 1974; Parent and Butcher, 1976; Satoh et al., 1983). However, because of the low degree of resolution offered by the histochemical techniques employed, many AChE-positive cells could only be visualized by pretreating the animals with bis-(1-methylethyl)phosphorofluoridate (diisopropylfluorophosphate—an irreversible inhibitor of AChE). Recently, 2 modifications of the existing histochemical techniques (Milner et al., 1982; Hedreen et al., 1985) have provided well-defined staining of AChE-containing axons, perikarya, and dendrites, eliminating the majority of diffuse background. Many of the regions (i.e., the cerebral cortex, reticular thalamic nucleus, and hippocampal formation) that contain AChE-positive perikarya also contain AChE-positive fibers and terminals, CAT-positive fibers and terminals, and cholinergic (muscarinic and nicotinic) receptors (Jacobowitz and Palkovits, 1974; Palkovits and Jacobowitz, 1974; Hunt and Schmidt, 1979; Rotter et al., 1979a, b; Arimatsu et al., 1981; Houser et al., 1983; Mesulam et al., 1983; Clarke et al., 1985). Alternatively, many of the PDHC-immunoreactive neurons found in regions enriched with AChE could be involved in the hydrolysis of other neurotransmitter candidates. AChE has been shown to hydrolyze both substance P and enkephalin (Chubb et al., 1980, 1982). In agreement with this alternative hypothesis, high concentrations of substance P and enkephalin have been reported in many of the areas containing high levels of PDHC and AChE e.g., the reticular thalamic nucleus, neocortex, and hippocampus (Ljungdahl et al., 1978; Finley et al., 1981).

Relation of PDHC-immunoreactive neurons to other neurotransmitter systems

Our results have shown that the majority of PDHC-immunoreactive neurons are not found in regions containing catecholaminergic nuclei. Exceptions to this are the lateral substantia nigra, locus coeruleus, and caudal ventrolateral medulla, all of which contain dopaminergic and noradrenergic neurons (Dahlström and Fuxe, 1964). However, the detection of perikarya with intense labeling for PDHC in the locus coeruleus and substantia nigra also may reflect intrinsic cholinergic or cholinceptive neurons in these regions. Unlike other catecholaminergic nuclei, the substantia nigra and locus coeruleus contain AChE-

positive cells (Butcher and Talbot, 1978; Albanese and Butcher, 1979).

Perikarya with moderate to intense labeling for PDHC also were found in nuclei that contained neither CAT, AChE, nor TH. The transmitter in these cells is unknown. In raphe nuclei, 5-HT is one likely putative transmitter (Steinbusch, 1981). In other cortical and subcortical regions, the distribution of perikarya with intense labeling for PDHC is similar to those containing somatostatin, neuropeptide Y, substance P, and vasoactive intestinal peptide (Ljungdahl et al., 1978; Eckenstein and Baughman, 1984; Johansson et al., 1984; Chronwall et al., 1985). The somatostatin- and neuropeptide Y-containing perikarya, like the noradrenergic neurons in the locus coeruleus and cholinergic neurons in the pons, also contain NADPH-diaphorase (Kauffman et al., 1974; Vincent et al., 1983a, b).

Functional implications

The present finding of regional variations in intensity of PDHC immunoreactivity in neuronal perikarya provides an explanation for variations in neuronal vulnerability either to extrinsic insults or metabolic disorders in the CNS. With regard to extrinsic damage, it is clinically of interest that selective groups of neurons, including some of those in the cerebral cortex containing moderately high intensities of PDHC, are among those damaged in ischemia (Pulsinelli et al., 1982). Further evidence for the involvement of mitochondria and glucose utilization in ischemia is supported by microvacuolation of mitochondria in the early stages of ischemia (Brierly, 1986) and the increased severity of neuronal damage following the administration of glucose prior to ischemia induced by 4-vessel occlusion in rats (Pulsinelli et al., 1982). The transmitter within most neurons showing vulnerability to ischemia is unknown but probably is not ACh since CAT has not been demonstrated in either of the neuronal populations affected by ischemia in the cerebral cortex or hippocampus (Armstrong et al., 1983). Other regions within the cortex and hippocampus do, however, receive major cholinergic afferents from the basal forebrain (Mesulam et al., 1983; Amaral and Kurz, 1985) and have been shown to have marked reductions of both PDHC and ACh in autopsied brains from Alzheimer's patients (Perry et al., 1980; Sorbi et al., 1983; Price, 1984; Sheu et al., 1985b). Furthermore, the striatum, which contains cholinergic neurons, also shows reduction of PDHC in Huntington's chorea (Sorbi et al., 1983; Butterworth et al., 1985; Graveland et al., 1985) and in Friedreich's ataxia (Blass et al., 1976). These coincident changes in PDHC and overlapping distributions between PDHC and cholinergic enzymes in some, but not all, brain regions, suggest that certain neurotransmitter-specific neurons, particularly those synthesizing ACh, may be more selectively vulnerable to mitochondrial diseases involving pyruvate utilization.

Appendix: Abbreviations Used in Figures

<i>ac</i> ,	Anterior commissure	<i>BL</i> ,	Basolateral amygdaloid nucleus
<i>AC</i> ,	Accumbens nucleus	<i>BLV</i> ,	Basolateral amygdaloid nucleus, ventral
<i>AHi</i> ,	Amygdalohippocampal area	<i>BM</i> ,	Basomedial amygdaloid nucleus
<i>Am</i> ,	Ambiguous nucleus	<i>bsc</i> ,	Brachium of superior colliculus
<i>AP</i> ,	Area postrema	<i>BST</i> ,	Bed nucleus of stria terminalis
<i>APT</i> ,	Anterior pretectal area	<i>C</i> ,	Cingulate cortex
<i>Arc</i> ,	Arcuate hypothalamic nucleus	<i>cc</i> ,	Corpus callosum
<i>B</i> ,	Basal nucleus of Meynert	<i>Ce</i> ,	Central amygdaloid nucleus
		<i>cg</i> ,	Cingulum
		<i>CG</i> ,	Central gray
		<i>Cl</i> ,	claustrum
		<i>CL</i> ,	Central lateral thalamic nucleus
		<i>CM</i> ,	Central medial thalamic nucleus
		<i>cp</i> ,	Cerebral peduncle, basal
		<i>CP</i> ,	Caudate putamen
		<i>Cu</i> ,	Cuneate nucleus
		<i>DA</i> ,	Dorsal hypothalamic area
		<i>DB</i> ,	Nucleus of the diagonal band
		<i>df</i> ,	Dorsal fornix
		<i>DG</i> ,	Dentate gyrus
		<i>DM</i> ,	Dorsomedial hypothalamic nucleus
		<i>DR</i> ,	Dorsal raphe nucleus
		<i>dsc</i> ,	Dorsal spinocerebellar tract
		<i>ECu</i> ,	External cuneate nucleus
		<i>En</i> ,	Endopiriform nucleus
		<i>Ent</i> ,	Entorhinal cortex
		<i>EP</i> ,	Entopeduncular nucleus
		<i>f</i> ,	Fimbria
		<i>fx</i> ,	Fornix
		<i>GP</i> ,	Globus pallidus
		<i>Gr</i> ,	Gracile nucleus
		<i>Hb</i> ,	Habenular nucleus
		<i>ic</i> ,	Internal capsule
		<i>IC</i> ,	Inferior colliculus
		<i>IF</i> ,	Interfascicular nucleus
		<i>IM</i> ,	Interstitial magnocellular nucleus posterior commissure
		<i>IO</i> ,	Inferior olive
		<i>La</i> ,	Lateral amygdaloid nucleus
		<i>LC</i> ,	Locus coeruleus
		<i>LD</i> ,	Laterodorsal thalamic nucleus
		<i>LDT</i> ,	Laterodorsal tegmental nucleus
		<i>LH</i> ,	Lateral hypothalamic area
		<i>LP</i> ,	Lateral posterior thalamic area
		<i>LPRM</i> ,	Lateral posterior thalamic nucleus, rostromedial
		<i>LRT</i> ,	Lateral reticular nucleus
		<i>LS</i> ,	Lateral septal nucleus
		<i>LSO</i> ,	Lateral superior olive
		<i>LTz</i> ,	Lateral nucleus of the trapezoid body
		<i>mcp</i> ,	Middle cerebellar peduncle
		<i>MD</i> ,	Mediodorsal thalamic nucleus
		<i>MdD</i> ,	Reticular nucleus medulla, dorsal
		<i>MdV</i> ,	Reticular nucleus medulla, ventral
		<i>Me</i> ,	Medial amygdaloid nucleus
		<i>MeV</i> ,	Nucleus mesencephalic tract of trigeminal nerve

<i>MG</i> ,	Medial geniculate nucleus
<i>ml</i> ,	Medial lemniscus
<i>MoV</i> ,	Motor trigeminal nucleus
<i>mp</i> ,	Mammillary peduncle
<i>MP</i> ,	Medial preoptic area
<i>MS</i> ,	Medial septal nucleus
<i>MTz</i> ,	Medial nucleus of the trapezoid body
<i>MVe</i> ,	Medial vestibular nucleus
<i>NTS</i> ,	Nucleus of the solitary tract
<i>oc</i> ,	Optic chiasm
<i>oct</i> ,	Olivocerebellar tract
<i>opt</i> ,	Optic tract
<i>OT</i> ,	Nucleus optic tract
<i>PaV</i> ,	Paratrigeminal nucleus
<i>PCRt</i> ,	Parvicellular reticular nucleus
<i>PMC</i> ,	Posteromedial cortical amygdaloid nucleus
<i>PMn</i> ,	Paramedian reticular nucleus
<i>PnC</i> ,	Pontine reticular nucleus, caudal
<i>PnO</i> ,	Pontine reticular nucleus, oral
<i>Po</i> ,	Posterior thalamic nucleus group
<i>PO</i> ,	Primary olfactory cortex
<i>P</i> ,	Prepositus hypoglossi
<i>PPn</i> ,	Pedunculopontine tegmental nucleus
<i>PPT</i> ,	Posterior pretectal nucleus
<i>PrV</i> ,	Principal sensory trigeminal nucleus
<i>py</i> ,	Pyramidal tract
<i>Re</i> ,	Reuniens thalamic nucleus
<i>RI</i> ,	Regio inferior of the hippocampus
<i>RLi</i> ,	Rostral linear nucleus raphe
<i>ROb</i> ,	Raphe obscurus nucleus
<i>RPn</i> ,	Raphe pontis nucleus
<i>RS</i> ,	Regio superior of the hippocampus
<i>RSp</i> ,	Retrosplenial cortex
<i>Rt</i> ,	Reticular thalamic nucleus
<i>RiT</i> ,	Reticulotegmental nerve
<i>S</i> ,	Subiculum
<i>SC</i> ,	Superior colliculus
<i>SG</i> ,	Supragenulate thalamic nucleus
<i>SNR</i> ,	Substantia nigra, reticular
<i>spV</i> ,	Spinal tract of trigeminal nerve
<i>SpV</i> ,	Nucleus of spinal tract of trigeminal nerve
<i>SpVe</i> ,	Spinal vestibular nucleus
<i>st</i> ,	Stria terminalis
<i>Te Aud</i> ,	Temporal cortex, auditory area
<i>ts</i> ,	Solitary tract
<i>Tu</i> ,	Olfactory tubercle
<i>VM</i> ,	Ventromedial thalamic nucleus
<i>VMH</i> ,	Ventromedial hypothalamic nucleus
<i>VP</i> ,	Ventral pallidum
<i>VPL</i> ,	Ventroposterior thalamic nucleus, lateral
<i>VPM</i> ,	Ventroposterior thalamic nucleus, medial
<i>VT</i> ,	Ventral tegmental nucleus
<i>VTa</i> ,	Ventral tegmental area
<i>X</i> ,	Dorsal motor nucleus of the vagus
<i>XII</i> ,	Hypoglossal nucleus

<i>XIIIn</i> ,	Roof of the hypoglossal nerve
<i>ZI</i>	Zona incerta

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