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Compartmentalization of NADPH-diaphorase staining in the developing human striatum

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Nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d) staining of striatal neuropil showed inhomogeneities in human fetal and adult brains. Highly reactive patches were seen during fetal and neonatal period, distributed in a lighter stained background matrix. In adult, zones of low NADPH-d reactivity appeared against darker background staining. NADPH-d reactive patches corresponded to and showed a similar shift in the intensity of staining during development as acetylcholinesterase (AChE) reactive striosomes.

Key words: Striatum; Nicotinamide adenine dinucleotide phosphate diaphorase; Acetylcholinesterase; Development; Human

Since the striosome-matrix compartmentalization was first recognized by acetylcholinesterase (AChE) histochemistry [7], work from a number of laboratories has demonstrated striosomal ordering for many transmitters and their related enzymes [10]. One of them is nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d) which has recently been discovered to be a calcium/calmodulin-dependent nitric oxide synthase (NOS) [11]. In adult human striatum, NADPH-d reactive neurons are predominantly located in a zone of the striatum called the matrix compartment [14] characterized by its high content of AChE. These neurons belong to a population of aspinal interneurons which colocalize somatostatin and neuropeptide Y [14]. Striosomal organization of NADPH-d staining of neuropil has been clearly shown in the adult cat striatum [18]. The NADPH-d staining is dense in the AChE-rich matrix of the caudate nucleus, but weak in the AChE-poor compartments known as striosomes. During development, remarkable changes in the disposition of striatal AChE reactivity have been demonstrated [8, 13]. Densely stained patches of AChE activity appear during fetal and early neonatal periods followed by rearrangement so that in adult striosomes of low AChE activity are present. Interestingly, striatal histochemical and cytoarchitectonic compartments are more clearly defined



Fig. 1. (18-week-old human fetus.) Note inhomogeneous patterns of diaphorase staining. Several patches of high diaphorase activity (arrowheads) within the lighter stained background. Bar=1 mm.

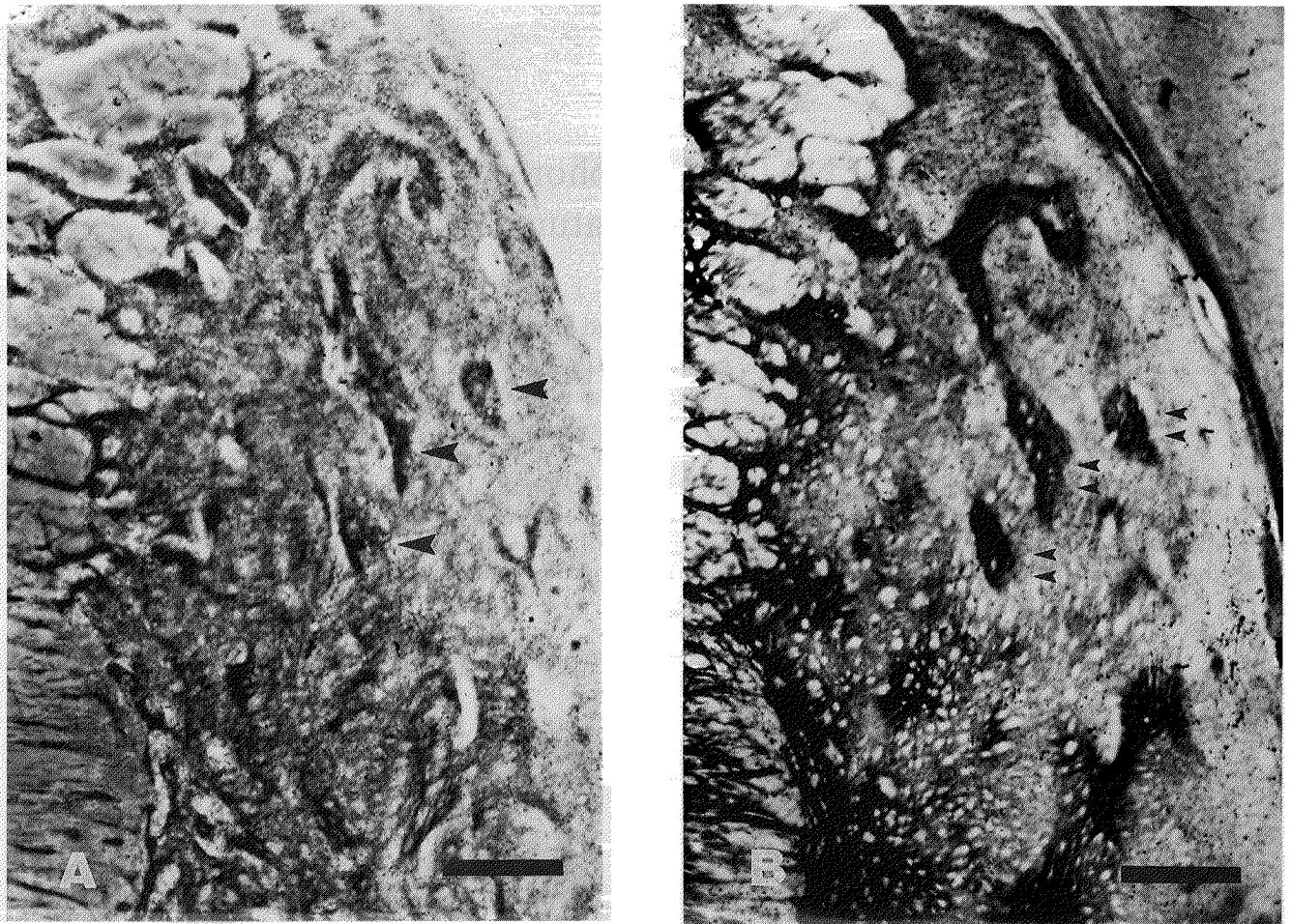


Fig. 2. Two adjacent transverse sections through the dorsal putamen of an 21-week-old human fetus illustrating patterns of NADPH-d (A) and AChE staining (B). Note diaphorase patches (arrowheads) correspond to AChE reactive striosomes (double arrowheads). Bar=1 mm.

during development [6, 8, 12]. To begin studying the development of the NADPH-d in human brain we have used fetal material to determine the question whether NADPH-d staining shows striatal compartmentalization and change in the distribution similar to that of AChE.

Postmortem striatal tissue from 5 human fetuses (postovulatory ages 18, 21, 25, 26 and 37 weeks), two infants (newborn and 14 months) and one young adult (12.5 years) was obtained at the autopsy (postmortem time less than 12 h) and fixed by the immersion for 24 h at 4°C in 4% paraformaldehyde in phosphate buffer. After cryoprotection, coronally orientied 80–120- μm -thick frozen sections were stained for NADPH-d using a modification of the direct method [2]. In the 21-week-old specimen the AChE histochemical method of Koelle (Lewi's modification) was also applied to compare distribution of NADPH-d and AChE staining in adjacent sections [15].

Our results indicate two aspects of the NADPH-d distribution in the striatum; the pattern of neuropil staining

and distribution of the individually labelled cells. There were clear inhomogeneities in diaphorase staining of the neuropil at all fetal ages studied. It seemed more well established in the putamen than in caudate nucleus, although the length of incubation was critical for demonstrating it; that is longer incubation time (2–4 h) is preferred. In the putamen the most conspicuous figures were oval to elongated diaphorase-rich (Figs. 1 and 2A) zones (dimensions 200–500 μm) distributed in a lighter stained background matrix. During development patches appeared first in the lateral parts of the putamen (Fig. 1). The background stain immediately around the patches was pale. During the neonatal period clumping pattern of diaphorase staining in the putamen was like that seen in the fetal brains: dark patches were distributed in a lighter background matrix (not shown). In the oldest infant (14 months) studied, the distribution of diaphorase staining had characteristics of the adult pattern (Fig. 4) with zones (dimensions 800–1500 μm) of low diaphorase staining appearing against a darker back-

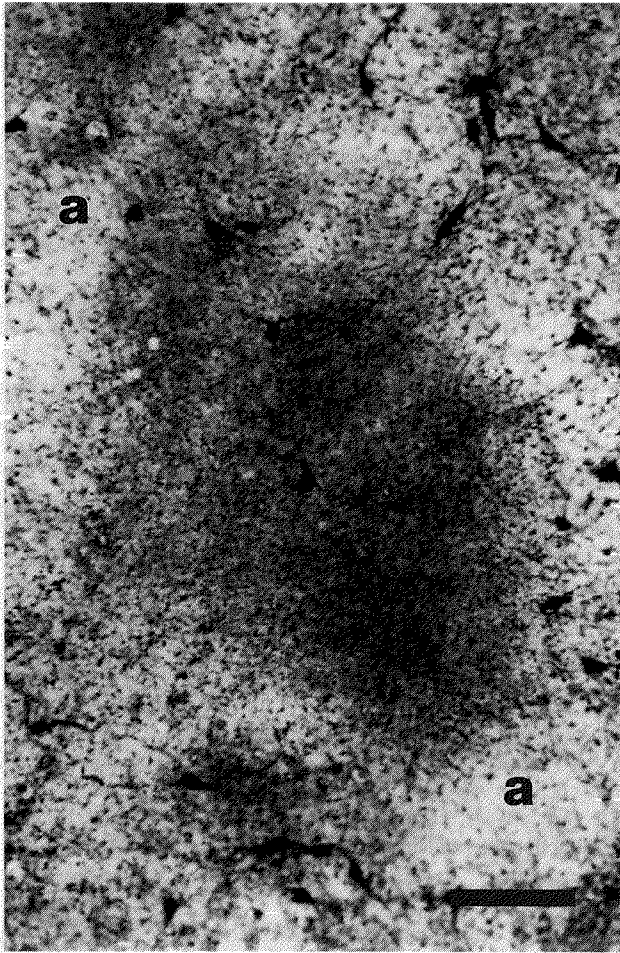


Fig. 3. (26-week-old human fetus.) High power of the NADPH-d reactive patch. Note that most of NADPH-d neurons are located outside the patch at the border of reactivity-free annulus (a). Bar=50 μ m.

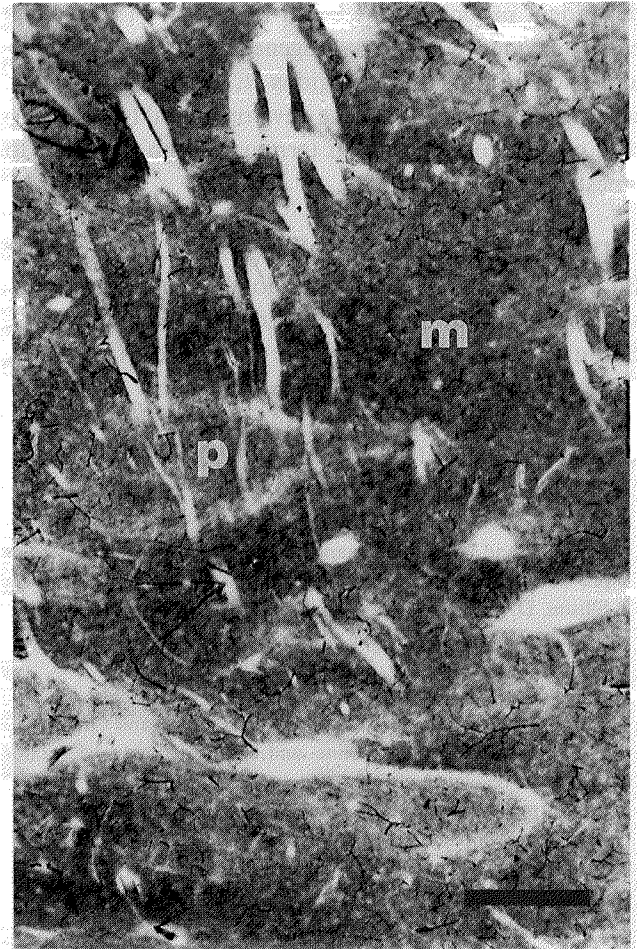


Fig. 4. (Young adult.) Patch of light diaphorase reactivity (p) surrounded by matrix of darker reactivity (m). Between them reactivity free annulus of lightest reactivity half-surrounding the patch. Bar=1 mm.

ground matrix. To study the relationship of diaphorase-rich patches to AChE reactive striosomes during fetal development, their distribution was compared in adjacent sections. We observed that diaphorase-rich patches closely corresponded to AChE reactive striosomes at least in 21-week-old specimen (Fig. 2A,B).

During fetal and early neonatal period NADPH-d neurons were located in the zones of low diaphorase staining and often at the interface of these zones with patches of high diaphorase staining. There were few reactive neurons within the patches of high reactivity (Fig. 3). Only at 18 weeks there seemed to be slightly more neurons located within these high reactive zones (not shown). This distribution resembles that observed in adult brain [14], with diaphorase neurons located mainly within the matrix compartment of high AChE reactivity. Striosomes of low AChE reactivity were largely devoid of diaphorase neurons.

These findings have shown that diaphorase staining has similar developmental trends in human striatum as

AChE [8, 13]; that is, from a patchy distribution of diaphorase stain to an adult pattern of dense stain interspersed with zones of low diaphorase staining. In addition, diaphorase-rich patches corresponded to AChE reactive striosomes during fetal development. It is not known in which cellular elements the reaction products reside, and whether AChE and NADPH-d are associated with the same elements. The possible candidates are dopamine terminals [9, 17, 19], corticostriatal afferents [5] or striatal patch neurons. The possible functional significance and developmental implications should be viewed in the light of recent evidence that NADPH-d is NOS [11]. According to one hypothesis [4] Ca^{2+} -dependent postsynaptic release of nitric oxide (NO) may be important in the establishment and activity-dependent refinement of axonal projection. The proto-striosomes lead the matrix developmentally in many characteristics. Striosomal neurons are the first to express two molecules that function in Ca^{2+} -regulated cascades and that are later widely expressed by striatal cells, Ca^{2+} -calmodulin-

dependent protein kinase 2 and DARPP-32 [3, 16]. The earliest evidence for synaptogenesis in the striatum is also shown in the island regions [1, 16]. NO might also serve as a mechanism for the developmental transformation of corticostriatal axon terminals from a diffuse to fenestrated distribution [6].

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