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Short Communicaion

Developmental changes in the distribution of acetylcholinesterase in the extrastriate visual cortex of the monkey

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Abstract

In the fetal and neonatal monkey, periodically organized regions of high activity of acetylcholinesterase were found in the visual cortical area V2 (Area 18). The acetylcholinesterase bands, like the thin and thick stripes of cytochrome oxidase, were found to run orthogonal to the area 17/18 border. During neonatal development these bands progressively narrow and finally disappear shortly after four months of age.

Key words: Ontogeny; Nervous system; Macaque; Vision; Area 17; Area 18

Transient expression of acetylcholinesterase (AchE) is a characteristic feature of the developing cerebral cortex. In the immature rat, high levels of AchE are transiently expressed in the somatosensory [18], auditory [26] and visual cortex [25,27] and disappear during the third postnatal week of life. This transient expression of AchE is thought to parallel the development of the thalamocortical pathway and is dependent on the integrity of the sensory relays [11,25]. This suggests, in rodents at least, that there is a link between AchE expression and the maturation of connectivity, and reinforces the hypothesis that the role of AchE in the CNS is not limited to cholinergic transmission [10].

In higher order mammals, the transient expression of AchE has been reported to show some important differences from that observed in rodents notably with respect to its timing. AchE expression exhibits a progressive increase in the forebrain of cat [2] and tree shrew [14]. In monkey Kostovic and Rakic [17] conducted a study on the expression of cholinesterase (chE) in the prestriate cortex during early fetal development. They reported that chE labelled axons were largely limited to extrastriate cortex and are first observed on embryonic day 80 (E80), reach a peak between E100 and E125, and later decrease progressively. In the present study we have examined the pattern of AchE expression at later developmental stages comparable to those when transient expression is detected in rodents. Our results show that high levels of AchE are transiently expressed in presumptive area V2 in an arrangement of bands or stripes running orthogonal to the border of area V2 with area 17. The disappearance of these AchE bands is not achieved until late in postnatal development around 5 months of age.

The distribution of AchE was investigated in the visual cortex of 13 immature and two adult cynomolgus monkeys (*Macaca irus*). Surgical procedures for fetal material have been described in detail elsewhere [6]. Animals were deeply anaesthetised before being perfused transcardially by a saline solution followed by a mixture of para- and glutaraldehyde (paraformaldehyde: 1%; glutaraldehyde: 1.25% and in BB98 paraformaldehyde: 8%) and a sucrose gradient (8–30%). Brains were immediately removed and 40-μm-thick sections cut on a freezing microtome. The cerebral hemispheres were sectioned either orthogonal to the cortical layers (in the parasagittal or horizontal plane) or tangential to the cortical layers after physically flattening the operculum and the cortex buried in the lunate sulcus (Table 1). AchE staining was performed

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Table 1
Age of animals, type of sectionning, and characteristics of the AchE bands in V2

Case	Age	Section	AchE band characteristics		
			Bands	Size (mm)	Separation (mm)
BB 83	E 94	P	−		
BB 31	E 121	P	+		
BB 98	E 129	T/P	+	2.23±0.48	0.50±0.11
BB 11	E 133	P	+		
BB 25	new-born	H	+		
BB 30	new-born	H	+		
BB 99	PND 6	T	+	2.43±0.36	0.51±0.22
BB 86	PND 15	T/P	+		
BB 88	PND 17	T	+	2.72±0.39	0.92±0.28
BB 82	1 month	P	+		
BB 94	4 months	T/P	+	1.13±0.25	1.71±0.46
BB 84	6 months	P	−		
BB 93	16 months	T	−		
M 38	adult	T	−		
M 45	adult	T	−		

The signs + or − indicate the presence or absence of AchE bands, respectively. The data are expressed ±S.D. E, embryonic day; PND, postnatal day; P, parasagital sections; H, horizontal sections; T, tangential sections on flattened cortex.

according to a protocol using acetylthiocholine iodide as the substrate [12]. Incubation times ranging from 2.5–3 h at 37°C or 3–5 days at 4°C were chosen to give optimal staining. In all cases, several sections were incubated in the presence of eserine, an inhibitor of the AchE, in order to test the staining specificity.

In both immature and adult cortex AchE histochemistry led to intensely stained axons with occasionally labelled somata. Outside of area V2 we did not detect any strong regional transient expression of AchE. Auditory cortex, which shows high levels of AchE activity in layer 4 in the adult [21], showed equally strong levels of labelling at all fetal ages examined. In area 17 there was a progressive increase in the intensity of labelling (Fig. 1). In the striate cortex of the E121 fetus, AchE activity was relatively weak and restricted to layers 4 and upper 6. Labelling in these layers progressively increased up to 4 postnatal months. At 6 months, the adult pattern of activity was apparent in layer 4 which is characterised by a cleft of low activity in the lower part of layer 4C as described by Fitzpatrick and Diamond [8]. The blobs of high AchE activity described by Horton [13] in layers 2/3 were found to emerge during the last month of gestation.

At all fetal and postnatal ages examined there was an abrupt transition corresponding to the border of area 17 with area V2. During development radical changes in the distribution of AchE stained axons are observed in the fundus and posterior bank of the lunate sulcus (PBLS) corresponding to the location of area V2 [9]. In the youngest fetus (E94) AchE forms a dense and continuous labeling throughout the lower part of the cortex in area V2. By E121, numerous AchE-labelled axons are found in layers 6,4, and the bottom of layer 3. In fetuses AchE activity in layer 4 is continuous while layer 3 staining forms dense patches as illustrated in the E121 fetus in Fig. 1A. During the last 45 days of gestation labelling in layer 4 decreases and is accompanied by a progressive reduction in the

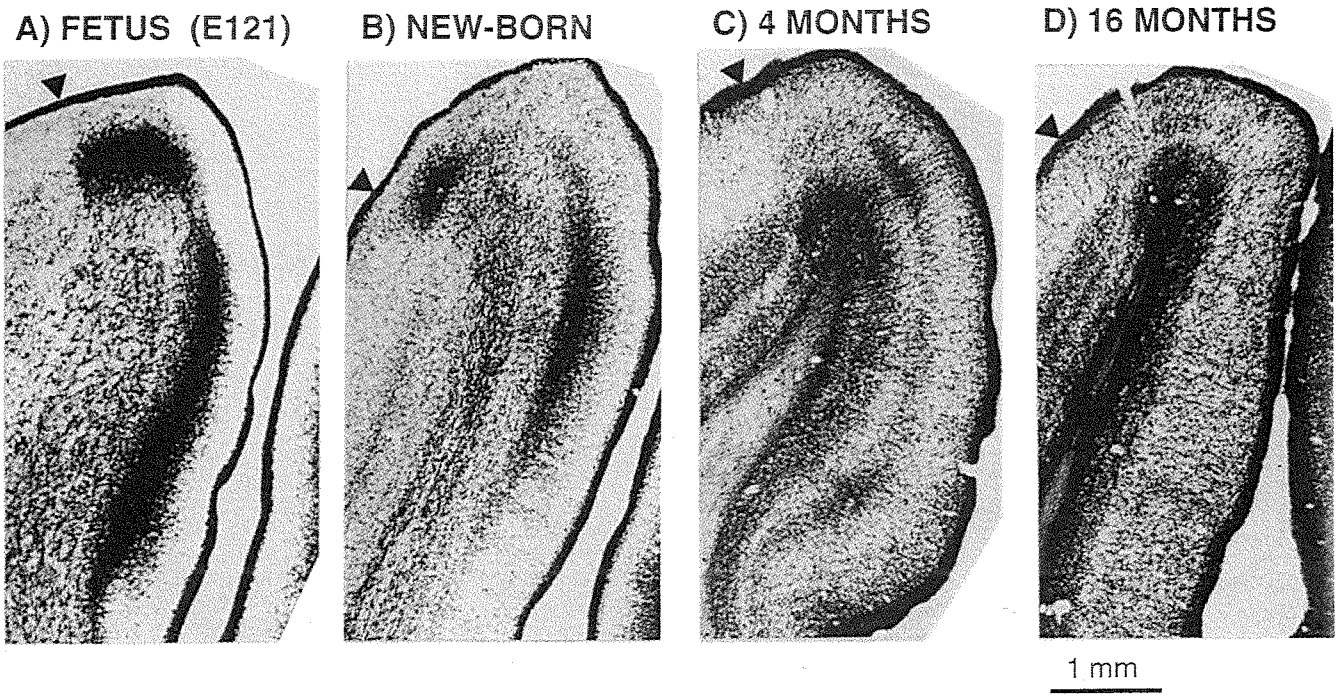

Fig. 1. AchE-stained parasagittal (A,C,D) and horizontal (B) sections of the operculum in a fetus (A), a new-born (B), a 4 month old (C) and a young adult monkey (D). Note that AchE staining delimitates clearly areas 17 and V2 (area 17/V2 border is indicated by a black arrowhead).

intensity of labeled patches in layer 3, so that by birth the continuous labelling in layer 4 is weaker and the patches in layer 3 do not extend as superficially as they do in the fetus (Fig. 1B). After birth, there is a gradual reduction in the intensity and distribution of labelling. At 4 postnatal months, layer 4 labelling is barely detectable and although patches can be clearly seen in layer 3 they appear smaller than at birth (Fig. 1C). The adult pattern of labelling emerges between 4 and 6 months and is characterised by a nearly uniform distribution of labelled axons throughout the cortex with layer 5 being just below threshold and layer 4 just above threshold (Fig. 1D).

By sectioning area V2 parallel to the cortical surface it is possible to examine the spatial organization of the transient expression of AchE in layer 3. This reveals that the regions of high AchE activity form a system of wide 1–3 mm bands which run perpendicular to the border of area V2 with area 17 (Fig. 2). The AchE bands disappear during postnatal development. In a 4 months old animal (Fig. 2C) AchE bands are still present but their limits are not as clear as in younger animals and their size is considerably reduced. At 6 and 16 postnatal months, as well as in the adults, we have not been able to detect AchE bands in the supragranular layers of area V2.

Developmental changes in the angle of the lunate sulcus with respect to the midline make it impossible to measure the width and the frequency of AchE bands in parasagittal and horizontal sections. We were able to make these measurements on tangential sections. Serial tangential sections were used to make reconstructions of AchE bands, so as to allow quantification of band width and frequency. Measurements of band width and separation on tangential sections could be made for approximately 8 bands per animal and the mean values are provided in Table 1. Significance was tested with a t-test. Band width and separation did not show any significant variation between the E129 fetus and the PND 6 and PND 17 neonates and showed a maximum value for band width of 2.7 mm and for separation of 0.9 mm. There was a significant change in these values at 4 months when band width dropped to 1.1 mm and separation increased to 1.7 mm ($P < 0.05$). These results demonstrate that there is a postnatal developmental narrowing of AchE bands which leads to their disappearance shortly after the fourth postnatal month.

The present results show that the periodic bands of high AchE activity emerge in area V2 between E94 and E121, show little change up to birth before disappearing between 4 and 6 months after birth. A major question concerns the origin of the transient expression of AchE in area V2. In the immature rat visual cortex, transient AchE is related to thalamic inputs as suggested by the loss of AchE activity following lesion of

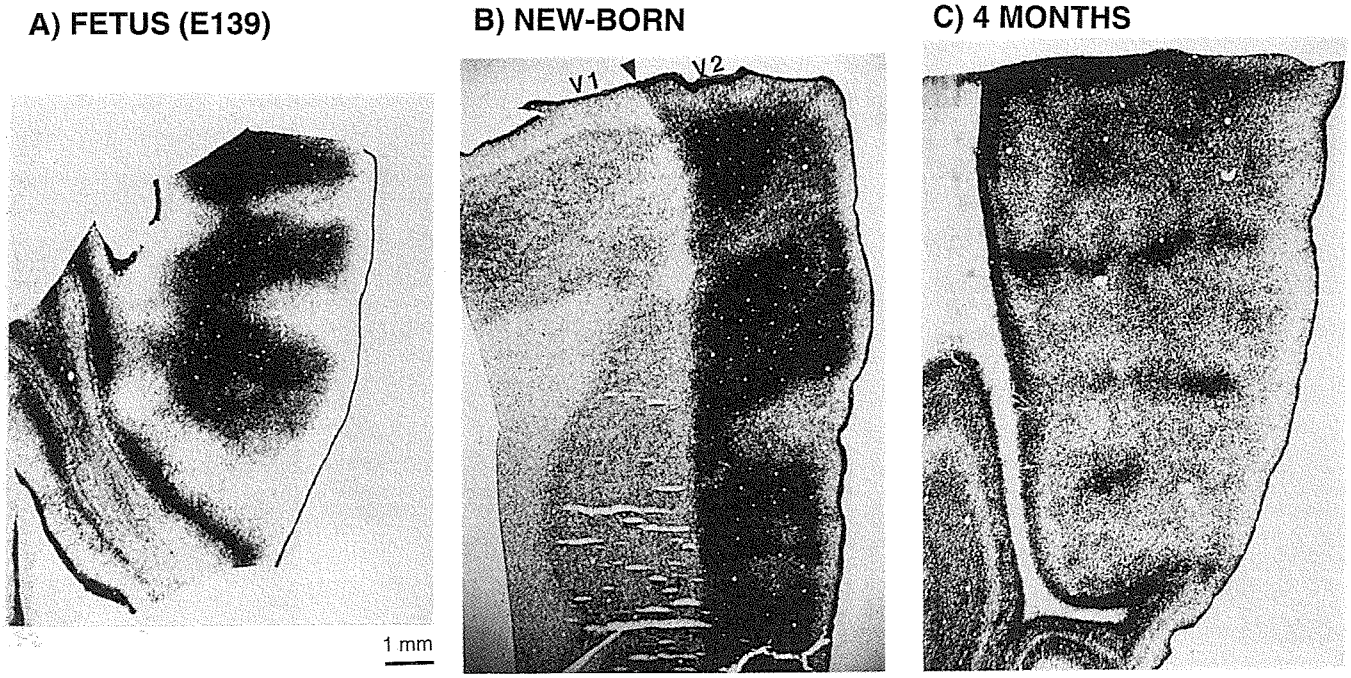

Fig. 2. AchE-stained tangential sections of area V2 obtained from a flattened cortex of a fetus (A), a 2 week old (B) and a 4 month old (C) monkey. In the fetus and the 2 week old animal AchE activity forms large bands perpendicular to the V1/V2 border (black arrowhead). In the 4 month old monkey AchE bands are narrower and present a lower level of activity.

the geniculate nucleus [11,25]. The inferior and lateral pulvinar are the main thalamic nuclei sending projections to area V2 [3,15,28]. These projections terminate in layer 4 and in the bottom of layer 3. These laminae correspond to the laminar compartments where AchE shows a transient expression. Pulvinar terminals in area V2 present a patchy organization [5,23] forming bands perpendicular to the area 17/18 border and are believed to be in register with the thick and thin cytochrome oxidase stripes [20]. Kostovic and Rakic [17] have shown that early outgrowth of the pulvinar towards the visual cortex can be explored using AchE histochemistry. Our results confirm that at E94 this projection is largely confined to the subplate and that invasion of the cortex occurs later at around E121. It could be that the thick bands of AchE in area V2 of the fetus correspond to maturing pulvinar input in this area, although direct experimental verification of this point is required. At present we do have indirect evidence as to the relationship of AchE bands with cytochrome oxidase bands. In the PND 15 and PND 17 neonates adjacent sections were processed for cytochrome oxidase histochemistry. Comparison of adjacent sections showed that the frequency of cytochrome oxidase bands was twice that of AchE bands.

The maturation of AchE expression is relatively prolonged compared to other developmental events. For example, the segregation of ocular dominance columns in V1 is accomplished around the sixth postnatal week [24]. Similarly, callosal and associative connectivity of area V2 is established during the first month of life [1,7]. Finally, cytochrome oxidase staining exhibits a characteristic pattern in areas 17 and V2 which reaches maturity during the first postnatal month [7,13,16]. The late maturation of AchE best correlates to synaptogenesis which has been shown to be incomplete at six months of age [4,22] and the overproduction of neurotransmitter receptors [19].

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[1] Barone, P., Dehay, C., Berland, M., Bullier, B. and Kennedy, H., Developmental remodelling of primate visual cortical pathway, *Cerebral Cortex*, in press.

[2] Bear, M., Carnes, K.M. and Ebner, F.F., Postnatal changes in the distribution of acetylcholinesterase in kitten striate cortex, *J. Comp. Neurol.*, 237 (1985) 519–532.

[3] Benevento, L.A. and Rezak, M., The cortical projection of the inferior pulvinar and adjacent lateral pulvinar in the rhesus monkey (*Macaca mulatta*): an autoradiographic study, *Brain Res.*, 108 (1976) 1–24.

[4] Bourgeois, J.-P. and Rakic, P., Changes of synaptic density in the primary visual cortex of the macaque monkey from fetal to adult stage, *J. Neurosci.*, 13 (1993) 2801–2820.

[5] Curcio, C.A. and Harting, J.K., Organization of pulvinar afferents to area 18 in the squirrel monkey: evidence for stripes, *Brain Res.*, 143 (1978) 155–161.

[6] Dehay, C., Horsburgh, G., Berland, M., Killackey, H. and Kennedy, H., The effects of bilateral enucleation in the primate fetus on the parcellation of visual cortex, *Dev. Brain Res.*, 62 (1991) 137–141.

[7] Dehay, C., Kennedy, H. and Bullier, J., Callosal connectivity of areas V1 and V2 in the new-born monkey, *J. Comp. Neurol.*, 254 (1986) 20–33.

[8] Fitzpatrick, D. and Diamond, I.T., Distribution of acetylcholinesterase in the geniculostriate system of *Galago senegalensis* and *Aotus trivirgatus*: evidence for the origin of the product in the lateral geniculate body, *J. Comp. Neurol.*, 194 (1980) 703–719.

[9] Gattass, R., Gross, C.G. and Sandel, J.H., Visual topography of V2 in the macaque, *J. Comp. Neurol.*, 201 (1981) 919–939.

[10] Greenfield, S., Acetylcholinesterase may have novel functions in the brain, *Trends Neurosci.*, 7 (1984) 364–368.

[11] Hanes, M.A., Robertson, R.T. and Yu, J., Transition from developing to mature patterns of acetylcholinesterase activity in rat visual cortex: implications for the time course of geniculocortical development, *Dev. Brain Res.*, 66 (1992) 97–108.

[12] Hardy, H., Heimer, L., Switzer, R. and Watkins, D., Simultaneous demonstration of horseradish peroxidase and acetylcholinesterase, *Neurosci. Lett.*, 3 (1976) 1–5.

[13] Horton, J.C. Cytochrome oxidase patches: a new cytoarchitectonic feature of monkey visual cortex, *Phil. Trans. R. Soc. Lond.*, B304 (1984) 199–253.

[14] Hutchins, J.B. and Casagrande, V.A., Development of acetylcholinesterase in the lateral geniculate nucleus, *J. Comp. Neurol.*, 275 (1988) 241–253.

[15] Kennedy, H. and Bullier, J., A double-labelling investigation of the afferent connectivity to cortical areas V1 and V2 of the macaque monkey, *J. Neurosci.*, 5 (1985) 2815–2830.

[16] Kennedy, H., Bullier, J. and Dehay, C., Cytochrome oxidase activity in the striate cortex and lateral geniculate nucleus of the new-born and adult macaque monkey, *Exp. Brain Res.*, 61 (1985) 204–209.

[17] Kostovic, I. and Rakic, P., Development of prestriate visual projections in the monkey and human fetal cerebrum revealed by transient cholinesterase staining, *J. Neurosci.*, 4 (1984) 25–42.

[18] Krist, D.A., Development of neocortical circuitry: histochemical localization of acetylcholinesterase in relation to the cell layers of rat somatosensory cortex, *J. Comp. Neurol.*, 186 (1979) 1–16.

[19] Lidow, M.S., Goldman-Rakic, P.S. and Rakic, P. Synchronized overproduction of neurotransmitter receptors in diverse regions of the primate cerebral cortex, *Proc. Natl. Acad. Sci. USA*, 79 (1991) 10218–10221.

[20] Livingstone, M.S. and Hubel, D.H., Thalamic input to cytochrome oxidase-rich regions in monkey visual cortex, *Proc. Natl. Acad. Sci. USA*, 79 (1982) 6098–6101.

[21] Morel, A., Garraghty, P.E. and Kaas, J., Tonotopic organization, architectonic fields, and connections of the auditory cortex in macaque monkey, *J. Comp. Neurol.*, 335 (1993) 437–459.

[22] O'Kusky, R. and Colonnier M., Postnatal changes in the number of astrocytes, oligodendrocytes, and microglia in the visual cortex (area 17) of the macaque monkey: a stereological analysis in normal and monocularly deprived animals, *J. Comp. Neurol.*, 210 (1982) 307–315.

[23] Ogren, M.P. and Hendrickson, A.E., The distribution of pulvinar terminals in visual areas 17 and 18 of the monkey, *Brain Res.*, 137 (1977) 343–350.

[24] Rakic, P., Prenatal genesis of connections subserving ocular dominance in the rhesus monkey, *Nature*, 261 (1976) 467–471.

[25] Robertson, R.T., Hanes, M.A. and Yu J., Investigation of the origins of transient acetylcholinesterase activity in developing rat visual cortex, *Dev. Brain Res.*, 41 (1988) 1–23.

[26] Robertson, R.T., Mostamand, F., Kageyama, G.H., Gallardo, K.A. and Yu, J., Primary auditory cortex in the rat: transient expression of acetylcholinesterase activity in developing geniculocortical projections, *Dev. Brain Res.*, 58 (1991) 81–95.
[27] Robertson, R.T., Tijerina, A.A. and Gallivan, M.E., Transient pattern of acetylcholinesterase activity in visual cortex of the rat: normal development and the effects of neonatal monocular enucleation, *Dev. Brain Res.*, 21 (1985) 203–214.
[28] Wong-Riley, M.T.T. Connections between the pulvinar nucleus and the prestriate cortex in the squirrel monkey as revealed by peroxidase histochemistry and autoradiography, *Brain Res.*, 134 (1977) 249–267.