Developmental Brain Research, 62 (1991) 137–141
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ADONIS 0165380691604107

BRESD 60410

Short Communications

The effects of bilateral enucleation in the primate fetus on the parcellation of visual cortex

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(Accepted 30 April 1991)

Key words: Monkey; Area 17; Development; Cell proliferation; Cell death

Bilateral enucleation in the macaque fetus causes an areal reduction of an otherwise normal striate cortex. Here we show that in early operated animals this reduction is accompanied by a separation of striate and prostriate cortices which are normally contiguous. However this induced separation does not correspond to the areal reduction of striate cortex, indicating that extrinsic signals regulate either the proliferation and/or survival of striate cortical neurons.

Early prenatal enucleation in the monkey results in an area 17 which is drastically reduced in its surface area although apparently normal in terms of its cortical lamination and the sharpness of its cytoarchitectonic borders[4,16]. Rakic[16] has suggested this might be due to a failure of striate cortex in the vicinity of the areal borders to acquire its normal cytoarchitectonic features. If the effect of enucleation is due to a smaller cortical region being claimed as striate cortex by a reduced number of geniculate fibers, then the unspecified cortex which has failed to acquire striate features should appear as a complementary expansion of adjacent cortex. However this is a difficult proposition to prove or disprove since it is virtually impossible to detect an expansion of area 18 as its anterior limits with area 19, unlike the border with striate cortex, are not precisely defined cytoarchitectonically[25]. What is required is an extrinsic landmark of the limits of area 17, other than area 18. This is available in the calcarine sulcus. Here, in the normal animal, area 17 shares a common border with prostriate cortex[18,22]. The distinctive cytoarchitectonics of prostriate cortex make it readily distinguishable from both area 17 and the surrounding areas 18 and 19. Thus, should prenatal enucleation result in a failure of specification of an uncommited cortical plate, area 17 will no longer be contiguous with prostriate cortex. Instead, cortex which was originally destined to become area 17 (and which would have subsequently failed to do so due to lack of peripheral input) will appear as a cortical region located between striate and prostriate cortex. To test this hypothesis we have used two-dimensional (2-D) reconstructions to measure the effect at birth of bilateral enucleation in the fetus on the relationship of striate cortex to area prostriata and to examine whether the reduction of striate cortex is accompanied by a proportional separation between striate and prostriate cortex.

Timed pregnant cynomolgus monkeys (*Macaca irus*) were prepared for surgery under ketamine (i.m.) followed by Alfatesin (i.v.) anaesthesia. After intubation, anaesthesia was continued with halothane in a N_2O/O_2 mixture (70/30). Expired CO_2 and heart rate were monitored. The body temperature was maintained using a thermostatically controlled heating blanket. A midline abdominal incision was made and uterotomy performed. After exposure of the fetal head and bilateral eye removal the fetus was replaced in the uterus and incisions closed using routine procedures. The mother was returned to the cage and given an analgesic (visceralgine, i.m.) twice daily for two days. Following either natural birth or delivery by caesarian section, control and preterm operated neonates were sacrificed on day E165 (the normal gestational period is 165 days). The infant monkey was anaesthetized with ketamine (i.m.) followed by nembutal (i.v.) and perfused through the heart with a

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TABLE I

Pial surface area of striate cortex in control and operated animals

	Adult		*Neonates*					
	1	*2*	*Normal*	*Enucleated*				
				E110	*E81*	*E77*	*E68*	*E59*
Surface area of striate cortex (mm^2)	808	894	804	543	693	496	227	224

mixture of 1.25% paraformaldehyde and 1.5% glutaraldehyde. Parasagittal sections (60 μm thick) were cut on a freezing microtome and one in 3 were stained for Nissl substance. Two-dimensional reconstructions of area 17 and adjoining cortex were made from the Nissl-stained sections. Maps were constructed in which topological and spatial relationships are conserved with a minimal distortion using the method of Van Essen and Maunsell[21].

Five fetal monkeys underwent bilateral enucleation. Following surgery, development was allowed to proceed to term at E165 when the brains were prepared for histological observation. Table I reports the pial surface area of striate cortex in control and operated animals. Areal measurements were made on back-projected counter-stained sections. Striate cortex in the normal neonate has a surface area similar to that in the adult[13]. In the enucleated animals the cytoarchitectonics of striate

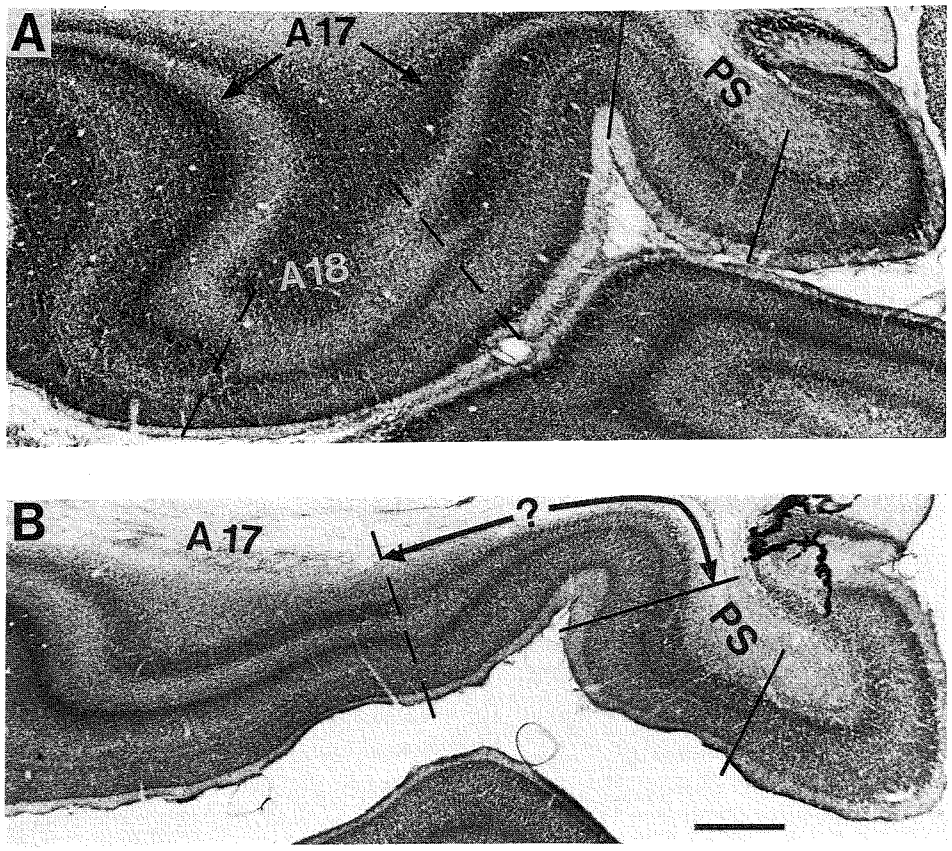

Fig. 1. Photomicrograph of Nissl-stained parasagittal sections of the rostral part of the dorsal bank of the calcarine sulcus showing cytoarchitectonic borders. A: section from the normal neonate. This section has been chosen because it illustrates both the area 17/18 border and the area 17/prostriata border and demonstrates that these borders are clearly distinguishable. The relationship of this section to the surrounding tissue is shown in the top of Fig. 2 (see white arrow). B: section from the E68 enucleated neonate. In this and the other early operated animal, striate cortex failed to contact prostriata. This section (indicated in the lower part of Fig. 2) was taken from the level where the neck of neocortex separating striate cortex from prostriata was at its narrowest. Dotted lines: limits of striate cortex. Continuous lines: limits of prostriata. The region of cortex marked with a question mark in the E68 operated neonate corresponds to the cortex separating prostriata and striate cortex and appears cytoarchitectonically like area 18. Bar = 500 μm.

cortex and its borders were clearly defined in Nissl-stained sections. In all 5 monkeys subjected to enucleation striate cortex was smaller than normal. The effect of enucleation was found to depend on the developmental stage of the cortex at the time of the operation. Production of the cells making up striate cortex commences at E45 and terminates around E100[14] and the cytoarchitectonic borders of striate appear around E85[23]. With respect to the normal neonate, late enucleation (E77, E81 and E110) gave an areal reduction of 14–38% and was not found to result in a greater reduction of striate cortex if carried out just before or just after formation of cytoarchitectonic boundaries. In contrast, the effect of enucleation during the early phase of cortical cell production (E59 and E68) led to a much more severe reduction of over 70%.

Prostriate cortex is characterised in the neonate, as in the adult, by a layer 3 of moderate density but lacking large pyramidal cells, and above all by a layer 4 which is poorly defined in comparison to that in striate cortex and area 18 (Fig. 1). Striate cortex has a sharp border with prostriata in the 3 normal animals examined (two adults and one neonate), as reported elsewhere[22].

The prostriate/striate boundary was found to be intact in the neonates which had been enucleated at E77, E81 and E110. The distances over which these two cortical areas were in contact (1–2.5 mm) were similar in the 3 late enucleated neonates (E77, E81 and E110) and the normal neonate.

Only in the neonates which were enucleated at E59 and E68 and which showed a reduction of over 70% did striate cortex fail to contact prostriate cortex. In these two animals, juxtaposed between striate and prostriate cortex was a cortical region which was indistinguishable from cortex which normally bounds striate cortex (Fig. 1). This cortex displays a pronounced layer 4 which readily distinguishes it from prostriate cortex.

By reconstructing the cortical surfaces buried in the calcarine sulcus it was possible to determine the topographical relationship between striate and prostriate cortex and to quantify changes induced by early enucleation (Fig. 2). The method used to construct these cortical maps seeks to reduce spatial distortions due to curvature of the cerebral cortex[21,22]. In the lower part of Fig. 2 is shown the reconstruction from the E68 enucleate. In the cortical region separating prostriate cortex from striate cortex the section contour lines run parallel to each other indicating that major changes in folding of the cortex have not occurred in this region. This is important because it makes it unlikely that there are any large topological distortions in our mapping of this region. The 2-D reconstruction also makes it possible to measure directly the minimum distance separating prostriata and

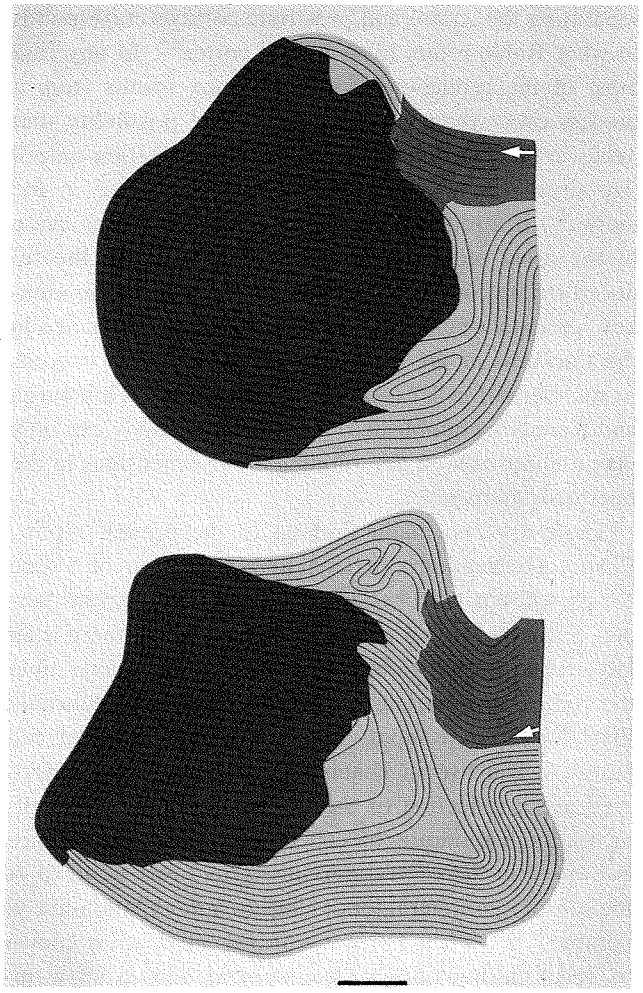

Fig. 2. Two-dimensional reconstruction of the calcarine sulcus in the normal neonate (top) and the neonate which had undergone enucleation at E68 (bottom). Each contour represents the outline of Nissl-stained sections. Maps were constructed using adjacent sections, those illustrated in Fig. 1 are indicated by white arrows. Green: allocortex including prostriate cortex; yellow: neocortex other than striate cortex; red: striate cortex. Bar = 3200 μm.

striate cortex which was found to be 1.5 mm in the E68 operated neonate. A similar separation of striate cortex and prostriata was found in the E59 operated neonate.

Our results test whether the areal reduction of striate cortex is due to the tissue originally destined to become striate cortex failing to acquire its normal cytoarchitecture, that is, whether altered peripheral input can result in the respecification of cortical areas. We find evidence for such peripheral control only in the two neonates enucleated at E68 or earlier. Further, the reduction of striate cortex is not accompanied by the insertion of an equivalent amount of cortex between striate and prostriate cortices. The configuration of the two experimentally separated borders which are parallel rules out the possibility that the reduced striate cortex has maintained even a small contact with prostriate cortex. This suggests — but does not prove — that the totality of the region

separating the striate and prostriate cortices corresponds to what Rakic refers to as hybrid cortex[17]. In any case most of the reduction of striate cortex results from a process other than a border shift. One possibility that needs to be considered is an effect of enucleation on cell size since this has been shown to be the case in the mouse[20]. If a reduction of cell size was the prime cause of the areal reduction then one would expect the reduced striate cortex to be thinner. However in the monkey, prenatal enucleation leads to little or no change in the thickness of the cortex (measured either in microns or numbers of cells) (refs. 2, 17 and Dehay, Horsburgh and Kennedy unpublished) so that cell atrophy can only play a minor role in determining areal dimensions in the operated animals.

There are two major findings in the present report. Firstly, enucleation can lead to a reduction of striate cortex without pertubation of the striate/prostriate border. Secondly, in the youngest cases in which there is an induced cortical region, it is considerably smaller than the reduction in striate cortex. We are led to conclude that the afferent input directly regulates cortical cell number. The two mechanisms that need to be considered are a reduction of the production of area 17 cells or an increased rate of cell death. There is evidence elsewhere in the central nervous system that removal of the sensory peripheral input leads to a modulation in cell number[1,3,5,7–9,11,12]. Further, cortical cell death is known to occur during normal development and to show increased levels under certain experimental conditions[6,24]. There is recent evidence that central neurogenesis is subject to peripheral control in invertebrates[1,19]. The proposition that peripheral input exerts an extrinsic control on the rate of cell proliferation in vertebrates is perhaps more controversial. Several authors have shown that cell production is at least partially regulated by the retinal input in the optic tectum of the frog[3,11,12] and the superior colliculus of the mouse[5]. The idea that extrinsic signals might regulate cell production in primate striate cortex is not incompatible with certain features of its development. For instance Kelly and Cowan[9] have pointed out that in incidences in which the periphery has been shown to control numbers of cells via an influence on the cell cycle, it has been shown that mitotic activity is prolonged over a considerable period. This is the case in monkey striate cortex where neuron production occurs between embryonic days 40 and 100[14]. Likewise, if afferents are going to regulate neurogenesis one would expect innervation to occur early in the developmental process. This is clearly the case since geniculate afferents are already present in the subplate zone by E78 if not earlier[15]. That is to say afferent fibers are in a position to exert an extrinsic control throughout most of the period of cortical neuron production.

To conclude, whereas the cytoarchitecture and periodicity of cytochrome oxidase blobs in area 17 develop independently of the sensory periphery, the areal dimensions are critically dependent on the presence of the retina — indicating two independent mechanisms of cortical specification[10]. The present results show that the effects of enucleation on the reduction of striate cortex and the formation of its border with prostriate cortex imply that extrinsic signals regulate the production and/or survival of striate cortical neurons.

This work was supported by an E.E.C. Science Grant 622. We are grateful to Ghislaine Clain for expert animal care, Sandrine Richard for histological expertise and to Pascale Giroud for her battle with the colour print.

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