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Maturation and connectivity of the visual cortex in monkey is altered by prenatal removal of retinal input

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In several species, the peripheral input from the eyes partly determines the pattern of interconnections between the visual areas of the two cerebral hemispheres through the fibre tract termed the corpus callosum[1–9]. In the macaque monkey, the neurons projecting through the callosum are largely restricted to area 18 throughout ontogeny, whereas area 17 is characterized by few or no callosal projections[10–12]. Here, we show that suppressing the peripheral input by prenatal removal of the eyes leads to a marked reduction in the extent of area 17, resulting in a large shift in the position of the histologically identifiable boundary between the two areas. Even so, the boundary continues to separate an area rich with callosal connections (area 18) from one poor in such projections (area 17), indicating there is no effect on the callosal connectivity of area 17. In contrast, in area 18, eye removal results in many more neurons with callosal projections than in normal animals. The results suggest that the factors that determine the parcellation of cortical areas also specify their connectivity.

Two fetal macaque monkeys (*Macaca cynomolgus*) had both eyes removed at 77±2 and 112±2 days after conception (days E77 and E112), and the fetuses were then replaced in the uterus. At about the normal time of birth the callosal connectivity of areas 17 and 18 was determined using horseradish peroxidase histochemistry.

The pattern of layers in area 17 seemed remarkably normal in both experimental animals. The border between areas 17 and 18, clearly defined in Nissl-stained sections, was as distinctive as in the neonatal control (Fig. 1 and ref. 13). The 17/18 border could also be detected in sections reacted for cytochrome oxidase activity (Fig. 1). Comparison of adjacent sections stained by the two methods reveals a double band of higher cytochrome oxidase activity in layer 4C that stops abruptly at the 17/18 border, as in the control animal[14]. Despite the weaker staining for cytochrome oxidase activity in the experimental animal its distribution within area 17 was, on the whole, similar[14].

In the monkey operated on day E77, the gyral pattern of the occipital lobe was unusual and the surface area of area 17 was considerably reduced (Fig. 2, see also ref. 13), particularly laterally where, in the intact animal, cortex subserving foveal and parafoveal vision is located. Later eye removal (on day E112) did not cause a noticeable reduction in the surface area of area 17 or a disturbance in the gyral pattern[13].

In both animals after eye removal, the disposition of callosally projecting neurons with respect to the border between areas 17 and 18 was normal (Fig. 2): they were largely confined to the extrastriate cortex and stopped abruptly at the 17/18 border. In both animals, only one or two labelled neurons per section were found in area 17 where they were located, as in normal animals, within 2-3 mm of the border[11,15,16]. In contrast, in area 18, callosally projecting neurons were more densely packed and stretched further rostrally from the 17/18 border than in normal neonates (Fig. 2). Counts of callosally projecting neurons were made in the part of area 18 that extends from the 17/18 border to the fundus of the lunate sulcus, in the part of the cortex that represents the parafoveal region of the visual field. In the normal animal there were 50–140 labelled neurons per section[10,12], whereas in both experimental animals the number was 5–6 times higher (280–875) than in either normal neonates or adults (Fig. 3). Although the age at which the eyes were removed did not influence the numbers of callosally projecting neurons in area 18, it did affect their spatial distribution. The animal operated on day E112 showed a continuous distribution of callosally projecting neurons but in the animal operated on day E77 the distribution of callosally projecting neurons was discontinuous and patchy (Fig. 2).

The demonstration that eye removal alters the callosal connectivity of area 18 but not of area 17 should be considered in the light of the ontogeny of callosal connections in the monkey. Several studies have shown that area 17 is largely free of callosal

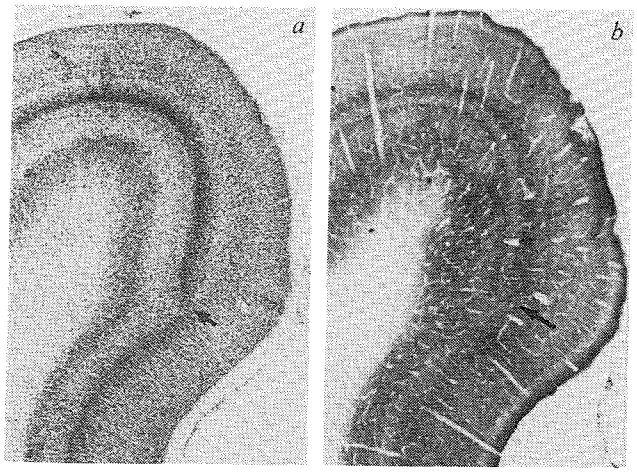

Fig. 1 Characteristics of the 17/18 border after early eye removal (E77). *a*, Section stained with cresyl violet *b*, Adjacent section reacted to show cytochrome oxidase activity. The arrows indicate the 17/18 border. Although the level of cytochrome oxidase activity is lower than in normal neonates the overall distribution in area 17 and the change in layer 4 at the 17/18 border is similar.
Methods. Timed pregnant monkeys 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 her cage and medicated for the first two days with analgesic (Visceralgine, i.m.). A muscular relaxant (Duvadilan, i.m.) was given twice daily until the fetus was delivered by caesarian section 1–2 weeks before term. The infant monkey was bottle fed until the axonal tracing experiments on day E159 when removal was at E77, and E164 when eye removal was at E112 (the gestation period is 165 days). Infant monkeys were anaesthetized with ketamine (i.m.) followed by Alfatesin (i.v.). Craniotomy was performed over the occipital lobe and 15–25 μl of 30–50% horseradish peroxidase (HRP) were injected using a Hamilton syringe equipped with a glass micropipette (60–120 μm tip diameter). Closely spaced injections were made over the occipital lobe so as to massively fill area 17 and the adjacent part of area 18. After 24 hours, the infant monkey was deeply anaesthetized and perfused through the heart with a mixture of 1.25% paraformaldehyde and 1.5% glutaraldehyde. Sections (60 μm thick) were cut parasagitally and one in three sections were processed for HRP histochemistry[18]. Intermediate sections were processed for cytochrome oxidase[19] and stained for Nissl substance (cresyl violet). The border of area 17 and 18 was clearly defined in Nissl and cytochrome oxidase-reacted sections and in the hemisphere contralateral to the injection of HRP, labelled neurons were counted and allocated to either area. Counts of labelled neurons were made in area 18 from the 17/18 border to the fundus of the lunate sulcus. Individual sections were traced out and the positions of labelled cells recorded using an *x-y* plotter electronically coupled to the microscope stage.

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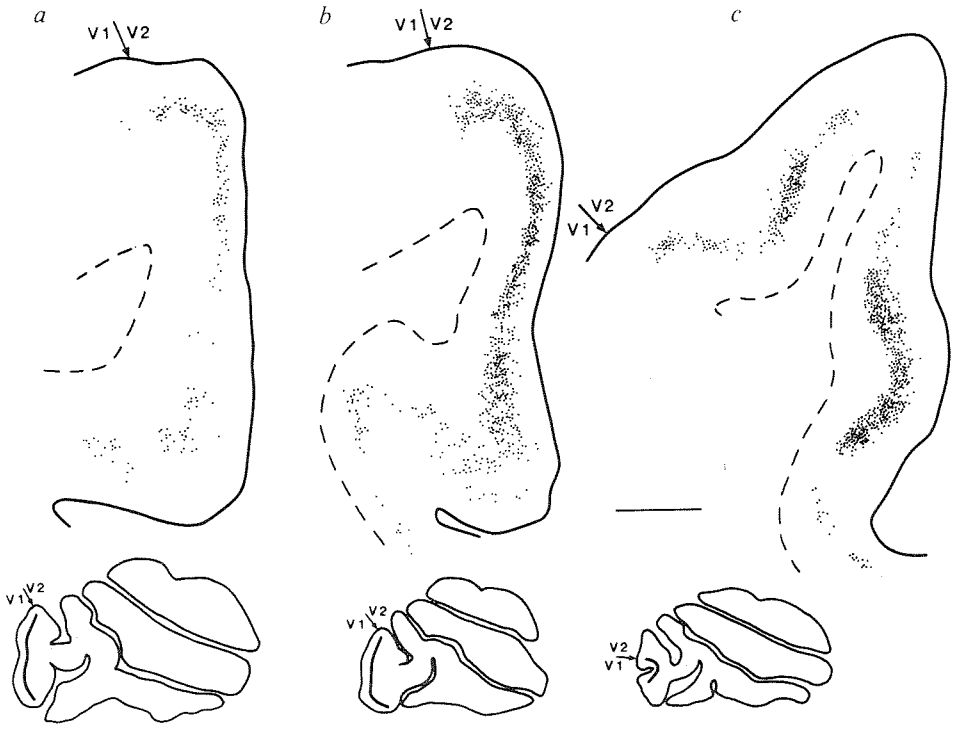

Fig. 2 Parasagittal sections of the area 17/18 border showing callosally projecting cells in the newborn monkey. *a*, Normal. *b*, Eye removal at E112. *c*, Eye removal at E77. Each dot represents a retrogradely labelled cell and arrows indicate the area 17/18 border. Inserts show low-power drawing at the level at which the high-power plots were made and where the continuous line in the operculum indicates the extent of area 17. Eye removal did not increase the numbers of callosally projecting neurons in area 17, but did increase the numbers of these cells in area 18. Whereas area 17 occupies most of the operculum of the normal animal and after eye removal at E112, after eye removal at E77, area 17 seems considerably shrunken. Scale bar, 1 mm.

connections throughout development (ref. 11; L. M. Chalupa and H. Killackey manuscript, in preparation; P. Rakic, personal communication). Eye removal, which stabilizes transient callosal connections in all other species examined[1-9], in monkey fails to reveal a transient callosal projection in area 17, emphasizing that the lack of callosal connections in area 17, unique to the monkey, is specified early in development. The normal maturation of the cortical connectivity of area 18 differs from that of area 17, as the distribution of callosally projecting neurons in area 18 changes during development[11]. Because eye removal both early and late in prenatal life leads to higher numbers of callosally projecting neurons in area 18, the elimination of the transient callosal projection, which normally occurs in the last month of gestation[11] must be regulated by epigenetic factors that depend at least partially on peripheral input.

The reduced size of area 17 after early eye removal could result from an increase in cell death. As the number of afferent fibres from the lateral geniculate nucleus in the thalamus is reduced after early eye removal (ref. 17; H. Kennedy, unpublished observation), the survival of neurons in area 17 may depend critically on the density of the input. It is also possible that the input from the thalamus contributes directly to determining cortical areas, so that the smaller number of afferents from the lateral geniculate nucleus after eye removal leads to reduction in the extent of cortex specified as area 17 and a consequent expansion of the adjacent area 18[13]. This raises the question of whether the cortex immediately adjacent to area 17 constitutes a hybrid area, that is, a region of cortex with characteristics of both areas 17 and 18[13]. As the absence of callosal connections from area 17 is determined early in development, it can be used to indicate whether tissue originates developmentally from area 17 or area 18. As callosally projecting neurons are found in area 18 immediately adjacent to area 17, the expanded area 18 seems to be a cortical region that was unspecified when the eyes were removed and, because of the subsequent lack of peripheral input, failed to mature into area 17.

These results indicate that retinal input has several, somewhat independent effects on cortical development. Early in development the factors that help to specify the limits of cortical visual areas also regulate the overall presence or absence of callosal connectivity. Once the boundaries of areas are established, the influence of peripheral input is limited to regulating the density of callosal projection neurons in extrastriate visual cortex.

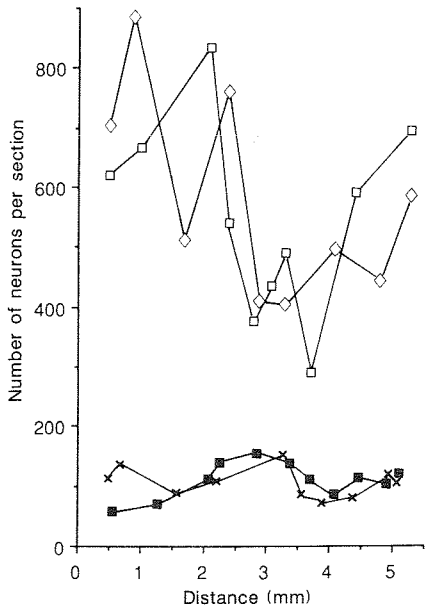

Fig. 3 Counts of HRP labelled callosally projecting neurons in area 18 of normal adult (■), neonatal control (×), after eye removal at E77 (□) and after eye removal at E112 (◇). In normal development, the numbers of callosally projecting neurons do not change after birth. Counts of callosally projecting neurons were considerably higher in the two animals whose eyes were removed. The age at which the eyes were removed had no influence on the numbers.

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