

Absence of interhemispheric connections of area 17 during development in the monkey

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Our understanding of the development of cortical connectivity largely stems from studies of the ontogeny of interhemispheric pathways in carnivores, rodents and lagomorphs¹⁻¹². Early in development, cortical neurons projecting to the contralateral hemisphere through the corpus callosum (callosal projection neurons) have a widespread distribution. As maturation proceeds, callosal projection neurons become restricted to those cortical regions that are connected in the adult. In newborn cats and rats, for example, callosal projection neurons are not restricted to the 17-18 border as in the adult, but are found throughout areas 17 and 18 (refs 2, 5 and 10). The macaque monkey is an exception, because at birth it has an adult-like distribution of callosal projection neurons in area 18, with practically none in area 17 (refs 13-15). Here we show that whereas area 17 is devoid of interhemispheric connections throughout prenatal development, the distribution of callosal projection neurons in area 18 shows the common sequence of an early widespread distribution followed by regression. The absence of callosal projection neurons in area 17 throughout ontogeny may well be a feature unique to Old World primates.

We have investigated the callosal connectivity of areas 17 and 18 using horseradish peroxidase (HRP) histochemistry in fetal macaque monkeys (*Macaca cynomolgus*) at 97 (± 2), 112 (± 2), 114 (± 2) and 133 (± 2) days after conception (gestation period is 165 days) (Fig. 1).

At a fetal age of 97 days (E97), anterogradely labelled callosal fibres had only just crossed the midline, and no retrogradely labelled neurons were found in the hemisphere contralateral to the injection. The lateral geniculate and pulvinar nuclei ipsilateral to the injected hemisphere were extensively labelled both retrogradely and anterogradely, indicating that pick-up and transport of HRP had occurred. By 114 days (E114), labelled neurons were found in the contralateral hemisphere. At this age the cytoarchitectonics of area 17 are quite distinctive, and its border with area 18 is as sharply defined as in the adult. Callosal projection neurons were found at the 17-18 border, but nowhere else in area 17. We have checked that HRP was taken up in the injected striate cortex by examining the ipsilateral lateral geniculate nucleus, which contained numerous labelled neurons. A dense band of labelled callosal projection neurons was found in anterior cortical regions stretching posteriorly throughout area 18, but terminating abruptly at the 17-18 border (Figs 2 and 3). Area 18 on the ventral surface of the hemisphere also contained large numbers of labelled neurons (Fig. 1b). All labelled neurons in this and the other animals were found in supragranular layers. Although, as often found in the neonate¹⁴, the density of HRP reaction product was relatively low in the cells, use of crossed polarized filters made it possible to visualize labelled cells and to chart their position using an x-y plotter electronically coupled to the microscope stage. This showed that densities of labelled cells were very high in extrastriate cortex and that there was no obvious periodic fluctuation of numbers of labelled neurons. The same results were obtained at embryonic day E112.

Callosal connectivity was examined in one animal at day

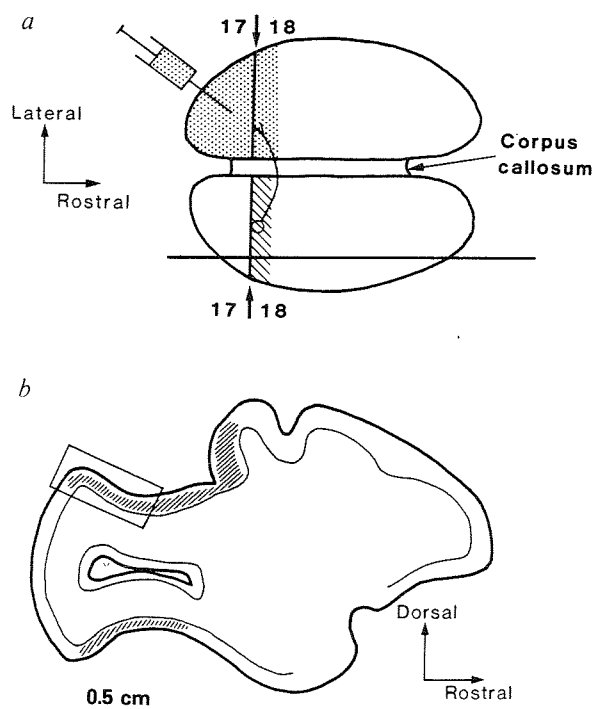


Fig. 1 a, The experimental paradigm. HRP is injected at multiple sites in the occipital lobe of one hemisphere so as to fill massively area 17 and the adjacent part of area 18 on the dorsal cortical surface. HRP is captured in the injected hemisphere and transported retrogradely to cell bodies in the contralateral hemisphere. After a suitable survival period to allow axonal transport, the contralateral hemisphere is examined for HRP reaction product. b, A parasagittal section (taken along horizontal line in a) of a prenatal monkey brain at embryonic day E114. At this age the lunule sulcus is not yet formed. Retrograde labelling of neurons is found in both the ventral and dorsal cortical surfaces, showing that at this age the interhemispheric pathway is in place. In this and all other embryonic ages examined, retrogradely labelled cells in the contralateral hemisphere were found extensively in area 18, but stopped abruptly at the 17-18 border. Square on dorsal surface shows location of high-power plot presented in Fig. 2.

Methods. Timed pregnant monkeys were prepared for surgery under Alfatesine (IV) anaesthesia. After intubation, anaesthesia was continued with halothane in N₂O/O₂ (70:30) mixture. Expired CO₂ and heart rate were monitored. After a midline abdominal incision, uterotomy was performed and the fetal head exposed. Craniotomy was performed over the occipital lobe and 3-5 μ l of HRP (30-50% solution) were injected by Hamilton syringe so as to fill massively areas 17 and 18 on the dorsal cortical surface. Injections (5-7) were made at a shallow angle so that each injection spanned 4-6 mm of cortex. All incisions were closed using routine procedures and the mother medicated with a muscular relaxant (Duvadilan) and analgesic (Visceralgine) before being returned to her cage. The fetus was delivered by caesarian section 36-48 h later, deeply anaesthetized and perfused through the heart with a mixture of 1.25% paraformaldehyde and 1.5% glutaraldehyde. Routine procedures were used for HRP histochemistry using tetramethylbenzidine (TMB) as the chromogen¹⁶.

E133. Again, no callosal projection neurons were found in area 17, despite the fact that the lateral geniculate nucleus contained numerous retrogradely labelled neurons. At day E133, the plots of retrogradely labelled neurons in extrastriate cortex showed an overall drop in density, compared with E112 and E114 (Fig. 2). The periodic spatial fluctuation of the density of callosal projection neurons in area 18, characteristic of the newborn and adult, was not present at E133 (Fig. 2).

Our data demonstrate that the callosal projection from area 18 completes its maturation between E133 and birth. This time course is similar, although delayed, to that of the development of callosal connections in the somatosensory cortex of the macaque monkey which begins around E119 and is completed

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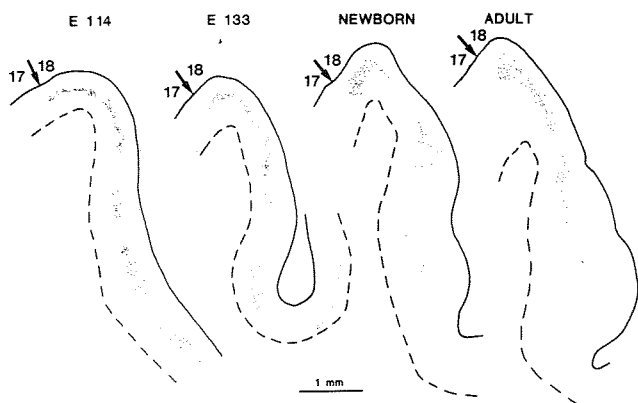


Fig. 2 Parasagittal 60 μm -thick sections of the area 17-18 border of fetal (E114, E133), newborn and adult monkeys. The arrow on the left of each brain section indicates the 17-18 border determined after counterstaining for Nissl substance. The dotted line indicates the boundary between grey and white matter. Each dot represents a retrogradely labelled neuron, following injection of HRP in the contralateral areas 17 and 18. In adult visual cortex, callosal neurons are largely confined to area 18 where they occur in clusters. The same distribution is observed in the newborn monkey. At E133, the distribution of callosal neurons in area 18 is continuous and no periodic fluctuation of the density of labelled neurons is observed. At E114, the density of labelled neurons appears considerably higher than at later ages and there is no suggestion of a periodic distribution. At all prenatal ages investigated, very few callosal projection neurons were found in area 17 where they were located on the 17-18 border. In all cases, callosal projection neurons were located in supragranular layers. For orientation of plot, see Fig. 1b.

by E133 (ref. 19). This delay in the reorganization of callosal connections of area 18, compared with somatosensory cortex, possibly reflects known rostro-caudal maturation gradients in the developing nervous system.

These results indicate that area 17 does not send axons to the contralateral hemisphere at any time in development in the macaque monkey. It is possible that callosal projection neurons are present in area 17, but for a much shorter period than that in which callosal projection neurons are undergoing reorganization in area 18. It seems unlikely that there are callosal projection neurons in area 17 before day E112. We found at E97 that callosal axons still had about 1-1.5 cm to grow before reaching the cortical grey matter of the contralateral hemisphere. Rates of axon growth range from 1.25 to 2.6 mm per day^{17,18} so that one could expect this pathway to be complete by about E107, leaving only a five-day period unexplored in this study. The possibility remains that callosal projection neurons are present in area 17 for only a brief period either before or after E133. In either case, both the onset and duration of the maturation of area 17 callosal connections would have to be very different from that found in other cortical areas, such as area 18 or the somatosensory cortex¹⁹.

The absence of callosal projection neurons in area 17 during ontogeny in the macaque is exceptional compared with other mammalian species. This raises the question of whether this is a general primate feature. There are no reports on the development of callosal connections of New World and prosimian primates. It is known, however, that numerous callosal projection neurons are found within area 17 in the adults of some species of these two suborders, and it is possible that these neurons constitute the remnants of an earlier extensive population of callosal projection neurons²⁰. The absence of callosal projection neurons in area 17 during development might therefore not be a common feature of all primates, but instead could be specific to Old World primates.

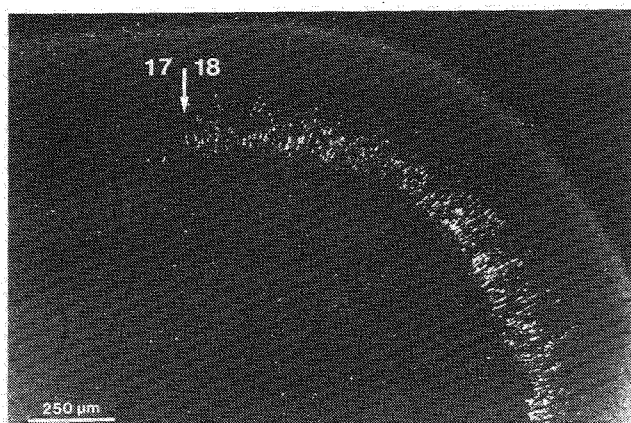


Fig. 3 Darkfield microphotograph of retrogradely labelled neurons from the section shown in Fig. 1b of the E114 fetal monkey. The white arrow indicates the 17-18 border. Callosal neurons are densely packed and lie in superficial layers. Scale bar: 250 μm .

Studies both on inter-¹⁻¹² and intrahemispheric²¹⁻²⁵ connections has led to the commonly accepted notion that cortical connections are more widespread during early development and that maturation is accompanied by a restriction of this connectivity. The present results suggest that this pruning of connections is not the only mechanism involved in the establishment of cortical connectivity. We cannot exclude the possibility that area 17 neurons send axons towards the corpus callosum, but over a very limited time so that they do not reach the contralateral hemisphere. Alternatively, the degree of specification of the connectivity of area 17 neurons might be much higher than for other cortical areas in the monkey, including the primary somatosensory cortex which does show exuberant callosal connections¹⁹. Should this be the case, area 17 of Old World monkeys might be expected also to be free of other transient connections which characterize the maturation of visual cortex in other species. These connections include transient projection to the pyramidal tract in carnivores²⁶, rodents^{27,28}, and lagomorphs¹⁷ and the projection of auditory cortex to area 17 in carnivores²²⁻²⁵.

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