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Incidence of visual cortical neurons which have axon collaterals projecting to both cerebral hemispheres during prenatal primate development

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Fast blue was injected massively in extrastriate cortex of one hemisphere and Diamidino yellow in area 17 of the other hemisphere, in adult and prenatal cynomolgus monkeys. After a suitable survival period the brains were processed for fluorescent dyes. Counts were made of the total number of labeled neurons and of those neurons which were labeled by both dyes and which project therefore to both hemispheres by means of bifurcating axon collaterals. At 122 and 135 days after conception (E122 and E135), shortly after cortico-cortical pathways are established, double-labeled neurons constituted 0.45% and 0.46% of the total population of labeled neurons in area V2. In V2 in the adult the range of values of double-labeled neurons was 0.03–0.08%.

The adult pattern of cortical connections is thought to emerge during development from a more widespread connectivity[6] (for a recent review see ref. 13). In the primate somatosensory cortex it has been shown that early in development when the density of callosal projecting neurons is very high, numerous neurons contribute bifurcating collaterals to the two major cortical pathways: the association (intrahemispheric) and callosal (interhemispheric) pathways[2]. Given the scarcity of bilateral projections in the adult[2,9,14] , this raises the question whether the loss of bifurcating projections is selective or alternatively parallels the overall rate of axonal elimination.

To test this hypothesis we have estimated quantitatively the segregation of callosal and association pathways in the developing primate visual cortex. Our results show that despite more extensive overlap of the parent neurons of the two pathways there is early in development only 0.5% of neurons which show bifurcated projections and this drops to 0.03–0.08% in the adult. Although a selective elimination does occur, it concerns a minute proportion of cells. These results show that a neuron's commitment to a particular cortical pathway is determined prior to axonal outgrowth by predominantly intrinsic factors.

The incidence during development of neurons which possess collaterals projecting to both hemispheres was investigated using two retrograde axonal tracers that can be distinguished within single cells[12] (Fig. 2). Fast-blue was injected in extrastriate cortex in one hemisphere and diamidino yellow was injected in a stereotyped fashion in area V1 of the other hemisphere. Two fetuses were used from timed pregnancies and two adults served as controls. Retrograde tracers were injected on embryonic day 122 (E122) and 135 (E135). In both cases there was a survival period of 10 days to allow retrograde transport. The fetal age refered to is the day of injection. Surgical procedures are more extensive in the fetal surgery and will be described in detail. Anaesthesia was similar in adult controls and pregnant females. Monkeys were prepared for surgery under ketamine (i.m.). After intubation, anaesthesia was continued with halothane in a N_2O/O_2 mixture (70:30). Heart rate, expired CO_2 and body temperature were monitored throughout. In the pregnant monkeys a midline abdominal incision allowed uterotomy to be performed and the fetal head exposed. Craniotomy was performed over the occipital, parietal and temporal lobes of one hemisphere. Contralaterally, a smaller craniotomy was made to expose the lateral part of the operculum. Callosal projecting neurons were labeled by extensive injections of fast-blue in the left hemisphere. Association projecting neurons were labeled by injection of diamidino yellow in a 3–5 mm tract 5–6 mm parallel to and behind the lip of the posterior bank of the lunate sulcus. Following injections, bone flaps were closed and the scalp stitched back in position. In prenatal material

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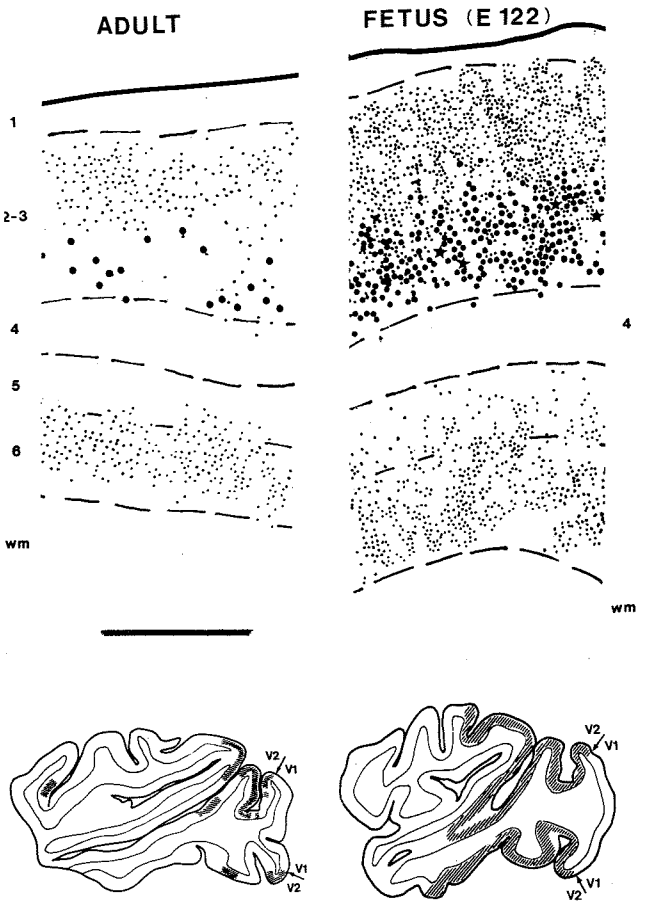

Fig. 1. Upper part: laminar distribution of association projecting neurons (small dots) and callosal projecting neurons (large dots) in area V2. Double-labeled neurons are shown by stars. Numbers refer to cortical layers and wm to white matter. (Scale bar = 0.5 mm.) Lower part: parasagittal section of the brain of the adult (left) and the E122 fetus (right) showing the distribution of callosal projecting neurons.

the fetus was replaced in the uterus and incisions closed using routine procedures. Following surgery the mother received post-operative medication (Visceralgine during 3 days) and a muscular relaxant (Duvadilan) until the fetus was delivered by caesarian section 10 days later. After the survival period, animals are deeply anaesthetized and perfused through the heart with 12% paraformaldehyde in 0.1 M phosphate buffer. Parasagittal sections (40 μm thick) were cut on a freezing microtome and 1 in 3 were kept for examination. Charts of labeled neurons were made in extrastriate cortex ipsilateral to the area V1 injection and their position recorded using an XY plotting table electronically coupled to the microscope stage. The histological borders were clearly defined with cresyl violet couterstaining to enable localization of labeled neurons in appropriate areas and layers. Percentages of neurons which were labeled by both dyes (double-labeled neurons) were calculated with respect to the total number of the two populations of single-labeled neurons. Since in the visual cortex of the macaque, callosal projecting neurons are located in supragranular layers[3,8] the percentages were calculated uniquely from those single-labeled neurons located in upper layers.

In the adult, callosal projecting neurons were found in restricted zones at the V1/V2 border, dorsally on the operculum or in the posterior bank of the lunate sulcus and ventrally in the inferior occipital sulcus, at the V3/V3a border in the anterior bank of the lunate sulcus, on the prelunate gyrus in a region including V4 and in the posterior bank of the superior temporal sulcus in a region corresponding to Zeki's movement sensitive area and widely known as MT (Fig. 1)[8,16,17]. The areal location of callosal projecting neurons extensively overlapped with that of association projecting neurons. Although the two populations were found in supragranular layers they were partially radially separated[9] since association projecting neurons were concentrated at the top of layers 2/3 and callosal projecting neurons at the bottom of these layers as reported in the visual cortex of other species[15]. In the adult double-labeled neurons were almost entirely restricted to area V2 where they comprised 0.03% and 0.08% of the total number of labeled neurons (Table I).

In the two fetuses, callosal projecting neurons were scarce in V1 where they were found within several millimeters of the border with V2[3]. In extrastriate cortex, callosal projecting neurons stretched as a dense continuous band throughout a large part of dorsal and ventral cortex (Fig. 1). The proportion of association projecting neurons which were located in supragranular layers in the immature cortex was higher than in the adult throughout extrastriate cortex as has been reported in the neonate[10]. The density of labeling of both populations of neurons was also higer in fetal cortex than in the adult (Fig. 1). Although the radial distribution of association projecting neurons was broader in the fetuses, overall a similar laminar distribution of the two populations of neurons was found in both sets of animals. Double-labeled neurons were easily identified in the fetus (Fig. 1). They were located in the overlap region of the two populations of single-labeled neurons in the lower part of layers 2/3 (Fig. 1) and preferentially near to the V1/V2 border. So as to determine quantitatively the incidence of double-labeled neurons, three cortical regions were distinguished. The first region stretched from the dorsal V1/V2 border to the fundus of the lunate sulcus, and largely corresponds to the future area V2. The second region went from the fundus of the lunate sulcus to the prelunate gyrus and covered region which will correspond to areas V3 and V3a in the adult, and lastly the posterior bank of STS where Zeki's movement sensitive area is located in the adult[16] (see Table I). Although the numbers of

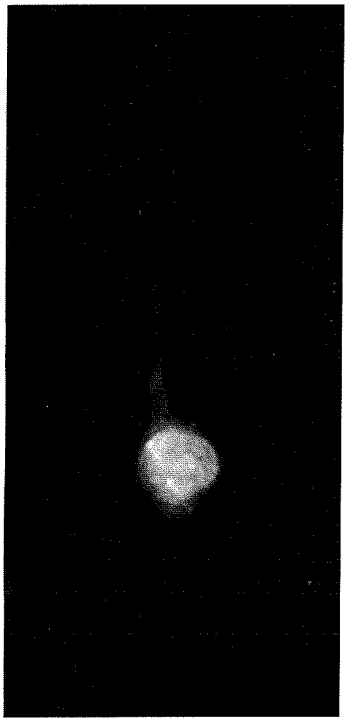

Fig. 2. An example of a double-labeled neuron in area V2 of the E122 fetus. Diamidino yellow gave a yellow colouring and was concentrated in the nucleus. Fast blue gave a blue colouring and was stored in the cytoplasm.

double-labeled neurons per section were higher than in the adult, when expressed as a percentage of the total population of labeled neurons they comprised only 0.45% at E122 and 0.46% at E135 (Table I). Further the incidence of double-labeling was found to drop at increasing distances from area V1 and was 0.2% in the anterior bank of the lunate sulcus and 0.06% in the posterior bank of the superior temporal sulcus.

Consideration of the time course of the maturation of visual cortex makes it unlikely that higher percentages of double-labeled neurons would be encountered before or after the dates examined here. E122 is the earliest age at which large numbers of callosal and association neurons can be labeled, since at this age callosal fibers linking the two hemispheres have been in place for 15 days[3] and association fibers for 7 days (Kennedy, unpublished). Further the percentage of double-labeled neurons will decrease after E135 since there is a reduction in the overlap zone of association and callosal projecting neurons. This is due to the fact that the supernumerary upper layer association connections reported in neonates[10] are already present by day E135 (Meissirel, C., Dehay, C., Berland M. and Kennedy, H., manuscript in preparation.) and callosal projections are beginning to disappear from extrastriate cortex after E135[3].

TABLE I

Percentages of double-labeled neurons

Figures in brackets refer to numbers of sections examined.

Animal	*Posterior bank of lunate sulcus*	*Anterior bank of lunate sulcus*	*Posterior bank of superior temporal sulcus*
E 122	0.45% n=29973 (14)	0.2% n=16810 (14)	0.06% n=9617 (14)
E 135	0.46% n=3485 (12)	–	–
Adult 1	0.03% n=16579 (13)	0.04% n=5033 (13)	0% n=4000 (13)
Adult 2	0.08% n=5852 (11)	0% n=1304 (11)	0% n=140 (11)

The quantitative results in the visual cortex are clear cut: despite the fact that the percentage of double-labeled neurons in early development is low there is a selective loss of bifurcated projections during maturation. The situation could be different in more rostral cortical regions. Double-labeling experiments in which nuclear yellow was injected in the dorsal bank of the principal sulcus and fast blue in the contralateral intraparietal sulcus led to an estimated 5% of double-labeled neurons in the uninjected parietal and frontal cortices[14]. However as pointed out by Chalupa and Killackey[2] there may be a selective elimination of bilateral projections prior to E132 during the period of callosal axon elimination in somatosensory cortex[2,11].

In non-primates the degree of radial separation of parent neurons of callosal and association pathways is less pronounced and this is accompanied by large proportions of bilateral projecting neurons during development[1,5,7]. The importance of the role of regressive phenomena (possibly predominantly axon elimination) characterizes the emergence of segregated cortical pathways in non-primates[5,7]. It would seem therefore that the infrequency of bilateral projecting neurons is a characteristic feature of the development of primate visual cortex.

Early during development many visual cortical neurons in the primate form callosal or association projections which do not persist into adulthood[3,10]. This allows factors related to the internal environment of the cortex to specify the final connectivity of some neurons. For example, removal of peripheral input allows many callosal projections which are normally eliminated to persist until birth[4]. However the present results show that, in addition to any extrinsic control, there is also an important degree of early specification of connectivity since throughout development neurons project to either the ipsilateral or the contralateral hemisphere and only a maximum of 0.46% project to both. It needs to be

pointed out that our injections in area 17 will only label a fraction of extrastriate neurons forming extrinsic, association connections. Therefore if all the ipsilateral targets of a cortical area were to be injected, one could expect a higher proportion of double-labeled neurons. Furthermore the early specification of connectivity concerns even those projections which are not conserved in adulthood, since amongst the population of area V1 projecting neurons in the region of MT which are scheduled to disappear, only 0.06% are double-labeled. The fact that during development the selective elimination of double-labeling concerns such a small proportion of labeled neurons argues against this being a major factor controlling the maturation of cortical connectivity. Rather it would seem that during the period of overall reduction of cortico-cortical connectivity, there is a decrease in the probability of a neuron with bilateral projections maintaining both collaterals.

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