The maturational status of thalamocortical and callosal connections of visual areas V1 and V2 in the newborn monkey*

Colette Dehay† and Henry Kennedy

Laboratoire de Neuropsychologie Expérimentale, INSERM-Unité 94, Bron (France)

(Received 7 July 1986)
(Revised version received 24 October 1986)
(Accepted 4 November 1986)

Key words: Development; Cortical connection; Vision; Primate

Cytochrome oxidase (CytOx) is known to preferentially stain those regions of the visual cortex which receive direct projections from the thalamus. The pattern of CytOx stain has been used to investigate the maturational development of thalamic inputs to areas V1 and V2 in the newborn monkey. In both areas, the intensity of CytOx activity was similar in newborns and adults. The distribution of CytOx in area V2 was not found to vary with age. In area V1, the only difference in CytOx activity in newborns was a relative immaturity of staining in layer 4C. The callosal connections of visual areas V1 and V2 were investigated by the axonal transport of wheat germ agglutinin conjugated to horseradish peroxidase and free horseradish peroxidase. In the adult, V1 was found to be reciprocally callosally connected for a distance of 1–2.5 mm from the V1/V2 border, whilst V2 was connected for a distance of 3–8 mm from the border. In both areas, callosal connections showed a certain degree of clustering, particularly in V2 which contained 97–98% of the total number of callosal connections of these two areas. In the newborn, the number, tangential extent and clustered distribution of callosal connections were as in the adult. In the newborn, as in the adult, callosal connections coincided with regions of high CytOx activity in area V2. The results showing a relative maturity of the tangential distribution of callosal projecting neurons on the one hand, and an immaturity of thalamic projections on the other, are discussed in terms of: (1) the maturational status of the newborn monkey compared to other mammals at the moment of birth and (2) the possible role of visual experience in shaping cortical connections.

INTRODUCTION

At the moment of birth, a considerable number of cortical connections which exist in the adult have yet to be formed. The fact that connections between neurons are being formed after birth, when the brain is responding to input from the sense organs, raises the possibility that experience may contribute to neural development. Should the evoked activity of neurons in any way influence their choice of synaptic contact then it would be quite reasonable to expect that it would do so throughout a limited period during which there is axonal growth and synapse formation.

Since the pioneering work of Wiesel and Hubel in the early 1960's, a considerable number of experiments have been carried out with the aim of elucidating the susceptibility of the visual cortex of the kitten to manipulations of the visual environment. It is now firmly established that two aspects of cortical connectivity, the input from the thalamus and the interhemispheric connections,

* First presented at the IBBS workshop on Hemispheric specialization and interhemispheric communication, held in Rotterdam on 20–21 March 1986.
† Present address: MRC Anatomical Neuropharmacology Unit, South Parks Road, Oxford OX1 3QT, U.K.
Correspondence: H. Kennedy, Laboratoire de Neuropsychologie Expérimentale, INSERM-Unité 94, 16 avenue du Doyen Lépine, 69500 Bron, France.
can be modified by the sensory experience of the animal. In both cases it has been difficult to decide categorically whether postnatal plasticity allows experience to exert an instructive role, or whether modifications of connectivity are merely the consequence of disuse (see ref. 39 for a discussion of these points).

The notion that plasticity of the visual cortex reflects the consequence of abnormal experience acting upon a developing system needs to be reconsidered with regard to the results of selective rearing experiments on primates. If it were not for the classical clinical observations on amblyopia\textsuperscript{38}, the demonstration in the late seventies, that the visual cortex of the young monkey is susceptible to abnormal sensory experience in much the same way as is the kitten, would have been rather surprising\textsuperscript{3,7,16,37}. In both kitten and monkey, early surgical closure of one eye causes a decrease in the cortical territory contacted by the lateral geniculate nucleus (LGN) layers receiving afferents from the closed eye. The similarity of the anatomical and physiological consequences of early eye closure in kitten and monkey is surprising in view of the difference in the degree of maturity at the moment of birth of these two species. Whereas kittens are born with an extremely immature visual system and do not open their eyes before 10 days, monkeys are visually responsive at birth and show relatively sophisticated visually guided behaviour.

However, in contrast to the advanced visual behaviour of the newborn monkey, it is known that the LGN afferents to the striate cortex are not fully mature at birth. Segregation of the left and right eye inputs to the striate cortex begins some 3 weeks before birth and is not complete until 6 weeks after birth\textsuperscript{7,16,30}. Susceptibility to deprivation in layer 4 outlasts the process of segregation of left and right eye afferents by about two weeks so that columnar development per se is not the sole factor limiting the plasticity in this layer\textsuperscript{16}. This does not mean, however, that plasticity is not specified by developmental events since synaptogenesis persists in area 17 in the monkey until 6 months\textsuperscript{26}. Certainly, the effectiveness of monocular deprivation is related to the segregation of LGN afferents: monocular deprivation carried out in the first 3 weeks of life causes a much more pronounced effect on layer 4 than does later deprivation\textsuperscript{16}.

The fact that both kittens and monkeys have a critical period for sensory deprivation lends support to the notion that susceptibility to the sensory input does indeed play an important role in establishing mature cortical function\textsuperscript{1}. This hypothesis is further strengthened when one considers the developmental basis of cortical plasticity. The timing of the sequence of developmental events appears to be very different in these two species. Proliferation of cortical neurons in kittens (gestation time 65 days) terminates at about embryonic day 57 (E57)\textsuperscript{22}, while the migration of later formed neurons is not complete until about 3 weeks after birth\textsuperscript{36}, and segregation of geniculate afferents begins at about 3 weeks after birth\textsuperscript{17}. Although neurogenesis of layer 3 neurons in the cortex of the monkey (gestation period 165 days) is complete by about E100\textsuperscript{29} and migration in the cortex by E125\textsuperscript{31}, segregation of left eye/right eye input to the cortex does not begin before E145\textsuperscript{30}. Segregation of left and right eye afferents in the kitten is therefore concomitant with neuronal migration, whereas in the monkey it begins some 20 days after migration has terminated. The lag between the migration of cortical neurons and segregation of geniculate input in the monkey ensures that development of ocular dominance columns occurs, as in the kitten, during the first few weeks of postnatal life.

The finding that the restriction of callosal connections in kittens occurs postnatally, raises the question of whether development of callosal connections also occurs postnatally in the monkey. By analogy with the development of ocular dominance columns, one might expect the elimination of exuberant callosal connections in the monkey to be delayed so that sensory experience would have a chance to act upon this developmental process. To address this question we have carried out injections of WGA–HRP in areas V1 and V2 of newborn monkeys, and examined the distribution of callosal connections in the contralateral areas V1 and V2. In the adult, callosal connections in area V2 are located in regions of high cytochrome oxidase (CytOx)
activity which are known to receive the major thalamic input from the pulvinar. The pattern of CytOx staining in striate cortex of the newborn monkey has been shown to be very different in layer 4C from that in the adult and to reflect the immaturity of LGN input to this layer at birth. In the present study, we have looked at the CytOx activity in area V2 of the newborn monkey to determine if a similar immaturity of the input to this area can also be detected using this histochemical technique.

METHODS

The anatomical techniques used in this study were as in the preceding paper and will not be described here. Quantitative and qualitative observations were made in 7 newborns and 3 adult controls.

RESULTS

In V1 of the newborn monkey (Fig. 1C, D), callosal connections did not extend further than in the adult (Fig. 1A, B). The 2-mm strip of V1 which was callosally connected contained, as in the adult, both anterograde labelled terminals and retrograde labelled cell bodies. The striate cortex in the neonate showed levels of CytOx staining similar to that found in the adult, although counterstaining showed that the laminar distribution in the layer 4C sublaminae was very different. In the newborn, CytOx did not fill the whole of layer 4C, but instead there was an upper band of label which occupied layer 4Cx, and a thin band of label at the bottom of 4Cβ. The upper two-thirds of 4Cβ showed low CytOx activity. This difference between layers 4Cx and 4Cβ is of particular interest since it is known that these two layers receive different types of input from the LGN. Whereas 4Cx receives input from the magnocellular layers of the LGN, 4Cβ is innervated by the parvocellular layers. The differential labelling of these two sublaminae suggests differences in the relative degree of maturity at birth of these two parallel pathways. The possibility that the magnocellular pathway is more mature than the parvocellular pathway was confirmed by the fact that the magnocellular layers in the LGN were also found to be more strongly labelled in the newborn.

Matching adjacent sections stained for CytOx and HRP showed that in the newborn, unlike the adult, callosal terminals did invade regions of high CytOx activity (Fig. 1C, D). Although labelled terminals were centered over layers 4B and 5, some terminals were found in layer 4Cx, and
there was only a narrow region devoid of callosal terminals. This zone free of callosal terminals was located just above the region of high CytOx activity running along the base of layer 4Cβ. As in the adult retrograde-labelled cells were absent from infragranular layers and were found mostly in layer 4B. In area V2 of the adult, the great majority of callosal projecting neurons are located at the bottom of layers 2 and 3, and axon terminals were distributed throughout all cortical layers although they were more concentrated at the bottom of layer 3 (ref. 12). In V2 of the neonate, the laminar distribution of both retrograde-labelled neurons and anterograde-labelled axon terminals was identical to that found in the adult.

Callosal neurons in V2 of the adult are not found evenly distributed but are grouped in patches or columns (Fig. 2B). Inspection of callosal neurons in V2 of the newborn reveals an equal tendency for a columnar distribution to that found in the adult (Fig. 2D). This has been repeatedly observed in all of the neonates studied where the brain has been sectioned parasagittally.

The patches of callosal connections in V2 of the adult are located in regions of high CytOx activity. This is shown in Fig. 2A, B which compares the location of retrograde-labelled cells with the CytOx distribution in the adjacent section. In the neonate, the tissue reacted as strongly for CytOx as in the adult, and CytOx was also distributed in clearly defined patches. When plots of retrograde-labelled cells were compared to adjacent sections, processed for CytOx activity, the correspondence between columns of callosal neurons and regions of dense CytOx activity was as clear as in the adult (Fig. 2C, D).

We shall now examine the important issue of whether the distribution of callosal neurons is more widespread at birth and whether there is a change in absolute numbers of callosal projecting neurons during postnatal development. In V1 and V2 of the adult, callosal projecting neurons have a more widespread distribution in cortex subserving the fovea than in peripheral cortex. In the newborn, the tangential extent of callosal connections was similar to that found in the adult and was also influenced by eccentricity in the visual field.
field. Should the surface area of the cortex increase between the moment of birth and adulthood, this would indicate that there is a wider distribution of callosal projection in the neonate. However, O’Kusky and Colonnier\textsuperscript{26} have shown that the surface area of V1 is the same in the neonate and the adult, so that one can conclude that the tangential distribution of callosal projecting neurons is not greater in the neonate than it is in the adult.

Although callosally projecting neurons do not have a more widespread distribution in the neonate, the possibility remains that the total number of neurons projecting to the contralateral hemisphere is greater at the moment of birth than it is in the adult. There are two difficulties associated with comparing numbers of callosal projecting neurons in the newborn and adult. Firstly, callosal projecting neurons are not evenly distributed but occur in patches and secondly, the extent of callosal connections varies with eccentricity in the visual field. To overcome these difficulties, it is necessary to compute the numbers of callosal projecting neurons over a wide expanse of cortex and to compare the newborn and adults at similar levels in the brain. To do this, we have made counts in V1 and V2 of labelled neurons in 20–30 sections in cortex subserving the parafoveal lower visual field. The results are presented in Fig. 3 where the maxima of the curves have been arbitrarily aligned on the higher value to facilitate comparison. In both sets of animals, the number of callosal projecting neurons changes from slide to slide, reflecting the equal tendency in adults and newborns for neurons to occur in patches. Furthermore, it can be seen that the total number of neurons in V1 and V2 which project to the contralateral hemisphere does not change during postnatal development. The possibility remains that the relative contribution of V1 and V2 is different for newborns and adults. We have tested for this by comparing in neonates and adults the proportion of callosal projecting neurons which are found in V1 with respect to the total number in V1 and V2. There is some variability from animal to animal. In the newborn,
0.6–1.2% of the total number of neurons in V1 and V2 which project across the callosum are located in V1. This range of values was similar to that found in the adult (0.6–3.2%). It would seem unlikely that the similarity of the numerical values of callosal projecting neurons in newborns and adults can be explained by changes in packing density of neurons. O'Kusky and Colonnier's study showed that in V1, the density of neurons in layer 4B, the principal layer of origin of callosal projecting neurons in that area, is similar in newborns and adults.

**DISCUSSION**

The present results show that the time course of maturation of CytOx activity is very different in V1 and V2. Whereas at birth the pattern of CytOx is immature in V1, it appears adult-like in V2. This maturation of CytOx activity probably reflects that of thalamic input. CytOx activity in the adult is known to label those regions of visual area V1 and V2 which receive a direct input from the thalamus. We have previously shown that the distribution of CytOx activity in layer 4C of area V1 in the neonate reflects terminal density distribution in this layer. Therefore it would seem that CytOx activity in the newborn, as in the adult, indicates thalamic input.

In V1, the immaturity of CytOx activity is accompanied by a more widespread distribution of callosal terminals. The result is that, in the newborn, callosal terminals are found in layer 4C, which is not the case in the adult. This contrasts with the situation in area V2 where CytOx activity, as well as callosal connectivity, exhibits a remarkable degree of maturity at the moment of birth. The mature features of callosal connections in V2 include their tangential extent, laminar location, columnar organization and absolute numbers. Immature aspects seem above all to concern the location of callosal terminals.

The postnatal maturation of the interhemispheric pathway in primates appears to be very different from that in rodents, lagomorphs and carnivores. In these species the restricted adult pattern of callosal connections emerges during postnatal life from a neonatal stage where callosal projecting neurons have a widespread distribution. It would not seem that this difference between primates and non-primates reflects postnatal changes in the number of axons in the corpus callosum. In the callosum both of the newborn monkey and the kitten there are about 3 times the number of axons found in the adult. From the present results one can conclude that, if there is an elimination of exuberant callosal connections in the monkey, then it occurs prenatally when numbers of callosal axons are in fact increasing.

At first glance, the restricted distribution of callosal projecting neurons would set the monkey neonate apart from non-primate mammals which exhibit a widespread distribution at birth. This raises the question of whether visual experience in non-primates has a greater opportunity to act upon callosal connectivity. Cats, rats and rabbits open their eyes between 11 and 17 days after birth. In each of these species, the tangential distribution of callosal connections, although immature at birth, is adult-like at the moment of eye opening. The sheep is the only animal which, like the monkey, has a restricted distribution of callosal connections at the V1–V2 border at the moment of birth. The sheep, like the monkey, has good visuomotor coordination on the day of birth, clear optic media and electrophysiological recordings show that area V1 neurons respond briskly to visual stimulation. It could be argued that the presence of exuberant callosal projecting neurons in V1 is symptomatic of a less sophisticated visual system. However, if this were the case then one would expect sheep to have a widespread distribution since this animal has a visual system far more similar to that of the cat than to that of the monkey. In conclusion, therefore, it would seem that it is the moment at which the animal is to use visual information and not that of birth which is significant in terms of callosal maturity.

It was Shatz who first suggested that retinal activity may play a role in establishing the adult pattern of callosal connectivity. Experiments have shown that this is certainly the case during the phase when callosal connections are being rapidly eliminated. If one or both eyes are removed at
birth, then there is a significant failure of callosal connections to be eliminated from V1 both in rodents and carnivores\textsuperscript{5,9,27,33}. Since eye opening occurs after the main phase of elimination, one would expect visual deprivation to have little or no effect, and indeed this is the case in rodents\textsuperscript{5,34}. It has been claimed that abnormal visual experience can modify, to a limited extent, the distribution of callosal connections in carnivores\textsuperscript{2,9,19,20,21}, although abnormal vision does not have such an effect in rodents\textsuperscript{5,33}. The difference in the effects of abnormal visual experience between rodents and carnivores is difficult to explain, although it might indicate a slightly more prolonged period of callosal maturation in the carnivore.

We can only speculate as to the role normal visual input might have on the postnatal maturation of the visual callosal pathway in the monkey. Given that counts of callosal neurons are already adult-like at birth, elimination, if it has occurred must have done so during the prenatal period, when both the normal pattern of impulse activity from the retina along with genetic factors may combine to shape callosal connectivity. Given the low number of callosal projecting neurons in area V1, visual experience could only exert a stabilizing influence on large numbers of callosal neurons in area V2. An interesting analogy exists with the role of experience on the development of ocular dominance columns. Wiesel and Hubel\textsuperscript{40} have shown that segregation of ocular dominance columns can occur in layer 4 in the absence of visual experience, but that outside of layer 4 the convergence of input from the two eyes to give binocular-driven neurons is dependent on visual experience\textsuperscript{7,16}. It could be that the elimination of callosal connections from the primary visual area V1 is largely dependent on genetic factors and that visual experience is required to maintain these connections at higher stages of visual processing in V2. Ongoing experiments on selectively reared and prenatal monkeys are aimed at resolving these issues.

ACKNOWLEDGEMENTS

These experiments were funded by MRT Grant 85.C.1148 and DRET Grant 85.210. We are grateful to Beatrice Allessant, Noëlle Boyer and Pascale Giroud for technical assistance and to Françoise Girardet for typing the manuscript.

REFERENCES

11a Kennedy, H. and Dehay, C., Functional implications of the anatomical organization of the callosal projections of visual areas V1 and V2 in the macaque monkey, Behav. Brain Res., in press.
13 Kennedy, H., Martin, K.A.C. and Whitteridge, D., The receptive field characteristics of neurons in striate cortex


32 Rao, V.M., Interhemispheric connections between primary visual areas in adult sheep and newborn lamb, *J. Physiol. (London)*, 296 (1979) 65P.


*Note added in proof*

Since submitting this paper we have shown that callosal connections are in fact absent from area 17 throughout prenatal development in the monkey (Dehay, C., Kennedy, H., Bullier, J. and Berland, M., Absence of interhemispheric connections of area 17 during development in monkey, *Nature (Lond.)*, 331 [1988] 348–350).