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Research Note

Transient projections from the fronto-parietal and temporal cortex to areas 17, 18 and 19 in the kitten

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Summary. Using the retrograde tracers, fast blue and horseradish peroxidase we have shown the presence of projections from extensive regions of the fronto-parietal and temporal cortex to areas 17, 18 and 19 in the newborn kitten. These projections are transitory as they do not exist in the adult cat. The anterograde transport of horseradish peroxidase conjugated with wheat germ agglutinin after injections in fronto-parietal and temporal cortex revealed that these transitory projections terminate in the gray matter and that they could therefore play a functional role in the development of the visual cortex.

Key words: Visual cortex – Development – Transient connections – Kittens

Introduction

The connectivity of the nervous system is known to undergo considerable reorganization during the early stages of development either by processes of axon elimination or by cell death (Cowan 1973; Purves and Lichtman 1980). Thus, it is possible that patterns of connectivity which are unknown in the adult could be present in the immature organism. This has been observed in the connections from the cortex to the contralateral hemisphere, the cerebellum and the pyramidal tract (Innocenti et al. 1977; Stanfield et al. 1982; Tolbert and Panneton 1983). Within the cortical mantle, Innocenti and Clarke recently reported that neurons from the auditory cortex project transiently to visual cortical areas in the kitten (Innocenti and Clarke 1984). In the present report, we demonstrate with the results of retrograde tracing experiments that transitory connections to the visual cortex arise from numerous cortical regions. We further show by using anterograde tracing that these transitory connections terminate in the gray matter of the visual cortex and that they are therefore in a position to play a role during the development of the visual cortex in the first few weeks of life.

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Methods

Kittens were premedicated with 0.5–1.0 mg/kg chlorpromazine (Largactil) and 1 mg dexamethasone (Soludecadron). Under ketamine anaesthesia (10–30 mg/kg) the brain was exposed so as to allow injection of axonal tracers. In each of 9 animals (0 to 57 days of age) a single injection of a 3% solution of fast blue (FB) (0.1–0.4 µl) was made with a Hamilton syringe into the gray matter of one of the three visual areas 17, 18 or 19 (Bentivoglio et al. 1980). After a survival period of 7 days, the kittens were perfused with 2.7% saline followed by 30% formalin in 0.1 M phosphate buffer and sucrose solutions of increasing concentrations ranging from 8 to 30%. Immediately after perfusion the brain was removed, blocked and cut on a freezing microtome at 40 µm. One section in two was mounted from saline onto gelatinized slides and observed under deep blue and UV light (355–425 nm) using a Leitz microscope equipped for epifluorescence. Sections of interest were counterstained with cresyl violet after observation.

HRP histochemistry was used to study both the origin and termination of transient cortical afferents to the visual cortex. Horseradish peroxidase conjugated to wheat germ agglutinin (WGA-HRP) was used at a concentration of 2% and injected by Hamilton syringe. Retrograde transport was studied in two animals (10 and 12 days old) following single injections of WGA-HRP (0.2 µl) in area 17 in one case and on the border of areas 17 and 18 in the other. Four kittens (2, 9, 14 and 18 days old) which were used to study the terminal configuration of transient fibers received a total of 0.4 to 0.8 µl of a 2% solution of WGA-HRP in several elongated injections. In the 18 day-old kitten injections were aimed at the lateral and medial banks of the ansate sulcus and the posterior sygmoid gyrus. In the 2 and 14 day-old kitten the same cortical areas were injected as well as a single injection in the anterior ectosylvian gyrus. In the 9 day-old kitten two injections were made in the anterior ectosylvian gyrus only. After a survival period of 15 to 48 h the kittens were perfused after a rinse with isotonic saline, by a mixture of 1% paraformaldehyde and 1.25% of glutaraldehyde in phosphate buffer followed by a 10% sucrose

solution. Following a period of at least 12 h in sucrose, frozen sections were cut and reacted by the TMB method (Mesulam et al. 1980). The positions of the injection sites were reconstructed after counter staining with cresyl violet. Cytochrome oxidase labels layer 4 in areas 17 and 18 and this histochemical technique was used to localise fibers in areas 17, 18 and 19 (Wong-Riley 1979).

The precision with which one can localise a cortical injection is directly dependent on the extent of the uptake zone of the tracer in the cortex. By making paired injections of two fluorescent dyes (FB and diamidino yellow) 2.5 mm apart within area 18 of a 17 day-old kitten, two separate populations of back filled cells were found in the A and A^1 laminae of the lateral geniculate nucleus (unpublished result). This shows that the uptake zone of these fluorescent dyes is less than 2 mm in diameter and is therefore very similar to that reported in the adult (Bullier et al. 1984). Histological reconstruction of the injection sites showed that the uptake zones as defined in Bullier et al. (1984) were in the present experiments restricted to the gray matter of a single cortical area. It is more difficult to estimate the extent of the uptake zone of WGA-HRP. For the purpose of the present report it is however important to note that the injection track never invaded the white matter. Since WGA-HRP is not taken up by undamaged fibers (Brodal et al. 1983), this implies that WGA-HRP uptake in our experiments was limited to the cortical gray matter.

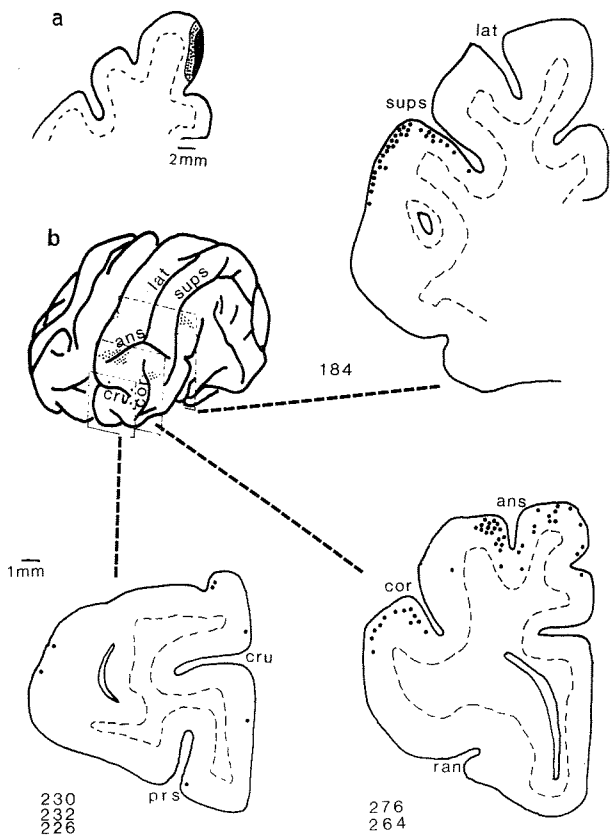

Fig. 1a and b. Distribution of transient afferent cortico-cortical cells labelled by retrograde transport of fast blue. **a** Frontal section showing the injection site of fast blue in area 17 of a 14 day-old kitten. The presumed uptake zone of the dye is represented by dots. **b** Surface view of the brain shows the overall distribution of FB retrograde labelled neurons which can be seen in more detail in representative frontal sections. Figures refer to section numeration. When several numbers are given, the data were pooled from the sections identified by the numbers. Abbreviations: ans: ansate sulcus; cor: coronal sulcus; cru: cruciate sulcus; ecsa: anterior ectosylvian sulcus; ecsp: posterior ectosylvian sulcus; lat: lateral sulcus; sups: superior suprasylvian sulcus; prs: presylvian sulcus; ran: anterior rhinal sulcus

Results

After injection in all three areas we found labelled neurons in several regions of the fronto-parietal and temporal cortex in kittens aged up to 25 days. FB positive neurons were found in varying numbers in the post sigmoïd and coronal gyri, the lateral and medial banks of the ansate and presylvian sulci, the medial aspect of the hemisphere, the ectosylvian gyrus and, in some animals, in the banks of the rhinal sulcus (Fig. 1). Apart from the banks of the ansate sulcus and the ectosylvian gyrus which were well labelled in all animals, the density of neurons in the presylvian sulcus and coronal gyrus was variable.

This variability could be related to a number of factors including the exact age at the time of injection, the area injected or even the region of the visual field represented at the site of injection. So far we have injected FB in similar numbers of kittens below 25 days of age in each area (2 in area 17, 2 in 18 and 3 in 19) and we have failed to reveal any characteristic differences in the pattern of labelled cells related to the cortical area injected. The results obtained with WGA-HRP injections in area 17 and at the 17–18 border are essentially identical to those obtained using fluorescent dyes (Fig. 2). Both retrograde techniques show that neurons labelled in nonvisual cortical regions are found principally in lamina 2 and upper 3 with only a few neurons in infragranular layers (Fig. 2).

After WGA-HRP injections in nonvisual cortex in all four kittens, anterograde and retrograde labelling was observed in various anterior thalamic nuclei, in the medial geniculate body and in the ventro-basal complex. Apart from the claustrum and the intralaminar nuclei, no label was found in subcortical structures which project to areas 17, 18 and 19 in the adult cat. Those regions (area 20, the suprasylvian gyrus and the banks of the splenial sulcus) which are known to exchange connections with the frontal and auditory cortex in the adult (Heath and Jones 1971; Kawamura 1973; Cavada and Reinoso-Suarez 1983) showed strong retrograde and anterograde labelling in the young animal. In contrast to these regions of strong anterograde labelling, the projections to areas 17, 18 and 19 were sparse (Fig. 3) and did not show the dust like deposits of HRP reaction product characteristically found in those regions of cortex

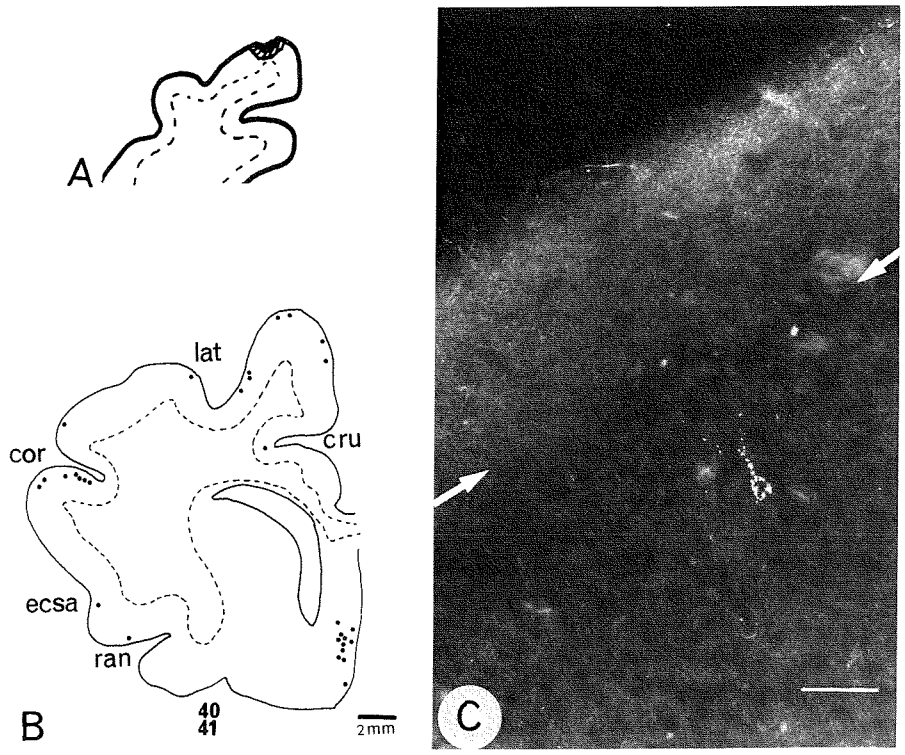

Fig. 2A–C. Transient afferent cortico-cortical cells labelled by retrograde transport of HRP-WGA. **A** Frontal section showing the injection site of HRP-WGA on the border of areas 17 and 18 in a 12 day-old kitten. **B** Distribution of WGA-HRP labelled neurons pooled from two adjacent frontal sections. Note that these neurons are almost exclusively located in the superficial layers of cortex. **C** HRP-WGA positive neuron located in the coronal gyrus. Arrows indicate the upper limit of layer 2. Scale: 40 microns

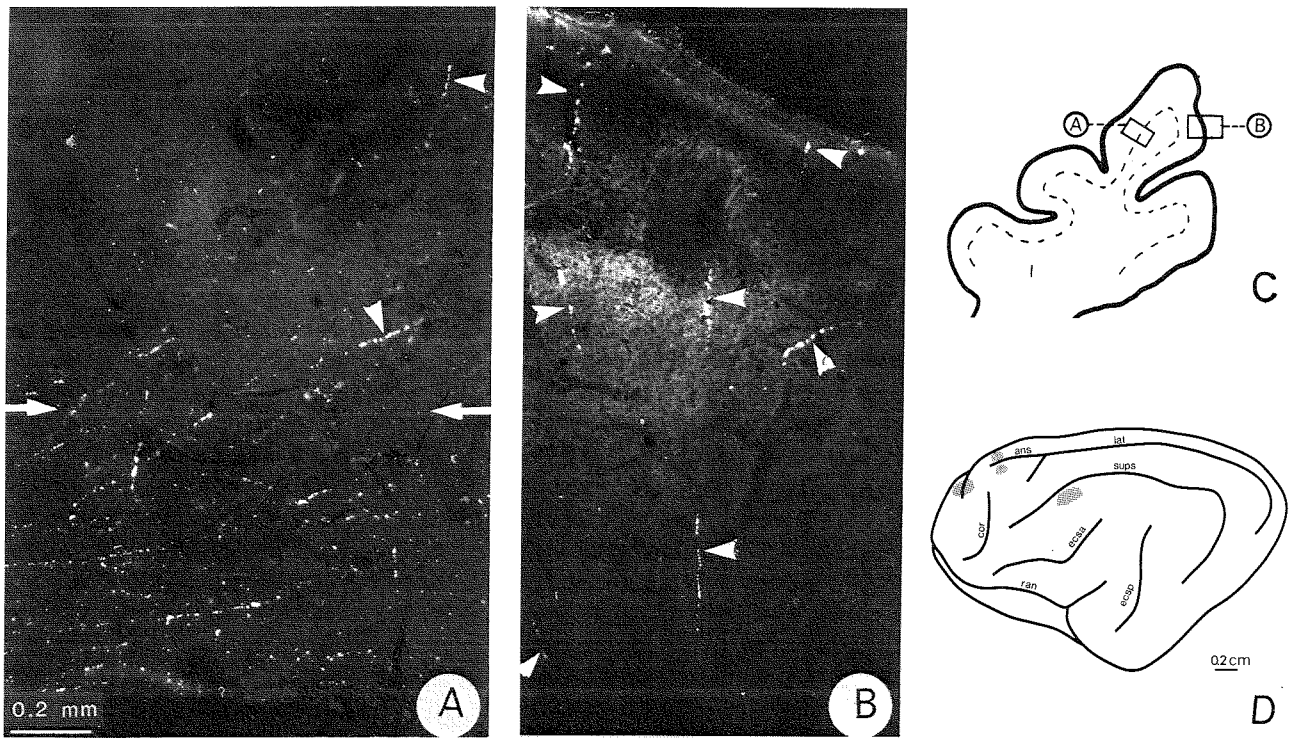

Fig. 3A–D. Anterograde WGA-HRP labelled transient fibers in the visual cortex of a 14 day-old kitten. **A** Transient fibers lying in the white matter (WM) beneath areas 18 and 19, with two fibers (arrow heads) penetrating the lower part of the cortex. Arrows: limits of white matter (lower) and gray matter (upper part) of figure. **B** Transient WGA-HRP labelled fibers (arrow heads) in the supragranular cortical layers. **C** Frontal section showing regions of cortex from which **A** and **B** were obtained. **D** Surface view of cortex showing WGA-HRP injection sites. Abbreviations: see Fig. 1

which receive projections from the frontal cortex in the adult. Numerous labelled fibers were observed in the white matter underlying areas 17, 18 and 19 and some fibers were found to penetrate the gray matter of all three areas, ascending up to lamina 1 (Fig. 3). In addition, very few retrograde labelled neurons were found in areas 17, 18 and 19, in contrast with the regions of adult-like labelling which contained large numbers of back filled cells. The number of neurons labelled in areas 17, 18 and 19 after large injections of WGA-HRP in fronto-parietal and temporal cortex was much lower than the number of FB labelled cells in these areas after small injections in areas 17, 18 or 19. It would seem therefore that transitory projections to areas 17, 18 and 19 can be considered as non reciprocal.

In addition to this ipsilateral retrograde and anterograde labelling, a few labelled cells were observed in fronto-parietal contralateral cortex after FB injection in 17, 18 or 19. Similarly, a small number of labelled fibers were found in contralateral occipital cortex after HRP injections in the frontal and temporal cortex. Thus, these fronto-occipital and temporo-occipital connections are principally ipsilateral with a small contralateral component.

The earliest age at which the presence of transient connections was noted was 4 days after birth when numerous HRP-WGA positive fibers were found in the white matter and some in the gray matter of areas 17, 18 and 19 after injections in fronto-parietal and temporal cortex. Thus, it appears likely that these connections are present very near the time of birth in contrast to the situation in transient cortico-cerebellar connections which appears around the 8th post-natal day (Tolbert and Panneton 1983).

The presence in the adult of projections from fronto-parietal and temporal cortex was tested by FB injections in area 17 of three animals. No labelled neuron was observed in frontal cortex and only three neurons were found in the ectosylvian gyrus of one animal. Thus, it appears that these connections are transitory and probably disappear around the 30th day of age as we observed labelled neurons in fronto-parietal and temporal cortex in all the youngest animals (9 kittens, 7 to 25 day old) and not in two older kittens aged 32 and 57 days. To investigate the mechanism of this disappearance, we injected FB in area 17 of a 7 day old kitten and perfused it when it was 55 days old. Since FB labelled neurons were still found in the fronto-parietal and temporal cortex of this animal, it is likely that collateral elimination rather than cell death is the principal mechanism for the disappearance of these transitory connections.

Discussion

The present results confirm and extend those of Innocenti and Clarke (1984). We found, as they did, numerous retrograde labelled neurons in the ectosylvian gyrus after injections in areas 17, 18 and 19. In addition, we found labelled cells in the frontal cortex which were restricted to particular regions of the cortical surface known to correspond in the adult to somatosensory, vestibular and motor regions (Liedgren et al. 1976; Guitton and Mandl 1978; Felleman et al. 1983). Thus, neurons in many nonvisual regions of the cortex send transitory connections to the visual cortex which, itself, does not appear to reciprocate these projections.

The presence of anterogradely labelled fibers in the gray matter of visual cortex after injections in fronto-parietal and temporal cortex demonstrate that these transitory connections are not restricted to the white matter and are in a position to make synapses with neurons in the visual cortex. These conclusions are reinforced by the fact that numerous labelled neurons were found in fronto-parietal and temporal cortex after FB and WGA-HRP injections which were restricted to the gray matter of cortex. Innocenti and Clarke's study using retrograde transport of fluorescent dyes could not address the issue of whether transient fibers terminate in cortical gray matter as all of their injections, with only one exception, extended into the white matter underlying areas 17 and 18 (Innocenti and Clarke 1984).

The postnatal maturation of cortical connectivity therefore includes a stage during which neurons in the visual cortex can be submitted to the influence of other sensory modalities. One could expect that the development of the cortical territory associated with other sensory modalities also involves a period of polysensory convergence. If this should be the case, it is possible that we observe this phenomenon only in the visual cortex because of the later development of this cortical region compared to that of auditory and somatosensory cortices (Marty and Scherrer 1964; Scheibel and Scheibel 1971). Alternatively the visual cortex could be unique in requiring polysensory and motor inputs for its normal development. In any case, polysensory convergence implying integration of neural activity is not the only possible role of these transient afferents. By penetrating the gray matter, these transitory connections will come into close proximity to the termination zones of other cortical afferents and could therefore compete for trophic substances or exert a stabilizing influence.

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