Topography of Developing Thalamic and Cortical Pathways in the Visual System of the Cat

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ABSTRACT

Adult patterns of connectivity could emerge during development by a process of selective elimination from an earlier, more widespread, connectivity. We have addressed this issue by examining the topography of developing projections to area 17 in the cat. At different postnatal ages, paired injections of the retrograde tracers diamidino yellow and fast blue were made in area 17. Interinjection separations were carefully controlled and the spatial distribution of the two populations of labelled neurones investigated.

Projections to the striate cortex from the lateral geniculate nucleus, area 18, as well as connections intrinsic to area 17 were analysed quantitatively with a graphic method that uses a two-dimensional model of the projection. This allows two parameters of the projection to be calculated: the divergence (the spatial extent of area 17 contacted by an infinitely small region of an afferent structure) and the convergence (the extent of an afferent structure that projects to an infinitely small region of area 17).

During postnatal development, the bulk of the connections making up the geniculostriate and corticocortical pathways showed no variation either in their convergence and divergence. However, the projection of area 18 to area 17 and the intrinsic area 17 connections (but not the geniculostriate projection) in the 3-15-day-old kittens were each found to contain a small subpopulation of widely scattered neurones with widespread axonal trajectories.

These results, showing that many initially formed connections display a high degree of topographical order, are discussed in terms of the control mechanisms specifying axonal trajectories during development. © 1994 Wiley-Liss, Inc.

Key words: axogenesis, fluorescent tracers, extrastriate cortex

The connectivity of the neocortex is the key anatomical feature underlying its physiological function, so that the precision and complexity of the connections linking neurones underlines the processing of information characteristic of this structure. Despite the prominence of the cortex in terms of phylogenetic and biological significance, it still remains unclear how the topography of the adult neocortex connectivity emerges during development. In the present study we tackle this issue by examining the changing geometry of connections formed by populations of neocortical neurones.

By using retrograde tracers, we have recently developed a graphic method, the connectivity graph, for the quantitative analysis of the topography of connections in the visual system of the cat (Salin et al., 1989). This involved defining two projection parameters: the divergence and the convergence. The divergence specifies the spatial extent of the target structure that is innervated by an infinitely small region of the source structure. The convergence defines the spatial extent of the source structure that innervates an infinitely small region of a given target (Fig. 1). Theoretical considerations (Salin et al., 1989; see Results) show that when the target and source structure have the same dimensions, as for instance is the case along the shared borders of adjacent cortical areas, then convergence equals divergence. When the target structure is considerably larger than the source structure, as for instance in the geniculostrate projection, then divergence will be much larger than convergence.

Studies of convergence and divergence in the adult cat show that these parameters, because they define the topographical relationships of interconnected structures, are in fact highly characteristic for a given pathway. Broadly speaking, divergence is generated by the sum of the scatter

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Fig. 1. Divergence (d) is defined as the spatial extent of the target structure (T) innervated by an infinitely small region of the source structure (S). Convergence (c) defines the spatial extent of the source structure that projects to an infinitely small region of the target. A: The effect of the dimensions of source and target structure on divergence and convergence. When the two interconnected structures have the same dimensions, as, for example, for adjacent cortical areas sharing a common border as is the case for the projection of area 18 to area 17 (left side), divergence and convergence will have identical values. When the source is smaller than the target, as is the case for the projection of the lateral geniculate nucleus (LGN) to area 17 (right side), divergence is larger than convergence. B: Representative connectivity graphs for connections between structures with similar magnification factors (left, areas 18–17) and target structure > source structure (right, LGN–area 17). The connectivity graph schematically represents neurones situated along a line connecting the two injection sites in the target (X1 and X2) and connecting the two populations of retrogradely labelled neurones (Y1 and Y2). When the limits of the injection sites and regions of retrograde labelling are plotted out as shown, the horizontal distance separating the two lines defines the divergence and the vertical distance the convergence.

plus the axonal spread at the target (Salin et al., 1989). The projection of the lateral geniculate nucleus (LGN) to area 17 shows low values of divergence and convergence, indicating that this projection is very nearly point-to-point. The divergence of the geniculostriate projection is larger than the convergence in accordance with the fact that area 17 is about twice the size of the LGN. In contrast, projections from extrastriate cortex to area 17 show large divergence
and convergence values, indicating that these connections are not strictly retinotopically organised (Salin et al., 1992), and in the case of area 18 projections to area 17 the anterior–posterior dimensions are similar so that the divergence and convergence values are nearly the same.

The developing nervous system is characterised by an overproduction of neurones, axons, and synapses, leading to a rapid turnover in connections. In this way, at each developmental stage there are large numbers of transient connections that are destined to disappear (for reviews, see Purves and Lichtman, 1980; Cowan et al., 1984; Easter et al., 1985). Furthermore, early formed connections in the nervous system are believed to be more widespread than in the adult. For example, connections between the cerebral hemispheres are restricted in the adult to well-defined territories, whereas early in development they originate from a more extensive cortical region (for a recent review, see Innocenti 1986).

The overabundance of connections observed at all levels of neural development and the fact that certain pathways have been shown to have a more widespread distribution has led to the notion that the adult pattern of connectivity evolves from a less precise connectivity and an initial low degree of order. Accordingly, the precision of adult connectivity is thought to be achieved by the selective elimination of certain connections and the stabilisation of others (Changex and Danchin, 1976; Cowan et al., 1984). In the present study, we have investigated whether the topographical precision of thalamicocortical and corticocortical pathways depends on such a developmental selection mechanism. By using quantitative techniques based on injections of retrograde tracers into area 17, we have determined whether there are developmental changes in divergence and convergence in three cat visual pathways: (1) the geniculostriate projection, (2) the extrinsic corticocortical connections, and (3) the intrinsic cortical connections of area 17. We shall now review the theoretical framework for the development of each of these three pathways.

Geniculostriate pathway

A developmental decrease in the divergence of the geniculostriate pathway could reflect the emergence of topographical order, but a decrease could also be part of the remodeling of axonal trajectories, which could occur during the formation of ocular dominance columns (Hubel et al., 1977). The transsynaptic transport of radioactive amino acids has shown that the segregation of separate territories in layer 4 receiving right and left eye afferents emerge from an initial state when the afferents from the two eyes are intermingled (LeVay et al., 1978). There is an interaction of the afferents from each eye, and the sorting into separate territories involves a pruning of an overabundant axonal arbor (LeVay and Stryker 1979). However, the evidence for this is actually quite indirect because transsynaptic autoradiograms do not allow the reconstruction of single axonal arbours. In a 4–5-week-old kitten, axonal filling showed that axonal arbours of geniculate fibres in area 18 are continuing increasing in tangential extent rather than decreasing (Friedlander and Martin, 1989; Anderson et al., 1992). Likewise, Antonini and Stryker (1993) failed to detect a significant increase in the tangential extent of the geniculostriate axonal arbours during development in the kitten. Although there could be a decrease in the size of the axonal arbour at ages prior to those that these researchers were able to study, they pointed out that would require pruning before arbour expansion and, furthermore, that such pruning would have to occur when numbers of synapses in the cortex are actually increasing (Cragg, 1975). This contradiction between the results of different laboratories may be due to the fact that the size of cortical territories labeled by axonal transport of tritiated amino acids reflects the divergence of populations of geniculostriate projections, whereas the intraxonal labelling reveals the size of individual axonal arbours. Hence, experiments looking at the divergence of small numbers of geniculostriate afferents, such as those of Friedlander and Martin (1989), may fail to detect small populations of geniculate neurones with very large cortical arbours. We have studied, therefore, the divergence and convergence values of geniculocortical projections to area 17 at regular intervals between 3 and 32 postnatal days.

Extrinsic corticocortical pathways

Studies of the development of connections between cortical areas within the same hemisphere have largely addressed the issue of the changes of the laminar distribution of cortical connections and their modular organisation (Bullier et al., 1984a, c; Price and Blakemore 1985; Kato et al., 1986; Kennedy et al., 1989). Studies that have specifically addressed the issue of the topography of developing connections have given somewhat conflicting conclusions. In a study using the anterograde transport of labelled amino acids, early formed projections from area 17 were found to be diffuse in the white matter but to have restricted access to the cortical gray matter, suggesting some degree of topographical precision (Price and Zumbroich, 1989). However, a study from the same group looking at the retrograde transport of fluorescent tracers after injections into area 18 reported widespread distributions of labelled cells in the suprasylvian sulcus and concluded that early formed connections undergo considerable refinement (Kato et al., 1991). Discrepancies in the conclusions drawn by these studies could be the consequence of differences in tracers used as well as the cortical links studied. A difficulty with the retrograde study is that the density of connections were not measured in this study, so that small numbers of neurones with widely divergent connections could be masking a high degree of topographical order. Both of these difficulties can be resolved by using the quantitative approach used in the present study.

Intrinsic cortical connections

It is now firmly established that long-range horizontal connections exist between visual cortical neurones within area 17 (Gilbert and Wiesel, 1979, 1983; Rockland and Lund, 1983; Martin and Whitteridge, 1984; Salin et al., 1992). In the adult, these intrinsic connections link cortex representing distant parts of the visual field and have a periodic clustered organisation (Gilbert and Wiesel, 1979, 1983, 1989; Martin and Whitteridge, 1984). Three studies to date have examined the development of the intrinsic connections of area 17. Luhmann et al. (1990) showed that injection of horseradish peroxidase into area 17 led to labelling spanning up to 10.5 mm within area 17 in the kitten and 3 mm in the cat. They concluded that the adult restricted pattern of intrinsic connectivity results from a pruning of an initially exuberant connectivity. Callaway and Katz (1990) made highly restricted injections of retrograde tracers and reported that the extent of intrinsic labelling was actually smaller in the neonate than in the
adult and that there was a modest postnatal increase in extent to reach adult levels at around 2 weeks of age. In their study it was the emergence of clustering rather than the decrease in tangential extent that characterised the development of intrinsic connectivity. This conclusion has been confirmed by Lubke and Albus (1992). It is conceivable that the differences reported with respect to the tangential spread of connections is related to the fact that the study by Luhrmann et al. (1990) used relatively large injections and that by Callaway and Katz, much smaller injections. If the wide tangential spread of labelling reported in the first study was the consequence of retrogradely labelling of a subpopulation of widely scattered neurons with highly divergent projections, then this might go undetected in a study such as that by Callaway and Katz, who used small injections. To resolve this issue, it is necessary to examine quantitatively the spread of labelling in area 17.

In the present study we have been able to show that the divergence and convergence values for the geniculostriate pathway remain invariant during postnatal development. Divergence and convergence of the intrinsic and extrinsic connections of area 17 hardly change during postnatal life. The somewhat more widespread labelling found in areas 17 and 18 after point injections of retrograde tracers into area 17 is due to the diffuse connectivity of a small number of neurons. Although the present findings argue against axonal pruning as a major means of achieving topographical precision, nevertheless they do show that, in one sense, connections are more widespread early in development. The fact that the young kitten has adult-like cortical divergence and convergence values in a smaller brain means that in the immature brain a point in area 17 interconnects with a larger proportion of areas 17 and 18 than is the case in the cat.

METHODS

Animals were premedicated for surgery with dexame- thasone (1 mg) and chlorpromazine (1 mg) injected intramuscularly. Animals were then anaesthetised with ketamine hydrochloride. A surgical plane of anaesthesia was maintained with 1–2% halothane in carbogene (O₂: 94%; CO₂: 6%). The adults were held in ear-bars. The young animals were held loosely in a head-holding device. In each animal, a small rectangular flap of bone was excised from the skull along the central sinus overlying the primary visual cortex. Injections of fluorescent tracers fast blue (FB) and diamino yellow (DY) were made into area 17 of the visual cortex at various postnatal ages from birth to adulthood (Table 1). The dyes (0.15 ml) were injected at a concentration of 3% with a glass micropipette (tip diameter approximately 100 μm) sealed to a Hamilton microsyringe. One injection of each dye was made in area 17 in the bank of the interhemispheric fissure. All injections were in cortex subserving the lower visual field according to published maps (Albus and Beckman, 1980). Injections were made so as to span the full depth of the cortex and oriented so as to be nearly perpendicular to the cortical surface and to minimise the anterior–posterior extent of the injection. Interinjection distances are given in Table 1.

Following a 6–10-day survival period, the animals were reanaesthetised and perfused through the heart with 2.7% saline, followed by 30% formalin in cacodylate buffer to give a final concentration of around 4%. After 45 minutes fixation, the brain was rinsed with increasing concentrations of sucrose (8–30%). The brains were blocked in the coronal plane, and 40-μm-thick sections were cut within 24 hours on a freezing microtome. One in two sections were mounted from saline onto gelatin-coated slides.

The sections were left without coverslips and were observed with oil-immersion objectives under UV light with a Leitz fluorescence microscope equipped with a D-filter set (355–425 nm). In the periphery of the projection field, the spatial locations of labelled neurons were charted by means of an X-Y plotter electronically coupled to the microscope stage. After plotting, sections were counterstained either for Nissl substance or for cytochrome oxidase activity (Wong-Riley, 1979) and back-projected onto the charts of labelled neurons so as to trace cytoarchitectonic borders. In central regions of the projection zones, maximum cell-density measurements were obtained by counting labelled neurons within a 0.41 × 0.41-mm² graticule, and the cell density was calculated by dividing the cell count by the surface area of the graticule.

Ten kittens of different ages were used to measure the dimensions of areas 17 and 18, the neocortex, and the LGN. These animals were perfused either with a mixture of 2% paraformaldehyde and 2% glutaraldehyde or with a solution of 4% paraformaldehyde. The type of fixative did not influence tissue shrinkage, which was found to be on the order of 10%. Dimensions of LGN and cortex as well as of labelling and uptake zones are not corrected for shrinkage.

### Table 1. Interinjection Separation Divergence (div), Convergence (con), and Extent of Labelling

<table>
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<tr>
<th>Case</th>
<th>Separation</th>
<th>Age at injection</th>
<th>Age at perfusion</th>
<th>LGN Area 17</th>
<th>Area 18</th>
<th>Extent of labeling</th>
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<td></td>
<td></td>
<td>con</td>
<td>div</td>
<td>con</td>
<td>div</td>
<td>FBA</td>
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<td>K124</td>
<td>1.5</td>
<td>3</td>
<td>9</td>
<td>0.5</td>
<td>2.5</td>
<td>5.9</td>
</tr>
<tr>
<td>K129</td>
<td>4.3</td>
<td>3</td>
<td>9</td>
<td>0.5</td>
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<td>4.2</td>
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<td>7</td>
<td>13</td>
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<td>5.0</td>
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<tr>
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<td>4.9</td>
<td>8</td>
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<tr>
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<td>14</td>
<td>21</td>
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<td>6.0</td>
</tr>
<tr>
<td>K126</td>
<td>3.6</td>
<td>16</td>
<td>21</td>
<td>0.45</td>
<td>2.55</td>
<td>6.0</td>
</tr>
<tr>
<td>K129</td>
<td>5.1</td>
<td>16</td>
<td>21</td>
<td>0.45</td>
<td>2.55</td>
<td>6.0</td>
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<tr>
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<td>21</td>
<td>27</td>
<td>0.42</td>
<td>1.95</td>
<td>5.0</td>
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<tr>
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<td>25</td>
<td>32</td>
<td>0.42</td>
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<tr>
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<td>32</td>
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<td>1.8</td>
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<tr>
<td>Adults</td>
<td></td>
<td>4.5</td>
<td>2.5</td>
<td>0.5</td>
<td>2.5</td>
<td>5.3</td>
</tr>
</tbody>
</table>

Values given in millimeters.
Sections were counterstained for Nissl substance or for cytochrome oxidase and the curvilinear length through layer IV of areas 17 and 18 as well as the total neocortex measured on parasagittal sections.

RESULTS

Two criteria had to be met for material to be selected for analysis in the present study. First, injections of tracers had to be restricted to the cortical gray matter and to span the depth of the cortex (Fig. 2). Second, both injections had to give uptake zones that did not involve the 17/18 border. The location of injection sites with respect to the areal borders was observed directly on the counterstained sections and was later confirmed by the pattern of thalamic labelling (Dehay et al., 1988).

Twelve kittens successfully fulfilled these criteria and were used for the quantitative analysis of the retrograde labelling. Two cats from a previous study were used as controls (Table 1). In all cases strong retrograde labelling was obtained in the cortical and subcortical regions, which are known to project to area 17 in the adult (Fig. 3; Bullier
et al., 1984a,b). Labelled neurones were also found in the primary auditory cortex, which is known to project transiently to the gray matter of area 17 in the kitten (Dehay et al., 1984, 1988; Innocenti and Clarke, 1988).

A detailed description of the quantitative analysis of topography in the cat visual system is provided elsewhere (Salin et al., 1989). A cortical injection of a retrograde tracer results in a three-dimensional distribution of labelled neurones in afferent structures. By simultaneously using two different tracers, it is possible to relate formally the separation of the two afferent populations to the interinjection separation. In the case of afferents from the LGN and area 18 to area 17 in the cat, the problem is greatly simplified because the vertical meridian in the lower visual field in areas 17/18 as well as in the LGN is represented rostrocaudally parallel to the medial plane. One therefore need only consider a two-dimensional distribution of retrogradely labelled cells. Thus, injections separated rostrocaudally in area 17, close to the border with area 18, will give rise to two populations of rostrocaudally separated populations of neurones, both in area 18 and in the A layers of the LGN. By plotting the number of labelled neurones per coronal section as a function of distance, one obtains “labeling curves” from which it is possible to estimate the separation of the two populations of labelled neurones. Values for the divergence and convergence of the projection are not directly available from the labelling curves because of the finite size of the injections sites. Hence, these curves are used to construct a two-dimensional model of the connections between those neurones lying along a line running parallel to the representation of the vertical meridian in the source and target structure. In the target structure (area 17), this line will interconnect the centres of the injection sites. In the source structure (LGN and area 18), the line will interconnect the centres of the maximum labelling zones. By plotting the extent of labelled neurones along the rostrocaudal axis against the extent of the uptake zones of the dyes, we obtain a “connectivity graph.” In the hypothetical case of zero divergence, convergence, and scatter, where each neurone of the source would project to a single point in the target, the connectivity graph would give a straight line with a slope determined by the ratio of the magnification factors of the source and target structures (Daniel and Whitteridge, 1981). With nonzero values of divergence and convergence, the connectivity graph gives two parallel lines, the vertical separation of which defines the convergence and the horizontal separation the divergence. In reality, the lines are not perfectly parallel, and an average divergence and convergence value is obtained by measuring separations midway between the injection sites (see the Discussion section for more details).

Convergence and divergence are interrelated parameters because their ratio defines the slope of the lines bounding the connectivity graph, which in turn corresponds to the ratio of the magnification factor of the source and the target structure. When the magnification factor of the source and target is equal, the slope will be 45°, and divergence is equal to convergence. This situation is encountered for projections linking adjacent cortical areas and intrinsic connections. Also, divergence approximates convergence when it is expressed as a percentage of the source or target (Fig. 1).

In the present study, divergence refers to a linear measure (in millimetres) of area 17 that receives projections from a point in an afferent structure. The convergence relates to the number of millimetres of an afferent structure that projects to a point in area 17.

**Geniculostriate pathway**

In the kittens as well as in the cats, retrogradely labelled neurones in the LGN formed two compact clusters. Figure 4 presents an example of labelling in the LGN of a 26-day-old kitten (injected on day 19) with an interinjection distance of 3 mm. In the sections illustrated in this figure, different populations of labelled neurones are located in different layers. Reconstructions show that labelling from
were largely separated. Furthermore, the extent of labelling for a given population of back-filled neurones was comparable in the adult and immature brains (kittens: 0.5–1.0 mm; cats: 1.0–1.5 mm). Because the spread of labelled neurones is related to the size of the injection site, it is necessary to construct the connectivity graph to obtain divergence and convergence values.

The connectivity graph relates the distance separating the two populations of retrogradely labelled neurones to the interinjection distance. The latter measurement requires a precise assessment of the uptake zone of the fluorescent tracers used. As discussed in detail elsewhere, this is relatively straightforward with DY and FB (Keizer et al., 1983; Bullier et al., 1984a; Kennedy and Bullier, 1985; Perkel et al., 1986; Ferrer et al., 1988; Salin et al., 1989). Connectivity graphs for the geniculostriate projections in two representative kittens and one cat are shown in Figure 6. After the corrections have been made for developmental rotation of the nucleus (see below), the divergence can be measured on these graphs by the horizontal separation midway between the two injection sites and the convergence by the vertical separation at the same location.

One major consideration in calculating the extent and separation of the two populations of labelled neurones in the LGN of the kitten is the rotation of the LGN in the parasagittal plane, which occurs during development (Kalil, 1978). Whereas in the adult the surface of the LGN runs approximately parallel to the cortical surface so that the separation along the representation of the vertical meridian can be directly measured from coronal sections, in the newborn kitten there is an angle with respect to the horizontal that progressively decreases from 72° to 6° in the adult (Kalil, 1978). Hence, the labeling curves constructed from coronal sections underestimate population separations in the kitten. To correct for this, we have assumed that the region of labelling in the A layers can be represented by parallel compartments at a specified angle, which is determined by the age of the animal (Table 2; Kalil, 1978). Knowing the angle makes it possible, by using simple trigonometry, to estimate the true sizes and separation of the regions of labelling in the A layers.

The validity of the correction procedure was checked in one kitten (K137). In this case, the LGN was sectioned parasagittally so that the true size and separation of the two populations of cells could be measured directly on the histologic section. This animal returned a convergence and a divergence of 0.42 and 2.65, which was very similar to the values obtained in kittens in which the correction procedure was required (Table 1).

The corrected convergence values in the kitten range between 0.35 and 0.5 mm (see Table 1) and are not significantly different (Mann Whitney U test, \( P = 0.5 \)) from adult values (0.4–0.5 mm). The corrected divergence values in the kitten range between 1.95–2.5 mm and are not significantly different from the cat (1.8–2.5 mm).

Corticocortical pathways

All the paired injections in the kitten resulted in a distinctive pattern of extrastriate labelling in which the degree of separation of the two populations of retrogradely labelled neurones was similar at all ages examined. In the present section we shall first give an overall, qualitative description of the pattern of extrastriate labelling before...
proceeding to compare the areal distribution of retrogradely labelled neurones in areas 17 and 18 in kittens and cats.

**Qualitative analysis.** The distributions of DY- and FB-labelled neurones are illustrated in the kittens injected at 8, 15, and 19 days (Figs. 7–9). The two populations show a rostral–caudal separation parallel to the representation of the vertical meridian on the 17/18 border, superimposed on a less marked medial–lateral separation. In areas 17 and 18, the two populations of labelled neurones showed partial overlap in all cases. In the kitten injected on day 8 (Fig. 7), large numbers of DY and FB neurones are intermingled on section 68 with a fall-off rostrally and caudally so that areas 17 and 18 in section 38 contain principally FB-positive neurones and section 98 principally DY-positive neurones. Similarly, in the oldest kitten (Fig. 9) extensive overlap in areas 17 and 18 is found in section 35 with predominantly DY-labelled neurones rostrally in section 40 and uniquely FB-positive neurones caudally in section 23.

Area 20 in the temporal cortex provides a clear example of a medial–lateral separation and is illustrated in the youngest kitten (section 38, Fig. 7) and the oldest kitten (section 23, Fig. 9). In this area, neurones projecting to area 17 are confined to infragranular layers (Bullier et al., 1984c). In both animals, retrogradely labelled neurones can be observed in the infragranular layers with DY-positive neurones lying lateral to the FB-positive neurones. In other cortical areas, the FB-positive neurones lie lateral (and posterior) to the DY-positive neurones. In the lateral bank of the suprasylvian gyrus, there is a variable degree of separation that, although only partially illustrated in the youngest kitten (Fig. 7, sections 68 and 98), is clearly shown in the two older animals (Fig. 8, section 47; Fig. 9, section 35 and 40). Mediolateral separations are also found in area 19 and is illustrated in the 2-week-old kitten (Fig. 8, section 47).

**Quantitative analysis.** The labelling curves have been prepared from the maximum densities in a restricted region of each section. In our previous study in the adult (Salin et al., 1989), we noted that the slopes of the density curves were steep, indicating a sharply delimited region of labelling. Nevertheless, even in the adult there are occasional ectopically placed neurones outside of the major projection zone. To estimate the extent of the region of labelling and avoid spuriously large values due to a few scattered cells, we defined the region of labelling as cortex containing more than 5% of the maximum labelling density. Once the zero level is reached, groups of neurones occasionally found in more distant sections are not taken into account for defining the extension of the labelled zone.

**Area 17: Intrinsic connectivity.** The density curves for the kittens injected on days 3, 15, and 19 are shown on the left-hand side of Figure 10. Density measures were made at distances beyond 150 μm from the uptake zone to minimise the effect of counting neurones that may have been labelled by uptake from the dendrites. Hence, the break in the middle of these curves corresponds approximately to the

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Fig. 5. Labelling curves for lamina A of the LGN in five kittens and one cat. These curves show the number of labelled neurones at successiverostrocaudal positions of the LGN. The abscissa shows the distance from the posterior pole of the LGN. Open symbols: numbers of neurones labelled by posterior injection. Solid symbols: neurones labelled by the anterior injection. Other conventions as in Figure 4.
Table 2. Percentage Distribution of Labeled Neurons in the Three Different Density Zones3

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<tr>
<th></th>
<th>A17</th>
<th>A18</th>
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<td>FB</td>
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<tr>
<td>≥50%</td>
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<td>&gt;5-50%</td>
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</tbody>
</table>

3n = number of labeled neurons counted.

In all three cases, the lines bounding the connectivity graph are approximately parallel and show similar separations leading to convergence values of 4.0–6.0 mm. Table 1 shows that these are representative of the populations of kittens studied.

**Area 18: Extrinsic Connectivity.** Peak labelling density values in area 18 range between 900 and 1,200 neurons/mm². The fall-off in density on either side of the maximum was only slightly less steep than in area 17 (left-hand side of Fig. 11). The connectivity graphs constructed from these labelling curves are shown in Figure 11 and give divergence values of 5.3–6.5 mm (median value, 5.7; adult range: 5.5–5.8 mm) and convergence values of 3.2–3.5 mm (median value, 4.5; adult range: 4.0–4.9 mm; see Table 1). The Mann Whitney U test showed that in area 18 that there was not a significant difference between cat and kitten convergence (P = 0.36) and divergence (P = 0.33) values.

The similar divergence and convergence values found in the adult and immature brains show that the bulk of extrinsic projections from area 18 to a point in area 17, as well as intrinsic connections within area 17, originate from a projection zone that remains the same size during development. However, close inspection of Figure 11 shows that in the kitten injected on day 3 and perfused on day 9 (K139) the 5% threshold is situated approximately 3 mm from the zero value for the caudal injection. In the kitten injected at 19 days of age, the 5% threshold was situated less than 0.5 mm from zero values for both fields of labelling. The kitten injected at 15 days showed intermediate distances between the 5% threshold and the zero value. These very low-density extensions of labelling were typical of the kittens injected at 3–15 days of age and were more pronounced for the FB-labelled population than for the DY population. To examine the areal extent of low-density levels around the projection zone, we have prepared two-dimensional surface-view reconstructions of labelling density.
K136 (15/21 PND)

Fig 8. Distribution of fast blue and diamidino yellow retrogradely labelled cells after small injections in area 17. Conventions as in Figures 4 and 7.
Fig. 9. Distribution of fast blue and diamidino yellow retrogradely labelled cells after small injections in area 17. Conventions as in Figures 4 and 7.
Fig. 10. Labelling curves and connectivity graphs for the intrinsic connectivity of area 17. **Left:** Density curves show numbers of labelled neurones/mm² plotted as a function of caudo-rostral distance in area 17. Breaks in the curve correspond to the location of the uptake zone plus approximately 160 μm. Open arrows: 5% density thresholds for the FB-labelled population. **Right:** Connectivity graphs show separation of the labelling in the source area plotted against the separation of the injection sites in the target area (area 17). Other conventions as in Figure 4.

The two-dimensional reconstructions of the areal distributions of retrogradely labelled neurones were constructed by using the area 17/18 border as a landmark (Fig. 12). Caudal FB injections are shown on the left-hand side, and the rostral DY injections on the right-hand side. Three levels of shading representing different density thresholds (2-5%, 5-50%, and >50% of maximum density) are indicated, the intermediate dark gray indicates the 5% level
used in the construction of the density curves. Furthermore, all retrogradely labelled neurones below the 2.0% level are represented as individual, scattered neurones irrespective of their distance from the major projection zone. The mediolateral and rostrocaudal extent of the 5% density labelling in both areas 17 and 18 is similar at all three ages for both sets of injections. The pattern in area 19 is more irregular, possibly due to problems in the reconstruction of this highly convoluted region of cortex. However, there is a noticeable difference in the kitten injected on day
Fig. 12. Two-dimensional surface view of the labelling density in areas 17, 18, and 19: caudal fast blue injections (left) and diamidino yellow injections (right). Three levels of areal densities of labelling are shown for each injection, each level being a percentage of the maximum density measured in each area. Single dots represent individual neurons. Conventions as in Figure 4.
To summarise, for the rostrocaudal direction the two-dimensional reconstructions complement the density profiles because they show the areal extent of the low-density labelling and confirm that the bulk of the labelled neurones in area 18 as well as in area 17 undergoes little topographical rearrangement during development. However, the surface view does show that in the two youngest animals there is a region of low labelling density that stretches unusually far caudally and medially to the injection sites. It is this low-density labelling that disappears during development and leads to the overall tangential extent of labelling being more restricted in the adult compared with the immature brain.

These results show that the majority of immature connections have similar convergence and divergence values to those found in the adult. To estimate the percentage of neurones in the young animals that have highly divergent and convergent projections, we calculated the proportions of labelled neurones in each of the four density zones shown in Figure 12. Relative proportions of neurones in each density zone were estimated by counting labelled neurones in each density zone at regular intervals throughout the projection zone. The limitations of this quantification related to the high levels of labelling in the centres of the projection zone, which lead to underestimating the numbers of neurones in the region of maximum density. This problem is particularly acute in the youngest animals where very high density levels are encountered. Consequently, one would predict that we in fact overestimate the percentage of labelled neurones in the low-density region in the immature animal. The results for three kittens are shown in Table 2 (in this table, the two lower density classes in Fig. 12 have been lumped together to give a <5% category). It can be seen that the numbers of neurones in the <5% zone is remarkably constant for a given age and for each pair of injections within each area. Overall, there is a steady decrease with age in the proportion of labelled neurones in the <5% density zones. In the kitten injected on PND8, the <5% density zone in area 18 contains 22.5–24.1% of the total number of labelled neurones found in this area. The <5% zone in area 17 of the same animal contains only 14.8–16.5% of the labelled neurones of the projection zone. In the animal injected on PND19, area 17 had 3.0–4.3% of its labelled neurones in the <5% density zone and in area 18 the figure was between 5.3–6.1%. The animal injected on PND15 returned intermediate values. This table shows that during development there is a significant reduction in the proportion of labelled neurones located in the periphery of the projection zone, in the region of low density of labelling.

The restriction of labelling during development is reflected in the population of kittens examined by the rostral and caudal limits at which a labelled neurone was found in the cortex. This is shown on the right-hand side of Table 1. It was possible to prepare scattergrams of these values for all populations except for the FB-labelled neurones because these very posterior neurones are located on the cortex, which is curving away from the midline and where it is not possible to directly measure the distance from the injection site. These scattergrams (Fig. 13) returned regression lines that confirm a significant reduction in the extent of labelling in area 17 during development.

Consequence of the expanding target size on divergence values. The present results show that divergence and convergence values show little or no variation with age (Fig. 8, both for the 2–5% density level and the scattered neurones. In this case, low neurone densities extend both caudally and medially in area 17 and rostrally and caudally in area 18. In the kitten injected on day 15, the 2–5% level is much reduced, and there are fewer scattered labelled neurones. In the kitten injected on day 19, the 2–5% level has virtually disappeared and the scattered neurones are much rarer than in the younger animals.

The two-dimensional reconstructions shown in Figure 12 confirm that the dorsomedial extent of labelling above 5% of the maximum density does not change during development. However, an unexpected finding is that the labelling extends farther medially in area 17 in the young animal than it does in the adult. This greater extent of labelling in the medial direction is due largely to an extension of the <5% level. In the kitten injected on postnatal day (PND) 8, labelling from the FB injection extends 6.9 mm medially and that from the DY injection 6.4 mm medially. In the oldest kitten, injected on PND19, the medial extent of the 5% level is reduced to 4.8 mm for the FB injection and 3.2 mm for the DY injection. Intermediate values were obtained for the animal injected on day 15.
Fig. 14. Graphic representation of the developmental changes in convergence (left) and divergence (right).

The fact that in the immature brain small, spatially restricted populations of cells interconnect with similar areal surfaces as in the adults needs to be considered with respect to brain growth. To investigate the consequence of the developmental expansion of the brain on topographical relationships, we measured the increase in length of the neocortex, cortical areas 17 and 18, as well as the LGN (see Methods). The results of this study show that there is a linear increase in brain size with steeper slopes in the cortex compared with the thalamus (Fig. 15). Overall, the rostro-caudal length of the cortex nearly doubles between birth and adulthood.

The divergence of a given projection to area 17 is defined as the rostral-caudal extent in millimetres of area 17 that receives projections from a virtual point in the source area. Because our results show that convergence and divergence
values do not change during development despite the fact that the brain is expanding, the early projections must cover a far greater portion of the target area than do mature projections. It is possible to quantify this changing topographical relationship by expressing the convergence and divergence values of a particular projection as a percentage of the rostral-caudal length of area 17 (Fig. 16). This shows a clear decrease in the relative divergence with age because there is a 30% reduction in the percentage of area 17 contacted by a point in the LGN and a 30% reduction in the extent of area 17 contacted by a point in area 18. In both cases adult-like values are obtained around 3 weeks of age. Percentage divergence and convergence theoretically should be the same. In Figure 16 the percentage divergence was found to be consistently larger than the percentage convergence. This is probably related to an overestimation of the size of the LGN.

**DISCUSSION**

The present study addresses the issue of quantitative changes in topography of the geniculostriate and corticocortical pathways during postnatal development. Our results show that the convergence and divergence values of the geniculate projection to area 17 show no detectable changes and that the bulk of intrinsic projections to a point in area 17 as well as extrinsic projections from area 18 also have adult-like convergence and divergence values. However, during the first 2 weeks of life we were able to detect a small subpopulation of cortical neurones showing a more widespread connectivity than in the adult, as well as a restriction of the medial extent of intrinsic area 17 connections. Before proceeding to relate these results to published findings, we shall discuss certain technical aspects of the present report.

**Relevance of the developmental stages examined**

The developmental stage that we have investigated is determined by when birth occurs in the cat. What are the possibilities that differences in the convergence and divergence values would be found if we were to examine earlier, prenatal stages? The cat is born after 65 days of gestation and still has a very immature geniculostriate pathway. Although geniculate fibres accumulate in the subplate at E36, penetration of fibres into the presumptive visual cortex does not begin before E50 (Shatz and Luskin, 1986; Ghosh and Shatz, 1992). LGN fibres begin to arrive in layer 4 between E55 and E60. Upon arrival in layer 4, there is a period of intense axonal growth that continues postnatally (Ghosh and Shatz, 1992). Final maturation of the pathway is not achieved before 5 weeks after birth when the ocular dominance columns appear adult-like (LeVay et al., 1978).

Less is known about the onset of axon growth in the cortex, so it is more difficult to define the period before birth during which cortical projecting neurones might exhibit larger divergence and convergence values than those found in the present study. What is known is that neurones destined for layers 2/3 are still migrating at birth and that they do not reach their definitive position in the cortex before the end of the second week of life (Shatz and Luskin, 1986). Hence, although cortical pathways are clearly established at birth (Bullier et al., 1984a,c; Price and Blakemore, 1985) and issue from neurones that are still migrating (Dehay and Kennedy, unpublished observation), one would predict that they would not exist much before the date when the parent neurones arrive in the vicinity of the cortex. Much of the corticocortical pathways originate from the supragranular layers, which are generated between E41 and E57 (Luskin and Shatz, 1985). Assuming 10 days for neuroblasts to reach the vicinity of the cortical plate (Rakic, 1974), one would expect axonal growth to start between E51–E67, with axons arriving at their targets at the earliest some 6 days after that date. Hence, one would predict that pathways between cortical areas are formed during the period from 9 days before birth and to some 7 days after birth. This timetable is in accordance with the emergence of the adult configuration of association projections at 3 weeks after birth (Bullier et al., 1984a,c; Price and Blakemore, 1985; Price and Zumbroich, 1989). Hence, although some cortical–cortical projections may be already established at birth, the maturation of this pathway is far from complete at the time of our earliest observations.

**Methodological considerations**

A major consideration is the choice of retrograde tracer. The ideal tracer has a relatively small pick-up zone that can span the entire depth of cortical grey matter without encroaching on the underlying white matter and that has a high visibility following retrograde transport in both adult and immature tissue. These criteria are both met by fast blue and diamidino yellow and are discussed elsewhere (Dehay, 1988).

The calculation of divergence and convergence makes use of the connectivity graph. Ideally, the lines making up the graph are parallel. However, in reality this is not always the case. Nonparallelism does not constitute a problem for the intrinsic connections, but it needs to be considered for the extrinsic cortical connections and to a lesser extent for the geniculostriate projections. Divergence and convergence are measured on the graph midway between the two injections, thereby averaging the extremes encountered at either injection site. We examined the variation that is encountered if measurements were made at the injection site. This shows that the error in most cases is less than
10% and the maximum error encountered is 19% for the extrinsic cortical connections of K139.

The presence of a small population of widely scattered neurones participating in the corticocortical pathways before 3 weeks of age can be explained at the single cell level in two ways. Compared with the adults, the mean size of the axonal arbour of the population as a whole could be slightly larger, or the precision of termination at the target could be slightly lower for the entire neuronal population participating in the projection. In this case, one would obtain a
shallower slope in the density labelling curves obtained from the cortex of the immature brain. In fact, this is not the case. The slopes in the immature material are, if anything, actually steeper than in the adult (Figs. 10, 11). Alternatively, a few neurones in the immature projection could have widely different arboreal sizes and/or scatter than the bulk of the projection. Our results strongly support the second hypothesis because the curve flattens at around the 5% threshold values (see arrows in Figs. 10, 11) in the immature brain.

The technique used in the present report can indirectly address the issue of the contribution of axonal arboreal size and scatter to changes in divergence. The proportion of double-labelled neurones in the overlap zone of the two populations of single-labelled neurones following a double, separate injection in a given target structure is related to axonal arboreal size (Perkel et al., 1986; Salin et al., 1989). High proportions of double labelling suggests that axonal arbours are large, and small proportions that the overlap is due to scatter. Although we did not specifically address this issue in the present study, we did estimate the percentage of double-labelled neurones in overlapping regions in the cortex and found that the proportion did not change significantly during development. This suggests, therefore, that the population of widely scattered cells observed in the youngest kittens corresponds to neurones with widely scattered terminal arbours.

It can be argued that the use of retrograde tracers coupled with the connectivity graph only gives an indirect indication of projection parameters that are directly examined in studies using anterograde tracers and reconstruction of single axons (e.g., Friedlander and Martin, 1989; Antonini and Stryker, 1993). However, the divergence and convergence as determined by the connectivity graph method is appropriate to investigating the projections of populations of cells and therefore directly informs us of the overall topography of the interconnections between two given structures. Although this is theoretically possible in anterograde studies, this would imply the highly impractical reconstruction of large numbers of axons.

**Geniculostriate pathway**

Prior to the present report the development of this pathway was investigated with respect to the formation of ocular dominance columns and not with respect to its topography. Transsynaptic transport following intracocular injections of tritiated amino acids shows that in the first few weeks of life the cortical columns in layer 4 appear less pronounced than in the adult (LeVay et al., 1978). Initially exuberant axonal arbours undergo selective pruning to give the adult pattern of ocular dominance columns (LeVay and Stryker, 1979). In support of this, LeVay and Stryker (1979) published a Golgi-impregnated LGN axon at PND17, which had a tangential extent of more than 1 mm. Similar axonal arboreal sizes have been described in layer 4 of PND7 kittens by using the lipophilic tracer Dil (Ghoseh and Shatz, 1992). The problem in interpreting this data is that LGN axonal arboreal sizes in area 17 in excess of a tangential extent of 1 mm exist in the adult (Freund et al., 1985, Humphrey et al., 1985). Our results showing that divergence of the geniculostriate pathway does not change during development is compatible with the observation that during development there is not a significant decrease in the size of arborization of LGN afferents to area 18 (Friedlander and Martin, 1989) and to area 17 (Anderson et al., 1992; Antonini and Stryker, 1993).

**Extrinsic cortical pathways**

The present study confirms and extends other reports that noted some degree of topographical order in early formed cortical projections. Price and Blakemore (1985) noted that injections of wheatgerm agglutinin hors eradish peroxidase (WGA-HRP) in area 18 revealed "a basic topographical arrangement" of connections in area 17. By using anterograde transport of H-proline, injections of the tracer in area 17 led to restricted labelling in areas 18, 19, and the suprasylvian cortex. The conclusions of this report are very different from those of Kato et al. (1991) who claimed that small injections of retrograde tracers in area 18 led to widespread labelling in the medial bank of the suprasylvian gyrus. The report by Kato et al. (1991) is also at odds with the present results where we note that side-by-side injections in area 17 lead to adjacent groups of FB- and DY-labelled neurones in the suprasylvian gyrus. We have not tried to measure quantitatively the divergence and convergence in this region because the multiple representation of the visual field in this part of the cortex makes this extremely hazardous (Salin et al., 1989). In any case, in the study by Kato et al. (1991), they noted that "even very small injections labelled the entire rostrocaudal length of one of the areas in the suprasylvian cortex." This is not the case in the present study where labelling is clearly restricted in the rostrocaudal direction. There are two possible explanations for the differences between our results and Kato et al.'s (1991) results. There may be a true areal difference, and projections from the lateral suprasylvian cortex to area 18 might not topographically organised, whereas those to area 17 might be. Alternatively, depth of injection in the cortex might be a critical factor. Injections involving the white matter lead to widespread labelling (Clarke and Innocenti, 1986). It is possible that in the Kato et al. (1991) study injections may have contaminated the white matter.

To conclude, Kato et al. (1991) reported a large reorganisation of corticocortical connections during development. In fact, the extent of labelling that they reported in the kitten is very similar to those we obtained in the cat (Salin et al., 1989). However, their conclusions differ from ours because they obtained much narrower projection zones in their adult controls than we did (Salin et al., 1989, 1992). In their paper, Kato et al. (1991) discussed why they obtained much narrower projection zones in the adult. They suggested that the more restricted labelling that they obtained in the adult compared with our study could be either due to differences in the size of injection or to our supposed use of multiple injections. However, this is not the case. In our work on topography of cortical connections using dual injections of fluorescent dyes, we invariably used single injections that were carefully controlled for their rostral-caudal extent (Salin et al., 1989, 1992, the present study). We can only suggest that Kato et al. might have overestimated the size of their injection sites in their adult controls and/or that they used nonoptimal conditions such as the use of very short survival times or, as they suggested, that their injections were too small to adequately label the entire set of afferents.

In their study on the development of efferent connections of area 17, Price and Zumbroich (1989) found evidence of topographical projections to the cortical gray matter. How-
ever, in their PND4 animal they found that labelling in the 
white matter was diffuse. They found evidence of solitary 
fibres penetrating into the cortical gray matter at inap-
propriate places in PND4 and 8 kittens. These isolated fibres 
were found at some distance from the injection site within 
area 17 as well as in extrastriate areas. These solitary 
fibres, which did not appear to be topographically organ-
ised, could correspond to the small population of highly 
divergent corticocortical neurones displaying very wide-
spread connections that we find up to 3 weeks of age.

Intrinsic connections

Two recent studies have specifically examined the postna-
tal development of long-range intrinsic connections in area 
17 in the kitten. Luhmann et al. (1990) made small injec-
tions of a variety of retrograde tracers (fast blue, fluoro 
gold, diamidino yellow, WGA-HRP, and rhodamine-conju-
gated latex beads). They found that tangential connections 
extended progressively farther at subsequent stages in 
development (distances from injection sites: PND5, 2.7 
mm; PND21, 10.5 mm; adult values reached at 7 weeks, 3 
mm). Concomitant with the expansion and reduction of 
tangential extent was the appearance of clustering that 
became progressively more pronounced from PND10 on-
ward.

Callaway and Katz's (1990) study used injections of 
rhodamine-conjugated latex beads. In the youngest kittens 
(PND5) they also found a smooth continuous distribution 
of intrinsic connections with no hint of clustering. At later 
steps they found crude clustering that became progressiv-
ely sharper up to when the adult configuration was 
achieved around 6 weeks after birth. Where these research-
ers do differ from the findings by Luhmann et al. (1990) is 
that they failed to detect an expansion during the first few 
weeks of life, followed by a retraction. They found that 
throughout development the range of distance was rostro-
caudal (2.5–4.2 mm) and mediolateral (1.9–4.0 mm). How-
ever, the youngest intrinsic labelling that they illustrate 
shows a tangential extent of 2 mm (Fig. 4A in Callaway and 
Katz, 1990). That this value is outside of the ranges they 
reported for either the immature or adult brains suggests 
that there might be a very modest increase in tangential 
extent in the first few weeks of life.

Recently, Lubke and Albus (1992) confirmed Callaway 
and Katz's (1990) study. They also showed that it is the 
appearance of clusters rather than a restriction of tangen-
tial extent that characterises the early development of 
cortical connections. Our findings suggest that these two 
sets of results can be reconciled. Possibly for technical 
reasons, the study by Luhmann et al. (1990) focussed on 
the maximum extent of cortical connections. Their findings 
of connections spanning 10 mm is in agreement with our 
findings, both in intrinsic and extrinsic connections. How-
ever, in our study we have shown that such widespread 
connections constitute the exception and not the rule and 
that the vast majority of cortical projecting neurones in the 
imature brain have similar connections as that found in 
the adult. If very small injections or single cell injections are 
used, as was the case in the studies by Callaway and Katz 
(1990) and Lubke and Albus (1992), then a more exact 
estimation of the range of tangential connections can be 
obtained for the majority of neurones participating in the 
projection. However, such an approach will overlook a small 
population of neurones with very widespread connections. 
On the other hand, large injections, as used by Luhman et

al. (1990), by disregarding differences in densities of label-
ing, will make it impossible to estimate the range of 
tangential connections of the majority of cortical connec-
tions. The possibility that the size and location of the 
injection site is related to the extent of labelling is sup-
ported by a recent publication that shows that transient 
intraneuronal axons span up to 9 mm but are largely confined 
to the white matter (Assal and Innocenti, 1993). Hence, 
retrograde-tracer injections, which are successfully limited 
to the gray matter, might reveal a much weaker transient 
connectivity.

The absence of far-ranging connections in the Callaway 
and Katz (1990) study could also be at least partially due to 
technical factors related to the fact that they used fluo-
rescent latex microspheres. Injections using this tracer lead 
to pick-up zones that are 100–400 μm in diameter. This 
means that the pick-up zone cannot span the full depth of 
the cortex, but, more importantly, such a small injection 
site can only concern a fraction of a given axon arbor, which 
one would expect to be of the order of a millimetre or more.
One consequence is that neurones with very divergent 
axons might only retrogradely transport very small num-
bers of beads. This problem could lead to the level of 
labelling being below threshold (3 fluorescent beads) so that 
the small population of neurones, which we find in the 
periphery of the immature projection zone and which we 
have argued have widespread axonal projections, would go 
undetected.

CONCLUSION

The corticocortical pathway in the adult shows large 
values of divergence and convergence so that a point in area 
18 projects to a region in area 17, measuring approximately 
32 mm². This contrasts with the adult geniculostriate pro-
jection where a point in the LGN projects to a region in 
area 17, measuring 3–6 mm². The consequence of the large 
divergence and convergence values found in the cortical 
projections is that, unlike the geniculostriate pathway, 
cortical connections link neurones with noncorresponding 
receptive fields (Salin et al., 1992). Kennedy et al. (1991) 
argued that understanding cortical mechanisms of informa-
tion processing will require functional explanations of its 
nonvisuotopic connectivity. These considerations relate to 
the developmental features described in this paper in that 
the cortical projections differ from the geniculocortical 
pathway in so far as the former possess small numbers of 
widespread connections that are selectively eliminated dur-
ing relatively late stages of development. Therefore, selec-
tive elimination of connections might be involved in the 
development of the highly divergent–convergent connectiv-
ity of the cortex but might not be involved in the develop-
ment of the strict point-to-point connectivity found in the 
geniculostriate pathway. If selective elimination of connec-
tions is involved in the refinement of the geniculostriate 
projection, it would have to occur prenatally, when thalamic 
axons reside in the subplate.

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TOPOGRAPHY OF DEVELOPING PATHWAYS IN CAT


