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"The Development of Projections from Cerebral Cortex"

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The Development of Projections from Cerebral Cortex

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1 Introduction

The intriguing and fascinating complexity of biological structures probably reflects constraints imposed by three processes: phylogenesis, development and function. The latter provides the strongest justification for the existence of development neurobiology as a distinct discipline, within the broader field of developmental biology.

The specific function of nerve cells is communication with other nerve cells and/or the physical world around them. Nerve cells communicate with each other mainly,

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although not necessarily uniquely, through connections established by, in general long, arborescent processes. The network established by these connections is essential to what the nervous system does: an "adaptive structuration" of the surrounding "world". Indeed, within limits compatible with survival and reproduction, the structure of the world an animal perceives probably reflects that of its brain networks.

In some fundamental way these networks must be genetically constrained in a precise manner; species differences in brain structure, and hence, according to the above assumption, in the structure of the perceived world, provide convincing evidence for this. However, there must be limits to genetic determination. One could be that the amount of information in the genome may be insufficient to specify all the details of nervous system organization, including individual synapses (Changeux and Danchin 1976). Another is that each individual is an unpredictable genetic mosaic – the consequence both of sexual reproduction and evolution. Although evolution probably eliminated disastrous incompatibilities between genes, no inbuilt harmony among genes can be expected either. The product of one gene, say any protein crucial for cell generation, migration, differentiation, recognition etc., may not match qualitatively, quantitatively or temporally that of other genes. One would expect coordination of gene actions to be a requisite for the construction of a functional brain. How is coordination achieved? Genes capable of regulating other genes' action do exist. However, coordination seems also to be achieved through adjusting interaction between the products of genetic expression, i.e. neurons or parts of neurons.

Two of these adjusting mechanisms are in the developing nervous system: (a) neurons, dendrites, axons and synapses are first produced in excess and then some are maintained while others are eliminated out of interactions with other neuronal and/or non-neuronal elements; (b) the activity of a neural network can back-regulate the structure of the network which generates it.

This paper will try to track some of these concepts in the analysis of the development of cortical projections. Cortical projections were studied light-microscopically with the anterograde and retrograde anatomical tracer techniques which have become available during the last 10–15 years. Extracellular injections of axonally transported tracers do not visualize single axonal arbors or neurons but populations of retrogradely labeled neurons or anterogradely labeled axons. The light-microscopic analysis of these preparations does not reveal synaptic connections. Instead it delineates the gross pattern of interrelations among brain structures, which underlies connectivity. How these interrelations emerge in development is relevant for the old and difficult problem of the development of neural connections.

Although the phenomenon of massive neuronal death occurring at around the time of the establishment of connections was well known (Hamburger and Levi-Montalcini 1949; Cowan 1973), it was only in the second half of the 1970s that doubts began to emerge as to whether the model of a precisely and "ab initio" correctly target-oriented axonal growth, which appeared well grounded for the development of retinotectal projections in the fish (Sperry 1963), could be generalized to the whole nervous system, and in particular to the cerebral cortex of mammals. Evidence against such a generalization stemmed from the work of Hubel

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and Wiesel (Hubel et al. 1977) and of Rakic (1976) indicating initial overlap of geniculocortical projections in the developing area 17 and lateral geniculate body of the monkey. In the same years, Changeux and Danchin (1976), mostly on the evidence of transient multiple innervation in the development of the neuromuscular synapse, proposed the selective stabilization of synapses as a general mechanism in the development of neural connections.In their model, selection for stabilization was based on activity. This was compatible with the well-demonstrated role of visual experience in the segregation of geniculocortical projections as well as with the role of visual experience in the development of other response properties of cortical neurons (reviewed by Blakemore 1978; Wiesel 1982; Frégnac and Imbert 1984). The studies on the visual system, particularly those on ocular dominance were a gargantuan step beyond the classic embryology of the nervous system, derived from development biology. By focussing on the development of connections of neuronal ensembles, characterized by common functional properties, these studies validated the notion that building blocks larger than the neuron exist in the basic organization of the central nervous system (Mountcastle 1978). On the other hand they demonstrated how detailed knowledge about the function of a neuronal ensemble provides the crucial theoretical framework for the analysis of its development.

Nevertheless, in those years, the implications of the above studies, for a general theory of the formation of neural connections were probably not fully appreciated. The chemoaffinity hypothesis was phrased in sufficiently general and occasionally unclear terms (see discussion in Innocenti 1988) to accommodate the existence of a few exceptions, some of which could be interpreted as development errors (reviewed in Clarke 1981). Developmental "errors" were known at least since Cajal but did not prevent that author from proposing one of the first theories of neural development based on chemotropism (Cajal 1909).

However, the studies mentioned above were but the tip of the iceberg. It was found that the emergence of neural networks, at least at the cortical level – although the same turned out to apply to several other systems – had little to do with the precisely target-aimed growth of one axon to its postsynaptic partner which would have been expected on the basis of a literal interpretation of chemoaffinity theory. Long corticocortical projections are generated in excess in the course of neural development and reach structures from which they will be subsequently eliminated (Innocenti et al. 1977; Distel and Holländer 1980; Ivy and Killackey 1981). The magnitude of the projections eliminated in development and their reliable occurrence in different species and individuals precludes them from being considered as developmental errors. The very concept of developmental error suffers from intrinsic semantic difficulties (see Innocenti 1988 for discussion).

As previously discussed (Innocenti and Clarke 1984b; Innocenti 1987) no single, monotonic process seems adequate to explain the development of cortical connections[1]. Rather, an architectonic analogy seems to apply. Cortical connectivity is

[1] Throughout this paper the term "connection" will imply that synapses have been formed between neurons; "connectivity" will not. The second term will often designate juvenile conditions in which the existence of synapses was not proven. "Projection" was operationally defined above.

originally organized according to a juvenile Bauplan[2] as different from the adult Bauplan as romanesque architecture is to gothic. A number of well-characterized events mark the transition from the juvenile to the adult Bauplan. How these events are regulated is the object of active investigations, since this knowledge may open the way to controlling a crucial step in cortical development and possibly the evolution of neocortex (for discussions on development and evolution see Katz and Lasek 1978; Innocenti 1988).

2 The Adult Organization of Cortical Connectivity

While the existence of structural anisotropies in the cortex along a dimension perpendicular to its surface has been recognized at least since Gennari (1782; quoted by Glickstein and Rizzolatti 1984), the identification of boundaries between layers and sublayers has been notoriously difficult even in areas such as visual 17 in the monkey, where the lamination is especially clear cut (Billings-Gagliardi et al. 1974). The fact that neocortex is commonly divided into six layers reflects the acceptance of a conventional frame of reference rather than an objective feature of cortical structure.

Nevertheless, the analysis of cortical connectivity with neuroanatomical tracers demonstrated that, in general different cortical afferents reach different cortical laminae (Lund et al. 1979; Gilbert and Wiesel 1981; inter alios). Moreover, the different corticofugal projections have different and characteristic laminar origins, although within a given layer distinct neuronal populations can give rise to different projections and the projections to a given target can originate from more than one layer (Gilbert and Kelly 1975; Catsman-Berrevoets and Kuypers 1978; Innocenti 1980; Swadlow 1983; Innocenti et al. 1986; inter alios). As a rule of thumb, the supragranular layers (II and III) are the main source of projections to other cortical sites. The infragranular layers (V and VI) project to subcortical structures. Layer IV, the main thalamic recipient layer, sends short radial connections. In addition, probably all neurons, with corticofugal axons, also establish local connections through axon collaterals and these connections have both radial and tangential specificity (Gilbert and Wiesel 1981). Each cortical layer also contains neurons with exclusively local axons (Golgi type II neurons: Cajal 1911; Peters and Jones 1984).

A tangential trajectory across the cortex reveals different types of anisotropy, all in some way related to the radial anisotropy. Neocortex can be parcellated in distinct cytoarchitectonic areas basically according to thickness of layers and their cell composition, but the details of this parcellation evoked different degrees of

[2] The term Bauplan (construction plan) has obvious anthropological undertones. This may be justified by the view that evolution could be considered the engineer of the brain (for a similar belief see Glassman 1987). The term was chosen because it implies architectural purposefulness. It is not necessarily related to the usage by 19th century comparative anatomists (see Alberch 1984).

consensus. Some areas are easily identified by objective criteria, while the identification of others requires art and skill. Other criteria for parcellation can be used; for example, an area can contain a sensory representation distinct from those of surrounding areas. But these criteria do not always match the cytoarchitectonic parcellation (for a discussion, see Van Essen, 1985).

In the adult brain, probably each area is the preferential site of termination of a characteristic set of thalamic nuclei (Caviness and Frost 1980; Graybiel and Berson 1981; inter alios). Each area establishes connections with a characteristic subset of other cortical areas and these connections define lines of processing within the cortex (Mishkin et al. 1983; Livingstone and Hubel 1987a, b; Hubel and Livingstone 1987). In general, reciprocal connections exist between two areas but the radial locations of the neurons of origin both of the connections and of their terminations define the reciprocal hierarchical position of two areas along a processing line (Van Essen and Maunsell 1983; Van Essen 1985). Connections between areas obey largely unexplored mapping rules. At least in one case, that of the interhemispheric connections of visual and to some extent, primary somatosensory areas, the rules seem clear. These connections link in an exclusive manner parts of the primary sensory representations corresponding to sensory territories near the midline (Berlucchi 1972; Innocenti 1986), i.e. the line which divides the sensory worlds of the two hemispheres. In another case, that of the connections between areas 17 and 18 of the monkey, the mapping seems to achieve separation of processing lines with different retinal origins and thalamic relays (Hubel and Livingstone 1987; Livingstone and Hubel 1987a, b).

In addition, each area receives from and projects to a characteristic set of subcortical structures.

A different type of tangential cortical anisotropy derives from the fact that most, probably all cortical areas appear to be organized in radially oriented "modules" or "columns". Columns have been recognized both on functional (Mountcastle 1978; Hubel 1982) and anatomical grounds (Heimer et al. 1967; Hubel and Wiesel 1969; Jones et al. 1975; Goldman-Rakic and Schwartz 1982). The anatomical columns consist of discrete accumulations of thalamocortical terminals (Hubel and Wiesel 1969), sometimes associated with cytoarchitectonic specializations (Woolsey and Van der Loos 1970), or of discrete accumulations of corticocortical terminals (Heimer et al. 1967; Jones et al. 1975; Imig and Brugge 1978; Goldman-Rakic and Schwartz 1982; inter alios). Occasionally, corticocortically projecting neurons are organized in radial columns (Jones et al. 1975; but see Caminiti et al. 1985; see Innocenti 1986; for discussion and references). Cortical columns can appear as tangentially oriented bands or "blobs". The functional meaning of geniculo-cortical columns is known (Hubel 1982), that of corticocortical projection columns usually not, with two notable exceptions: (1) callosal termination columns in A1 are related to "ear dominance" bands (Imig and Brugge 1978); (2) origin and termination of projections from 17 to 18 in the monkey are related to afferent connectivity and functional properties of visual cortical neurons (Hubel and Livingstone 1987; Livingstone and Hubel 1987a, b).

Thus it appears that all the main principles of cortical organization recognized hitherto have their counterpart in some aspect of cortical connectivity.

3 The Juvenile Organization of Cortical Connectivity

Four features characterize juvenile cortical connectivity: (1) the existence of numerous transient projections; (2) radial specificity in the origin of the various projections; (3) some degree of topographical order in the tangential organization of the projections; (4) axons take specific routes in the white matter and cortex and exhibit a characteristic behavior at their site of termination.
A recent review by Payne et al. (1988b) complements this section in many respects.

3.1 The Transient Projections

Hubel et al. (1977) and Rakic (1976) provided the first evidence of transient overlap, in the visual cortex, of geniculocortical axons carrying information from the two eyes. This overlap is probably due to transient short branches of thalamocortical axons (Le Vay and Stryker 1979; Wiesel 1982) and therefore resembles the transient hypercollateralization of Purkinje cell axons and of climbing fibers (Cajal 1911) and the multiple innervation of the muscles (for references see Purves and Lichtman 1985).
A different type of exuberance, characterized by the production of long transient projections, was discovered in area 17 of the cat (Innocenti et al. 1977). It was found that while in the adult only the region near the border between areas 17 and 18 projects into the corpus callosum, the whole of areas 17 and 18 do so in the young kitten. Similarly, exuberant callosal projections were later found in somatosensory areas of the cat, where most of SI is devoid of callosal projections in the adult but not in the neonate (Innocenti and Caminiti 1980), as well as in the visual areas of the hamster, rat (Mooney et al. 1984; Olavarria and Van Sluyters, 1985) and rabbit (Chow et al. 1981), and in area 18 (Dehay et al. 1988b) and the somatosensory areas (Killackey and Chalupa 1986) of the monkey. Work in the somatosensory cortex of the rat (Ivy et al. 1979; Ivy and Killackey 1981) and in the auditory cortex of the cat (Feng and Brugge 1983) showed that in areas where callosal projections originate from more or less discrete cortical "columns", a continuous distribution of callosally projecting neurons is found in development. Apparently, in all areas and species where the development of callosal connections was studied, transient projections could be demonstrated. One interesting exception to this rule may be area 17 of the monkey, which either never projects into the corpus callosum (Dehay et al. 1988b) or does so but not as much as do the other areas (Chalupa et al. 1989), although the callosal projections from area 18 undergo the usual phase of exuberance. Unfortunately, at the moment, this negative finding is supported by the study of only three age points, embryonic days (E) 112, 114 and 133. The development of callosal connections has not yet been studied between E 133 and E 165, when exuberant callosal connections can be expected. Projections from area 17 seems to be missing at the time when the adjacent area 18 has already established them, but nearby cortical areas can establish callosal projections with remarkable time lags (Floeter and Jones 1985). In addition, the possibility that the transient callosal projections from area 17

may either fail to extend far beyond the midline or reach areas other than 17 and/or 18 was not explored. Neither possibility is unlikely, since the same transient projections can grow different distances in different species (O'Leary and Stanfield 1986), and exuberant callosal projections to heterotopic areas have been demonstrated (Innocenti and Caminiti 1980; Innocenti and Clarke 1984a, b).

Transient intrahemispheric projections were also discovered in the cat (Innocenti and Clarke 1984a; Dehay et al. 1984; Price and Blakemore 1985a, b; Clarke and Innocenti 1986; Price and Zumbroich 1989). Two of these projections have been particularly well characterized. One runs from the auditory to the visual cortex (Innocenti and Clarke 1984a, b; Innocenti et al. 1988; Dehay et al. 1988a), i.e. two areas between which only weak projections (Innocenti et al. 1988) exist in the adult. The other one is from area 17 to area 18 (Price and Blakemore 1985a, b). Like the auditory callosal projections mentioned above, this projection originates from discrete, vertically oriented modules in the adult, and from a tangentially continuous neuronal zone in the neonate. Shorter-range intra-areal or inter-areal transient projections have also been described in areas 17 (Luhmann et al. 1986; Katz and Wiesel 1987) and 18 (Price 1986) of the cat.

Transient corticosubcortical, and not only transient corticocortical projections, are formed in development (Distel and Hollander 1980). They include projections to the spinal cord (D'Amato and Hicks 1978; Distel and Hollander 1980; Stanfield et al. 1982; Bates and Killackey 1984; O'Leary and Stanfield 1986; Joosten and Van Eden 1989), the superior colliculus (Tsumoto et al. 1983; Thong and Dreher 1986), the cerebellum (Distel and Hollander 1980; Tolbert and Panneton 1983), the pons (Adams et al. 1983; Mihailoff et al. 1984), the nucleus ruber (Leonard and Goldberg 1987), the trigeminal nuclei (Tolbert et al. 1984) and other structures in the medulla (Iriki et al. 1988).

In summary, the selective connectivity between one area, or part of one area, and other cortical or subcortical centers, as well as the mapping rules of the projection, including their columnar origin, differ in the juvenile and adult brain.

Till recently, the extravagance of exuberant projections established by cortical neurons contrasted with the seemingly parsimonious development of thalamocortical connectivity (Payne et al. 1988a, b). In addition to the lack of segregation of geniculocortical afferents, mentioned above, only a moderate degree of laminar exuberance, a transient projection to layer I, was described (Kato et al. 1984; 1986). Lately, however, long transient thalamocortical projections have also been found (Bruce and Stein 1988; Minciacchi and Granato 1989). Indeed, the number of transient projections discovered in the developing brain increases at a fast rate and their comprehensive list is bound soon to become obsolete.

One may ask if the fact that a projection is visualized by pathway tracing experiments in the young animal, but not in the adult, provides sufficient evidence that the projection is eliminated in development. Given that a tracer may not have the same uptake and transport efficiency in the young and in the adult, the elimination should preferentially be corroborated by: (a) multiple tracers or experimental approaches other than axonal transport, for example, estimates of number of axons and (b) the stabilization of the transient projection into adulthood by experimental manipulation, but under conditions in which "de novo" growth of

projections induced by the manipulation can be excluded. Only a few of the transient projections reported thus far pass both criteria, namely the callosal and intrahemispheric projections to areas 17 and 18, the corticospinal projection from the visual cortex and the corticocerebellar projection from the frontal and somatosensory cortex (see Sects. 5 and 6), although other projections pass the first criterion.

Conversely, the failure to demonstrate transient projections in the development of a given structure does not necessarily preclude its existence. Some of the factors which can lead to false negative results were discussed elsewhere (Berbel and Innocenti 1988).

3.2 The Radial Origin of Juvenile Cortical Projections

By and large, juvenile cortical projections originate from layers which will also give rise to the same kind of projection in the adult. Thus, in kittens, as in adult cats, the bulk of callosal projections from areas 17 and 18 originates from layers III and upper IV, with a few from layer VI. Layers II and V do not contribute substantial amounts of callosal projections, neither in the adult nor in kittens (Innocenti 1980; Caminiti and Innocenti 1980; Segraves and Innocenti 1985). Even more striking is the differential radial distribution of supragranular neurons with different targets in the auditory areas, including neurons with different transitory targets (Innocenti and Clarke 1984a; Dehay et al. 1988a). Similarly, the transient projections to the cerebellum (Tolbert and Panneton 1983), spinal cord (Stanfield and O'Leary 1985b) and tectum (Tsumoto et al. 1983) originate from layer V, which is also the main source of cortico-mesencephalic and cortico-pontine projections in the adult.

This is not to say that no changes occur in the relative contributions of different layers to a given projection in development. On the contrary, the interhemispheric projection from the posteromedial lateral suprasylvian cortex to areas 17 and 18 originates nearly exclusively from layer VI in adults (Segraves and Innocenti 1985) but also from layer III in the kitten (Innocenti and Clarke 1984b). Similarly, in the rat parietal cortex, the earliest callosal neurons are in layers Va and Vc-VIa. Later, the projection from layer III appears and the density of callosally projecting neurons greatly decreases in Vc and VIa (Ivy and Killackey 1981). Selective loss of projections from supragranular layers, but maintenance of the projection from infragranular layers was described in area 17 of the rat (Olavarria and Van Sluyters 1985).

The radial changes described above could just be another facet of the type of exuberance described in the preceding section. In the cat, subsets of layer III and layer VI neurons distributed throughout the cortex appear to be determined to send an axon through the corpus callosum. At a later stage, in certain areas, for example in most of area 17, neurons of both layers eliminate their callosal axon; in other areas and projections, e.g. the projection from suprasylvian areas to area 17, only neurons in layer III do so, and this shifts the origin of the projection to infragranular layers (Innocenti and Clarke 1984b). In addition, changes in the radial origin of a projection may be due to the fact that some neurons send an axon through the

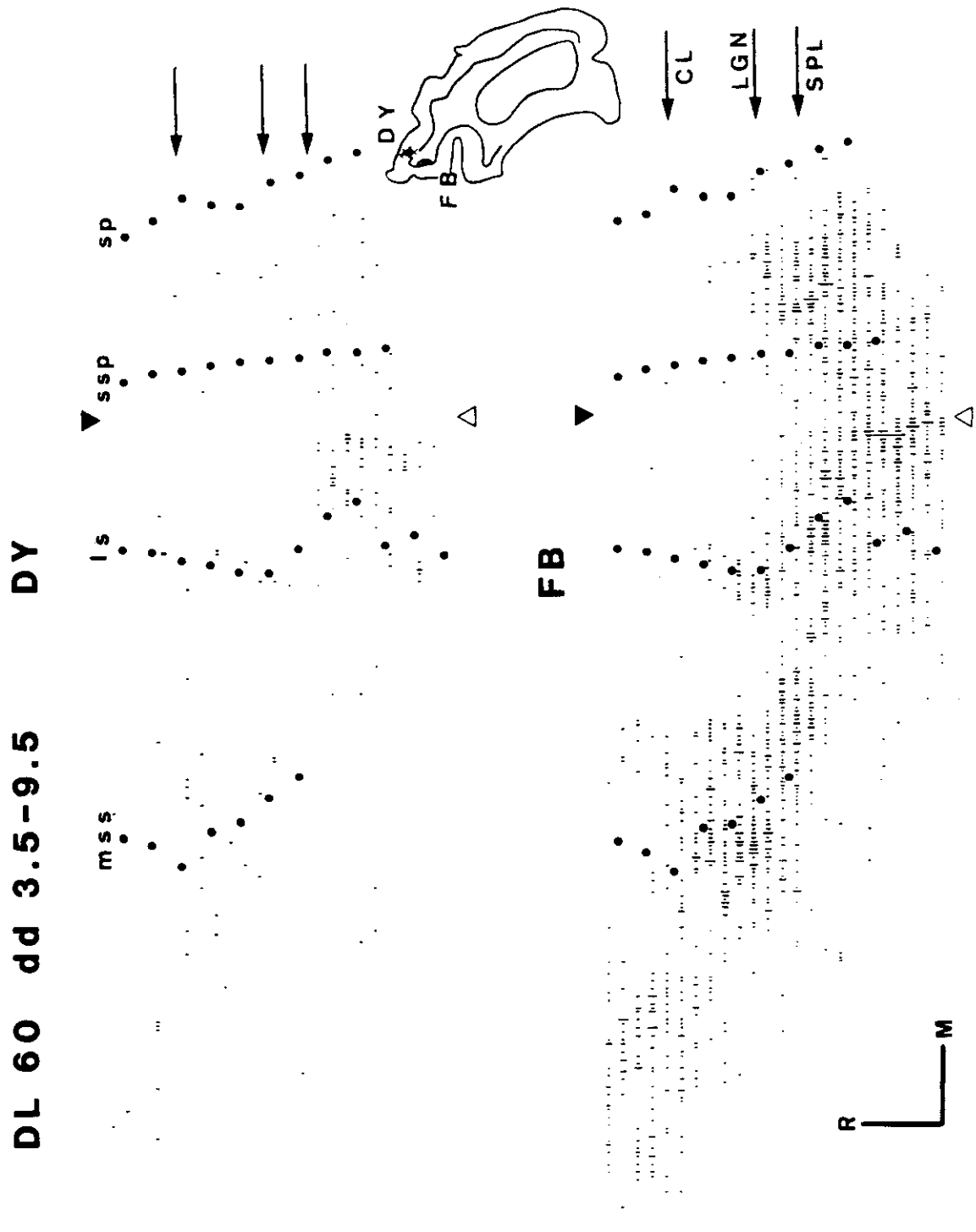
corpus callosum before they have reached their final radial position (Schwartz and Goldman-Rakic 1986), and that different layers establish projections at different times (Ivy and Killackey 1981).

As we discussed elsewhere (Innocenti and Clarke 1984b; for similar concepts see Rakic 1988) it appears that where a neuron sends its axon is determined rather early in development and is related to a neuron's radial position in the cortex.

This initial determination may condition pathway choice rather than target choice; the fact that axons reach a given target may be the consequence of having chosen a given pathway. The cortical white matter is organized in compartments related to the origin and termination of cortical projections, and individual tracts are topographically organized (Innocenti et al. 1983b; Nelson and Le Vay 1985; Nieuwenhuys et al. 1988; inter alios). There is also evidence that juvenile axons, including those which will be eliminated, travel in specific compartments in the white or gray matter (Distel and Holländer 1980; Katz andWiesel 1987; Innocenti et al. 1988).

It is well known to general embryologists that a cell position within the embryo can determine aspects of its differentiation, which may indicate that a cell's fate depends on "positional information" (Wolpert 1969). It appears improbable that positional information determines a neuron in a certain layer to send an axon to a given target/pathway. Firstly, neurons with identical radial position in the cortex are found in the adult to project to different targets, and neurons in different layers can project to the same target (Segraves and Innocenti 1985; Innocenti et al. 1986; inter alios). Secondly, neurons in the frontal cortex of the monkey appear to send an axon into a given pathway, namely the corpus callosum, before they have acquired their final radial position (Schwartz and Goldman-Rakic 1986). Thirdly, in the reeler mouse (Caviness 1977), and after X-irradiation or treatment with cytotoxic drugs (Jones et al. 1982; Jensen and Killackey 1984; Innocenti and Berbel 1989a, b) a neuron's radial position, but not its target/pathway choice, can be profoundly altered, although the usual relation seems to exist between the latter and the neuron's birthdate. Experiments aimed at testing directly the hypothesis that a neuron's birthdate also determines its pathway/target choice by etherochronic transplantation have given results compatible with this notion (McConnell 1988). Nevertheless, birthdate does not fully predict where a neuron sends its axon. The projection to a given structure can originate from several layers and the same layers also give rise to projections to other structures. For example, callosal neurons in the cat originate from layers VI, II and IV (Innocenti 1986) and these layers are generated at different times (Luskin and Shatz 1985). The callosally projecting neurons are a minority among neurons in each layer (Innocenti 1986).

Possibly, a neuron's clonal origin or serial position in a clone, rather than its birthdate is the crucial element in the initial pathway choice. Indeed, work in invertebrates has demonstrated that the serial position of a neuron in a clone determines or restricts its phenotype (Sulston and Horvitz 1977). On the other hand, neurons which are near each other in a cortical layer do not necessarily derive from the same germinal cell (Walsh and Cepko 1988; but see Luskin et al. 1988) but may owe their proximity to sideways displacement during migration along the fasciculating and defasciculating adjacent glial channels shown by Gadisseux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et al. (1987). It is possible that clonal heterogeneity and intermixing plays a major role in determining the differential axonal growth of adjacent neurons in the cortex.

3.3 The Tangential Organization of the Juvenile Projections

A third characteristic feature of the juvenile projections is that they are neither totally diffuse nor random. One aspect of this is that although each area projects to and receives from a broader territory than in the adult, certain projections fail to form. A surprising absence is that of a projection from visual to auditory areas in kittens at ages when the auditory to visual projection and other transient projections are well developed (Innocenti and Clarke 1984a, b). Equally surprising is the apparent absence of transient projections from area 17 in the newborn monkey (Dehay et al. 1988b) already discussed above.

A second aspect of the tangential, juvenile organization is that in young kittens, an injection in areas 17 and 18 and involving the subcortical white matter in order to reach transient corticocortical axons, labels a characteristically shaped territory in the contralateral hemisphere (Innocenti and Clarke 1984a, b; Figs. 1, 2). This territory includes regions which project to areas 17 and 18 in the adult, as well as regions which lose this projection during maturation. The labeled territory is elongated, its greater extension running roughly mediolaterally. Its anteroposterior location changes systematically with the position of the injection site. The shape and location of this territory may reflect principles of axonal order in the callosal pathway and the direction of axonal growth. The anteroposterior cortical axis is

◀ **Fig. 1.** Flat reconstructions of the occipital portion of the left hemisphere of a kitten injected on postnatal day 3.5 (P 3.5) and killed on P 9.5, showing distribution of layers II–VI callosal neurons retrogradely labeled by diamidino yellow (*DY, top*) or by fast blue (*FB, bottom*). Injection sites are shown on the section drawing. In each coronal section the labeled neurons were plotted with a computer microscope and projected onto a line running 400 μm below the pial surface. The line was divided into 100-μm segments and the number of neurons per segment represented by vertical lines whose lengths are proportional to the number of neurons (shortest line, one neuron). *Filled* and *empty triangles* denote the axis along which coronal sections were aligned. *Arrows* point to coronal levels through the caudal end of the lateral geniculate nucleus (*LGN*), splenium of the corpus callosum (*SPL*) and coronal end of the claustrum (*CL*). *Dots*, sulci: *mss*, middle suprasylvian sulcus; *ls*, lateral sulcus; *ssp*, suprasplenial sulcus; *sp*, splenial sulcus. Calibrations are 2 mm. *R*, rostral; *M*, medial; *DL60*, code number; *dd*, age in days. Note that the *DY* injection was restricted to the gray matter near the 17/18 border and labeled neurons almost exclusively in a cluster near *ls*, corresponding to the contralateral 17/18 border which projects into the corpus callosum in the adult; only a few neurons were labeled in medial area 17 (between *ssp* and *sp*) or in the auditory cortex (lateral to *mss*), which do not project to contralateral areas 17 and 18 in the adult. In contrast, the *FB* injection extended into the white matter under area 17 and labeled neurons at the 17/18 border, but also in the two regions which do not project to contralateral areas 17 and 18 in the adult. (From Innocenti and Clarke 1984b)

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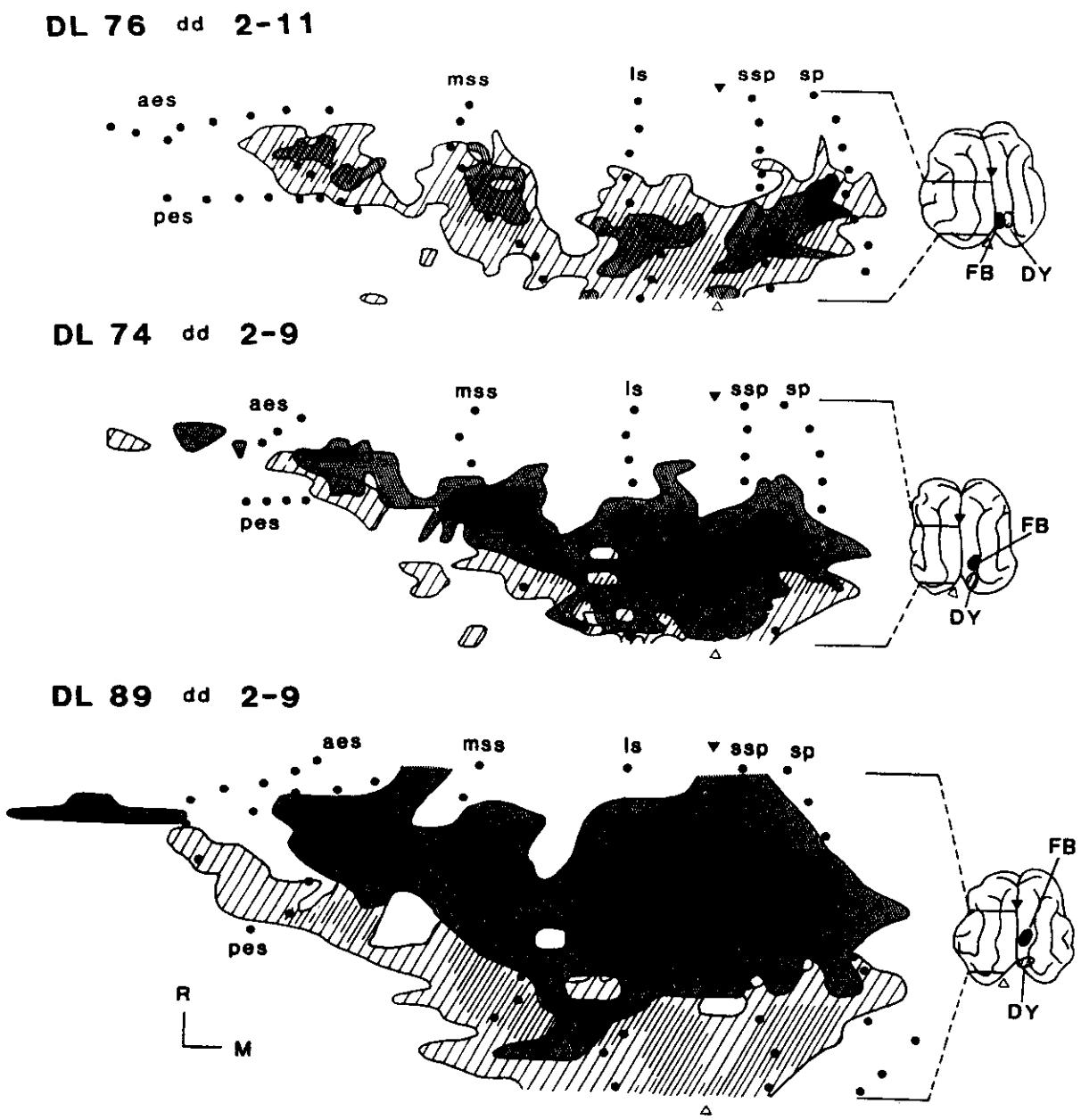

Fig. 2. Flat reconstructions of the occipital portion of the left hemisphere of three kittens (identified by code number and by age at injection and at death), showing regions containing layers II–VI callosal neurons retrogradely labeled by DY (*hatching*) or by FB (*shading*). The region reconstructed is that between *bars* on brain insets. Unlike in Fig. 1, these reconstructions are based on hand-drawn sketches of cell distributions. This procedure is less precise. Therefore, only two arbitrary levels in density of labeled neurons are indicated, i.e. high density (*dark* shading or hatching) and low density (*light* shading or hatching). Locations of injection sites are marked on brain insets. *Dots*, sulci: *aes*, anterior ectosylvian sulcus; *pes*, posterior ectosylvian sulcus; *R*, rostral; *M*, medial. Calibration bars are 2 mm. Notice that the labeled territories progressively separate, proportionally to the distance between injection sites. (From Innocenti and Clarke 1984b)

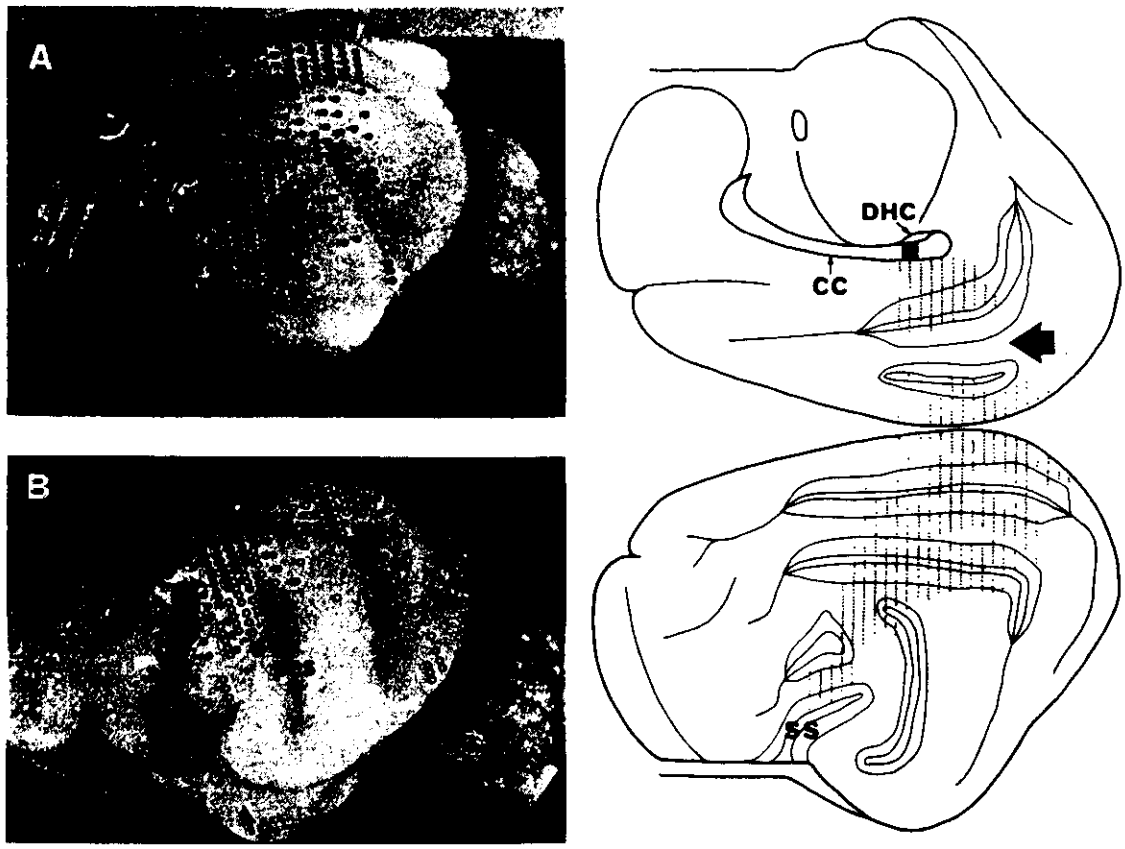

Fig. 3 A, B. *Left*, lateral view photographs of two kitten brains showing the distributions of neurons retrogradely labeled by *FB* (*filled dots*) or *DY* (*open dots*), from injections in contralateral areas 16 and 18 and the underlying white matter. *Above*, kitten DL 39, injected on P 2.5, killed on P 9.5; *below*, kitten DL 74, also shown in Fig. 2, injected on P2 killed on P9. In each photograph, *arrows* mark the rostral and caudal limits of the reconstructed regions. *Dots* are arrayed parallel to the plane of sectioning; their density along an array gives a rough indication of the density of labeled neurons. In the lower example, symbols for *FB* and *DY* labeled neurons from the same section are staggered. Sulci are abbreviated as in Figs. 1 and 2. (From Innocenti and Clarke 1984a). *Right*, reconstructions showing the distributions of neurons retrogradely labeled from applications (*in black*) of horseradish peroxidase to the presplenial part of the body of the corpus callosum (*CC*) of an adult cat. The bottom drawing shows a lateral view of the hemisphere, the top drawing an inverted, medial view; some sulci are open. *DHC*, dorsal hippocampal commissure; *SS*, sylvian sulcus. (From Nakamura and Kanaseki 1989). Notice the similarity in the shape and location of the labeled territories in the two types of experiments. However, no projections from medial area 17 (*arrow*) are labeled in the adult

mapped anteroposteriorly in the adult corpus callosum (Innocenti 1980; Pandya and Seltzer 1986), and such a topographical order seems to appear early in development (Olavarria and Van Sluyters 1986). In a recent study in which the topographic origin of callosal axons was investigated by discrete applications of HRP to the corpus callosum in adult cats (Nakamura and Kanaseki 1989), applications of the tracer to the presplenium retrogradely labeled a territory whose

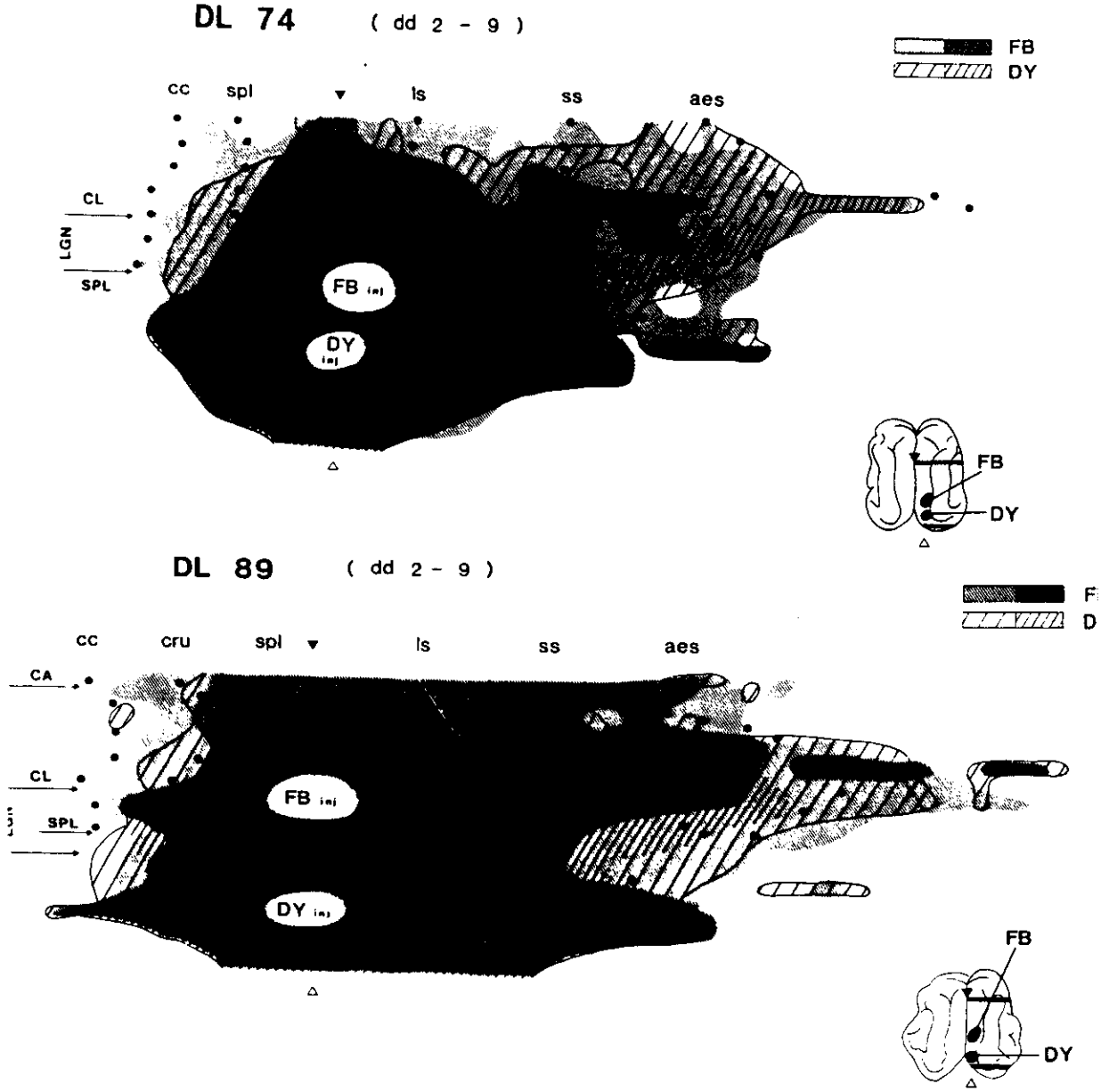

Fig. 4. Flat reconstructions of the occipital portions of the right hemispheres of two kittens, showing regions containing layers II–VI association neurons retrogradely labeled by DY (*hatching*) or FB (*shading*). The region reconstructed is that between *bars* in brain insets. All conventions as in Fig. 2, where the distribution of retrogradely labeled callosal neurons in these two kittens are also shown. Note (compare with Fig. 2) the broader rostrocaudal distribution of labeled association neurons than of callosal neurons. (From Clarke and Innocenti 1986)

location and extent were amazingly similar to that labeled by area 17 and 18 injections in the kitten (Fig. 3). As discussed earlier (Clarke and Innocenti 1986), the fact that intrahemispheric juvenile projections are less clearly topographically organized (Fig. 4) could reflect the lack of a preferential axis of growth of intrahemispheric projections.

To what extent order in the pathway is critical for the establishment of the projection is unclear. As in the case of the retinotectal projection, errors due to disordered growth may be corrected to some extent by redirection of axonal growth near the target (Thanos and Bonhoeffer 1986). Evidence that in partially "acallosal" mice the topography of callosal connections is close to normal but the trajectory of the axons may be abnormal and the projection is reduced (Olavarria et al. 1988b) does not clarify the issue in the absence of more detailed knowledge of the development of the agenesis, and the nature of the postulated disorder in axonal growth.

Some degree of topographical order, corresponding to the mediolateral cortical direction, was documented for the transient corticocerebellar projection (Tolbert 1989). The interpretation given was that this order matches the somatotopy of SI, from where part of the projection originates, to that of the vermis and paramedian lobule where part of the projection terminates. Nevertheless, the order found in this transient projection may also reflect the influence of factors ordering cortico-cerebellar axons along their pathway.

What may be responsible for the order along the pathway is unknown. In principle, axoaxonal interaction may suffice, although the growth of the first axons in the pathway and the subsequent addition of axons may not be unconstrained. Cell adhesion molecules may be involved (Edelman 1987), since in other systems the antibodies against these molecules cause loss of axoaxonal contacts and disorderly growth (Thanos et al. 1984).

3.4 Relations Between Juvenile Cortical Axons and Their Targets

Studies on several systems including neocortex suggest that "in vivo" axonal growth is not a continuous phenomenon but that it rather proceeds in spurts separated by pauses.

In the development of corticopetal afferents, one of the last pauses occurs at the bottom of the gray matter, where ingrowing axons appear to "rest" in a special compartment, usually referred to as the "subplate" (Rakic 1977; Lund and Mustari 1977; Wise et al. 1977; Wise and Jones 1978; Killackey and Bedford 1979; Shatz and Luskin 1986). This compartment contains transient neurons (Marin-Padilla 1978; Kostovic and Rakic 1980; Chun et al. 1987), descending dendrites of layer VI neurons (Marin-Padilla 1971, 1978), and astrocytes (Innocenti and Berbel 1990a) and is traversed by radial glia and migrating neurons. Whether any of these components causes or allows ingrowing axons to stop is presently unknown, although the transient neurons appear to receive transient synapses (Chun et al.1987). The pause of the geniculocortical axons in the subplate may be terminated by the positioning and possibly maturation of layer IV neurons, the main recipient of thalamocortical projections (Shatz and Luskin 1986). Thus, the waiting may be caused by the fact that the arrival of axons and the maturation of their target are not synchronous. In truth, what axons do during this waiting phase is unknown and one can think of additional or alternative roles for it.

Corticocortical axons, in particular callosal axons, were also found to pause in the subplate (Wise and Jones 1976; Goldman-Rakic 1982; Price and Zumbroich 1989),

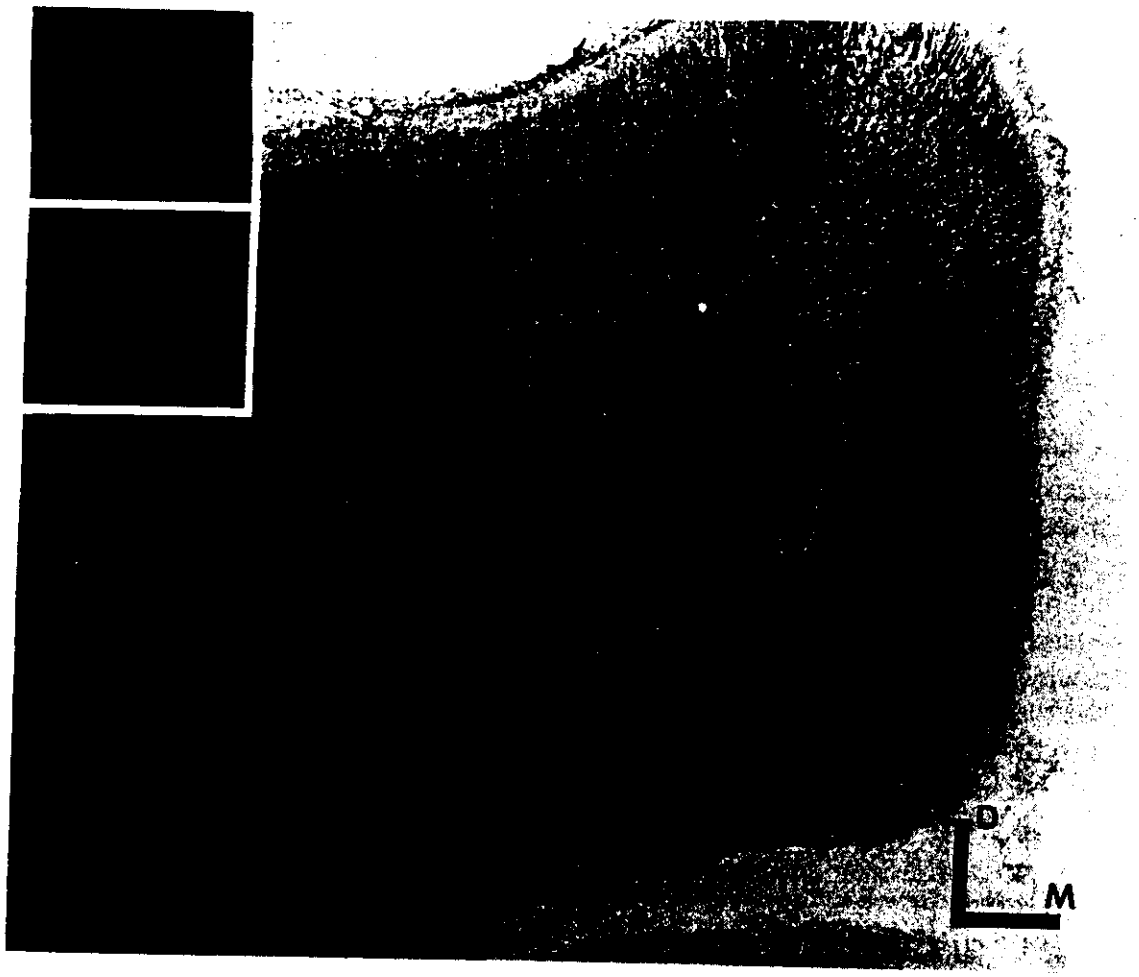

Fig. 5. Photomicrograph of a tetramethylbenzidine reacted section from the hemisphere contralateral to a wheat germ–horseradish peroxidase injection in a kitten injected on P1.5, killed on P2.5. Notice the continuous distribution of labeled neurons and the bundle of axons (from the corpus callosum), fanning out in the white matter. In the *upper right* portion of the section, faintly labeled axons have entered the gray matter in the region of the area 17/18 border. Other axons are directed to the gray matter of area 17 but without entering it. High-power photomicrographs of two of these axons from an adjacent section reacted with a modification of Hanker–Yates' technique are shown in the *inset*, oriented as in the full section. Calibration bars are 0.5 mm for the low-power and 10 mm for the high-power photomicrographs. *D*, dorsal; *M*, medial. (From Innocenti and Clarke 1984b)

although the significance of this pause is even less clear. Characteristically, the waiting phase of corticocortical axons is terminated by growth into specific "columnar" patterns resembling those of the adult (Wise and Jones 1976; Ivy et al. 1979; Innocenti 1981a; Goldman-Rakic 1982; Feng and Brugge 1983; Miller and Vogt 1984; Innocenti and Clarke 1984b; Olavarria and Van Sluyters 1985). Axons appear to enter at specific tangential locations, while they avoid others (Figs. 5, 6). This is clear in the case of callosal projections to the visual areas whose termination bears characteristic, fixed relations to the cytoarchitectonic borders (Innocenti 1981a; Innocenti and Clarke 1984b; Olavarria and Van Sluyters 1985).

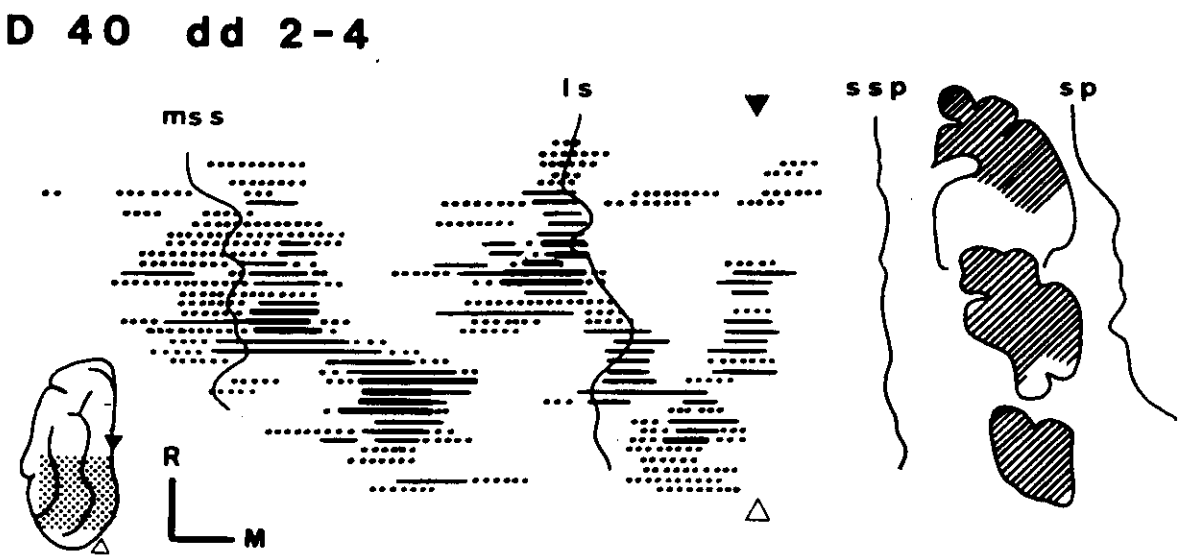

Fig. 6. Flat reconstruction of the occipital portion of the brain of a kitten injected on P2, killed on P4, showing the tangential distribution within the gray matter of terminating callosal axons. The region reconstructed corresponds to the *shaded portion* of the brain inset but it also comprises the cortex within the interhemispheric fissure. *Heavy lines*, heavy labeling; *light lines*, medium labeling; *dots*, light labeling. Outlines of coronal sections show the spread of horseradish peroxidase (*hatched*) at three rostrocaudal levels, in the injected hemisphere. Calibrations are 1 mm. *R*, rostral; *M*, medial. Note, in comparison with Figs. 1 and 2, the selective tangential distribution of terminating callosal axons. (From Innocenti and Clarke 1984b)

Juvenile callosal axons also reach other parts of the visual areas, notably most of area 17 and 18, where they do not terminate in the adult (Innocenti 1981a). At these locations, however, the overwhelming majority of callosal axons remains confined to the subplate or to the bottom of the gray matter (Innocenti 1981a; Innocenti and Clarke 1984b; Olavarria and Van Sluyters 1985).

Most of the callosal axons which enter the gray matter at the sites of adult termination originate from tangential sites destined to maintain the callosal projection (Innocenti and Clarke 1984b; Fig. 1). Most of the axons which remain confined to the white matter come from tangential sites from where callosal projections will be eliminated (Innocenti and Clarke 1984b). A few axons, however, possibly those whose fate depends on visual experience (Lund et al. 1978; Innocenti and Frost 1978, 1979; Cynader et al. 1981; see also Sect. 6.1) may enter the visual cortex and establish synapses.

Thus, callosal projections appear to acquire their adult pattern at their site of termination first and this may involve selection based on mutual recognition between the juvenile axons and some aspects of their targets.

Although the model above is based on the development of callosal projections, its general features may apply to the development of other corticocortical and corticosubcortical projections. Transient projections from auditory to visual areas enter only occasionally the gray matter and when they do, they do not appear to develop terminal arbors in it (Clarke and Innocenti 1986; Innocenti et al. 1988;

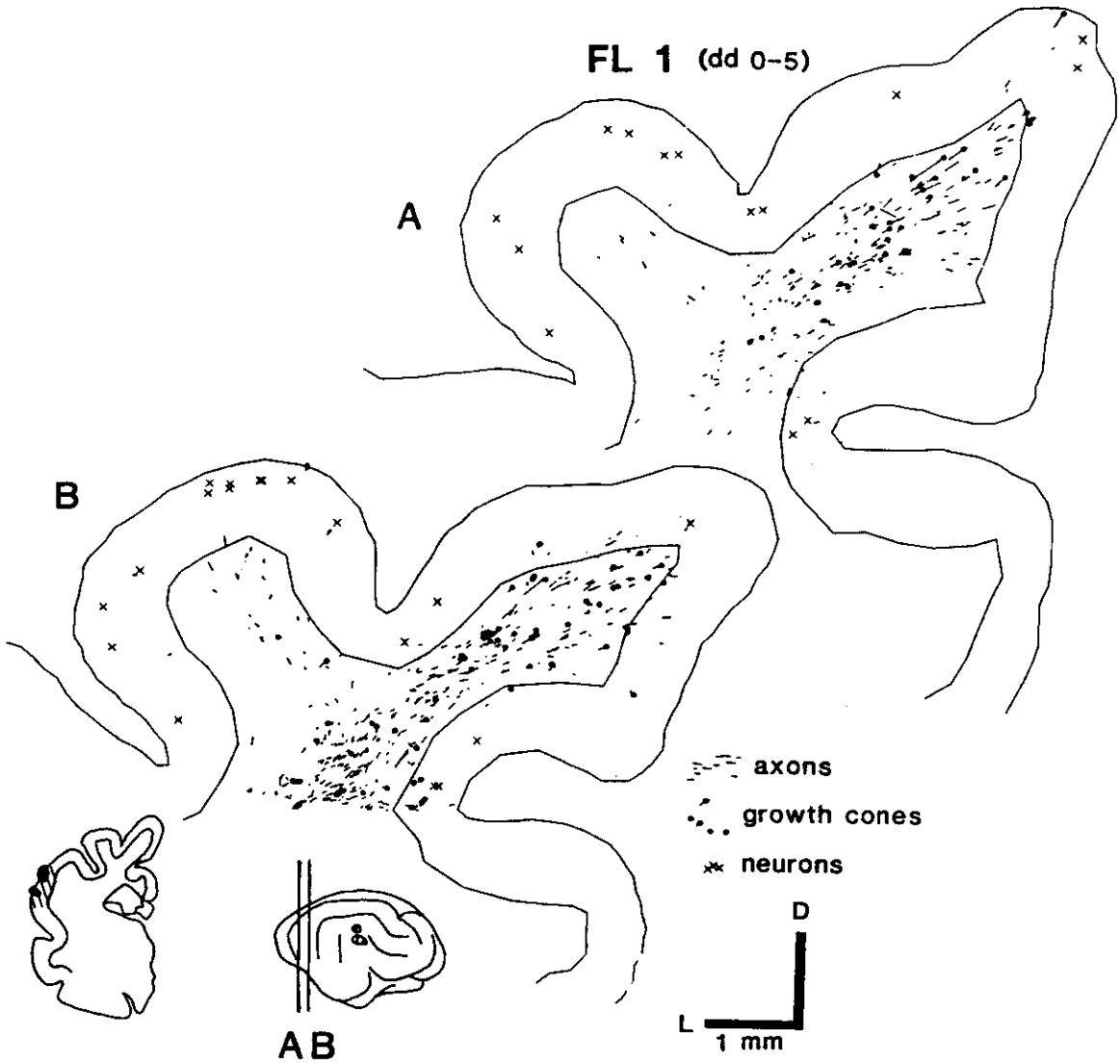

Fig. 7A, B. Computer-aided charts of coronal sections through areas 17 and 18 of the right hemisphere of a kitten, identified by code number and age at injection and sacrifice, showing the distribution of axons (*lines*) and neurons (*crosses*) labeled by a deposit of rhodamine-B-isothiocyanate (RITC) in ipsilateral AI and AII. Growth-cone-like endings are represented by *dots* on one end of the corresponding axon segment. The levels of the charted sections are indicated in the brain inset, together with the locations of the RITC deposits, which are also shown in the section drawing. Note that only a few of the transient axons from auditory cortex enter the visual cortex. (From Clarke and Innocenti 1986)

Fig. 7). Transient intrahemispheric projections between visual areas also fail to grow into the cortex (Price and Zumbroich 1989), and the analysis of axons from intracellularly filled single neurons in area 17 indicated that although these axons may extend tangentially over longer distances than in the adult, their radial growth takes, from the beginning, adult-like patterns (Katz and Wiesel 1987). Thus, transient intrahemispheric projections seem to lack some feature which allows target colonization. The fact that some transient axons do enter the cortex was

stressed by Dehay et al. (1988a). Unfortunately, in their study of the transient auditory to visual projection, large injections of wheat germ agglutinin – horseradish peroxidase involved not only the auditory cortex but also the lateral suprasylvian cortex which is known to send projections to areas 17 and 18 in the adult (Symonds and Rosenquist 1984). The transient corticosubcortical projections do also not appear to enter regions of neurons to any significant extent and/or develop terminal fields in them (Distel and Hollander 1980; Stanfield and O'Leary 1985b). A few transient corticocerebellar axons may enter the deep cerebellar nuclei (Tolbert and Panneton 1983).

It should be stressed that at least in the case of the corticocortical projections, the fact that transient axons do not enter the cortex does not exclude the possibility that they may form synapses, since the subplate contains dendrites and interstitial neurons and the latter bear synapses (Chun et al. 1987).

4 Hypotheses on the Genesis of the Juvenile Organization: Cellular Specificities, Temporal Mismatches, Target-Directed Vs Pathway-Directed Axonal Growth

The evidence discussed above suggests that three factors (or groups of factors) may be crucial in organizing the juvenile projections.

The first factor appears to direct axonal growth into one of the several pathways available to the neuron. This pathway "choice" is probably conditioned by some kind of cellular specificity whose most probable determinant, by exclusion of others, seems to be either the clonal origin of a neuron or its serial position whithin a clone. The nature of the pathways and how they differ from each other cannot be specified. The hypothesis is compatible with the well-demonstrated selective nature of axonal growth, under the control of local cues (reviewed by Weiss 1955; Letourneau 1983; Purves and Lichtman 1985).

The second factor appears to be the topographical order of axons within the pathway. This has been long suspected to be a crucial factor in the establishment of orderly retinotectal projections (Cook and Horder 1977; Rager 1980), although it may not reflect with absolute precision the topological relations between the parent neurons, since individual axons change neighborhood relations along their course (Williams and Rakic 1985).

The third factor seems to be a mismatch between the time axons begin to grow or even arrive at the target and the maturation of either the target itself or of the competence of axons in recognizing the target. This can have two consequences: (a) axons pause near the target before entering it or (b) axons grow past the target and then either retract or grow an axon collateral into it. The first may occur when axons grow perpendicularly with respect to the cortex, the second when they grow tangentially. The second condition may be the cause of much of the exuberance observed in the developing nervous system (exuberance in the "elongation mode" according to Schneider et al. 1987).

This model has a certain number of implications, raises several questions and needs qualifications.

One implication is that that the juvenile connectivity is organized by axon-pathway and possibly ax-axonal interactions, while the adult connectivity is determined by axon-target interactions. As will be discussed below (see Sect. 7), the latter may include specific mechanisms for axon-target recognition as well as axo-axonal interactions and neural activity. These mechanisms do not prevent the occurrence of exuberance of axonal branches and synapses near the sites of axonal termination (exuberance in the "arborization mode" according to Schneider et al. 1987).

Although they may appear superfluous, arguments exist which refuse the possibility that the organization of the juvenile connectivity is directed from distant (i.e. several mm) signals released from the target, as earlier theories suggested (Cajal 1909). The first argument is development exuberance itself, i.e. the fact that axons grow towards brain sites which they will later abandon. Second, as mentioned above, several corticofugal or corticopetal axons begin to grow towards a target often before the target has been generated, has matured or reached its adult position.

The strongest arguments suggesting that axon-target interactions become decisive only after axons have reached their cortical targets, however, come from the fact that the adult organization appears first at the target and seems determined by the selective growth of axons into it. These interactions could be mediated by target-released factors (Heffner et al. 1990).

The main difficulty with this "dualistic" interpretation of the formation of connections is that one needs to postulate an additional mechanism relating pathways to targets in order to explain why axons, by choosing a certain pathway, reach an appropriate target, and, on the other hand, why many do not. Perhaps, an explanation could be sought in phylogenesis. Possibly, up to a certain level of complexity, target structures and pathways leading to them appeared in a one-to-one ratio. They may have carried similar markers, as Sperry proposed (1963). In such an "ideally primitive" brain, axons which chose a certain pathway reached one target only. In evolution, new structures may have appeared in excess to the available pathways. Consequently, a pathway gave access to several targets. In other words, in more evoluted brains, pathway-directed axonal growth uses more general cues than target-directed axonal growth. In evolution, pathway, pathway identification rules and rules for axonal ordering along the pathway may be relatively conserved while the neuronal groups which send into a pathway or receive from it are modified.

The model predicts that both exuberance and the waiting of axons near the target may become more conspicuous in structures which have undergone more drastic evolution. Such a model supports the relative importance that both phenomena seem to have acquired in the development of cerebral cortex, a structure which has undoubtedly undergone massive evolution.

The model neither implies nor excludes the formation of transient synapses. Furthermore, the fact that axonal "waiting" near the target and exuberance may have the same cause does not imply that they also have the same function. In addition to compensating for a time gap between developmental events which

probably proceed independent of each other, axonal exuberance seems to have other "adjusting" functions in neural development (see Sect. 6) and is possibly involved in transient behavior (Iriki et al. 1988). The possibility that both axonal "waiting" and exuberance may have yet other functions must remain an open question.

5 Transition from the Juvenile to the Adult Organization

Two different mechanisms could be responsible for the partial elimination of juvenile projections characteristic of the transition from the juvenile to the adult corticocortical connectivity: neuronal death and selective axonal elimination. The first mechanism cannot be ruled out and indeed cell death seems to occur in the development of cerebral cortex, (Heumann and Leuba 1983; Finlay and Slattery 1983; Ferrer et al. 1989), including death of well-identified neuronal populations (Kostovic and Rakic 1980; Price and Blakemore 1985a; Chun et al. 1987). Neuronal death may be responsible for the elimination of the transient projection from deep layers in area 17 to area 18 (Price and Blakemore 1985a).

Nevertheless, the majority of the transient cortical projections appear to be lost by axonal elimination, in the absence of neuronal death. The experiment on which this concept is based involved labeling the neurons of origin of a juvenile projection which would be partially eliminated with a long-lasting retrograde tracer. The animal was allowed to survive until the projection was partially eliminated and then a different tracer was injected at the same location as the first tracer, or elsewhere. In the first experiment, differential distribution of the neurons labeled by the early and late injection indicated that the juvenile projection had been eliminated but the neurons from which it originated were still alive. After injection of the second tracer some of the neurons which had eliminated the juvenile projection could be double-labeled and this identified the location of their final target (Innocenti 1981a; O'Leary et al. 1981; Ivy and Killackey 1982; Tolbert and Panneton 1984; Price and Blakemore 1985a; see also below).

A second experiment was used to investigate whether a systematic relation exists between the site to which a neuron sends its transient axon and the target of its permanent axon. In this respect, supragranular and infragranular neurons seem to behave differently.

In the cat, supragranular neurons in visual area 17, which send a transient axon to contralateral areas 17 and 18 (Innocenti et al. 1986), and neurons in the auditory cortex which send a transient axon to areas 17 and/or 18 in the same or in the contralateral hemisphere (Clarke and Innocenti 1990) were investigated. In each of these populations, neurons were found with late, presumably permanent projections within the area where their cell body is located or to areas nearby, but none appeared to establish permanent projections to more distal areas (Fig. 8). Neurons with transient callosal axon in area SI of the rat and monkey also establish short permanent projections (Ivy and Killackey 1982; Chalupa and Killackey 1989).

In contrast, neurons with transient corticospinal axons from the visual cortex of the rat to the spinal cord, a population of layer V neurons, establish permanent

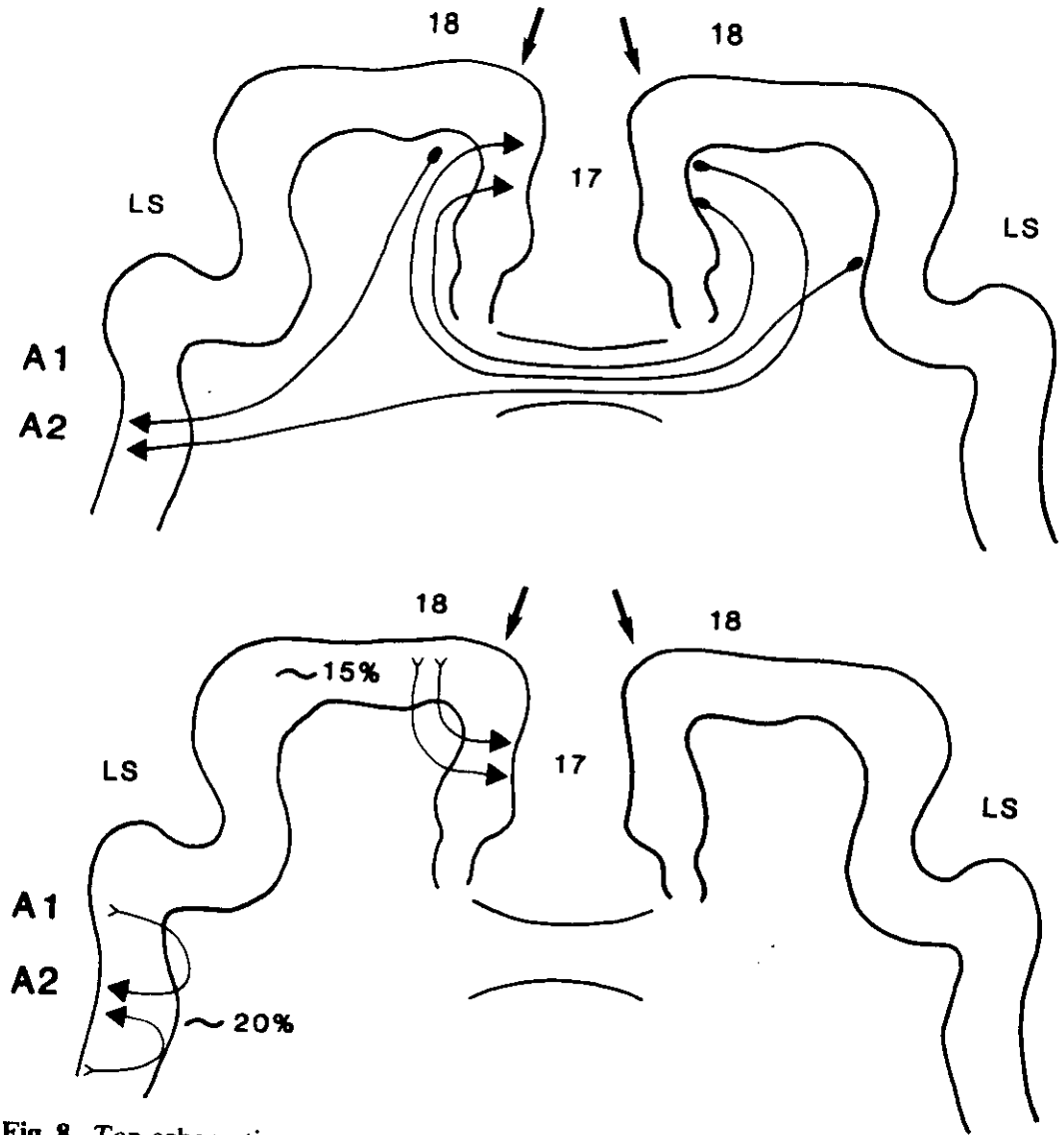

Fig. 8. *Top*, schematic representation of four types of neurons (*filled triangles*), characterized by different locations and site of transient projection. Two of these neurons are in the medial part of area 17 and their axons respectively reach the white matter under contralateral area 17 and the lateral suprasylvian areas (*LS*). Two other neurons are in areas A1 and A2 and their axons respectively reach the white matter under ipsi- and contralateral areas 17–18. *Bottom*, schematic representation of the presumed final projection of the same neurons shown above, after elimination of the transient projections. Notice that they all form relatively short projections. Percent values refer to the fraction of areas 17 and A1, A2 neurons with transient projections for which the local, final projection could be documented

projections to the superior colliculus or to the pons (O'Leary and Stanfield 1985). In the cat, neurons in the frontoparietal cortex with transient projection to the cerebellum, also layer V neurons, establish permanent projections to the spinal cord or brainstem (Tolbert and Panneton 1984).

Thus, the initial overproduction of axoplasm which will subsequently be eliminated seems to be greater for supragranular than infragranular neurons. Interestingly,

though, the total axoplasmic production is probably similar in the two cases, and, in both, the connection to the final target may be through a modest collateral of the transient axon. More important, in all cases the initial transient projection seems to overshoot the final target, although to different degrees for different neuronal populations. Studies with retrogradely transported tracers suggested that at some stage, neurons in supra- and infragranular layers have axonal branches directed to their permanent target, in addition to their transient axon (Ivy and Killackey 1982; O'Leary and Stanfield 1985; Innocenti et al. 1986). Anterograde filling of the axons of both supragranular and infragranular neurons showed that, in both, the permanent axon forms as a side branch of the transient axon and the part distal to it will be deleted (Katz and Wiesel 1987; O'Leary and Terashima 1988). Therefore, the apparent lack of unique relations between sites reached by transient and permanent projections of supragranular and infragranular layer neurons may however be dictated by identical constraints on axonal growth and maintenance.

The emergence of the adult Bauplan of cortical connectivity, outlined above, seems to imply axonal loss in the pathways involved. Indeed, in the cat, during the first postnatal month, precisely at the time when transient callosal projections are being eliminated, the corpus callosum loses at least 70% of its axons (Berbel and Innocenti 1988). Similarly, the pyramidal tract of the rat eliminates in development at least 50% of its axons (Reh and Kalil 1982); this elimination coincides with that of corticospinal projections as shown with retrograde transport (O'Leary and Stanfield 1986). The decrease in the number of axons in the corpus callosum and in the pyramidal tract directly reflects the elimination of the transient projections and may be caused by the latter. In the monkey, however, the number of callosal axons decreases postnatally (La Mantia and Rakic 1984), while transient callosal projections from somatosensory areas and visual area 18, the only two transient projections thus far studied, are eliminated prenatally (Killackey and Chalupa 1986; Dehay et al. 1988b). The reason for this cross-species difference is not known (for discussion see Berbel and Innocenti 1988). Basically more complete and detailed information on the development of cortical connections in the monkey is needed.

In the cat, the axonal loss in the corpus callosum is so massive that it provokes a decrease in its cross-sectional callosal area (Berbel and Innocenti 1988), already noticed (Innocenti and Caminiti 1980) in a study by Fleischhauer and Schlüter (1970) (Fig. 9). Maximal axonal elimination and the associated decrease in the cross-section of the callosum occur before the onset of myelination and during the fast phase of synaptogenesis in the visual cortex (Fig. 9). These temporal relations between morphogenetic events are interesting because they suggest that the fate of a juvenile callosal axon is decided before its myelination and that it may actually be influenced by the synaptogenesis in its target territory or by the signal(s) which trigger the latter.

The cross-sectional callosal area decreases in man during the last 2 gestational months and the first 2 postnatal months (Clarke et al. 1989). This decrease bears temporal relations to myelination of the corpus callosum (as determined by Yakovlev and Lecours 1967) and to the fast synaptogenesis in the cortex (as determined by Huttenlocher et al. 1982) similar to those observed in the cat (Fig. 10). It is therefore probably also an indicator of the occurrence of axonal elimination.

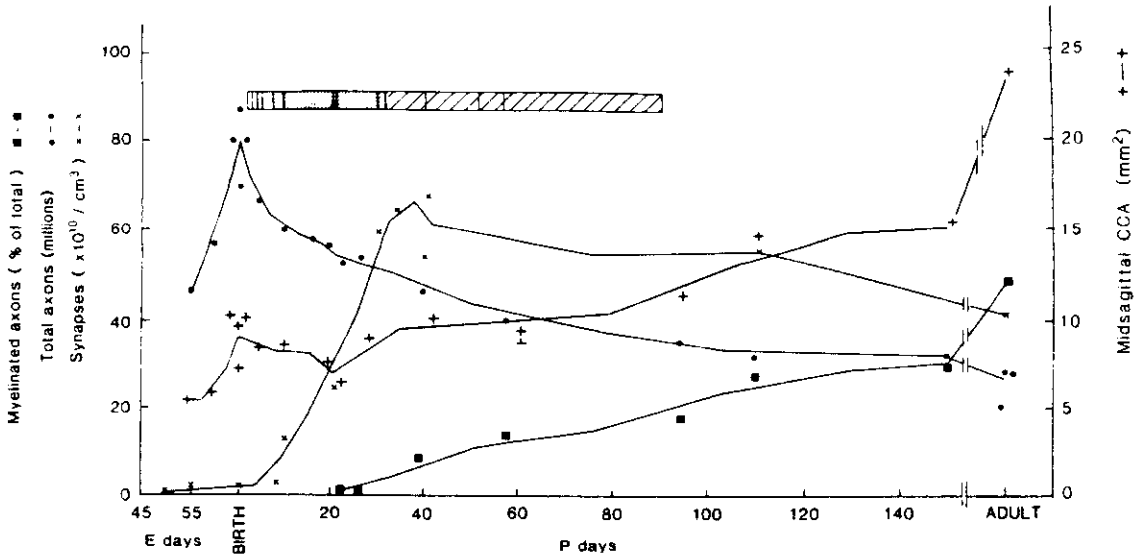

Fig. 9. Temporal relations of different aspects of cortical and callosal development in the cat, i.e., number of axons in the corpus callosum (*dots*), midsagittal cross-sectional callosal area (*CCA, crosses*), myelination of callosal axons (*squares*), density of synapses in the visual cortex (*Xs*, Cragg 1975). The *horizontal rectangle* represents the period of elimination of callosal projections from area 17, demonstrated with retrograde transport by Innocenti and Caminiti (1980). Each *vertical line* in the rectangle indicates the age of one kitten at the time of horseradish peroxidase injection. *Stippling* indicates the period of elimination of the projection, considered to extend from the age of the oldest kitten in which the projection was still fully exuberant to that of the youngest kitten in which the projection seemed to be as in the adult. Two phases are distinguished: the first (*heavy stippling*) corresponds to the bulk of the elimination, from most of area 17; the second (*light stippling*), corresponds to the elimination of projections close to the 17/18 border. Notice that most of the callosal axons and projections are eliminated before the onset of myelination. This massive elimination coincides with a pause in the growth of CCA and occurs during the fast increase in synaptic density. The peak in synaptic density is probably reached around P70, not around P37 as indicated here (Winfield 1981). Thus, the elimination of callosal axons and projections is most conspicuous during the initial 1 third to 1 fourth of synaptogenesis in area 17. (Modified from Berbel and Innocenti 1988)

Biochemical and immunohistochemical analysis of callosal axons during the postnatal period indicated that the three subunits of neurofilaments progressively increase during the first month (Figlewicz et al. 1988). In particular, a monoclonal antibody which recognizes a phosphorylated epitope of the heavy (approx. 200K) subunit shows that only between P11 and P18 have significant numbers of callosal axons acquired this protein, although possibly, until P29, in smaller amounts than in the adult (Figlewicz et al. 1988; Guadano-Ferraz 1990; Fig. 11). Even more interestingly, a second monoclonal antibody, which in the rat recognizes an unphosphorylated epitope of the heavy subunit of neurofilaments, begins to show significant axonal labeling only around P39 (Guadano-Ferraz et al. 1990; Fig. 11). The transition between the juvenile and the adult forms of the microtubule-

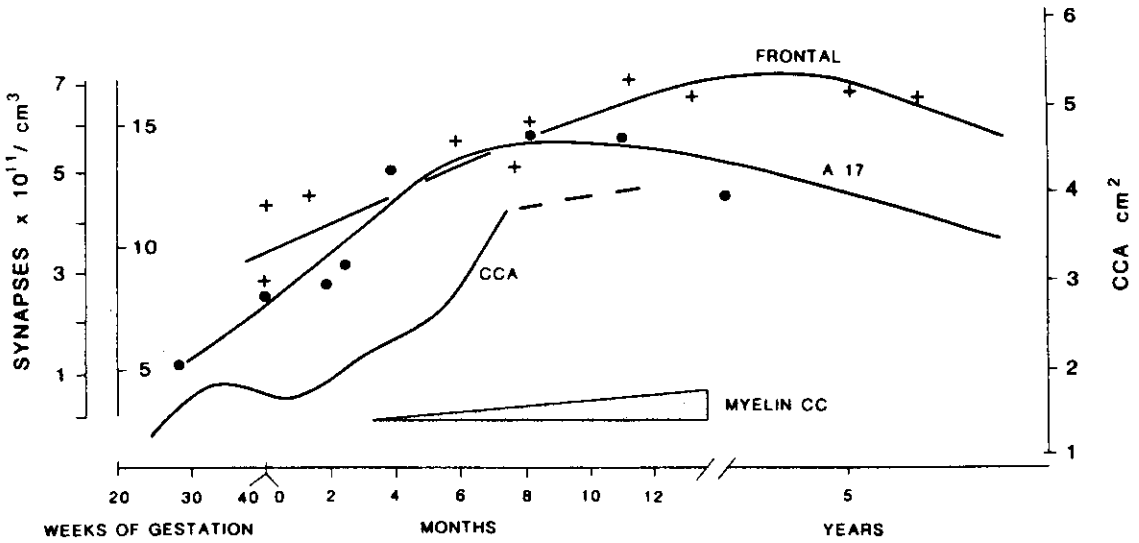

Fig. 10. Temporal relations of different aspects of cortical and callosal development in man, i.e. midsagittal cross-sectional callosal area (*CCA*, Clarke et al. 1989), synaptogenesis in area 17 (*dots*, curve fitted by eye; Huttenlocher et al. 1982) and in frontal cortex (*crosses*, curve fitted by eye; Huttenlocher 1979), and myelination of callosal axons (light microscopic observation of Yakovlev and Lecours 1969). Notice that CCA appears to decrease between the end of gestation and the first postnatal month. This period may correspond to the massive elimination of transient callosal projections and axons in man: as in the cat, most of the suspected elimination occurs before myelination and during the initial phase of fast synaptogenesis in the cortex

associated proteins Tau also occurs between P19 and P28 (Riederer and Innocenti, unpublished; Fig. 11).

These changes in the cytoskeletal proteins of callosal axons occur simultaneously to ultrastructural changes (Berbel et al. 1989). The number of microtubules and neurofilaments per axon increases, although at different rates. The increase in the number of microtubules precedes that in the number of neurofilaments and also slightly precedes the increase in axon diameter to which the number of neurofilaments and/or microtubules is correlated. Changes in the minimal distance between microtubules or neurofilaments are temporally correlated with the changes in microtuble associated proteins and the appearance of the phosphorylated and partially dephosphorylated variants of the heavy subunit.

Thus, the time of axonal elimination, in the corpus callosum, coincides with profound modifications of the cytoskeleton of the remaining callosal axons. It is tempting to speculate that these modifications may correspond to their transition from a juvenile-labile state into an adult-stable state (for a similar concept in the case of synaptogenesis see Changeux and Danchin 1976). This hypothesis requires evidence that the fate of a juvenile callosal axon is not already decided before the elimination begins and also, that the fate of an axon and its cytoskeletal modifications are regulated by the same factors. This evidence exists (see below) since the period of elimination of callosal axons coincides with a "sensitive period" during which various manipulations can either stabilize axons which would

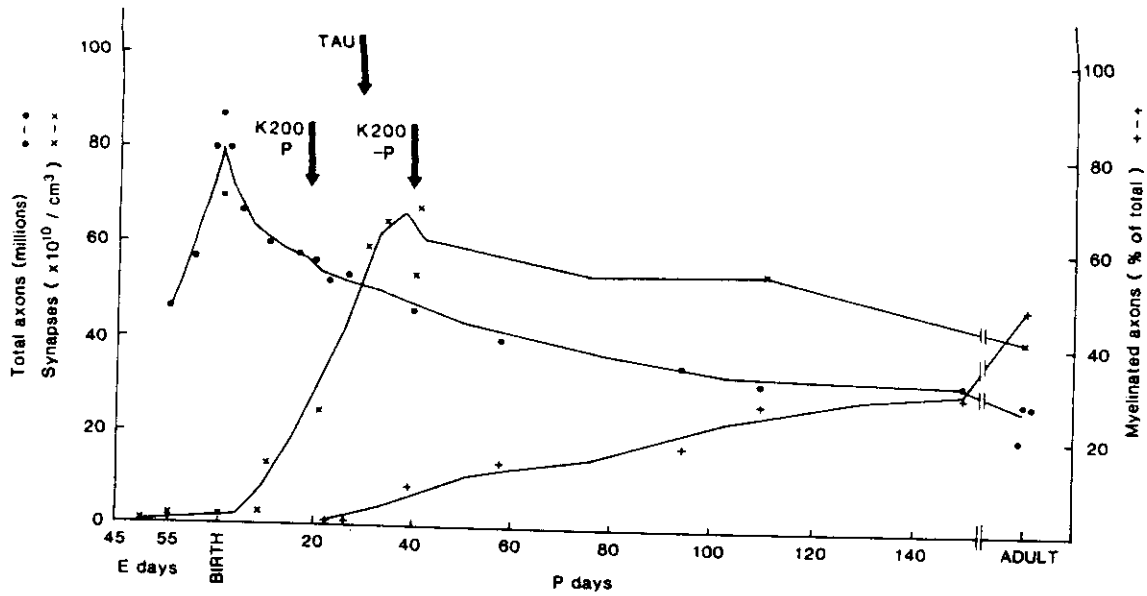

Fig. 11. Temporal relations of morphological and biochemical aspects of cortical and callosal development in the cat. The curves of number and myelination of callosal axons and synaptic density are the same as shown in Fig. 9. *Arrows* point to the first unequivocal appearance of three cytoskeletal proteins in callosal axons: the phosphorylated heavy subunit of neurofilaments (K200 P; Figlewicz et al. 1988), the adult forms of the juvenile microtubule associated proteins Tau (Riederer and Innocenti, unpublished) and a partially dephosphorylated form of the heavy neurofilament subunit (K200-P Guadano-Ferraz et al. 1990)

normally be eliminated or eliminate axons which would normally be maintained. At least one condition, hypothyroidism, appears to interfere both with the elimination of callosal axons and the maturation of their cytoskeleton, in particular the appearance of the heavy neurofilament subunit (Gravel and Hawkes 1990).

Less is known about cellular events underlying the elimination of transient cortical axons. Although often referred to as "axonal retraction", the phenomenon most probably involves axonal degeneration followed by phagocytosis of the degenerating elements. Indeed, at the time of axonal elimination, macrophages appear in the white matter at sites traversed by transient axons of cortical origin. Electronmicroscopically, some of these macrophages appear in the process of phagocytosing axons (Innocenti et al. 1983a, b; Berbel and Innocenti 1988). However, no clear instances of electron opaque degeneration of callosal axons were noticed in a study where they were specifically sought (Berbel and Innocenti 1988). The degeneration may take more subtle forms as suggested by the appearance of vacuoles and swollen mitochondria in some callosal axons at the time of their elimination (Berbel and Innocenti 1988). Alternatively, the degenerating debris may be cleared before they acquire distinctive ultrastructural features.

6 The Regulation of Axonal Survival in the Developing Cortex

Probably, much of the interest evoked by the discovery of the existence of transient projections in the developing cortex derived from the fact that they appeared to reveal an enormous potential for developmental plasticity in this structure. This view, if justified, could provide new tools for understanding brain development as well as the relations between structure and function in the cerebral cortex.

Alternatively, however, the transient projections could be inexorably doomed to elimination, either because they subserve a transient function or because they represent a phylogenetic relict.

This alternative has implications for the difficult question of the teleology of developmental exuberance (Changeux and Danchin 1976; Katz and Lasek 1978; Innocenti 1981b, 1988; Katz 1983; Ebbesson 1984; Cowan et al. 1984 inter alios). If transient projections are inexorably eliminated, then the possibility that transient structures "may allow developmental decisions to be made when the necessary information is not available or not usable in the system" (Innocenti and Clarke 1984b) or, differently phrased, may play the role of an "ontogenetic buffer mechanism" (Katz 1983) could be rejected. Indeed, developmental exuberance may have no function. Its widespread occurrence across species could just indicate that evolution preserved this mode of development as an extravagant but not detrimental way of putting a brain together.

The available evidence is clear-cut. Neither the fate of transient projections, nor that of the projections which are normally stabilized is rigidly predetermined in ontogenesis. Nevertheless, in spite of nearly 10 years of efforts we seem far from understanding what determines this fate and how.

A posteriori, the theoretical basis for most of the work may have been naïve in the sense that we searched for "the" factor responsible for the elimination/stabilization of juvenile projections. The more or less explicit assumptions were that, by appropriate perturbation experiments, as in the case of the formation of ocular dominance columns, one or few factors would be identified and that they would be the same across systems and species. The studies reviewed below weaken both assumptions.

First, the same perturbation experiment has occasionally had different outcomes in different species and systems. Second, we appear to be dealing with several, possibly interacting factors. A more realistic view may be that which juvenile axons are maintained and which are eliminated "emerges" as a property of a network of causal interactions (for the roots of this concept see Weiss 1955), to a large extent determined by the neural network the axons are embedded in, but also by more general signals, for example hormones. Key properties of this network, are that (a) the effects of local perturbations may spread through the network and thus affect distant brain sites and (b) activity may regulate the development of its structure.

This hypothesis does not exclude the possibility that one crucial step, for example, a biochemical modification of cytoskeletal components, may control the transition

from the juvenile to the adult connectivity. But this hypothetical step appears to be regulated by multiple, possibly interdependent factors, some of which at least have been identified. These are:

1. Sensory experience
2. Integrity of the sensory periphery and of the pathways originating from it
3. Factors dependent on the target and/or the afferents it receives.

6.1 Sensory Experience

The first experiments aimed at testing the role of visual experience on the development of corticocortical connectivity were inspired by the fact that callosal connections between the area 17/18 borders in the two hemispheres appear to be involved in establishing anatomical and functional continuity across the vertical meridian of the visual field (Choudhury et al. 1965; Berlucchi et al. 1967; Berlucchi and Rizzolatti 1968; Hubel and Wiesel 1967). In normal vision, the line of decussation of the ipsi and contralateral retinofugal projection, i.e. the line which divides the visual world of the two hemispheres and the geometrical midline of the binocular visual field (the vertical meridian) coincide and are superposed in visual space. The effects of horizontal strabismus were studied, since it shifts the geometrical midline of the binocular visual field onto the nasal or temporal hemiretina, depending on whether the eyes diverge or converge. This new vertical meridian is separated from the decussation line proportionally to the degree of squint. The underlying hypothesis was that synchronous activation of cortical loci in the two hemispheres may be necessary for the stabilization of the juvenile callosal connections and that strabismus allowed synchronous stimulation, although through different eyes, of cortical sites whose callosal connections would normally be eliminated because they "viewed" different parts of the visual field.
These experiments produced moderately enlarged callosally projecting zones in area 17 of the cat (Innocenti and Frost 1978, 1979), and the finding was confirmed (Bermann and Payne 1983; Elberger et al. 1983). Expansion of the callosal terminal territory in the same area was also observed (Lund et al. 1978). However, no systematic relation was found between the degree of expansion of the callosally projecting zone in area 17 and the angle of strabismus induced in the animal. The enlargement of the callosal zone was modest when compared with the wealth of callosal projections at birth, and most of the projection still originated at the 17/18 border. Finally, similar expansion of the callosal zone near the 17/18 border was produced by other rearing conditions such as monocular deprivation or enucleation, binocular enucleation (Innocenti and Frost 1979, 1980) and short periods of normal vision followed by binocular deprivation (Innocenti et al. 1985). Rearing conditions which induce expansion of the callosally projecting zone seem to have in common that they interfere with the normal binocular vision during the 2nd postnatal month (see Innocenti et al. 1985 for discussion). One tentative explanation for these results is that the stabilization of callosal connections requires synchronous activation of the callosal axon and of its target neuron and that this is achieved by proximity or overlap, as well as similar orientation and direction specificity of the

receptive fields of the callosally projecting neurons and of the neurons which receive from them. This mechanism is similar to that proposed for the maturation of binocular properties of cortical neurons (Wiesel 1982). As discussed previously (Innocenti et al. 1985), during the 1st and 2nd postnatal months this mechanism may refine callosal connections by stabilizing certain connections and eliminating others. Abnormal binocular vision interferes with this refinement either because normal binocular vision is required or because it results, at the cortical level, in broader and less sharply orientation tuned receptive fields (Chino et al. 1983). Modifications of callosal connections, broadly related to those described in kittens, may occur in humans. John and Timney (1986) described increased interhemispheric transmission times for targets within 5° of the fixation point, i.e. within a part of the visual field whose cortical representation is callosally connected in most animals thus probably in man.

A decrease in the number of retrogradely labeled callosally projecting neurons in area 17 was obtained in the cat by binocular eyelid suture (Innocenti and Frost 1980; Innocenti et al. 1985; Fig. 12) and by dark rearing (Frost and Moy 1989). This

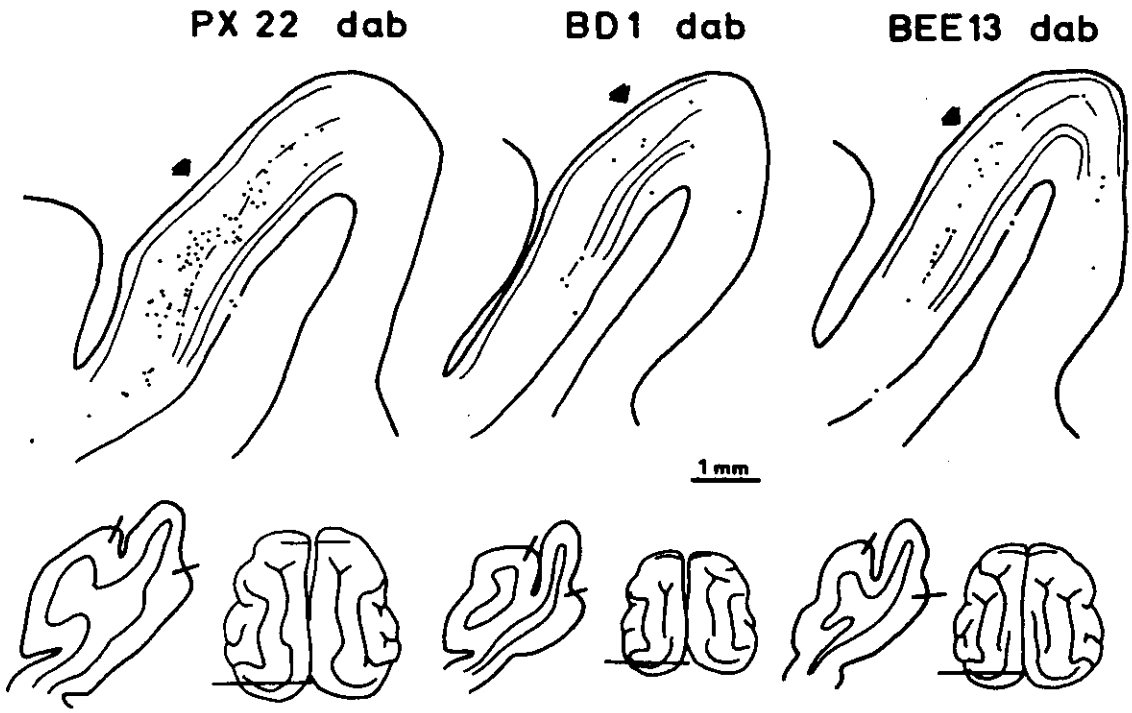

Fig. 12. Computer-microscope charts of the distributions of horseradish-peroxidase (HRP)-labeled callosal neurons in coronal sections at corresponding rostrocaudal levels of the postlateral gyri of a normal adult cat (*PX 22*), a cat (*BD* 1) raised with bilaterally sutured eyelids and a cat (*BEE* 13) which had been bilaterally enucleated on postnatal day 2. All sections were reacted with *DAB*. Each HRP-labeled neuron is represented by one dot. The drawings at the top are enlargements of the regions denoted in the corresponding low-power drawings. The rostrocaudal levels are indicated on drawings made from dorsal view photographs of the corresponding brains. In each section, *thinner lines* mark the lower boundaries of layers I, III, IV, V; a *filled arrow* points to the cytoarchitectonic boundary between areas 17 and 18. (From Innocenti and Frost 1980)

confirmed the role of vision in regulating maintenance or elimination of juvenile projections. However, there were limits to the influence of visual experience: (a) a non-negligible complement of callosally projecting neurons was stabilized in primary and secondary visual areas even under the severest conditions of visual deprivation; (b) these neurons remained centered around the 17/18 border; (c) in some of the experiments, different effects were obtained in supragranular and infragranular layers, the latter being, for example, more resistant to bilateral eyelid suture (Innocenti et al. 1985).

This unequivocally points to the existence of control mechanisms independent of vision, and this conclusion agrees with the recent finding that maturation of callosal connections in primary visual areas may be a largely prenatal event in monkey (Dehay et al. 1988b) and probably man (Clarke et al. 1989).

These control mechanisms are not necessarily independent of activity. Spontaneous activity of retinal or central origin may provide the necessary synchronization in the activity of cortical sites which become callosally connected. Spontaneous retinal activity appears early enough in development for this to be possible (Galli and Maffei 1988).

Visual experience may have a more general role in the development of cortical connections, since binocular deprivation was found to disrupt the pattern of intrinsic connections in area 17 (Luhmann et al. 1986) and may decrease the number of neurons which maintain intrinsic connections in area 18 (Price 1986).

6.2 The Afferent Periphery

Other experiments on the visual system in cats and rodents tested the role of the sensory periphery in the development of callosal and other cortical connections. That the periphery may play a role in organizing callosal connections was strongly suggested by Shatz's observations (1977) that the callosally projecting and receiving zones in area 17 of Siamese cats were expanded compared to normal cats (for a different result, see Tremblay et al. 1987). A systematic relation was found between the abnormality in the cortical mapping due to the abnormal crossing of retinal axons at the chiasm, and the rearrangement of visual callosal connections. At that time, the developmental exuberance of callosal connections was not known and therefore the phenomena leading to the reorganization could not be interpreted. Furthermore, since the Siamese cats are also strabismic, the possibility that the reorganization of their visual callosal connections may be due to visual experience, rather than to the structural changes in the retino-geniculocortical projection, cannot be excluded. Finally, although the results in the Siamese cats clearly pointed to a role of either the periphery or of vision in the organization of callosal connections, the possibility that the same genetic defect which is responsible for abnormal crossing at the chiasm may, independent of the latter, induce abnormalities in either the visual cortex or the corpus callosum could not be ruled out.

The role of afferents from the sensory periphery in the regulation of the selection of juvenile callosal axons could be deduced from experiments on binocularly or

monocularly enucleated kittens (Innocenti and Frost 1979; 1980), mouse, rat and hamster pups (Rhoades and Dellacroce 1980; Rothblat and Hayes 1982; Rhoades and Fish 1983; Olavarria and Van Sluyters 1984) and monkey embryos (Dehay et al. 1989) as well as on anophthalmic mice and rats (Olavarria and Van Sluyters 1984; Olavarria et al. 1988a; Rhoades et al. 1984). All these conditions produced enlargement of the callosally projecting zone (Fig. 13), usually interpreted as stabilization of callosal projections which would otherwise be eliminated, but also more subtle changes in the distribution of callosally projecting neurons (Olavarria et al. 1987). The results of the various experiments differed in certain details. Binocular enucleation in the cat also caused a decrease in the number of retrogradely labeled neurons projecting into the corpus callosum (Innocenti and Frost 1980). This is apparently not the case in rodents and monkey, where, however, no quantification was attempted. In rodents, but probably not in cats (Innocenti and Frost 1979 and unpublished), the callosally projecting zone and the callosal terminal territory increase on the side of the remaining eye but remain normal on the other side (Rhoades and Dellacroce 1980; Rothblat and Hayes 1982; Cusick and Lund 1982; Olavarria et al. 1987). In the monkey, the increase seems to be due exclusively to stabilization of projections coming from area 18, while in the cat projections coming from area 17 were stabilized; they were, however, the only ones studied.

To some extent, these differences may reflect the particular emphasis of the different studies. However, they may also be related to differences in the organization of the visual system of monkeys, cats and rodents and in particular to the different degree of crossing of the optic pathways in these three species. In addition, in rodents but not in cats, the callosal connections contribute significantly to the binocularity of cortical neurons (see Innocenti 1986 for references). Differences in the function of callosal connections and in their mode of development, in particular for the monkey (see Sect. 3.1), could also account for differences in the results of peripheral lesions.

To what extent the concept of a role for the periphery and/or sensory experience in callosal maturation can be generalized is unclear. Auditory callosal connections appear not to be modified by neonatal bilateral destruction of the cochlea (Brugge et al. 1983). Similarly, the development of the transient auditory to visual connections is not significantly modified by bilateral enucleation of the eyes (Innocenti et al. 1988).

In rodents, callosal connections develop before eye opening suggesting that the changes after peripheral lesion may not be due to altered visual experience (see for discussion Rhoades and Fish 1983; Olavarria et al. 1987). In the cat, however, binocular visual deprivation by eyelid suture, a condition which prevents form vision but not the detection of changes in diffuse illumination (Innocenti and Frost 1980; Innocenti et al. 1985), dark rearing (Frost and Moy 1989) and binocular eye enucleation (Innocenti and Frost 1980) led to qualitatively and quantitatively distinguishable abnormalities in the callosal connections, suggesting that integrity of the periphery and vision may play distinct roles. As discussed above these results do not exclude activity of retinal origin as a mechanism.

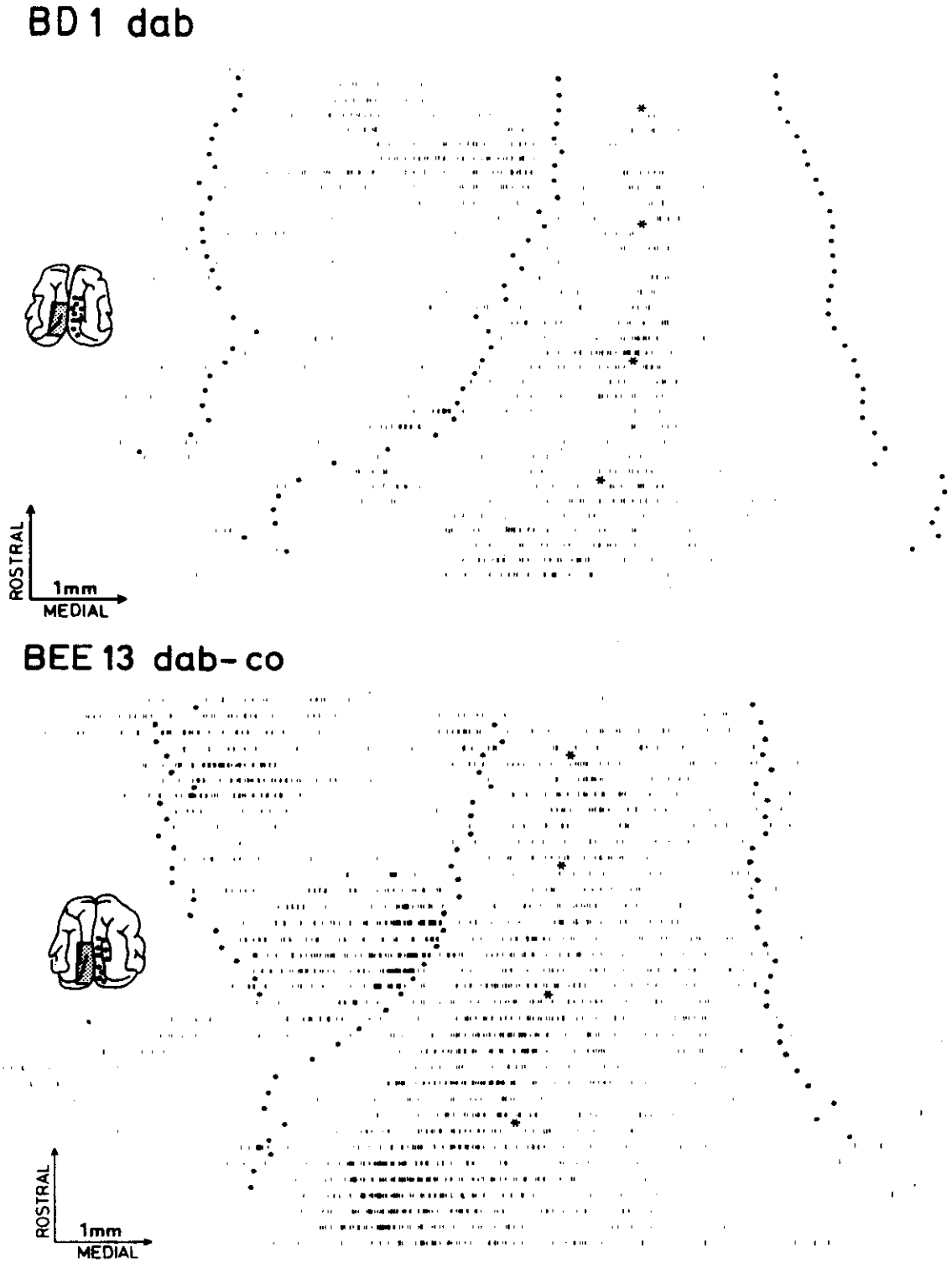

Fig. 13. Computer reconstructions showing labeled callosal neurons in layers II–IV in a cat (*BD* 1) raised with bilaterally sutured eyelids, and in another (*BEE* 13), which had been binocularly enucleated on P2. Brains processed with *DAB*. Flattened representations of postlateral and lateral gyri (corresponding to *stippled area* in the inset). *Dotted lines* indicate, from left to right, fundi of the lateral, postlateral and suprasplenial sulci. The *asterisks* mark the boundary between areas 17 (medially) and 18 (laterally).

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As in the visual deprivation experiments, peripheral lesions resulted in minor changes of callosal connections, compared to their wealth at birth. Since most of the enucleations were performed at birth, one possibility is that callosal connections are too mature to be modified further, i.e. that by birth the fate of most juvenile projections is irreversibly determined. This possibility seems to be ruled out by the finding that the stabilization is no greater in congenitally anophthalmic than in neonatally enucleated mice and rats (Olavarria and Van Sluyters 1984; Olavarria et al. 1988a).

Thus, even higher controls of the developmental selection of juvenile corticocortical axons probably exist. Activity-based controls cannot "a priori" be ruled out. In particular, the synchronization of EEG activity between the hemispheres in the adult appears to be, at least to some extent, independent of callosal connections (Berlucchi 1966; Singer and Creutzfeldt 1969; Susic and Kovacevic 1974). If this is the case in development, a "diffuse" cortical activator located below the cortex could provide sufficient synchronous input to the hemispheres to stabilize a somewhat normal complement of callosal connections. A condition which presumably reduces or eliminates this hypothetical activator as well as other possible controls of thalamic origin is the transection of the thalamic radiation.

This experiment produced different results in different species and systems. In the somatosensory system of the rat the callosal termination to SI was studied on the side of a large thalamic lesion including the ventrobasal complex and was found to be unaffected (Wise and Jones 1978). In contrast, a comparable study in the visual system reported tangential expansion of the callosal terminal territory in 17/18 (Cusick and Lund 1982). In the hamster, transection of the optic radiation also resulted, in the deafferented hemisphere, in expansion of the territory of callosal termination, loss of the callosal projection from supragranular layers and maintenance of the projection from the infragranular layers (Rhoades et al. 1987). This finding seems to rule out the hypothesis that the expansion of the callosal terminal territory found on the side of the remaining eye in the case of monocular enucleation may be due to "abnormal instructions emanating from the thalamus ipsilateral to the remaining eye" (Olavarria et al. 1987). Alternatively, the expansion of the callosal terminal territory in both types of lesion may indicate that the intact thalamus normally sharpens the tangential distribution of callosal axons, either through a competitive interaction or through synchronous firing as proposed above. On the other hand, the suggestion that the "initial development of callosal axon" by supragranular callosal neurons, "may depend on thalamic input" (Rhoades et al. 1987) seems based on a somewhat implausible developmental mechanism whose demonstration requires analysis of the callosal projections at short survival after the lesion in order to rule out the alternative mechanism, i.e. that the callosal projections from the 17/18 border are formed, but not maintained, in the absence of thalamic

◀ **Fig. 13** (*continued*). The neurons were projected onto a line running parallel to the pial surface and 400 μm deep; the line was divided into bins of 50 μm and the number of neurons in each bin represented by a corresponding number of vertical line segments. Each *horizontal row* of line segments represents one section. Sites of injection are represented by *dots* on the inset. (From Innocenti and Frost 1980)

input. Contrary to what was found in the hamster, similar experiments in the cat led to maintenance of the projection from the deafferented to the intact hemisphere, but striking reduction of the projection from the intact to the deafferented hemisphere (Melzer et al. 1987).

Taken at face value, these differences may indicate that the stabilization of a juvenile callosal axon depends in cats more on the thalamic input at the target and in rodents more on the thalamic input at the origin of callosal axons. Unfortunately, the results of neither experiment are easy to interpret since the transection of the optic radiation interrupts not only the geniculocortical projection but also other subcortical afferents to the visual cortex, and it also transects the axons of corticosubcortically projecting neurons. The consequences of these lesions are unknown but may differentially affect the development of callosal connections in the two species.

6.3 The Target

Although the role of the afferent periphery and/or visual experience on the maturation of visual callosal connections appears well established, the mechanism of action is far from being clear. One uncertainty is whether the afferents affect callosal neurons and axons, or their target, or both. The recent work of Stanfield and O'Leary (1985a) showed that occipital cortical neurons, presumably the same which normally establish transient projections to the spinal cord, maintain this projection if grafted in the somatomotor region from where the normal projection to the spinal cord originates. If it could be convincingly shown that the neurons which establish maintained projections to the spinal cord are the same that would have established the transient projection had they remained in the occipital cortex, these experiments would strongly support the hypothesis that the tangential position rather than some other "innate" quality of a cortical neuron may be critical in determining whether its axon will be maintained or eliminated.

A number of experiments tested the possibility that the fate of a juvenile axon may depend on events at its target and in particular on competition with other axons. The task is difficult, since the fact that one projection is maintained when another is experimentally eliminated is probably not sufficiently rigorous evidence that competition occurs in normal development between the two projections (Guillery 1988). A modest stabilization of normally transient callosal axons from the forepaw representation in SI to contralateral SII was obtained when the SI on the same side was neonatally lesioned. Lesion of the remaining SII did not provoke a similar effect nor did it increase the effects of the SI lesion (Caminiti and Innocenti 1981). These experiments suggested that competition between callosal and ipsilateral axons from SI to SII may affect survival of the former. Unfortunately, the possibility that the SI lesion may also have modified the thalamic input to SII and that this might have caused the abnormal stabilization of the callosal projection could not be ruled out.

Stabilization of the transient cortical projection to the deep cerebelar nuclei was obtained by neonatal lesions of the cerebellar cortex (Panneton 1986), suggesting

that competition with the axons of the Purkinje cells may normally be involved in the elimination. Unfortunately, as in the case of SI lesion, the possibility of more indirect effects secondary to modifications of other inputs to the cerebellar nuclei or even unspecific trophic effects of the lesion (see below) could not be ruled out. The fact that crossed corticothalamic and corticorubral projections are maintained into adulthood in cats with neonatal unilateral cortical lesion (Leonard and Goldberg 1987) may also be interpreted as evidence that competition plays a role in the normal elimination of this transient projection.

Intriguing evidence of activity-driven competition between visual callosal projections and thalamocortical projections or the neurons on which the latter impinge was reported by Cynader et al. (1981), who raised monocularly deprived, split chiasm kittens and reported loss of callosal inputs and probably of projections originating in the hemisphere receiving from the deprived eye.

The studies above illustrate the characteristic difficulty of interpreting perturbation experiments in complex systems with a network structure. Because it cannot be excluded a priori that the local disturbance may have remote and cascading effects elsewhere in the network, the network must be fully characterized both in its final structure and in its development. Furthermore, since the function of the network can condition its structure information about the latter is also necessary.

An attempt in this direction was made in a recent series of experiments (Innocenti et al. 1987; Innocenti and Berbel 1989a, b; Assal et al. 1989) whose initial motivation was to study whether trophic dependence of transient axons on their transient target existed, by means of the early destruction of the latter. Areas 17 and 18 of newborn kittens were injected with ibotenic acid, an "axon-sparing" excitotoxin which binds to aspartate receptors. Unexpectedly, these neonatal injections did not provoke complete destruction of the cortex but rather its reorganization into a microcortex consisting of neurons normally destined for layer II and III, in the absence of granular and infragranular layers. These neurons have not finished migration at the time of injection and appear to be spared by the injection. The structure of the microcortex is similar to that of microgyria, a congenital malformation of the human cortex whose most probable cause appears to be an ischemic insult around the end of the period of neuronal migration. The ibotenic acid-induced microcortex possess several features of normal visual cortex, including connections with the lateral geniculate and with other cortical areas and orientation- and direction-specific responses to visual stimuli. Surprisingly, the microcortex maintains the normally transient projections from ipsi- and contralateral auditory cortex (Fig. 14) but not the transient projections from contralateral area 17. The specificity and apparent long duration of the stabilization suggests that it may not be due to generalized trophic effects of the lesion (for this concept see Nieto Sampedro et al. 1983), although this possibility cannot be fully ruled out in view of the increase in the number of astrocytes in the white matter of the microcortex. It appears more probable that the absence of the granular and infragranular layers is directly responsible for the stabilization. Layer VI or perhaps subplate neurons may be more specifically involved, since parts of cortex where only these structures are injured maintain axons from the auditory cortex.

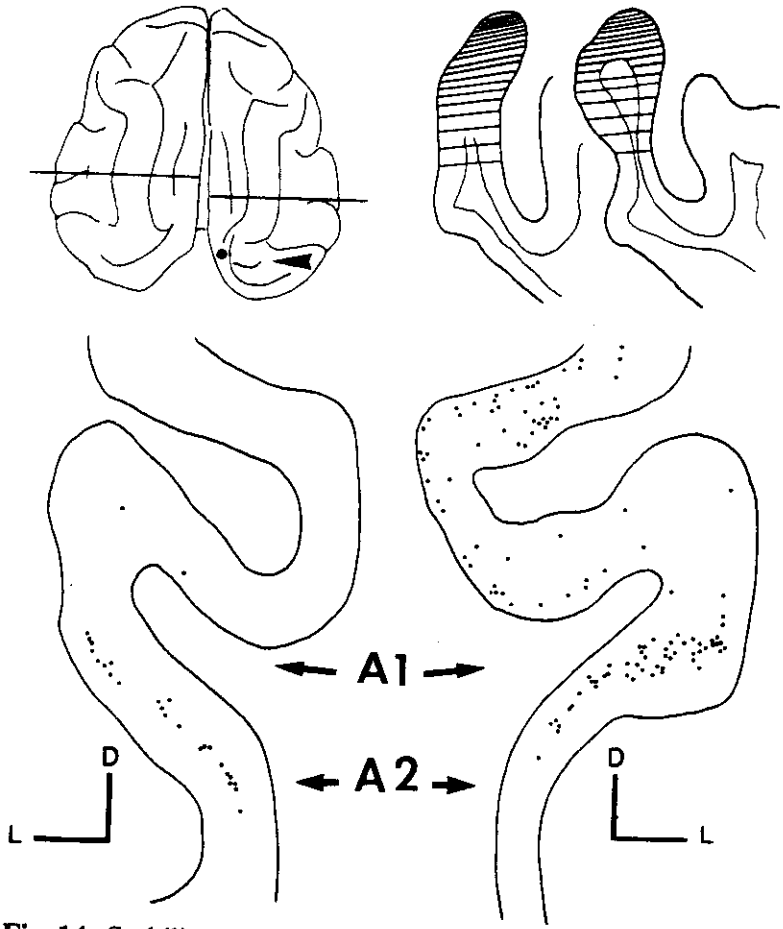

Fig. 14. Stabilization of projections from auditory cortex to frontal cortex in an adult cat in which microcortical regions were created in areas 17 and 18 by neonatal injections of ibotenic acid. The microcortex was injected with wheat germ agglutinin–horseradish peroxidase (*dot* in brain drawing; note narrower gyri in the right occipital cortex). The injection site is shown by the *hatching* on the low-power frontal sections. Labeled neurons are shown in the auditory areas (*A1* and *A2*) ipsi- and contralateral to the injection, which would normally have been eliminated. (See Innocenti and Berbel 1990b for details)

6.4 Others

The factors discussed above are almost certainly not an exhaustive list of those which may influence the fate of juvenile axons. For example, lesioning the superior colliculus was also found to maintain callosal projections which would otherwise be eliminated (Mooney et al. 1984). If in the hamster, as in the cat, visual callosal projections transfer information to the superior colliculus (Antonini et al. 1979), probably by contacting corticocollicular projection neurons in layer V, this finding may indicate that, in development, the fate of an axon is not only affected by its target neurons but also, indirectly, by events affecting the neuronal population to which the latter project.

Factors intrinsic to the neuron undoubtedly can affect both formation and maintenance of axonal arbors (Schneider et al. 1987). These factors may include intrinsic differences among axonal branches in sustaining critical neuronal functions such as survival and target recognition (Sharkey et al. 1986; Tolbert 1987).
General signals, in particular hormones, may play a role. Both the elimination of exuberant callosal axons and the maturation of their cytoskeleton, i.e. the expression of the heavy neurofilament subunit, were prevented by hypothyroidism (Gravel and Hawkes 1990). Gender may affect callosal size and shape in animals and man (Berrebi et al. 1988; Clarke et al. 1989), suggesting a possible role of steroids in callosal maturation.
Understanding the respective roles of the factors discussed above and their interactions will be not be an easy task. But it may be a worthwhile one. Failures in the elimination of normally transient projections may occur in circumstances other than the well-documented situations described above, in for example an experimental model of the fetal alcohol syndrome (Miller 1987) or even in schizophrenia (Feinberg 1982). The opposite type of pathology, i.e. an exaggerated elimination of axons in pathways of cortical origin such as the corpus callosum and the pyramidal tract, can also occur (Fig. 15) and might have a genetic origin (Lyon et al. 1990).

7 Conclusions

One may wonder if, besides their contribution to the understanding of the development of cerebral cortex, and therefore ultimately of higher brain functions, the studies above may also be relevant for the old and more general problem of the formation of neural connections.
Certainly, the formation of exuberant projections linking in a transient way neural structures which will be disconnected in the adult is not restricted to the cerebral cortex. For example, major transient retinofugal (Land and Lund 1979; McLoon and Lund 1982; Frost 1984; Bagnoli et al. 1987 inter alios) and retinopetal (Clarke and Cowan 1976; O'Leary and Cowan 1982; Catsicas et al. 1987) projections have been described, together with others at higher levels in the visual pathway (Stein et al. 1985). Although some of these projections may not involve the formation of long transient axons but only of locally widespread terminal arbors (Rakic 1976; Sretavan and Shatz 1987), the regulation of the survival of some of these projections appears to obey some of the same factors, for example competition and activity, which are crucial in the development of cortical connectivity (Rakic 1981; Frost 1986; Sretavan et al. 1988). One of the fascinating perspectives emerging from these studies is that of redirecting major sensory pathways within the brain with at least partially preserved function (Frost and Metin 1985).
The artificial control of developmental exuberance by selective lesions, constrained sensory experience, chemical manipulations and grafting, in addition to the more obvious potential of genetic manipulations, make a new type of "neuronal engineering" possible, and through the latter the repair of the developing and adult brain.

Fig. 15. A case of human congenital atrophy of the corpus callosum accompanied by atrophy of the white matter and absence of the pyramidal tract, considered to be a primary disorder of axonal development, occurring during the late fetal and early postnatal period, i.e. roughly during the time when normal elimination of cortical projections is suspected to occur in man (see Fig. 10). Girl deceased at 10 months. Coronal sections (dorsal is up, medial to the left) of the hemispheres. Myelin stain. Notice the white matter atrophy in the gyrus cinguli and the extremely thin, but myelinated corpus callosum. No Probst bundle exists, and the pathology is therefore different from the classical agenesis of the corpus callosum. This anomaly may be a pathological exaggeration of the normal developmental elimination of cortical axons, possibly a cytoskeletal defect. (From Lyon et al. 1990)

Still, one seems to be only nagging at the main theoretical question: to what extent is brain connectivity determined by prespecified axon-target recognition mechanisms?

At first glance, the development by exuberance of many neural connections seems to exclude prespecification as a plausible mechanism for their formation. Indeed, this mode of development seems at odds with the parsimonious and infallible growth to

the target that prespecification, in particular chemoaffinity, seems to imply (see also Innocenti 1988, for discussion). Furthermore, the selection of juvenile axons for maintenance or elimination depend on multiple factors, including functional criteria whose logic seems hierarchically higher than that of the molecular interactions at the site of neuron-target interaction.

Appealing as it may be, this view of neural development must nevertheless be qualified by several other considerations. First, in some systems, projections topographically similar to those of the adult are established from the beginning. This is the case with afferent projections to the cerebellum (Sotelo et al. 1984; Arsenio Nunes and Sotelo 1985; Mariani et al. 1987), although recently transient projections to this structure were found as well (Bower and Payne 1987). In the case of neuromuscular connections, the elimination of multiple innervation results only in small topographical rearrangements (Bennett 1987). Thus, in these systems, some kind of "prespecification" cannot be excluded and its role may be preponderant in the formation of the connections. Second, even in the case of corticofugal projections, where exuberance is impressive, there are early signs of specificity in the projection, including topographic specificity. Third, the possibility that some "preformed" specific cell recognition mechanism, presumably chemical in nature, may be involved in the selection of the juvenile connections cannot be excluded, although contrary to what chemospecificity theories suggest, it probably does not play the primary role.

Possibly the most important lesson of the developmental studies of cortical connectivity is the demonstration that no unique mechanism is responsible for the final shape of a neural circuit. Possibly, like the brain itself, the mode of its development is the piecemeal product of pragmatic evolution. In the course of phylogenesis, the complexity of regulatory developmental mechanisms may have increased because new mechanisms were introduced and were maintained when and where they had adaptive value.

The isolation and analysis of fragments of the whole developmental process is certainly a legitimate procedure, as far as these pieces can then be modeled in the complex network of causal interactions they belong to. This consideration may warn against the continually resurrected reductionistic vs holistic sectarianism in the field of science.

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