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Research report

Neuronal differentiation patterns in the optic tectum of the lizard *Gallotia galloti*

Jesús Báez[a], Maximina Monzón-Mayor[a,*], Carmen Yanes[b], Maria del Mar Romero-Alemán[a], Juan Francisco Arbelo-Galván[a], Luis Puelles[c]

[a]*Department of Morphology (Cell Biology), University of Las Palmas of Gran Canaria, Las Palmas of Gran Canaria (Canary Islands), Avda Dr Pasteur s/n. A.C. 550, Las Palmas, Canary Islands, 35016, Spain*
[b]*Department of Microbiology and Cell Biology, University of La Laguna, La Laguna, Tenerife, Canary Islands, 38071, Spain*
[c]*Department of Morphological Sciences, University of Murcia, Murcia, 30071, Spain*

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Abstract

This study examines in detail the sequences of morphological differentiation and deduces mode of migration into specific layers of all types of neurons present in the optic tectum of the lizard *Gallotia galloti*. It complements previous similar work on tectal histogenesis in the chick. It was found that the neuronal population diversity in the lizard tectum can be reduced by developmental analysis to three neuroblast classes, called Types I, II and III. These classes correspond closely to those present in the developing avian tectum. Neurons belonging to each developmental class were characterized by their initial polarity, mode of translocation into the mantle layer and pattern of sprouting of primary axonal and dendritic processes. Each class produced along time a subset of the cell types distinguished in the mature tectum. Some aspects of sauropsidian tectal histogenesis are also common of other vertebrates, suggesting that fundamental mechanisms of tectal neuronal differentiation are conserved in tetrapods. Analysis of evolutive differences of tectal structure points to changes affecting the layering and perhaps the population size of specific cell types. Whereas tectal cell-type homology can be easily fundamented on embryological evidence and seems to be consistent with hodological and, to some extent, functional homology, the periventricular, central and superficial strata of the tectum are heterogeneous in cellular composition in different species and therefore represent analogous, rather than homologous entities.

Theme: Cell differentiation and migration

Topic: Process outgrowth, growth cones, and sprouting

Keywords: Neurohistogenesis; Neuroblast; Migration; Golgi-impregnation; Reptile

1. Introduction

The optic tectum of sauropsids (birds and reptiles) shows various degrees of migrated cell layering [18,47,51,55] and a variety of recognizable neuronal cell types [2,4–6,18,19,28,32–34,50–53,55,56,73]. There is less migration and cell layering in the optic tectum of amphibians [31,60], elasmobranchs [10,35] and cyclostomes [22], while teleosts [41] and mammals [72] show a highly layered tectal structure. This raises the question of which developmental processes and changes in cell typology and circuitry characterize the separate trends to an increased cell migration and lamination in sauropsidian, teleostean and mammalian tectal evolution [46,47]. Knowl-

Abbreviations: CP, cortical plate; I, postmitotic type I neuroblasts; I_a, arciform type I cells; I_{dm}, displaced multipolar type I cells; I_m, multipolar type I cells; I_p, periventricular type I cells; I_t, tangential type I neuroblasts; IZ, intermediate zone; II, postmitotic type II neuroblasts; II_{br}, bipolar radial type II cells; II_h, horizontal type II cells; II_m, mitral type II cells; II_r, radial type II cells; II_s, stellated type II cells; III, postmitotic type III neuroblasts; III_a, arciform type III cells; III_e, elongated type III cells; MZ, marginal zone; PVZ, periventricular zone; SAC, stratum album centrale; SAP, stratum album periventriculare; SGC, stratum griseum centrale; SGFS, stratum griseum et fibrosum superficiale; SO, stratum opticum; SGP, stratum griseum periventriculare; V, ventricular cells; VZ, ventricular zone; 1–14, laminae of Ramón

*Corresponding author. Tel.: +34-928-451-466; fax: +34-928-453-420.
E-mail address: mmonzon@dmor.ulpgc.es (M. Monzón-Mayor).

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edge in this field remains obscured by lack of relevant comparable developmental data.

This report aims to fill in some developmental data needed to understand the evolutive transition between anamnia, sauropsids and mammals, studying a representative of reptiles. We have only very general developmental data on the reptilian tectum, which come from studies on the ontogeny of tectal lamination [62–67]. In particular, we lack Golgi-impregnation studies of immature neuronal cell shapes and their changing spatial distribution with time, to give insight on the critical processes of neuronal migration and differentiation.

Puelles and Bendala [49] used the Golgi impregnation method to study the development of tectal cell types in the chick, and cross-correlated these data with the autoradiographic observations of LaVail and Cowan [29] on neurogenetic and layering patterns. This study suggested that at least two main classes of tectal neurons, classified according to distinct differentiation sequences, are produced with partial temporal overlap (cell types I and II). Early to late elements of these two cell forms varied in the range of cell migration (or somatic translocation) and in the spatial growth and final arrangement of neurites. The resulting developmental schema accounts for most mature cell types and the layering complexity described in the avian tectum [1,7,8,36,37].

We report here a similar Golgi-impregnation analysis of neuronal differentiation patterns in the embryonic and postnatal tectum of a Type I lizard (*Gallotia galloti*). We investigated whether cells comparable to the avian Type I and II cells (with their characteristic differentiation and migration patterns) could be detected in this reptilian species, as would be expected if basic tectal histogenetic processes are conserved, at least among sauropsids. Our study indeed supports the existence of remarkably similar differentiation processes and suggests the distinction of yet a third developmental class of tectal neurons, present in both the chick and *G. galloti*. Our data serve to compare differentiation sequences and migration patterns for all the mature neuron cell types in the lizard and avian tectum, allowing also tentative extrapolation to other vertebrates. This analysis provides new insight into evolutive trends in the layering of the optic tectum in sauropsids.

This basic study complements the research of our group on development [42–45,78] and regeneration [26,27] of the visual pathway in the lizard *G. galloti* in which we are involved at present.

2. Materials and methods

Eggs of *G. galloti* were obtained in the field in Tenerife (Canary Islands, Spain) during several yearly laying seasons. The eggs were incubated further at room temperature in the laboratory, enveloped in slightly humified cotton in periodically ventilated containers. At appropriate intervals, the embryos were fixed and staged according to the developmental table of Dufaure and Hubert [9] and Ramos [57]. A number of them were immersed in Bouin's solution for ulterior paraffin embedding and hematoxylin–eosin staining of 5- to 10-μm-thick sections in transverse, sagittal and horizontal planes. Other embryos were placed in the Golgi-Stensaas fixative [49,69] and processed according to the standard procedure in most cases. Embryos smaller than stage 35 impregnated better with less glutaraldehyde in the fixative (half the normal amount) and a reduced fixation time (14–18 h), as well as with the whole procedure carried out at 15–16 °C. Postnatal brain specimens were also impregnated. We found that in this case the Golgi-Colonnier's technique [3] gave better results than the Stensaas' variant. On the whole, this study comprises abundant material sampled from practically all stages between stage 32 and hatching.

The Golgi impregnated specimens (representing the whole embryos, or only the heads or the dissected brains enveloped in duramater, depending on the stage of development) were sectioned into transverse or, occasionally, sagittal 60- to 80-μm-thick sections with a tissue chopper or a vibratome, after embedding in agar. Other specimens were embedded in celloidine and sectioned with a sledge microtome. All the sections were mounted with Araldite. Most of them were stabilized according to Lavilla's procedure [49] and some of them were counterstained with cresyl violet.

Mapping of neuronal typology for each stage studied implied visual microscopic analysis of all the sections, camera lucida drawing and photography of well-impregnated cells (marking observable layer boundaries and location within the tectum) and final synthetic reconstruction of typical cell forms and position, referred to the slightly more advanced rostral part of the optic tectum.

3. Results

3.1. Development of tectal lamination

We studied cytoarchitectural maturation of the tectum of *G. galloti* in hematoxylin-eosin stained specimens ranging from stage 30 (E30) to the adult. The changes may be grouped into three periods: (1) early stages (E30–31) show scarce cytoarchitectural differentiation, having only a thin marginal zone with a few postmitotic neurons (MZ; Fig. 1A); (2) intermediate stages (E32–37), in which the main cellular and plexiform strata develop; (3) late embryonic and postnatal stages, in which the definitive lamination appears.

An incipient periventricular neuronal stratum appears at E32. This becomes progressively thicker at E33–34, coincidental with gradual exhaustion of the ventricular zone (PVZ; Fig. 1B). By E34, a sparsely populated intermediate stratum containing migrated neurons appears

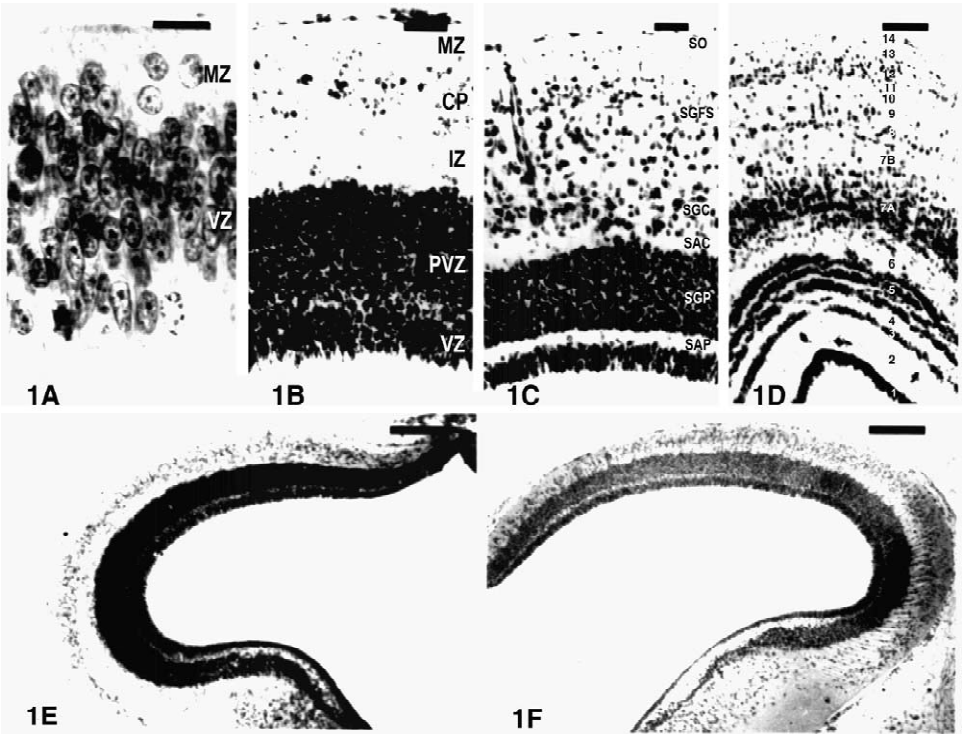

Fig. 1. Cytoarchitectural development of the tectum at various stages. (A) At E30, the midbrain alar plate consists solely of undifferentiated neuroepithelium or ventricular zone plus marginal zone (VZ,MZ). (B) At E34, there is a large periventricular stratum (PV), a moderately populated intermediate zone (IZ) and an incipient cortical plate (CP). (C) At E37, the six strata of Huber and Crosby [23] are present and migrated cells have increased in number. (D) At the eclosion stage, the 14 laminae of Ramón [58] are visible. (E) A transversal section at E34 illustrates the ventrodorsal gradient (dorsal up). (F) A horizontal section at E34 shows rostrocaudal differences (rostral to the left). Bars: A=50 μm; B,C=100 μm; D=250 μm; E,F=500 μm.

superficial to the thick periventricular zone (IZ; Fig. 1B). Most of these cells are disposed circumferentially and tend to be less abundant at the innermost levels, which contain many circumferential fibers. The intermediate stratum therefore seems to foreshadow the central album and gray (layers 6–7). Some smaller cells accumulate more superficially, building the earliest anlage of the cortical plate (CP; Fig. 1B), or prospective superficial stratum [62]. An incipient stratum opticum also appears subpially in the marginal zone.

Between E35 and E37, migrated cell bodies regroup as they become more numerous, establishing the main tectal strata of Huber and Crosby [18] (Fig. 1C). Retinal afferent plexuses develop during the late embryonic stages, as studied in this lizard species by Medina [38]. This leads to the definitive tectal lamination. The 14 layers of Ramón [51] can be clearly distinguished at E40, the hatching stage (Fig. 1D).

Tectal cytoarchitectural development proceeds asynchronously, due to overall histogenetic gradients along the latero(ventro)dorsal and rostrocaudal directions. In transversal sections, ventrolateral tectal portions are more advanced than dorsomedial ones (Fig. 1E) and in horizontal sections, rostral parts show more advanced lamination than caudal parts (Fig. 1F).

3.2. Cellular morphology

In the following sections, we describe results obtained from E32 to E40 in Golgi-Stensaas-impregnated specimens and these are compared with postnatal data. We identified cells that showed a ventricular attachment as non-neuronal ventricular or glial cells. Cells clearly freed from the ventricle but still lacking one of the mature neuronal forms seen postnatally were classified as postmitotic or differentiating neuroblasts.

At E32, which was the earliest stage at which we obtained useful impregnations, two distinct types of neuroblasts were present that closely corresponded to the Type I and Type II neuroblasts of the avian tectum [49]. Our description accordingly follows this terminology, though we also introduce a novel Type III neuroblast class, separating into this category cells subsumed under Type II by Puelles and Bendala [49]. Basically, postmitotic Type I neuroblasts are first very similar to interphase ventricular cells, since they reach the tectal surface with a subpial endfoot, but are already detached from the ventricle. The subpial endfoot thereafter directly transforms into an axonal growth cone, whose growth becomes deflected laterally. While the axon elongates, the cell body moves out of the ventricular zone (by means of somatic transloca-

tion within the cell) and dendritic processes start to grow out either before or after the perikaryon reaches the level of the axon origin.

In contrast, Type II neuroblasts detach from the ventricular zone shortly after mitosis and become freely migrating, smaller cells, which are presumably apposed to radial ventricular processes for guidance. They display during migration a short leading process, which transforms into the main dendritic process after the migration ends. The axon, preceded by a small growth cone, appears at the trailing end of the cell and other dendrites may appear there too at a later stage.

A distinct subgroup of freely migrating cells show a leading process at least twice as long as that of Type II cells, and are also distinct in that the tip of the leading process generates both a terminal dendritic bouquet and an ascending axonal process; these ascending axons course into the diencephalon through the marginal stratum, or trace characteristic wide arched courses into the central tectal layers. These cells may be conceived to represent a Type III group of neuroblasts, which gives rise to specific subtypes of tectal neurons. Figs. 2–4 show representative examples of the Type I, Type II and Type III cell forms at the characteristic radial levels in which they appear, as seen at the most advanced part of the tectum in successive stages. The inferred differentiation sequences are described in detail below.

3.3. Initial differentiation pattern of Type I neuroblasts

3.3.1. Tangential (prospective multipolar) neuroblasts

At stages E32 and E33, the simplest Type I neuroblasts have piriform or fusiform cell bodies, which appear mixed with the ventricular cells. They are detached from the ventricular lining, but show an outer radial cytoplasmic process that ends with a subpial endfoot (I; Figs. 2A,B and 5A). Slightly more advanced cells of this type bend their outer processes into a circumferential course of variable length (I; Figs. 2A,B and 5B). We observed a number of transitional forms with a subpial endfoot partially transformed into an axonal growth cone and starting to grow laterally under the tectal surface (Figs. 5A,B, insets). The cells which have a longer axon have also slightly more superficial cell bodies and a correspondingly shorter radial body, at the same level as their circumferential axons, adopting thus a tangential configuration (I_t; Figs. 2A,B and 5C). The larger tangential cells occupy relatively deeper positions and show a thick, scarcely ramified dendritic process that grows from the ab-axonal pole of the soma and a few thinner filopodia-like cell processes (I_t; Figs. 2B and 5D). These tangential cells later transform into the well-known multipolar cell type.

3.3.2. Type I periventricular cells

A variant of the previous cell form is represented by radial piriform cells with their circumferential axons placed just outside the periventricular stratum, at the same level or deeper than the tangential cells described above (I_p; Figs. 2B and 6A). At E34 a number of such cells already show incipient dendritic outgrowths both at the point where the radial outer process bends into the circumferential axon and at the ventricular pole of the deep cell body (I_p; Figs. 2B,C and 6B). This suggests that these cells do not translocate their somata outwards as actively as the cells becoming tangential more superficially. They apparently differentiate as Type I pyramidal cells of the periventricular stratum.

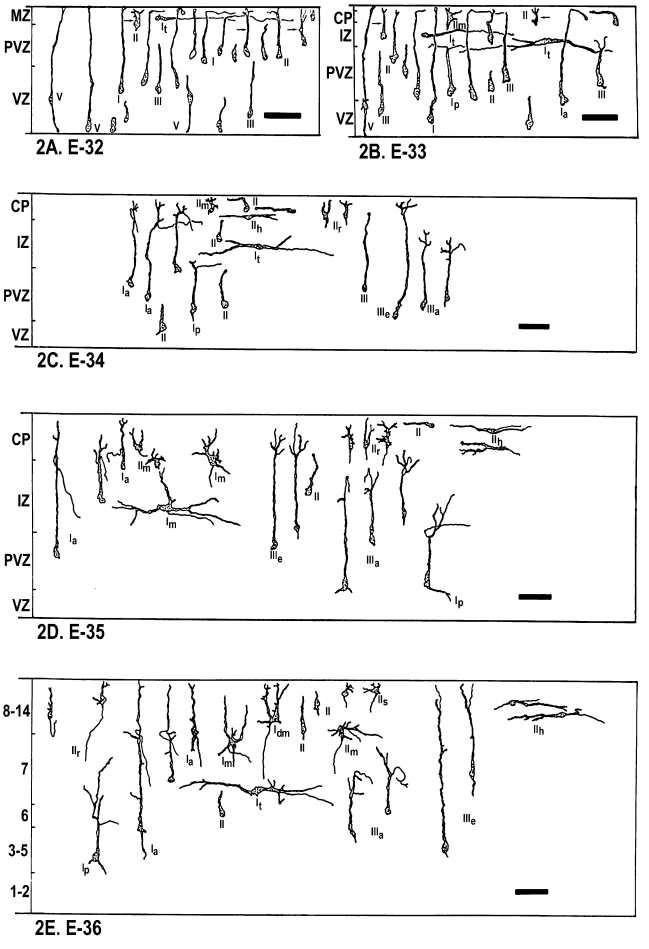

Fig. 2. Schematic illustration of the cell populations observed in the tectal wall of *G. galloti* sectioned transversely. Individual cell examples were rearranged from multiple camera lucida drawings. The developmental stage (E32–40 and hatching stage) is indicated and the tectal laminae are marked on the left (see abbreviations). Bar=50 μm.

3.3.3. Type I arciform cells

A second variant Type I sequence is represented already at E32–33 by some young neuroblasts with cell bodies occupying a periventricular level. Their incipient superficial axons do not grow tangentially, but form a tight arch directed inwards towards the deep part of the intermediate stratum (I_a; Figs. 2B and 7A). At E34, cells displaying this behavior impregnate more frequently. Many of them now

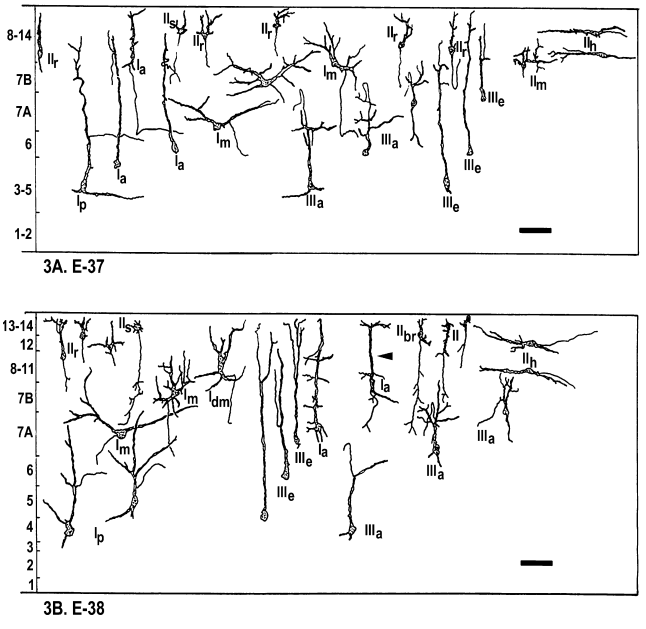

Fig. 3. Schematic illustration of the cell populations observed in the tectal wall of *G. galloti* sectioned transversely. Individual cell examples were rearranged from multiple camera lucida drawings. The developmental stage (E32–40 and hatching stage) is indicated and the tectal laminae are marked on the left (see abbreviations). Bar=50 μm.

show one or several ascending dendritic outgrowths originating from the superficial aspect of the arciform initial axonal segment (I_a; Figs. 2C and 7B). These neurons are named here Type I arciform cells; they later transform into the well-known shepherd's crook cells of Ramón y Cajal [55]. Later, the cell bodies of many of these Type I arciform neurons translocate all the way upwards within the primary radial cytoplasmic process and additional basal dendrites appear (I_a; Fig. 2C).

3.4. Late development of multipolar Type I neurons

At E34, practically all tangential neuroblasts lie within the central stratum and have dendritic outgrowths extending in the direction opposite to that of the axon. The dendritic arbor length increases in complexity from the more superficial tangential neuroblasts to the deeper ones (I_t; Fig. 2C) and includes in the latter vertically or obliquely ascending dendritic processes. Such ascending processes are considerably more frequent at E35 (I_t, I_m; Fig. 2C,D). More complex transitional forms found within the outer central stratum at E35 gradually adopt a multipolar shape (I_m; Figs. 3B and 5E). The axon of such cells has a short descending course before it enters the inner central stratum and becomes circumferential (I_m; Figs. 2D and 5F). These observations are consistent with the interpretation that the somata of these neurons translocate into one of the ascending dendritic processes; with the growth of additional ascending or descending dendrites, the cells acquire a multipolar shape; the axon always trails behind.

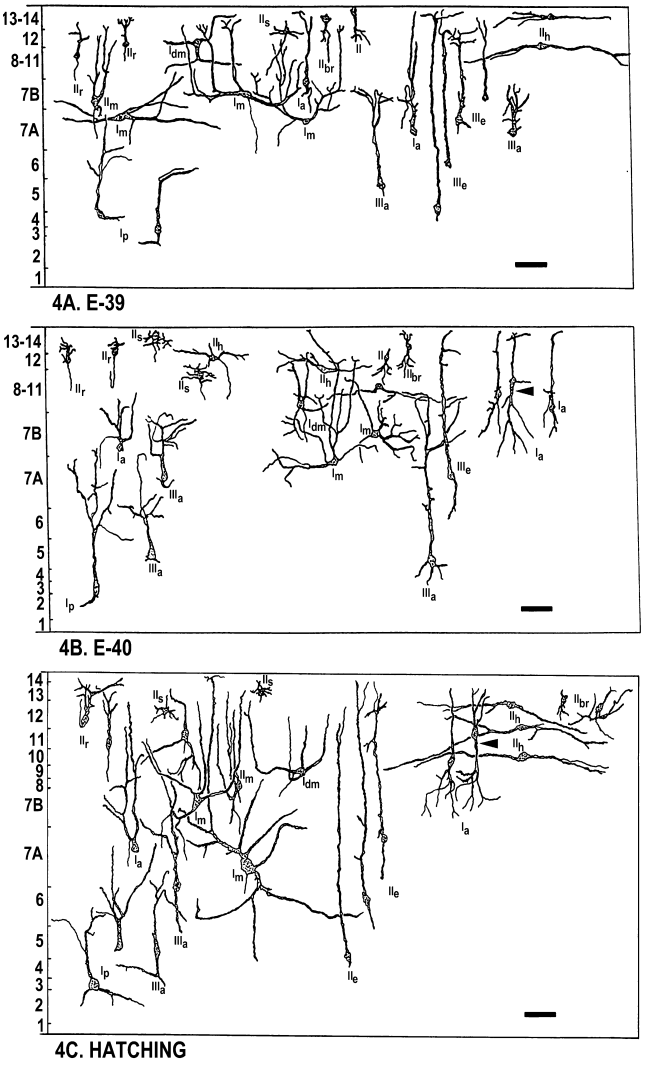

Fig. 4. Schematic illustration of the cell populations observed in the tectal wall of *G. galloti* sectioned transversely. Individual cell examples were rearranged from multiple camera lucida drawings. The developmental stage (E32–40 and hatching stage) is indicated and the tectal laminae are marked on the left (see abbreviations). Bar=50 μm.

At E36, only few tangential neuroblasts were impregnated within the inner part of the central stratum (prospective layer 6). These all had ascending dendritic processes (I_t; Fig. 2E). The outer part of this stratum (prospective layer 7) contains many multipolar cells. Some of these cells extend dendritic processes into the superficial strata and a few even seem to have translocated their somata into the innermost superficial stratum layers. We identified them as displaced multipolar cells (I_{dm}; Fig. 2E).

One stage later (E37), the SGC may be subdivided into two sublayers: lamina 7a becomes increasingly populated by migrated cell bodies, including other cell types apart from the multipolar cells (see below), whereas lamina 7b remains sparsely populated (Fig. 3A). At stage E38, impregnated multipolar neurons appear within lamina 7b and some of them start to grow additional dendrites

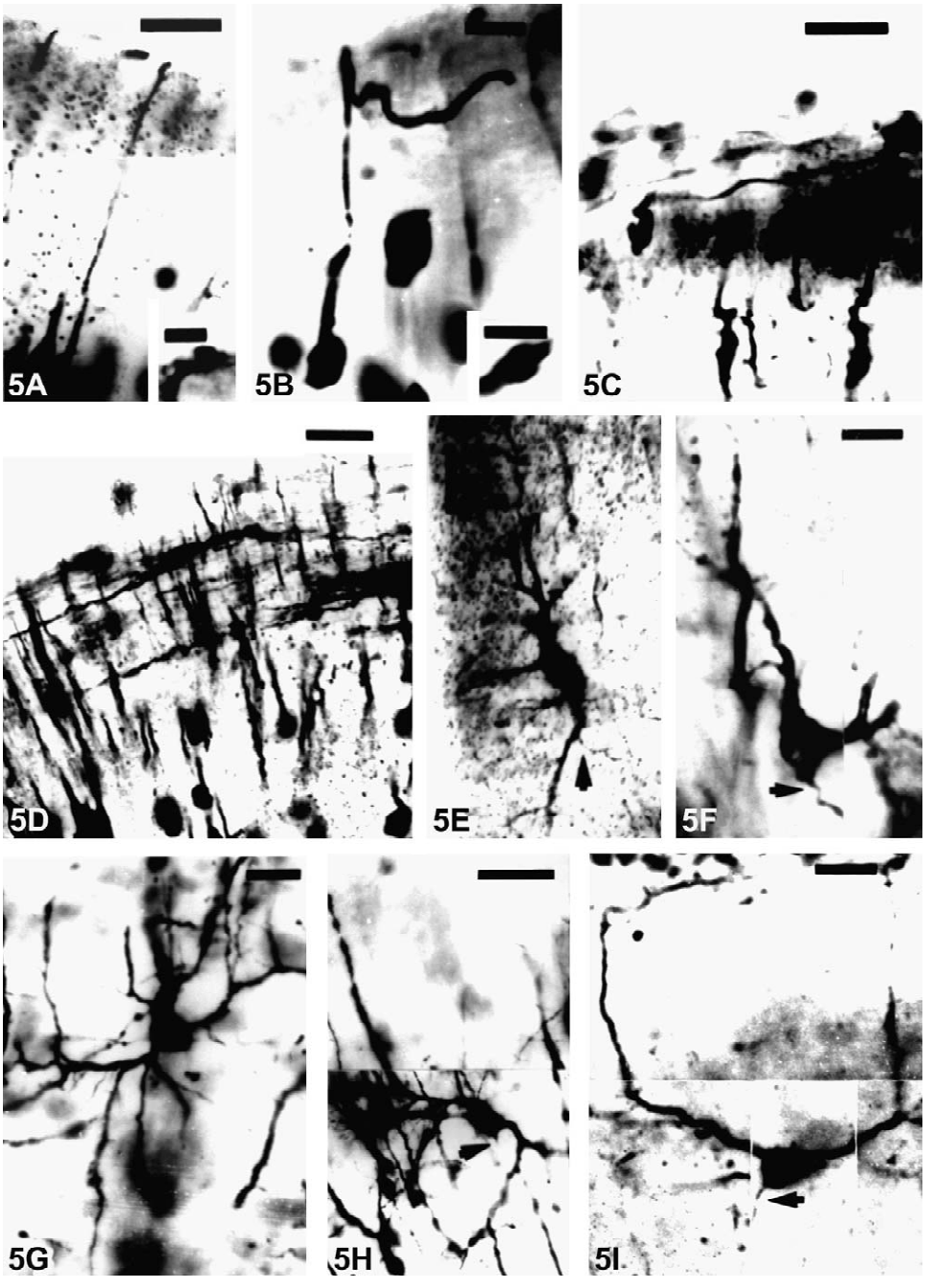

Fig. 5. Microphotographs illustrating the differentiation sequence of the multipolar Type I neurons. (A and inset) Type I neuroblast at E32, beginning to transform its subpial endfoot in an axonal growth cone. (B,C) Tangential elongation of the axon and gradual translocation of the soma, leading to a tangential configuration of the cell at E33. (D,E) Transient cell forms between the tangential and multipolar cell shapes at E35; note the radial dendrites oriented towards the tectal surface (to the left) (E). (F) Multipolar neuron next to the cortical plate (SGFS) at E35. (G) Multipolar neuron in the SGC at E38. Note the expanded dendritic tree. (H,I) Multipolar neurons in different layers at the postnatal stage (H=layer 7a; I=layer 12). Arrowheads in E,F,H,I, show the initial segment of the axon. Bars: A=50 μm (inset=15 μm); B=25 μm (inset=20 μm); C=20 μm; D,F=25 μm; E,G,I=50 μm; H=100 μm.

directed inwards (I_m; Fig. 5G). Occasional displaced multipolar cells were found within laminae 8–11 of the superficial stratum and sometimes even in lamina 12 (I_{dm}; Fig. 3B).

Subsequent late embryonic and postnatal growth of the multipolar cells mainly implies lengthening and branching of the dendrites (I_m; Fig. 4A,B), leading to at least two slightly different mature forms: (a) the innermost multipolar cells have rounded or polygonal cell bodies of large or medium size; they are placed within lamina 7a (I_m; Figs. 4C and 5H); (b) the outer multipolar cells have medium-sized polygonal cell bodies, occupy preferentially lamina 7b (I_m; Fig. 4C). The large deep multipolar cells may correspond to the tufted neurons described in other lizards

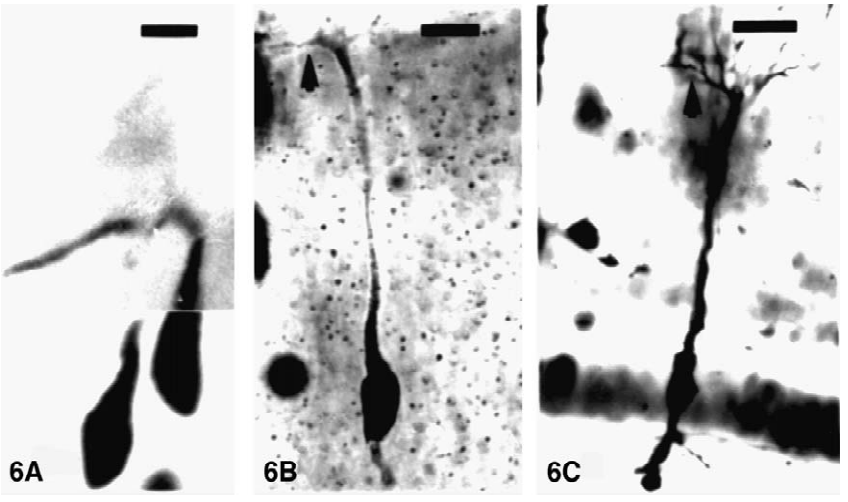

Fig. 6. Microphotographs showing differentiating periventricular cells. (A) Type I neuroblast at E32 with a tangential axon elongating within the periventricular zone. (B) Periventricular Type I neuron at E34 showing incipient growth of apical and basal dendrites. (C) Periventricular Type I neuron at E38, with a mature morphology. Arrowheads in B,C show the initial segment of the tangentially coursing axon. Bars: A=10 μm; B=25 μm; C=50 μm.

[50,51] and the medium-sized ones clearly represent the so-called ganglionar neurons [4,47]. Some displaced multipolar cells can still be found within the superficial laminae, with shapes similar to those in lamina 7b (I_{dm}; Figs. 4C and 5I). In all cases, the axon originates from the inner side of the cell body and descends into the SAC.

3.5. Late development of periventricular Type I neurons

From E35 onwards, the superficial and deep dendritic outgrowths of the periventricular Type I cells become longer and more branched (I_p; Figs. 2C-4 and 6C), leading to a pyramidal cell shape. Typically, the cell bodies remain placed within the periventricular cell layer. The apical dendritic tufts have few primary branches, which only rarely exceed the external limit of the SGC (I_p; Fig. 3A, at left). The inner dendrites are shorter and usually extend circumferentially within one of the plexiform sublaminae of the periventricular stratum. The axons of these cells extend within the SAC. While most of these fibers seem to exit the tectum laterally, others are oriented towards the tectal commissure.

3.6. Late development of Type I arciform neurons

At E34 the cell bodies of the arciform and pseudo-

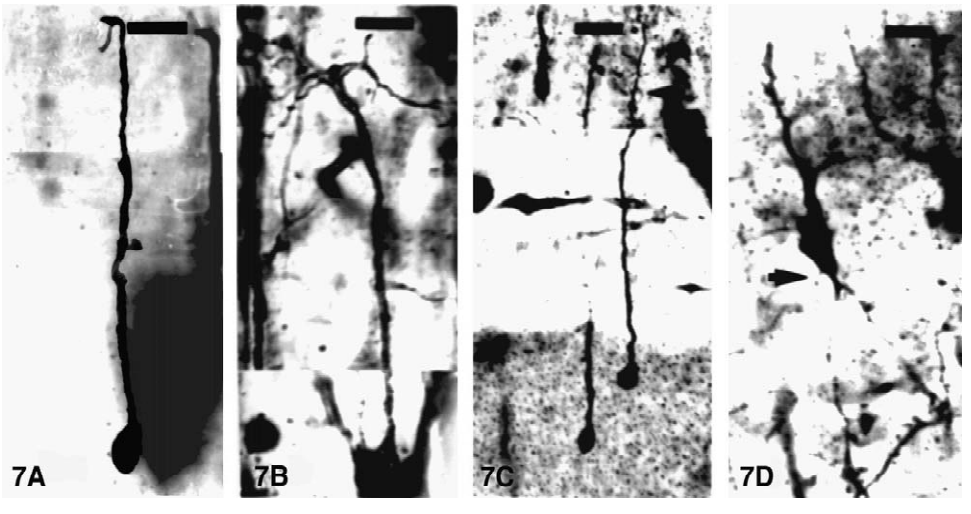

Fig. 7. Microphotographs showing differentiating Type I arciform neuroblasts. (A) Type I arciform neuroblast at E34 showing initial dendritic sprouting at the convexity of the axonal crook. (B) More developed dendritic sprouts in a slightly more advanced cell. (C) Arciform Type I neuron at E37, with its soma within the external layer of the SGP. (D) Arciform Type I neuron at E38; note its soma lies in the SGFS and has translocated higher than the axonal origin (arrow). Bars: A,B,D=25 μm; C=50 μm.

arciform Type I cells still occupy different levels of the periventricular stratum (I_a; Fig. 2C). One stage later (E35), many of the arciform cells have their soma within the intermediate zone; some of them even have translocated their somata far up, near to the cortical plate (I_a; Figs. 2D and 7C). The apical dendritic outgrowths become longer, in parallel with the increase in thickness of the cortical plate, and show incipient collateral branchlets. The arciform axons typically have their shepherd's crook bend just under lamina 8 and reach the SAC. Some arciform cell perikarya are still found within the central or periventricular strata at stages E36–37 (I_a; Fig. 2D on the left, and Fig. 3A).

We postulate that a peculiar cell form which suddenly appears in the SGFS at these later stages also corresponds to a further step in the Type I arciform maturation sequence. These mature looking neurons have a pyramidal cell shape and their axons originate from the side of the cell body or from a basal dendrite just below lamina 8 and show the typical crook-shaped course of other Type I arciform axons into the white matter (I_a with arrowhead; Figs. 3, 4 and 7D). No transitional younger forms were found, in which such an axon was seen growing out, preceded by a growth cone, of a primarily piriform superficial cell. This leads us to interpret these cells as extreme translocated cases of the Type I arciform cells, in which the cell body comes to lie at the same level or higher than the primary axonal origin. Such cells accordingly partly lose the characteristic arciform morphology. At E39, many of these cells occupy the inner laminae of the SGFS and show extensive development of the apical dendrites. Postnatally, there occurs an increase in the extent of additional internal dendrites, which gives many of these neurons a bitufted appearance (I_a with arrowhead; Fig. 4C). Their cell bodies become stabilized mainly at lamina 10 of the mature structure.

3.7. Development of Type II neurons

Cells classified as postmitotic Type II neuroblasts were observed already at E32 (II; Fig. 2A). Golgi impregnations were unsuccessful earlier than that. Type II neuroblasts characteristically are small piriform cells with a single apical cytoplasmic process that extends radially a few cell diameters and ends with a smoothly rounded tip; these processes never reach the pial surface (II; Figs. 2A,B and 8A). These small cells are found within the ventricular stratum of the rostral tectum only up to E34 (II; Fig. 2C). This suggests that production of such elements may stop thereafter. Additional cells with an identical size and shape were found at E32–34 throughout the thickness of the growing tectum and are visible in the developing SGFS up to E36 (II; Fig. 2A–E). Generally, only the most superficial Type II cells show morphological changes revealing the selection of one of several divergent differentiation pathways. These changes affect initially only the surface-oriented leading process, which soon acquires a dendritic character, previous to the typical sprouting of the axon at the opposite (internal) pole of the cell:

(a) A number of superficial Type II cells present at E32 retain a radial piriform shape, but the tip of their former leading process shows short lateral sprouts that apparently mark its transformation into a growing dendrite (II with small arrow; Figs. 2A,B and 8B). The neuronal subtypes named *deep radial* and *mitral* can be distinguished by E34, depending, respectively on the formation of either a single radial dendrite (often bifurcated peripherally), or of several main dendrites that spread radially or obliquely outwards from the cell body (II_r; II_m; Figs. 2C, 8C and 9A). The axon of these cells arises at the inner pole of the cell body and extends radially into the deeper strata (II_r; II_m; Figs. 2A,B and 8C,D). The axon is preceded by a small and scarcely ramified growth cone.

Mitral neurons seem to develop slightly in advance of the more abundant radial neurons. Subsequently, they are usually found at the radial level marking the boundary between central and superficial strata, immediately below the levels occupied by the crooks of the Type I arciform axons in the upper part of lamina 7B (II_m; Figs. 2C,D, 3A, 4A, 9A,B). At later stages, the perikarya of mitral cells

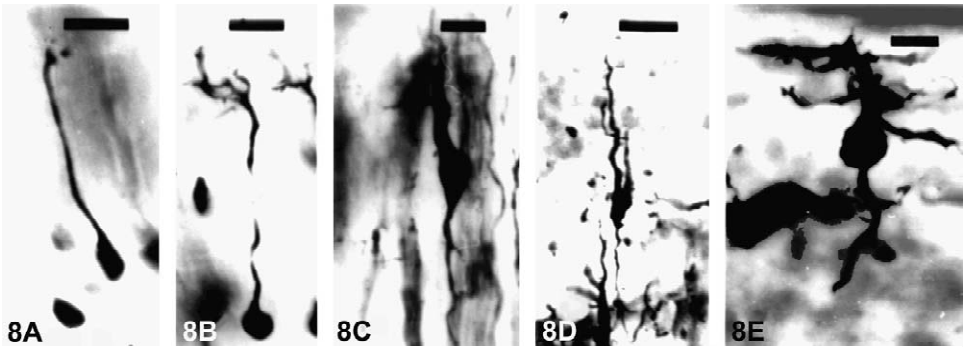

Fig. 8. Microphotographs of Type II radial neurons. (A) Type II postmitotic migratory neuroblast at E32. (B) Incipient dendritic sprouting at the subpial apical tip of the leading process of a radial neuron at E33. (C) Immature radial cell with a descending axon at E35. (D) Radial cell with a recurrent axon at E36. (E) Small superficial radial cell at E38. Bars: A,B,D=25 μm; C,E=50 μm.

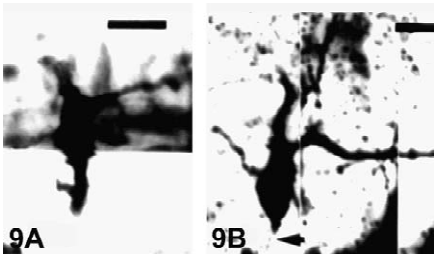

Fig. 9. Microphotographs of differentiating Type II mitral cells. Bars: A,B=50 μm.

may translocate upwards within one of their oblique dendrites or new internally growing dendrites may be added, producing cells of multipolar shape (II_m; Fig. 4C). These are always smaller than the underlying Type I multipolar cells.

After E35, the development of deep radial cells implies mostly lengthening of the apical dendrite. Additionally, some deep radial neurons develop ascending collaterals of their descending axons. In some cases, the whole axon has a recurrent ascending course and distributes within the superficial laminae (II_r; Fig. 2D; 3A). In the mature structure, deep radial cells appear mainly below layer 8, at the same level as the crooks of the Type I arciform axons, or lie superficial to that level (II_r; Figs. 4C and 8E).

(b) Other superficial Type II cells show transitional shapes suggesting their gradual migratory transformation into *horizontal* neuroblasts. The leading process remains short and smooth, but bends laterally under the pial surface and extends circumferentially (II; Fig. 2B); the cell body eventually translocates to the same circumferential level (II_h; Fig. 2C). These cells are thus morphologically identical to the radially migrating younger forms but lie horizontally within the cortical plate primordium, and are oriented in various directions (II_h; Figs. 2B,C and 10A). Dendritic outgrowths first appear in the deepest lying horizontal neurons at E34 (II_h; Fig. 2C), in such a way that the leading process becomes branched and dendrite-like, whereas a tangential axon and eventually also secondary dendrites later sprout from the trailing pole of the cell (II_h; Figs. 2C,D and 10A–D). Subsequently, deeper horizontal cells are always larger and more developed than more superficial horizontal cells (II_h; Figs. 2C,D, 3A,B and 4A,B). This suggests that successive Type II migratory cells producing this cell type become layered and differentiate in an inside-out gradient. The latest migratory Type II neuroblasts found just adopting a horizontal morphology were observed subpially at E34/35 (Fig. 2C,D). Outermost horizontal cells later come to lie in lamina 12 of the postnatal tectum, whereas the innermost ones occupy laminae 8–10 (II_h; Fig. 4C).

(c) Finally, postmigratory Type II neuroblasts reaching the tectal surface at E36 seem to form *outer radial piriform* neurons, *stellate* neurons and *bipolar radial* neurons. The outer radial piriform cells have a superficially directed radial dendrite and grow an inward directed short axon that remains within the outer tectal laminae (II_r; Figs. 2E, 3 and 4). Prospective stellate cells apparently first become more or less rounded subpially, once their initial long migration finishes, and then grow multiple dendrites in all directions, as well as a descending axon that usually reaches the SGC (II_s; Figs. 2E and 11A–D). These cells finally occupy laminae 12 and 13 (II_s; Figs. 3–4). Prospective bipolar radial cells are detected first at E38 as rounded subpial cell bodies with a single, inward directed dendritic process. From one of its innermost branchlets arises a short descending axon (II_{br}; Figs. 3B, 4A and 12A,B). By E40, the cell bodies of these cells lie inside the upper levels of lamina 14 and they grow slender outer dendritic branchlets which finally give them a bipolar appearance (II_{br}; Figs. 4B and 12B).

3.8. Development of Type III neurons

A distinct cell population present up to stage 34 is represented by the *elongated* neuroblasts, which are here proposed to represent a separate developmental class of tectal neurons. They are simple radially disposed piriform cells displaying a leading process which is twice or three-times longer than that of the normal migrating Type II neuroblasts. The elongated cell's leading process also ends initially with a rounded tip short of the pial surface, either inside the cortical plate or below it. The cell bodies of these cells lie mostly within the periventricular stratum (III; Fig. 2A–C). We interpret these forms as free migrating neuroblasts whose cell bodies somehow lag behind in

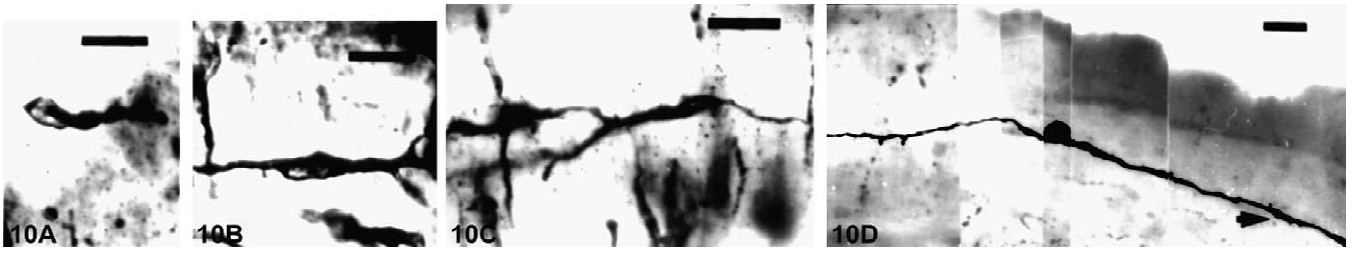

Fig. 10. Microphotographs of differentiating Type II horizontal neurons. (A) Typical monopolar migrating horizontal neuroblast at E33. (B) Horizontal cell starting to grow its axon at the cell pole opposed to the dendrite at E34. (C) More differentiated horizontal neuron at E35. (D) Longer horizontal cell within the SGFS at stage 40. Bars: A,B,C=25 μm; D=50 μm.

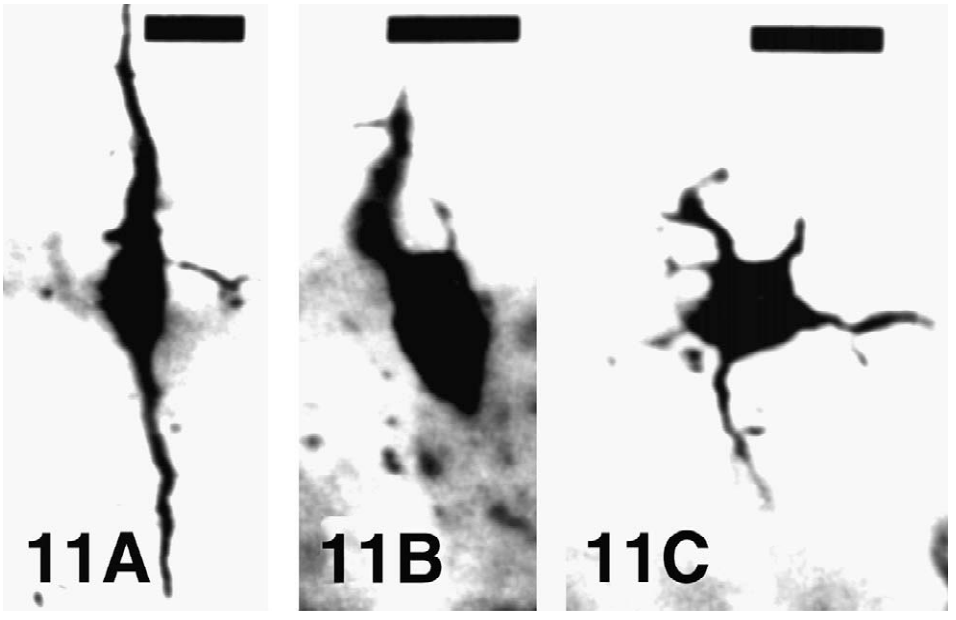

Fig. 11. Microphotographs of differentiating Type II stellate cells. (A,B) Late Type II migratory neuroblasts reaching the marginal zone of the tectum at E36. (C) Dendrites of the stellate cells at E36 sprout all around the rounded cell body, as well as a vertically descending axon. (D) More mature stellate cell with axon descending into the SGC at E38. Bars: A,B=50 μm; C,D=100 μm.

their outward translocation, while their leading processes grow unimpeded into the incipient cortical plate. At E34, some of these elongated cells start to differentiate a characteristically sparse dendritic tuft at the tip of their former leading process. They remain momentarily axonless (III_e; Figs. 2C and 13A). The differential characteristics, compared to Type II cells, is that elongated cells eventually sprout an ascending axon from the outer tip of the primary leading process, or sometimes from the side of one of the ascending dendrites, a phenomenon which apparently starts at E35 (III_e; Fig. 2D and 13B). This coincides with gradual translocation of many of their cell bodies out of the periventricular stratum into the central layers, though some remain periventricular at the latest stage studied by us (III_e; Figs. 2E, 3A,B and 4A–C).

As development proceeds, many Type III cells develop mainly one apical dendrite (less frequently two or more), which approaches the pial surface, showing scarce short lateral branches. Their axon generally appears to start at the side of the dendrite and courses upwards, disappearing

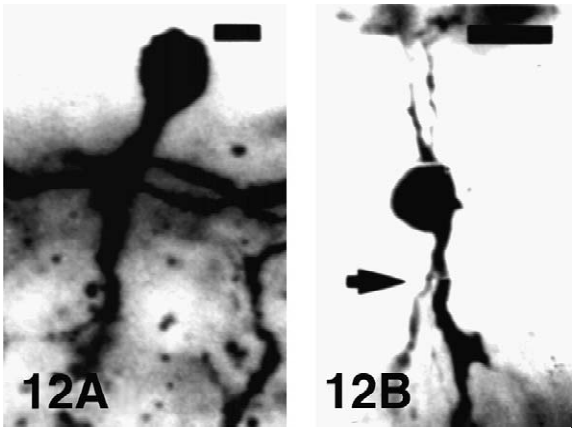

Fig. 12. Microphotographs of bipolar radial neurons. (A) Cell with a monopolar shape at the external part of the SGFS at E39. (B) Typical bipolar radial cell at the lamina 13 at E40. Bars: A=15 μm; B=50 μm.

Fig. 13. Microphotographs illustrating Type III elongate neurons. (A) Elongate Type III neuron showing initial differentiation of sparse dendritic branches at E34. (B) Elongate neuron with an incipient axonal process appearing at the base of the main dendritic branches at E34 (arrow). Bars: A,B=50 μm.

from the section near the tectal surface; we call these cells *elongate* Type III neurons (III_e; Fig. 2D). Their axons apparently exit the tectum through the dorsal tectothalamic tract. There are also deeper Type III cells, which develop shorter divergent dendrites more deeply in the superficial tectal stratum; their ascending axons do not traverse the whole cortical plate and bend back in a recurrent course, often forming a wide arciform loop that ends in several terminal axonal branches within the SGC (layer 7). Following the terminology of Puelles and Bendala [49], who also found such cells in the chick, we name these latter cells *arciform* Type III neurons, thus distinguishing them from both the *elongated* Type III projection neurons and the unrelated arciform Type I cells (III_a; Fig. 2C–E, 3A,B, 4A–C and 14A,B). We never observed dendrites growing out from the convexity of Type III arciform axons, this being an exclusive feature of the Type I arciform cells. Moreover, the arciform axonal loops of the Type III cells develop later under the tectal surface, and usually are ampler (larger radius) than the crook-like axonal loops of the Type I arciform cells. They never extend into layer 6. Cell bodies of some Type III arciform cells also translocate out of the periventricular stratum into the upper layer 7 levels by E37 (III_a; Figs. 3, 4 and 14B), though others remain periventricular (III_a; Figs. 3, 4 and 14A). Their axonal terminals seem restricted to the 7A and 7B central laminae.

3.9. Dendritic spines

In general, Type I, Type II and Type III cells with dendritic branches extending into or passing through the retinorecipient laminae start to show development of dendritic spines at E37–38. This coincides with the period in which three bands of retinal terminals are established in this species [38].

4. Discussion

4.1. Developing cell types in the reptilian and avian tectum

The sequence of layer development observed in *G. galloti* is basically the same as in other reptiles [64–67] and also is very similar to that of birds [28,49]. Cell birthday data are not available for the lizard tectum, but our observations on the diverse cell populations, summarized in Fig. 15, are consistent with a layering pattern similar to that of the chick tectum [29], in which alternating inside-out and outside-in distributions of cells appear.

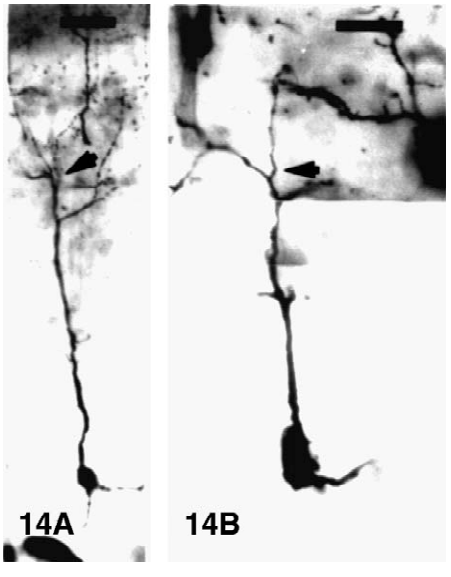

Fig. 14. Micrographs of arciform type III neurons. (A) Arciform Type III neuron with the soma in the SGP, showing its frail dendritic branches and arciform axon at E40 (arrow). (B) Arciform Type III neuron with its cell body translocated out of the SGC at E39. Note the initial ascending segment of its axon (arrow). Bars: A,B=100 μm.

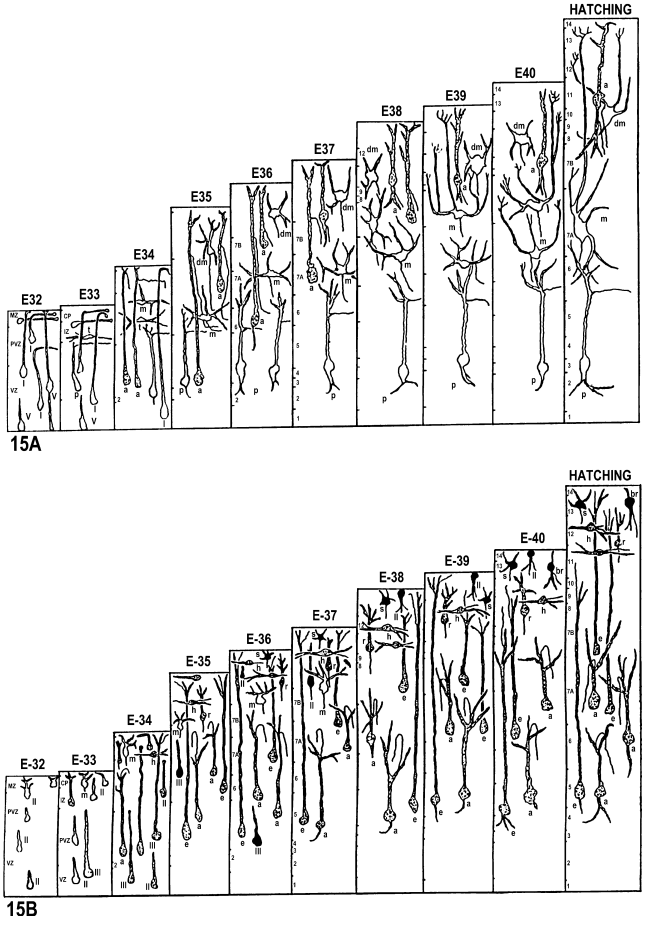

Fig. 15. Schematic overview of the characteristic radial stratification of the tectal cell types derived from the Type I, II and III classes as the tectum grows in thickness. (A) Type I cells. (B) Type II and III cells. Temporal sequence is represented by the filling of the cell profiles: white=early developing; dotted=intermediate; black=late developing.

Each stratum behaves independently and the SGFS, in particular, shows an inside-out layering within its outer part and may have an outside-in layering in its inner part. Given that both SGFS parts and the underlying SGC and SGP contain several mixed populations of cells, it probably should be assumed that the complexity of layering is actually higher than reported, if understood as the addition of several simpler patterns in terms of individual cell types (e.g. layering of horizontal cells, independent of that of radial, stellate and bipolar cells within the outer SGFS). The forementioned global gradients simply represent rough neurogenetic profiles across the temporal overlap in the production and differentiation of several cell types.

To understand such complex layering of the changing tectal structure at least three independent parameters need to be contemplated (Fig. 15): (1) different *neuroblast classes* (Types I–III) are produced independently and in part simultaneously at the ventricular zone; these differ in initial relative *cell polarity* and *mode of migration*; the polarity may be circumstantial to the moment in the postmitotic period in which the cells detach from the ventricle, as speculated by Puelles and Bendala [49], and may be induced by bound or soluble molecules; (2) both the microenvironment and the postmitotic cells probably change in molecular constitution with time, due to effects of previously differentiated neuronal and glial cells, incoming afferents and eventual cell-autonomous transcription-factor-induced changes in gene expression; this produces differences in axonal and dendritic growth patterns between early and late members of the same cell class (*cell subtypes*); and (3) various degrees of *secondary (late) somatic translocation*, depending on the specific cell type involved. Accumulated Golgi data in the chick and now in the lizard support the following synthesis:

Type I cells start to differentiate their axonal pole at the subpial surface of the neuroepithelium and their axons grow uniformly into layer 6, which connects them with the tegmentum and with the tectal commissure [48]. Depending on parameters 2 and 3 (above), such cells subsequently may transform into periventricular pyramidal, multipolar (tufted and ganglion cells), displaced multipolar and arciform or pseudoarciform Type I neurons [7,8,49, present results]. Fig. 15A shows that they become distributed to all three primary strata of the lizard tectum; this occurs without a clear-cut layering pattern, due to different range and direction of the somatic translocation processes for each cell type. The arciform Type I neurons are the latest to differentiate in this class and show diverse ranges of upward somatic translocation in the lizard, while they enter massively the SGFS in the chick; some of them even translocate their somata higher than the origin of the axon, becoming pyramidal cells of layer 8 (Fig. 15A). Similar cells have been described in the inner laminae of the SGFS in the Japanese quail [17]. Each of these cell subtypes may originate specific efferent projections of the tectum: among them, the massive crossed tectobulbar tract, the possibly collateral intertectal commissural fibers [48], the ventral tectothalamic (or tectorotundic) tract and the tectoisthmic tract. However, in reptiles, experimental connectivity data correlated with specific neuronal types in the tectum are still too fragmentary to fully confirm this point [19,71]. Type I arciform cells, Ramón y Cajal's shepherd's crook cells [55], in birds clearly project specifically to the isthmic region [21,53,61,77] and populate exclusively the SGFS [8,49,55]. These characteristic cells populate the SGP, SGC and SGFS strata in the lizard, depending on how far they translocate secondarily their cell bodies [6,18,50,51, present results].

Type II cells first migrate radially in an undifferentiated form into the SGFS. It is probable that their advance towards the tectal cortical plate is related to contact guidance at the surface of either ventricular cells, Type I immature neurons or radial glia. Their subsequent divergent pattern of differentiation (parameters 2 and 3 above) variously transforms them into mitral, radial piriform, horizontal, stellate, outer radial or radial bipolar neuronal forms of the outer SGFS (Fig. 15B), essentially in parallel with data on chick embryos [15,36,49,75]. Type II neurons seem to be largely tectal interneurons, though it remains unclear whether the deepest and earliest differentiated elements of this class (mitral cells or deep radial piriform/pyramidal cells) may have axons projecting out of the tectum.

Type III cells have a short free radial migration. Their postmitotic forms are *typologically* similar to Type II cells, but have a distinctively more elongated leading process which, some time later, develops both ascending dendritic outgrowths and an ascending axonal process; this is the second differential characteristic. Depending on parameters 2 and 3 (above), these elements transform into the mature elongate and arciform Type III neurons, which mostly have their cell bodies in the SGP and SGC; Fig. 15B). Elongate Type III cells were discovered in birds by Ramón y Cajal [55] and in teleosts, anura, lizard and chamaleon by his brother [51]. They seem to send their axons through layer 14 into the dorsal tectothalamic tract [4,12,58] and are thus projection neurons. Hunt and Künzle [20] in the pigeon showed such neurons project to the pretectum and ventral thalamus, as seems to be the case in reptiles (see references above). In contrast, arciform Type III neurons become tectal interneurons.

Note that Puelles and Bendala [49] did not regard the elongated neuroblasts as a Type III class by themselves and classified them as a late subtype of the Type II cells. However, the peculiar differences in cell shape, locus of axonal origin and axonal course are significant enough to warrant postulation of a separate Type III class.

In conclusion, subdivision of the differentiating tectal cells of *G. galloti* into the Type I–III classes closely correlates with observations in the chick [1,7,8,36,49,75]. This supports a fundamental conservation of tectal differentiation patterns and related control mechanisms

among sauropsids, as well as the morphological homology of specific cell types one to another, within the limits of the class. The identification of cell types as being individually homologous, independently of the radial locus adopted by the cell soma, agrees as well with Ramón y Cajal [56], Ramón [51–53], Wilm and Fritzsch [71], Roth et al. [59] and Nieuwenhuys [46]. The present results simply suggest what may be the developmental basis for that conclusion. Further advance might lie in confirming the distinct possibility that such homologous cell types, or subsets among them, have comparable patterns of connectivity and neurotransmitter metabolism. This sort of analysis is not straightforward, as revealed by data on the avian tectum, suggesting that different subsets of horizontal Type II cells may be either glutamatergic or GABAergic [19] and different elongate Type III in the inner SGFS cells may be either GABAergic or cholinergic [39,68].

4.2. Are tectal strata homologous?

Senn [62] compared tectal cytoarchitectural development in reptiles and concluded on histological grounds that at least the periventricular, central and superficial strata are comparable among them and probably among sauropsids as well [47]. However, there are differences in the distribution of mature cell types in the lacertid periventricular, central and superficial strata, compared with the avian pattern. Basically, in birds the periventricular stratum (SGP) is greatly reduced, the central stratum (SGC) is largely populated by multipolar neurons and most other cell types are found in the superficial stratum (SGFS). In *G. galloti*, a type I lizard, the SGP and SGC clearly contain the somata of some cell types which normally are only found in the avian SGFS (for instance, arciform Type I, III and elongate cells). On the other hand, most or all Type II cells seem to migrate into the SGFS, in the lizard, or into the outer SGFS, in the chick (see more comments on this below). A number of Type I arciform and displaced multipolar neurons invade the SGFS in both animals; it is unclear whether there are quantitative differences in this between them. From the point of view of the developmental lineage of the constituent neurons, therefore, the main tectal strata are partly heterogeneous in adult sauropsids and thus should be regarded as analogous morphological entities, rather than homologous ones.

The avian inner SGFS laminae (layers 10–12) are occupied by mitral and deep radial piriform Type II cells, arciform Type I cell bodies and numerous elongate and arciform Type III cells. These layers may well be analogous as a whole to the outer division of the reptilian layer 7 (conventionally assigned to SGC), in which bipolar fusiform cells of mixed embryological origin likewise predominate, together with some multipolar cells [47,50, present results]. It would thus seem possible to postulate that the upper part of layer 7 in Type I lizards is an incipient analog of the inner, late-developing part of avian SGFS. Alternatively, it may at least be thought that these reptiles have adopted an evolutive status in which the SGFS is incompletely separated from the SGC.

The present comparative analysis between a bird and a Type I lizard needs to be extended to additional sauropsidian species. Nevertheless, existing descriptions of Golgi-impregnated or Golgi-like-labeled tectal neurons in several sorts of adult reptiles supports the general accuracy of our description and classification [2,6,18,47,50,51]. Reexamination of all these data with the Type I–III categories in mind easily reveals that examples of all the relevant neuronal forms have been recorded repeatedly.

4.3. Type I/II/III cells in other vertebrates

Some Golgi developmental data reported for other vertebrates can be drawn for comparison with present data: lamprey [22]; teleost [76]; frog [30]; rat/mouse [11,25]. The lamprey data, however, are too fragmentary to be useful in the present context.

Wilm and Fritzsch [76] studied some of the developing tectal cell types in a perciform teleost, referring to the previous classification of tectal neurons in the goldfish by Meek and Schellart [41]. They expressly left out of their analysis all cells having cell bodies in the thick internal (periventricular) layer. This apparently led to exclusion of most immature premigratory cell forms, hampering a detailed comparison with our data. However, Wilm and Fritzsch [76] interestingly concluded that migrated efferent cell types usually develop their axons before dendrites differentiate, a Type I characteristic in sauropsids [49, present results], whereas intrinsic neurons seem to extend axon and dendrites simultaneously (analogously to Type II and Type III cells, though we wonder whether dendritic growth can perhaps be shown to shortly precede axonal sprouting in such cells after a more detailed analysis). Wilm and Fritzsch [76] also emphasized invariant tectal laminar levels at which specific sorts of axons originate, irrespective of changing positions of the neuronal somata. They convincingly argued that there exists in some cell types (similar to arciform Type I cells) marked secondary somatic translocation, which takes the soma past the initial axonal origin.

Data on the developing frog tectum [24,30,70] indicate that stratification of tectal neuronal types, both by somatic translocation of Type I or III cells and free migration of Type II cells, may operate at least minimally in anurans, though this feature may be secondarily lost in paedomorphic urodeles [47,59]. Lázár's developmental results in larvae of *Xenopus laevis* [30] are incomplete with respect to earliest stages of cell migration and differentiation, when compared to the present ones. Nevertheless, they do suggest a fundamental identity of cell types in this frog with those observed in chick and lizard, particularly if they are subdivided into our Type I/II/III groups according to the reported topography of axonal process origin and its

course relative to the dendritic arbors. Periventricular cells clearly include both Type I and Type III efferent subtypes (axons in SAC vs. SO, as described also by Ramón [54] and Lázár [31]). The Type I elements seem to represent both the multipolar and the arciform subtypes of sauropsides. Superficial elements seem to include several subtypes of our Type II neurons.

Differentiating neuronal types have been reported in the mouse superior colliculus, in the context of birthday data and lamination patterns [11]. Displacement of postmitotic neurons into the mantle layer did not follow a strictly continuous radial order, suggesting the existence of qualitative layering differences between early neurons born at E11, intermediate neurons born at E13 and late neurons born at E15. Cells were simultaneously and differentially added to the superficial and deep strata, which agrees with the independent behavior of hypothetical Type I–III cell classes. It is conceivable that reclassification of the different mammalian immature neurons according to their developmental type (similar to Types I/II/III of the present report) might uncover some of the rules governing this cell mixing pattern.

The comparative analysis of the cellular types in the embryonary tectum of the mouse reveal that development of the superior colliculus, as reported by Edwards et al. [11], is comparable in a general way to that of sauropsids in many cellular details (temporal sequence of types, migratory processes, axonal growth, efferent pathways and targets, dendritic typology). One of the largest differences lies in the much increased contribution of late-born neurons to the most superficial layers (E15 group). The fact that these layers are known to predominantly project into the pretectum, ventral thalamus and dorsal thalamus opens the possibility that these late cells may be comparable to elongate Type III neurons, which would then have translocated all the way into the upper SGFS, in contrast to the partial migration seen in sauropsids. It is also relevant that mammalian tectal cells comparable to sauropsidian tectoisthmic neurons (arciform Type I in birds) lie in the superficial stratum, whereas crossed tectotegmental tract neurons, ipsilateral tectopontine neurons and cells projecting into the intralaminar and posterior dorsal thalamus lie mainly in the intermediate and deep strata [74].

The comparative considerations on the whole suggest that the fundamental tectal histogenetic pattern, schematized in terms of the Type I–III cell classes, may be plesiomorphic in tetrapods and, perhaps, in all vertebrates [10,22,23,40,46,47].

4.4. Cell type, free migration and perikaryal translocation as independent variables in tectal evolution

There is probably no single variable that accounts for all the observed variations among the optic tecta of vertebrates. The existence of species in which the full tectal neuronal population is periventricular, but still retains comparable cellular and hodologic diversity, for instance urodeles [46,59], indicates that the number of primary cell type classes probably is invariant and thus does not represent an essential variable for qualitative variation. Nevertheless, possible variation in the ratios of Type I–III cells produced in diverse vertebrate lineages needs to be investigated. The main evolutive variation seems to lie in the layering of the different neuronal subtypes and presumably in their synaptic relationships with afferents and local circuitry.

Nieuwenhuys [46] discussed the mechanisms which might contribute to the evolution of novel tectal cell layering patterns and progressive migration of cells out of a primitive periventricular location. He limited his comments to tectal structure in teleosts and amphibians, but they seem relevant in a wider context. He developed the viewpoint that such changes may be selected in diverse instances due to evolutive pressure to increase the functional effectiveness of plesiomorphic axodendritic input sites found primitively in topologically distant radial positions from the deep somata. A long interposed dendritic stalk greatly reduces the capacity of axodendritic inputs to modify the axonal output signal. Apart from increasing directly the number and/or size of the relevant synaptic contacts, an evolutive feature which presumably promotes the development of specific plexiform laminae, Nieuwenhuys [46] cited two other alternatives, namely transposition of the axonal origin site or the whole soma from their primitive deep position nearer to the distant axodendritic input. Examples cited for the transposition of the axonal origin in several vertebrates clearly refer to a mixture of arciform Type I and elongate or arciform Type III cell types.

However, data on sauropsides [49, present study] indicate that such cells do not develop from early forms having an axonal origin in a deep tectal stratum. In the particular case of the shepherd's crook cells (Type I arciform neurons), the apical dendrites grow at or near a pre-existent axonal origin site, so that it becomes impossible to conceive the axon origin moving out of the soma and nearer to the dendrites. Similar considerations may be made for the elongate and arciform Type III cells, where the axons directly sprout at the pre-existent apical dendritic bouquet. In all these cells, the ascending dendrites later elongate considerably into the outer SGFS laminae, actually leaving the fixed axonal origin behind. It is true that avian and reptilian elongate Type III cells develop dendritic and axonal collateral arborizations within the deepest retinorecipient lamina [19,51], thus potentially increasing the surface of afferent stimulation. Translocation of the cell body upwards in these cases would only contribute increased effectiveness to eventual synapses on the soma (not described so far) or on the basal dendrites. Our developmental data thus do not support the selective transposition of the axonal origin as an evolutive mechanism for the tectum.

In contrast, results on free migration of Type II cells into the SGFS and on variable degrees of somatic translocation of Type I–III cells in the avian and lizard tectum corroborate the general contribution of these phenomena to the evolution of tectal cell layering. The apparent density of migrated Type II cells in the superficial stratum increases from anamnia to amniotes. Free migration of neuroblasts implies coordination of leading process protrusion with perikaryal translocation, these being parallel mechanisms advancing at a similar rate. Leading edge protrusion and somatic translocation do dissociate in some cases, however, as occurs in stellate cells (soma translocation is at the end stronger than leading process advance, which leads to rounding up of the cell), or in elongate cells (soma translocation lags behind leading process advance). Free migration is thus a special case of somatic translocation that occurs in very immature neurons. Differential quantitative and directional control of somatic translocation in different cell types thus seems to be a primary and general cause of variation in the mature neuronal and laminar typology in the vertebrate optic tectum, as discussed above.

Though unreported by Puelles and Bendala [49], their study also produced results suggestive of late somatic translocation of some Type I arciform neurons into laminar levels of the SGFS higher than that occupied by their initial axonal segments (Puelles, unpublished results). Such movements were also deduced to occur in the lizard, in a pattern which appears entirely comparable in teleost [76]. Such cases of very extensive somatic translocation may entail secondarily a relocation of the axonal hillock. Other instances of the same phenomenon are found in the sequence of the multipolar Type I cells. The axonal origin of these cells apparently relocates as they migrate tangentially [36] and as the somata translocate upwards into layer 7 or higher up (displaced multipolar cells). Somatic translocation of Type III cells seems largely restricted to transposing the somata from the periventricular into the central/superficial strata and occurs incompletely in *G. galloti* when compared to the chick (see comments above on upper layer 7 as an analog of the avian inner SGFS).

The functional effects of such widespread somatic translocation remain obscure, though it is obvious that there is a trend among tetrapods to increase the range and incidence of this phenomenon at least in Type I and Type III cells, paralleling a similar trend to increase the number of Type II cells which migrate freely into the superficial and central tectal strata. The hypothesis of Nieuwenhuys [46] on a selective evolutive pressure to approximate sites of important synaptic input on distal dendrites to the soma or axonal hillock may be thus valid in many cases and may also explain diverse successive phases of translocation, which become eventually superposed one upon another as the tectal structure grows, the distances between layers increase and, eventually, novel afferents arrive (particularly in mammals, where cortical inputs increase considerably). Notwithstanding all these considerations, it is still baffling that the targeting of eye, head and tongue movements, the best understood function of the optic tectum, is conserved in urodeles, in which tectal radial migration of neurons is practically absent [47,59].

4.5. Temporal heterogeneity of cell types

An aspect we can address here only collaterally relates to the mechanisms which control the partly overlapping appearance of the three cell classes. Our earliest data showed that all three Types I–III were found at E32. In the chick, the Type I cells are produced between 3.5 and 7.5 days of incubation, Type II cells between 5.5 and 9 days of incubation and Type III cells start to appear at 7–7.5 days of incubation [29,49, Puelles, unpublished results]. In the lizard, the last postmitotic Type I cells were seen at E33 and the last postmitotic Type II and Type III cells at E34 (note that global incubation time of lizards is around 40 days [9], against 21 days in the chick, so that consecutive stages are days apart). Though they are incomplete, our data do suggest there is more temporal overlap of the diverse postmitotic cell types in the lizard than in the chick. This pattern is difficult to analyse statistically in Golgi-impregnated material, due to the essential unpredictability of this reaction.

Clonal data obtained in the chick tectum by Sanes's group with retroviral lacZ labeling show that early clones labeled at 3–4 days of incubation contain all cell types, including glial cells [13–15]. This indicates that early precursors for Type I neurons are also precursors for most, if not all, later appearing cell classes and subtypes. Gray and Sanes [16] reported tectal clones labeled on successive days up to 8 days of incubation, thus coinciding with the production peaks for Type II–III neurons. Though the authors center their analysis of the results on radial glia (tentatively identified as stem cells) and astrocytes, several of their figures show clones which lack neuronal derivatives below the SGFS (their Figs. 3A,B and 4B), or whose neuronal components correspond in shape or position exclusively to Type II cells (their Fig. 6A) or a mixture of Type II and Type III cells (their Figs. 6B and 7A,B). It is therefore possible that a cascade of specification decisions causes the different cell classes to be segregated from an initially common precursor. This mechanism may be regulated differently across vertebrates, notably by gradual differentiation of the stem cells.

It is further remarkable that anamniotes display a mode of tectal cell production, in which patterning and differentiation practically follow the end of proliferation at each tectal locus, so that differentiation of any cell type largely overlaps with the others [76], while proliferation continues on at the tectal edge during a long time or even during the whole life of the animal. In contrast, amniotes have adopted a mode of cell production in which proliferation is limited in time and overlaps considerably with parallel fate

patterning and differentiation processes; the latter may become partially non-overlapping in time for specific cell classes, as occurs in birds [49, present results]. The considerable developmental, structural and hodological homology persisting after this evolutive change suggests that the basic aspects of tectal histogenesis (the gradual production of Type I/II/III cells across the tectum and their subsequent mutual histogenetic and trophic interactions, among themselves as well as with efferent pathways and incoming afferents) may be more strongly influenced by intercellular molecular signalling, than by cell birthday patterns, cell clock mechanisms and/or clonal relationships. Or, in other words, the above underlined difference in proliferative mode may be irrelevant from the point of view of the invariant fundamental processes controlling the tectal histogenetic field in all vertebrates.

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