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Cytoarchitectonic subdivisions in the subtectal midbrain of the lizard *Gallotia galloti*

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Summary

Contemporary study of molecular patterning in the vertebrate midbrain is handicapped by the lack of a complete topological map of the diverse neuronal complexes differentiated in this domain. The relatively less deformed reptilian midbrain was chosen for resolving this fundamental issue in a way that can be extrapolated to other tetrapods. The organization of midbrain centers was mapped topologically in terms of longitudinal columns and cellular strata on transverse, Nissl-stained sections in the lizard *Gallotia galloti*. Four columns extend along the whole length of the midbrain. In dorsoventral order: 1) the *dorsal band* contains the optic tectum, surrounded by three ventricularly prominent subdivisions, named griseum tectale, intermediate area and torus semicircularis, in rostrocaudal order; 2) a subjacent region is named here the *lateral band*, which forms the ventral margin of the alar plate and also shows three rostrocaudal divisions; 3) the *basal band* forms the basal plate or tegmentum proper; it appears subdivided into medial and lateral parts: the *medial part* contains the oculomotor and accessory efferent neurons and the medial basal part of the reticular formation, which includes the red nucleus rostrally; the *lateral part* contains the lateral basal reticular formation, and includes the substantia nigra caudally; 4) the *median band* contains the ventral tegmental area, representing the mesencephalic floor plate. The alar regions (dorsal and lateral) show an overall cellular stratification into periventricular, central and superficial strata, with characteristic cytoarchitecture for each part. The lateral band contains two well developed superficial nuclei, one of which is commonly misidentified as an isthmic formation. The basal longitudinal subdivisions are simpler and basically consist of periventricular and central strata.

Introduction

Recent embryological and molecular advances on the isthmic organizer and on dorsoventral patterning have thrown new light on some position-dependent causal aspects of midbrain structure (see Discussion). It is widely assumed that early gene patterns and diverse gradients of morphogenetic molecules establish two-dimensional developmental fields within which specific neuronal populations and sets of axonal connections eventually develop as tridimensional complexes. Such studies aim to unravel the emerging rules of midbrain dorsoventral and anteroposterior patterning. However, experience in this field indicates that frequently the power of these approaches is handicapped by insufficient understanding of the topological structure and cytoarchitectonic diversity of the vertebrate midbrain, irrespective of previous efforts to systematize available data. For instance, it is not clear whether the midbrain shows transverse anteroposterior subdivisions and we lack a modern model of midbrain organization, which may help both the predictions and deductions made when interpreting gene functions during midbrain morphogenesis, and the comparative study of the differential evolution of midbrain structure in the diverse vertebrate lineages (i.e., distinction of conserved versus evolved features).

We aimed to provide a solution to this issue by reexamining in detail the morphology and apparent embryonic derivation of all detectable cytoarchitectonic elements of a reptilian midbrain. For practical reasons, we chose the lizard *Gallotia galloti*. The reptilian midbrain offers advantages for an inquiry on basic structural patterns, due to its simpler construction and limited topological deformation, as compared, for example, with the midbrain of birds or mammals (Senn, 1979). On the other hand, its intermediate range of cytoarchitectural differentiation, cell migration and cell aggregation in

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the mantle zone provides characteristics not generally available in anamniotes. It is thus one aim of this report to explore how far a purely cytoarchitectural analysis can help to define midbrain subdivisions which may be of general relevance in tetrapods and may be taken as the end result of midbrain molecular patterning.

The adult reptilian midbrain is delimited conventionally by coronal planes passing through the posterior commissure and behind the trochlear nerve root, without express reference to embryological data (ten Donkelaar, 1998). It is widely accepted that the midbrain is subdivided dorsoventrally into tectal and subtectal or tegmental portions, which possibly implies a longitudinal subdivision into alar and basal columns like in other vertebrates (Palmgren, 1921; Bergquist, 1953, 1954; Kuhlenbeck, 1975). However, the absence of a sulcus limitans in the midbrain of reptilian embryos and adults handicaps the usual procedure for locating the alar-basal boundary. Various maps of the reptilian mesencephalon in fact do not draw such a boundary (Frederikse, 1931; Senn, 1968, 1979; Cruce & Nieuwenhuys, 1974; ten Donkelaar *et al.*, 1987). From the developmental point of view sketched above, it should be of interest to know which parts of the subtectum may be attributed respectively to the alar or basal longitudinal columns, and what is their respective structural complexity. There is also ambiguity in the literature on which neural centers actually develop within the midbrain vesicle and thus are properly called "mesencephalic", as well as on what is their respective location and mutual relations within the midbrain.

The subtectum is reported to contain some very characteristic grisea, like the torus semicircularis, the oculomotor complex, the nucleus ruber and the substantia nigra, which are recognized as mesencephalic by all authors. There are also some less well defined cytoarchitectural entities, like the "torus semicircularis pars laminaris" or the "nucleus profundus mesencephali", whose localization by different authors is not unanimous. Other grisea are often expressly or implicitly included in the mesencephalon, even though there is good separate evidence that they belong either to the pretectum or to the isthmus (e.g., the trochlear, isthmic and interpeduncular nuclei, which develop in the isthmus and the n.lentiformis mesencephali, n. of the basal optic root and n.fasciculi longitudinalis medialis, which develop in the pretectum; see Discussion). Finally, there are a number of blank (uncharted) spaces in published maps of the reptilian and other vertebrate midbrains. On comparison with histological preparations, these correspond to well populated but still scarcely investigated areas of the subtectum. It was accordingly our aim to provide a full description of neuronal groups found strictly within the embryological limits of the lizard midbrain, classifying them consistently according to longitudinal, rostrocaudal and radial position. Examination of these points in a reptile is relevant for the understanding of changes in midbrain structure in other reptiles, and more generally in tetrapods.

The literature records three embryologically-based approaches which might help to resolve questions on the detailed structural organization of the mesencephalon in reptiles and other vertebrates. In the first place, Palmgren (1921) firmly established the rostral and caudal embryological boundaries of the mesencephalon in various vertebrates. His conclusions were corroborated by Rendahl (1924), Vaage (1969, 1973), Keyser (1972) and others (i.e.: Puelles & Martínez-de-la-Torre, 1987, Puelles *et al.*, 1987). Palmgren (1921) also put forward a rather detailed scheme for the longitudinal subdivision of the subtectum, distinguishing in it four columns (dorsal, lateral, medial and ventral) and several smaller parcellations. His material included cartilaginous and teleost fishes, amphibians, birds and mammals, but very few reptilian specimens. Unfortunately, Palmgren's seminal contribution on longitudinal parcellation of the vertebrate midbrain was largely disregarded by subsequent workers (the only exception known to us is Rendahl's 1924 study of the developing chick diencephalon, in which he corroborated Palmgren's longitudinal columns and extended them into the forebrain). The simpler schema preferred by Bergquist (1953), for example, divides longitudinally the subtectum only into dorsal and ventral areas. Bergquist and Källen (1953a,b, 1954), who, together with Vaage (1969), conceived a rostrocaudal division of the midbrain into two segments, named the two subtectal columns ventrolateral and ventral areas, respectively. Most authors, however, conceive a poorly structured midbrain tegmentum.

Separately, Senn (1970, 1979) developed a midbrain-based general conception of stratification in the reptilian brain. His periventricular, central and superficial layers are inspired in tectal stratification (Ramón, 1896; Huber & Crosby, 1933). Senn's studies highlighted the analysis of stratification as a useful tool for understanding the changing structure of the brain wall along the radial dimension. Actually, a general radial subdivision of the neural tube into three strata was also conventionally contemplated by previous embryological workers (e.g., stratum cellulare internum/intermedium/superficiale of Palmgren, 1921; Rendahl, 1924).

Finally, Nieuwenhuys (1974) developed a procedure for topological representation of neural formations upon a two-dimensional map, irrespective of their different adult radial positions. This consists basically in projecting graphically the elements to be mapped into the ventricular contour, using the deformed internal radial lines revealed by radial glia and/or blood vessels as guides. Theoretically, this graphic operation visualizes the nuclei close to the place where they originated. Topological maps of the midbrain in a turtle and a lizard are available (Cruce & Nieuwenhuys, 1974; ten Donkelaar & Nieuwenhuys, 1979).

The present investigation reexamines the structural subdivision of the adult mesencephalon of the lizard *Gallotia galloti*, using jointly the interpretational systems offered in the work of Palmgren (1921), Senn (1970, 1979) and Nieuwenhuys (1974). This led us to search for transverse segmental boundaries, longitudinal columns and radially stratified grisea. Our study emphasizes subtectal structure, since the optic tectum has been described extensively (Ramón, 1896; Senn, 1968, 1979; see for reviews Northcutt, 1984; ten Donkelaar, 1998).

Methods

Eight young lizards (*Gallotia galloti*) were used for this study. Some of these animals ($n = 3$) were deeply anesthetized with ether, perfused with 4% phosphate-buffered paraformaldehyde, and then postfixed overnight. The others ($n = 5$) were perfused with phosphate-buffered saline and the brains were extracted for *in vitro* HRP-labelling experiments (not described in this paper; protocol as in Diaz *et al.*, 1992a). These specimens were immersion-fixed overnight after 24–36 hours in the culture medium. All the brains were stripped of meninges. After cryprotection in 30% sucrose, the fixed brains were serially sectioned in a cryostat in sagittal ($n = 4$), horizontal ($n = 1$) and transverse ($n = 3$) planes. Sections were cut at 10 μm from the perfused brains and at 50 μm from the immersion-fixed brains. All sections were stained with acetate-buffered cresyl violet (in the experimental cases, as a counterstain to the HRP reaction). Other *Gallotia* brains sectioned in paraffin, stained with the luxol-fast-blue myelin procedure and counterstained with cresyl-violet were available for comparison.

A series of selected transverse 50 μm-thick sections through one midbrain served to prepare a detailed atlas of cytoarchitectonic subdivisions, and to obtain a topological midbrain reconstruction according to Nieuwenhuys (1974), in which the diverse boundaries were projected along curved radial lines onto the ventricular surface. The nomenclature used in this report follows Huber and Crosby (1926, 1933) and Senn (1970, 1979) in the cell strata, while the names of cell groups and fiber tracts follow in general the conventional terminology (Cruce & Nieuwenhuys, 1974; ten Donkelaar & Nieuwenhuys, 1979; ten Donkelaar, 1998), although we introduced some changes according to the embryological origin of the cell masses (i.e., excluding those nuclei which are isthmic or pretectal). Several cell groups and other features are first identified in the subtectum of the lizard *Gallotia* and new terms are proposed for them.

Results

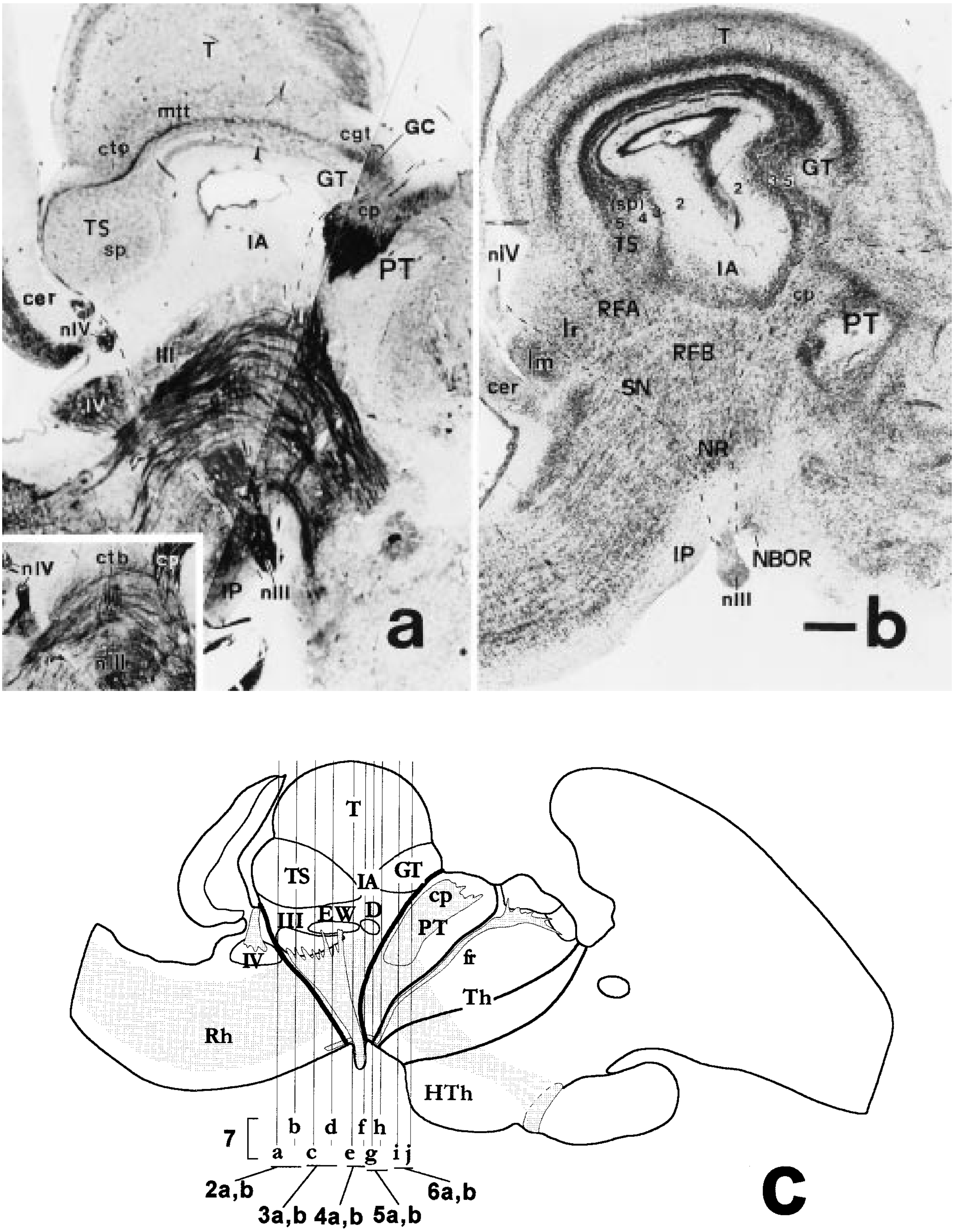
BOUNDARIES OF THE MESENCEPHALON

The embryological and comparative definition of midbrain limits given by Palmgren (1921) agrees with recent data on gene expression (Rubenstein & Puelles, 1994). Notably, the isthmo-mesencephalic boundary is now accepted to coincide with the caudal boundary of expression of the gene *Otx-2* in the midbrain (Millet *et al.*, 1996) and the pretecto- or diencephalo-mesencephalic boundary coincides with the caudal boundary of expression of the gene *Pax-6* in the diencephalon (Puschel *et al.*, 1992; MacDonald *et al.*, 1994; Stoykova *et al.*, 1996; Mastick *et al.*, 1997; Warren & Price, 1997; Puelles *et al.*, 2000). Corresponding embryological evidence for these boundaries in the lizard *Gallotia galloti* was obtained by Trujillo and Puelles (unpublished). These transverse boundaries are best observed in sagittal sections, since their topography is modified during development by the progressive bending of the longitudinal axis of the brain at the cephalic flexure.

The rhombo-mesencephalic (or isthmo-mesencephalic) limit is marked in the mantle zone by a clearcut gap (gap "z", or fissura rhombo-mesencephalica of Palmgren, 1921) that separates clearly the neighboring neuronal populations and the fibers coursing transversely in both region (Fig. 1a–c). Dorsally, the gap passes behind the external bulge of the torus semicircularis, just in front of the trochlear nerve decussation (Fig. 1a); dorsolaterally it courses between the magnocellular and rostral isthmic nuclei (Im, Ir; Fig. 1b; the latter actually lies in the midbrain and therefore is not isthmic; see this issue resolved for the chick in Vaage, 1973; Puelles & Martínez-de-la-Torre, 1987; Marín and Puelles, 1994). Ventrally, the gap divides the oculomotor nucleus from the trochlear nucleus (Fig. 1a) and extends behind the substantia nigra and in front of the rostralmost raphe nucleus, surfacing behind the oculomotor root, just in front of the interpeduncular nucleus (Fig. 1a–c). This boundary is also clearly marked at the ventral midline by the end of the palisade-like floorplate raphé of the rhombencephalon at the rostral end of the fovea isthmi; rostral to this, the median radial glia cells suddenly change into the less conspicuous floorplate cells of the midbrain and diencephalon.

The diencephalo-mesencephalic (or pretecto-mesencephalic) limit is less obvious cytoarchitecturally, because mesencephalic grisea are juxtaposed to the pretectal ones without a separating cell-poor gap. However, embryological evidence (Palmgren, 1921; Rendahl, 1924; Bergquist, 1953, 1954; Vaage, 1969; Trujillo & Puelles, unpublished) suggests a plane passing just behind and parallel to the posterior commissure, dorsally, and just behind the nucleus of the basal optic root, ventrally, as the most appropriate boundary (Fig. 1a and b). This plane lies slightly in front of the oculomotor nerve root.

These topologically transverse limits of the midbrain converge basally (as seen in sagittal sections), due to the inflexion of the axis at the cephalic flexure and the disproportionate growth of the optic tectum dorsally. This gives to the mesencephalon a wedge shape (Fig. 1c). The oblique topography of these boundaries in the adult brain explains the tendency of many authors studying cross-sections to incorporate erroneously isthmic or pretectal cell masses into the midbrain.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The ventricular cavity of the mesencephalon is subdivided into a caudal narrow "aqueductal" portion, coinciding with the region occupied by the torus semicircularis, and a rostral enlarged portion, which displays wide lateral recesses inside the optic lobes (Fig. 1a).

LONGITUDINAL, TRANSVERSAL AND RADIAL SUBDIVISIONS: OBSERVATIONS IN TRANSVERSE SECTIONS

Our cytoarchitectonic analysis of the subtectum in *Gallotia galloti* is presented in a series of sectioning levels, illustrated in photographs (Figs. 2–6) and line drawings (Fig. 7). These sections (and also others intercalated in between) served to draw a topological map of the midbrain grisea (Fig. 8b), using the procedure of Nieuwenhuys (1974). There are various graphical conundrums caused by the invaginated ventricular relief at the rostral and caudal poles of the mesencephalon, as well as by its global wedge shape, which necessarily leads to successive changes in the topological section plane (oblique to transverse to oblique). We therefore only claim to provide a tentative solution in the mapping of our Fig. 8b. However, we think that this reconstruction, and the more schematic version in Fig. 8a, correctly visualizes the relative connectedness of the diverse longitudinal and transverse regions, as revealed by their rather distinct cytoarchitectural properties.

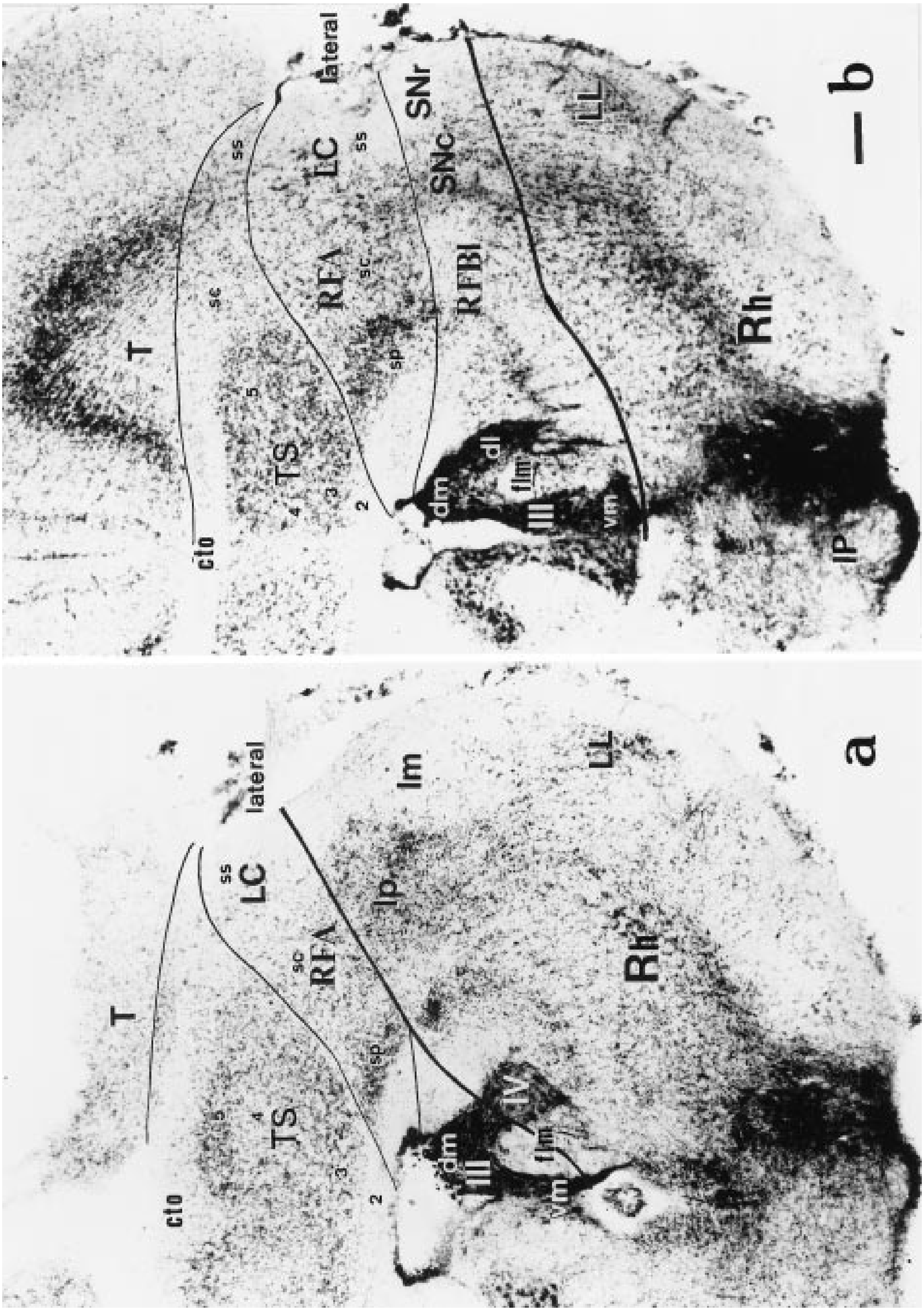
We found there are at least two alar longitudinal parts of the midbrain, named here *dorsal* and *lateral* bands. The optic tectum is predominant in the dorsal band, though it is complemented rostrally, ventrally and caudally by the griseum tectale, the intermediate area and the torus semicircularis, respectively. The "lateral band" represents the topological ventral margin of the alar plate (Figs. 2–8); curiously, this band expands dorsalward close to the isthmo-mesencephalic boundary, where it approaches the roof plate just caudal to the torus semicircularis (see schema in Fig. 8a). This laterocaudal midbrain domain contains the so-called nucleus isthmi rostralis (Sereno, 1985; Sereno & Ulinski, 1987). Since the latter term is topographically wrong—proper "isthmic" nuclei lie outside the midbrain—and this may be confusing for causal developmental analysis, we propose to rename it "laterocaudal mesencephalic nucleus" (LC; Figs. 2–4, 7a–e, and 8a and b). The alar mesencephalon is thus built by the griseum tectale/tectum/toral complex and an underlying lateral band with distinct structure. On the other hand, the mesencephalic tegmentum proper appears subdivided longitudinally into a basal band (basal plate) and a median band (floor plate) (Fig. 8a and b). These are described in more detail below.

Dorsal band

The optic tectum will not be described here. We concentrate instead on the other formations within this band. These are characterized by protruding more or less into the ventricle, in contrast with the evaginated tectum. We distinguish at least three different grisea along this bulge: the torus semicircularis, the intermediate area and the griseum tectale (Figs. 1 and 8). These are next described in caudorostral order.

The torus semicircularis is more massive caudomedially, where it replaces the tectum at the dorsal midline. This portion bulges outwards behind the caudal pole of the optic tectum, forming an inferior-colliculus-like structure (TS; Figs. 1a, 2a, and 7a). The torus also protrudes into the ventricular cavity, where the right and left tori semicirculares are partly fused together (Figs. 2a and b and 7a and b). Structurally, the caudomedial part of the torus is formed by a much enlarged, spherical periventricular stratum, that is separated from a thinly populated central/superficial stratum by the myelinated fibers of the mesencephalic trigeminal tract and the intertoral commissure (Figs. 1a and 2a and b). The periventricular stratum is divided into a thin, pale-staining, deep lamina (continuous with tectal layer 3; terminology of Senn, 1979) and a bilayered outer cell mass. The inner layer of the latter has a neuropile with thinly myelinated fibers, terminal arborizations and some interspersed small cells, while the outer layer has slightly larger and more numerous basophilic cells and seems comparable to the paratorus described in *Natrix* (Senn, 1979) (Figs. 1a and 2a and b). This bilayer is globally continuous with tectal layer 5. A thick fibrillar layer 2, containing some thin myelinated fibers, and a thin plexiform layer 4 are also present within the caudomedial torus (Fig. 1a). However, the neuropile of the toral layer 4 is characteristically separated from the adjacent tectal layer 4 by a small transitional zone in which the cellular laminae 3 and 5 are fused together (arrow; Figs. 3b and 4a).

Fig. 1. General layout of the lizard midbrain. (a, b) Microphotographs of two sagittal sections from different young *Gallotia* lizards, showing the location of the caudal and rostral transverse boundaries of the mesencephalon (dash lines), as well as several midbrain formations and tracts. Rostral is to the right. The bar = 200 μm. The section in (a) is myelin-stained and (b) shows a Nissl-stained section. A cell- and myelin-poor gap marks the limit between the isthmus and the midbrain (a, b). Note that some myelinated tracts run parallel to the transverse boundaries, as is the case of the posterior commissure (cp), or the crossed tectobulbar tract (cp,ctb; in a, and in the inset); the fibers of the longitudinal bundles follow the bent brain longitudinal axis (a). (c) Schematic drawing illustrating the relevant transverse boundaries and general topographic relationships of some midbrain centers. The posterior commissure, longitudinal fibers and oculomotor/trochlear nerves are shaded in gray. The section levels illustrated in Figs. 2–6, whose interpretation is also collected in Fig. 7a–j, are indicated.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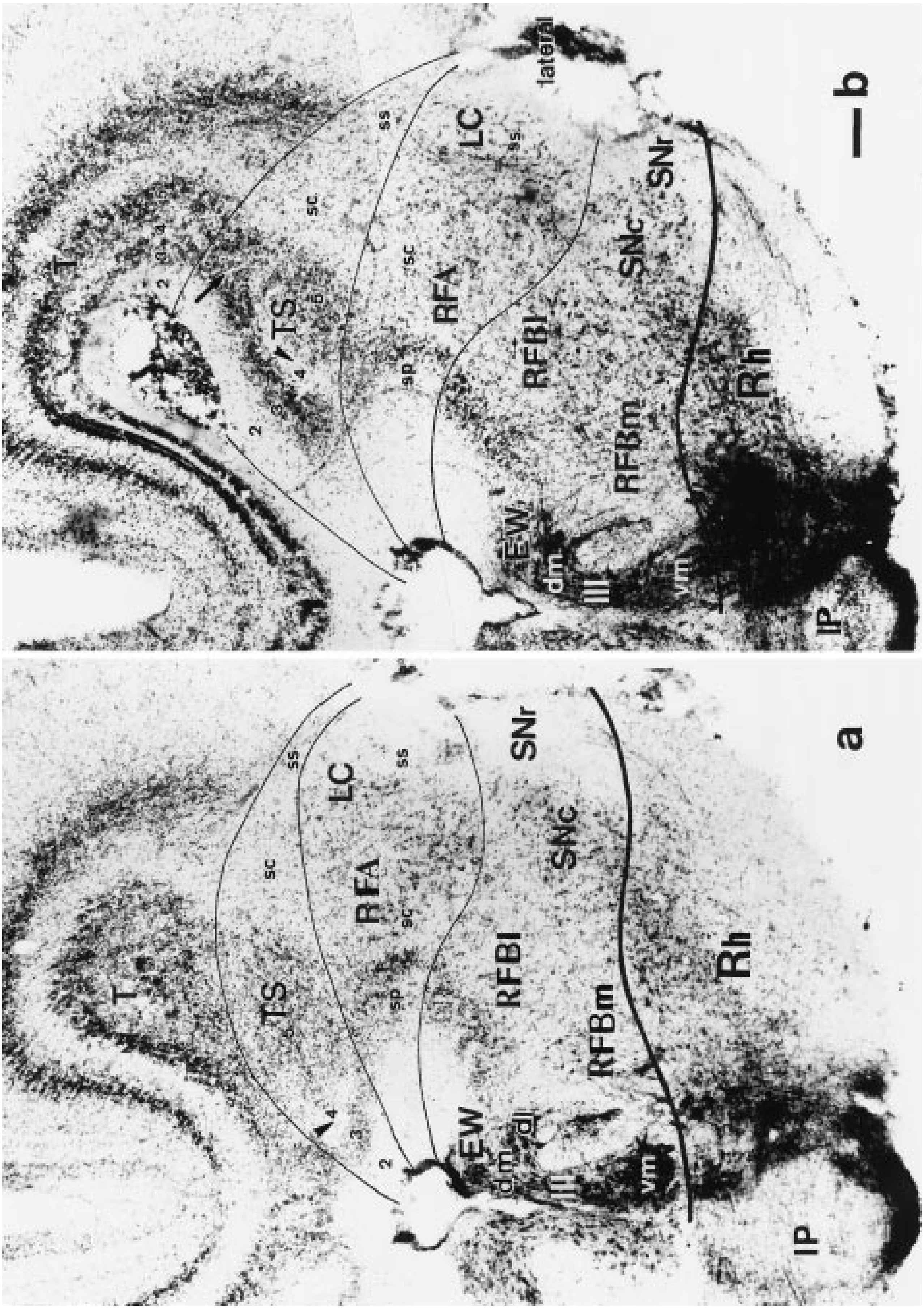
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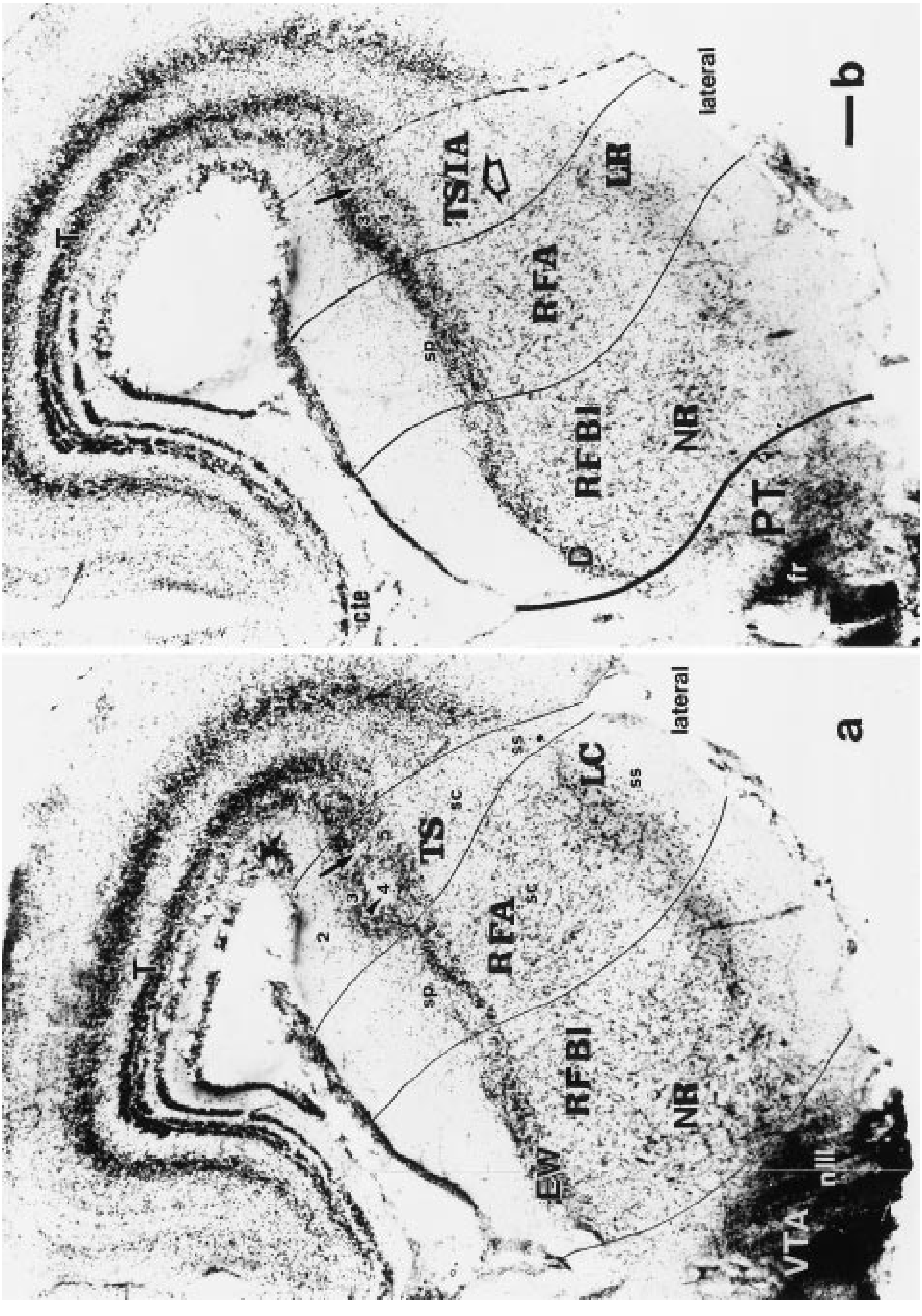
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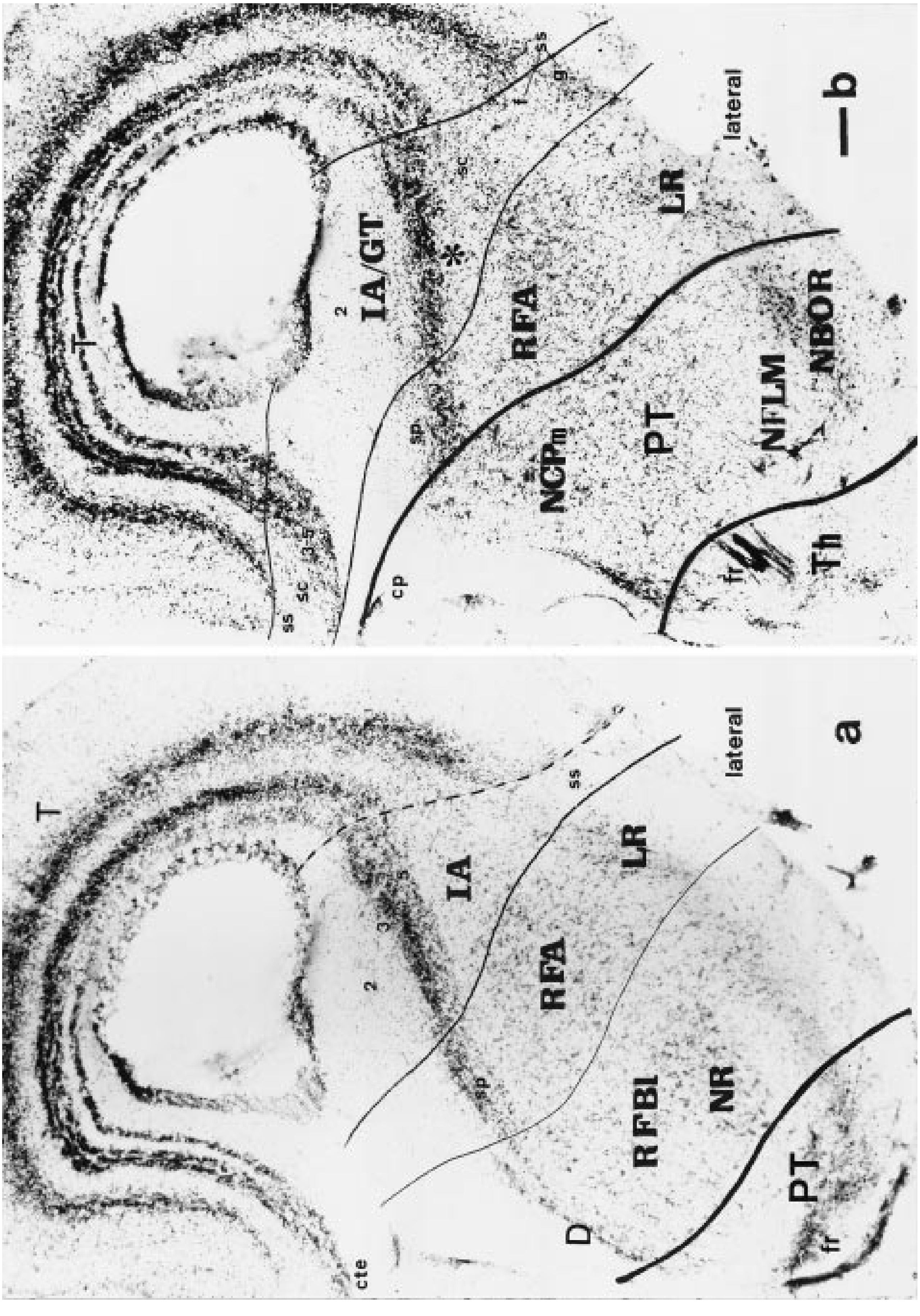
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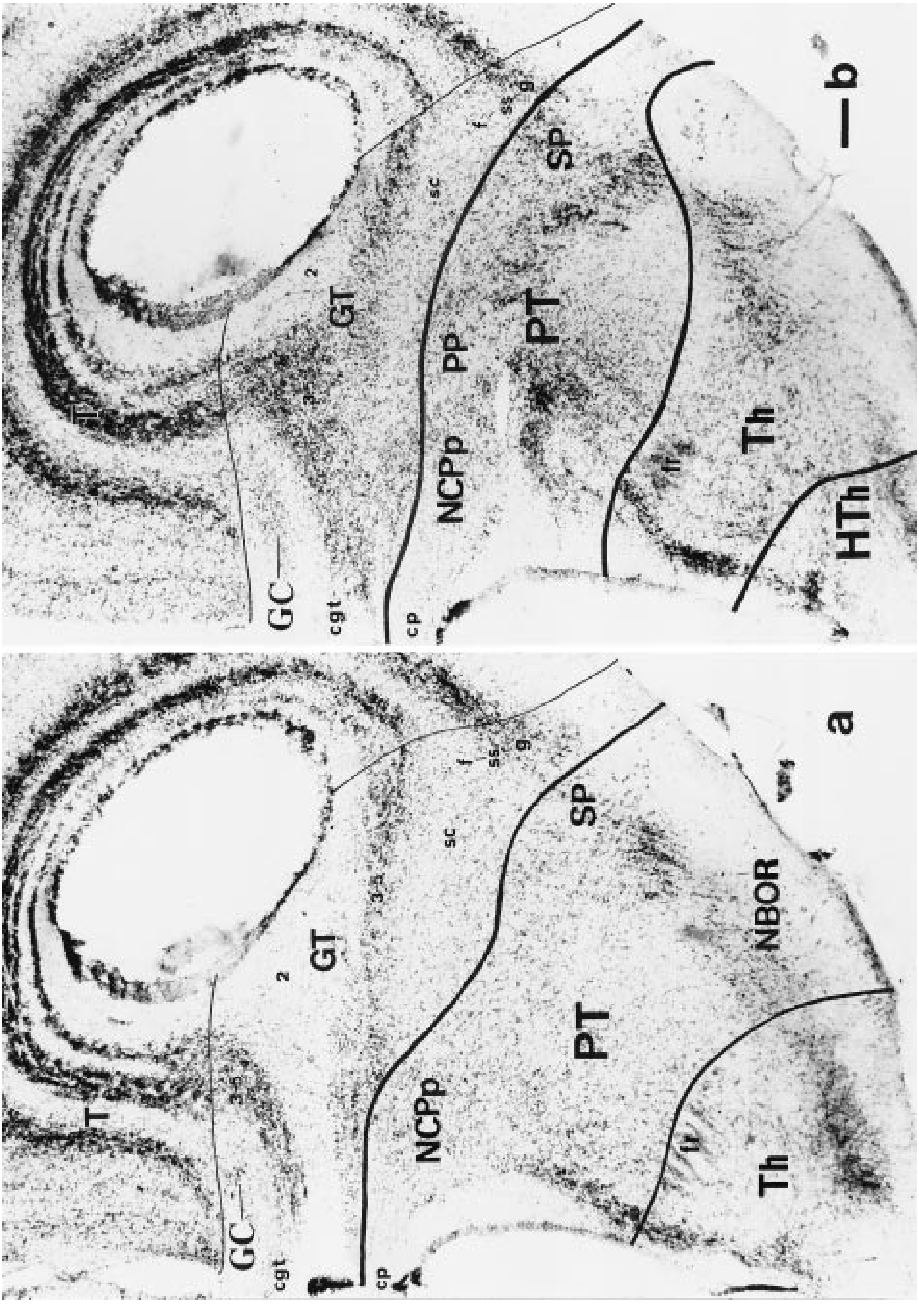
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The torus semicircularis complex thins out gradually as it extends lateroventrally under the tectum (topologically ventralwards–see Fig. 8a), the chief change being a drastic reduction in its surface extent, probably as a result of the circumferential expansion of the adjacent tectum (Figs. 3a and b, 4a, and 7c–e). The toral stratum centrale simultaneously expands with a reticular appearance, laxer than the tectal central stratum (Figs. 2b, 3a and b, 4a, and 7b–e). The periventricular cell layer 3 becomes thicker and more densely populated in the lateroventral torus. The torus layer 4 neuropil remains separated from the tectal plexiform layer 4 by a short portion in which layers 3 and 5 are fused together (marked by an arrow in the figures). The layer 4 neuropil diminishes progressively in size in the rostralmost sections (arrowhead; Figs. 3a and b and 4a). The periventricular outer cellular lamina (layer 5) also becomes gradually poorer in cells and less overtly bilayered, and can be distinguished from the adjacent layer 5 of the tectum by its lower cell density (Figs. 3a and b and 4a).

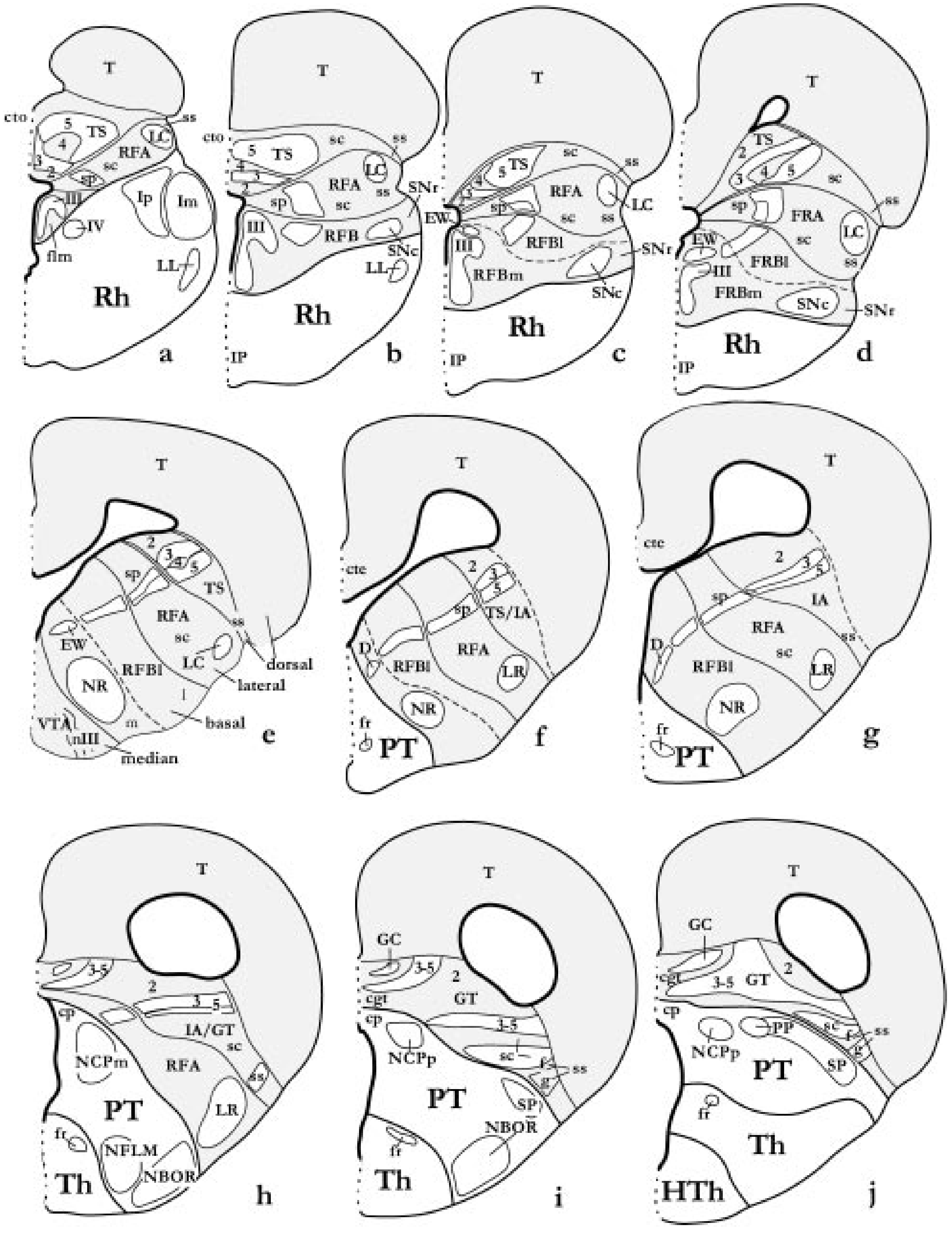
The intermediate area is found laterally in the middle of the midbrain and may be conceived as a transitional region between the torus and the griseum tectale (IA; Figs. 4b, 5a and b, 7f–h, and 8a and b). It differs from the torus semicircularis and the optic tectum in various aspects: the periventricular stratum is modified, with a thicker plexiform layer 2, a more dense and basophilic cellular layer 3, practically no layer 4 neuropile, and the cellular layer 5 is much reduced in thickness and cell density, to the point that both its cellular laminae seem continuous with tectal layer 3, instead of with layer 5 (IA; Fig. 4b). Additionally, the IA stratum centrale keeps the typical shape and position adjacent to the central tectal stratum that was described for the torus, but shows a number of large-sized neurons in its deeper part (open arrow; Fig. 4b). Such cells are not present in the stratum centrale of the torus semicircularis.

The griseum tectale is the rostral end of the dorsal band. It extends dorsomedially up to the midbrain roof plate, where it replaces the tectum at the rostral constriction separating the optic lobe and the pretectum (GT; Figs. 5b, 6a and b, and 8). The griseum tectale is a stratified complex that protrudes into the rostral part of the midbrain ventricle (GT; Figs. 5b, 6a, and 7h and i). Laterally, its superficially compressed shape is similar to that of the rostrolateral torus semicircularis and intermediate area (Fig. 6a and b), but its lamination pattern is different. It has a reduced periventricular stratum that is limited to a rather thick plexiform layer 2 and a single compact cellular layer 3 (these form the stratum griseum et fibrosum periventriculare of the GT), thus lacking the cell-sparse layer 5 observed in the intermediate area (compare Figs. 6a and 4b). Its central stratum displays an inner stratum album centrale with few neurons and an outer, more populated stratum griseum centrale, as occurs in the tectum. Myelinated fibers in the stratum album centrale cross the dorsal midline in a "commissure of the griseum tectale" that lies in between the posterior commissure and the tectal commissure (cgt; Figs. 1a, 6a and b, and 7i and j; see also Pérez-Santana *et al.*, 1996). The stratum griseum centrale is populated by small and medium sized neurons; a particular condensation of similar cells found dorsomedially near the cgt may be defined as a separate 'griseum commissurale' (GC; Figs. 1a, 6a and b, and 7i and j). The griseum tectale also has a superficial stratum or stratum griseum et fibrosum superficiale (sgfs) that is simpler than the tectal sgfs. It consists of an inner, scarcely populated plexiform superficial layer, and an outer cellular superficial layer with medium sized neurons placed below a subpial axonal stratum (Figs. 5b and 6a and b). The latter two laminae of the GT are traversed by optic tract fibers; these laminae are not present over the griseum commissurale. The area occupied by the griseum commissurale expands superficially as it extends dorsomedially towards the cgt commissure; here it has maximal superficial area and even bulges a little outwards medial to the border of the optic tract and caudal to the posterior commissure. This mimics the external bulge of the torus semicircularis at the caudal pole of the mesencephalon in a minor size range (Figs. 1a, 5b, 6a and b, and 7h–j).

Lateral band

This distinct longitudinal band corresponds essentially to Palmgren's (1921) lateral column, with some

Figs. 2–6. Series of 10 selected transverse 50 μm-thick sections through the midbrain of *Gallotia galloti* (see section levels in Fig. 1c). This brain received a HRP deposit in the rostral interpeduncular nucleus at the level of Fig. 2, which mainly labeled ascending axons into the diencephalic floor and scarce neurons in the midbrain reticular formation; moreover, label difused into the oculomotor nerve root, which serendipituously served to label precisely the oculomotor nucleus (Figs. 2 and 3). The order of the sections within the series is caudorostral. The microphotographs show one side of the Nissl-counterstained sections, dorsal being oriented upwards. Thick lines drawn in mark the transverse boundaries of the midbrain, either with the rhombencephalon (Rh), caudally (Figs. 2a and 3b), or the pretectum (PT), rostrally (Figs. 4b–6b). Note that caudal sections intersect obliquely the isthmomesencephalic limit, showing isthmic structures underneath it. Likewise, sections rostral to the oculomotor root intersect obliquely the pretecto-mesencephalic limit, showing various diencephalic grisea underneath it. The thin lines trace the boundaries between the observed midbrain longitudinal subdivisions. Each longitudinal column within the mesencephalon is subdivided in periventricular (sp), central (sc) and, eventually, superficial (ss) strata (Senn, 1979). Bar $= 100$ μm.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modifications. According to our observations, the lateral band represents a layered complex whose typical structure runs longitudinally all along the mesencephalon, just below the dorsal band, but it also extends dorsally behind the caudomedial torus formation. That is, the lateral band represents the margin of the mesencephalic alar plate both with regard to the subjacent tegmentum (basal plate) and the isthmo-mesencephalic boundary (Fig. 8a and b). Like the dorsal band, the lateral band can be roughly subdivided in larger caudal and rostral sectors, with a small transitional zone in between.

Caudally, the lateral band first appears just caudal to the external torus bulge, separating it from isthmic and cerebellar centers. As occurs generally in the alar plate (Senn, 1979), the lateral region consists of periventricular, central and superficial strata. The periventricular stratum is formed by a sizeable deep plexiform layer and a dense cellular plate (lateral; Fig. 2a). The latter is distinctly separated from the paler-staining periventricular stratum of the torus semicircularis by a thin cell-poor gap. The lateral central stratum is a thick intermediate layer, populated by a mixture of small-, medium- and large-sized neurons, which apparently includes the vaguely defined “n.profundus mesencephali” (Cruce & Nieuwenhuys, 1974). This intermediate layer may be conceived as forming the caudal part of an alar reticular formation (RFA). The overlying superficial stratum is formed by a magnocellular nucleus which lies immersed in a subpial fiber tract, the ventral tectothalamic tract. This superficial nucleus is known as “nucleus isthmi (magnocellularis) rostralis” (Sereno & Ulinski, 1987), though it obviously forms within the mesencephalon. To avoid confusion and properly indicate its topography, we propose to name it ‘laterocaudal mesencephalic nucleus’ (LC; Fig. 2a).

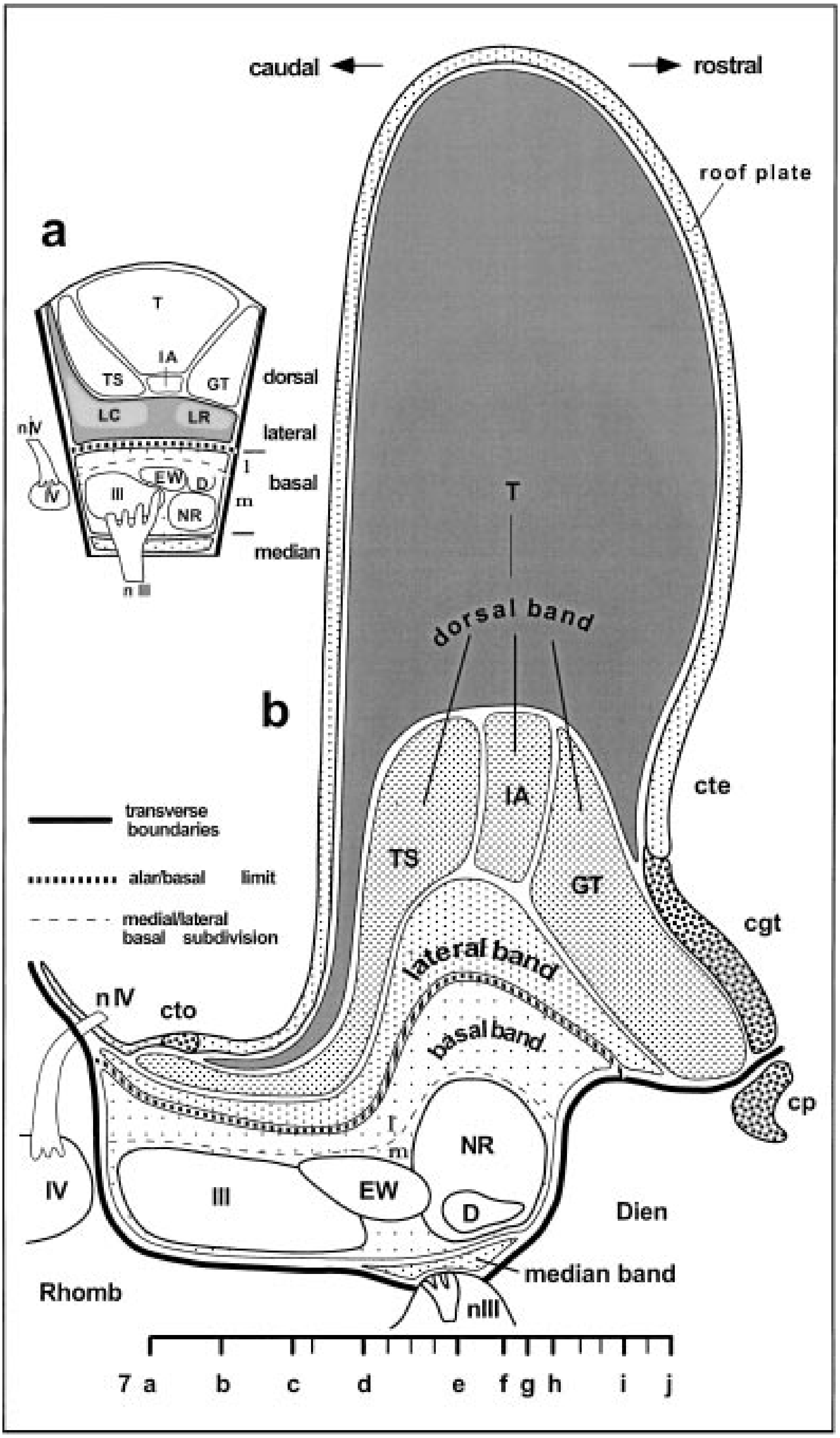
In sections rostral to the point where the caudal lateral band first contacts the mesencephalic basal plate, its periventricular cell plate has strongly basophilic cells and a neuropile populated by few small neurons appears deep to this cell plate (lateral; Figs. 2b, 3a and b, and 4a). There are also numerous large neurons in the corresponding ventral part of the lateral central layer or alar reticular formation (RFA; Figs. 2b and 3a and b). The laterocaudal mesencephalic nucleus ends in an enlarged rostroventral portion (LC; Figs. 3a and b and 7c and d) and, after a brief transition occupied by less distinct, smaller neurons, is substituted in more rostral sections by another compact superficial nucleus, which also contains slightly smaller neurons. We called it the laterorostral mesencephalic nucleus. This extends to the rostral limit of the lateral band at the mesencephalic border with the pretectum (LR; Figs. 4b, 5a and b, and 7f–h).

Basal plate

The analysis of this structural region is most affected by the wedge-shape deformation of the mesencephalon. Thus, in our selection of transverse sections only one of them represents integrally the midbrain basal plate, passing at the level of the oculomotor nerve root (Fig. 4a; see section level in Fig. 1c). More caudal sections intersect obliquely the isthmo-mesencephalic boundary, thus apparently showing various isthmic structures “ventrally” (Figs. 2, 3, and 7a–d). Similarly, sections rostral to the oculomotor root intersect obliquely the pretecto-mesencephalic limit and show various basal diencephalic grisea apparently “ventral” to it (Figs. 4b and 7f; compare with Fig. 1c). Following Palmgren (1921), we divided the basal plate into medial and lateral regions.

The topological mapping indicates that the medialmost basal nuclei are the oculomotor nucleus, the accessory nucleus of Edinger-Westphal and the small-celled nucleus of Darkschewitsch (Fig. 8b). These form a caudorostrally slightly overlapping series of periventricular cell masses, with the somatomotor III nerve neurons being caudalmost (III; Figs. 2a and b, 3a and b, and 7a–d), the accessory nucleus lying in an intermediate position (EW; Figs. 3a and b, 4a, and 7c–e) and the n.Darkschewitsch rostralmost (D; Figs. 4b and 7f). Ventral to these periventricular grisea, that is, in the corresponding central stratum, there is the fasciculus longitudinalis medialis (flm) and the medial reticular tegmental region (RFBm). The latter is characterized by a dispersed neuronal population, except in the rostral half of the mesencephalon, where the magnocellular red nucleus appears (NR; Figs. 4a and b, 5a, and 7e–g). Lateral to this medial tegmental region there is a lateral basal tegmentum. This shows a rather compact periventricular cell plate and a reticular central stratum extending radially to the ventrolateral mesencephalic surface (RFBl; Figs. 2b, 3a and b, 4a and b, and 5a). The midbrain part of the substantia nigra, a migrated cell population (Medina *et al.*, 1994), appears superficially

Fig. 7. (a–j) Line drawings of the 10 transverse sections photographed in Figs. 2–6. The location of the transverse sections is indicated in Fig. 1c. The shaded areas represent regions found within the transverse limits of the midbrain. The main distinct nuclei or compact cellular laminae of the subtectum were left unshaded for easier localization. The four longitudinal bands of distinct structure are labeled in (e) only, for clarity, but are uniformly delimited one from another by continuous thin lines. The dash lines show secondary subdivisions. The basic periventricular, central and superficial strata are labelled as sp, sc and ss, respectively. The sublaminae of the toral periventricular stratum are indicated with the usual numbering system (Ramón, 1896). Transverse intersegmental boundaries of the midbrain and diencephalon are drawn as thicker black lines.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dorsal band
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transverse boundaries
alar/basal limit
medial/lateral basal subdivision
lateral band
basal band
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within the caudal part of this tegmental region, in the immediate neighborhood of the isthmo-mesencephalic boundary and behind the oculomotor root fibers (SN; Figs. 2b, 3a and b, and 7b–d).

It is difficult to visualize rostrocaudal subdivisions of the basal plate in cross-sections, but such subdivisions are suggested by our sagittal myelin-stained sections. These show that the fan-shaped, crossed tectobulbar tract (ctb; Fig. 1a and inset) collects ventrally into a compact bundle at the center of the midbrain tegmentum, thus dividing the caudal part of the basal plate from the rostral one. In many sections this gives the impression of a third, ctb-related, intermediate partition of the mesencephalic basal reticular formation (Fig. 1a and inset; Fig. 1b). A tripartite constitution is also supported by connectivity data (Díaz, 1991). We therefore postulate caudal, intermediate and rostral distinct regions of the basal plate; these are held to be aligned with the three corresponding regions of the alar plate.

Median band

We distinguish this very small territory (Figs. 4a and 8b) as a separate longitudinal entity for coherence with developmental studies. These suggest a retarded heterochronic pattern of neurogenesis in a band adjacent to or overlapping the mesencephalic midline raphe (and continuing rostrally into the hypothalamus). This floorplate-related band is therefore histogenetically distinguishable from the typically precocious basal plate (paramedian band of Puelles *et al.*, 1987). A clearcut derivative of the median band in the midbrain is the ventral tegmental area, a ventral aggregate of small neurons which partially migrate subpially lateral to the oculomotor root (VTA; Figs. 4a and 7e; Díaz & Puelles, 1992a,b; Medina *et al.*, 1994). The VTA is only found rostral to the interpeduncular nucleus.

Discussion

In the present report we studied the internal organization of the subtectum of the lizard *Gallotia galloti*, exploring the usefulness of tridimensional topological characterization of grisea in the antero-posterior, dorso-ventral and radial dimensions. In the following sections, the delimitation approach used, the resulting subdivisions and the specific midbrain cell populations identified will be discussed.

MIDBRAIN DELIMITATION

One of the problems we addressed was to reexamine what should be regarded as mesencephalic and what not, consistent with the best available embryological information. We adopted Palmgren's (1921) definition of the midbrain boundaries, which was based on the comparison of series of embryonic and adult brains of several vertebrates. We corroborated that a cell-poor gap formed at the isthmo-mesencephalic boundary neatly separates the isthmic derivatives from the midbrain. Fate maps performed at early stages in the chick show that the initial mesencephalic vesicle includes the prospective midbrain and a part of the prospective isthmocerebellum (although the prospective boundary is already clearly marked by the caudal limit of *Otx-2* expression; Millet *et al.*, 1996; Puelles *et al.*, 1996b). Morphological changes subsequent to anteroposterior patterning induced by the isthmic organizer and the resulting differential growth give rise to transverse regions that contribute separately to the mature midbrain and to the isthmic and cerebellar structures (Vaage, 1973; Puelles & Martínez-de-la-Torre, 1987; Martínez & Alvarado-Mallart, 1989; Hallonet *et al.*, 1990; Marín & Puelles, 1994; Millet *et al.*, 1996; Puelles *et al.*, 1996b; Joyner, 1996). Vaage (1973) convincingly showed that all components of the alar isthmic nuclei in the chick originate from the rostralmost rhombencephalon, except the nucleus isthmi principalis pars magnocellularis, which has a mesencephalic origin; this nucleus clearly corresponds to the laterocaudal mesencephalic nucleus in the lizard (rostral isthmic nucleus of Sereno & Ulinski, 1987). Vaage's conclusions on isthmic nuclei were experimentally corroborated by Marín and Puelles (1994), whose data also located the rostralmost raphé nuclei and the interpeduncular nucleus in the isthmus. Likewise, the trochlear nucleus arises from the rhombencephalic isthmus (Vaage, 1973; Müller & O'Rahilly, 1988; Lumsden & Keynes, 1989; Noden, 1991;

Fig. 8. (a) Simplified flatmap diagram of topological relationships in the lizard midbrain. The thick black lines represent the transverse rostral and caudal boundaries of the midbrain. Each area represents a tridimensional histogenetic complex extending from the ventricle to the pial surface of the midbrain, irrespective of whether in some cases we indicated specific nuclei lying at a particular depth level. In this schema only topological relationships are respected and the relative sizes of the regions have been normalized to the possible proportions at early embryonic stages (no morphogenetic deformation of the midbrain vesicle). (b) Topological flatmap of midbrain structures (commissures and distinct grisea), represented as projected along curved radial-glial lines onto the graphically reconstructed ventricular surface. Note midbrain boundaries (thick black lines) deformed into a wedge shape. This map is based on the sections shown in Figs. 2–7, plus other 7 intercalated sections (positions shown as thin lines in the bottom ruler). Whereas size relationships theoretically are more realistic than in (a), the differential extent of the ventricular surface, particularly in the caudal midbrain (where midline fusion of the toral protrusions may have further decreased the available surface), possibly leads to underrepresentation of the nuclei and tectal portions associated to the torus semicircularis. The positions of several commissures in the brain roof are indicated.

Gilland & Baker, 1993; Fritzsch *et al.*, 1995; Bass & Baker, 1997).

The pretecto-mesencephalic boundary poses a similar problem. Often the pretectum has been taken as a "transitional area" between the diencephalon and the mesencephalon in reptiles (Huber & Crosby, 1926; Butler & Northcutt, 1973; Senn, 1979; Smeets *et al.*, 1986a), or has been divided into "mesencephalic" and "diencephalic" parts (Kuhlenbeck, 1973). The tentative postulation by His (1893) of a diencephalo- mesencephalic boundary that passed across the posterior commissure and close to the mammillary bodies in human embryos probably was determinant for this viewpoint, since it was widely used without any experimental support in the subsequent comparative neuroanatomical literature. This is also the original source for the idea that the midbrain tegmentum (basal plate) extends far rostral to its alar plate into the mammillary area, thus forming the so-called prerubral tegmentum (Kuhlenbeck, 1973, 1975; Senn, 1979). Curiously, His (1893) intended to provide only a very tentative, provisional reference until more data accrued. In contrast, Palmgren (1921), Rendahl (1924) and Vaage (1969) embryologically redefined the rostral boundary of the midbrain as passing just behind the posterior commissure and parallel to its fibers; this view has been corroborated by various additional embryologic evidence (Keyser, 1972; Puelles *et al.*, 1987; Puelles & Trujillo, unpublished). Moreover, this boundary correlates with recent molecular data on the caudal limit of the expression domain of the gene *Pax-6* in several vertebrates (Puschel *et al.*, 1992; MacDonald *et al.*, 1994; Stoykova *et al.*, 1996; Mastick *et al.*, 1997; Warren & Price, 1997; Puelles *et al.*, 2000).

According to these definitions, a few pretectal nuclei often are incorrectly assigned to the mesencephalon. For instance, the nucleus lentiformis mesencephali is located superficially within the juxtacommissural/commissural pretectum, rostral to the griseum tectale (see Butler & Northcutt, 1973; Smeets *et al.*, 1986a). The pretectal tegmental area lies rostral to the red nucleus and includes the nucleus of the basal optic root and the nucleus of the medial longitudinal fasciculus, which often are considered part of the mesencephalic tegmentum (see i.e. Kuhlenbeck, 1973; Cruce & Nieuwenhuys, 1974; Schwab, 1979; ten Donkelaar, 1998). It has been shown several times that this tegmental area develops within the caudal diencephalon of vertebrates (Palmgren, 1921; Rendahl, 1924; Keyser, 1972; Puelles *et al.*, 1987, 1996a; Pombal & Puelles, 1999).

LONGITUDINAL, TRANSVERSAL AND RADIAL SUBDIVISIONS

The structure of the adult brain reflects how it was built during the embryonic period, though sometimes in a non overt way. The neuroepithelium is specified molecularly into primary histogenetic subdivisions which are longitudinal (i.e., roof, alar, basal and floor plates) or transversal (neuromeres; Puelles & Rubenstein, 1993; Rubenstein & Puelles, 1994; Puelles, 1995). Each primary parcellation becomes subdivided into smaller histogenetic fields by primary and secondary anteroposterior and dorsoventral regionalization effects, which are sometimes dependent on specific organizer regions. For instance, the basal plate owes its existence to ventralizing inductive effects arising from the axial mesoderm and the neural floor plate (Placzek *et al.*, 1993; Yamada *et al.*, 1993; Tanabe *et al.*, 1995; Marti *et al.*, 1995), whereas the alar plate is affected by dorsalizing effects stemming from the roof plate and early non-neural ectoderm (Liem *et al.*, 1995, 1997). Tectal, toral and isthmic structures are known to be set in place in their mutual anteroposterior relationships by the isthmic organizer, which forms at the isthmo-mesencephalic boundary (Martínez & Alvarado-Mallart, 1990; Gardner & Barald, 1991; Martínez *et al.*, 1991; Nakamura & Itasaki, 1992; Marín & Puelles, 1994; see also the reviews by Alvarado-Mallart, 1993; Martínez *et al.*, 1995; Puelles *et al.*, 1996b). Each histogenetically distinct area generates a characteristic sequence of radially migrating young neurons, and further differentiation, layering and trophic stabilization of these elements gives rise to the specific morphological and functional populations observed in the adult. Insofar as the position of neuronal derivatives in the mantle zone remains undisturbed, covering the respective matrix areas in the neuroepithelium, the topology of cell populations found in the adult can be assumed to reflect regionalization events which took place during development. This assumption needs checking for eventual tangential migration of some neurons, which might complicate the analysis (Puelles & Martínez-de-la-Torre, 1987; Martínez *et al.*, 1992).

In the present study, we found evidence for distinct longitudinal, transversal and radial midbrain subdivisions in the lizard *Gallotia*. The roof plate, the alar and basal subdivisions (the dorsal and lateral bands, plus the medial and lateral subdivisions of the basal plate), and the median floor-plate band can therefore be understood as alternative dorsoventral histogenetic fates of the midbrain neuroepithelium. These fates probably are positionally dependent on the crossed dorsalizing and ventralizing mechanisms cited above. On the other hand, the main transverse boundaries of the midbrain, and the differential histologic structure found at rostral, intermediate and caudal levels of most of the longitudinal components (Fig. 8b) may be explained as anteroposterior patterning effects possibly influenced by their relative distance from the isthmic organizer (known to induce at least caudal midbrain structures in the chick; see Martínez & Alvarado-Mallart, 1990; Gardner & Barald, 1991; Martínez *et al.*, 1991; Nakamura & Itasaki, 1992; Marín & Puelles, 1994;

reviews by Alvarado-Mallart, 1993, Martínez *et al.*, 1995; & Puelles *et al.*, 1996b) and/or from the diencephalo-mesencephalic boundary. There is only circumstantial evidence that the latter may function also as a secondary organizer (Martínez *et al.*, 1992; Marín & Puelles, 1994).

An intriguing observation is the dorsal expansion of the caudal part of the lateral band along the isthmo-mesencephalic border, separating the toral complex from the isthmus proper. Some published evidence and work in course indicates that there are some mouse and chick developmental genes whose expression pattern duplicates this peculiar fate distribution—i.e., *Nkx-2.2* and *Sim-1* (Puelles & Rubenstein, 1993; Fan *et al.*, 1996; Puelles, E. & Puelles, L. unpublished), but a causal connection can not be proposed at present.

Longitudinal subdivisions

We found four main longitudinal cytoarchitectonic columns in the lizard midbrain. These largely correspond to the dorsal, lateral, medial and ventral bands detected by Palmgren (1921), but we chose to name them more descriptively, consistent with recent developmental literature, as dorsal, lateral, basal and median bands.

The dorsal and lateral bands constitute the alar plate, while the basal and median bands constitute the mesencephalic tegmentum proper. Using this definition, the alar plate correlates in dorsoventral position with the isthmic nuclei and the sensory columns of the rombencephalic alar plate (cochlear, vestibular and somatosensorial/visceral fields), caudally, and with the alar diencephalon (pretectum, dorsal and ventral thalami), rostrally. We corroborated also Rendahl's (1924) finding that Palmgren's (1921) dorsal and lateral bands continue from the midbrain into the caudal diencephalon; this suggests the existence of common dorsoventral patterning mechanisms, in agreement with the relevant data on gene functions cited above. The basal and median bands of the midbrain are also caudally continuous with the somato-/visceromotor columns of the hindbrain basal and floor plates, comprising motor nuclei, reticular formation and raphe nuclei. The mesencephalic tegmentum contacts rostralwards the diencephalic tegmentum (i.e., prerubral or pretectal tegmentum, posterior tuberculum and retromammillary region), with which similarity resides at least in the development of catecholaminergic populations (Puelles & Medina, 1994; Puelles & Verney, 1998).

Our postulate that the midbrain alar/basal boundary lies between the lateral band and the lateral part of the basal tegmentum is tentative and needs corroboration by means of molecular markers (for instance, the alar plate marker genes *Pax3/Pax7*, not yet cloned for the lizard). Puelles and Rubenstein (1993) and Shimamura *et al.* (1995) suggested that the linear expression of the gene *Nkx-2.2* in the midbrain and diencephalon may approximate the location of the alar/basal boundary. Recent detailed analysis of Nkx-2.2 in chick embryos (Puelles *et al.*, 1999) suggests however that this gene is expressed in a three-dimensional band across the midbrain neuroepithelium and mantle layer, which probably overlaps with the equivalent of our present lateral part of the basal plate.

In the columnar approach, the sulcus limitans of His (1893) is widely identified as the delimiting landmark between the alar and the basal territories of the hindbrain and midbrain (His, 1893, 1904; Herrick 1910, 1917; Kuhlenbeck, 1973), but it is an unreliable landmark (Röthig, 1923; Nieuwenhuys & Bodenheimer, 1966; Keyser, 1972). Moreover, the sulcus limitans, even when it is visible, lacks exact correlation with gene-expression domains (Bulfone *et al.*, 1993). In the *Testudo* topological map, Cruce and Nieuwenhuys (1974) could not follow the sulcus limitans from the hindbrain into the midbrain.

Although in our cytoarchitectonic analysis of this point we have not found a definitive answer, we think that the characteristic pluristratified structure of the lateral band places it in the same side of morphospace as the dorsal band, whose alar nature seems undisputable. The lateral periventricular stratum is only subtly different from that seen at the dorsal band. It is to be noted, though, that the lateral central stratum, and perhaps also the laterorostral and laterocaudal mesencephalic nuclei, which form its superficial stratum, have an appearance that has favoured in the past their inclusion in the reticular formation (sometimes as nucleus profundus mesencephali; i.e., Schwab, 1979; Wang *et al.*, 1983). This does not pose any particular difficulty as regards classification of the lateral band as an alar derivative, since the lateralmost (dorsal) part of the hindbrain reticular formation in mammals also clearly belongs to the alar plate, as indicated by its radial position underneath the trigeminal sensory column (Paxinos & Watson, 1986; Paxinos *et al.*, 1990; Voogd *et al.*, 1998).

The idea of an alar midbrain portion distinct from the tectum and the torus semicircularis was considered by others besides Palmgren (1921), including Cruce and Nieuwenhuys (1974), who saw their 'nucleus profundus mesencephali rostralis and caudalis' (probably equivalent to our lateral central stratum) as a rostral extension of the special somatic sensory zone of the alar rhombencephalon (see also comments on the "tegmentum sensorium", on page 2147 of Nieuwenhuys *et al.*, 1998).

Our dorsal band corresponds to the dorsal column described by Palmgren (1921) in fishes, amphibians, birds and mammals, and by Rendahl (1924) in the chick embryo. However, these authors did not identify the intermediate area nor the griseum tectale. The dorsal mesencephalic column of Bergquist and Källén (1953) instead seems to correspond with the dorsal band plus the lateral band, which indicates that we also agree with them on the alar/basal boundary. Our data agree basically with the schema proposed by Puelles *et al.* (1994) for reptiles, as regards relative positions of the griseum tectale, tectum and toral complex (compare their Fig. 14—or the copy in Nieuwenhuys *et al.*, 1998—with our Fig. 4). However, we are still uncertain which alar subdivision in the lizard may correspond to the avian intercollicular area, since we have two candidates, namely the intermediate area of the dorsal band and the intermediate part of the lateral band. The cholinergic terminal neuropil identified in a potential lizard intercollicular area by Medina *et al.* (1993) is comparable in location to the periventricular stratum of our lateral band at an intermediate area level (compare their Figs. 4n and 15B with our Figs. 2f–h and 1, respectively). Further analysis will be needed, nevertheless, keeping in mind other potential markers (Martínez-de-la-Torre, 1985; Robles, 1995).

On the other hand, the somatosensory recipient area of the reptilian midbrain, identified by Foster and Hall (1978) as 'area intercollicularis' in *Iguana iguana*, also identified previously by Ebbesson (1967) in *Tupinambis nigropunctatus*, clearly does not have a topological position comparable to the avian 'intercollicular area', as defined by Puelles *et al.* (1994). Foster and Hall's (1978) area intercollicullaris constitutes basically a caudolateral portion of the central stratum of the torus semicircularis, comparable to the avian spinorecipient 'preisthmic superficial area' (see Puelles *et al.*, 1994). The same can be said of the 'midbrain somatosensory area' identified by Pritz and Stritzel (1989, 1990) in *Caiman crocodilus*.

In agreement with Palmgren (1921), we divided the basal band longitudinally in lateral and medial parts, although the agreement is not complete. Our lateral basal tegmentum does correspond roughly with his medial column (Palmgren's term 'medial' is confusing, since the intended meaning was 'intermediate', as seems clear from the context). However, our medial basal tegmentum, including the oculomotor and red nuclei, only corresponds with the lateral part of his ventral column, whereas our median band, where the ventral tegmental area arises, corresponds to the medial part of Palmgren's ventral column.

The basal reticular formation of the midbrain has remained poorly defined in reptilian cytoarchitectural studies, particularly because the often cited NFLM nucleus actually belongs to the pretectal tegmentum. At a rhombencephalic level, the reptilian reticular formation was subdivided in medial and lateral parts; the medial part was held to extend into the midbrain, while the lateral reticular formation was thought to be restricted to the hindbrain (Cruce & Nieuwenhuys, 1974; ten Donkelaar *et al.*, 1987). In contrast, Newman and Cruce (1982) extended both portions of their superior reticular domain into the mesencephalon, thus apparently agreeing with our observations. Contrarily, the lateral and medial midbrain reticular formations of Reiner *et al.* (1984) correlate with our alar and basal reticular formations, respectively. Our concept of an alar reticular formation added to medial and lateral basal parts of the midbrain reticular formation, suggests a common structural pattern with the dorsal, intermediate and medial parts of the hindbrain reticular formation, as distinguished by Paxinos and Watson (1986), Paxinos *et al.* (1990) and Voogd *et al.* (1998) in mammals.

Various connectivity data show a more complex structure of the reptilian midbrain reticular formation than has been rendered by the cytoarchitectural studies so far (see i.e. Hoogland, 1982; Welker *et al.*, 1983; Bruce & Butler, 1984; Künzle & Schnyder, 1984; Sereno, 1985; Diaz, 1991; ten Donkelaar, 1998). This suggests that this extensive area should be a subject for more detailed studies.

The median band (floor plate of Kingsbury, 1920, 1922, 1930; Vaage, 1969) originally was not recognized in the midbrain (i.e., Rendahl, 1924; Vaage, 1969; Keyser, 1972; Kuhlenbeck, 1973). However, histochemical and gene expression data subsequently have shown that a modified sort of floor plate (divided into a median epichordal strip and paramedian bands) extends all along the mesencephalon and caudal diencephalon in vertebrates (though it is less massive than that found in the hindbrain); this "epichordal strip" ends just behind the mammillary region (Newgreen *et al.*, 1981; Wallace, 1982; Teitelman *et al.*, 1983; Puelles *et al.*, 1987; Matsui *et al.*, 1990; Puelles, 1995; Shimamura *et al.*, 1995; Verney *et al.*, submitted).

Transversal subdivisions

Our results suggest an internal rostrocaudal subdivision of the lizard midbrain that had not been reported previously. The caudorostral series of three sensory grisea of the dorsal band (GT, T + IA, TS) is correlatively aligned with three less distinct transverse regions each in the lateral and basal bands (see Figs. 1 and 8). The intermediate transverse subdivision is an area of scarce cells, which is selectively crossed by the dorsoventral fibers of the tectobulbar tract. Our transversal subdivision of the lizard midbrain therefore does not coincide with Vaage's (1969, 1973) proposal of two mesomeres in the midbrain. His descriptive evidence supporting such a neuromeric subdivision is tenuous in the light of recent chick fate-mapping experiments mentioned above, indicating that caudal parts of the early midbrain vesicle contribute later to isthmocerebellar structures (this argument affects as well the generalized midbrain schema proposed by Nieuwenhuys (1998), where the isthmus—without the corresponding cerebellar part—is appended to the midbrain). Whatever interpretation is given to Vaage's (1969, 1973) 'atrophic' m2 area, it would seem that only his m1 neuromere contains prospective midbrain material. Our tripartite rostrocaudal subdivision occurs thus wholly inside Vaage's m1. Further research may resolve whether these partitions represent three mesomeres or just secondary transverse parts within a single mesomere. The functional meaning of the internal transverse subdivision of the midbrain remains to be studied. Preliminary data from HRP injections at different rostrocaudal levels of the fore-, mid- and hindbrain basal or median bands in *Gallotia* reveal that some of these transverse internal subdivisions can be labelled specifically depending on the site of label injection (Diaz, 1991; Díaz & Puelles, unpublished data; see also Diaz *et al.*, 1999).

Radial subdivisions

We also reported here on radial subdivisions in the lizard midbrain, basically following the brain stratification system and terminology of Senn (1970). The most complex radial structure was found in the alar plate. Each histogenetic areal compartment displayed a characteristic variant of the fundamental three-layered schema (periventricular, central and superficial strata). The torus semicircularis has a predominant development of its periventricular stratum, as was already pointed out by Senn (1970, 1979), in contrast to the optic tectum, where central and superficial strata are better developed. Nevertheless, individual toral cellular and plexiform laminae can be unequivocally related to the neighboring tectal laminae, independently of their particular thickness or cellular density, as revealed by the feasibility of using Ramón's (1896) tectal layer numeration system (Senn, 1970, 1979). The same can be said for the intermediate area and the griseum tectale, the latter showing a tectum-like, more equilibrated development of the three basic strata, as compared with the torus. The periventricular stratum of these last two regions, together with that of the lateral band, is indistinctly referred to in the literature as "laminar portion of the torus semicircularis" (ten Donkelaar, 1998). We suggest that this term is confusing due to the differences between the areas encompassed within it, and it arbitrarily incorporates into the toral complex regions which seem to have a completely different nature (note that GT is retinorecipient; Martínez-de-la-Torre, 1985; Medina, 1990, and the intercollicular area homolog—if we follow the definition issued by Puelles *et al.*, 1994—is related to vocalization rather than to hearing). We thus propose that use of this term be discontinued.

An issue raised here that we are unable to resolve is how far stratification and lamination similarities depend on development of similar classes of neurons, which would interact under different local conditions to generate the particular histic phenotypes observed. The only alar midbrain area that has been studied in minimally sufficient detail as regards neuronal typology is the optic tectum.

Radial positioning of neurons in the basal plate is simpler, as also underlined by Senn (1979). There are periventricular elements that include the oculomotor neurons, and migrated central stratum elements that contribute to the basal part of the reticular formation, including the rostrally placed red nucleus. The partly superficial position of the substantia nigra neurons may be strictly due to radial migration, too, though a potential contribution of some cells migrated tangentially from the ventral tegmental area cannot be discarded on the basis of our data (see Puelles & Medina, 1994).

CELL POPULATIONS

Contrary to the idea of Cruce and Nieuwenhuys (1974) of the midbrain tegmentum, defined as "a continuous zone of diffuse gray" in which "individualized cell masses can be distinguished but . . . no complete parcellation is possible", we presented here a more detailed "parcellation" of the lizard tegmentum. This serves to fill in the relevant blank spaces that usually appear in the published maps of the midbrain in vertebrates (i.e., reptiles: Cruce & Niewenhuys, 1974; birds: Stokes *et al.*, 1974; Karten & Hodos, 1967; mammals: Paxinos & Watson, 1986). We focus the following discussion on the less well-known cell groups.

Dorsal band

In addition to the tectum and the torus semicircularis, we found two other laminated grisea in the dorsal band, the intermediate area and, rostrally, the griseum tectale. The intermediate area is still poorly understood. More research is needed to examine the possibility that it may relate to a vocal center variously identified as "dorsomedial nucleus" or intercollicular area in birds (see Puelles *et al.*, 1994).

The laminated griseum tectale was previously identified in *Caiman sclerops* (Repérant, 1975), *Vipera aspis* (Repérant & Rio, 1976) and several other reptiles including *Gallotia galloti* (Martínez-de-la-Torre, 1985; Medina *et al.*, 1993, 1994), based on its identical topography compared to the avian griseum tectale (Puelles & Zabala, 1982; Puelles *et al.*, 1988; Gamlin & Cohen, 1988a,b). The reptilian griseum tectale receives retinal afferents (Repérant *et al.*, 1978; Medina, 1990; Medina & Smeets, 1992; Medina *et al.*, 1993), as in birds (Puelles & Zabala, 1982; Gamlin & Cohen, 1988a). De Lange (1913) included this area within his nucleus lentiformis mesencephali. In mammals, the posterior pretectal nucleus was compared to the griseum tectale of reptiles and birds due to its similar position caudal to the posterior commissure and rostral to the superior colliculus, its histochemical features and its connectivity (Martínez-de-la-Torre, 1985; Caballero-Bleda, 1988; Caballero-Bleda *et al.*, 1992; Gamlin & Cohen, 1988b). A griseum tectale formation has been distinguished as well in *Rana* and *Xenopus* frogs (Puelles *et al.*, 1996a;

Milán & Puelles, 2000). Whereas the amphibian GT complex remains largely periventricular, the reptilian GT develops periventricular, central and superficial strata, and the avian and mammalian GT homologs largely constitute superficially migrated formations.

Lateral band

The magnocellular laterocaudal mesencephalic nucleus was apparently misidentified as nucleus profundus mesencephali in several reptiles (Foster & Hall, 1978; Wang *et al.*, 1983; Wolters *et al.*, 1985; ten Donkelaar *et al.*, 1987), if we accept Cruce and Nieuwenhuys' (1974) mapping, that locates nucleus profundus (as the name indicates) at a deeper locus, corresponding to our lateral intermediate stratum, or alar part of the midbrain reticular formation. Dacey and Ulinski (1986) apparently identified the conspicuous LC nucleus as 'a caudal part of the nucleus of the tectothalamic tract plus the mesencephalic isthmi nucleus' in the snake *Thamnophis sirtalis*. Sereno (1985) and Sereno and Ulinski (1987) identified it as "nucleus isthmi, pars magnocellularis rostralis" in the turtle *Pseudemys scripta*. However, it clearly belongs to the midbrain, as occurs with its avian counterpart, the magnocellular isthmic nucleus (Vaage, 1973; Puelles & Martínez-de-la-Torre, 1987), both being located rostral to the trochlear nucleus and the trochlear decussation. This nucleus has reciprocal connections with the optic tectum in reptiles (Künzle & Schnyder, 1984; Sereno & Ulinski, 1987; Pérez-Santana, 1993) and birds (Puelles, unpublished observations; see Fig. 3 in Martínez & Puelles, 1989).

The superficial laterorostral mesencephalic nucleus is a practically unknown cell group, which may correspond to the nucleus confusingly called 'profundus mesencephali rostrolateralis' by Sereno (1985). This author described in the turtle *Pseudemys scripta* a caudorostral sequence of nuclei sandwiched between the sensorially specialized dorsal mesencephalic grisea and the tegmental structures. These seem to correspond to our two superficial lateral nuclei (see his Figs. 2, 3, and 12). We were led to propose new names for them—*laterorostral and laterocaudal mesencephalic nuclei*—because we think that the terms found in the literature for these superficial nuclei are extremely confusing and easily lead to misidentification. It should be interesting to explore the possible homology of the laterorostral mesencephalic nucleus with the 'torus lateralis' formation found so prominently in the midbrain of teleosts, with a similar topological position (Palmgren, 1921; Nieuwenhuys *et al.*, 1998).

The periventricular stratum of the lateral band is a badly studied locus in most vertebrates. The mammalian counterpart perhaps might be found in the lateral portion of the periacueductal gray. In lizards, it may correspond at least in part to the torus semicircularis, pars laminaris, since ten Donkelaar and Nieuwenhuys (1979) and others described this area as restricted to "the compact periventricular cell layer." In other lizards, the nucleus laminaris of the torus semicircularis receives spinal afferents (Künzle & Woodson, 1982; ten Donkelaar *et al.*, 1987) while the central nucleus of the torus semicircularis relates to the auditory pathway.

Median band

A restricted concept of the ventral tegmental area (VTA) as a floor plate derivative was previously defined in *Gallotia* (Martínez-de-la-Torre, 1985; Puelles & Medina, 1994). This view considers the VTA proper as a reduced median small-celled group which lies medial to the oculomotor nerve roots rostral to the isthmic interpeduncular nucleus, and partially migrates tangentially lateral to the oculomotor roots (Díaz & Puelles, 1992a,b; Medina *et al.*, 1994). The VTA receives bilateral innervation from the habenula (Díaz & Puelles, 1992a) and a few of its cells are habenulopetal, being perhaps comparable to the interfascicular nucleus of the VTA in rats. The catecholaminergic VTA described in reptiles has a much broader extension and may partially overlap with our substantia nigra (see Smeets, 1994; Medina *et al.*, 1994). On the other hand, a VTA-like formation extends outside the midbrain itself, into the diencephalic prerubral tegmentum, posterior tuberculum and retromammillary areas (Smeets, 1994; Puelles & Medina, 1994). The similarity of the midbrain VTA with the more rostral analogues probably results from the operation of similar inductive effects (Ericson *et al.*, 1995; Hynes *et al.*, 1995a,b).

Abbreviations

cer	cerebellum
cgt	commissure of griseum tectale
cp	posterior commissure
ctb	crossed tectobulbar tract
cte	commissure of optic tectum
cto	commissure of the torus semicircularis
D	nucleus of Darkschewitsch
Dien	diencephalon
EW	accessory oculomotor nucleus of Edinger-Westphal
flm	medial longitudinal fasciculus
fr	retroflex fasciculus
GC	griseum commissurale
GP	pretectal geniculate nucleus
GT	griseum tectale (tectal gray)
HTh	hypothalamus
IA	intermediate area
III	oculomotor nucleus
IIIdl	oculomotor nucleus, dorsolateral part
IIIdm	oculomotor nucleus, dorsomedial part
Im	magnocellular isthmic nucleus
IP	interpeduncular nucleus

Ip	parvicellular isthmic nucleus
Ir	rostral isthmic nucleus
IIIvm	oculomotor nucleus, ventromedial part
IV	trochlear nucleus
l	lateral part of basal band
LC	laterocaudal mesencephalic nucleus
LL	lateral lemniscal nucleus
LR	laterorostral mesencephalic nucleus
m	medial part of basal band
mtt	mesencephalic trigeminal tract
NBOR	nucleus of the basal optic root
NCPm	magnocellular nucleus of the posterior commissure
NCPp	parvocellular nucleus of the posterior commissure
NFLM	nucleus of the medial longitudinal fasciculus
NR	red nucleus
nIII	oculomotor nerve
nIV	trochlear nerve
PP	principal pretectal nucleus
PT	pretectum
RFA	alar reticular formation
RFB	basal reticular formation
RFBl	basal reticular formation, lateral part
RFBm	basal reticular formation, medial part
Rh	Rhomb, rhombencephalon
sc	central stratum
SN	substantia nigra
SNc	substantia nigra, pars compacta
SNr	substantia nigra, pars reticulata
SP	subpretectal nucleus
sp	periventricular stratum
sp2-5	layers 2–5 of the periventricular stratum
ss	superficial stratum
ss-g	stratum griseum superficiale
ss-f	stratum fibrosum superficiale
T	optic tectum
Th	thalamus
TS	torus semicircularis
VTA	ventral tegmental area

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