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The evolutionary origin of the mammalian isocortex: Towards an integrated developmental and functional approach

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Abstract: The isocortex is a distinctive feature of mammalian brains, which has no clear counterpart in the cerebral hemispheres of other amniotes. This paper speculates on the evolutionary processes giving rise to the isocortex. As a first step, we intend to identify what structure may be ancestral to the isocortex in the reptilian brain. Then, it is necessary to account for the transformations (developmental, connectional, and functional) of this ancestral structure, which resulted in the origin of the isocortex. One long-held perspective argues that part of the isocortex derives from the ventral pallium of reptiles, whereas another view proposes that the isocortex originated mostly from the dorsal pallium. We consider that, at this point, evidence tends to favor correspondence of the isocortex with the dorsal cortex of reptiles. In any case, the isocortex may have originated partly as a consequence of an overall "dorsalizing" effect (that is, an expansion of the territories expressing dorsal-specific genes) during pallial development. Furthermore, expansion of the dorsal pallium may have been driven by selective pressures favoring the development of associative networks between the dorsal cortex, the olfactory cortex, and the hippocampus, which participated in spatial or episodic memory in the early mammals. In this context, sensory projections that in reptiles end in the ventral pallium, are observed to terminate in the isocortex (dorsal pallium) of mammals, perhaps owing to their participation in these associative networks.

Keywords: basolateral amygdala; claustrum; Emx-1; endopiriform nucleus; dorsal cortex; dorsal ventricular ridge; hippocampus; homology; olfactory cortex; Pax-6; ventral pallium

1. Introduction

The study of brain evolution has been hampered by difficulties related to the complexity of this organ, which makes difficult the comparisons among different taxa; and by the relative lack of plausible scenarios proposing specific mechanisms by which transformations of brain structure may have taken place. The first attempts in comparative neuroanatomy date from at least two centuries ago, and were mainly based on analyses of distinct cell masses from histological sections of developing and adult brains of different species (Ariëns Kappers et al. 1936; Ramón y Cajal 1995). With the advent of tract-tracing techniques in the mid-twentieth century, a new era appeared which permitted visualization of the connectivity between these brain components and identification of similarities and differences in network organization among species (Heimer 1970; Nauta & Gygax 1951; 1954; Nauta & Karten 1970). Powerful histochemical and immunochemical techniques subsequently have been applied to the nervous system. These procedures permitted identification of neurochemical markers (neurotransmitters, cell adhesion molecules, cytoskeletal components, and other elements) that labeled specific neuronal populations, thus providing finer-grained observations on the anatomical arrangements and development of different cell masses (Parent & Olivier 1970). More recently, molecular techniques based on the analysis of gene expression have proved to be particularly fruitful for determining genes involved in the development of distinct brain components and for making cross-species correspondences.

Although each of these different strategies has a value of its own, unfortunately some of these techniques have been used to validate discrepant interpretations of brain evolution. For example, when searching for homologue structures, one may look for nuclei with similarities in connectivity, or for nuclei with a common developmental origin. If these two criteria agree, there will obviously be no problem, but if they disagree, one has to think that only one (or neither) of these criteria is valid. Furthermore, if, say, connections are considered to be more reliable indicators of ho-

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mology, then one should explain how the structures acquired different developmental origins during evolution. Conversely, if developmental criteria are found to be stronger, then one should explain how the connectivity of these nuclei has changed in evolution. In this sense, when different criteria for homology disagree, one is forced to propose an explanation of how only some homology criteria are valid in that particular case, and must also offer a plausible mechanism (a scenario) to account for the observed differences.

In this target article, we address the issue of the evolutionary origin of the mammalian isocortex, which has been amply debated in recent years. We first discuss different proposals about this structure's homology with specific brain components in reptiles and birds (these two taxa together are called *sauropsida*), and provide arguments to validate the criteria that we will embrace. Next, we propose a scenario of isocortical origins, integrating both developmental and connectional/functional evidence, to account for some of the discrepancies in the homology criteria. In other words, we present an integrative hypothesis, combining different lines of evidence into a coherent proposal about the early evolution of the mammalian isocortex. Briefly, our suggestion is that the mammalian isocortex originated in large part as a consequence of a "dorsalizing" influence in pallial development. This means that (1) genes specifying the fate of dorsal territories in the embryonic pallium may have increased their domains of expression, perhaps at the expense of the expression of genes specifying lateral or ventral phenotypes; (2) cells from other brain compartments contributed to the developing dorsal pallium (although perhaps not massively); and (3) there may have been an increased production of progenitor cells in the dorsal pallium and other brain regions, leading to an increase in brain size. This may have occurred as a consequence of the development of olfactory-hippocampal associative networks in primitive mammals, and the progressive incorporation of the dorsal cortex into this network. This process was accompanied by major changes in the pattern of termination of some sensory afferents into the pallium, especially those sensory pathways that use the mesencephalon as a relay to access the thalamus.

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Daniver Morales received his B.Sc. degree in Biology from the Faculty of Sciences, University of Chile. He then joined Francisco Aboitiz's laboratory, where he conducted research on the cortical development of reptiles and mammals. Currently he is a graduate student at the Rockefeller University in New York, under the supervision of Mary E. Hatten. He is studying histogenesis of the cerebellum, focusing on the generation and specification of distinct cell types, using mouse and chicken as model systems. Morales is a recipient of a Presidential Fellowship from the Government of Chile.

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2. The problem of isocortical origins

The mammalian isocortex is a character unique to mammals in several respects. First, it has undergone an enormous expansion, especially in the tangential domain (Rakic 1988). Second, it has a six-layered architecture, which differs from the three-layered array of simpler telencephalic laminar structures such as the hippocampal formation, the olfactory cortex, and the reptilian cortices (Supèr et al. 1998b). Although in other vertebrates there are some expanding telencephalic structures that receive a similar sensory input, in no case has such a conspicuous laminar arrangement been observed (Striedter 1997).

There have been important disagreements as to which components of the non-mammalian telencephalon can be compared to the isocortex. This problem is complicated by the intricate topography of the hemispheres in some vertebrate classes and by the absence of a single criterion to establish the homology of neural structures. Commonly used criteria for similarity are connectivity (Bruce & Neary 1995; Butler 1994a; 1994b; Karten 1969; 1997; Medina & Reiner 2000; Reiner 2000), neurochemistry (Reiner 1991; 1993), and embryonic origins (Aboitiz 1992; 1995; Källén 1951; Puelles et al. 1999; 2000; Smith Fernández et al. 1998; Striedter 1997). Unfortunately, when intending to identify structures homologous to the isocortex, there have been discrepant conceptions derived from these different approaches.

In order to understand its origins, we first need to establish which structure gave rise to the isocortex. For this, we need to select some homology criteria as the most reliable, while rejecting other criteria. However, while doing this, we also need to address the fact that the rejected criteria may indicate important differences between the isocortex and its homologue. Below, we briefly outline some aspects of brain organization in vertebrates and discuss the different hypotheses of homology that have been proposed. After that, we offer a developmental and a connectional/behavioral scenario, which may account for early isocortical evolution. Finally, we review some evidence on fossil mammals which bears relation to isocortical origins.

3. The cerebral hemispheres of vertebrates

3.1. Taxonomical relations

Vertebrates – or craniates – are divided into *agnathans* (jawless vertebrates like the lamprey) and *gnathostomes* (jawed vertebrates like most fish and all terrestrial verte-

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brates). Among gnathostomes, there are the *chondrychthian* fish (sharks, rays, and related cartilaginous fish) and the *osteichthies* or bony fish. *Sarcopterygians* are a group of bony fish that are considered related to the ancestors of terrestrial vertebrates. Terrestrial vertebrates are divided into anamniotes (amphibians) and amniotes (reptiles, birds, and mammals). Amniotes are those vertebrates whose embryological development occurs within an amniotic cavity (either within the egg as in reptiles, birds, and monotreme mammals, or inside the maternal uterus as in marsupials and placental mammals), and thus are able to reproduce outside the water (see Fig. 1). The basic stock of amniotes is considered to be represented by the stem reptiles, from which different groups diverged in the Paleozoic period. Reptiles have been usually classified on the basis of the pattern of openings in the dermal skull behind the orbits. In primitive amniotes (subclass *anapsida*), there are no openings and the roof of the skull is completely covered by bone. Members of this class include fossil reptiles, and some authors have placed turtles among them, based on the absence of cranial openings in this group (see Carroll 1988). For this reason, turtles have been considered in many instances to be the reptiles closest to the point of reptilian-mammalian divergence, and their brains have been considered as models of the ancestral amniote brain. However, some paleontologists have considered the inclusion of turtles into the subclass anapsida somewhat arbitrary, as it disregards many other aspects which strongly suggest that this group is rather a highly derived one (see Carroll 1988). Moreover, recent phylogenetic analyses based on morphology and on molecular evidence place turtles as a rather modified group of reptiles, with no direct relation to the ancestral anapsids (Mannen & Li 1999; Rieppel & Reisz 1999; Zardoya & Meyer 2001). Therefore, the absence of temporal openings in the skull of turtles may be secondary and not reflect an ancestral condition.

Another reptilian subclass is represented by the *synapsid* condition (with one cranial opening), which is exemplified by the mammal-like reptiles from which the first mammals emerged (Fig. 1). Synapsid reptiles were the first group of amniotes to be abundant and diversified, and their relations with the presumed ancestral anapsid stock are obscure. It is likely that the mammal-like reptiles emerged quite early from the ancestral amniote stock. The *diapsid* reptilian condition (Fig. 1), in which there are two postorbital openings, possibly originated from a group of anapsid reptiles. This subclass represents most living reptiles and includes two main groups: *lepidosaura,* with lizards, snakes, and a primitive New Zealand reptile called the tuatara; and *archosaura,* which includes crocodiles, dinosaurs, and birds. According to some phylogenetic analyses mentioned above, turtles may also belong to the diapsids. Finally, there is the subclass *parapsida,* represented by fossil ichthyosaurs and plesiosaurs (Carroll 1988).

3.2. A brief history of the pallium

The cerebral hemispheres can be subdivided into a dorsal part or pallium (divided into medial, dorsal, lateral, and ventral pallium; see below), and a ventral part or subpallium (see Fig. 2). In agnathans, the olfactory bulbs project heavily upon the whole pallial surface (Northcutt 1996a; Northcutt & Puzdrowski 1988; Wicht & Northcutt 1992; 1993; Wicht 1996), and there is evidence that a true dorsal pallium may be lacking at least in some species (Myojin et al. 2001). In gnathostomes, the olfactory projection occupies a

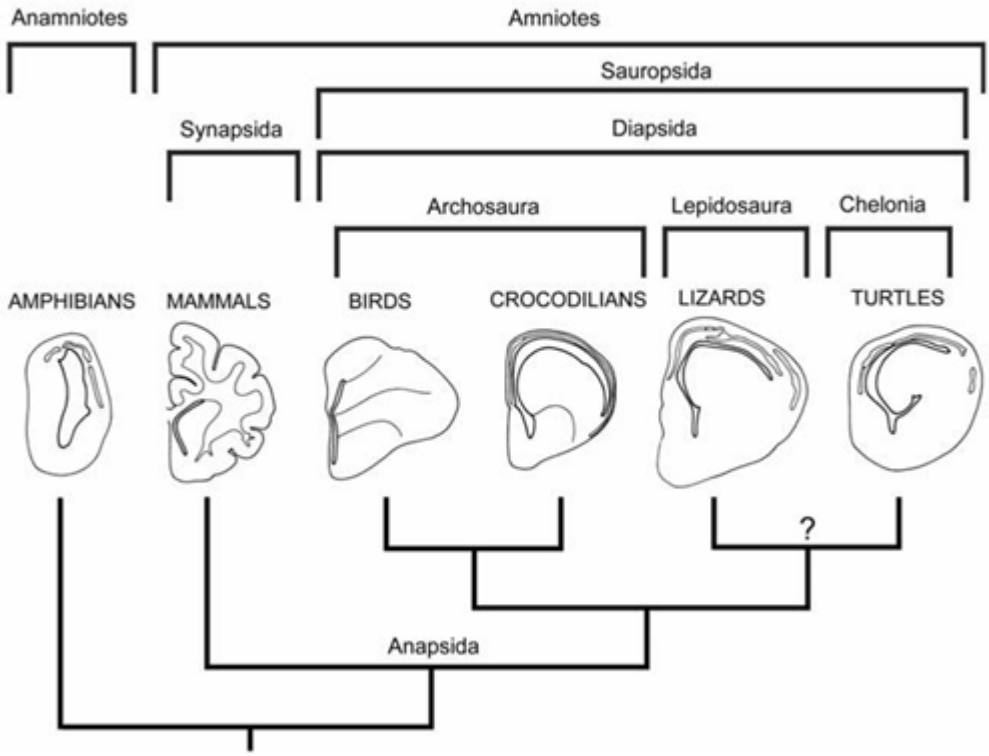

Figure 1. Cladogram indicating phylogenetic relationships in tetrapods. In each crown taxon, a figure of a coronal section of one cerebral hemisphere (only one hemisphere is shown; lateral is to the right and medial is to the left) of a representative species is included.

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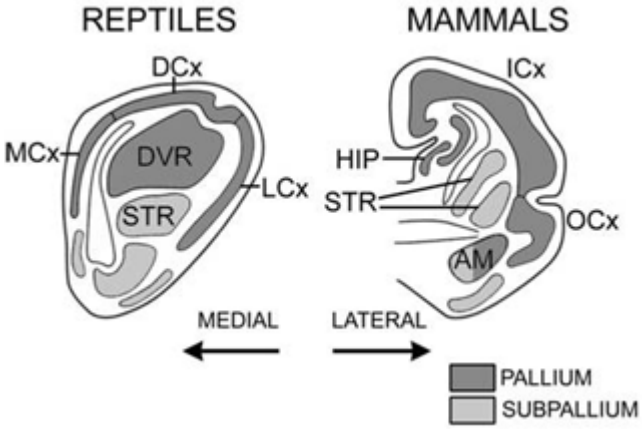

Figure 2. The main components of the amniote cerebral hemispheres (coronal sections of only one hemisphere are shown; lateral is to the right). The pallium of reptiles consists of a medial cortex (MCx), a dorsal cortex (DCx), a lateral cortex (LCx), and a large part of the periventricular dorsal ventricular ridge (DVR). Interposed between the medial and the dorsal cortex, a dorsomedial cortex is observed in many reptiles. In mammals, the pallium consists of the hippocampal formation (HIP, which is comparable to the MCx and the dorsomedial cortex), the isocortex (ICx), the olfactory cortex (OCx, comparable to the reptilian LCx), and part of the claustroamygdaloid complex (AM). The subpallium of both reptiles and mammals include the basal ganglia, of which a main component is the corpus striatum (STR). In reptiles, part of the posterior DVR may be of subpallial origin, while in mammals part of the amygdalar complex is also subpallial.

much more restricted portion of the pallium, being usually confined to the lateral aspect of this structure (Ebbesson & Heimer 1970; Northcutt & Kaas 1995; Smeets 1983). The acquisition of predatory lifestyles by the early gnathostome vertebrates, involving the further development of other sensory modalities, implied the progressive development of ascending visual, somatosensory, and lateral line afferents to the pallium (Northcutt & Puzdrowski 1988; Wicht 1996; Wicht & Northcutt 1992; 1993). The expansion of these sensory projections, which are relayed to the hemispheres via the diencephalon, was concomitant with the enlargement of the telencephalic components receiving the respective inputs (Northcutt 1981; Northcutt & Puzdrowski 1988; Striedter 1997; Wicht 1996; Wicht & Northcutt 1992; 1993). With the exception of amphibians, which are considered to have a secondarily simplified brain (Neary 1990; Northcutt 1981), this phenomenon is also evident among terrestrial vertebrates.

The pallium of amphibians consists in large part of periventricular cells that show a limited degree of radial migration, and do not make up a true cortical architecture. The amphibian pallium has been subdivided into lateral, dorsal, and medial components (Bruce & Neary 1995; Neary 1990). Based in large part on the relative absence of direct olfactory input and on the presence of at least thalamic visual and somatosensory projections, the medial pallium has been considered to be comparable to both the medial/dorsomedial cortex and the dorsal cortex of reptiles (or the hippocampus and the isocortex of mammals, respectively; Bruce & Neary 1995; see also Ten Donkelaar 1998a; 1998c). On the other hand, the dorsal pallium receives substantial input from the main olfactory bulb, and has been compared to parts of the lateral and olfactory cortices of reptiles and mammals. The lateral pallium of amphibians is subdivided into a dorsal component, also comparable to parts of the lateral cortex of amniotes, and a basal part which is comparable to the basolateral amygdalar complex of mammals and perhaps to the dorsal ventricular ridge (DVR) of birds and reptiles. In amphibians, there is also a caudal striatum (subpallial), which has been considered to be homologous to the striatal amygdala of reptiles and to the central amygdalar complex of mammals (Bruce & Neary 1995).

The reptilian pallium (Fig. 2) has a three-layered cortex, consisting of a medial and a dorsomedial moiety (both comparable to the mammalian hippocampal formation), plus a lateral (olfactory) cortex (Ulinski 1990), and finally a dorsal cortex (equivalent to the Wulst of birds) located between these two. Part of the dorsal cortex receives visual projections from the dorsal lateral geniculate nucleus, as well as some somatosensory input (Medina & Reiner 2000). In reptiles and birds, many non-olfactory sensory projections terminate in a prominent periventricular structure called the dorsal ventricular ridge (DVR; Fig. 2) (Ten Donkelaar 1998c; Ulinski 1983). The DVR is the most expansive telencephalic component of reptiles and birds, and is a main integratory center in their brains. It consists of an anterior part (ADVR) and a posterior or basal part (PDVR). The ADVR receives much of the sensory input, and its output is directed mainly to the subpallial corpus striatum and to the PDVR. The latter (corresponding to the archistriatum in birds) has been compared to parts of the mammalian amygdala and projects mainly to the hypothalamus (Lanuza et al. 1998; 1999; Ten Donkelaar 1998c).

Mammals are characterized by the possession of the isocortex (Fig. 2), which, during development, originates at least in large part from the dorsal pallium (Northcutt & Kaas 1995; Rakic 1988; 1995). Recent studies in mammals indicate that additionally, cells originating in the embryonic ganglionic eminences migrate tangentially in a dorsal direction and become incorporated into the isocortex, mostly as GABAergic interneurons (Anderson et al. 1997a; 1999; 2001; Lavdas et al. 1999; Marín & Rubenstein 2001; Nadarajah & Parnavelas 2002; Nery et al. 2002; Parnavelas 2000). The fact that tangentially migrating interneurons have also been observed migrating into the DVR and other pallial regions of birds (Cobos et al. 2001) suggests that the acquisition of this character predates the origin of mammals and the isocortex. The isocortex receives ascending sensory input from the thalamus and projects to the hippocampus and to the amygdala, as well as sending output to many lower brain centers including the thalamus, corpus striatum, various brainstem nuclei, and the spinal cord. Medial to the isocortex is the hippocampal formation, and lateral to it is the olfactory cortex. Finally, there is a claustroamygdaloid complex in the ventral pallium, containing both pallial and subpallial elements (Fig. 2).

3.3. The basal ganglia (subpallium)

A main component of the subpallium are the basal ganglia, which embryologically derive from the medial, lateral, and caudal ganglionic eminences (MGE, LGE, and CGE), which give rise to the corpus striatum, to the globus pallidus, and to some amygdalar components, respectively (see Fig. 2). The evolution of the basal ganglia has been rather conservative in the history of vertebrates (Marín et al. 1998; Medina & Reiner 1995; Smeets et al. 2000). However, a few changes in striatal output have taken place in mammals. One of these consists of the pathways connecting the basal ganglia with the mesencephalic optic tectum. There are

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multiple pathways for this connection, of which the most important are a ventral route via the substantia nigra which is present in all tetrapods, and a dorsal route via the pretectal nuclei. The pretectal pathway is most developed in anurans, some lizards, turtles, crocodiles, and birds, although it is weak or absent in urodeles, some lizards, snakes, and mammals, suggesting that it is a highly variable trait (Marín et al. 1998). In addition, some authors have described in mammals an emphasis of projections from the basal ganglia to the dorsal thalamus, which in turn projects to the isocortex (Brauth 1990; Medina & Reiner 1995). This perhaps has some relation to the loss of the pretectal pathway from the basal ganglia to the optic tectum (in mammals, the optic tectum is significantly reduced in relative size), and may be concomitant with the development of the mammalian corticospinal tract. In reptiles, projections from the basal ganglia to the dorsal thalamus appear to be less developed, although there has been an independent development of pallido-thalamic connections in birds (Brauth 1990). Finally, one important difference between reptiles and mammals is that the corpus striatum, whose more prominent input may be from the ADVR in reptiles, receives a major projection from the isocortex in mammals.

4. Diverging concepts of homology in pallial organization

Two alternative hypotheses have been raised regarding the origins of the mammalian isocortex, which have been elegantly summarized by Northcutt and Kaas (1995) as the "recapitulation hypothesis" and the "out-group hypothesis" (Fig. 3). Proponents of the recapitulation hypothesis suggest that a dorsal ventricular ridge (DVR)-like structure existed in the common ancestor of mammals and reptiles, which became somehow transformed into parts of the isocortex in the origin of mammals. According to the outgroup hypothesis, the common ancestor of reptiles and mammals would have had a cerebral hemisphere similar in its topographic organization to that of present-day amphibians. In this case, the most likely candidate for homology with the isocortex is the reptilian dorsal cortex, which derives from the dorsal pallium. Proponents of the recapitulation hypothesis mostly focus on similarities of sensory projections between the DVR and the isocortex, while proponents of the outgroup hypothesis have usually put weight on developmental evidence. Below, we will consider some of the evidence argued in favor of each of these hypotheses.

4.1. Connectional and neurochemical similarities between reptilian pallial fields and the mammalian isocortex

The dorsal cortex of reptiles and its avian equivalent, the Wulst, are considered to be homologous to both the striate or primary visual cortex and the somatosensory cortex of mammals, as all these structures receive similar sensory projections (Karten 1997; Medina & Reiner 2000; Nauta & Karten 1970; Shimizu & Karten 1993). More precisely, the visual projections from the retina to the thalamic dorsal lateral geniculate nucleus (the so-called thalamofugal visual pathway; see Fig. 4) terminate in the posterior dorsal cortex/Wulst of sauropsids and in the striate cortex of mammals, respectively. In addition, the somatosensory spinothalamic and the dorsal column-medial lemniscus pathways project to the anterior dorsal cortex/Wulst of sauropsids and to the somatosensory cortex of mammals (Medina & Reiner 2000; Wild 1997).

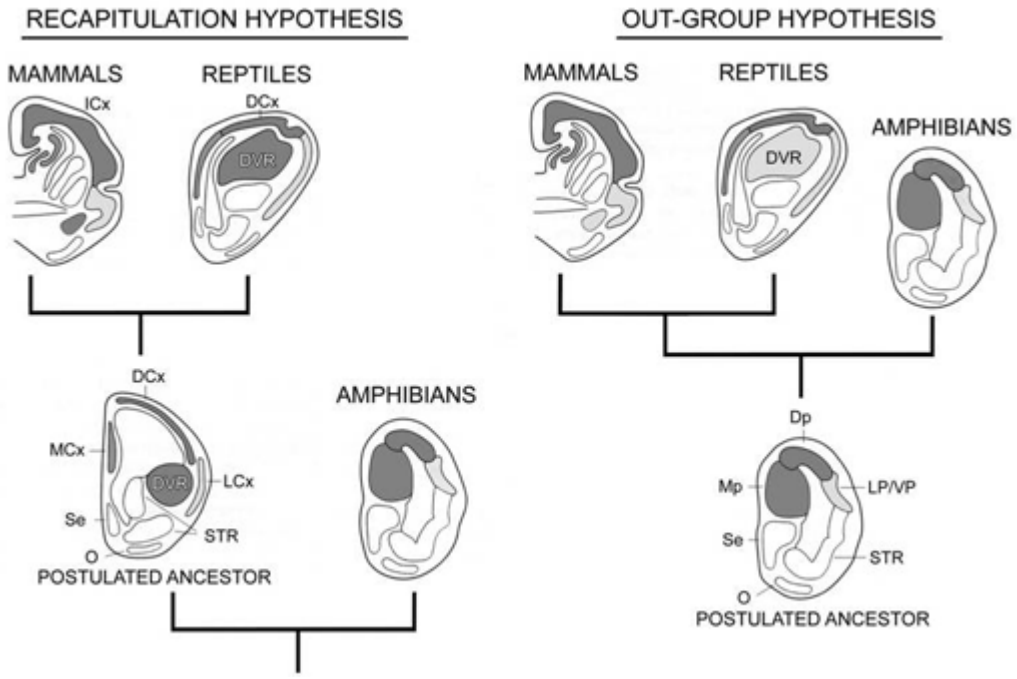

Figure 3. In the recapitulation hypothesis, the common ancestor of mammals and reptiles had a DVR-like structure which evolved into the isocortex in mammals and into the DVR of reptiles. In the out-group hypothesis, the DVR appears as a derived structure of reptiles and birds. O = olfactory tuberculum; Se = septum. Other abbreviations as in Figure 2.

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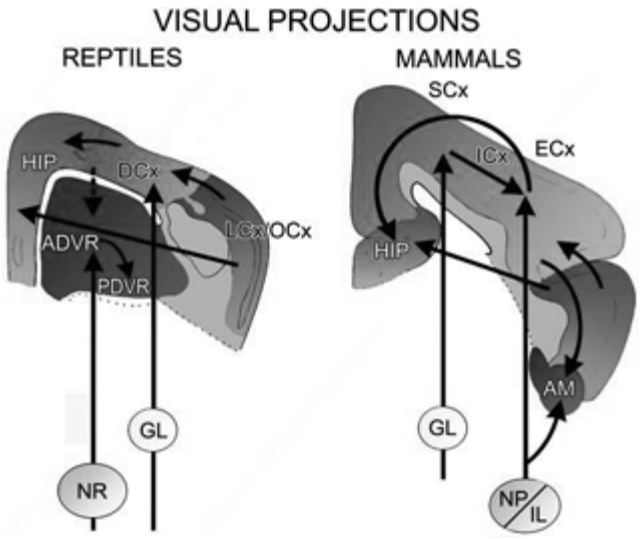

Figure 4. Diagrams summarizing some visual projections in mammals and in reptiles. Visual projections originate in the retina and project in two separate pathways: the thalamofugal or lemniscal path (LEM/TAF), which is directed to the dorsal lateral geniculate nucleus (GL), and from there to the dorsal cortex (DCx) in reptiles and to the primary or striate visual cortex (SCx) in mammals. The second visual route is the mesencephalic or tectofugal pathway (MES/TEF), that projects from the retina to the mesencephalic optic tectum (or superior colliculus). In reptiles, this pathway then synapses in the thalamic nucleus rotundus (NR) and from there projects to the reptilian anterior dorsal ventricular ridge (ADVR). In mammals, this pathway projects to the pulvinar nucleus (NP) and to the extrastriate visual cortex (ESt). There is some controversy as to whether the NR and the NP can be considered homologous (see text). Other two important sensory pathways (not shown) are the somatosensory pathways and the auditory pathway. In both mammals and reptiles, the somatosensory system contains a lemniscal component (spinothalamic and dorsal column pathways), that does not synapse in the mesencephalic colliculi before reaching the thalamus. These pathways end in the reptilian dorsal cortex and in the mammalian somatosensory cortex. On the other hand, the auditory pathway is a "mesencephalic" or collicular one that has an important mesencephalic relay (in the reptilian torus semicircularis or mammalian inferior colliculus) before reaching the thalamus. The auditory pathway ends in the reptilian ADVR and in the mammalian auditory cortex.

Additionally, the lateral cortex (LCx) or olfactory cortex (OCx) projects to the medial/dorsomedial cortex (MCx) in reptiles and to the hippocampus (HP) in mammals. The reptilian dorsal cortex (DCx) has connections with the MCx. In mammals, the primary visual cortex (SCx) exerts control over the extrastriate visual areas (ECx); all sensory isocortical areas indirectly project to the hippocampus (HIP). The reptilian posterior dorsal ventricular ridge (PDVR) is related to parts of the mammalian amygdalar system and receives projections from the ADVR. All other abbreviations as in Figure 2.

In addition to the thalamofugal visual pathway described above, there is a tectofugal visual pathway, which originates in the retina and projects to the mesencephalic optic tectum or superior colliculus. From there, this pathway is directed to the reptilian/avian thalamic nucleus rotundus and to the mammalian pulvinar nucleus, to end in the cerebral hemispheres (see Fig. 4). In the telencephalon, the tectofugal visual pathway ends in the avian/reptilian dorsolateral ADVR and in the mammalian extrastriate visual cortex. Furthermore, the auditory pathway terminates in the ventromedial ADVR of reptiles and in the mammalian auditory cortex. This similarity in sensory connectivity has been claimed to support the concept of homology between the DVR of reptiles and the visual extrastriate and auditory cortices of mammals (Karten 1968; 1997; Nauta & Karten 1970; Shimizu & Karten 1993). Furthermore, relying on similarities in intrinsic connectivity, it has been proposed that the avian ectostriatum (a component of the neostriatum), the rest of the neostriatum and the archistriatum correspond to the mammalian visual extrastriate cortical layers IV, II-III and V-VI, respectively (Karten 1997; Nauta & Karten 1970; Shimizu & Karten 1993; Veenman et al. 1995). Both the ectostriatum of birds and the isocortical layer IV of mammals receive thalamic afferents; these structures project to other parts of the avian neostriatum and to the layers II-III of the mammalian isocortex respectively, which themselves project to the archistriatum and to isocortical layers V-VI. Summarizing, this hypothesis (recapitulationist; Fig. 3) implies that the mammalian isocortex has a dual origin, one from the dorsal cortex of reptiles and corresponding to the striate visual cortex and the somatomotor cortex, the other from a structure homologous to the DVR of reptiles and corresponding to the auditory cortex (receiving the auditory projection) and to the extrastriate visual cortex (associated to the tectofugal visual pathway).

In agreement with this interpretation, Butler (1994a; 1994b) has classified dorsal thalamic nuclei as either *lemnothalamic* or *collothalamic. Lemnothalamic* nuclei receive projections from lemniscal systems, which do not synapse in the mesencephalic colliculi, like the visual thalamofugal pathway (which relays on the lateral geniculate nucleus), and the somatosensory, spinothalamic, and dorsal column pathways (see Fig. 4). *Collothalamic* nuclei receive sensory projections from the mesencephalic colliculi (like the visual tectofugal and the auditory pathways). Lemnothalamic nuclei project to the dorsal cortex of reptiles and birds, and to the more medial/dorsal aspects of the isocortex of mammals (such as the striate or primary visual cortex and the somatosensory cortex), whereas collothalamic nuclei project to the ADVR of reptiles and birds, and to more lateral/ventral regions of the mammalian isocortex (such as the extrastriate visual cortex and the auditory cortex).

In terms of neurotransmitter contents, the DVR resembles the reptilian dorsal cortex and the mammalian infragranular isocortical layers (Reiner 1993). The granular and supragranular isocortical layers differ from these structures in that some of their cells possess neurotransmitters (CCK8, VIP, and acetylcholine) that are absent in reptiles and in the infragranular isocortical layers.

4.2. Differences in connectivity between the DVR and the isocortex

Other connectional evidence points to important differences between the reptilian DVR and the mammalian isocortex. First, the mammalian extrastriate visual cortex receives an important input from the primary or striate visual cortex (Montero 1993; Rosa & Krubitzer 1999). Although in reptiles, projections from the dorsal cortex to the DVR have been described (Ten Donkelaar 1998b; Ulinski 1990), these projections do not exert a significant influence. Secondly, the mammalian isocortex projects reciprocally to the entorhinal cortex and from there to the hippocampus (Haberly 1990; Insausti 1993; Rosene & Van Hoesen 1987;

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Van Hoesen 1982), while in reptiles few connections have been reported from the DVR to the medial/dorsomedial cortex or hippocampus (Ten Donkelaar 1998b; Ulinski 1983; 1990). Note that, in this regard, the mammalian isocortex resembles more the reptilian dorsal cortex, which has important connections with the medial/dorsomedial cortices (Ulinski 1990; Fig. 4). Finally, the main termination of sensory projections is not always in comparable structures. In amphibians, the auditory and tectal visual pathways terminate mainly in the corpus striatum, which is clearly not homologous to either the isocortex or the DVR (Ten Donkelaar 1998a; Wilczynski & Northcutt 1983).

Moreover, based on comparisons of connectivity, Bruce and Neary (1995) have argued that the reptilian DVR is most similar to the mammalian lateral amygdala, since both structures receive projections from collothalamic nuclei and both project to the corpus striatum, the striatal amygdala, and the ventromedial hypothalamus. (More precisely, the reptilian ADVR would correspond to the mammalian basolateral amygdala, while the whole DVR might correspond to the whole lateral amygdalar nucleus.) On the other hand, the isocortex projects to many other brain regions in the brainstem and spinal cord, and does not project to the hypothalamus. These authors claim that fewer changes in connectivity are required, by assuming homology between the lateral amygdala of mammals and the DVR of reptiles, than by considering homology between the isocortex and the DVR.

One particularly relevant aspect in this discussion concerns the thalamic pulvinar nucleus of mammals. This nucleus receives projections from the superior colliculus and projects to the extrastriate visual isocortex, and has been considered to be homologous to the reptilian or avian nucleus rotundus, which receives projections from the optic tectum and sends efferents to the ADVR (Fig. 4; Butler 1994b). Furthermore, Major et al. (2000) recently described marked similarities of the dendritic morphology of motion-sensitive tectopulvinar neurons in mammals and birds. In both groups, such neurons have dendritic arborizations that end in monostratified arrays of spiny terminal specializations called "bottlebrush" endings. The presumed homology between the pulvinar and the rotundus nuclei has been a strong element in the theory of homology between the lateral isocortex and the DVR, since these two nuclei have been considered to form part of the collothalamic, tectofugal visual pathway in mammals and reptiles, respectively. According to Bruce and Neary (1995), because in mammals the thalamic nuclei projecting to the lateral amygdala belong to the intralaminar complex, these nuclei and not the pulvinar nucleus should be considered homologous to the thalamic nucleus rotundus of reptiles and birds. Supporting this interpretation, recent reports have emphasized the fact that the mammalian pulvinar nucleus receives a different type of tectal projections than the reptilian rotundus; the pulvinar receives axons from late-born, superficial collicular layers, whereas the rotundus receives axons from early-born, deep collicular layers (Dávila et al. 2000; 2002; Guirado et al. 2000; Redies et al. 2000; Yoon et al. 2000). These authors subdivide the thalamic nuclei into three tiers: the intermediate and ventral tiers receive projections from the mesencephalic colliculi, and the dorsal tier receives projections from lemniscal systems. According to this view, the reptilian nucleus rotundus and the mammalian intralaminar nuclei might correspond to intermediate tier nuclei, while the mammalian pulvinar might be a dorsal tier nucleus which acquired a tectal input in the origin of mammals. Whichever interpretation of pulvinar homology is correct, the outgroup hypothesis implies that this nucleus must have undergone important changes in connectivity in the origin of mammals: either its efferents were re-routed to the dorsal pallium (Shimizu & Karten 1993), or it expanded from a subgroup of intralaminar nuclei and received invading afferents from the superior colliculus (Bruce & Neary 1995). On the other hand, the recapitulation hypothesis specifies little connectional changes for the origin of the pulvinar nucleus, as it assumes it to be the homologue of the reptilian nucleus rotundus.

4.3. Developmental criteria

Another criterion to establish homology is the comparison of the developmental origin of structures across taxa. Northcutt (1996b) has already pointed out the importance of the ontogenetic context in evolutionary considerations, as originally proposed by Garstang (1922). More precisely, in a lucid discussion of the different approaches to the problem of homology, Striedter (1997) quotes Russel's (1916/1982) considerations favoring similarity of development as a strong criterion for homology. In Russel's view, the generalized morphology and topographic relations are shown most clearly in early developmental stages; this view facilitates cross-species comparisons. This assumption is valid in cases in which there is cross-species conservation of embryonic processes, whereas adult morphology tends to diverge. Alternatively, in cases of embryological diversity with adult conservation, perhaps adult structures and relations may be a better criterion for homology (Aboitiz 1995; 1999b). In a similar line, Striedter and Northcutt (1991) and Striedter (1997) have called attention to the importance of combining embryological information with phylogenetic data in order to discern true homologies from instances of independent evolution or homoplasy, and emphasize phyletic continuity as a strong requirement for homology. In the case of the amniote telencephalon, phylogenetic evidence points to a notable conservation of early embryonic structure with adult diversification (Aboitiz 1995; 1999b; Striedter 1997), which adds weight to embryological comparisons as a reliable criterion for homology.

During embryogenesis, the DVR (especially its anterior part) develops from a position deep inside the olfactory cortex (Källén 1951; Striedter et al. 1998), whereas most of the isocortex originates from the dorsal pallium. This is supported by studies of expression patterns of regulatory homeobox-like genes in the embryonic forebrain, which have revealed a conserved mosaic organization in which the different compartments develop into specific brain components in the adult (Gellon & McGinnis 1998; Moens et al. 1998; Puelles & Rubenstein 1993; Seo et al. 1998). In the embryonic mammalian telencephalon, distinct markers for pallial and subpallial regions have been detected. The embryonic lateral and medial ganglionic eminences (GEs), which are located in the lateral subpallium, express the marker genes Dlx-1 and Dlx-2 (Anderson et al. 1997b). The cerebral cortex arises mostly from the embryonic pallium and is characterized by the expression of genes of the Emx and the Otx families (Acámpora & Simeone 1999; Mallamaci et al. 1998; Pannese et al. 1998; Puelles & Rubenstein 1993; Simeone et al. 1992; see Fig. 5). Smith Fernández et

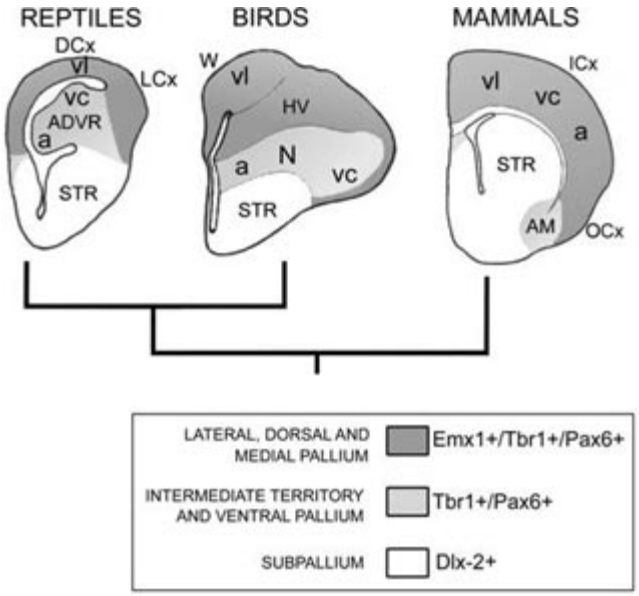

Figure 5. The cerebral hemispheres of reptiles, birds, and mammals, indicating the medial, dorsal and lateral pallium (dark grey), which, during development, expresses the marker genes Emx 1/2, Otx 1/2, Pax-6, and Tbr-1, and gives rise to the Wulst (W, equivalent to the reptilian DCx) and hyperstriatum ventrale (HV) of birds, and to cortical structures in reptiles and mammals. The subpallium (white) expresses Dlx-type genes during embryogenesis and gives rise to the corpus striatum (STR) among other structures. Light grey indicates the intermediate territory or ventral pallium, which is largely positive for the genes Emx-2, Pax-6 and Tbr-1 and gives rise to the anterior dorsal ventricular ridge (ADVR) of reptiles, to the neostriatum (N) of birds (which corresponds to a large part of the reptilian ADVR), and to the basolateral amygdala (AM) of mammals. In each vertebrate class, the projection sites of the auditory pathway (a) and the visual pathways via the lateral geniculate (vg, thalamofugal) and via the optic tectum (vt, tectofugal), are indicated. D, dorsal cortex; ISO ICx, isocortex. (Based on Smith Fernández et al. 1998 and Puelles et al. 1999.)

al. (1998) identified for the first time an intermediate territory (IT) in the equatorial region of the hemisphere, between the pallium and the subpallium of amphibians, reptiles, birds, and mammals, which does not express either the Emx-1 or Dlx-1 markers of the pallium and subpallium, respectively, but is largely positive for the gene Pax-6 (Smith-Fernández et al. 1998; see Fig. 5). More recent reports (Puelles et al. 1999; 2000) confirmed the existence of the IT (which has been termed ventral pallium, VP, by these authors), and extended the previous findings by showing that Pax-6 is expressed mainly near the ventricular zone of the whole pallium including the IT/VP; another gene, Tbr-1, is also expressed in both the pallium and the IT/VP but has a more superficial domain of expression. Thus, the medial, the dorsal, and part of the lateral pallium express Emx-1 and Tbr-1 superficially and Pax-6 more internally, whereas the IT/VP expresses Tbr-1 superficially and Pax-6 deeply, but not Emx-1 (Fig. 5).

In sauropsids, an important part of the ADVR (including the neostriatum and ectostriatum of birds) and part of the lateral cortex develop from the IT/VP (Fig. 5), whereas in mammals, the basolateral amygdalar complex, part of the claustral complex, the endopiriform nucleus, and parts of the lateral or olfactory cortex – among other structures – derive from this region (Puelles et al. 1999; 2000; Smith-Fernández et al. 1998). In this context, two mammalian structures have recently been proposed to be homologous to the ADVR: the basolateral amygdala (Bruce & Neary 1995; Puelles et al. 1999; Smith Fernández et al. 1998) and the endopiriform nucleus (Striedter 1997). Developmental evidence favors both the basolateral amygdala and the endopiriform nucleus as homologous to the ADVR (Puelles et al. 1999; Smith Fernández et al. 1998). On the other hand, connectional evidence indicates similarity between the ADVR and the basolateral amygdala (Bruce & Neary 1995), while the connections of the endopiriform nucleus parallel quite closely those of the olfactory cortex (Behan & Haberly 1999).

Smith-Fernández et al. (1998) have argued that, in reptiles and birds, the IT/VP remains as a distinct neuroepithelial zone until late development, the period in which it gives rise to most of the ADVR. On the contrary, in mammals, this territory has been described as producing only the above-mentioned, early-generated components, becoming obliterated between the Emx-1-positive and the Dlx-½-positive zones in later development (Smith-Fernández et al. 1998). Swanson (2000) and Künzle and Raddtke-Schuller (2001) suggest that the mammalian claustral complex has developmental timing similar to early-produced cortical elements, which in part agrees with the concept that the IT/VP gives rise mostly to early-produced brain components. This evidence may suggest that in mammals there are no structures comparable to those late-generated components in the avian/reptilian intermediate territory, which also agrees with the concept that in mammals there is no strict homology to the reptilian ADVR (Aboitiz 1992).

Puelles et al. (1999) seem to disagree with the concept of the IT/VP disappearing from the neuroepithelial surface in mammals, although they admit that this territory is considerably compressed between the lateral pallium and the developing striatum. Undoubtedly, further studies are urgently needed to clarify the developmental fate of the IT/VP in mammals. In any case, for this component to contribute to isocortical development as predicted by the recapitulation hypothesis, a massive tangential migration of Emx-1-negative neurons should take place from the IT/VP into the dorsal pallium, making up the visual extrastriate and the auditory cortices. There is evidence that many isocortical GABAergic cells originate in the subpallial GEs (ganglionic eminences) and migrate dorsally into the isocortex (Anderson et al. 1997a; 2001), which raises the possibility that some excitatory cells from the IT/VP also migrate to the dorsal pallium. Therefore, it cannot be discounted that the IT/VP becomes reduced as a consequence of cell emigration to the dorsal pallium during late development. Just as cells migrating from the GEs into the isocortex keep expressing their subpallial molecular markers (Anderson et al. 1997a), cells migrating from the IT/VP might make the visual extrastriate and auditory cortices largely Emx-1-negative (something that has not been observed), unless they somehow begin to express Emx-1 as they invade the dorsal pallium. Nevertheless, no evidence yet exists for a massive migration from the IT/VP into the isocortex.

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5. The origin of the isocortex: I. Developmental aspects

Considering the evidence reviewed in the above sections, we have argued that the structure that most likely corresponds to a large part of the mammalian isocortex is the reptilian dorsal cortex, which expanded both tangentially (increase in area) and radially (increase in thickness) during the origin of mammals. As mentioned earlier, at this point we intend to propose some hints as to how this structure has changed from a small, three-layered structure in reptiles into a large, six-layered component in the mammalian brain.

5.1. Dorsoventral gradients and expansion of the dorsal pallium

During embryogenesis, telencephalic differentiation is patterned by dorsoventral gradients of regulatory genes such as sonic hedgehog (Shh), which specifies a ventral phenotype by inducing subpallial markers like Nkx-2.1, Gsh-2, and Dlx-2. On the other hand, dorsal pallial markers and phenotypes are at least partly determined by the gene Gli-3 (for review, see Monuki & Walsh 2001; Wilson & Rubenstein 2000). Furthermore, mutations in some genes can produce displacements in compartment boundaries during development. In mutants for the gene Nkx-2.1, the corpus striatum becomes enlarged at the expense of the more ventral globus pallidus (Sussel et al. 1999). In the Emx-2 −/− mutant mouse, presumptive cortical regions develop into a basal ganglia phenotype (Muzio et al. 2002a). Perhaps more interesting for our purposes, mutants of the gene Pax-6 show a dorsal expansion of the medial ganglionic eminence (MGE) into the territory of the lateral ganglionic eminence (LGE; Chapouton et al. 1999; Stoykova et al. 2000). In this mutant, the expression limits of marker genes such as Emx-1, Tbr-1, Shh, Dlx-1, and Nkx-2.1 become displaced dorsalwards, producing a dorsal shift in the pallial-subpallial boundary, which is associated with dysgenesis of the claustrum, endopiriform nucleus, insular cortex, and piriform cortex. This dorsal displacement of boundaries may be, in part, the consequence of an enhancement of ventro-dorsal migration of cells (mostly inhibitory interneurons) from the GEs into pallial territories. Normally, Pax-6 upregulates R-cadherin, which acts as a barrier for the migration of many cells from the MGE and the LGE into the cortex (Chapouton et al. 1999). The release of this barrier in the Pax-6 mutant may imply an increased number of cells migrating into the dorsal telencephalon. Another important possibility is that the boundaries expand by changing the identity of the cells previously belonging to neighboring compartments. That is, the lack of Pax-6 signal may transform future pallial cells into subpallial phenotypes. In this context, the gene Gsh-2, a marker for the GEs (Szucsik et al. 1997), has been proposed to play a complementary role with Pax-6 in the specification of the GEs and the IT/VP; in mutants for Gsh-2, part of the LGE may differentiate as IT/VP, whereas in Pax-6 mutants, the IT/VP is re-specified to become a LGE-like structure (Yun et al. 2001). More recently, Butler and Molnár (2002) reported the development of a DVR-like structure (termed the pallial "mound") in the Pax-6 −/− mutant, which they consider to consist of cells from the lateral migratory stream that failed to migrate into the lateral isocortex. However, in addition to not expressing Pax-6, this mutant has been described to lack, or express very poorly, other ventral pallial markers such as Dbx-1 (Osumi 2001; Yun et al. 2001), and its ventral pallial structures have been reported to be, in general, dysgenic. Therefore, it is not clear to what extent this pallial "mound" can be directly compared to the reptilian DVR. It could be that the mound is formed by the accumulation of cells in the ventricular and subventricular zones of the lateral and dorsal pallium, which depend on Pax-6 for proper migration (Heins et al. 2002; Tarabykin et al. 2001). In any case, we agree with Butler and Molnár (2002) in that the Pax-6 −/− mutant may represent a ventralized phenotype somehow similar to that of the reptilian brain, although not in some specific details.

Other gene systems have also been found to be expressed in gradients in the developing telencephalon. For example, the genes of the Wnt family are required for hippocampal development and are expressed strongly in the caudomedial margin of the cortical pallium (Kim et al. 2001). Wnt receptors of the Frizzled family are most concentrated in the isocortical neuroepithelium, while being sparse or entirely absent in the more medial hippocampal neuroepithelium (Kim et al. 2001). Similarly, two Wnt inhibitors (secreted Frizzled-related proteins 1 and 3) are expressed in opposing anterolateral to caudomedial gradients in the telencephalic ventricular zone. In addition, the gene Emx-2, which is similar to Emx-1 but has a more widespread domain of telencephalic expression (including pallium and subpallium; Gulisano et al. 1996), has been found to be arranged in a gradient with maximal concentrations in the posteromedial isocortex and minimal concentrations in the anterolateral isocortex (Mallamaci et al. 2000). Conversely, the gene Pax-6 is expressed in a complementary gradient, being maximally expressed in the anterolateral isocortex and minimally expressed in the posteromedial isocortex (Bishop et al. 2000). Interestingly, mutants of Emx-2 show reduction of the posteromedial cortical areas (i.e., visual), and, concomitantly, there is significant expansion of anterolateral cortical regions (i.e., somatosensory and frontal), whereas Pax-6 mutants evidence a reduction of anterolateral areas and expansion of posteromedial regions (Bishop et al. 2000; Mallamaci et al. 2000).

The above evidence suggests that the modulation of overall dorsoventral, frontocaudal, and/or mediolateral gradients of regulatory gene expression may have profound effects in the development of specific telencephalic components. It is therefore possible that the expansion of the dorsal pallium in primitive mammals occurred partly as a consequence of the enhancement of a dorsalizing signal in telencephalic development. Since the structure that expanded the most in mammalian evolution is the dorsal pallium (isocortex) rather than the medial pallium, the lateral pallium, and the IT/VP, there may be some yet unknown genes exclusively determining the fate of this structure, which increased their domains of expression and enhanced cell proliferation specifically in this region. In particular, genes involved in the regional specification of the cortical ventricular zone, like Wnt-3a, BMP, Neurogenin, and others, may have been fundamental in the expansion of this structure (Monuki & Walsh 2001). The expansion of the presumptive dorsal pallial territory may have produced a ventrolateral displacement of the lateral pallium, which perhaps differentiated in territory originally destined to the

IT/VP. In this way, through shifts in the boundaries of the territories of regulatory gene expression, cells that initially differentiated in one specific compartment may have acquired patterns of differentiation of other telencephalic areas. The obliteration or compression of the IT/VP that has been described in the mammalian telencephalon (Smith-Fernández et al. 1998), might result from tangential expansion of the expression domain of Emx-1 and related markers, from invasion of the IT/VP by tangentially migrating Emx-1 positive cells, or simply from its elimination by cell exhaustion or cell death. Of course, this may not be the whole story because, in addition to the displacement of pallial boundaries, there has also been an overall increase in brain size, which possibly was largely produced by an increase in proliferative activity within the dorsal pallium and other regions. In this context, a recent report indicates that transgenic mice expressing b-catenin in neural precursors develop an enlarged cortical surface area, while maintaining a normal cortical depth (Chenn & Walsh 2002). Another report indicates that ASPM, a gene essential for mitosis in embryonic neuroblasts, is required for attaining a normal cortical size (Bond et al. 2002). Perhaps these genes are downstream elements in the cascade triggered by the dorsalizing elements in early development. On the other hand, the pallium of reptiles may present an enlarged IT/VP because of a ventralizing pallial influence that maintains a restricted expression of Emx-1 and other dorsal pallial genes.

Another, not alternative possibility is that dorsalizing factors somehow triggered the tangential migration of excitatory neurons from the IT/VP into the dorsal pallium, thus contributing to the expansion of this brain region. As said, this mechanism might imply that these neurons acquired Emx-1 expression as they invaded the dorsal pallium. Increased tangential migration of inhibitory cells toward the dorsal pallium is observed in ventralized phenotypes like the Pax-6 mutant (Chapouton et al. 1999), but yet there are no observations on tangential migration of excitatory cells or their regulation.

Reiner (2000) has proposed that, in the common ancestor to reptiles and mammals, there was a structure that diverged into the lateral isocortex of mammals and into the ADVR of reptiles. This structure was either Emx-1 negative and acquired Emx-1 expression in mammals, or, alternatively, was Emx-1 positive and lost Emx-1 expression in reptiles and birds. This possibility is certainly consistent with the concept of a dorsalized pallium in mammals with respect to reptiles. However, in many reptiles the topographic relation between the IT/VP and the dorsal cortex is such that a large part of the olfactory cortex is interposed between them. Perhaps a mere shift in gene boundaries may not be sufficient to transform the IT/VP into an isocortical area, and a tangential migration of cells from the IT/VP through the lateral pallial territory into the dorsal cortex would also be needed. Exceptions to the topographic relations between DVR, lateral cortex, and dorsal cortex are found in turtles and in Sphenodon. In turtles, a structure termed the pallial thickening (a dorsal cortex-derivative located beneath the lateral cortex) bridges the ADVR with the dorsal cortex (Ten Donkelaar 1998b; Ulinski 1990); whereas in Sphenodon there is continuity between the ADVR and the dorsal cortex in the rostral hemisphere (Reiner & Northcutt 2000). It is not clear if cellular continuity between the ADVR and the dorsal cortex is an ancestral character of reptiles. If so, the possibility of a mechanism as proposed by Reiner (2000), might depend on the precise topographic relations between ADVR and dorsal cortex (recall that auditory and extrastriate visual cortices are located in the posterior hemisphere and that the continuity between ADVR and dorsal cortex tends to be found anteriorly).

Summarizing, two possibilities (not necessarily exclusive) may account for the relative growth of the mammalian dorsal pallium in relation to the IT/VP. The first is, that the dorsal pallium expanded tangentially, perhaps invading territory destined to the lateral pallium, and the latter was displaced into territory destined to the IT/VP. The second possibility is that of a massive migration of cells from the IT/VP into the dorsal cortex. This mechanism would make the visual extrastriate and auditory cortices largely negative for Emx-1 (and positive for Dbx-1; Yun et al. 2001), something that has not been observed. Still, there is the possibility that Emx-1-negative cells from the IT/VP migrate dorsally very early in development, and acquire Emx-1 expression after they arrive in the dorsal pallium. Unfortunately, at this point there is no evidence indicative of a migratory process from the ventral pallium into the dorsal pallium. Further studies oriented to identify regulatory genes specific for the dorsal pallium and their regulatory mechanisms, as well as analyses of cell migration into and out of the IT/VP, will help to determine to what extent each of these two possibilities accounts for the expansion of the isocortex.

5.2. Laminar development of the isocortex

The mammalian isocortex develops through successive waves of neuronal migration following the pathway imposed by radial glia from the ventricular zone of the hemisphere (as mentioned, there is also contribution of tangentially-migrating cells). The first migratory waves make up a transient, embryonic cell layer known as the preplate (Fig. 6). This is later split into a superficial marginal zone (future layer I) and a deep subplate by the arrival of older cells that make up the future cortical plate (cortical layers VI to II). That is, cells of the cortical plate become positioned within the preplate, dividing the latter into a superficial marginal zone and a deep subplate. Within the cortical plate, cells become arranged in an inside-out neurogenetic gradient, in which late-produced cells migrate past early-produced ones and locate above them. Thus, the more superficial layer II contains the youngest neurons of the cortical plate, and the deepest layer VI contains the oldest neurons of the cortical plate. In many species, cells of the preplate (subplate and marginal zone) die during late development (for full review, see Rakic 1988).

Marín-Padilla (1978) originally proposed that the mammalian preplate represented the ancestral reptilian cortex. In mammals, Swanson (2000) and Künzle and Radtke-Schuller (2001) have recently emphasized the developmental relation (in terms of similar timing of origin) between the subplate and other early-produced cell populations such as the claustrum. Considering that part of the claustral complex corresponds to the IT/VP and has been considered homologous to part of the reptilian ADVR, by extension the possibility is open that some subplate cells can be comparable to some reptilian embryonic or adult cortical cells.

However, there may be some problems comparing an embryonic structure like the mammalian preplate with an

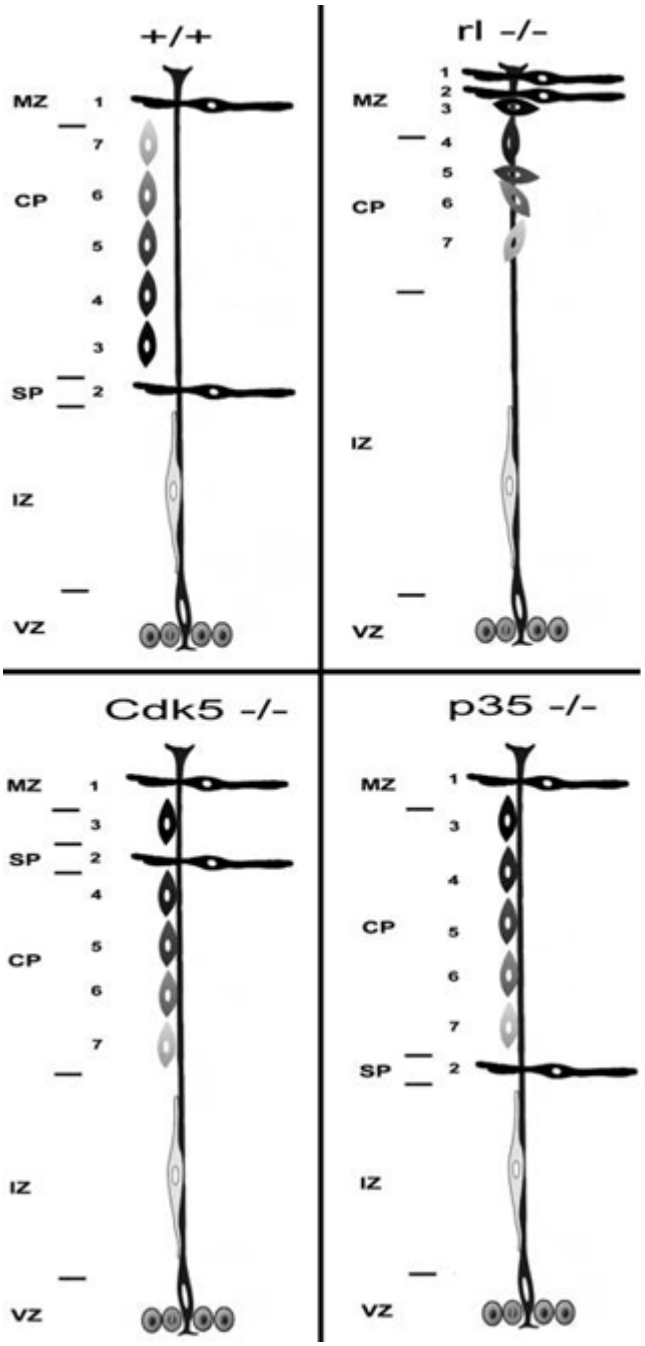

Figure 6. Laminar isocortical arrangement in normal and mutant mice. In the normal mouse (+/+), the preplate (consisting of the marginal zone, MZ, and the subplate, SP) is split by the cells of the younger cortical plate (CP), which cross the SP and remain between the latter and the MZ, in an inside-out gradient (where late-born cells become positioned above early-born cells). Numbers indicate order of origin of the different cell layers. In the reeler mouse (rl −/−), CP cells are unable to split the preplate and accumulate below it, invading layer I. In addition, CP layers are arranged in an abnormal outside-in gradient (where late-born cells become positioned below early-born cells). In the cdk5 −/− mutant, CP layers are arranged in an outside-in gradient. Furthermore, only the earliest-born CP cells are able to cross the SP and reach their normal position; subsequently born CP cells accumulate below the SP. In the p35 −/− mutant, all CP cells are able to cross the SP, but arrange in an outside-in gradient. The difference between the cdk5 −/− mutant and the p35 −/− mutant may be that in the latter, some other activator of cdk5 (perhaps p39) permits cortical plate neurons to migrate past the subplate. IZ, intermediate zone; VZ, ventricular zone.

adult one like the reptilian cortex. Perhaps it is more meaningful to compare the developing mammalian isocortex with the developing reptilian cortex. Supèr et al. (1998b) argue that an incipient preplate, including a subplate, may be already present in reptiles, and we have observed horizontal cells above and below the developing cortical plate of reptiles (cf. Aboitiz 1999a), suggesting a preplate-like arrangement. Nacher et al. (1996) described the presence of somatostatin-positive cells, appearing first in the inner plexiform layer and later in the outer plexiform layer of the medial and dorsal cortices of the developing lizard brain. Nevertheless, Cordery and Molnár (1999) argue that no simple homology can be found between the early cortical cells in the turtle and cells in the mammalian preplate, since the former have very low levels or simply lack the markers reelin and calbindin (which are present in the mammalian preplate). However, they also observed cells positive to neuropeptide-Y, scattered in all regions of the ventral and dorsal pallium of the turtle (Cordery & Molnár 1999). We also need to note that Kostovic and Rakic (1990) consider that much of the subplate is not ancestral; instead, this structure can be viewed as an acquisition of the mammalian brain, since it is most complex in those areas that appear later in mammalian evolution, and also is more complex in mammals with more complex brains.

Thus, there seem to be early cell populations in the developing reptilian cortex, which may appear before the development of the reptilian cortical plate. However, these early cells are not totally equivalent to the mammalian preplate cells. The question is to determine to what extent the reptilian early cells form a preplate-like structure that is split into a marginal zone and a subplate by the arrival of younger cortical neurons. It is important to note that, if in reptiles there is a subplate-like structure, cortical plate cells might have to migrate past these cells as they do in the mammalian brain.

Comparing the adult isocortex with the reptilian cortex, Ebner (1969) and Reiner (1991; 1993) proposed that the reptilian cortex mostly corresponds to the deep isocortical layers VI and V of the mammalian cortical plate. Cells in the superficial layers IV–II are morphologically and neurochemically different from reptilian cells, and can be considered as a derived character of the mammalian isocortex.

Summarizing the above points, some cells in the embryonic preplate and in layers VI–V of the adult mammalian isocortex may be comparable to the early-produced and late-produced cortical cells of reptiles. In the evolution of the mammalian isocortex, new cell types have appeared both in the preplate (including reelin- and calbindin-positive cells) and in the cortical plate. In particular, the late-born, superficial isocortical layers IV–II may have originated as an extension of the neurogenetic period in cortical development, and largely consist of phenotypically new cell types (Aboitiz 1999a; Aboitiz et al. 2001a; 2001b).

Note that, in mammals, the difference between the embryonic subplate and the deepest layers of the adult cortical plate are not absolutely clear-cut, and that these structures may be developmentally related. For example, mutations in the gene Tbr-1 cause defects in both the preplate and the deepest layer VI of isocortex (Hevner et al. 2001), suggesting similarities in genetic organization between these structures. Moreover, considering that in the rat and other mammals with a limited or moderate degree of isocortical expansion, many subplate cells survive into

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adulthood, forming the layer VII (Reep 2000; Woo et al. 1991), it is also possible that in some reptiles there are early cortical cells that remain until adulthood.

Further molecular evidence supports the concept of the superficial isocortical layers IV–II as developmentally distinct from the inferior layers VI and V. In addition to specifying pallial territories, the gene Pax-6 participates in the laminar differentiation of the isocortex. Mutants for Pax-6 exhibit cortical migration defects – consisting of the inability to migrate and differentiate, on the part of superficial, late-generated isocortical neurons belonging to layers IV to II (Caric et al. 1997; Fukuda et al. 2000). It has been proposed that Pax-6 specifies a cellular environment that permits late-born cells to express their developmental potential. Furthermore, a recent report shows that early-produced cortical plate neurons destined to isocortical layers VI and V are produced in the embryonic ventricular zone and express the marker gene Otx-1. On the other hand, late-produced neurons, which depend on Pax-6 for their development and are destined to superficial layers IV–II, are produced in the subventricular zone and express the marker gene Svet-1 (Tarabykin et al. 2001). Thus, it is perhaps likely that Pax-6 somehow participated in the origin of the isocortical superficial layers (see Aboitiz et al. 2001b), and that this may have involved the recruitment of the subventricular zone in the neurogenetic process. In this context, the recent findings that radial glial cells can generate neurons, and that their neurogenic potential depends on Pax-6 expression (Heins et al. 2002) is of great interest. It will also be of interest to determine the contribution of the subventricular zone and of the genes Pax-6 and Svet-1 to dorsal cortical development in reptiles.

5.3. The inside-out neurogenetic gradient

Another feature that characterizes the isocortex is its inside-out neurogenetic gradient (Angevine & Sidman 1961; Rakic 1974), in which late-produced neurons migrate past layers of early-produced neurons and become located in more superficial layers. In the reptilian cortex, laminar development proceeds according to an outside-in neurogenetic gradient, where late-produced neurons become positioned below early-produced ones and thus are not able to migrate past older cells (Goffinet et al. 1986). In the mammalian isocortex, there are at least two signaling cascades (which may be related to some degree) involved in the generation of the inside-out neurogenetic gradient. Mutations in components of these two cascades produce an inversion of the normal inside-out neurogenetic gradient, positioning cells in an outside-in gradient reminiscent to that of reptilian cortex (Fig. 6). One of these signaling pathways is related to the extracellular protein reelin, to its receptors (which include the low density and the very low density lipoprotein receptors, integrin receptors, and N-cadherin) and to its downstream intracellular components such as Dab-1 (Curran & D'Arcangelo 1998; Dulabon et al. 2000; Frotscher 1998; Senzaki et al. 1999; Trommsdorff et al. 1999). Reelin, which is secreted by the Cajal-Retzius cells and related cell types in the embryonic marginal zone (future layer I), has been proposed to work by binding to its receptors in the surface of the migrating neurons, detaching them from the radial glia (Aboitiz et al. 2001b; Dulabon et al. 2000; Pinto-Lord et al. 1982). If reelin signaling is defective as in the reeler mutant, neurons do not separate from the radial glia and keep migrating into the marginal zone, compressing the preplate above them. Thus, in the reeler, cells might arrange in an abnormal outside-in pattern because the early-born cells remain attached to the radial glia and do not leave space for younger neurons to migrate past them (Pinto-Lord et al. 1982). Interestingly, the reelin gene is faintly expressed in the developing cortex of reptiles (Bar et al. 2000), indicating that early cells in the marginal zone have undergone important transformations in the origin of mammals, and that reelin may have been an important element in isocortical evolution. Recently, it has been observed that the role of reelin may be more complex than previously thought. For example, in organotypic cultures, neurons may migrate across the reelin-positive marginal zone to differentiate in a cortical plate at a distance from the cortical slice (Hedin-Pereira et al. 2000). Somehow, neurons in this preparation are prevented from arresting migration in their proper place; perhaps this results from blocking reelin function, the reelin cascade, or other factors which may be altered in the organotypic culture. A more recent study found that some of the wild characters – like the ability to split the preplate – were rescued by artificially expressing ectopic reelin in the ventricular zone of reeler mice (Magdaleno et al. 2002). However, in the cortical plate, many cells still show an inverted lamination and migrate into the marginal zone as in the standard reeler mutant. Likewise, in a mutant for Dab-1 (p45), a downstream component of the reelin cascade, late-born neurons are observed in the marginal zone (Herrick & Cooper 2002), suggesting that they are not prevented from migrating into it. Another study shows that migration of neural stem cells is impaired in the reeler mutant (Kim et al. 2002). Tabata and Nakajima (2002) found that abnormalities in the internal plexiform zone may cause some of the deficits observed in the reeler. Finally, in the olfactory bulb, Hack et al. (2002) observed that reelin acts as a detachment signal but not a stop or guidance clue. Despite these recent findings, we believe the evidence indicates, overall, that in natural conditions, reelin has a role in the final stages of neuronal migration, perhaps specifically in the generation of a cell-free marginal zone. Any proposed mechanism for reelin action must take into account the facts that in the reeler mouse migrating neurons invade the marginal zone, and remain abnormally attached to the radial glia (Pinto-Lord et al. 1982).

The other signaling cascade depends on the cyclin-dependent, serine-threonine protein kinase 5 (cdk5) and its neural-specific activator p35 (Chae et al. 1997; Ohshima et al. 1996; cf. Fig. 6). It has been suggested that cdk5/p35 permit cortical neurons to migrate through preexisting cell layers within the cortical plate (Kwon & Tsai 1998), since in the mutants (more specifically, in the p35 mutant) cortical cells apparently respond normally to reelin in the marginal zone and yet arrange in an abnormal outside-in gradient (in this mutant, the preplate is correctly split but the cortical plate arranges in an inverted sequence). Furthermore, cdk5/p35 inhibit N-cadherin cell aggregation in migrating neurons (Kwon et al. 2000). This provides a possible mechanism by which migrating cells may bypass a migratory-suppressing signal (N-cadherin or a related molecule) present in postmigratory neurons of the cortical plate, thus allowing them to migrate through the latter to reach the inferior border of the marginal zone (Aboitiz 2001; Aboitiz et al. 2001a; 2001b). In the present context, it is of interest to note that in cdk5 mutants, cells belonging to the deepest

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cortical layer VI (which are also the earliest-produced of the cortical plate) are the only ones that can reach their proper position above the subplate (Fig. 6). Later-produced cells (destined to layers V–II) are not able to cross the embryonic subplate and arrange below it, in an outside-in gradient. This may be partly because mutant cells migrate very slowly, and since the cdk5 mutant dies perinatally, these cells may not have time to arrive to the cortical plate (Gupta et al. 2002; Nadarajah et al. 2001). Considering that early-produced cortical plate cells may represent an ancestral phenotype, while late-produced cells are considered to be an evolutionary acquisition, we have suggested that the cdk5/p35 pathway is somehow related to the migration of the phylogenetically new cell types of the mammalian isocortex and to the origin of the inside-out neurogenetic gradient of the isocortex (Aboitiz 1999a; 2001; Aboitiz et al. 2001a; 2001b). Cells in isocortical layer V might represent an intermediate condition between the ancestral phenotypes and the new ones, as they originate in the ventricular zone and are morphologically and neurochemically reptilian-like; but like younger cells, they depend on cdk5 to cross the subplate and layer VI. Again, the study of cdk5/p35 expression in the developing cortex of reptiles may provide substantial information on isocortical evolution.

Recently, two modes of cell migration have been observed in the developing cerebral cortex (Nadarajah et al. 2001). One is called *translocation,* and is observed in early-produced cells of the cerebral cortex, including those of layer VI, and also in the latest stages of migration of late-produced cells within the cortical plate. In this modality, the apical process of the cell contacts the subpial layer, and the cell body is dragged toward the surface while the apical process shortens. The other mode, *locomotion,* consists of the continuous lengthening and shortening of the apical process as the cell migrates along the radial glia. It is highly interesting that in the cdk5 and the p35 mutants, only cells that move by locomotion are affected, whereas cells in layer VI and other early-produced cells that migrate mainly by translocation are unaffected (Aboitiz 2001; Gilmore & Herrup 2001). This suggests that cdk5 and its activators p35 and p39 participate in helping migration by locomotion. It is then possible that cdk5, activated by p35, allows migrating neurons to move by locomotion within the cortical plate, thus allowing the generation of the inside-out gradient.

To summarize: in isocortical origins, the cdk5/p35 pathway may have facilitated the migration by locomotion of late-born, phylogenetically new neurons, and also (particularly by the activation of cdk5 by p35) permitted the migration across cells of the cortical plate, producing the inside-out gradient. At this stage, reelin may have become important in somehow preventing these migrating cells from penetrating into the marginal zone, and also in detaching neurons from the radial glia, thus allowing later-produced cells to use the same glial pathway to migrate across layers of older neurons. In reptiles, perhaps there are other factors different from reelin that maintain a cell-free marginal zone. Besides the reelin and the cdk5 cascades – which, although they are partly independent, may well have cross-talk among them (Gupta et al. 2002) – there are several other genes regulating radial migration (such as Lis-1), which may also have had important roles in isocortical origins. It is quite possible that several other genes are discovered that participate specifically in mammalian cortical cell migration and in the generation of the outside-in gradient. Our attempt has been to point out the (at this point) most likely genetic mechanisms involved in the generation of this unique structure and its developmental sequence.

Previously (cf. Aboitiz 1999a), we proposed a hypothesis for the origin of the inside-out neurogenetic gradient, based on the fact that, in the cortex of reptiles, cortical afferents are arranged in a tangential direction in layer I. In mammals, on the other hand, most isocortical afferents enter the cortex radially from the underlying white matter. In the mammalian isocortex, late-produced cells participate in cortico-cortical connections of large and short range, while the early-produced, deep layers are mostly output layers. Thus, the addition of late-produced cells may have provided the emerging isocortex with a tier of intracortical processing before the output was produced by the early-born neurons. However, a requisite for this function is to gain access to the axons providing input to the cortex, which were located in the most superficial layer I. Hence, the inside-out gradient may have originated as a strategy to gain synaptic contact with cortical afferents by the late-produced neurons. Eventually, cortical afferents may have found a "shortcut" to reach the cortex: traveling through the subcortical white matter and entering the cortex radially instead of running tangentially. In this process, the transient subplate may have become a fundamental element as it projects to the thalamus, and its axons serve as substrates for axonal growth toward the isocortex (Molnár & Blakemore 1995). Furthermore, the subplate serves as a "waiting compartment" for thalamocortical axons, which arrive before the maturation of the cortical plate. Once the cortical plate is mature, thalamocortical axons leave the subplate and enter radially into the cortical plate (Allendoerfer & Shatz 1994). Thus, the subplate may have been important to guide axons through the subcortical white matter and allowing them to enter the isocortex in the radial direction. In addition, the tangential expansion of the isocortex which resulted in an explosive increase in cortical area may have also put a constraint for the tangential growth of thalamocortical axons through the marginal zone, as fibers would have to run excessively long distances to reach the cortical fields on which they were supposed to synapse.

6. The origin of isocortex: II. Evolution of connectivity

As we have just discussed, developmental biology can provide important clues about the mechanisms involved in evolutionary transformations. However, we strongly consider that major evolutionary transitions have been a result of the process of natural selection, therefore, there must be a functional or behavioral context in which these developmental changes proved to be adaptive. In the following sections, we will argue that assuming that the mammalian isocortex originated largely from the reptilian dorsal cortex, implies that a major divergence in brain connectivity has occurred in the evolution of reptiles and mammals. More specifically, in mammals, the sensory pathways that relay in the mesencephalon (collothalamic) are directed to the dorsal pallium, whereas, in reptiles and birds, these pathways terminate in the ventral pallium. The dorsal and the ventral pallium have different domains of connectivity and are involved in different functions, which implies diverging strategies of sensory processing in sauropsids and mam-

mals. In the following sections, we will provide a hypothesis relating the expansion of the dorsal cortex in early mammals, with functional demands associated to the development of associative networks between the olfactory cortex, the dorsal cortex, and the hippocampus. This hypothesis intends to provide a partial account for the major difference in sensory connectivity in the major groups of amniotes.

6.1. Sensory processing in reptiles and mammals

In mammals, the primary visual cortex projects to the extrastriate cortical areas (which receive the tectofugal visual projection), and these, together with the auditory and somatosensory cortical areas, project (through a series of successive cortico-cortical projections) to the hippocampus and the amygdala, in order to process different types of mnemonic information (spatial/episodic and emotional, respectively; Lynch 1986; Maren 1999). In reptiles and birds, although a circuit exists associating the olfactory, the dorsal (visual thalamofugal), and the medial/dorsomedial (hippocampal) cortices, the more important projections from somatosensory, auditory, and visual tectal systems end in the ADVR, which is in turn connected primarily to the PDVR (comparable to parts of the mammalian amygdalar complex). In other words, in reptiles and birds, two relatively (but not totally) separate systems exist for processing thalamic sensory information. One receives thalamofugal visual information and somatosensory projections (lemniscal systems), which are connected primarily with the hippocampus. The other receives mesencephalic or collothalamic auditory and visual information, which is more related to the amygdalar system (see Fig. 4). Thus, in mammals, the hippocampus may receive a much heavier sensory projection than in reptiles and birds, who may rely more on amygdalar components (PDVR/archistriatum) than on the hippocampus to process certain types of sensory and mnemonic information. Further comparative behavioral studies on hippocampal function and spatial memory in mammals and reptiles are needed to verify this intriguing possibility. In this context, it has been found that, in homing pigeons, hippocampal lesions disrupt certain types of spatial learning such as using the sun for directional information, but the capacity to learn on the basis of landmark beacons remains intact (Gagliardo et al. 1996). This suggests that not all forms of spatial memory depend on the hippocampus in birds, as they do in mammals.

An additional difference between sauropsids and mammals is that the corpus striatum receives its major projection from the ADVR and from the isocortex, respectively. Nevertheless, the basolateral amygdala of mammals and the dorsal cortex of reptiles also project to the corpus striatum (Bruce & Neary 1995; Ten Donkelaar 1998b), thus implying similarity of connections between the ADVR and the mammalian basolateral amygdala, and between the dorsal cortex of reptiles and the mammalian isocortex. The fact that most collothalamic inputs terminate in the sauropsidian ADVR and in the mammalian isocortex may account for the quantitative differences in striatal input between these groups. In any case, the basal ganglia of reptiles receive their major input from the ADVR (related to collothalamic inputs), and project to the optic tectum or superior colliculus. On the other hand, in mammals the basal ganglia receive their major input from the isocortex (related to both lemnothalamic and collothalamic inputs), and possibly send an important projection back to it, via the dorsal thalamus (Brauth 1990).

6.2. Olfaction in early mammals

Although an explanation for the evolutionary development of the reptilian DVR is still needed, meanwhile, we will suggest a scenario for the behavioral context of isocortical origins. This proposal is based partly on Sagan's (1977) and Lynch's (1986) original hypotheses of the origin of the isocortex. We suggest that associative networks between the dorsal cortex and the olfactory system, via the hippocampus, became increasingly important to develop multisensorial maps of space and behavior. This may have triggered the expansion of the dorsal cortex as a recipient of not only the visual thalamofugal but also the auditory and visual tectofugal sensory projections.

It has been repeatedly proposed that, in ancestral mammals, olfaction was an important sensory modality (Jerison 1973; 1990; Kemp 1982). Endocasts of mesozoic mammals indicate relatively large olfactory bulbs and perhaps an elevated rhinal fissure (Jerison 1990), suggesting a large olfactory cortex in relation to the rest of the pallium. Likewise, in small-brained insectivores and in some marsupials, olfactory-related structures occupy a much larger proportion of the volume of the brain than is the case in larger-brained species with a well-developed isocortex (Finlay & Darlington 1995; Finlay et al. 1998; Stephan 1983; Voogd et al. 1998). Since early mammals were most likely nocturnal animals (Carroll 1988; Jerison 1973; Kemp 1982; Sagan 1977) the exploration of their environment may have depended heavily on the olfactory system.

6.3. Olfaction and the hippocampus

In amphibians, there are projections from both the lateral and dorsal pallium (receiving olfactory input) into the medial pallium (Ten Donkelaar 1998a; 1998c). These projections may have served as precursors for the development of olfactory-hippocampal circuits in amniotes. In reptiles, there is a well-defined circuit connecting the medial (hippocampal), the dorsal (receiving the lemnothalamic pathways), and the olfactory cortices (Lynch 1986; Ten Donkelaar 1998b; see Fig. 4). Furthermore, there is evidence that in reptiles and in other vertebrates, the medial and the dorsal cortices participate in spatial learning (Rodríguez et al. 2002). Active foraging lizards tend to have larger medial and dorsal cortices than species that hunt with a sit-and-wait strategy (Day et al. 1999). In addition, lesions in these regions impair spatial learning in these animals (Day et al. 2001; Rodríguez et al. 2002). These researchers further argue that these cortical areas use non-spatial clues for spatial navigation, which is important in relation to new concepts of hippocampal function described below.

The mammalian olfactory cortex (which is really a mosaic of areas, including the piriform cortex, the entorhinal cortex, and the perirhinal cortex, among others; Shipley & Ennis 1996) is reciprocally connected with the hippocampus through the entorhinal cortex (Haberly 1990), and has been postulated to engage in associative interactions with other sensory modalities which are mapped in the isocortex and project to the hippocampus (Lynch 1986; Shipley & Ennis

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1996). Thus, hippocampal activity is strongly dependent on olfactory and other sensory information.

A classical understanding of hippocampal function is that this structure creates a Cartesian representation of space, in which the different places and coordinates are mapped onto the structure itself (O'Keefe & Nadel 1978). This concept was partly proposed on basis of the discovery of "place-cells" in the hippocamus of experimental animals, which are activated by specific positions of the animal in a given space (O'Keefe & Dostrowsky 1971). However, evidence has shown that many hippocampal cells fire in response to non-spatial determinants such as odors, and that the activity of the cells depends on the behavioral state of the animal (see Eichenbaum 1999; Eichenbaum et al. 1999; Frank et al. 2000). Hippocampal cells can recognize rewarded and non-rewarded cues, spatial configurations of odors, differences between odors, or fire at specific behavioral instances (Wiener et al. 1989; Wood et al. 1999; 2000). In addition, it has been found that spatial and non-spatial (olfactory) information are segregated in interleaved, oblique stripes along the hippocampus (Hampson et al. 1999).

The hippocampus has also been shown to be required for non-spatial olfactory tasks, such as tests of transitive inference, in which if A>B, B>C, C>D, and D>E, then B>D (Dusek & Eichenbaum 1997). If A–E are distinct olfactory cues, rats can be trained to prefer A over B, B over C, and so on. Furthermore, rats can also infer that B>D, but only if their hippocampus is intact (A>E can be learned by intact and lesioned rats, but this can be solved by noticing that A is always chosen and E is never chosen, and thus does not require transitive inference).

Another example of hippocampal-dependent, non-spatial learning is the social transmission of food preferences, in which rats in contact with other rats who have recently eaten some scented food will prefer this food over others. This is learned by an association between the scent of the food and a constituent of the rat's breath, carbon disulfide (Galef 1990). Again, hippocampal-lesioned rats perform poorly in this task (Bunsey & Eichenbaum 1996). (This latter finding was called into question; Burton et al. 2000; but see reply by Alvarez et al. 2001.)

In summary, there is substantial evidence that the hippocampus participates in olfactory memory. Although it may not be essential to memory for single odors, it seems to be critical for establishing the relations among odor memories, and for the expression of odor memory representations in novel situations (Eichenbaum 1998).

Partly based on this evidence and on the finding that place cells are more consistently controlled by local cues (see Eichenbaum et al. 1999), it has been proposed that the representation of space in the hippocampus consists of the specification of behaviorally-relevant spots. These spots are identified by cues such as odors and other (visual) characteristics of the environment, and include a collection of independent representations of places, linked among them by the behavioral context in which the animal explores its environment (Eichenbaum 1999; 2000b; Eichenbaum et al. 1999). For these authors, a fundamental function of the hippocampus is its participation in episodic memory, that is, memory of the events that take place during a particular behavioral action. Spatial memory emerges as a consequence of the integration of successive episodes during an exploration task, and involves associations between different sensory modalities, including vision and olfaction. This proposal could reconcile the apparently discrepant findings that, in animals, the hippocampus participates in spatial memory, whereas in humans, it participates in declarative memory. Instead of spatial memory, Eichenbaum prefers to speak of a "memory space," which is an organized representation of memory episodes linked by their common features (Eichenbaum 2000b; however, see alternative view by O'Keefe 1999). We suggest that, in the process of generating this memory space, olfaction (which is an important sense used to investigate the environment by many small mammals such as rodents and insectivores) may participate as a "glue" that helps to link many of these spots, creating a cohesive map of the behaviorally-relevant points. This may have been especially important in early mammals or mammaliaforms, which had not yet developed a strong cortical visual system. In present-day mammals such as the rat, visual input is necessary for the firing of a large number of hippocampal cells, while olfactory information can be used to compensate for the lack of visuospatial information (Save et al. 2000). Therefore, visual information may have progressively been involved in the associative hippocampal-olfactory networks during the origin of mammals, thus triggering the expansion of the dorsal cortex.

The olfactory-hippocampal-dorsal cortex circuit may have been put to use by the first mammals to make relatively elaborate, largely olfactory-based representations of space, in which specific odors labeled particular places and routes. Nevertheless, the contribution of the visual system undoubtedly became necessary in the elaboration of more precise maps of space, especially when mammals invaded diurnal niches after the decline of dinosaurs. The dorsal cortex, receiving visual information from the thalamofugal visual pathway, may have become an important sensory processing system in the early mammalian brain (Aboitiz 1992).

In this context, a dorsalizing effect on dorsal pallial development as we have suggested before, triggering an expansion of the dorsal cortex, may have been of great benefit for the development of olfactory-hippocampal-cortical networks. This expansion may have permitted the arrival of "mesencephalic" sensory routes to the dorsal pallium, originating the visual extrastriate and auditory cortices (Northcutt 1969; Northcutt & Kaas 1995). In addition, the auditory projection to the cerebral cortex may have benefited from the cortical representation of space by developing a more elaborate sound localization system. In reptiles, on the other hand, the thalamofugal/lemniscal visual pathway does not play a dominant role for processing visual information (Ulinski 1990). In this vertebrate class, the more important tectofugal pathway and the auditory system project into the DVR, which apparently does not participate in such extensive associative networks with the hippocampus.

Thus, we postulate that a major innovation in the origin of the mammalian brain has to do with the confluence of the lemnothalamic and the collothalamic pathways in the dorsal pallium, in order to process spatial information which, among other things, participated in spatial learning and episodic memory. In this process, the hippocampus may have become a fundamental component in which both types of sensory pathways eventually converged. Strictly, this particular proposal is consistent with both the recapitulation and the outgroup hypotheses, since the merging of the two pathways is independent of the embryonic origin of

the ventrolateral isocortex. However, it may be more parsimonious to consider that for this confluence to occur, only the axonal projections changed their route instead of producing a massive cellular migration that dragged the collothalamic axons to a more dorsal position.

6.4. Other circuits: Frontal cortex and basolateral amygdala

An additional olfactory pathway influencing isocortical activity consists of the projections from the piriform cortex to the dorsomedial thalamic nucleus – which then sends axons to the frontal cortex – and of direct projections from the piriform cortex to the frontal cortex (Haberly 1990; Haberly & Price 1978). It is interesting that these projections are better developed in so-called "primitive" mammals like the opossum, whose dorsomedial nucleus has much denser olfactory projections than placental mammals (Benjamin et al. 1982; Lynch 1986). Furthermore, the orbitofrontal cortex of the rat is reciprocally connected with perirhinal and entorhinal areas and participates in odor memory (Ramus & Eichenbaum 2000). In the rat there is also a hippocampo-prefrontal/orbitofrontal circuit, which is connected with the nucleus accumbens, a structure involved in motivation (Aboitiz & Montiel 2001; Thierry et al. 2000). Thus, the olfactory cortex and the hippocampus may have been connected with parts of the dorsal cortex through several routes, which may have also participated in motivational aspects of behavior.

In this context, the basolateral amygdala of mammals has important connections with orbitofrontal cortex (McDonald 1991) and participates in olfactory memory, perhaps by encoding the motivational significance of stimuli used to guide behavior. On the other hand, the orbitofrontal cortex may use this information to select an appropriate behavioral strategy (Schoenbaum et al. 1998; 1999). The basolateral amygdala of mammals, instead of receiving and processing collothalamic sensory information as the ADVR does in reptiles, acquired a perhaps more restricted, modulatory role over motivation and emotional behavior.

7. Fossil mammals and their brains

The first radiation of mammal-like reptiles (synapsids) gave rise to the *pelycosaurs,* which were relatively large, lizard-like reptiles. In the upper Permian, pelycosaurs were gradually replaced by their descendants, the *therapsids.* The hands and feet of these animals faced more directly forward instead of being oriented sidewards as in other reptiles, which gave those animals a more mammalian-like gait. Some therapsids grew to achieve large sizes, and they are classified into carnivorous and herbivorous therapsids. Most therapsids became extinct by the end of the Triassic, but one group of carnivorous therapsids, the *cynodonts,* survived well into the Jurassic (Carroll 1988; Kemp 1982).

Cynodonts had a more mammal-like jaw musculature, but the ear ossicles were still attached to the lower jaw, as they are in reptiles. From cynodonts arose the *eucynodonts* or *mammaliaforms,* which include Jurassic fossils like Sinoconodon and Morganucodon, whose gross morphology resembled that of some present-day insectivores (Rowe 1996a; 1996b). True mammals descend from eucynodonts, and are defined by the presence of a single dentary bone making up the inferior mandible and the complete detachment of the middle ear ossicles, as in the fossils Hadrocodium (Luo et al. 2001), Gobiconodon and Repenonamus (Wang et al. 2001; however, according to these authors, Hadrocodium is a juvenile form and it is not clear whether it had a fully mammalian middle ear). Further evolution of mammals includes the origin of monotremes, marsupials, and placental mammals. Triconodon is another interesting fossil, originally considered to be close to Morganucodon (Carroll 1988), but, according to newer analyses, this fossil has been classified as a true mammal, perhaps belonging to the *therians* (marsupials and placental mammals; Rowe 1996a; 1996b).

Endocasts are molds of the cranial cavity of fossil animals. Analysis of these casts indicates that early mammal-like reptiles (therapsids) had narrow, tubular hemispheres with no signs of telencephalic expansion (Hopson 1979; Kemp 1982; Quiroga 1980). Increase in brain size, resulting from a generalized growth of the isocortex, occurs in the recent fossil mammals Triconodon and Hadrocodium (Luo et al. 2001; Rowe 1996a; 1996b; Fig. 7). In these fossils, the detachment of the auditory bones from the mandible to form the mammalian middle ear coincides with enlargement of the brain (Rowe 1996a; 1996b). However, in other fossil mammals like Repenomanus and Gobiconodon, braincases are narrow despite detachment of the ear ossicles (Wang et al. 2001). Therefore, brain expansion may have occurred after the origin of the middle ear, more than the reverse, that is, brain enlargement triggering ossicle detachment for mechanical reasons (Wang et al. 2001). As we propose, the development of collothalamic sensory projections (including the auditory pathway) into the isocortex was an important factor in the expansion of the isocortex. Although this may have been related more to the develop-

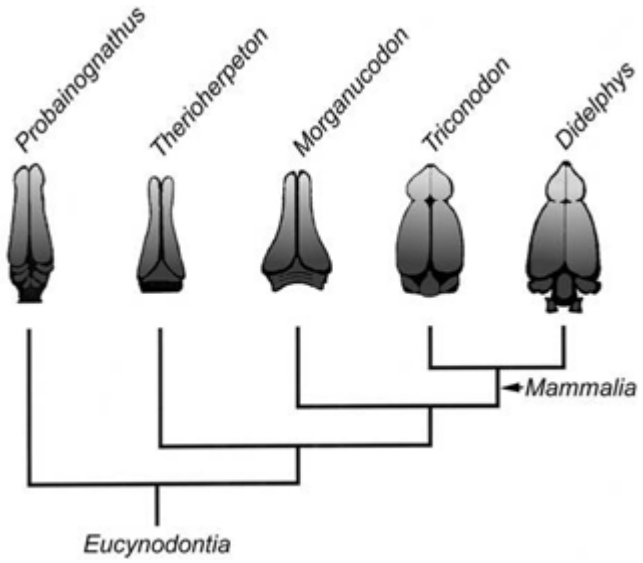

Figure 7. Endocasts of mammal-like reptiles and primitive mammals, indicating the progressive increase in brain size. Note that in Morganucodon, expansion of the posterior part of the hemisphere can be observed. More advanced mammals like Triconodon show a more complete expansion of the hemispheres. The first true mammals, possibly represented by Hadrocodium, Repenomanus, and Gobiconodon (not shown), are proposed to be in an intermediate position between Morganucodon and Triconodon. Based on Rowe (1996a; 1996b).

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ment of the hippocampal, multimodal association circuits described above, it may have also contributed to enhanced hearing. In this context, the mammalian auditory cortex contains binaural cells, many of which are interconnected interhemispherically by fibers of the corpus callosum (Pallas 2001) and may participate in spatial localization of sounds. On the other hand, the DVR of birds and reptiles has few or no interhemispheric connections, which may limit telencephalic auditory spatial processing in these animals.

Interestingly, Morganucodon, a primitive mammaliaform taxonomically intermediate between Triconodon and smaller-brained, more primitive therapsids, shows only partial expansion of the brain. In this species, widening of the occipital parts of the hemispheres can be observed (Rowe 1996a; 1996b; Fig. 7). Admittedly, endocast information can be difficult to interpret, since there are few anatomical details to identify as landmarks. Nevertheless, this feature may perhaps be attributed to the early development of the dorsal cortex in Morganucodon.

8. Final comments

The main point of this article is that the origin of the mammalian isocortex may have been largely due to an overall dorsalizing effect during early pallial development in mammal-like reptiles. This may have implied (1) tangential expansion of the ventricular zone corresponding to the dorsal pallium, (2) increased duration of the neurogenetic period, and perhaps also (3) tangential migration of excitatory cells from the ventral pallium (although there is no evidence yet of such a process).

Current developmental evidence suggests separate origins of the mammalian isocortex and the reptilian DVR. The latter appears to be related to ventral pallial structures of mammals such as the basolateral amygdala and/or the endopiriform nucleus (ventral claustrum), while the isocortex originates largely from the dorsal pallium. There is a possibility that some cells that originally differentiated into the IT/VP, became part of the lateral or perhaps dorsal pallium, either by changing territorial identity or by undergoing tangential migration. In our view, this alternative is not to say that the lateral isocortex derives from an ancestral DVR. Rather, we consider that the two structures originated in parallel, in two different lineages (in mammal-like reptiles and in stem reptiles, respectively), neither of which is ancestral to the other. Evidence supporting this interpretation is the fossil endocasts of mammal-like reptiles, which indicate that brain expansion (and possibly the origin of the isocortex) was a late event in mammalian evolution.

Connectional evidence indicating similarity of sensory input in the reptilian ADVR and the mammalian auditory and extrastriate isocortex is contrasted with other hodological evidence showing important connectional differences between these structures. If the ADVR and the isocortex have different embryonic origins, this implies that the main mesencephalic sensory input has been directed to different targets in the lineages leading to sauropsids and to mammals. For example, the ectostriatum, a component of the avian ADVR, receives thalamic visual input from the thalamic nucleus rotundus, whereas layer IV of extrastriate visual cortex of mammals receives projections from the thalamic pulvinar nucleus. Furthermore, the intrinsic connections of the avian DVR (ectostriatum to neostriatum to hyperstriatum) are similar to the connections between the different isocortical laminae (layer IV to layers III-II to layers VI-V; Karten 1969). Many of these similarities in intrinsic connectivity may perhaps have been independently acquired in the evolution of birds and mammals. In fact, there may be strong parallelisms in sensory processing in the brains of birds and mammals, which does not imply that there cannot have been an important divergence earlier on. One important point in these considerations is that perhaps one of the major innovations in mammalian sensory processing was the confluence of the lemniscal and mesencephalic sensory pathways (especially visual) in the dorsal pallium, and their convergence in the amygdala and hippocampus. The concept that, in mammals, the collothalamic and the lemnothalamic processing routes merge in the telencephalon is compatible with both the recapitulation and the outgroup hypotheses. However, in terms of developmental mechanisms, it may be more parsimonious to consider that there has been a re-routing of the collothalamic inputs (outgroup hypothesis) rather than a migration of cells from the ventral to the dorsal pallium (recapitulation hypothesis).

One important problem concerns the issue of determining a strict homology to the isocortex in the reptilian brain. As we discussed, at least some cells in the superficial isocortical layers may have no counterpart in sauropsids. Likewise, if the outgroup hypothesis turns out to be correct, there may be many isocortical regions (i.e., extrastriate visual and auditory) that perhaps have no specific correspondence in the brains of sauropsids (although they may have emerged from some embryonic component present in the reptilian brain, like the dorsal cortex). One possibility is to consider the dorsal pallium of sauropsids and mammals as "field homologues." We consider that, although there may be a true homology in the embryonic structures, perhaps the best consideration is to assume that only some components of the adult isocortex may have homology in the adult reptilian brain, whereas others may be viewed as evolutionary innovations (see Northcutt 1999). Likewise, many parts of the sauropsidian ADVR may have no counterpart in the adult mammalian brain (Aboitiz 1992; 1999b).

The above proposals have been complemented with a scenario describing the origin of the isocortex from the dorsal pallium, based on the reciprocal relations of this structure and the hippocampus. The scenario proposed here is an attempt to describe the sequences of developmental and functional changes that may have led to this early separation of the reptilian and mammalian brain architectures. In our view, the main significance of these scenarios is that they provide an evolutionary framework which may guide future studies of the embryology and structure of the cerebral cortex.

Strictly speaking, our "hippocampal-olfactory" theory for the origin of isocortex might also agree with the recapitulation hypothesis. In this case, there would have been cell migration from the IT/VP into the dorsal cortex, and collothalamic afferents would have followed those cells; all this might have contributed to the development of associative networks with the olfactory cortex and hippocampus. In other words, cells originally from the IT/VP would have been transformed into dorsal pallial phenotypes. However, we consider that the outgroup hypothesis fits better with the concept of expansion of the dorsal cortex, in the sense

that it requires less and less dramatic developmental transformations. Furthermore, as mentioned, at this point there is no evidence indicative of the migratory process required by the recapitulation hypothesis.

Rejecting the recapitulation hypothesis and accepting the outgroup hypothesis implies the assumption that the reptilian and the mammalian brains diverged very early in their evolution, which would be consistent with the concept of therapsids diverging at the earliest points of the amniote radiation. An explanation of the expansion of the DVR in reptiles is still needed. However, we will preliminarily suggest that the reptilian solution was a more conservative outcome, in which emphasis in sensory processing was given by the mesencephalic projection systems (collothalamic), perhaps as it happens in other vertebrate classes. In mammals, the main innovation consisted of the early dominance of olfaction in sensory processing (possibly associated with nocturnal habits and with a new respiratory system; Carroll 1988; Kemp 1982), which triggered the development of olfactory-dorsal cortex-hippocampal associative networks, eventuallly facilitating the confluence of the lemnothalamic and collothalamic sensory streams in the dorsal pallium and hippocampus.

Finally, the present perspective raises questions that impinge directly on several lines of research. For example, the study of dorsoventral gradients in isocortical specification, the studies of tangential cell migration into the isocortex, and the role of Pax-6 in reptilian cortical lamination may have immediate relevance to the hypotheses proposed here. On the functional side, comparative studies on hippocampal and amygdalar functions in amphibians, reptiles, and mammals may be of special relevance in relation to the concept of isocortical expansion triggered by hippocampo-olfactory-cortical associative networks.

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APPENDIX

Glossary of Abbreviations

A	termination site of the auditory projection
ADVR	anterior dorsal ventricular ridge
AM	amygdala; basolateral amygdala
cdk5	cyclin-dependant protein kinase 5
CP	cortical plate
DCx	dorsal cortex
Dp	dorsal pallium
DVR	dorsal ventricular ridge
GEs	ganglionic eminences
GL	thalamic dorsal lateral geniculate nucleus
HIP	hippocampus
ICx	isocortex
IL	thalamic intralaminar nucleus
IT	intermediate territory
IT/VP	intermediate territory/ventral pallium
IZ	intermediate zone
LCx	lateral cortex
LEM/TAF	lemniscal/thalamofugal visual pathway
LGE	lateral ganglionic eminence
Lp	lateral pallium
MCx	medial cortex
MES/TEL	mesencephalic/tectofugal visual pathway
MGE	medial ganglionic eminence
Mp	medial pallium
MZ	marginal zone
NP	thalamic pulvinar nucleus
NR	thalamic nucleus rotundus
O	olfactory tuberculum
OCx	olfactory cortex
PDVR	posterior dorsal ventricular ridge
Se	septum
SP	subplate
STR	corpus striatum
vl	termination site of the lemniscal, thalamofugal projection
vc	termination site of the collicular, tectofugal projection
VP	ventral pallium
VZ	ventricular zone
W	Wulst

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From axis to triangle: The role of orbital cortex

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Abstract: This commentary focuses on the "olfactory cortices–hippocampal formation" axis, proposed by Aboitiz et al. to be that network which allowed the first mammals to create elaborate representations of space. I argue here that this neural axis can be extended to a triangle of structures which also includes the orbital cortex.

Aboitiz et al. present compelling evidence that the mammalian isocortex appeared on account of a dorsalizing effect during the early development of the pallium of the first mammals. The authors argue that the olfactory-hippocampal-dorsal cortex circuit was needed to create complex olfactory-based representations of space.

Though the data cited by the authors support the olfactory cortex–hippocampus theory (OHT), I propose that this network is extendable to the orbital cortices and that these form a necessary component of the network of brain regions responsible for complex representations of space.

The orbital cortex in the rat receives connections from the subiculum (Canteras & Swanson 1992; Jay & Witter 1991), and a light projection from the CA1 field of the hippocampus (Jay & Witter). Unilateral ablations of the ventral lateral orbital cortices in rats lead to spatial neglect (King et al. 1989), and bilateral lesions of the same area impair rats performing tasks that require spatial maps of the environment (Corwin et al. 1994). The orbital cortex of the rat also receives connections from such olfactory-related structures as the perirhinal cortex (Ramus & Eichenbaum 2000a) and the entorhinal cortex (Deacon et al. 1983; Swanson &