Expression of the *Emx-1* and *Dlx-1* homeobox genes define three molecularly distinct domains in the telencephalon of mouse, chick, turtle and frog embryos: implications for the evolution of telencephalic subdivisions in amniotes

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**SUMMARY**

Homologies between vertebrate forebrain subdivisions are still uncertain. In particular, the identification of homologs of the mammalian neocortex or the dorsal ventricular ridge (DVR) of birds and reptiles is still a matter of dispute. To get insight about the organization of the primordia of the main telencephalic subdivisions along the anteroposterior axis of the neural tube, a fate map of the dorsal prosencephalon was obtained in avian chimeras at the 8- to 9-somite stage. At this stage, the primordia of the pallium, DVR and striatum were located on the dorsal aspect of the prosencephalon and ordered caudorostrally along the longitudinal axis of the brain. Expression of homeobox-containing genes of the *Emx*, *Dlx* and *Pax* families were used as markers of anteroposterior developmental subdivisions of the forebrain in mouse, chick, turtle and frog. Their expression domains delineated three main telencephalic subdivisions in all species at the onset of neurogenesis: the pallial, intermediate and striatal neuroepithelial domains. The fate of the intermediate subdivisions diverged, however, between species at later stages of development. Homologies between forebrain subdivisions are proposed based on the conservation and divergence of these gene expression patterns.

Key words: Evolution, Forebrain, Telencephalon, Brain, Vertebrates, Mouse, Chick, Turtle, Frog, Neural tube, *Emx-1*, *Dlx-1*, *Pax-6*

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**INTRODUCTION**

Several features of brain organization have been conserved during vertebrate evolution, in particular in the midbrain and hindbrain of higher vertebrates. Structures like the optic tectum or the cerebellum that have phylogenetically conserved position, cytoarchitecture, connections, function and neurochemistry are considered as homologous in different species. In contrast, the organization of the forebrain is more divergent and there is still no consensus regarding the homology of the different telencephalic subdivisions in mammals, birds and reptiles. This question has led, since the beginning of the century, to a wide variety of interpretations and controversies (reviewed in Striedter, 1997).

The telencephalon of adult mammals comprises two major subdivisions: the cerebral cortex, dorsally, and the deeply located basal ganglia (composed of striatum and pallidum). The telencephalon of living birds and reptiles (sauropsids) is subdivided into three major domains, a dorsal cortical-like pallium, a subpallial formation called the dorsal ventricular ridge (DVR), which bulges into the lateral ventricle and a basally located striatal domain. At the beginning of this century, the location and extent of the basal ganglia in birds and reptiles were a matter of dispute. These uncertainties are still reflected in the nomenclature of telencephalic subdivisions and in their variations between species in both birds and reptiles (see Fig. 1). More recent findings (Reiner et al., 1984; Medina and Reiner, 1995; Northcutt and Kaas, 1995) have shown that there is a great similarity between the neuronal populations that form the basal ganglia in living species of birds, reptiles and mammals with regard of neurotransmitter contents, physiology and connections. Therefore, most modern comparative neuroanatomists now agree that the evolution of basal ganglia has been more conservative than previously believed.

The six-layered neocortex or isocortex of mammals is a recent evolutionary acquisition not found in birds and reptiles. It has been claimed that, in the synapsid line (mammal-like reptiles and mammals), the isocortex is derived from the ancestral dorsal pallium comparable to that of living amphibians (Herrick, 1948; Northcutt, 1981; Northcutt and Kaas, 1995). An alternative hypothesis proposes that the lateral portion of the isocortex and the anterior part of the DVR (ADVR) have a common origin and
are thus homologous (Karten, 1991; Butler, 1994). This hypothesis is based on evidence that the ADVR is not, as previously thought, a striatal formation, but shares many neurochemical, connectional and physiological similarities with the mammalian isocortex. In contrast, the posterior basal part of the DVR (BDVR) displays characteristics that resemble those of the amygdala (Ulinski, 1983, Streidter, 1997 for review).

These theories of an isocortical ADVR have not gone unchallenged (Lohman and Smeets, 1991; Smeets and Gonzalez, 1994; Northcutt and Kaas, 1995) and the ADVR has been considered as related to various mammalian non-cortical structures: claustrum (Filimonoff, 1964; MacLean, 1990), lateral amygdala (Bruce and Neary, 1995) and endopiriform nucleus (Striedter, 1997).

It is now well established that the structure and cellular functions of many Drosophila developmental genes have been conserved during evolution. In addition, it has been realized that the arrangement along the main body axes of the expression domains of many of these genes, notably the Hox genes but also genes of the Otx, Emx, Dlx and Pax families, has been maintained to a large extent during evolution. This finding was unexpected because of the great morphological diversity of adult animals. At early stages, these developmental genes are supposed to demarcate relative positions in animals rather than to specify particular structures (Carroll, 1995). This early role seems to have been conserved in vertebrates, in particular in the developing neural tube. In the embryonic mouse brain, the expression of homologs of Drosophila developmental genes have been shown to delineate a map comprising potential morphogenetic units that correspond in part to the neuromeres of earlier authors (Puelles and Rubenstein, 1993; Rubenstein et al., 1994).

The aim of the present study was to try to understand better the formation of divergent telencephalic structures in higher vertebrates by taking advantage of the similarity of early developmental processes and gene expression between species. Using quail-to-chick transplantation, we first determined that the primordia of the three main subdivisions of the avian telencephalic vesicle are aligned along the anteroposterior axis of the early neural tube. We then compared the telencephalic expression of Emx-1, Dlx-1 and Pax-6 at successive embryonic stages in a series of species belonging to different tetrapod taxa: mammals (Mus), birds (Gallus), reptiles (Emys) and amphibians (Xenopus). We found that the developing mouse telencephalic vesicle is similar at early developmental stages to those of the other species studied in that its germinative neuroepithelium contains transiently an intermediate third subdivision. We discuss the possible homologies between telencephalic subdivisions in light of these observations, which make some of the previous interpretations unlikely.

MATERIALS AND METHODS

Embryos

OF1 mouse (Iffa Credo) embryos were considered E0.5 the day where vaginal plug was detected, White Leghorn chick embryos (Morizeau) and Japanese quail embryos (La Caille de Chanteloup, Corps-Nuds, France) were staged according to Hamburger and Hamilton (1951. designed HH), turtle embryos (Emys orbicularis) according to Pieu and Dorizzi (1981), and frog embryos (Xenopus laevis) according to Nieuwkoop and Faber (1956).

BrdU administration

Pregnant mice were injected intravenously (20 mg/kg) with a solution of 5-bromo-2′-deoxyuridine (BrdU) (Sigma) in 0.9% NaCl on embryonic days (E) 10.5 to E14.5. They were killed at E18.5 and the fetuses were fixed in paraformaldehyde 4% in sodium phosphate buffer 0.12M pH 7.4 overnight and washed in PBS. The prosencephalon was dissected out, and coronal sections (100 μm) were cut using a vibrating slicer (Campden Instruments).

Fate map of the telencephalic vesicle

The quail-to-chick transplantations were performed as described (Bally-Cuif and Wassef, 1994). The same portion of the telencephalic vesicle was cut out from a 7- to 9-somite quail embryo and transported to a stage-matched chick host with a glass pipette, to replace the ablated portion. The egg was then closed with tape and returned to the incubator for 3 to 6 days, after which time the embryos were fixed with 4% paraformaldehyde and processed for QCPN immunocytochemistry. The present study is based on a series of 180 grafts 65 of which survived and were successfully immunostained.

Immunocytochemistry

The quail-specific QCPN monoclonal antibody, developed by B. and J. Carlson, was obtained from the Developmental Studies Hybridoma Bank maintained at Johns Hopkins University, Baltimore and University of Iowa, Iowa City under contract NO1-HD-2-3144 from the NICHD. For BrdU detection, sections were preincubated for 45 minutes in 2 N HCl, followed by washes in PBS and then treated with proteinase K at 20 mg/ml. After several rinses in PBS, the sections were postfixed in paraformaldehyde 4% glutaraldehyde 0.2% in sodium phosphate buffer 0.12 M pH 7.4. In toto dissected neural tubes or 200 μm thick vibratome sections were incubated overnight in QCPN (1/50 dilution) or anti-BrdU antibody (Becton Dickinson, 1/400 dilution) in PBS-0.2% gelatin-1% Triton. After washing, they were incubated with a biotinylated secondary antibody (Jackson Lab 1/200) followed by a streptavidin-biotin-peroxidase complex (Amersham 1/400) and revealed with diaminobenzidine (DAB).

Fig. 1. Telencephalic subdivisions in representative species of mammals (mouse), birds (chick), reptiles (turtle) and amphibians (frog). Schematic representation of transverse sections at comparable anteroposterior levels of the rostral telencephalon in adult mouse, chick, turtle and frog brains. The mouse telencephalon contains two main subdivisions the cortex (cx) and striatopallidum (st); the septum (s) and amygdalar (a) regions are also indicated. The putative homologs of the mouse striatopallidum (st) in the other species are depicted in green. This region is known as paleostriatum (pst) in the chick. The DVR of reptiles is a large structure of the lateral telencephalic wall which bulges into the ventricle. It separates the dorsal cortex (dc) from the striatum (st). The complex formed by the neostriatum (n) and the hyperstriatum ventrale (hv) in birds is considered to be the homolog of the reptilian DVR. a, amygdala; cx, cerebral cortex; dc, dorsal cortex; dp, dorsal pallium; DVR, anterior dorsal ventricular ridge; hv, hyperstriatum; lp, lateral pallium; mp, medial pallium; n, neostriatum; pst, paleostriatum; s, septum; st, striatum.
Cloning of a fragment of mouse, chick and turtle *Dlx-1* cDNAs

*Dlx-1* probes were isolated by RT-PCR using the following oligonucleotides, designed by comparing the mouse *Dlx-1* (Price et al., 1991) and the *Xenopus* Xdll4 subclone (Asano et al., 1997) cDNA sequences.

Oligo a: 5′ GCGGTGTCGGCTTGTTTAACGG 3′
Oligo b: 5′ CTGCTAGCTTCACGTCGCG 3′
Oligo c: 5′ CATCAGTTGAAGGCTGTCGAT

They amplified a 401 bp fragment from mouse and chick *Dlx-1* and a 416 bp fragment from *Emys orbicularis* *Dlx-1* including the homeobox domain. These fragments were subcloned in the PCR II vector (Invitrogen) and sequenced.

Cloning of a fragment of the chick and turtle and *Xenopus* orthologs of *Emx-1*

*Emx-1* probes were isolated by PCR using the following oligonucleotides, already used to clone the *Xenopus* cognate (Paternello et al., 1997):

emx1F: CAGGTAAAAGTTTGGTTTCA
emx1R: TAATCGTCTGAGGTGACGTC

The amplified 376 bp fragments were subcloned and sequenced (Paternello et al., 1997).

Probes for *in situ* hybridization

The chick and turtle *Dlx-1* subclones were linearized with *XbaI* and transcribed using SP6 RNA polymerase or linearized with *HindIII* and transcribed with T7 to generate the antisense and sense probes, respectively (*XbaI*, SP6: antisense; *HindIII*, T7: sense). The other subclones were used as follows: mouse *Dlx-1* subclone (*HindIII*, T7: antisense; *XbaI*, SP6: sense; Price et al., 1991), *Xenopus* Xdll4 subclone (*PstI*, T3: antisense; Papalopulu and Kintner, 1993), mouse *Emx-1* subclone (*EcoRI*, SP6: antisense; Simeone et al., 1992b), chick and turtle *Emx-1* subclones (*HindIII*, T7: antisense; *EcoRI*, SP6: sense; Patarnello et al., 1997), *Xenopus Emx-1* subclone (*HindIII*, T7: antisense; *EcoRI*, SP6: sense), mouse *Emx-2* subclone (*HindIII*, T7: antisense; Simeone et al., 1992b), chick *Emx-2* subclone (*HindIII*, T7: antisense; Patarnello et al., 1997). The mouse *Pax-6* subclone (*PstI*, T3: antisense; *HindIII*, T7: sense; gift of Dr S. Saule) consisted of a 1.8 kb fragment including part of the paired box domain, and the whole homeobox domain, subcloned in pBluescript KS. The quail *Pax-6* subclone (*EcoRI*, T7: antisense; Turque et al., 1994). This probe cross-reacted with chick *Pax-6* RNA under the conditions used.

Single-colour and double-colour *in situ* hybridization

Mouse, chick, turtle or frog embryos were fixed by immersion or perfused through the heart with 4% paraformaldehyde, and kept at 4°C until being dehydrated in methanol/PBT. After dehydration, the telencephalon was dissected out and embedded in gelatin/albumin (30% albumin, 0.5% gelatin, in phosphate buffer 0.1 M pH 7.3 hardened by adding 1.25% glutaraldehyde). Sections, 200 μm thick, were cut in the desired orientation on a vibrating slicer (Campden Instruments) and kept at −20°C overnight after dehydration in methanol/PBT.

Single *in situ* hybridization was performed as described for whole-mount preparations (Wilkinson, 1992). Probes were labeled with digoxigenin-UTP (Boehringer Mannheim) using the Riboprobe...
Gemini System II kit (Promega). They were added to the hybridization buffer at a concentration of 1–2 mg/ml; sections were hybridized at 70°C overnight in a water bath. An anti-digoxigenin alkaline-phosphatase-coupled antibody (Boehringer Mannheim) was used diluted 1/2000; alkaline phosphatase activity was revealed using NBT/BCIP as substrate.

For double in situ hybridization, the probes were synthesized either with digoxigenin-UTP or fluorescein-UTP; they were added together to the hybridization buffer and revealed sequentially as described in Bally-Cuif and Wassef (1994) except that the anti-fluorescein antibody was used at a concentration of 1/2000 and that between the revelation of the first and second probe the sections were incubated for 10 minutes in 0.1 M glycine-HCl pH 2.2 plus 0.1% Tween-20 in order to eliminate the first alkaline-phosphatase-coupled antibody (Hauptmann and Gerster, 1994).

RESULTS

A schematic representation of the main neuroanatomical subdivisions of mouse, chick, turtle and frog rostral telencephalon is given in Fig. 1. The telencephalic subdivisions are illustrated on transverse sections of adult brains, an orientation adopted in most previous neuroanatomical comparative studies. In green, the territories considered as homologs of the mouse striatopallidum (Reiner et al., 1984; Medina and Reiner, 1995; Northcutt and Kaas, 1995) are designed thereafter as striatum. This term should not be confused with the so-called ‘neostriatum’ a component of the chick ADVR (see below). The ADVR, a thickening of the lateral telencephalic wall, was initially described on morphological grounds in reptiles. The ADVR is bounded by two sulci, which can be detected early in development. Many investigators (Karten, 1969; Ulinski, 1983) considered the ADVR to be homologous to avian neostriatum and ventral hyperstriatum, which are therefore collectively referred to as ADVR. In the following descriptions, unless specified, DVR refers to ADVR.

Organization of the telencephalic subdivisions in the 8-somite chick embryo

Earlier fate maps in chick (Coulby and Le Douarin, 1987) and Xenopus (Eaglestone and Harris, 1990; Eaglestone et al., 1995) suggested that the primordium of the striatum is more anterior in the neural plate, rather than more basal, than the primordium of the dorsal pallium. In order to better understand how the telencephalic subdivisions are organized in the closed neural tube before the bulging out of the telencephalic vesicle, we performed a fate-mapping study of the dorsal chick prosencephalon at the 8-somite stage. Quail neural tube fragments were transplanted homotopically and isochronically into the dorsal prosencephalon of 7- to 9-somite chick embryos (HH9). Unilateral transplants were taken from the dorsal aspect of the neural tube as illustrated in Fig. 2. Our aim was to identify the relative positions of the telencephalic primordia on a rough fate map. The embryos were fixed after 1, 3 or 5 days survival, the grafts were identified using a quail-specific monoclonal antibody (QCPN).

At the 8-somite stage, the primordium of the telencephalic vesicle was entirely located in the anterior portion of the dorsal prosencephalon. The presumptive striatum, DVR and dorsal pallial anlagen occupied successive transversal domains along the anteroposterior axis of the neural tube (Fig. 2), the presumptive striatopallidal territory being the most anterior (Fig. 2C). The prospective limits of these three major telencephalic subdivisions were roughly transversal to the axis of the neural tube (Fig. 2J). It was not possible, however, based on these grafts to delineate precisely each territory. Between E4 and E6, the basal ganglia were displaced from their early anterior to their traditional ‘basal’ position.

As noted previously (Balaban et al., 1988), postmitotic cells arising from the grafted neuroepithelium migrated tangentially and spread over the host neuroepithelium. In a few instances, accumulations of quail cells were observed in regions that were distant from the grafted neuroepithelium. Grafts giving rise mostly to pallium also produced a stream of quail cells extending in the lateral telencephalic vesicle (Fig. 2E). Grafts giving rise mostly to rostral pallial + intermediate territory produced a row of cells in the basal telencephalic vesicle (Fig. 2F-I).

Subdivisions defined in the vertebrate telencephalon by Emx-1 and Dlx-1 expressions

The Emx and Dlx genes code for homeodomain-containing transcription factors related to Drosophila ems and dll, which are also expressed in restricted head domains in Drosophila (Price et al., 1991; Simeone et al., 1992a, 1994; Bulfone et al., 1993, 1994; Porteus et al., 1991, 1994; Gulisano et al., 1996; Yoshida et al., 1997). In mouse embryos, Emx-1 and Dlx-1 are expressed in complementary domains in the proliferative layer of the cortical and striatal anlagen. In order to localize the origin of the DVR in relation to the expression of the Emx-1 and Dlx-1 orthologs, we examined, at successive developmental stages, the expression domains of these genes in the forebrain of mouse, chick, turtle and frog embryos. Thick sections or dissected brains were treated for the detection of either or both transcripts. Although this method does not allow for high resolution analysis, it is well suited for the examination of large brain subdivisions.

The first stages examined, mouse E11.5, chick HH28 and turtle stage 17 (Pieau and Dorizzi, 1981) corresponded to the production of the first postmitotic neurons in the dorsal pallium. At this stage in all three species, both Emx-1 and Dlx-1 are already expressed at high levels in complementary territories: Emx-1 in a dorsal pallial region and Dlx-1 ventromedially. In order to facilitate comparison with the other two species, the pattern of expression of Emx-1 and Dlx-1 in the mouse telencephalon will be first briefly summarized, even if more complete descriptions have already been published (Simeone et al., 1992a,b; Price et al., 1991; Bulfone et al., 1993).

A transient gap separates the expression domains of Emx-1 and Dlx-1 in the telencephalon of mouse embryos

At E11.5, in the ventricular zone of the dorsal (pallial) part of the telencephalic vesicle, the Emx-1 expression domain extends from the prospective hippocampal region, medially, to the paleocortical anlage, laterally (Fig. 4A, Simeone et al., 1992). At E12.5, the pallial anlage was more clearly defined. The Emx-1 expression domain extended also into the lateral aspect of the dorsal telencephalon. The Dlx-1 expression domain was restricted to the caudal part of the telencephalon, ventromedially to the Emx-1 expression domain.
is an anatomical sulcus that has been generally considered as separating the prospective territories of the cortex dorsally and the basal ganglia ventrally (Smart and McSherry, 1982). Interestingly, the limit of $Emx-1$ expression in the ventricular zone (upper arrowhead in Fig. 3A) lies clearly dorsal to the palliobasal angle.

The $Dlx-1$ territory includes the broad subventricular zone characteristic of the prospective basal ganglia and septum. $Dlx-1$-positive cells constitute young postmitotic neurons and mitotically active progenitors in the subventricular and ventricular zones (Bulfone et al., 1993). The $Dlx-1$ expression domain do not reach the palliobasal angle (lower arrowhead in Fig. 3B). Ventricular cells expressing neither gene can be clearly distinguished around the palliobasal angle between the $Emx-1$ and $Dlx-1$ domains (between arrowheads in Fig. 3A,B).

At E13.5, the relative position of the $Emx-1$- and $Dlx-1$-positive ventricular zone with respect to the palliobasal angle (arrowheads in Fig. 3C, D) has not changed much, although, at this stage, the negative gap is less conspicuous than at E11.5.

At E15.5, the $Emx-1$- and $Dlx-1$-negative ventricular zone at the level of the palliobasal angle is no longer distinguishable (Fig. 3E,F).

**In chick, turtle and frog the $Emx-1$ and $Dlx-1$ telencephalic domains are separated laterally by a growing intermediate territory**

**Chick**

Stage HH 28-29 (about E6, Fig. 3G) is broadly comparable to mouse E11.5. At this stage, $Emx-1$ is expressed in ventricular and postmitotic cells in a dorsal, pallial territory, whose position is comparable to that of mouse $Emx-1$ at E11.5. $Emx-1$ is also expressed in the whole caudal pole of the telencephalic vesicle. Some $Emx-1$-expressing cells are found in a ventral territory, corresponding probably to the olfactory tubercle. $Dlx-1$ is expressed in a basal telencephalic territory corresponding to the paleostriatum and septum anlagen (not shown). The paleostriatum is considered to be the equivalent in birds of the striatum of mammals (Reiner et al., 1984). Most $Dlx-1$-positive cells are, as in the mouse, located in the subventricular zone.

A small territory, in which the ventricular zone and the overlying postmitotic cells are negative for both markers, separates the $Emx-1$ and $Dlx-1$ domains at stage HH 28-29. Its position is equivalent to that of the mouse intermediate territory although there is no palliobasal angle in birds.

At stage HH35 (E9, Fig. 3H), $Emx-1$ is still expressed in the neuroepithelium of the dorsal pallium and hyperstriatum as well as in postmitotic cells. In addition, $Emx-1$ expression is maintained in a stream of superficial postmitotic cells (Fig. 3H, asterisk; compare with Fig. 2E, arrow) which were probably generated at early stages in the dorsal territory (Tsai et al., 1981a,b, Fig. 6E). The limit of the $Dlx-1$ territory coincides with the dorsal medullary lamina, a sheet of axons first detected at this stage which marks the boundary between the paleostriatum and neostriatum. At this stage, the primordium of the neostriatum lies in the gap between the $Emx-1$ and $Dlx-1$ expression domains whereas the hyperstriatum ventrale is located in the $Emx-1$ domain.

Beginning from stage HH 36-37 (E10-11, Fig. 3I), the main subdivisions of the adult telencephalic vesicle are marked by anatomical landmarks (Tsai et al., 1981b). The $Emx-1$ territory is delimited by the axons of the lamina hyperstriatica which, in adults, mark the limit between the neostriatum and hyperstriatum. $Emx-1$ expression is maintained in the hyperstriatum but downregulated in the dorsal pallium. The parahippocampus and the hyperstriatum accessorium, which originated from the early $Emx-1$ dorsal domain, no longer express this gene. $Dlx-1$ expression is restricted to the subventricular layer of the paleostriatum and septum. As in the mouse (Bulfone et al., 1993; Porteus et al., 1994, Anderson et al., 1997), $Dlx-1$-expressing cells are also detected in the subventricular zone of the chick dorsal telencephalon.

Thus, the neostriatum and hyperstriatum ventrale, two classical subdivisions of the avian DVR, arise from distinct neuroepithelial domain: the $Emx-1$-positive and the intermediate domain, respectively. Because the DVR was initially identified in reptiles and the term was secondarily extended to specific regions of the brain in birds (Uliński, 1983), we decided to examine the relationship of the developing DVR with the $Emx-1$ and $Dlx-1$ expression domains in turtle embryos.

**Turtle**

The development of the telencephalic vesicle is comparable in stage 16-17 (Pieau and Dorizzi, 1981) $Emys orbicularis$ embryos to that of mouse E11.5 and chick HH 28-29 embryos with a row of postmitotic neurons detected in a subpial position in the pallium. The expression domains of $Emx-1$, in the pallium, and $Dlx-1$, in a basal territory, are also very similar (not illustrated). However, the sulci in the lateral telencephalic wall used in previous studies (Uliński, 1983) to delimit the DVR anlage are not formed yet at this stage.

Between stages 19 and 21, the dorsal ventricular ridge begins to bulge between the dorsal and lateral sulci. $Emx-1$ is confined to a dorsal pallial region (Fig. 4A) and to the caudal part of the telencephalic vesicle. Its expression domain in the ventricular layer extends beyond the dorsal sulcus. $Emx-1$ is also expressed in postmitotic cells in the lateral pallium. $Dlx-1$ is expressed in a basal telencephalic domain comparable to that observed in chick and mouse (Fig. 4B). Interestingly, in the absence of a clear subventricular zone in turtle embryos, $Dlx-1$ is expressed in cells of the ventricular layer. The sharp limit of the $Dlx-1$ expression domain lies slightly dorsal to the lateral sulcus (Fig. 4B). In the most prominent part of the DVR bulge, a negative ventricular zone is intercalated between the expression domains of $Emx-1$, dorsally, and $Dlx-1$, basally. This intermediate domain is narrow at stage 19 and widened by stage 21 (Fig. 4A,B). At stage 21, the $Emx-1$-positive domain in the caudal telencephalon can be identified as the basal dorsal ventricular ridge (BDVR). Some isolated cells in the cellular layers of the medial and dorsomedial cortices in the medial pallium are $Dlx-1$ positive at this stage.

At stage 23, $Emx-1$ expression is restricted to postmitotic cells of various pallial derivatives. $Dlx-1$ expression has decreased. Their expression domains are now delimited by cytoarchitectonic discontinuities.

Thus, as in mouse and chick embryos, a narrow territory intervening between the $Emx-1$ and $Dlx-1$ domains is also detected in early turtle embryos. At later stages, although the width of the intermediate territory increases, it is not bounded by the ventricular sulci, which classically delimit the DVR but include most of the DVR bulge. These observations indicate that the major part of the DVR, as classically defined, does not belong to the $Emx-1$ territory.
The presence of an intermediate telencephalic territory in the telencephalon of mouse chick and turtle embryos suggested that it could represent an archetypic brain subdivision already present in their common ancestor. To test this possibility, we examined the expression patterns of\textit{Emx} and\textit{Dlx} genes in\textit{Xenopus} embryo, an amphibian species.

\textbf{Frog}

The expression patterns of\textit{XEmx-1}, \textit{Xdll-4} were compared in\textit{Xenopus} embryos between stages 41 and 50 (Nieuwkoop and Faber, 1956).

A negative intermediate territory was observed between the faintly positive caudal \textit{XEmx-1} region and the rostral \textit{Xdll-4} telencephalic domain on stage 41 embryos treated for whole-mount in situ hybridization (not shown). In stage 43 brains reacted in toto, the \textit{XEmx-1} and \textit{Xdll-4} expression domains were broadly complementary (see Papalopulu and Kintner, 1993). Later on, at stage 50, the intermediate telencephalic domain is composed of a large negative region in the lateral telencephalic wall and a smaller medial one. These domains are likely to give rise to the lateral pallium and to a septal domain, respectively. Thus, as in amniotes, an intermediate neuroepithelial domain, inserted between the \textit{Emx-1} and \textit{Dlx} telencephalic domains, is also observed in frog embryos (see Fig. 4C).

\textbf{Pax-6 and Emx-2 expression in the intermediate territory of mouse and chick embryos}

Because a specific marker of the telencephalic intermediate territory was not available, we examined the expression patterns of the \textit{Pax-6} and \textit{Emx-2} genes, which are known to be expressed in the dorsal and lateral telencephalic vesicle (Walther and Gruss, 1991; Stoykova and Gruss, 1994;

\begin{figure}[h]
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\caption{\textit{Emx-1} and \textit{Dlx-1} expression patterns in the telencephalic vesicle of mouse and chick embryos. Transverse vibratome sections through the forebrain region were treated for the detection of \textit{Emx-1} (A,C,E,I), \textit{Dlx-1} (B,D,F) or both (G,H). (A-F) Mouse embryos. At E11.5, the expression domains of \textit{Emx-1} in the pallium and \textit{Dlx-1} in the striatum are separated by a thin intermediate territory (between arrowheads) expressing neither gene. At E13.5, this intermediate territory is still visible (compare C and D) and surrounds the palliobasal angle (arrowheads). By E15.5, the domains of \textit{Emx-1} and \textit{Dlx-1} expressions are roughly complementary. (G-I) Chick embryos. At E6, there is a gap (arrowheads) between the limits of the \textit{Emx-1} (purple) and \textit{Dlx-1} (red) expression domains. At E9, a wide domain corresponding to the so-called neostriatum (between arrowheads) separates the \textit{Emx-1} (purple) and \textit{Dlx-1} (red) territories. The \textit{Emx-1} expression domain extends superficially in the lateral part of the telencephalic vesicle (asterisk). At E11, the hyperstriatum (H) and hippocampus (b) express \textit{Emx-1}, which has been downregulated in other pallial regions. The position of the axon tracts that delimit the neostriatum (not visible at this magnification) is marked by arrowheads. The scale bar represents 400 \textmu m in A,B; 550 \textmu m in C,D,H; 700 \textmu m in E,F,G; 1250 \textmu m in I.}
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\includegraphics[width=\textwidth]{fig4}
\caption{Expression of \textit{Emx} and \textit{Dlx} in turtle. (A,B) Transverse vibratome sections cut through the forebrain of a stage 21 turtle (\textit{Emys orbicularis}) embryo and treated for the detection of \textit{Emx-1} (A) and \textit{Dlx-1} (B) transcripts. \textit{Emx-1} is expressed in the pallial regions and \textit{Dlx-1} in the basal telencephalon. In between, an intermediate negative domain (between arrowheads) comprises most of the bulging part of the DVR but is not coextensive with the DVR. (C) Diagram summarizing results in A and B; \textit{Emx-1} (red) and \textit{Dlx-1} (green) expression patterns in the neuroepithelium. (D) Schematic drawing summarizing the patterns of \textit{Emx-1} (red) and \textit{Dlx-1} (green) expression patterns in the neuroepithelium of the telencephalic vesicle in different species in relation to the various sulci which usually serve as landmarks. The intermediate neuroepithelium is labeled in blue. Scale bar 300 \textmu m.}
\end{figure}
Stoykova et al., 1996; Simeone et al., 1992a,b; Gulisano et al., 1996; Yoshida et al., 1997). In addition, Pax-6 has been shown to be important for the segregation of telencephalic domains in the mouse (Stoykova et al., 1996, 1997). We will concentrate on features of Pax-6 and Emx-2 expression in the telencephalic vesicle, which may be relevant for the present study.

In the E10.5-E11.5 mouse embryo, Pax-6 is expressed in a dorsocaudal telencephalic territory (Fig. 5A,B), considered in previous studies as the cortical anlage and encompassing the Emx-1 expression domain (compare Figs 5B and 3A). The Pax-6 expression domain surrounds the palliobasal angle extending, beyond that of Emx-1, in the intermediate territory. At later developmental stages, both the Pax-6 (Fig. 5A-F) and Emx-2 (Fig. 5J) expression domains extend beyond the palliobasal angle. Pax-6 is expressed at a high level in the ventricular zone of the intermediate domain (Fig. 5A, arrowheads, Fig. 5B, between arrowheads), both in the lateral telencephalic wall and in its thin medial extension located in the septum (Fig. 5C,E, arrowheads). At E11.5, a group of Pax-6-expressing postmitotic cells (Fig. 5B) lies close to the striatopallial angle between arrowheads. At E11.5, a group of Pax-6-expressing postmitotic cells lies close to the striatopallial angle (Fig. 5B). From E12.5 on, the Pax-6-expressing nuclei are displaced superficially but maintain a topographical relationship with the striatopallial angle (Fig. 5C-F, arrows). These neurons can now be identified as belonging to several subnuclei, from medial to lateral, of the septal region, the diagonal band of Broca, part of the amygdala and a lateral population which could be part of the paleocortex. Interestingly, these structures maintain their expression of Pax-6 in adult mice (Stoykova and Gruss, 1994). Emx-2, but neither Emx-1 nor Dlx-1, is expressed in a slightly different pattern in the same nuclei as Pax-6 (Fig. 5I-J).

In chick embryos, Pax-6 and Emx-2 are expressed in the ventricular zone of the dorsal telencephalic vesicle. At HH28 (E6), the Pax-6 and Emx-2 expression domains include that of Emx-1 but do not overlap with the Dlx-1-positive region. Medially, the dorsalmost part of the septum is Pax-6 positive, whereas laterally Pax-6 is expressed at high level in the neuroepithelium of the neostriatum, which belongs to the intermediate territory (see above). A group of postmitotic Pax-6-positive neurons surmounts the lateralmost Pax-6-positive neuroepithelium. A row of basally located nuclei contains both Pax-6 and Emx-2-expressing neurons (not illustrated).

Pax-6 is strongly expressed in the ventricular zone of the neostriatum by E9 but its expression is progressively

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**Fig. 5.** Pax-6 and Emx-2 expression in the telencephalic vesicle of mouse and chick embryos. (A) Whole mount and (B–K) vibratome sections through the telencephalic vesicle of mouse (A–F,J) and chick (G,H,K) embryos treated for the detection of Pax-6 (A–H) or Emx-2 (I–K) transcripts. (A) Whole mount of a E10.5 mouse embryo. The Pax-6 domain in the caudal part of the telencephalic vesicle is delimited by a stripe of high intensity of expression (arrowheads); this band corresponds to the region delimited by arrowheads in B. (B) Transverse section through the telencephalon at E11.5 (orientation indicated in A). Pax-6 is expressed in the pallial region and most intensely in the ventricular zone of the intermediate territory (between arrowheads; compare with Fig. 3A and B). A group of Pax-6-positive postmitotic cells is located more basally than the lateral stream of Emx-1-expressing neurons (Fig. 3A, asterisk). (C–F) Rostral (C,E) and caudal (D,F) transverse sections through the telencephalic vesicle of E12.5 (C,D) and E15.5 (E,F) mouse embryos. The neuroepithelium of the pallial and intermediate territories express Pax-6. The intermediate neuroepithelium is linked to the populations of Pax-6-positive neurons of the basal telencephalon through a thin stripe of Pax-6-positive cells, both laterally (arrows) and medially (arrowheads). (G) E6 chick: Pax-6 is expressed in the ventricular zone of the dorsal pallium including the intermediate region where the labeling is more intense both laterally (delimited by arrowheads, compare with Fig. 3G) and medially (arrow). Two groups of Pax-6-positive postmitotic cells one lateral and one basal are located near the basal limit of the Pax-6-positive neuroepithelium. (H) E9 chick: Pax-6 expression is still detectable in the ventricular zone of the region corresponding to the neostriatum (delimited by arrowheads) and the ventralmost part of the hyperstriatum. (I–K) Rostral (I) and caudal (J,K) transverse sections through the telencephalic vesicle of E13.5 mouse (I,J) and E6 chick (K) embryos treated for the detection of Emx-2 transcripts. In the neuroepithelium, the domains of Emx-2 and Pax-6 expressions are very similar. In addition, in both mouse and chick, Emx-2 labels populations of postmitotic neurons in the same regions as Pax-6 (asterisks in F,J, arrows in D,F,J). Arrowheads in J,K point to the ventral limit of Emx-2 expression in the neuroepithelium. The scale bar represents 500 μm in A,B,G,J; 670 μm in C,D,E,F,H,K,L; 400 μm in I.
from this stage on. From E14.5 on, the labeled nuclei (Fig. 6C). BUdR injected at E15.5 or later is not
nuclei appear as a BUdR-negative island containing few
density of cortical and striatal cells whereas the
most prominent.

Our observations indicate that, at the onset of neurogenesis, the
earliest to become postmitotic (Tsai et al., 1981a,b, compare
Figs 5G and 6E).

The pattern of Pax-6 expression in the ventricular zone is
thus similar in both chick and mouse and provides a good
marker of the intermediate telencephalic territory. On the
contrary, similar early populations of postmitotic cells
expressing Pax-6 and Emx-2 are observed close to the
neuropilethium of the intermediate territory. At later stages,
however, the regulation of Pax-6 expression in postmitotic
neurons seems to diverge between species.

**Time of origin of the Pax-6-positive nuclei in the
mouse telencephalon**

In order to delineate the early generated basal telencephalic
structures, we performed bromodeoxyuridine (BUdR)
injections at different stages of development. All animals were
killed at E18.5.

Most of the early postmitotic cells, born at E10.5, belonged
to the Pax-6-positive structures of the basal telencephalon (Fig.
6A). BUdR injections at E11.5, labeled a large number of cells
in these Pax-6-positive nuclei (Fig. 6B); in contrast, only a few
scattered cells in the cortical and striatal territories become
postmitotic at this stage. It is worth noting that this is the time
when the gap between Emx-1 and Dlx-1 expression domains is
most prominent.

BUdR injections at E14.5 resulted in the labeling of a high
density of cortical and striatal cells whereas the Pax-6-positive
nuclei appear as a BUdR-negative island containing few
labeled nuclei (Fig. 6C). BUdR injected at E15.5 or later is not
incorporated in the Pax-6-positive nuclei (Fig. 6D) indicating
that these structures are produced before E15.5. The Pax-6-
positive structures already display some of their adult
morphological features by E15.5 and can be easily identified
from this stage on. From E14.5 on, the Emx-1 and Dlx-1
expression domains seem to be contiguous.

These birthdating experiments indicate that most neurons in
the Pax 6-positive nuclei are produced between E11.5 and
E13.5, when the neuroepithelium of the intermediate territory
can be detected. Previous neuronal birthdating experiments in
chick embryos have shown that the neurons that transiently
express Pax-6 in the basal telencephalon are also among the
earliest to become postmitotic (Tsai et al., 1981a,b, compare
Figs 5G and 6E).

**DISCUSSION**

In the present study, we compared the expression patterns of
Emx-1 and Dlx-1/2 in chick, mouse, turtle and frog embryos.
Our observations indicate that, at the onset of neurogenesis, the
developing telencephalic vesicle can be subdivided into three
conserved neuroepithelial domains: the dorsal pallium, the
ventral striatopallidum and an intermediate territory. These
territories, as deduced from our fate-mapping studies,
corresponded to successive rostrocaudal domains of the dorsal
neural tube. We propose that this initial partition in three
territories should be considered as a developmental homology.

The fate of the intermediate territory differed between
species. Whereas in mouse its neuroepithelium rapidly became
vestigial and could not be detected beyond E13.5, its width
increased in the lateral wall of the telencephalic vesicle in chick
turtle overlapping with the neuroepithelium lining the
growing DVR. In mouse and chick, we identified discrete
neuronal populations that are located in similar positions,
maintain a topographical relationship with the intermediate
territory and express similar markers. We propose that these
populations can also be considered as developmentally
homologous. Conversely, we propose that, because of the
protracted proliferation of the intermediate neuroepithelium in
chick and turtle as compared to mouse, other neuronal
populations belonging in particular to the chick neostriatum
and to the turtle DVR have no developmental homolog in
mouse.

**The telencephalic vesicle derives from the dorsal aspect of the prosencephalic vesicle**

The position of the presumptive territory of the telencephalon in
the early neural tube is still controversial. Most classical
neuroanatomists implicitly consider that it derives from the
rostralmost tip of the neural tube, being composed of a pallial
part, dorsal in nature, and a basal part, issued from the ventral
half of the tube (Altman and Bayer, 1995; Alvarez-Bolado et
al., 1995). More recent descriptions of brain organization and
in particular of telencephalic subdivisions were based on the
prosomeric model (Puelles and Rubenstein, 1993; Rubenstein
et al., 1994; Puelles, 1995). These authors assume that the
telencephalic vesicle arises from the dorsal aspect of the tube
(Bulfone et al., 1993, 1995). However, because most of their
early schemes were based on gene expression patterns at E12.5
in the mouse, the striatal anlagen was depicted as more basal
than the cortical anlagen. In a more recent study, based on gene
expression patterns at earlier stages of mouse development,
Shimamura et al. (1997) depict the presumptive cortex and
striatum as aligned along the anteroposterior axis of the neural
tube as we find in the chick fate map, instead of being
successive dorsoventral structures. The transverse boundary
that separates the Emx-2/Pax-6 and Dlx-2 domain receives the
name of zona limitans intratelencephalica.

Our observations confirm earlier fate map studies performed
at the neural plate stage in chick (Couly and Le Douarin, 1987)
and Xenopus (Eagleson and Harris, 1990; Eagleson et al.,
1995), which showed that the whole telencephalic vesicle is
issued from the dorsal anterior part of the neural tube. In
addition, in chick embryos, the primordium of the striatum
(‘floor of the telencephalon’ in Couly and Le Douarin, 1987)
is located more anteriorly than the pallial region (‘roof of the
telencephalon’). Similarly, in Xenopus (Eagleson et al.,
1995), the striatal anlage is located more medially on the anterior
neural ridge of the open neural plate (i.e. more anteriorly in the
closed neural tube) than the primordium of the cortex. Our fate-
mapping study indicates that the three major telencephalic
subdivisions, delineated on the basis of differences in gene
expression (see also Shimamura et al., 1997), originate from
successive transversal stripes in the dorsal anterior
prosencephalon which are arranged along the anteroposterior
axis and correspond, from rostral to caudal, to the basal ganglia
(septum/pallidum/striatum), the intermediate region
(composed more prominently in the chick of the medial
septum, a set of ‘basal’ nuclei and the neostriatum) and the
pallial region (including the hippocampus, hyperstriatum accessorium and hyperstriatum).

**Emx-1 and Dlx-1 as markers of early telencephalic regionalization**

In the proliferative cell populations of the telencephalon, the Dlx-1, Emx-1 and Pax-6 transcripts were used as regional markers with the aim of identifying and following early telencephalic subdivisions in the absence of other landmarks. The expression of these transcripts, at later developmental stages, in several populations of postmitotic neurons were a priori considered as an independent issue needing complementary data to be interpreted.

The present study relies on two assumptions: (i) the early Emx-1 and Dlx-1 expression domains delineate homologous territories in different vertebrate species and (ii) the onset of neuronal production can be considered as a reliable reference for comparison of developmental stages between species.

There is a concern that gene duplications during evolution could have partially released the constraint on their spatial regulation resulting in a variability of their expression patterns in different species. As discussed below, it seems that, in the Dlx gene family, the variations in expression patterns between orthologs antedates the formation of amniotes (Morasso et al., 1995). Less is known about the expression patterns of genes of the Emx family. The available data in mouse, chick, and zebrafish, however, indicate that the spatial regulation of Emx-1 and Emx-2 has probably been fixed early in evolution.

The Dlx gene family comprises at least 6 members (Stock et al., 1996) in the mouse. Except for mDlx-3 (Robinson and Mahon, 1994) and mDlx-7 (Shimamoto et al., 1997), which are not expressed in the central nervous system, all (mDlx-1, mDlx-2, mDlx-5 and mDlx-6) are expressed in the subventricular zone of the medial and lateral ganglionic eminences and in the septum. At early developmental stages, their expression domains share a common limit at the border of the striatopallidal anlage (Porteus et al., 1991; Price et al., 1991; Simeone et al., 1994). This feature is likely to have been evolutionarily conserved between orthologs of these genes. In *Xenopus*, the X-dll3 and X-dll4 genes which belong to the mDlx-5 and mDlx-2 subfamilies, respectively, are expressed in the ventral forebrain (Papalopulu and Kintner, 1993; Dirksen et al., 1993), whereas X-dll2 and mDlx-3, which belong to the same subfamily, are not expressed in the brain. Furthermore, frog regulatory sequences from the X-dll2 promoter confer appropriate Dlx-3 like expression on a lacZ reporter gene in transgenic mice (Morasso et al., 1995).

The vertebrate ems homologues that have been cloned in mouse (Simeone et al., 1992b), zebrafish (Morita et al., 1995), chick, frog and turtle (Paternello et al., 1997) are all expressed in the forebrain. In mouse, chick and zebrafish embryos, Emx-1 and Emx-2 are similarly expressed in overlapping domains, Emx-2 being expressed earlier and more broadly than Emx-1.

Thus, at least at early developmental stages, the expression domains of the Emx-1 and Dlx-1/2 genes in the telencephalic vesicle have been conserved during evolution.

Our concern here was to use a way of comparing developmental stages which would be based on a parameter of differentiation and not on the pattern of gene expression. At the onset of neuronal production, the telencephalic vesicles of mouse chick and turtle are still very similar although they have already acquired species-specific morphological traits. This stage also corresponds to the sharpening of the limits of Emx-1 expression and is late enough to be close to the production of the neuronal structures that we wanted to compare. The fact that the patterns of Emx-1, Dlx-1 and Pax-6 expression are very similar at this stage in mouse chick and turtle and change progressively thereafter can be taken as a validation of this choice.

**Derivatives of the transient intermediate territory in the mouse**

Structures derived from the mouse intermediate territory are expected to have two properties: first, they should be located laterobasally in the telencephalic vesicle on the basis of their site of origin and of the result of our grafting experiments in chick, and, second, because they are produced by a transient domain of the neuroepithelium, they are expected to become postmitotic at early developmental stages.

The radial glia provides a record of early topographical proximity between the ventricular and pial domains. Late in embryonic development, a subset of the glia fibers follows a curved trajectory at the border of the basal ganglia linking the neuroepithelium of the striatopallial angle to the ventrolateral part of the cerebral cortex; this glial palisade could serve as a substratum and a guidance cue for migratory cells (De Carlos et al., 1996 and references therein, Striedter and Beydler, 1997). It is unclear whether the Pax 6 transcripts that line the striatum, linking the palliobasal angle with the Pax-6-positive amygdalar nuclei, are contained in radial glia or in migrating neurons. In any case, this pattern makes the laterobasal Pax-6 structures a likely derivative of the transient intermediate neuroepithelium.

Besides their location and the fact that they maintain a connection with the striatopallial angle through a line of Pax-6-positive cells, the Pax-6-positive nuclei of the paleocortex, amygdala and diagonal band and medial septum are good candidates as derivatives of the intermediate telencephalic on the basis of their early generation and also because, in Pax-6 (sey) mutants, both the intermediate domain and the ring of Pax-6-positive nuclei fail to form (Stoykova et al., 1996).

**Derivatives of the intermediate territory in chick and turtle.**

According to Ulinski (1983), two ventricular sulci designed MVS and DVS mark the lateral wall of the telencephalic vesicle and demarcate the DVR. Their presence is a common feature in all reptiles and birds. It is interesting to note that the intermediate territory, defined as a gap between the Emx-1 and Dlx-1 expression domains, lies into the DVR neuroepithelium of chick and turtle embryos. It is, however, not bounded by the MVS and DVS sulci. In fact, sulci in the ventricular wall are not reliable markers of the limits of histogenetic subdivisions (Puelles and Rubenstein, 1993). These boundaries are in general marked by local histological discontinuities or coincide with limits of gene expression domains; they are also often marked by the course of major axonal tracts (Puelles and Rubenstein, 1993).

Such histological discontinuities are indeed observed in *Emys orbicularis* as step variations in both the thickness and the proliferative activity of the DVR neuroepithelium (Goffinet et al., 1986). These discontinuities do not coincide with the
MVS and DVS sulci but define a subdomain in the middle of the classical DVR, which may correspond to our intermediate domain.

In E6 chick embryos (Tsai et al., 1981b), a distinct boundary is delineated by a difference in the distribution of cells labeled by \(^{3}H\)thymidine injections at E5. This limit preFigss the lamina medullaris dorsalis, which marks the basal limit of the DVR.

A great part of the chick and turtle DVR is thus likely to derive from the intermediate territory. Our results indicate, however, that the classical definition of the avian DVR (Ulinski, 1983), considered as an homolog of the reptilian DVR, is misleading. By analogy with reptiles, the bird DVR was delimited by two early sulci appearing in the lateral wall of the telencephalic vesicle. We propose that the structures that derive from the lateral portion of the intermediate telencephalic territory including most of the classical DVR of reptiles and exclusively the neostriatum in birds should be considered as DVR in other species; the hyperstriatum should thus be considered as a pallial derivative.

Besides the DVR, additional structures located in the basal telencephalon of adult animals could arise from the intermediate territory. In chick embryos, this sheet of basally located cells is not produced by the overlying neuroepithelium (Fig. 2 F-I). The scale bar represents 300 \(\mu m\).

Are the structures that derive from the intermediate territory in different vertebrate species homologous?

During the early ‘phyloytic’ phase, the intermediate territory generates a population of neurons belonging to nuclei that, in chick and mouse, share their lateral and basal positions, their
Archetypic embryonic stage

![Diagram of embryonic stages](image)

**Fig. 8.** Interpretative scheme of the evolution of telencephalic regionalization. The adult structures produced by the three main subdivisions of the telencephalic vesicle neuroepithelium are depicted in different species based on the results of the present study (see text for details). The ‘pallial’ territory is shown in red, the ‘intermediate’ territory in blue and the ‘basal’ territory in green. Same abbreviations as in Fig. 1.

early birthdates and their expression of Pax-6 and Emx-2. Each of these nuclei does not necessarily derive entirely from the intermediate telencephalon. Indeed, several rhombomeres contribute to the formation of single cranial nerve motor nuclei (Marin and Puelles, 1995). In the telencephalon, neurons produced in the Dlx-1/2 domain have been shown to migrate dorsally and differentiate as GABAergic interneurons in the neocortex and olfactory bulb (Anderson, 1997a,b).

Our data, and those of others (Stoykova and Gruss, 1994; Gulisano et al., 1996), in mouse embryos, support the case for a dual origin of the nuclei constituting the mouse amygdalar complex: the corticomedial and central nuclei (both expressing Emx-1) are likely derivatives of the dorsal or ‘pallial’ territory, while those nuclei of the laterobasal complex (which express Pax-6) could derive from the intermediate territory. Taking into account our data in the other species studied, the search for a homolog of the reptilian DVR and the avian neostriatum in mammals should be centered in the laterobasal part of the amygdala, while traditional homologs of the amygdala in birds (the archistriatum) and reptiles (the BDVR) should now be tested as homologs of the corticomedial and central part of the amygdala (all these structures express Emx-1). Bruce and Neary (1995) reached a similar conclusion based on a detailed analysis of the connections of amygdalar subnuclei in different species.

We argue that the early generated populations of neurons can be considered as homologous in chick and mouse and probably in other vertebrate species and that, on the contrary, later generated structures, although produced by homologous developmental brain subdivisions, should not be considered properly as homologs (illustrated in Fig. 7). An argument in favor of this interpretation comes from the analysis of mouse mutants in which defined populations of early produced neurons can be detected at late embryonic stages although their neuromere of origin is ‘deleted’ or failed to develop. The Kreisler and Krox-20 genes, for example, are necessary for rhombomere development in mouse and zebrafish. In mutants, the affected rhombomeres express early position-specific markers but fail to develop further and either disappear in the case of Krox-20 homozygous mutants (Schneider-Maunoury et al., 1993, 1997) or fail to acquire fully differentiated traits as in Kreisler mutants (Cordes and Barsh, 1994; McKay et al., 1994; Moens et al., 1996). In both Krox-20 and Kreisler mutants, it has been noted that a normal contingent of early generated neurons is produced before the mutant phenotype is detected (Schneider-Maunoury et al., 1997; Moens et al., 1996). Similarly, BF-1 function is required for normal development of the telencephalon and the size of the telencephalic vesicles is severely reduced in BF-1 null mutants. Nevertheless, in the vestigial cerebral cortex of BF-1 mutants, a neuronal population is detected that expresses markers of the preplate neurons, the earliest to be born in the wild-type cerebral cortex (Xuan et al., 1995). In Dlx-1/Dlx-2 double mutants, the early population of striatal neurons is formed whereas late born striatal neurons fail to differentiate (Anderson et al., 1997a,b).

Alternatively, the whole set of intermediate telencephalon derivatives comprising the diagonal band of Broca, the DVR in chick and turtle and the mouse septal and amygdalar nuclei could be considered as developmental homologs (Fig. 8). We are less in favour of this interpretation because it underestimates the constraint on timing in the generation of neuronal structures. Two observations indicate that brain developmental time is constrained at a local as well as at a more global level. In the mammalian cerebral cortex, despite large variations in the duration of corticogenesis, the same proportion of the total time is devoted to the production of defined populations of neurons (Caviness et al., 1995). Furthermore, in mammalian species, the order of neurogenesis in various brain divisions is conserved across a wide range of brain sizes resulting in a strong constraint on the relative growth of each structure. Only late-generated structures show disproportional enlargement across mammalian species (Finlay and Darlington, 1995). Similar studies across different vertebrate lineages are hampered by their disparate brain organization, which precludes the comparison of brain divisions between species belonging to different lineages. It may however be supposed that the constraint on the relative growth of brain subdivisions is conserved in reptiles, birds and other non-mammalian lineages but not across lineages. Because there are no fossil records of brain structures and the duration of neurogenesis in a given brain subdivision may successively expand and shorten during evolution, its seems that only the structures that have been conserved across species can be considered as homologous and that the early generated structures are the most likely to have homologs in distantly related species.

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