Development/Plasticity/Repair

Delineation of Multiple Subpallial Progenitor Domains by the Combinatorial Expression of Transcriptional Codes

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The mammalian telencephalon is considered the most complex of all biological structures. It comprises a large number of functionally and morphologically distinct types of neurons that coordinately control most aspects of cognition and behavior. The subpallium, for example, not only gives rise to multiple neuronal types that form the basal ganglia and parts of the amygdala and septum but also is the origin of an astonishing diversity of cortical interneurons. Despite our detailed knowledge on the molecular, morphological, and physiological properties of most of these neuronal populations, the mechanisms underlying their generation are still poorly understood. Here, we comprehensively analyzed the expression patterns of several transcription factors in the ventricular zone of the developing subpallium in the mouse to generate a detailed molecular map of the different progenitor domains present in this region. Our study demonstrates that the ventricular zone of the mouse subpallium contains at least 18 domains that are uniquely defined by the combinatorial expression of several transcription factors. Furthermore, the results of microtransplantation experiments *in vivo* corroborate that anatomically defined regions of the mouse subpallium, such as the medial ganglionic eminence, can be subdivided into functionally distinct domains.

Key words: **telencephalon; subpallium; patterning; cell specification; interneuron diversity; cortex**

Introduction

Understanding the mechanisms underlying neuronal diversity in the mammalian telencephalon is a major challenge in developmental neurobiology, because this region holds the most heterogeneous collection of neurons in the entire nervous system. In other structures of the CNS, neural diversity arises from regional and/or temporal differences in the specification of neural progenitors. In the spinal cord, the best characterized system so far, graded signaling by morphogenetic molecules defines distinct domains of progenitor cells characterized by the expression of a specific combination of homeodomain proteins. Subsequently, these factors initiate a cascade of transcriptional interactions that lead to the specification of the precise classes of postmitotic neurons that derive from each individual progenitor domain (Jessell, 2000; Shirasaki and Pfaff, 2002). In contrast, retinal cell diversity is apparently not achieved by spatial patterning into distinct progenitor domains, but rather by the sequential production of cell types in a defined order. Thus, retinal progenitors go through a series of changes in intrinsic properties that control their competence to make different cell types during development (Cepko, 1999; Marquardt and Gruss, 2002).

Initial progress has been made in identifying the origin of neural diversity in the mammalian telencephalon, in particular in the subpallium (Marín and Rubenstein, 2002; Rallu et al., 2002; Campbell, 2003; Flames and Marín, 2005; Guillemot, 2005; Wonders and Anderson, 2006). In this region, which gives rise to the basal ganglia, parts of the amygdala and interneurons in the olfactory bulb and cortex, three main proliferative domains have been identified: the lateral, medial, and caudal ganglionic eminences (LGE, MGE, and CGE, respectively) (Bulfone et al., 1993). The telencephalic stalk (i.e., the nonevaginated telencephalon, including the preoptic area) and parts of the septum also belong to the subpallium and are thought to give rise to specific populations of telencephalic neurons (Puelles et al., 2000).

To a large extent, the definition of different progenitor regions in the subpallium has been based on anatomical landmarks, such as sulci and bulges. Unfortunately, despite the convenience of

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anatomical references, morphological boundaries do not always coincide with molecular limits and are often misleading when they change in position over time. This is the case, for example, of the pallial–subpallial boundary, located perceptibly ventral to the physical ventricular sulcus between the LGE and the cortex (Puelles et al., 2000). Similarly, the CGE is solely defined on the basis of morphological criteria, which change during development (Nery et al., 2002). Thus, it seems clear that a more accurate description of the subpallium should not only rely on anatomic landmarks but also take into account the expression of defined molecular markers.

More recent evidence supporting the division of the subpallium into different progenitor domains comes from gene expression studies and analysis of mouse embryos with mutations in homeodomain proteins expressed in the subpallium. For example, the LGE ventricular zone (VZ) is characterized by the expression of *Pax6*, whereas the MGE distinctly expresses *Nkx2-1* (also known as *Titf1*) (Sussel et al., 1999; Stoykova et al., 2000). The expression of several other genes further subdivides the LGE VZ into dorsal and ventral domains, which appear to give rise to different neuronal populations (Toresson et al., 2000; Yun et al., 2001, 2003; Stenman et al., 2003a; Waclaw et al., 2006). In addition, analysis of interneuron populations derived from the MGE and CGE suggests that these two regions may contain different progenitor pools (Xu et al., 2004; Butt et al., 2005; Wonders and Anderson, 2006). Thus, the notion of multiple distinct progenitor domains is not far from our present understanding of the mammalian subpallium.

Considering that neuronal diversity has reached its peak in the mammalian telencephalon, it is likely that many more progenitor pools than previously recognized exist in the subpallium. Here, we comprehensively compared the expression patterns of multiple transcription factors in the VZ of the developing subpallium. With this information, we generated a detailed molecular map of the different domains possibly present in this region. Each of these VZ domains is characterized by the expression of a unique combination of genes and a coherent anatomical location within the subpallium. Furthermore, using a microtransplantation paradigm, we demonstrate that functionally distinct progenitor domains do exist within the classically defined regions of the subpallium. Based on these findings, we propose a new nomenclature to designate VZ domains in the mouse subpallium, which we believe integrates previous findings, provides a comprehensive view of the different telencephalic structures, and can be expanded as new findings arise from future studies. We argue that this novel definition of histogenetic domains in the mouse subpallium will contribute to a better understanding of the patterning mechanisms underlying the development of the telencephalon.

Materials and Methods

Animals. Wild-type and green fluorescent protein (GFP)-expressing transgenic mice (Hadjantonakis et al., 1998), maintained in a CD1 background for at least 10 generations, were used in this study. The day of vaginal plug was considered embryonic day 0.5 (E0.5). Animals were kept at the Instituto de Neurociencias de Alicante under Spanish and European Union regulation.

In situ *hybridization.* Pregnant females were killed under anesthesia by cervical dislocation and embryos (E11.5–E13.5) were fixed overnight in 4% paraformaldehyde (PFA), cryoprotected in 30% sucrose in RNase free PBS, embedded in Tissue-Tek OCT compound (Sakura Finetek, Tokyo, Japan) and stored frozen at −80°C. Twenty-micrometer coronal or horizontal sections were cut on a cryostat and subsequently postfixed in 4% PFA for 10 min. After three washes in PBS, sections were treated with 10 μg/ml proteinase K in PBS (Roche, Indianapolis, IN) for 5 min, briefly transferred to 4% PFA, and then washed with 0.01% Tween in PBS (PBT). Subsequently, sections were acetylated for 10 min (1.3% triethanolamine, 0.25% acetic anhydride, and 17.5 mM HCl). Slides were then transferred to a hybridizing chamber (Thermo-Shandon, Pittsburgh, PA) where they were incubated for 1 h at 62°C with 500 μl of prehybridization solution [50% formamide (Ambion, Austin, TX), 10% dextran sulfate, 0.2% tRNA (Invitrogen, Carlsbad, CA), 1× Denhardts solution (from a 50× stock; Sigma, St. Louis, MO), 1× salt solution (from a 10× stock containing 2 M NaCl, 0.1 M Tris, 50 mM NaH_2PO_4, 50 mM Na_2HPO_4, 50 mM EDTA, pH 7.5)]. Digoxigenin (DIG)-labeled RNA probes were heated to 80°C for 5 min, cooled in ice, and added to prewarmed (62°C) prehybridization solution to a final concentration of 200–400 ng/ml (typically 0.2 μl of probe in 100 μl of hybridization solution). The prehybridization solution was then poured off the slides and 75 μl of hybridization solution containing the appropriate probe was added to each slide, which was subsequently covered with a coverslip and incubated overnight at 62°C. The next day, the slides were washed for 15 min with 50% formamide, 0.5× SSC, and 0.1% Tween at 62°C. Coverslips were gently removed and slides were incubated in 50% formamide, 0.5× SSC, and 0.1% Tween three times for 30 min each at 62°C. Slides were then washed three times in MABT (0.1 M maleic acid, 0.2 M NaOH, 0.2 M NaCl, 0.01% Tween), incubated for 1 h in blocking solution [10% blocking solution (Roche) and 10% sheep serum in MABT], and subsequently incubated overnight with anti-DIG antibody (1:5000; Roche) diluted in a solution containing 1% sheep serum and 1% blocking solution in MABT. Slides were next washed 10–15 times each for 30 min in MABT, with a final overnight wash in the same solution. Next, the slides were washed three times for 5 min in reaction buffer (0.1 M Tris, pH 9.5, 0.1 M NaCl, and 50 mM $MgCl_2$) and incubated in the dark in nitroblue tetrazolium (NBT)/5-bromo-4-chloro-3-indolyl phosphate (BCIP) solution [3.4 μl/ml from NBT stock and 3.5 μl/ml from BCIP stock in reaction buffer (100 mg/ml NBT stock in 70% dimethylformamide; 50 mg/ml BCIP stock in 100% dimethylformamide; Roche)]. Slides were checked periodically and the reaction was stopped in Tris-EDTA buffer. Finally, slides were dried overnight, dehydrated, and covered with Eukitt. Images were obtained using a cooled CCD camera (DC500; Leica, Nussloch, Germany). The following cDNA probes were used in this study: *Dbx1* (Lu et al., 1992), *Dlx2* (Liu et al., 1997), *Couptf1* (Qiu et al., 1994), *Er81* (Lin et al., 1998), *Gsh2* (Szucsik et al., 1997), *Lhx2* (Rincon-Limas et al., 1999), *Lhx5* (Zhao et al., 1999), *Lhx6* and *Lhx7* (Grigoriou et al., 1998), *Ngn2* (Gradwohl et al., 1996), *Nkx2-1* (Shimamura et al., 1995), *Nkx2-2* (Sussel et al., 1998), *Nkx6-2* (Qiu et al., 1998), *Pax6* (Walther and Gruss, 1991), and *Shh* (Echelard et al., 1993).

Analysis of gene expression. The expression of every gene reported in the study was analyzed in at least five different embryos for each stage. In each case, the expression was analyzed throughout the whole telencephalon, but only selected genes were chosen to illustrate each rostrocaudal level. In general, the expression of an individual gene was selected for a particular figure only when it contained novel information for the definition of at least a specific domain within the subpallial VZ. The description of the level of expression of individual genes was performed according to the following qualitative standards. When the gene was expressed by a majority of the cells in a domain (>75%), gene expression was reported as weak (+, signal just above background levels) or strong (++, signal intensity at least twofold above that defined as weak for the same gene). When the gene was expressed by a minority of the cells in a domain (<25%), gene expression was reported as scattered (+/s), independently of the intensity of the signal in individual cells. Analysis of the intensity of gene expression for each gene was performed using the open domain NIH ImageJ analysis software (http://rsb.info.nih.gov/ij/).

The boundaries between the different domains were built with the information provided by all genes showed. In general, each individual gene provided specific information related to only a few progenitor domains (for example, *Pax6* expression clearly delineates the boundary between LGE and MGE). Nevertheless, all boundaries were depicted in each panel to help the comparison among different genes.

In utero *transplantation.* For whole MGE *in utero* transplants, the MGE of six to eight E13.5 GFP-expressing embryos were dissected under

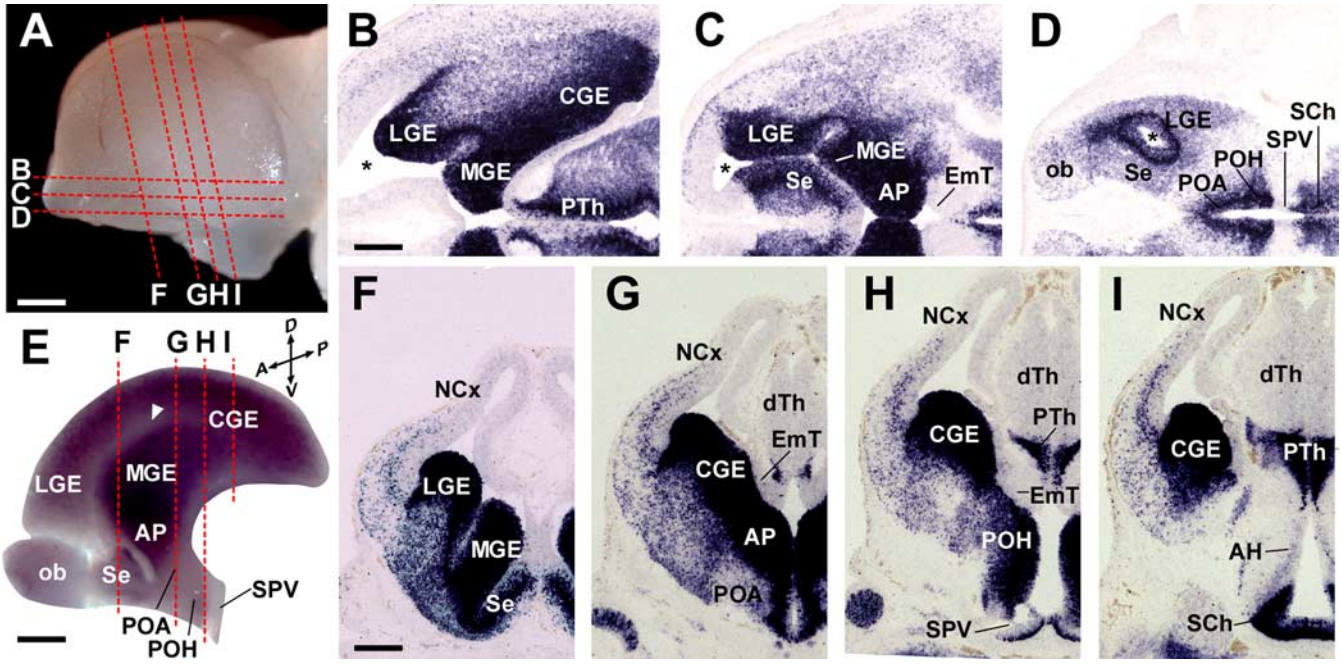

Figure 1. Anatomical organization of the embryonic telencephalon. *Dlx2* ventricular expression delineates the embryonic subpallial territory. ***A***, Lateral view of the forebrain at E13.5 of development. The dotted lines represent the level and orientation of photographs in ***B–D*** and ***F–I***. ***B–D***, Horizontal sections of the E13.5 telencephalon showing the expression pattern of *Dlx2* mRNA. ***E***, Whole-mount *in situ* hybridization of *Dlx2* at E13.5. The arrowhead points to the caudal end of the sulcus between the LGE and the MGE, which is typically referred as the rostral limit of the CGE. The dotted lines indicate the level and orientation of photographs (***F–I***). The crossed arrows mark the dorsoventral (D–V) and anteroposterior (A–P) axes. ***F–I***, Coronal sections through the E13.5 telencephalon showing *Dlx2* expression. The asterisks in ***B–D*** mark the lateral ventricle. AH, Anterior hypothalamus; dTh, dorsal thalamus; EmT, eminentia thalami; NCx, neocortex; ob, olfactory bulb; PTh, prethalamus; SCh, suprachiasmatic nucleus; SE, septum; SPV, supraoptic paraventricular region. Scale bars: ***A***, 300 μm; ***B–D***, 250 μm; ***E***, 250 μm; ***F–I***, 200 μm.

a stereomicroscope. Explants were washed in 0.5 ml of L-15 medium (Invitrogen) containing DNase I (100 μg/ml) and cells were mechanically dissociated by repeated pipetting (20–30 times) through a 200 μl plastic pipette tip. Dissociated cells were then pelleted by centrifugation (5 min; 1000 rpm), resuspended in 6 μl of L-15 medium with DNase I, and kept on ice until injection. For MGE domain microtransplants, a small cube of the MGE of $\sim$0.0035 mm^3 and approximately corresponding to the domain designated here pMGE1 ($n = 3$) or pMGE4 ($n = 4$) was isolated from a 250-μm-thick coronal slice of the telencephalon from an E13.5 GFP-expressing embryo. In each case, the GFP microtransplant was then mixed with the MGE of six E13.5 wild-type embryos, and the cells were dissociated and prepared for transplantation as above.

High-density cell suspensions ($\sim$25,000 cells/μl) were front-loaded into beveled glass micropipettes ($\sim$50 μm diameter) prefilled with mineral oil and mounted in a pressure microinjector (Visualsonics, Toronto, Ontario, Canada), as described previously (Pla et al., 2006). Recipient pregnant females (E13.5) were anesthetized with sodium pentobarbital (0.625 mg/10 g, i.p.), and their uterine horns were exposed and mounted under an ultrasound microscope (Visualsonics). The tip of the micropipette was inserted into the MGE under real-time ultrasound guidance and 36–54 nl of cell suspension was injected. The position of the embryo and the path of the micropipette insertion were recorded for each embryo at the time of the injection.

Immunohistochemistry. For embryonic analysis, pregnant females were killed as described above. E13.5 embryos were fixed for 1.5 h in ice-cold 4% PFA, cryoprotected in 30% sucrose in ice-cold PBS, embedded in Tissue-Tek OCT compound, and cut frozen in 20-μm-thick coronal sections. Sections were incubated with primary antibodies overnight, followed by appropriate secondary antibodies. Fluorescent stainings were counterstained with Hoechst (Sigma). Primary antibodies used were as follows: rabbit anti-Dlx2 (1:200), rabbit anti-Lhx6 (1:2000; kindly provided by V. Pachnis, National Institute for Medical Research, London, UK), rabbit anti-Nkx2-1 (1:2000; Biopats, Piedimonte Matese, Italy), goat anti-Nkx6-2 (1:2000; kindly provided by T. Jessell, Columbia University, New York, NY), and rabbit anti-Olig2 (1:2000; kindly provided by D. H. Rowitch, University of California at San Francisco, San Francisco, CA). Secondary antibodies used were as follows: Alexa 488 anti-rabbit and Alexa 546 anti-goat (Invitrogen), both diluted 1:200.

For the analysis of transplantation experiments, postnatal day 14 (P14) mice were anesthetized with an overdose of sodium pentobarbital and transcardially perfused with 4% PFA. Brains were removed, postfixed for 3 h at 4°C, and cryoprotected in 30% sucrose in PBS. Brains were then cut frozen transversally at 40 μm on a sliding freezing microtome, and the sections were stored at −20°C in ethylene glycol solution until used. The following antibodies were used: chicken anti-GFP (1:1000; Aves, Tigard, OR), rabbit anti-parvalbumin (PV) (1:5000; Swant, Bellinzona, Switzerland) and rat anti-somatostatin (SST) (1:200; Chemicon, Temecula, CA). The following secondary antibodies were used: rabbit anti-chicken Alexa 488, Cy5-conjugated donkey anti-rabbit (Jackson ImmunoResearch, West Grove, PA), and Cy3-conjugated donkey anti-rat (Jackson ImmunoResearch). Analysis was performed using a confocal microscope (Leica DM-R/TCS-SL), and data were statistically analyzed using χ^2 tests.

Results

It is widely accepted that the developing subpallium includes the floor region of the evaginated telencephalic hemispheres and some parts of the nonevaginated telencephalon anterior to the optic region. In mammals, the evaginated subpallium consists of the LGE, MGE, and CGE, whereas the telencephalic stalk (the nonevaginated telencephalon) includes the peduncular region (AP, also known as the anterior entopeduncular region, AEP) (Bulfone et al., 1993), the anterior preoptic area (POA), and the preoptic–hypothalamic (POH) border region, close to the chiasmatic region (Fig. 1). In addition, embryological and gene expression analyses suggest that a large part of the septum also belongs to the subpallium (Puelles et al., 2000).

The anatomical delineation of the mammalian subpallium holds important limitations, because the limits of this region do not precisely coincide with any clearly defined morphological

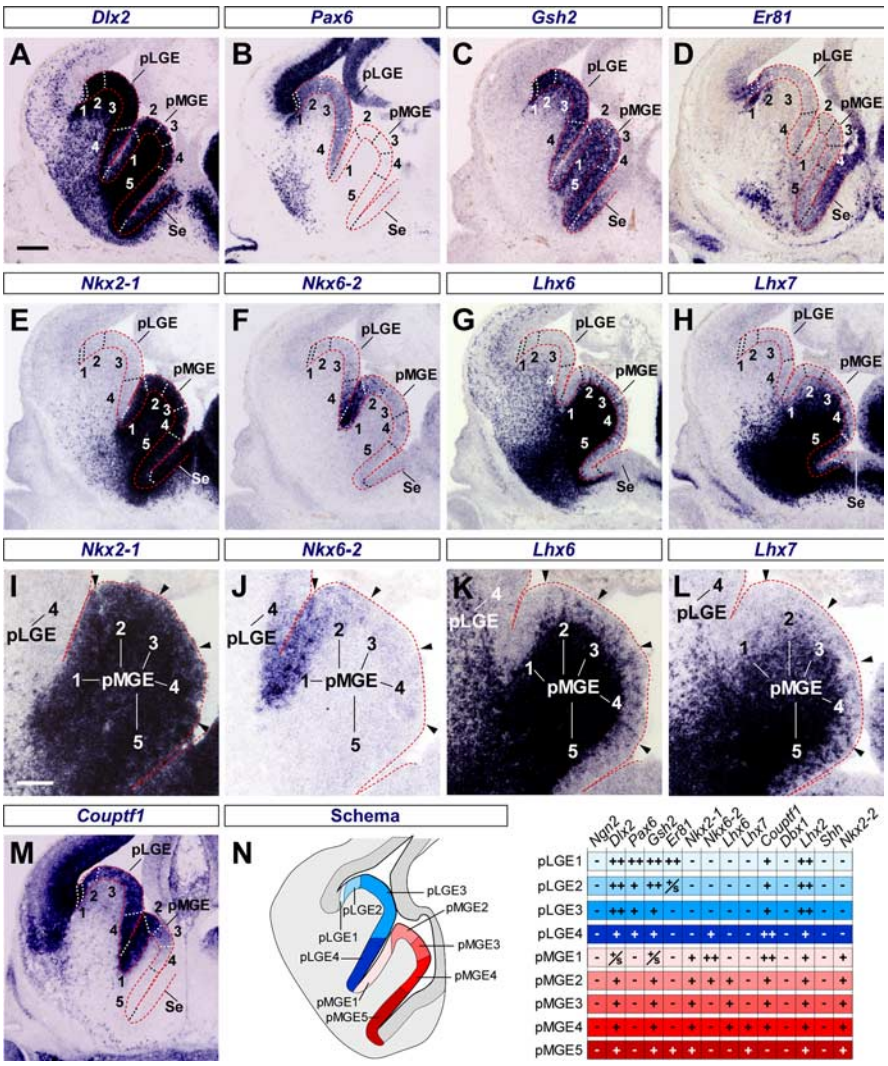

Figure 2. Molecular identification of distinct ventricular domains in the E13.5 rostral subpallium. ***A–M***, Coronal sections through the rostral telencephalon at E13.5 showing the expression of *Dlx2* (***A***), *Pax6* (***B***), *Gsh2* (***C***), *Er81* (***D***), *Nkx2-1* (***E***, ***I***), *Nkx6-2* (***F***, ***J***), *Lhx6* (***G***, ***K***), *Lhx7* (***H***, ***L***), and *Couptf1* (***M***) RNA. All sections were taken from the same level and plane of section. The red dashed lines delineate the VZ. Progenitor domains are named after the anatomical region they belong to, followed by a number given in dorsoventral order. The white or black dashed lines mark the limits between ventricular domains, as inferred from the combined information of the expression of all transcription factors shown in the figure. ***A***, The dorsal limit of the *Dlx2* mRNA expression in the VZ delineates the pallial/subpallial boundary. The most dorsal domain of the MGE has only scattered *Dlx2* expression (pMGE1). ***B***, *Pax6* mRNA is expressed at high levels in the pallial VZ and in pLGE1, at low levels in the remaining of the LGE VZ (pLGE2, pLGE3, and pLGE4), and is not expressed in the MGE VZ. ***C***, *Gsh2* mRNA is expressed both in the LGE and MGE VZ. The most dorsal part of the LGE has stronger expression of *Gsh2* (pLGE1 and pLGE2). As in the case for *Dlx2*, *Gsh2* mRNA is only found in scattered cells in pMGE1. ***D***, *Er81* mRNA is expressed in the most dorsal part of the LGE, overlapping with the domain of strong *Gsh2* expression (pLGE1 and pLGE2); it is also expressed in ventral parts of the pallium. ***E***, *Nkx2-1* mRNA expression defines the limit between pLGE4 and pMGE1. ***F***, *Nkx6-2* mRNA is expressed around the LGE/MGE boundary. The most ventral part of the LGE (pLGE4) expresses low levels of *Nkx6-2*, the dorsal part of the MGE (pMGE1) expresses high levels of *Nkx6-2*, and pMGE2 expresses low levels of *Nkx6-2*. ***G***, Scattered cells expressing *Lhx6* mRNA are found in the core of the MGE VZ (pMGE2, pMGE3, pMGE4), but are rare or not present in the most dorsal and ventral domains (pMGE1 and pMGE5). ***H***, *Lhx7* is also expressed by scattered cells in the ventricular zone of the ventral half of the MGE (pMGE4, pMGE5). ***I–L***, Higher magnification of ***E–H***, respectively. The arrowheads point the domain boundaries. ***M***, *Couptf1* is expressed at high levels in the ventricular zone of the LGE and in a dorsoventral gradient in the MGE but is not detected in pMGE5. ***N***, Schematic summary depicting the anatomical disposition of the proliferative domains present at this level. The table summarizes the molecular identity of each domain: −, absent; +, present; ++, present at high levels; and +/s, scattered expression. pLGE, Progenitor domain from the LGE; pMGE, progenitor domain from the MGE; SE, septum. Scale bars: ***A–H***, ***M***, 140 μm; ***I–L***, 70 μm.

boundaries. For example, it is now well established that the PSB (pallial–subpallial boundary) in the lateral aspect of the lateral ventricle lies ventral to the angle formed by the LGE bulge and the cortex (for review, see Campbell, 2003). In contrast, subpallial progenitor regions are more precisely delineated by the area of expression of several genes, among which *Dlx2* is best characterized (Fig. 1) (Liu et al., 1997; Puelles et al., 2000). In view of this, it seems obvious that an accurate description of distinct progenitor domains within the subpallium should not rely on anatomic landmarks (e.g., LGE, MGE, CGE) but rather on the expression of specific genes. To test this idea, we have analyzed the expression patterns of tens of genes in the developing mouse telencephalon at midembryonic stages (E10.5–E13.5). Based on combinatorial patterns of expression in the VZ, we identified distinct progenitor domains within the subpallium. We focus our analysis on E13.5, but data for E11.5 are also shown in supplemental Figure S1 (available at www.jneurosci.org as supplemental material).

The lateral ganglionic eminence contains four distinct progenitor domains

During development, the LGE is anatomically defined as the bulge that forms between the cortex and the MGE (Fig. 1*B*,*C*,*E*,*F*). From a molecular point of view, however, the most dorsal aspect of the LGE VZ contains a pallial territory. This small domain, named the VP (ventral pallium), is characterized by the expression of *Trb2*, *Ngn2*, and *Dbx1*, and by the absence of *Dlx2* expression (Puelles et al., 2000; Yun et al., 2001). The rest of the LGE VZ is subpallial in nature, and is characterized by the expression of *Dlx2*, *Gsh2*, and *Pax6*, and lack of *Nkx2-1* expression (Stoykova et al., 1996; Szucsik et al., 1997; Sussel et al., 1999) (Figs. 2*A*,*B*,*C*,*E*,*N*, 3*A*,*B*,*C*,*E*,*M*).

Previous studies have recognized that the VZ of the subpallial LGE contains at least two different progenitor pools, the dorsal and ventral LGE (Puelles et al., 2000; Toresson et al., 2000; Yun et al., 2001, 2003; Stenman et al., 2003a,b). Detailed examination of the dorsal LGE, initially defined by the subpallial expression of *Er81* mRNA (Puelles et al., 2000; Toresson et al., 2000; Yun et al., 2001, 2003; Stenman et al., 2003a,b), suggest that this territory is further subdivided in two distinct progenitor pools. The first domain is a small territory only a few cell diameters wide that express high levels of *Pax6*, *Gsh2*, and *Er81* (Fig. 2*A–D*,*N*) (Yun et al., 2001). VZ cells in this domain, which we have named progenitor (p) LGE1 (pLGE1), co-express high levels of Pax6 and Gsh2 protein (Yun et al., 2003, their Fig. 1*d*). The remaining of the *ER81* subpallial territory contains low levels of *Pax6* mRNA, high levels of *Gsh2* mRNA, and scattered cells expressing *ER81* mRNA. We designated this

second progenitor domain as pLGE2. Of note, pLGE1 and pLGE2 are not restricted to rostral levels of the LGE, but extend along the entire rostrocaudal extent of the subpallium, from the rostral end of the LGE into the CGE (Figs. 3–5).

Our analysis showed that the remaining LGE VZ is further subdivided in two additional domains, pLGE3 and pLGE4. Both domains lack *Er81* expression and appear to give rise to *Isl1*-expressing cells in the subventricular zone (Figs. 2*D*, 3*D*, 4*D*, 5*D*) (Stenman et al., 2003a). However, pLGE4 contains progenitor cells that express *Nkx6-2* (Figs. 2*F*,*J*,*N*, 3*F*,*M*; supplemental Fig. S2, available at www.jneurosci.org as supplemental material), whereas pLGE3 does not (Figs. 2*F*,*N*, 3*F*,*M*, 4*C*,*N*). As in the case of pLGE1 and pLGE2, pLGE3 extends along the entire rostrocaudal extent of the subpallium (Figs. 2–5). In contrast, the rostrocaudal extension of pLGE4 is much more limited than that of the other LGE domains, because it ends shortly after the end of the intereminential ventricular sulcus that separates LGE and MGE.

The medial ganglionic eminence consists of multiple progenitor domains

The MGE emerges during development as a prominent bulge between the LGE and the septum (Fig. 1*B*,*C*,*E*,*F*), but its caudal and ventral limits are very difficult to define because there are not anatomical landmarks that clearly discriminate the MGE from the CGE or from the telencephalic stalk region. Analysis of gene expression patterns, however, revealed that the MGE VZ is globally defined by the strong expression of *Nkx2-1*, weak expression of *Nkx2-2*, and lack of *Pax6* and *Shh* expression (Figs. 2*B*,*E*,*N*, 3*B*,*E*,*L*,*M*, 4*F*,*G*,*H*,*N*) (Sussel et al., 1999). Using this unequivocal molecular definition, it is clear that the MGE VZ expands caudally into regions that have been anatomically considered part of the CGE or the peduncular regions (Figs. 3, 4).

A detailed analysis of the expression patterns of multiple genes at E13.5 revealed the existence of at least five different progenitor domains within the MGE. First, the ventricular zone of the most dorsal aspect of the *Nkx2-1*-positive region (pMGE1) expresses high levels of *Nkx6-2* (Figs. 2*E*,*F*,*I*,*J*, 3*E*,*F*). Strikingly, the number of VZ cells that express *Dlx2* in this domain is reduced compared with adjacent progenitor domains (Figs. 2*A*, 3*A*). Moreover, no *Lhx6* strongly positive cells were detected in the VZ of pMGE1 (Figs. 2*G*,*K*,*N*, 3*G*,*M*). Both pLGE4 and pMGE1 are mostly associated with the LGE/MGE sulcus (the intereminential sulcus); these domains expand briefly beyond the end of this sulcus (Fig. 3) and do not reach the most caudal aspect of the subpallium (Fig. 4).

In contrast to pMGE1, pMGE2 contains many cells that express *Dlx*2. In addition, scattered cells in this domain express low levels of *Nkx6-2* and *Lhx6* (Fig. 2*A*,*F*,*G*,*J*,*K*,*N*). This

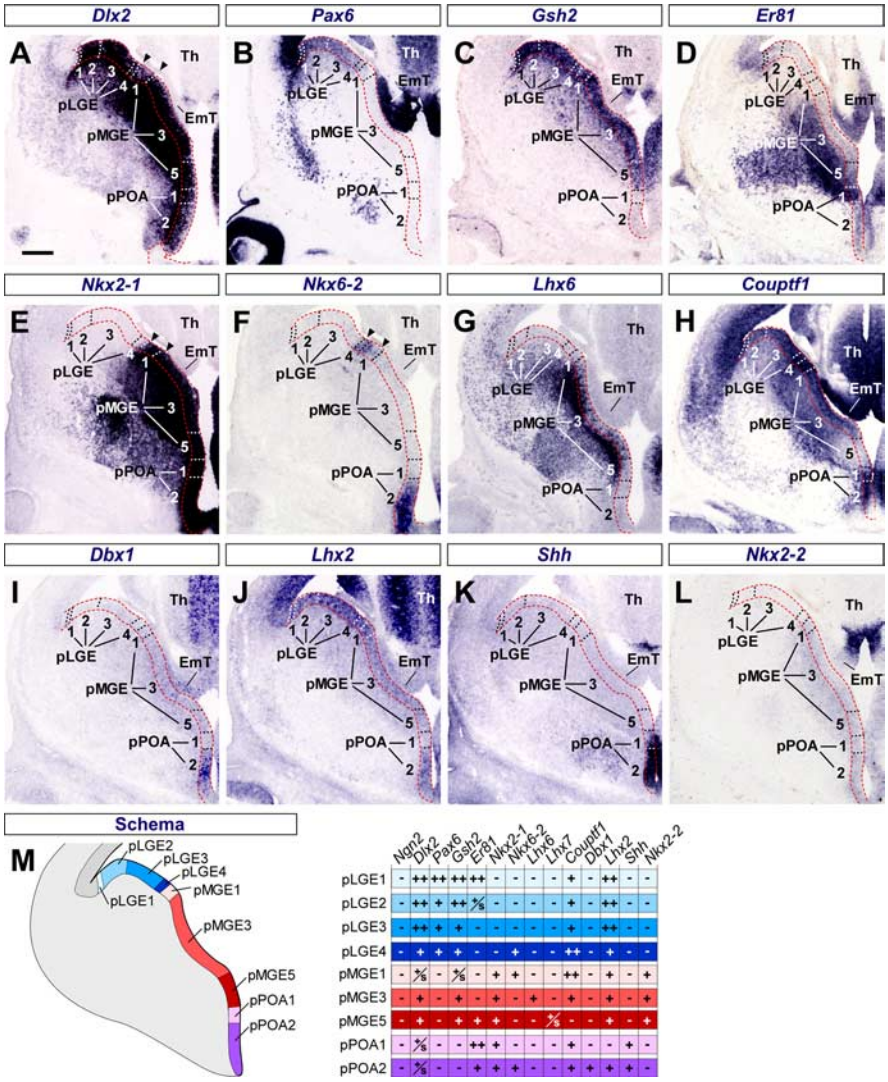

Figure 3. Molecular identification of distinct ventricular domains in the E13.5 rostral-intermediate subpallium. ***A–L***, Coronal sections through the rostral-intermediate telencephalon at E13.5 showing the expression of *Dlx2* (***A***), *Pax6* (***B***), *Gsh2* (***C***), *Er81* (***D***), *Nkx2-1* (***E***), *Nkx6-2* (***F***), *Lhx6* (***G***), *Couptf1* (***H***), *Dbx1* (***I***), *Lhx2* (***J***), *Shh* (***K***), and *Nkx2-2* (***L***) mRNA. All sections were taken from the same level and plane of section. The red dashed lines delineate the VZ. Progenitor domains are named after the anatomical region they belong to, followed by a number given in dorsoventral order. The white or black dashed lines mark the limits between ventricular domains, as inferred from the combined information of the expression of all transcription factors shown in the figure. ***A***, The ventricular domain of low *Dlx2* expression (arrowheads) defines the caudal extension of the pMGE1. *Dlx2* also shows scattered expression in the POA ventricular zone. ***B***, *Pax6* mRNA is expressed at high levels in the pallial VZ and pLGE1, and at low levels in pLGE2 and pLGE3. ***C***, *Gsh2* mRNA is expressed both in the LGE and MGE VZ but is absent from the POA VZ. ***D***, *Er81* mRNA is expressed in the VZ of the most dorsal parts of the basal ganglia (pLGE1 and pLGE2) as well as in the ventral MGE (pMGE5) and POA. Interestingly, the dorsal POA expresses higher levels of *Er81* (pPOA1) than the ventral POA (pPOA2). ***E***, *Nkx2-1* is expressed in the ventral half of the basal ganglia (MGE and POA). As in more anterior levels, *Pax6* and *Nkx2-1* ventricular expression patterns are complementary. ***F***, *Nkx6-2* is expressed in three domains, the caudal pole of pLGE4, pMGE1, and the ventral part of the POA (pPOA2). ***G***, *Lhx6* is expressed by scatter cells in pMGE3 and pMGE5. ***H***, *Couptf1* mRNA is absent from the ventricular zone of the ventral MGE (pMGE5). ***I***, *Dbx1* mRNA is exclusively expressed in the ventral part of the POA (pPOA2). ***J***, *Lhx2* mRNA is expressed in a dorsoventral gradient in the LGE and MGE VZ, is absent from the dorsal POA (pPOA1), and is expressed at high levels in the ventral POA (pPOA2). ***K***, *Shh* is expressed in the VZ of the dorsal and the ventral POA. ***L***, *Nkx2-2* is weakly expressed in the ventral half of the basal ganglia (MGE and POA). ***M***, Summary of the figure. The schema depicts the anatomical disposition of the proliferative domains present at this level, and the table summarizes the molecular identity of each domain: −, absent; +, present; ++, present at high levels; and +/s, scatter expression. EmT, Eminentia thalami; pLGE, progenitor domain from the LGE; pMGE, progenitor domain from the MGE; pPOA, progenitor domain from the POA; Th, thalamus. Scale bar, 130 μm.

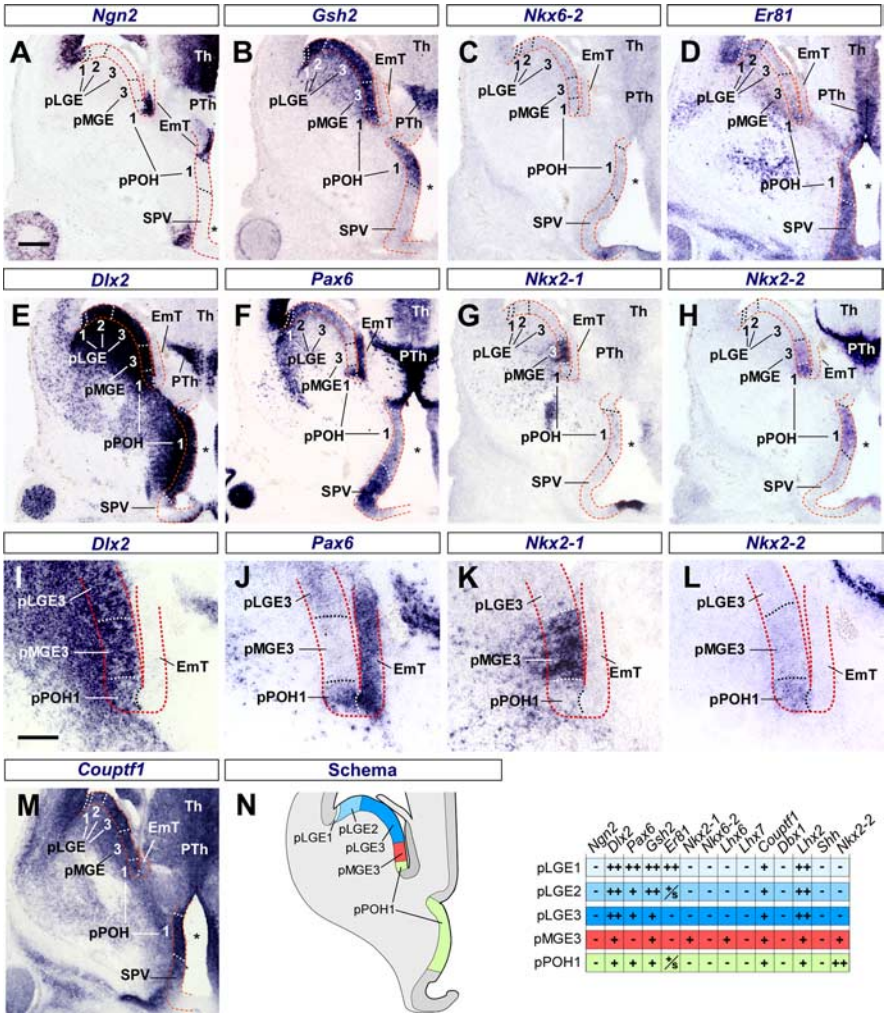

Figure 4. Molecular identification of distinct ventricular domains in the E13.5 caudal-intermediate subpallium. ***A–M***, Coronal sections through the caudal-intermediate telencephalon at E13.5 showing the expression of *Ngn2* (***A***), *Gsh2* (***B***), *Nkx6-2* (***C***), *Er81* (***D***), *Dlx2* (***E***, ***I***), *Pax6* (***F***, ***J***), *Nkx2-1* (***G***, ***K***), *Nkx2-2* (***H***, ***L***), and *Couptf1* (***M***) mRNA. All sections were taken from the same level and plane of section. The red dashed lines delineate the VZ. Progenitor domains are named after the anatomical region they belong to, followed by a number given in dorsoventral order. The white or black dashed lines mark the limits between ventricular domains, as inferred from the combined information of the expression of all transcription factors shown in the figure. At this caudal level, the VZ of the ganglionic eminences and the VZ of the telencephalic stalk are separated by the prethalamus (PTh). ***A***, *Ngn2* mRNA expression (a pallial and eminentia thalami marker) is absent from the subpallial ventricular zone including the ganglionic eminences and the telencephalic stalk. ***B***, *Gsh2* is expressed in all proliferative subpallial domains, indicating the absence of pPOA at this caudal level. As in anterior levels, *Gsh2* expression is stronger in the most dorsal parts of the basal ganglia (pLGE1 and pLGE2). ***C***, *Nkx6-2* is not expressed in the telencephalon at this level, indicating the absence of pMGE1 or pPOA2. ***D***, *Er81* is expressed in pLGE1, pLGE2, and POH. ***E***, *Dlx2* mRNA is expressed in all proliferative subpallial domains; the absence of a domain with scattered *Dlx2* expression suggests that pMGE1 is not present at this level. ***F***, *Pax6* expression defines the progenitor domains of the LGE (pLGE1, pLGE2, and pLGE3) and the POH (pPOH1), whereas *Pax6* is absent from the progenitor domains of the MGE (pMGE3). ***G***, *Nkx2-1* ventricular expression shows the presence of MGE progenitor domains at this caudal level (pMGE3). ***H***, *Nkx2-2* mRNA is expressed at low levels in the MGE VZ (pMGE3) and at high levels in the POH VZ (pPOH1). ***I–L***, Higher magnification of ***E*** and ***H***, respectively, showing the presence of a small piece of the POH ventricle between the MGE and the EmT. The POH VZ expresses *Dlx2*, *Pax6*, and *Nkx2-2* and lacks *Nkx2-1* expression. ***M***, *Couptf1* mRNA is expressed in all proliferative subpallial domains at this level, suggesting that pMGE5 is absent at this posterior level. ***N***, Summary of the figure. The schema depicts the anatomical disposition of the proliferative domains present at this level, and the table summarizes the molecular identity of each domain: −, absent; +, present; ++, present at high levels. EmT, eminentia thalami; pLGE, progenitor domain from the LGE; pMGE, progenitor domain from the MGE; pPOH, progenitor domain from the hypothalamic preoptic area; PTh, prethalamus; Th, thalamus; SPV, supraoptic paraventricular region; *, third ventricle. Scale bars: ***A–H***, ***M***, 150 μm; ***I–L***, 75 μm.

domain is restricted to the rostrocaudal extent of the intereminential sulcus, because it disappears caudal to the level in which this sulcus can be distinguished (Fig. 3*F*,*G*,*M*).

pMGE3 is characterized by scattered *Lhx6* expression, lack of *Nkx6-2*, and weak or absent *Lhx7* (also known as *Lhx8*) expression in the VZ (Figs. 2*F–H*,*J–L*,*N*, 3*F*,*G*,*M*, 4*C*) (data not shown). pMGE3 occupies a large portion of the MGE VZ at midtelencephalic levels (Fig. 3) and extends caudally in the subpallium more than any other MGE domain (Fig. 4).

pMGE4 is characterized by expression of both *Lhx6* and *Lhx7* in scattered VZ cells (Fig. 2*G*,*H*,*K*,*L*,*N*). Similarly to pMGE2, pMGE4 is only present in the rostral MGE, because it is not found caudal to the end of the intereminential sulcus.

Finally, pMGE5 is the most ventral MGE progenitor domain; it is characterized by strong expression of *Lhx7* in many VZ cells, whereas *Lhx6* is barely detected in this region. Moreover, pMGE5 lacks *Couptf1* expression (Figs. 2*G*,*H*,*K*,*L*,*M*,*N*, 3*H*,*M*). Because this transcription factor is expressed both dorsal and ventral to pMGE5, the lack of expression of *Couptf1* in this region clearly delineates this domain. Of note, pMGE5 lies in the telencephalic stalk in the anatomical region previously defined as the peduncular area or the anterior entopeduncular area (AP or AEP, respectively) (Figs. 1, 3) (Bulfone et al., 1993; Puelles et al., 2000). It represents the transition between the evaginated and the nonevaginated (preoptic) subpallial telencephalon.

The preoptic region contains at least two distinct progenitor domains

During development, the POA is defined anatomically as the region immediately in front of the optic recess, at the limit between the telencephalon and the diencephalon. From a molecular perspective, the gene expression profile of the POA is particularly different from the rest of the subpallium. The POA VZ is uniquely defined by the simultaneous expression of *Nkx2-1*, *Nkx2-2*, and *Shh* (Fig. 3*E*,*L*,*K*,*M*), and most prominently by the lack of detectable levels of *Gsh2*, *Lhx6*, *Lhx7*, or *Olig2* expression (Fig. 3*C*,*G*,*M*) (data not shown).

Two distinct proliferative domains can be distinguished within the POA. In the dorsoposterior POA, the VZ lacks *Nkx6-2*, *Dbx1*, and *Lhx2* expression (Fig. 3*F*,*I*,*J*,*M*). Conversely, in the ventroanterior POA, the VZ expresses these three genes (Fig. 3*F*,*I*,*J*,*M*). We named these progenitor domains pPOA1 and pPOA2, respectively.

The preopto-hypothalamic region constitutes the border of the subpallium

The POH is the outermost structure of the *Dlx2*-positive telencephalic stalk. It has been defined recently in the chick as a thin territory separating longitudinally the POA from the magnocel-

lular hypothalamus (Fig. 1*D*,*E*,*H*) (Bardet et al., 2006). From a molecular perspective, it is clear that the POH constitutes a progenitor region that is distinct from the POA. Thus, the POH VZ (pPOH1) contains progenitor cells that express *Dlx2*, *Pax6*, *Olig2*, and *Gsh2*, and it lacks expression of *Nkx2-1*, *Shh*, *Nkx6-2*, and *Dbx1* (Fig. 4*B*,*C*,*E–G*,*I–K*,*N*) (data not shown). In addition, the pPOH1 expresses high levels of *Nkx2-2*, which is also weakly expressed throughout the MGE territories (Figs. 4*H*,*L*, 6*I*).

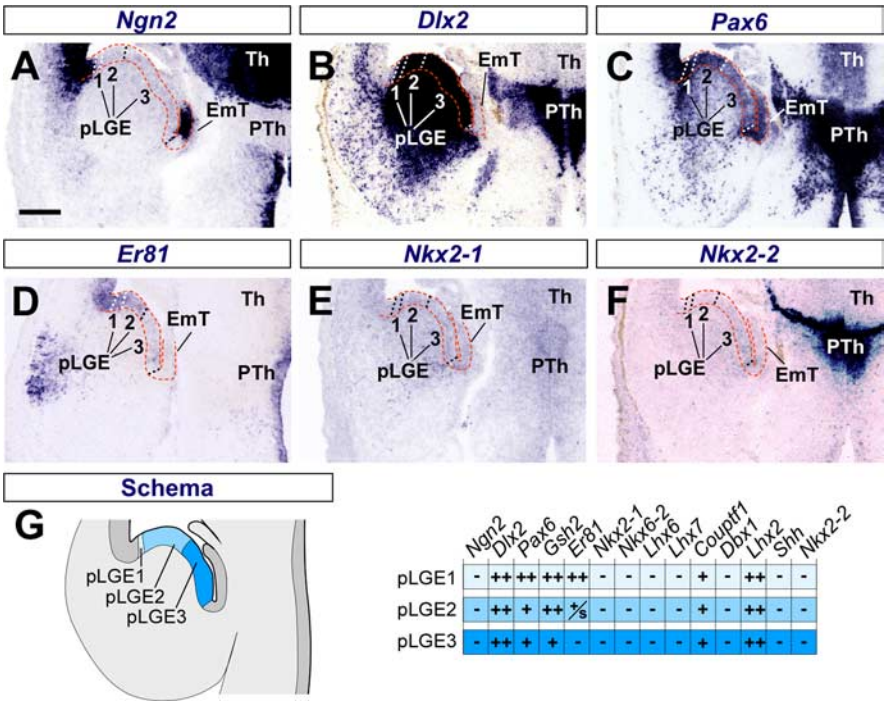

Figure 5. Molecular identification of distinct ventricular domains in the E13.5 caudal subpallium. ***A–F***, Coronal sections through the caudal telencephalon at E13.5 showing the expression of *Ngn2* (***A***), *Dlx2* (***B***), *Pax6* (***C***), *Er81* (***D***), *Nkx2-1* (***E***), and *Nkx2-2* (***F***) mRNA. All sections were taken from the same level and plane of section. The red dashed lines delineate the VZ. Progenitor domains are named after the anatomical region they belong to, followed by a number given in dorsoventral order. The white or black dashed lines mark the limits between ventricular domains, as inferred from the combined information of the expression of all transcription factors shown in the figure. ***A***, *Ngn2* expression, a pallial marker, is absent from the subpallium. ***B***, *Dlx2* is expressed in a complementary manner to *Ngn2*, labeling the VZ of the subpallium. ***C***, *Pax6* is expressed throughout the subpallial VZ. ***D***, *Er81* is expressed in the most dorsal domains of the subpallium (pLGE1 and pLGE2). ***E***, Lack of *Nkx2-1* mRNA expression indicates the absence of pMGE domains. ***F***, *Nkx2-2* is also absent from the VZ, suggesting the absence of POH. ***G***, Summary of the figure. The schema depicts the anatomical disposition of the proliferative domains present at this level, and the table summarizes the molecular identity of each domain: −, absent; +, present. EmT, eminentia thalami; pLGE, progenitor domain from the LGE; PTh, prethalamus; Th, thalamus. Scale bar, 140 μm.

The subpallial septum contains several distinct progenitor regions

The septum constitutes the paracommissural part of the telencephalic medial wall, and it consists of both pallial and subpallial regions (Puelles et al., 2000). Its subpallial region is defined by the expression of *Dlx2* (Figs. 1*C*,*F*, 6*A*,*J*) (Puelles et al., 2000). Analysis of other gene expression patterns in the VZ of the septum revealed that different domains of progenitor cells in this region share many characteristics with their counterparts on the other side of the lateral ventricle and are continuous with them under the ventricle (i.e., pLGE, pMGE, and pPOA domains). Thus, the part of the septum adjacent to the pallial–subpallial boundary is continuous with the LGE and shares some characteristics with this latter region, such as expression of *Pax6* and lack of *Nkx2-1* transcripts (Fig. 6*B*,*E*,*J*). Three different domains (pSe1, pSe2, and pSe3) can be distinguished within this region, which differ in their levels of *Pax6* and *Gsh2* expression. pSe1 expresses high levels of both *Pax6* and *Gsh2*, pSe2 contains high levels of Gsh2 and low levels of *Pax6*, and pSe3 expresses low levels of both genes (Fig. 6*B*,*C*,*J*). Three additional domains exist within the septum, all of which express *Nkx2-1* (Fig. 6*E*,*J*). pSe4 and pSe5 are continuous laterally with the pallidum; pSe4 contains cells that express *Nkx6-2*, a property that distinguishes it from pSe5, which lacks such transcripts (Fig. 6*F*,*J*). pSe6 is a thin median domain continuous with the POA; like this area, it expresses *Shh* (Fig. 6*H*,*J*).

Despite the partial similarities in gene expression patterns between septal domains and their lateral counterparts in the LGE, MGE, or POA, there are several genes expressed in these latter regions that are not expressed in the E13.5 septum, such as *Lhx6*, *Lhx7*, nor *Couptf1* (data not shown). On the contrary, the septum expresses several genes, such as *Lhx5*, that are unique to this region (compared with other subpallial domains) and are likely conferring these domains with their septal identity (Fig. 6*G*,*J*).

Multiple distinct progenitor domains already exist at early embryonic stages

The gene expression analysis described above shows that the VZ of the E13.5 subpallial telencephalon has multiple molecularly distinct progenitor domains. We next examined whether these subpallial VZ subdivisions are established at earlier stages of development. Thus, we analyzed at E11.5 the patterns of expression of the most significant genes found in our previous analysis. As it is summarized in supplemental Figure S1 (available at www.jneurosci.org as supplemental material), we found that all of the progenitor domains described at E13.5 were identified at E11.5. Thus, early patterning of the subpallium appears to confer identity to multiple domains in this region, which could then be recognized and correlated with the ganglionic eminences at later stages of development. Of note, although the progenitor pools defined at E13.5 were readily distinguishable at E11.5, the boundaries between different domains were, in some cases, less clearly defined than at later stages.

Analysis of transcription factor expression in single progenitor cells

Our analysis of gene expression patterns suggests that multiple domains exist in the subpallial VZ during development. However, the expression of multiple transcription factors in a specific domain does not necessarily imply that these factors are coexpressed in individual cells. Moreover, scattered cells expressing a given factor were sometimes found in several adjacent domains (e.g., *Nkx6-2* in pLGE4, pMGE1, and pMGE2), and the analysis of mRNA expression could not clarify whether those cells coexpress other markers specific of each domain (and therefore truly represent genetically distinct progenitors) or they exclusively coexpress markers of a single domain and are somehow misplaced in the adjacent territory. To address this issue, we analyzed the

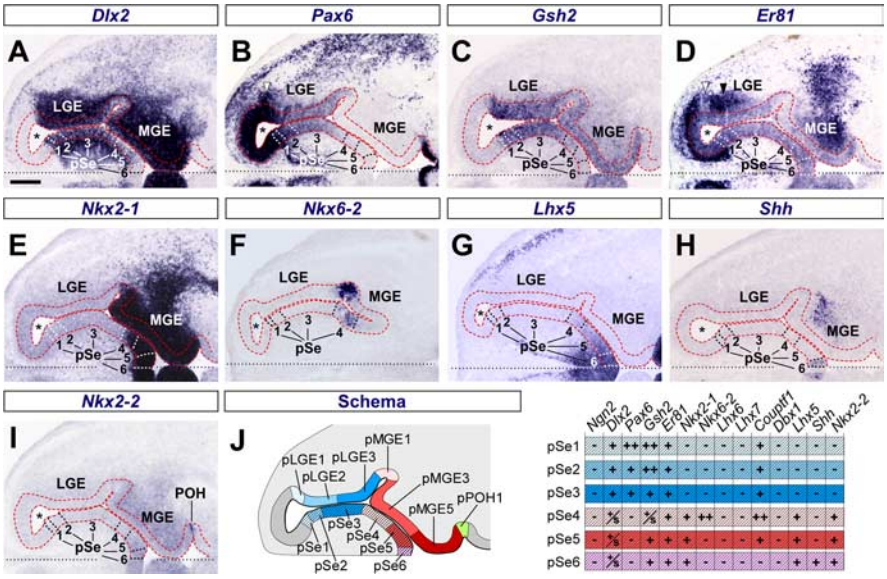

Figure 6. Molecular identification of distinct ventricular domains in the E13.5 subpallial septum. ***A–I***, Horizontal sections through the telencephalon at E13.5 showing the expression of *Dlx2* (***A***), *Pax6* (***B***), *Gsh2* (***C***), *Er81* (***D***), *Nkx2-1* (***E***), *Nkx6-2* (***F***), *Lhx5* (***G***), *Shh* (***H***), and *Nkx2-2* (***I***) mRNA. All sections were taken from the same level and plane of section, except for ***F***, which is more ventral. The horizontal dotted line marks the midline. The red dashed lines delineate the VZ. Progenitor domains are named after the anatomical region they belong to, followed by a number given in anterodorsal to caudoventral order. The white or black dashed lines mark the limits between ventricular domains, as inferred from the combined information of the expression of all transcription factors shown in the figure. ***A***, *Dlx2* mRNA is expressed in all septal domains except for pSe5, where only occasional cells expressing *Dlx2* can be observed. ***B***, *Pax6* marks the LGE counterpart domains of the septum (pSe1, pSe2, and pSe3). ***C***, *Gsh2* is expressed in most parts of the septum but is absent from the most ventromedial part. ***D***, *Er81* is expressed throughout the septum. ***E***, *Nkx2.1* is expressed in the most ventromedial part of the septum (pSe4 to pSe6). ***F***, *Nkx6.2* is expressed in a small septal domain (pSe4). ***G***, *Lhx5* mRNA is exclusively expressed in the septum forming a medial-to-lateral gradient. ***H***, *Shh* is expressed in a very small domain in the most medial part of the septum (pSe6), corresponding to the *Dlx2*- and *Gsh2*-negative domain. ***I***, *Nkx2-2* is expressed at low levels in two domains of the medial septum (pSe5 and pSe6). ***J***, Summary of the figure. The schema depicts the anatomical disposition of the proliferative domains present at this level, and the table summarizes the molecular identity of each septal domain: −, absent; +, present; ++, present at high levels; +/s, scatter expression. Septal color codes match with their ganglionic eminences progenitor domain counterparts but are represented with a lined pattern to symbolize their singular characteristics. The white arrowheads in ***B*** and ***D*** indicate the possible postmitotic output of pLGE1, which is complementary to the postmitotic output of pLGE2 (***D***, black arrowhead). pLGE, Progenitor domain from the LGE; pMGE, progenitor domain from the MGE; pPOH, progenitor domain from the hypothalamic preoptic area; pSe, progenitor domains from the septum; *, lateral ventricle. Scale bar, 180 μm.

expression of several transcription factors in individual cells using immunohistochemistry. We focused our analysis in the LGE/MGE boundary, around which several domains have been defined by gene expression analysis (Fig. 2). Virtually every cell in the MGE expresses Nkx2-1 (Fig. 7*A*) (S. Nobrega and O. Marín, unpublished observations), whereas none of the cells in the LGE do. In contrast, most cells in both the LGE and the MGE express Olig2, although at different levels (Fig. 7*C*). Expression of Nkx6-2 and Nkx2-1 defines two different types of cells in pMGE1 and pMGE2: Nkx2-1+/Nkx6-2+ cells and Nkx2-1+/Nkx6-2− cells (Fig. 7*A*,*B*). This analysis suggests that cells expressing Nkx6-2 in pMGE1 and pMGE2 may represent a unique population that is dispersed through the dorsal aspect of the MGE. Analysis of Olig2 expression, however, demonstrates that cells in pMGE1 and pMGE2 are different to some extent. Thus, VZ cells in pMGE1, as in pLGE4, express consistently lower levels of Olig2 than VZ cells in pMGE2 (Fig. 7*B–D*). Finally, the expression of Lhx6 in some cells in pMGE2 but not in pMGE1 also reinforces the view that these two domains contain distinct pools of progenitors. In sum, analysis of transcription factors at the single-cell level in this region corroborates the boundaries determined by mRNA expression, and reveals that at least two distinct types of VZ cells exist in pLGE4, pMGE1, and pMGE2: Olig2+/Nkx6-2+ and Olig2+/Nkx6-2− in pLGE4, Nkx2-1+/Olig2+^{low}/Nkx6-2+ and Nkx2-1+/Olig2+^{low}/Nkx6-2− in pMGE1, and Nkx2-1+/Olig2+^{high}/Nkx6-2+ and Nkx2-1+/Olig2+^{high}/Nkx6-2− in pMGE2.

In utero transplantation reveals functionally distinct MGE progenitor domains

Our experiments reinforce the view that the embryonic subpallium consists of many more progenitor domains than previously recognized. However, the mere molecular characterization of these domains does not demonstrate that they indeed represent functionally distinct progenitor pools. To test this idea, we designed a proof of concept experiment in which we aimed to identify the cortical derivatives of a small MGE domain. It has been previously shown that the MGE gives rise to two major populations of cortical neurons, PV- and SST-containing interneurons (Xu et al., 2004; Butt et al., 2005; Ghanem et al., 2007). In agreement with these observations, we performed *in utero* transplantation of E13.5 GFP-expressing MGE cells into isochronic host embryos and found that slightly more than 50% of cortical interneurons derived from the E13.5 MGE contain PV in the neocortex of P14 host animals, whereas ~30% contain SST and the remaining transplanted cells do not stain for any of these markers (n = 330 counted cells) (Fig. 8; supplemental Figs. S3*A*, S4, available at www.jneurosci.org as supplemental material) (Butt et al., 2005). To establish whether each of these two major neurochemical populations arise from different progenitor domains within the MGE, as defined in our study, we next transplanted cells from a small cube of tissue obtained from either the pMGE1 or pMGE4 domains (Fig. 8*A*,*B*,*E*). In brief, dissociated cells from the pMGE piece were mixed with cells obtained from a GFP-negative MGE and injected into the MGE of isochronic host embryos; the distribution of GFP-expressing cells was subsequently analyzed in the neocortex of P14 host animals. pMGE1 and pMGE4 microtransplants consistently gave rise to a relatively small population of interneurons in the neocortex of P14 host mice (Fig. 8*C*,*F*). In the case of pMGE1 transplants, triple immunohistochemistry against GFP, PV, and SST revealed that >60% of the transplanted neurons contained SST, whereas <25% stained for PV (n = 94 counted cells from three independent experiments) (Fig. 8*D*,*D*′,*H*; supplemental Figs. S3*B*, S4, available at www.jneurosci.org as supplemental material). Conversely, analysis of pMGE4 transplantation experiments revealed that the majority of cells derived from this domain express PV (~50%), with only a minor fraction of the transplanted neurons expressing SST (n = 128 counted cells from three independent experiments) (Fig. 8*G*,*G*′,*H*; supplemental Figs. S3*C*, S4, available at www.jneurosci.org as supplemental material). Interestingly, the laminar distribution of interneurons derived from

pMGE1 and pMGE4 was similar; most transplanted cells occupied deep layers of the cortex, as expected from their birth date (Pla et al., 2006). In sum, these results demonstrate that the progeny of small MGE domains is selectively enriched in discrete neurochemical populations of cortical interneurons, reinforcing the view that distinct progenitor pools exist within this structure.

Discussion

In the spinal cord, different neuronal populations arise from distinct progenitor domains characterized on the basis of restricted expression of transcription factors (for review, see Jessell, 2000). Such organization is also thought to underlie the generation of subpallial neuronal diversity in the telencephalon, and several molecularly distinct progenitor domains have been identified in the subpallium through differential gene expression (for review, see Marín and Rubenstein, 2002; Campbell, 2003). In most cases, however, analysis of gene expression patterns focused on specific coronal section levels and therefore did not systematically assess the combinatorial expression of multiple transcription factors across the entire subpallium. In addition, previous attempts to subdivide the subpallium into different progenitor regions mostly relied on anatomical landmarks, such as sulci and bulges. Because these structures transform continuously during morphogenesis, a definition of progenitor domains based exclusively on these anatomical references is both ambiguous and unreliable. Consequently, the current view of the areal organization of the mammalian subpallium is fragmentary.

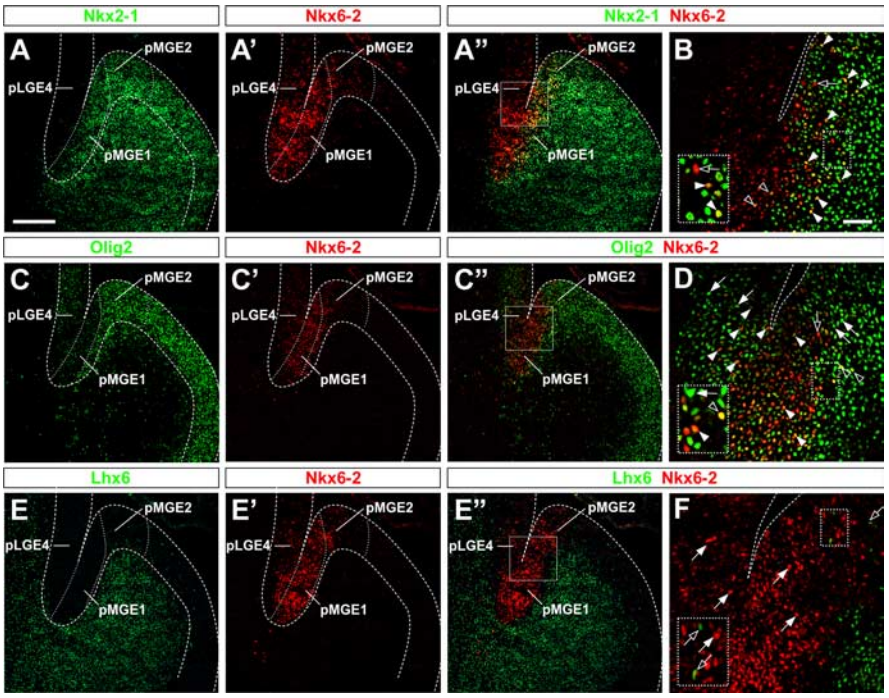

Figure 7. Characterization of ventricular zone cells at the intereminential (LGE/MGE) sulcus in the E13.5 subpallium. ***A–A″***, ***C–C″***, ***E–E″***, Adjacent coronal sections through the rostral telencephalon at E13.5 showing the expression of Nkx2-1 (***A***, ***A″***, ***B***), Nkx6-2 (***A′***, ***A″***, ***B***, ***C′***, ***C″***, ***D***, ***E′***, ***E″***, ***F***), Olig2 (***C***, ***C″***, ***D***), and Lhx6 (***E***, ***E″***, ***F***) proteins. ***B***, ***D***, and ***F*** are high magnification confocal photographs of the areas boxed in ***A″***, ***C″***, and ***E″***, respectively. The insets in ***B***, ***D***, and ***F*** show a high magnification of the boxed areas. The dashed lines delineate the VZ. Progenitor domains are named after the anatomical region they belong to, followed by a number given in dorsoventral order. The dotted lines mark the limits between ventricular domains pLGE4, pMGE1, and pMGE2. ***A***, ***B***, Expression of Nkx2-1 and Nkx6.2 at the LGE/MGE sulcus. Virtually every cell in the MGE expresses Nkx2-1. pMGE1 and pMGE2 contain cells that express both Nkx2-1 and Nkx6-2 (arrowheads). Double-labeled cells are more abundant in pMGE1 than in pMGE2. Only occasionally Nkx6-2+/Nkx2-1− cells are observed in pMGE1 (open arrows) or Nkx6-2+/Nkx2-1+ in pLGE4 (open arrowheads). ***C***, ***D***, Expression of Olig2 and Nkx6.2 at the LGE/MGE sulcus. Olig2 is expressed in many cells in pLGE4, pMGE1, and pMGE2; however, Olig2 is consistently expressed at higher levels in pMGE2 than in pMGE1 and pLGE4. pLGE4 contains cells that express Olig2 but not Nkx6-2 (arrows) and double-labeled cells (arrowheads). pMGE1 and pMGE2 contain cells that express Olig2 but not Nkx6-2 (open arrows) and double-labeled cells (arrowheads and open arrowheads). Olig2 levels are consistently higher in pMGE2 cells (open arrowheads) than in pMGE1 cells (arrowheads). ***E***, ***F***, Expression of Lhx6 and Nkx6.2 at the LGE/MGE sulcus. Lhx6 is expressed in very few cells in the VZ of the pMGE, but it is consistently absent from pMGE1 (***E***). Lhx6 (arrows) and Nkx6-2 (open arrows) are expressed in different VZ cells. Scale bars: ***A***, ***C***, ***E***, 100 μm; ***B***, ***D***, ***F***, 20 μm.

Our study demonstrates that the mouse subpallium contains at least 18 progenitor domains that are each uniquely defined by the combinatorial expression of several transcription factors and other genes. Based on these findings, we propose a new nomenclature to designate progenitor domains in the mouse subpallium, which we believe integrates previous and novel findings, provides a comprehensive view of the different telencephalic structures, and has the possibility to expand to accommodate new discoveries. Analysis of transcription factor expression at the cellular level suggests that boundaries between domains may slightly overlap and that certain heterogeneity in the population of progenitor cells present in each domain may exist. Although this novel definition of the VZ of the subpallium is based on molecular expression patterns, the results of our microtransplantation experiments strongly suggest that the defined domains function as distinct progenitor domains giving rise to different neuronal cell types.

LGE, MGE, and POA each contain several progenitor domains

It has been previously recognized that the dorsal aspect of the LGE contains progenitor cells that express a unique combination of transcription factors. Originally described as the dorsal LGE, the pLGE1 and pLGE2 progenitor domains abut the ventral pallium and are thought to give rise to interneurons that migrate to the olfactory bulb (Toresson et al., 2000; Yun et al., 2001; Stenman et al., 2003a; Waclaw et al., 2006). Interestingly, both domains may contribute to distinct populations of postmitotic neurons. Thus, pLGE1 appears to give rise primarily to postmitotic cells that maintain the expression of Pax6, whereas pLGE2 may contribute primarily to postmitotic neurons that maintain the expression of ER81 (Stenman et al., 2003a; Carney et al., 2006; Waclaw et al., 2006). The remaining part of the LGE VZ amplifies through a *Dlx1/2*$^+$ and *Isl1*$^+$ cell population in the SVZ and is likely to represent striatal progenitors (Stenman et al., 2003a). In this context, the expression of *Nkx6-2* in the ventral aspect of the LGE suggests that this structure is subdivided in at least two additional domains, pLGE3 and pLGE4 (*Nkx6-2*$^-$ and *Nkx6-2*$^+$, respectively) (Stenman et al., 2003c).

The existence of different progenitor domains within the

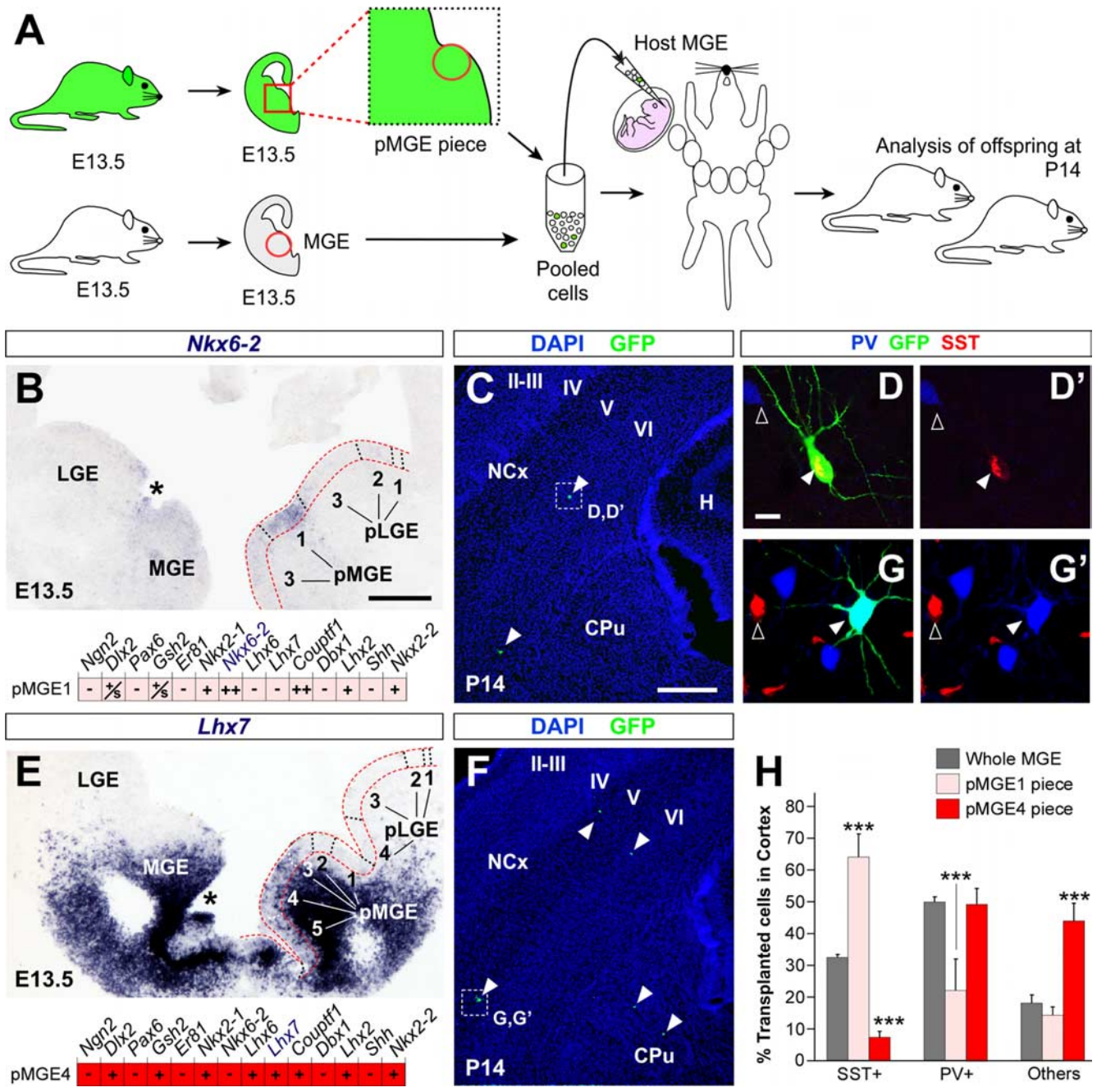

Figure 8. Fate of cortical interneurons derived from pMGE1 and pMGE4. ***A***, Schematic diagram of the experimental design. Coronal slices were prepared from the telencephalon of E13.5 GFP+ donor embryos, and progenitor cells were isolated from a small cube of tissue approximately corresponding to pMGE1 at rostral-intermediate telencephalic levels. In parallel, the MGE was dissected from wild-type embryos and dissociated. Pooled donor GFP+ pMGE1 and MGE cells were then injected into the MGE of E13.5 host embryos. Host embryos were analyzed at P14. ***B***, Coronal section obtained from a donor GFP+ slice showing the expression of *Nkx6-2* mRNA (experiment T106). The asterisk indicates the original location of transplanted cells within the MGE (approximately pMGE1 at this level). The inset summarizes the molecular profiling of pMGE1. ***C***, Coronal section through the somatosensory cortex of a transplanted P14 mouse showing two pMGE1-derived cells (arrowheads) after nuclear staining [4′,6′-diamidino-2-phenylindole (DAPI)] and immunohistochemistry for GFP (green). ***D***, ***D′***, The arrowhead points to a neuron expressing GFP and SST, but negative for PV. The open arrowhead points to a PV+ cell. ***E***, Coronal section obtained from a donor GFP+ slice showing the expression of *Lhx6* mRNA (experiment T129E). The asterisk indicates the original location of transplanted cells within the MGE (approximately pMGE4 at this level). The inset summarizes the molecular profiling of pMGE4. ***F***, Coronal section through the somatosensory cortex of a transplanted P14 mouse showing two pMGE4-derived cells (arrowheads) after nuclear staining (DAPI) and immunohistochemistry for GFP (green). ***G***, ***G′***, The arrowhead points to a neuron expressing GFP and PV, but negative for SST. The open arrowhead points to a SST+ cell. ***H***, Quantification of the percentage of SST+, PV+, or PV/SST− in the P14 cortex after whole MGE, pMGE1, and pMGE4 transplantation. GFP+/SST+ cells: 30.12 ± 2.27% (whole MGE), 63.88 ± 7.34% (pMGE1), and 7.24 ± 1.73% (pMGE4); GFP+/PV+ cells: 50.66 ± 1.26% (whole MGE), 21.96 ± 9.7% (pMGE1), and 48.96 ± 5.07% (pMGE4); GFP+/SST−/PV− cells: 19.2 ± 2.02% (whole MGE), 14.14 ± 2.52% (pMGE1), and 43.78 ± 5.7% (pMGE4). Error bars indicate SEM. ***$p < 0.001$, χ^2 tests between whole MGE and pMGE1 or whole MGE and pMGE4. Scale bars: ***B***, ***E***, 200 μm; ***C***, ***F***, 300 μm; ***D***, ***D′***, ***G***, ***G′***, 10 μm.

MGE has also been previously suggested. For example, the differential expression of *Nkx6-2* and *Gli1* in the dorsal aspect of the MGE VZ led to the proposal that this region may contain at least two different progenitor pools (Stenman et al., 2003c; Xu et al., 2005; Wonders and Anderson, 2006). Our analysis, however, suggests that the MGE VZ (encompassing the previously separated anterior entopeduncular area) may contain up to five molecularly distinct progenitor pools, which we have designated

pMGE1 to pMGE5. The MGE is known to be the origin of multiple neuronal types, including striatal and cortical interneurons (Lavdas et al., 1999; Sussel et al., 1999; Marín et al., 2000). Among the later type, current evidence suggests that the MGE is the origin of at least two chemically distinct populations: PV- and SST-containing interneurons. In addition, it has been suggested that cortical NPY (neuropeptide Y)-containing interneurons may also derive from the MGE (for review, see Wonders and Anderson, 2006). Our *in utero* transplantation experiments strongly suggest that these subtypes of cortical interneurons may arise from the different progenitor pools described in this study. Specifically, it seems likely that most SST+ MGE-derived cortical interneurons originate from progenitor pools located in the dorsal aspect of the MGE (pMGE1 and/or pMGE2), suggesting that the combinatorial expression of Nkx2-1 and Nkx6-2 may underlie the specification of SST+ interneurons. Our experiments also suggest that PV+ MGE-derived cortical interneurons derive from more ventrally located domains, such as pMGE4. Because the level of resolution of the transplantation experiments is inherently limited, it seems evident that genetic fate mapping experiments will be necessary to more precisely identify the origin of this and other populations of cortical interneurons in relation to the proposed progenitor domains defined here.

Cholinergic neurons in the striatum and basal forebrain are also likely to originate from the MGE (Sussel et al., 1999). Considering that Lhx7 function is essential for the development of the vast majority of the cholinergic neurons in the basal telencephalon (Zhao et al., 2003; Fragkouli et al., 2005), and *Lhx7* expression in the VZ appears to be restricted to the pMGE4 and pMGE5 domains, we hypothesize that cholinergic neurons may derive from these progenitors pools. There is an interesting parallelism with the development of cholinergic neurons in the spinal cord, where they originate from a progenitor pool located close to floor plate, the source of Shh (for review, see Jessell, 2000). In the telencephalon, pMGE5 is immediately adjacent to the preoptic area, whose progenitors also express high levels of Shh.

The preoptic region is defined as the part of the subpallium whose VZ coexpress *Nkx2-1* and *Shh*. The expression pattern of several other genes, such as *Dbx1*, *Lhx2*, and *Nkx6-2*, suggests that the preoptic region consists of at least two different progenitor domains, POA1 and POA2, although the potential contribution of these domains to neuronal diversity in the telencephalon remains to be elucidated. In the chick, *Shh*-expressing neurons migrate tangentially from the POA into the pallidal and striatal mantle (Bardet et al., 2006), but the nature of these cells remain to be elucidated.

The CGE contains the caudal extension of LGE and MGE progenitor domains

Recent studies have shown that the CGE is the origin of several neuronal populations that migrate and colonize specific regions of the telencephalon, including the amygdala, neocortex, and hippocampus (Nery et al., 2002; López-Bendito et al., 2004; Medina et al., 2004; Xu et al., 2004; Tole et al., 2005; Yozu et al., 2005). To date, the definition of the CGE has been solely based on morphology (i.e., the region of the ganglionic eminences caudal to the end of the intereminential sulcus) (Bulfone et al., 1993). This definition, however, has obvious caveats. First, the sulcus that separates LGE and MGE forms progressively, so that the rostral limit of the CGE changes as development proceeds. Second, using this definition, the CGE cannot be identified in mouse mutants in which the sulcus separating the MGE from the LGE is compromised, as it is the case of *Nkx2-1* mutants. Third, the CGE contains both pallial and subpallial territories (Kim et al., 2001; Medina et al., 2004; Remedios et al., 2004; Tole et al., 2005). Thus, in the same way that LGE and MGE has proven to be ambiguous terms when referring to distinct progenitor pools, the term CGE can only bring confusion to this issue.

Our analysis of gene expression patterns corroborate that many progenitor pools extend across the entire subpallium, from a septal representative to the preopto-hypothalamic and amygdaloid regions of the telencephalic stalk (Fig. 9). Thus, progenitor pools that exist at rostral levels, such as pLGE1, pLGE2, or pMGE3, extend throughout the subpallium into the amygdaloid end of the hemisphere. In that sense, the notion that the CGE shares features of both the LGE and MGE, as previously recognized (Nery et al., 2002; Xu et al., 2004), is partially correct: the subpallial CGE just represents the caudal end of some of the LGE and MGE domains that can be found at more rostral levels. Remarkably, the CGE also holds a small portion of the POH ventricular zone, which may also contribute to different neuronal cell types in the caudal telencephalon. Furthermore, the CGE also contains the caudal prolongations of the pallial territories that are also found rostrally in the most dorsal aspect of the LGE and immediately adjacent to it. These pallial components are characterized by the expression of *Dbx1*, *Fgf7*, *Sfrp2*, and *Pax6* (and several other markers) and the lack of *Emx1* at early development stages (Smith-Fernández et al., 1998; Puelles et al., 2000; Kim et al., 2001; Assimacopoulos et al., 2003; Medina et al., 2004). Because pallial, LGE, and MGE domains are organized concentrically in the telencephalic hemispheres (Puelles et al., 2000), the CGE contains progressively less progenitor pools as it extends caudally, until eventually the most caudal CGE appears to only consist of pallial progenitors (Kim et al., 2001, their Fig. 1*D*).

In our study, none of the transcription factors analyzed showed specific expression patterns in the CGE compared with the LGE or the MGE (with the only exception of the intrahemispheric high Nkx2-2 expression in pPOH). Thus, our analysis suggests that the CGE does not contain pools of progenitors that are intrinsically different from those also found in the LGE or MGE. Is this compatible with the notion that the CGE is the origin of specific populations of neurons? For example, it has been reported that calretinin (CR)-containing interneurons derive from the CGE, but not from the MGE or LGE (Xu et al., 2004; Butt et al., 2005). Specifically, it has been suggested that CR interneurons derive from the dorsal aspect of the CGE, which most likely corresponds to the caudal pole of pLGE2, as defined in our analysis. Interestingly, the rostral, striatal part of pLGE2 has been proposed to give rise to CR interneurons (Waclaw et al., 2006). The main difference between both sets of CR neurons may just be their final destination: cells from the rostral pLGE2 become CR interneurons in the olfactory bulb, whereas cells from the caudal pLGE2 become CR interneurons in the cerebral cortex. Considering the fact that the olfactory bulb is also a pallial structure (i.e., projections neurons in the olfactory bulb derive from the pallium, as is the case for the rest of the cortex), it is simpler to interpret that pLGE2 extends across both LGE and CGE and produces CR interneurons throughout its rostrocaudal extent, which are then guided into different parts of the pallium (i.e., olfactory bulb vs cerebral cortex).

In the spinal cord, motor neuron columnar fate (i.e., rostrocaudal motor neuron identity) is assigned through a Hox transcriptional regulatory network that is induced by graded fibroblast growth factor signaling (Dasen et al., 2003, 2005). There is increasing evidence for an analogous process in the telencepha-

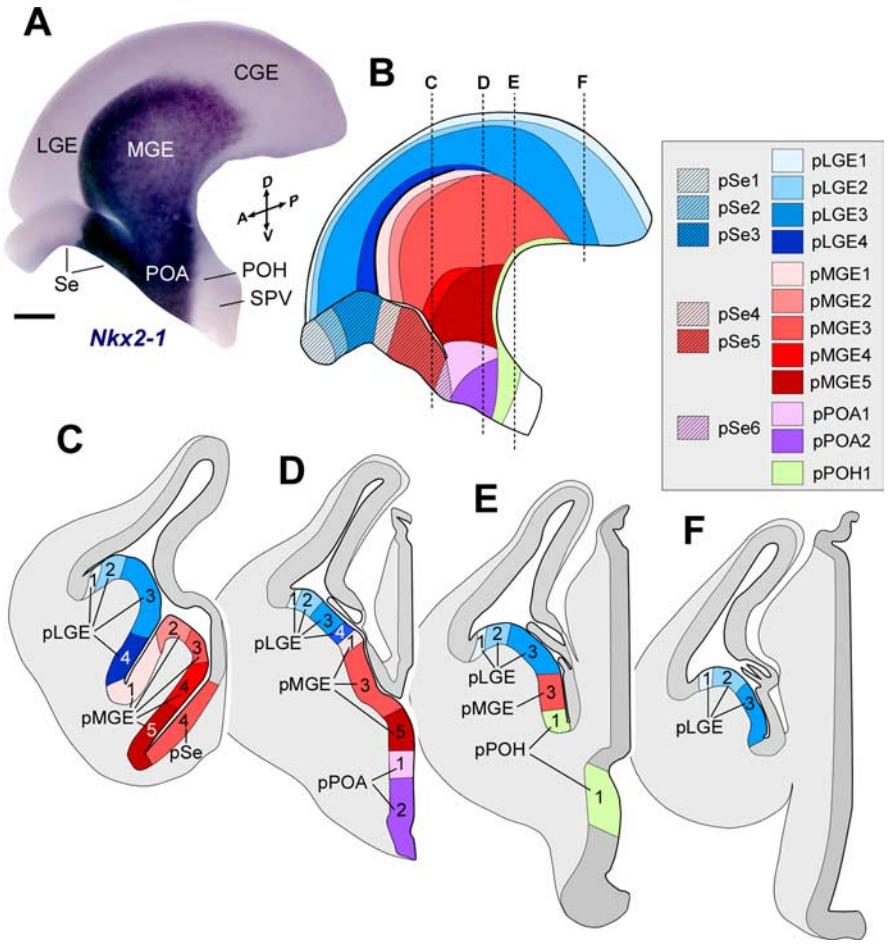

Figure 9. A model of organization for the ventricular zone of the mouse subpallium. ***A***, Whole-mount *Nkx2-1* expression. *Nkx2-1* is expressed in the MGE, POA, and part of the CGE and the septum. ***B***, Schema of the subpallial progenitor domains represented by a color code. Each dotted line represents the plane and level of section of schemas ***C–F***. ***C–F***, Schema of the four different anterior–posterior levels described in this work. All different proliferative domains are shown with a color code. pLGE, Progenitor domain from the LGE; pMGE, progenitor domain from the MGE; pPOA, progenitor domain from the preoptic area; pPOH, progenitor domain from the hypothalamic preoptic area; SE, septum; SPV, supraoptic paraventricular region. Scale bar, 200 μm.

lon; fibroblast growth factor signaling also participates in rostral–caudal patterning of the entire telencephalon through regulating the graded expression of transcription factors such as *Couptf1*, *Emx2*, *Dbx1*, and *Sp8* (Garel et al., 2003; Storm et al., 2006). Because the expression pattern of multiple genes in the subpallium does not seem to respect clear boundaries, and instead distributes in gradients, it is conceivable that differential rostrocaudal positional identity along the described domains may be acquired in the telencephalon through mechanisms similar to those described in the spinal cord.

The septum shares features of the LGE, MGE, and POA progenitor domains

Our analysis corroborates and further expands the idea that gene expression patterns correlative with those in lateral parts of the subpallium are also found within the subpallial septum [the striatal and pallidal septal regions of Puelles et al. (2000)]. In addition, we also discovered that the commissural median region of the septum shares features with the POA, such as the ventricular expression of *Shh*. Consequently, the different septal progenitor domains represent the topologically dorsal continuation (into the roof plate) of the three major subdivisions of the subpallium, namely the LGE VZ (*Nkx2-1*$^-$ and *Shh*$^-$), the MGE VZ (*Nkx2-1*$^+$ and *Shh*$^-$) and the POA VZ (*Nkx2-1*$^+$ and *Shh*$^+$). Despite these similarities, however, there are several genes that are uniquely expressed in the septum and not in the rest of the subpallium (e.g., *Lhx5*), which may thereby contribute to defining the identity of neurons derived from this region. These observations clarify our understanding of the general morphological structure of the subpallium (Fig. 9), because it is apparent that relatively simple patterning mechanisms operating at neural plate stages and afterward may account for this organization.

Conclusions

Our analysis of gene expression patterns in the mouse subpallium demonstrates the existence of a diversity of progenitor domains that are uniquely defined by the combinatorial expression of several transcription factors. These domains exist within each of the three major subdivisions of the subpallium, the LGE, MGE, and POA. Each of these VZ domains extends along the secondary rostrocaudal axis of the telencephalon to different degrees; some of them stretching from the septum to the caudal end of the telencephalic hemispheres. Furthermore, analysis of the expression of transcription factors in single cells suggest that distinct progenitor pools may exist within each of the VZ domains described in this study. Because variations in the expression pattern of some of the transcription factors analyzed in this study has been reported for different strains of mice, it remains to be determined to what extent the proposed model applies to other background strains. In addition, we hope this model may contribute to standardizing findings from different laboratories, because it may easily adapt to new discoveries (for example, pMGE1 can be split in two new domains named pMGE1a and pMGE1b if new evidence arises supporting such subdivision). We believe that the molecular identification of multiple progenitor domains in the subpallium and their correlation with fate-mapped primordia at neural plate and early neural tube stages will also contribute to increasing our understanding of the mechanisms underlying patterning of this region and, most importantly, the generation of neuronal diversity in the mammalian telencephalon.

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