Origins of Cortical GABAergic Neurons in the Cynomolgus Monkey

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In human most cortical γ-aminobutyric acidergic (GABAergic) neurons are produced in the proliferative zones of the dorsal telencephalon in contrast to rodents. We report that in cynomolgus monkey fetuses cortical GABAergic neurons are generated in the proliferative zones of the dorsal telencephalon, in addition to the proliferative region of the ventral telencephalon, the ganglionic eminence (GE), however, with a temporal delay. GABAergic neuron progenitors labeled for Mash1 and GAD65 were present mainly in the GE at embryonic days (E) 47–55, and in the entire dorsal telencephalon at E64–75. These progenitors within the dorsal telencephalon are generated locally rather than in the GE. The ventral and dorsal lineages of cortical GABAergic neurons display different laminar distribution. Early generated GABAergic neurons from the GE mostly populate the marginal zone and subplate, whereas cortical plate GABAergic neurons originate from both ventral and dorsal telencephalon. A differential regulation of the two GABA synthesizing enzymes (GAD65 and GAD67) parallels GABAergic neuron differentiation. GAD65 is preferentially expressed in GABAergic progenitors and migrating neurons, GAD67 in morphologically differentiated neurons. Therefore, the dorsal telencephalic origin of cortical GABAergic neurons is not human-specific but appears as a former event in the ascent of evolution that could provide GABAergic neurons to an expending neocortex.

Keywords: ganglionic eminence, glutamic acid decarboxylase, interneurons, migration, neurogenesis, ventricular zone

Introduction

γ-Aminobutyric acidergic (GABAergic) neurons that represent roughly 15–30% of cortical neurons in human (Houser et al. 1984; Hendry et al. 1987; Jones 1993; Gabbott and Bacon 1996; DeFelipe 2002), play a crucial role in regulating the activity of neuronal networks and the complex interactions among principal neurons, including the emergence of spatio-temporal synchrony of the pyramidal cells (Somogyi and Klausberger 2005; Skaggs et al. 2007). Therefore, the coordination and integration of cortical function depends on the number and diversity of GABAergic neurons. In fact, dysfunction or cell death of several specific types of GABA neurons is a hallmark of various psychiatric and neurological diseases such as schizophrenia (Lewis 2000; Lewis et al. 2005) and epilepsies (Houser 1999; DeFelipe 2004) to which defect in neurogenesis or migration of these neurons can be a predisposing factor (Rakic 2000; Powell et al. 2003; Levitt et al. 2004; Levitt 2005a, 2005b).

Interestingly, increased structural complexity of the brain during mammalian evolution (Uylings and van Eden 1990; Rakic 1995; Kornack 2000) parallels an increased number and

diversity of GABAergic neurons (Jones 1993; Gabbott and Bacon 1996; Gabbott et al. 1997; Letinic and Rakic 2001; Zecevic and Rakic 2001; DeFelipe 2002; Wang et al. 2004). For instance, whereas in rat and mouse GABAergic neurons are absent in most dorsal thalamic nuclei excepted the reticular nucleus (Houser et al. 1980; Esclapez et al. 1993), they are present in all thalamic nuclei in human and nonhuman primates (Smith et al. 1987; Arcelli et al. 1997). In cortex, despite many interneurones appear to be common to all species, some like in layer I display more elaborate features in primates that may represent evolutionary specializations (Zecevic and Rakic 2001; DeFelipe 2002; Rakic and Zecevic 2003a).

One could question whether evolution uses the same or different developmental rules to provide that increased population of GABA neurons? Rakic and colleagues have proposed species-specific programs for generation of these neurons in human brain (Letinic et al. 2002). This hypothesis is mostly based on the demonstration of a migratory pathway (previously suggested, Sidman and Rakic 1973; Letinic and Kostovic 1997) of GABAergic neurons from the ganglionic eminence (GE) of the human telencephalon to the thalamus (Letinic and Rakic 2001) that is not observed in macaque and mouse (Letinic and Rakic 2001). More recently the authors suggested that the source of cortical GABAergic neurons might also be human-specific (Letinic et al. 2002). Indeed, in human most neocortical GABA neurons are produced in the proliferative region of the dorsal telencephalon, prospective neocortex (Letinic et al. 2002; Rakic and Zecevic 2003a), whereas in rodents, cortical GABAergic neurons are generated in the subcortical proliferative zone of the ventral telencephalon, the GE, and migrate tangentially to the dorsal telencephalon (Van Eden et al. 1989; Del Rio et al. 1992; DeDiego et al. 1994; De Carlos et al. 1996; Anderson, Eisenstat, et al. 1997; Tamamaki et al. 1997, 1999; Anderson et al. 1999; Lavdas et al. 1999; Parnavelas 2000, 2002; Parnavelas et al. 2000, 2002; Pleasure et al. 2000; Anderson et al. 2001, 2002; Corbin et al. 2001; Marin and Rubenstein 2001, 2003; Jimenez et al. 2002; Nadarajah and Parnavelas 2002; Nadajarah et al. 2002, 2003; Nery et al. 2002; Polleux et al. 2002; Xu et al. 2003, 2004; Wonders and Anderson 2006).

A puzzling question is whether the dorsal telencephalic origin of neocortical GABAergic neurons is human-specific or reflects a boosting of preexisting developmental mechanisms? In favor of the later hypothesis, several lines of mice, knockout for genes expressed specifically in the ventral telencephalon, show a striking, but however not total decrease in the number of cortical GABAergic neurons at birth. These include mutants for the transcription factors Dlx1 and Dlx2 that show 75% decrease in neocortical GABAergic cells at postnatal day

0 (Anderson, Qiu, et al. 1997; Anderson et al. 1999); Nkx2.1—50% decrease in neocortical GABAergic cells at embryonic day (E) 18.5 (Sussel et al. 1999) and Mash1 that show 50% decrease in neocortical GABAergic cells at E18.5 (Casarosa et al. 1999; Horton et al. 1999). These data suggest that in lower mammals some GABA neurons could also be generated in the proliferative zones of dorsal telencephalon.

On the other hand, it is still debated following the work of Letinic et al. (2002) whether in human, progenitors of GABAergic neurons are produced directly by the proliferative zones of the prospective neocortex or whether some of them might migrate at early stages from the GE to the proliferative zones of the dorsal telencephalon where they would continue to divide locally, giving rise to postmitotic GABA neurons. This later hypothesis was supported by data obtained in the mouse, showing that some progenitors appear to migrate from the GE directly into the subventricular zone (SVZ) of the prospective neocortex after E14.5, but in fact they do not proliferate in their new position (Anderson et al. 2001).

In order to address these questions, we have studied the sources and migratory pathways of neocortical GABAergic neurons in a nonhuman primate, the cynomolgus monkey. For this purpose, we performed in fetuses from E47 to E90 (neurogenesis period, term 165 days), immunohistochemical (IHC) experiments for the following proteins: 1) the basic helix–loop–helix (bHLH) transcription factor Mash1, a marker of GABAergic neuron progenitors (Casarosa et al. 1999; Schuurmans and Guillemot 2002); 2) The two forms of glutamic acid decarboxylase (GAD, GAD65, and GAD67), two synthesizing enzymes for GABA which are coexpressed in almost all GABAergic neurons of the rat, monkey and human adult brain (Kaufman et al. 1991; Esclapez et al. 1994; Hendrickson et al. 1994; Sloviter et al. 1996). In order to further characterize the developmental stage and neural lineages of the different populations of Mash1- and GAD-containing cells, double IHC labelings were performed for 1) the nuclear antigen Ki67 expressed in all proliferating cells and 2) the platelet-derived growth factor receptor-α (PDGFR-α) a marker of oligodendrocyte progenitors (Lu et al. 2000) since it is known that Mash1 promotes also the generation of oligodendrocytes (Parras et al. 2007) that occurs in the ventral and dorsal telencephalon in rodents (Kessaris et al. 2006) as in humans (Ulfig et al. 2002; Rakic and Zecevic 2003b; Jakovcevski and Zecevic 2005).

We demonstrate that in the monkey as in human, cortical GABAergic neurons are generated in the proliferative cortical zones of the dorsal telencephalon, in addition to the proliferative subcortical regions of the ventral telencephalon, the GE. Our data further suggest that the first morphologically differentiated GABAergic neurons observed in the marginal zone (MZ) and the subplate (SP) of the dorsal telencephalon are likely generated in the GE, whereas GABA neuron progenitors in the dorsal telencephalon likely contribute to provide neurons of the cortical plate (CP).

Preliminary reports of some of the findings have been published previously in abstract form (Esclapez et al. 2004).

Materials and Methods

Animal and Tissue Preparation

Twelve cynomolgus monkeys (*Macaca fascicularis*) ranging in age from E47 to E90 (term 165 days) were obtained from the breeding colony of the Institut National de la Recherche Agronomique (INRA) Primate Center at Jouy en Josas, France. This series included 47- (*n* = 2), 55- (*n* = 1), 56- (*n* = 1), 64- (*n* = 1), 68- (*n* = 1), 70- (*n* = 2), 75- (*n* = 1), 83- (*n* = 1), 88 (*n* = 1), 90- (*n* = 1), day-old fetal animals, delivered by cesarean section under anesthesia with ketamine HCl (10 mg/kg, i.p.) and diazepam (1 mg/kg, i.p.). The study was conducted under the approval and guidelines of the Ethical Committee of the INRA Primate Center and the European Communities Council Directive (86/609/ EEC). All fetuses were deeply anesthetized with ketamine HCl (10 mg/ kg, i.p.) and quickly perfused transcardially with a cold fixative solution of 4% paraformaldehyde in 0.12 M sodium phosphate buffer (PB), pH 7.4, for 30 min. The brains were then removed from the skull and quickly sectioned in the coronal plane. Blocks of brain were postfixed in the same fixative for 6 h at room temperature, rinsed in PB for 1 h, immersed overnight at 4 °C in cryoprotective solutions of 10% and then 20% sucrose in PB and frozen at –50 °C in isopentane cooled with dry ice.

Thick sections (40–150 μm) were cut in a coronal plane on a cryostat, rinsed in 0.01 M phosphate-buffered saline (PBS), pH 7.4, collected sequentially in tubes containing an ethylene glycol-based cryoprotective solution and stored at –20 °C until histological processing.

Every tenth section was stained with cresyl violet in order to determine the general histological characteristics of the tissue within the rostral–caudal extent of each fetal brain. Adjacent sections were processed for 1) single IHC labeling for the detection of GAD65, GAD67, and Mash1; 2) double IHC labeling for simultaneous detection of GAD65 and Mash1; GAD65 and Ki67; Mash1 and PDGFR-α.

Immunohistochemistry

Antibodies

The rabbit polyclonal antiserum K2, raised against GAD67 (Kaufman et al. 1991; obtained from Chemicon International, Temecula, CA) and the mouse monoclonal antibody GAD-6 directed against GAD65 (Chang and Gottlieb 1988; obtained from Chemicon) were used to localize GAD-containing cells. The characteristic and specificity of these antibodies for the two GAD isoforms have been described previously (Chang and Gottlieb 1988; Kaufman et al. 1991; Esclapez et al. 1994). In addition, the K2 and GAD-6 antibodies recognized respectively the GAD67 and GAD65 isoforms in human and monkey tissues (Sloviter et al. 1996).

A monoclonal antibody (kindly provided by Dr Jane Johnson) was used to localize Mash1-containing cells. This antibody was produced by an hybridoma line obtained after immunization of a mouse with the human protein achaete-scute homolog 1 cloned from human medullary thyroid cancer cDNA library that displayed 95% similar amino acid sequence to Mash1 (Ball et al. 1993).

A rabbit monoclonal antibody raised against a synthetic peptide derived from the human cell cycle associated protein Ki67 (clone SP6; Vector Laboratories, Burlingame, CA) was used to identify proliferating cells.

A mouse monoclonal antibody raised against the human PDGFR-α (clone αR1, BD Biosciences, San Diego, CA) was used to label oligodendrocyte progenitors.

IHC Methods

Free-floating sections to be processed for single or double IHC labeling were first rinsed for 30 min in 0.02 M potassium PBS (KPBS; 16 mM K_2HPO_4, 3.5 mM KH_2PO_4, 150 mM NaCl, pH 7.4), incubated in 1% hydrogen peroxide for 30 min to block endogenous peroxidase and rinsed in KPBS.

Single IHC labelings for GAD65, GAD67, and Mash1 were performed with unlabeled primary antibodies and standard avidin-biotin–peroxidase method (Vectastain Elite ABC, Vector Laboratories,) at 4 °C for all steps. Sections processed for GAD67-IHC labeling were incubated for 1 h in KPBS containing 3% normal goat serum (Vector Laboratories) and overnight in K2 antiserum diluted 1:4000 in KPBS containing 1% normal goat serum. Sections processed for GAD65- or Mash1-IHC labeling were incubated for 1 h in KPBS containing 3% normal horse serum (Vector Laboratories), and overnight in GAD-6 (1:100) or Mash1 (1:200) monoclonal antibody diluted in KPBS containing 1% normal

horse serum. After these steps, sections were rinsed for 30 min in KPBS and incubated for 1 h in biotinylated goat anti-rabbit immunoglobulin G (IgG) or biotinylated horse anti-mouse IgG diluted 1:200 in KPBS containing 3% normal goat serum or normal horse serum. Sections were then rinsed in KPBS, incubated for 1 h with an avidin–biotin–peroxidase solution prepared according to manufacturer's recommendations (Vector Laboratories). After several rinses in KPBS, sections were processed for the same time (10 min) in 0.04% 3-3′-diamino-benzidine-HCl and 0.006% hydrogen peroxide diluted in KPBS. The sections were then rinsed in KPBS, mounted on gelatin-coated slides, dried, dehydrated, and coverslipped with Permount (Fisher scientific, Electron Microscopy Sciences, Washington, PA).

IHC controls: No labeling was detected when primary monoclonal antibodies were replaced with normal mouse IgG, or when primary antibodies were omitted. When K2 polyclonal antiserum was replaced with normal rabbit IgG, vessels, pia and microglia-like cells were observed. However, this staining was easily delineated from specific GAD67-immunolabeling.

Double IHC labelings for GAD65 and Mash1; GAD65 and Ki67; PDGFR-α and Mash1 were performed by sequential immunodetection at 4 °C. Sections were rinsed for 30 min in KPBS, incubated for 1 h in KPBS containing 3% normal horse serum and first processed for detection of GAD65 or PDGFR-α. They were incubated overnight in GAD-6 (1:50) or PDGFR-α (1:50) mouse monoclonal antibody diluted in KPBS containing 1% normal horse serum (Vector Laboratories). Following several rinses in KPBS, sections were incubated for 1 h in biotinylated horse anti-mouse IgG (1:200), rinsed in KPBS and incubated for 2 h in Alexa 488–conjugated Streptavidin (1:200, Jackson Immunoresearch, West Grove, PA). After intensive rinses in KPBS (3 × 30 min), the sections were processed for detection of Mash1 or Ki67. For Mash1 immunodetection the mouse on mouse (MOM) kit (Vector Laboratories) was used according to manufacturer's instruction. Sections were incubated for 1 h in Mouse IgG blocking reagent and overnight in Mash1 mouse monoclonal antibody diluted 1:100 in MOM diluent. Then they were rinsed 3 × 30 min in KPBS and incubated for 2 h in Cy3-conjugated goat anti-mouse IgG (1:200, Jackson Immunoresearch) diluted in KPBS. For Ki67 immunodetection, sections were incubated for 1 h in KPBS containing 3% normal donkey serum (Jackson Immunoresearch), and overnight in Ki67 rabbit monoclonal antibody diluted 1:100 in KPBS containing 1% normal donkey serum (Jackson Immunoresearch). They were rinsed 3 × 30 min in KPBS and then incubated for 2 h in Cy3-conjugated donkey anti-rabbit IgG (1:200, Jackson Immunoresearch) diluted in KPBS. After being rinsed in KPBS all sections were then mounted on gelatin-coated slides, dried and coverslipped with Gel/Mount (Biomedia, Foster City, CA). The specimens were analyzed with Olympus fluoview-500 laser scanning microscope.

IHC controls: In all cases, no cross reactivity was detected when at the second step, the primary antibody was omitted. In addition, the antibodies used for double labeling targeted distinct compartments of the cell body (nuclear for Mash1 and Ki67 versus cytoplasmic for GAD65 and membrane for PDGFR-α), and the cell was confirmed as double labeled only if distinct compartments of the same cell were labeled.

Results

E47–E55

Mash1-Containing Cells

From E47 to E55, the bHLH transcription factor Mash1 was expressed mainly in the proliferative zones of the ventral telencephalon including the GE and the septal ventricular zone (VZ) (Fig. 1*A,C*), and at a lower extent in the most dorsal part of the dorsal telencephalon, on the roof of the lateral ventricle (Fig. 1*A*).

In the GE, almost all cells displayed a nuclear immunolabeling for Mash1 (Figs 1*A,C*, 2*A*, 3*A*). However the distribution of these Mash1-containing cells was different in the ventral and dorsal parts of the GE, presumed to correspond respectively to the medial and lateral GEs (Fig. 1*C*). These cells were organized in two bands in the VZ of the ventral GE (Figs 1*C*, 2*A*), whereas they formed a homogenous band in the dorsal part of the GE (Fig. 1*C*). In addition, the cells located in the proliferative VZ of the GE were strongly labeled, whereas the majority of the cells in the SVZ were less intensively labeled (Figs 1*C*, 2*A*, 3*A*). A similar difference in labeling intensity was observed among the numerous Mash1-containing cells of the prospective septal region. These cells were highly labeled in the VZ, whereas they were moderately labeled in the SVZ (Figs 1*C*, 2*B*).

In the dorsal telencephalon, some Mash1-labeled cells were also observed in the VZ and SVZ (Figs 1*A*, 2*E,F*) but their number and labeling intensity were much lower than in the proliferative zones of the GE and septal region (compare Fig. 2*F* with Fig. 2*A,B*). Furthermore, in these prospective cortical regions, a clear decreasing gradient of Mash1 labeling intensity was evident in all sections processed at E47 and E55 (Fig. 1*A*). The moderate level of labeling observed in the most dorsal part of the dorsal telencephalon, on the roof of the lateral ventricle (Figs 1*A*, 2*F*), decreased in the lateral and medial wall (Fig. 1*A*), and a striking feature was the absence of Mash1 immunoreactivity in the most ventral part of the dorsal telencephalon close to the GE (Figs 1*A*, 3*A*). No labeling was observed in the intermediate zone (IZ), SP, CP, and MZ.

GAD65-Containing Cells

Numerous GAD65-labeled cells were present in the telencephalon. The vast majority of these GAD65-labeled cells were observed in the GE and septal proliferative zones and likely corresponded to neuronal progenitors (Figs 1*D*, 2*C,D*). They displayed a round-shape, a thin cytoplasmic ring, a rather large nucleus and were closely apposed to each other (Fig. 2*D*, insert). Their pattern of distribution corresponded mainly to that of the moderately Mash1-labeled cells (compare Fig. 2*C* with *A*; *D* with *B*). Thus, in the GE as well as in the septal proliferative zone, the GAD65-labeled cells were mainly located in the SVZ, only few labeled cells being present in the VZ (Fig. 2*C,D*).

In contrast to GE and septal region, no GAD65-containing progenitor-like cells were observed in the VZ and SVZ of the dorsal telencephalon (Fig. 2*G*). Instead, numerous GAD65-labeled cells exhibiting the characteristic morphology of early migrating neurons, including either a leading or both leading and lagging processes were present in the lateral and medial wall, (Figs 2*G*, 3*B–E*). These migrating-like neurons likely originated from the GE, since a dense band of migrating cells leaving the GE was also observed in all sections. The density of these migrating GAD65-containing neurons varied within the different zones. A large stream of tangentially migrating neurons was located in the lower IZ/upper SVZ of the dorsal telencephalon, on all coronal sections from rostral to caudal levels (Fig. 3*B–D*). Smaller streams of migration were present in the MZ, whereas a few tangentially oriented migrating GAD65-labeled neurons were also observed in the SP (Fig. 3*E*). Finally, none of these tangentially migrating GAD65-containing neurons were observed in the proliferative zones of the dorsal telencephalon (VZ and deep SVZ) (Fig. 3*B*).

Double Labeling Experiments for GAD65 and Mash1; GAD65 and Ki67

These experiments clearly showed that at E47–E55, most GAD65-containing cells in the proliferative zones of the GE and
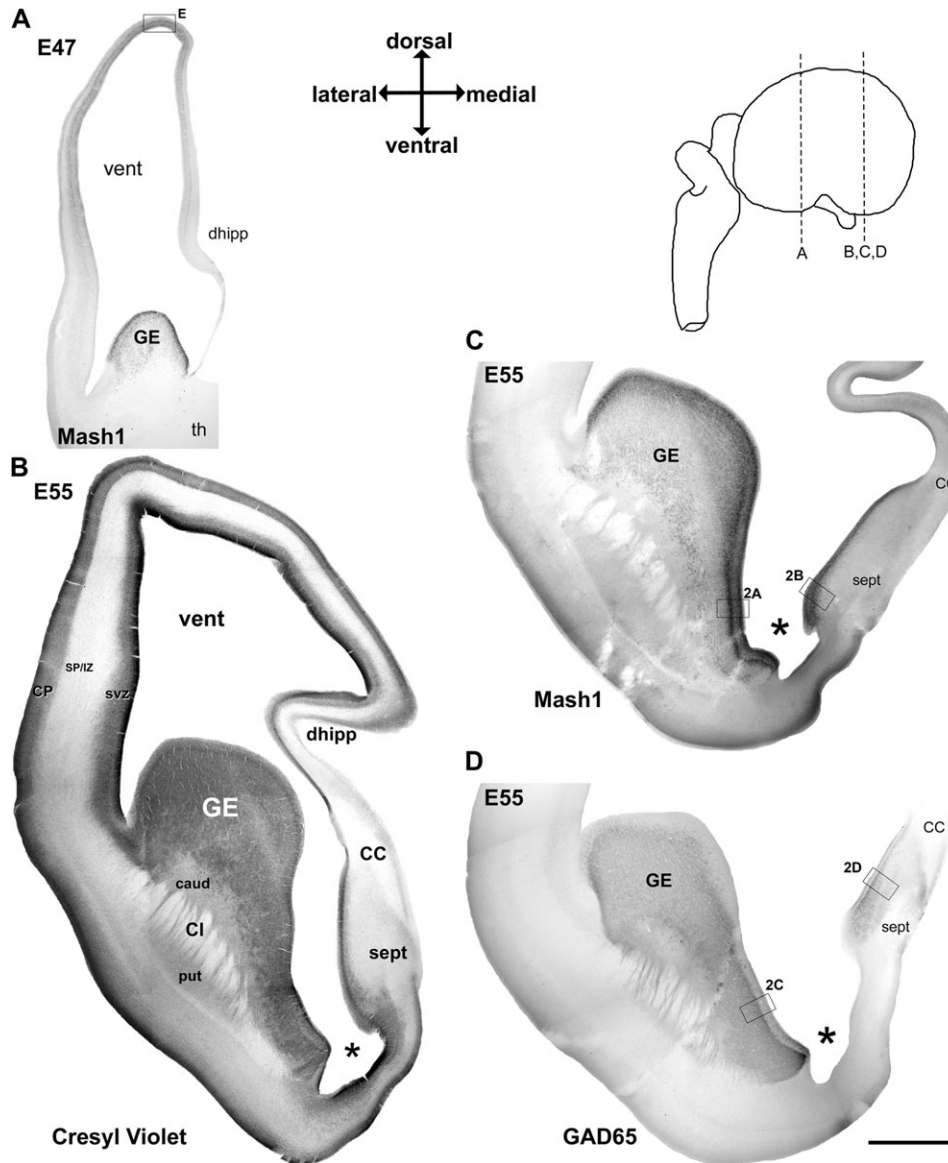
Figure 1. Mash1 (*A*, *C*) and GAD65 (*D*) labeling patterns in coronal sections of monkey fetal telencephalon at E47 (*A*) and E55 (*B–D*). (A) At E47 Mash1 was highly expressed in the proliferative zone of the ventral telencephalon, the GE. Mash1 immunoreactivity was also observed in the proliferative zones of the dorsal telencephalon and followed a clear dorso-ventral decreasing gradient in the labeling intensity within the entire cortical wall. Whereas moderate level of labeling was observed in the most dorsal part of the telencephalon, on the roof of the lateral ventricle (vent) (indicated by square and magnified in Fig. 2E), the intensity of staining decreased in the lateral and medial wall. No Mash1 immunoreactivity was observed close to the GE, nor in the most ventral part of the medial telencephalic wall that corresponds at this level to dorsal hippocampus (dhipp). No Mash1 immunoreactivity could be seen in the thalamic nuclei (th). (*B–D*) Adjacent sections from E55 fetal brain at a rostral level in which the lateral ventricle displayed its rostral dilatation (paleocortical ventricle indicated by a star). (B) Cresyl violet labeled section, in which the following regions were specified: corpus callosum (CC), septum (sept), caudate nucleus (caud), putamen (put), capsula interna (CI), and primordium of the dorsal hippocampus (dhipp). (C) Strong intensity of labeling for Mash1 was present in the GE (box magnified in Fig. 2A) and septal proliferative zone (box magnified in Fig. 2B). (*D*) GAD65-immunolabeling was observed in the same regions of the ventral telencephalon as Mash1, the GE and the septal eminence (boxes enlarged in Fig. 2C,D). The levels of sections (*A*, *B*, *C*, *D*) are indicated by dotted lines on the schematic drawing of monkey fetal brain. Crossed arrows indicated the orientation of sections (*A*, *B*, *C*, *D*). Scale bar = 1 mm (same for all the figures).

septal region were labeled for Mash1 (Fig. 5*A*) and Ki67, a marker of proliferative cells (Fig. 5*E,F*) and therefore confirm that these cells likely correspond to progenitors of GABAergic neurons. In contrast, migrating-like GAD65-labeled cells leaving the GE or tangentially oriented in the upper SVZ/lower IZ of the dorsal telencephalon did not contain detectable levels of Mash1 (Fig. 5*A*) and the vast majority of these cells were not labeled for Ki67 (Fig. 5*E,E'*). However, these migrating-like GAD65-containing neurons were surrounded by many mitotic cells labeled for Ki67. These mitotic cells were particularly

numerous in the IZ and the VZ of the dorsal telencephalon (Fig. 5*E*).

Double Labeling Experiments for Mash1 and PDGFR-α

At this developmental stage some PDGFR-α–containing cells were present in the GE and all these presumed oligodendrocyte progenitors were clearly colabeled for Mash1 (Fig. 6*A*). These double-labeled cells for PDGFR-α and Mash1 in the GE were clearly less numerous than double-labeled cells for GAD65 and Mash1 (compare Fig. 5*B* with Fig. 6*A*). Many
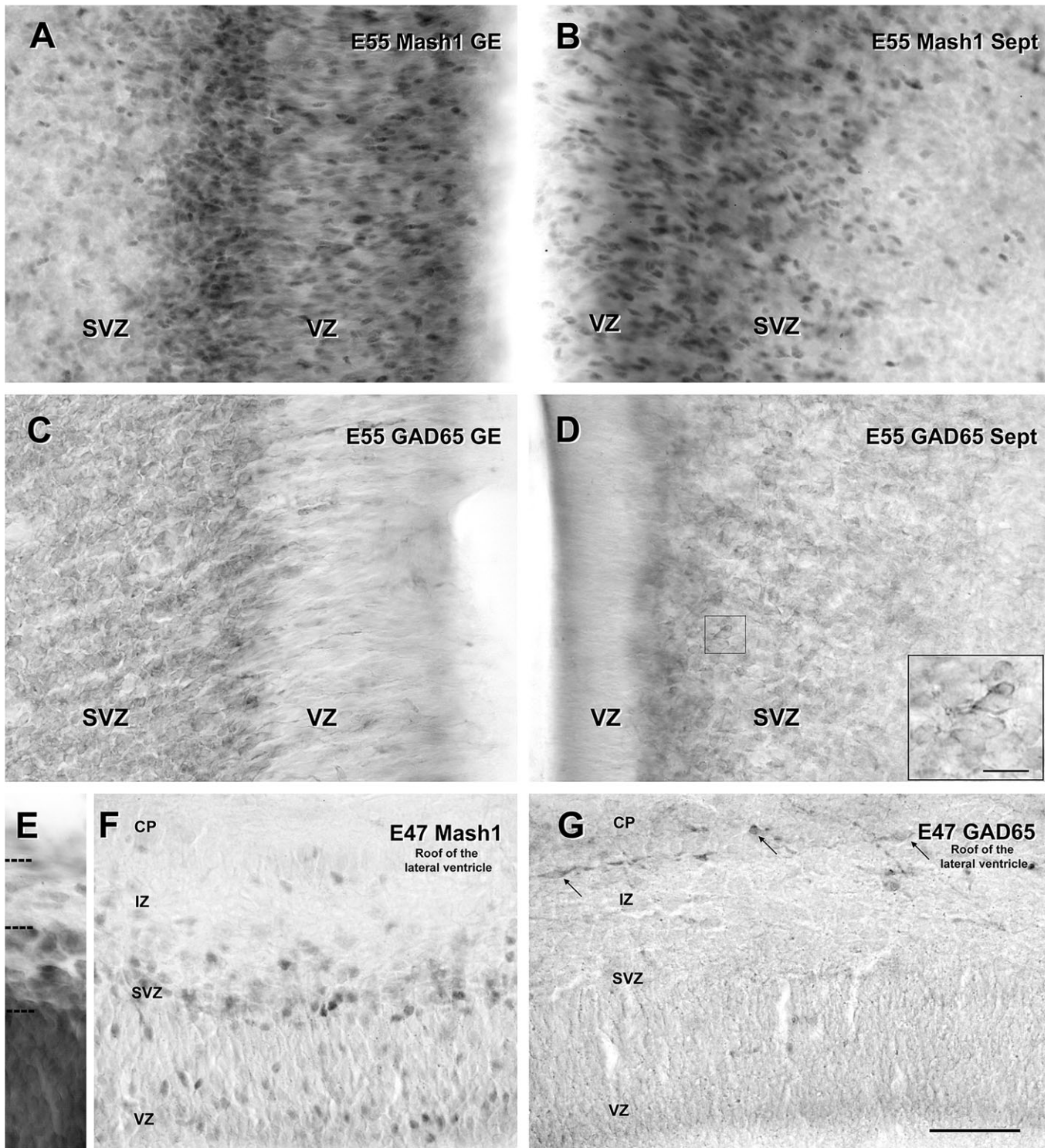
Figure 2. Comparison of labeling for Mash1 (*A*, *B*, *E*) and GAD65 (*C*, *D*, *F*) in the proliferative zones of GE (*A*, *C*), septum (*B*, *D*) and dorsal telencephalon (*E*, *F*) in coronal sections of monkey fetal telencephalon at E47 (*E*, *F*) and E55 (*A*–*D*). Figures correspond to magnified regions indicated by boxes in Figure 1*A,C,D*. (*A*) E55. In the GE many cells were labeled for Mash1 in the VZ and the SVZ, the intensity of labeling being much higher in the VZ than in the SVZ. Moreover, the cells highly labeled for Mash1 in the VZ of the ventral part of GE were organized in two bands in contrast to the dorsal part where the highly labeled cells for Mash1 were distributed in a homogeneous band (see Fig. 1*C*). (*B*) E55. In the prospective septal region (Sept) numerous cells highly labeled for Mash1 were observed in the VZ whereas the cells in the SVZ displayed a moderate level of labeling. (*C*) E55. Many cells labeled for GAD65 were observed in the GE. These cells were mainly located in the SVZ, only a few cells being present in the VZ. (*D*) E55. Many cells labeled for GAD65 were present in the SVZ of the septal region. Box: magnified region. (*E*) E47. Cresyl violet labeled section, in which the different cellular zones of the most dorsal part of the dorsal telencephalon are delineated. (*F*) E47. Some cells moderately labeled for Mash1 were observed in the proliferative zones of the most dorsal part of the dorsal telencephalon on the roof of the lateral ventricle. Most of these Mash1 positive cells were present in the upper part of the SVZ, on the border to the IZ. Some cells were also observed in the deepest part of the VZ. (*G*) E47. In contrast to Mash1 no GAD65-containing cells were observed in the VZ and SVZ of the most dorsal part of the dorsal telencephalon. However in this region, some GAD65-containing cells with a characteristic morphology of early postmitotic migrating neurons, including a leading process, and tangentially oriented, were observed in the IZ and the CP (arrows). Scale bars = 50 μm (*A*–*G*), 10 μm (box in *D*).
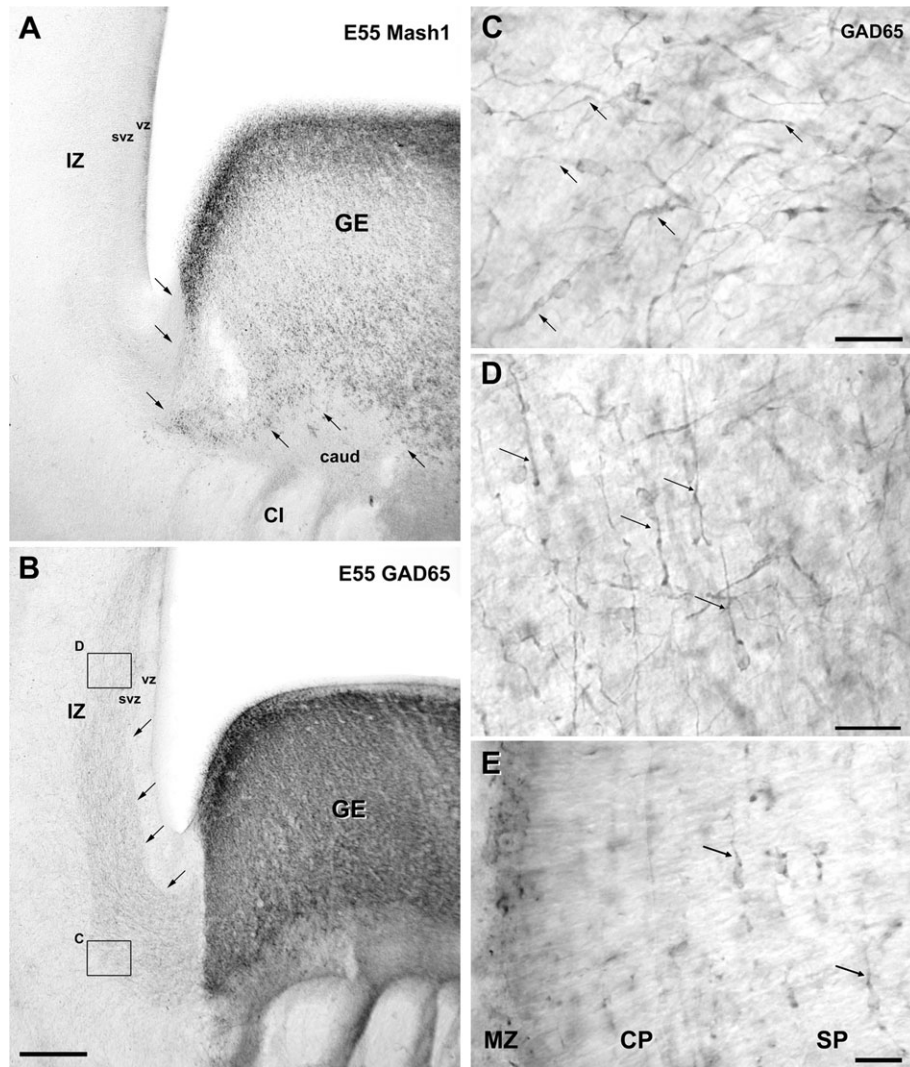
Figure 3. Labeling patterns for Mash1 (*A*) and GAD65 (*B–E*) in coronal sections at E55. (*A*) IHC labeling for Mash1 is present in the GE but not in the neighboring structures including the caudate nucleus (caud), capsula interna (CI), and adjacent zones of the dorsal telencephalon: VZ, SVZ, and IZ. Arrows indicated border between GE and adjacent structures. (*B*) A high level of GAD65 labeling is present in the GE. In addition a stream of postmitotic like GAD65-containing neurons extending from GE to dorsal telencephalon was observed in the lower IZ/upper SVZ. Arrows indicate the deeper border of this stream, showing that at E55 none of the cells leaving GE migrate through VZ and lower SVZ. Boxes: magnified regions in (*C*) and (*D*). (*C*) GAD65-containing cells with a characteristic morphology of postmitotic migrating neurons, including a leading process (arrows) leaving the GE. (*D*) In the IZ and upper SVZ of dorsal telencephalon, the majority of GAD65 migrating neurons are tangentially (parallel to pia) oriented (arrows). (*E*) Tangentially oriented GAD65 migrating neurons are also observed in the SP (arrows), CP, and MZ of the dorsal telencephalon. Scale bars = 350 μm (*A, B*), 20 μm, (*C, D, E*).

PDGFR-α containing cells were also observed in the SP (Fig. 6*B*) but in contrast to those present in the GE they displayed very low (Fig. 6*D*) or no detectable levels of Mash1 (Fig. 6*C*). No PDGFR-α containing cells were observed in the VZ and SVZ of the dorsal telencephalon.

E64–E75

Mash1-Containing Cells
In GE and septal region of the ventral telencephalon, the pattern of labeling for Mash1 displayed strong similarities with that observed at E47 and E55. On the contrary, in the dorsal telencephalon it was very different from that observed at earlier stages. A massive expression of Mash1 was observed now in the VZ and SVZ, which extended to the entire dorsal telencephalon including its most ventral part close to the GE (compare Fig. 4*A–C* with Fig. 3*A*). These Mash1-labeled cells

present in the VZ and SVZ of the whole dorsal telencephalon, displayed a strong intensity of labeling (Fig. 4*A–C*) similar to that observed in the proliferative zones of the GE (Fig. 4*A*) and septal region. In addition numerous cells lightly labeled for Mash1 were present in the lower part of the IZ and upper part of the SVZ (Fig. 4*A*) whereas Mash1-labeled cells were still not observed in the SP, CP and MZ (Fig. 4*B*).

GAD65-Containing Cells
The labeling pattern for GAD65 at E64–E75 (Fig. 4*E,F*) was also very different from that described at earlier stages (Figs 3*B–E*, 2*C,D,F*, 1*D*) especially in the dorsal telencephalon. GAD65-containing cells were not only numerous in the GE and septal region (Fig. 4*E–H*), as at earlier stages (Figs 1*D*, 3*B*), but they were also present in great number in the VZ and SVZ in all regions of the dorsal telencephalon (Fig. 4*E*), in contrast to the earliest stages (compare Fig. 4*E* with Fig. 3*B*). In these
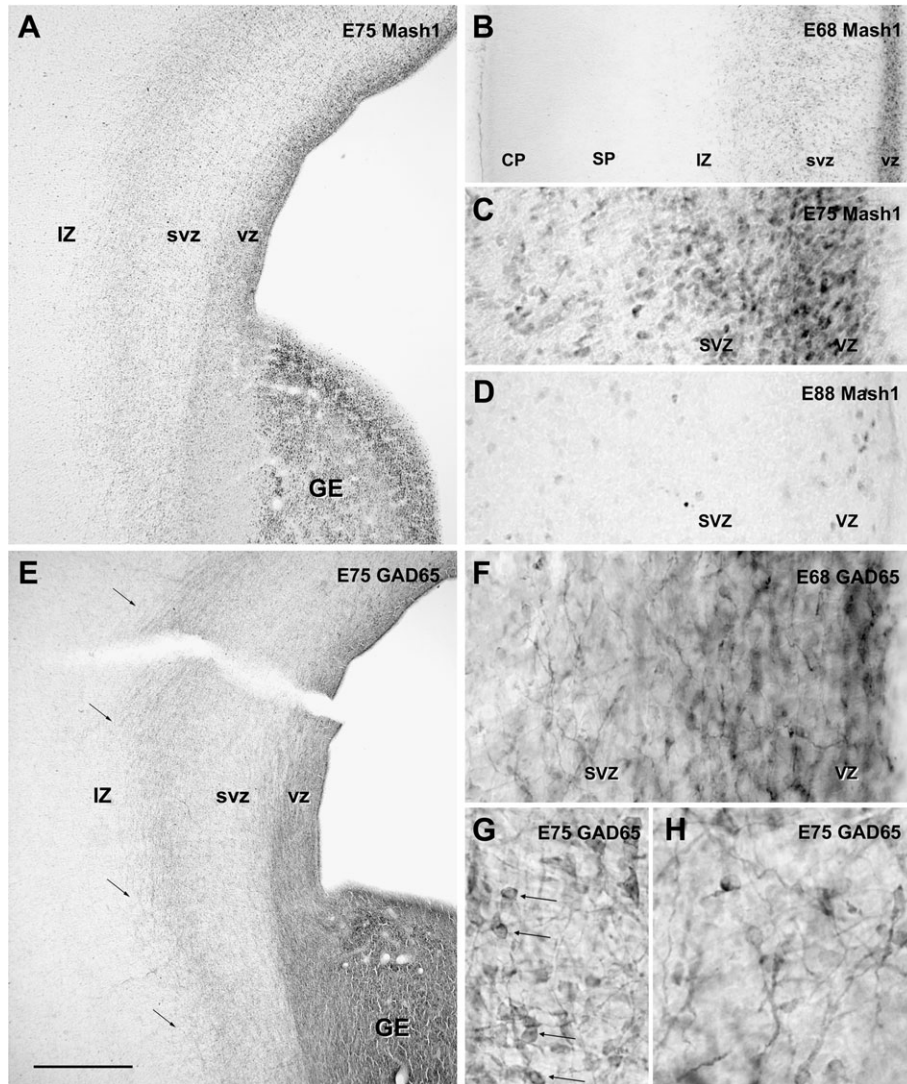
Figure 4. Labeling patterns for Mash1 and GAD65 in coronal sections at E68, E75 and E88. (A–D) Mash1. (A) At E75, presence of Mash1 immunoreactivity in GE as well as in ventricular (VZ) and subventricular (SVZ) zone of the entire dorsal telencephalon. In both areas, the intensity of labeling for Mash1 decreased from the VZ to the SVZ. Few cells very lightly labeled for Mash1 were observed in the lower part of the IZ. (B) At E68, the same pattern of labeling for Mash1 is already present in the VZ and SVZ of the dorsal telencephalon. No labeling is observed in the upper part of IZ, the SP and the CP. (C) E75 Higher magnification of panel A. The numerous Mash1 highly labeled cells located in the VZ and SVZ of the dorsal telencephalon are round-shaped. (D) At E88, end of neurogenesis, the intensity of labeling for Mash1 was strongly decreased as compared to E68 (B) and E75 (A and C). (E–H) GAD65. (E) At E75, GAD65 immunolabeling was present in the GE, but also in the proliferative VZ and SVZ of the dorsal telencephalon in contrast to earlier stages (Figs 1D, 3B). The stream of migrating GAD65-containing neurons observed at E55 (Fig. 3B) in the lower part of IZ and upper part of SVZ was still present (arrows). (F) At E68, similar labeling was already observed. In contrast to previous stages (E47–E55 compare with Figs 2F and 3B) many migrating-like postmitotic neurons with one leading process were now observed in the VZ and SVZ of the dorsal telencephalon. (G) At E75, in addition to migrating-like postmitotic neurons, many round-shaped cells without any process were labeled for GAD65 (arrowheads). These cells displayed a similar morphology to that observed in the GE and septal region (compare with Fig. 2C,D, and box in Fig. 2D). (H) At E75, many migrating-like GAD65 neurons in the SVZ of the dorsal telencephalon displayed multidirectional orientations. Scale bar = 550 μm (A, E), 250 μm (B), 60 μm (C, D) and 30 μm (F–H).

proliferative zones of the prospective cortex, two main populations of GAD65-containing cells were observed. The first one, corresponded to round-shaped cells without any evident processes, highly labeled for GAD65 like the cells in the GE. They were often distributed by groups of 2 or 3 closely juxtaposed cells and likely corresponded to GAD65-containing progenitors (Fig. 4G). The second population corresponded to migrating-like GAD65-containing neurons. However the orientation of these postmitotic migrating neurons also differed strikingly from that observed at E47–E55. Instead of being tangentially oriented, they displayed multidirectional orientations at E64–E75 (Fig. 4H). These nontangentially oriented GAD65-containing neurons were observed in all layers of the

dorsal telencephalon including IZ, SP, CP, as well as the SVZ and VZ.

Double Labeling for Mash1 and GAD65; GAD65 and Ki67

At E64–E75, GAD65-containing cells that coexpressed Mash1 were still numerous in the GE and septal region of the ventral telencephalon (Fig. 5B), but now also in the VZ and SVZ of the prospective cortex (Fig. 5C,D). At this stage, in contrast to the previous one, almost all Mash1-labeled cells in the VZ and SVZ of this prospective cortex were labeled for GAD65. These colabeled cells in the cortical wall, often formed groups of 2 or 3 closely associated cells (Fig. 5D) suggesting ongoing divisions further supported by the presence of many double-labeled cells
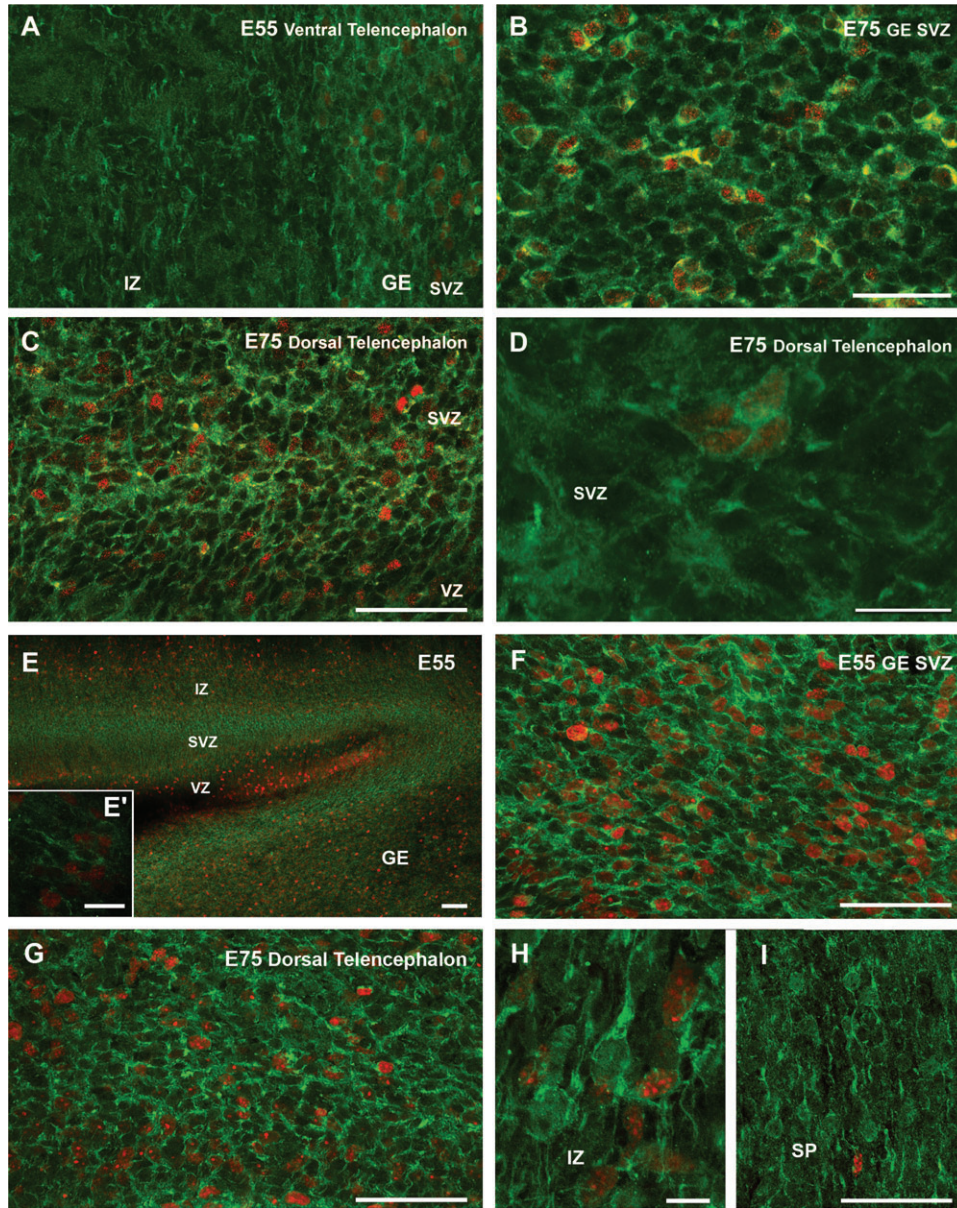
Figure 5. Confocal micrographs of coronal sections of monkey fetal telencephal at E55 and E75 double-labeled (*A–D*) for Mash1 (red, nuclear) and GAD65 (green, cytoplasmic) or double-labeled (*E–I*) for GAD65 (green, cytoplasmic) and Ki67 (red, nuclear). (*A*) At E55, almost all Mash1-labeled cells in SVZ of the GE showed cytoplasmic labeling for GAD65, whereas none of the migrating GAD65 neurons in the IZ displayed detectable level of Mash1. (*B*) At E75, in the SVZ of GE many Mash1-containing cells were labeled for GAD65. (*C*) At E75, in the dorsal telencephalon, most Mash1-labeled cells of SVZ also contained GAD65, whereas many Mash1-labeled cells in the VZ did not. (*D*) At E75, aggregates of cells double-labeled for Mash1 and GAD65 were frequently observed in the SVZ of the dorsal telencephalon, suggesting cells ongoing division. (*E*) At E55, in the SVZ of the GE many GAD65-labeled cells were labeled for Ki67 and therefore corresponded to proliferating cells. In contrast none of the migrating GAD65-containing cells in the IZ displayed detectable level of Ki67 as illustrated at higher magnification (inset E′) and therefore likely corresponded to early postmitotic neurons. (*F*, *G*) At E55 in the SVZ of GE (*F*) like at E75 in the SVZ of the dorsal telencephalon (*G*) most GAD65-containing cells were labeled for Ki67 and therefore likely corresponded to progenitors of GABAergic neurons. (*H*, *I*) At E75, many migrating like GAD65-containing cells in the IZ (*H*) and SP (*I*) were not labeled for Ki67. Scale bars = 50 μm (*A*, *C*, *E*, *F*, *G*, *I*), 30 μm (*B*, *E′*) and 10 μm (*D*, *H*).

for GAD65 and Ki67 in these cortical proliferative zones (Fig. 5*G*). In contrast to these progenitor-like cells, migrating-like GAD65-containing neurons did not express Mash1 or Ki67 (Fig. 5*H,I*).

Double Labeling Experiments for Mash1 and PDGFR-α
At this developmental stage, PDGFR-α–containing cells were present in the GE but also now in the SVZ and IZ of the dorsal telencephalon and all presumed oligodendrocyte progenitors were clearly colabeled for Mash1 (Fig. 6*E*). Many PDGFR-α containing cells were also observed in the SP (Fig. 6*F*) and some

in the CP (Fig. 6*G*), these cells displayed morphological feature of oligodendrocytes and did not display detectable levels of Mash1 (Fig. 6*F,G*).

E83–E90
A clear decrease of Mash1 immunolabeling in the proliferative zones of the telencephalon was observed at E88 (Fig. 4*D*) as compared to E64–E75 (Fig. 4*B,C*). On the other hand, the morphological types (progenitors and postmitotic neurons), distribution and orientation profiles of GAD65-containing cells were similar to those described at E64–E75.
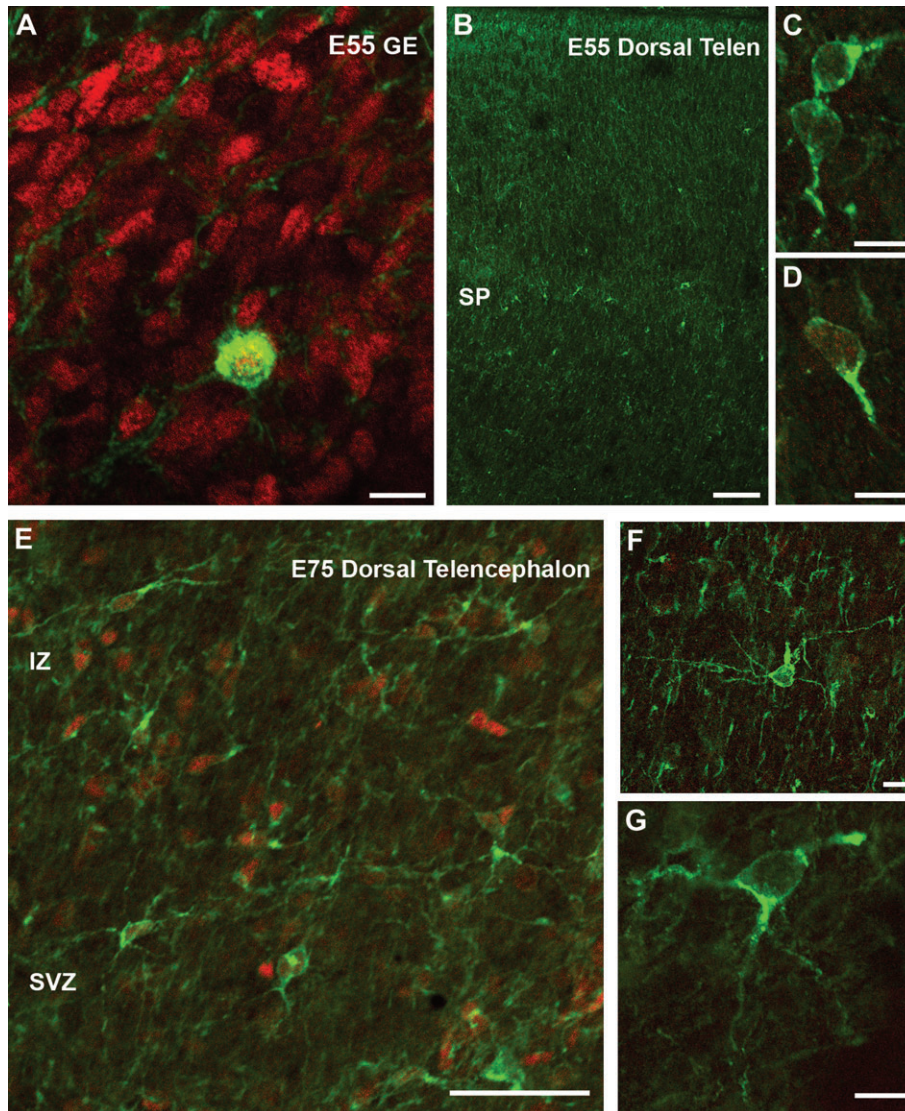
Figure 6. Confocal micrographs of coronal sections of monkey fetal telencephalon at E55 (*A–D*) and E75 (*E–G*) double-labeled for Mash1 (red, nuclear) and PGDFR-α (green, cytoplasmic). (*A*) Few cells double-labeled for PGDFR-α and Mash1 were present among the numerous cells only labeled for Mash1 observed in the SVZ of the GE. (*B, C, D*) Some PDGFR-α cells were observed in IZ and SP of the dorsal telencephalon, these cells contained very low or no detectable level of Mash1 in contrast to PDGFR-α in the GE (compare with *A*). (*E*) At E75 many cells double-labeled for PDGFR-α and Mash1 were now observed in the SVZ and IZ of the dorsal telencephalon. (*F, G*) In the SP (*F*) and CP (*G*) presumed PDGFR-α containing oligodendrocytes did not show detectable level of Mash1. Scale bars = 10 µm (*A, C, D, F, G*), and 50 µm (*B, E*).

GAD67 IHC Labeling

Many cell bodies and processes were labeled with GAD67-specific antiserum K2, however the pattern of labeling differed from that obtained with GAD65-specific antibody. The vast majority of GAD67-containing cells corresponded to more differentiated neurons, that is, neurons with strongly labeled cell bodies and proximal dendrites (Fig. 7*a′*, *b*, *c*, *c′*). Interestingly, a few labeled neurons were observed already in the cortical wall from E47 on, then their number increased considerably with different time courses according to the different layers (Fig. 7*A–C*). They were located in the MZ and just under the CP, in the developing SP, at E47–E55 (Fig. 7*a′*). It is mainly in that layer that their number increased from E55 to E75 (Fig. 7*A–C*). In contrast in the developing CP, very few GAD67-containing neurons were observed before E75. Their number increased significantly at E75–E88 and they displayed

morphological features of more differentiated maturing neurons (Fig. 7*c′*).

A few migrating-like GAD67-containing neurons were also clearly observed from E47 to E88 in the IZ, SP, MZ, and CP (Fig. 7*A*, *a*, *b*), but in a much lower number than migrating-like GAD65-labeled neurons at all developmental stages studied.

Finally, progenitor-like GAD67-labeled cells were rarely detected in the proliferative region of the entire telencephalon including the GE, septal region and VZ and SVZ of the cortical wall at all developmental stages studied.

Discussion

A major goal of the present study was to determine whether in nonhuman primates neocortical GABAergic neurons originate from two different sites of origin (i.e., dorsal and ventral telencephalon) as in human (Letinic et al. 2002; Rakic and
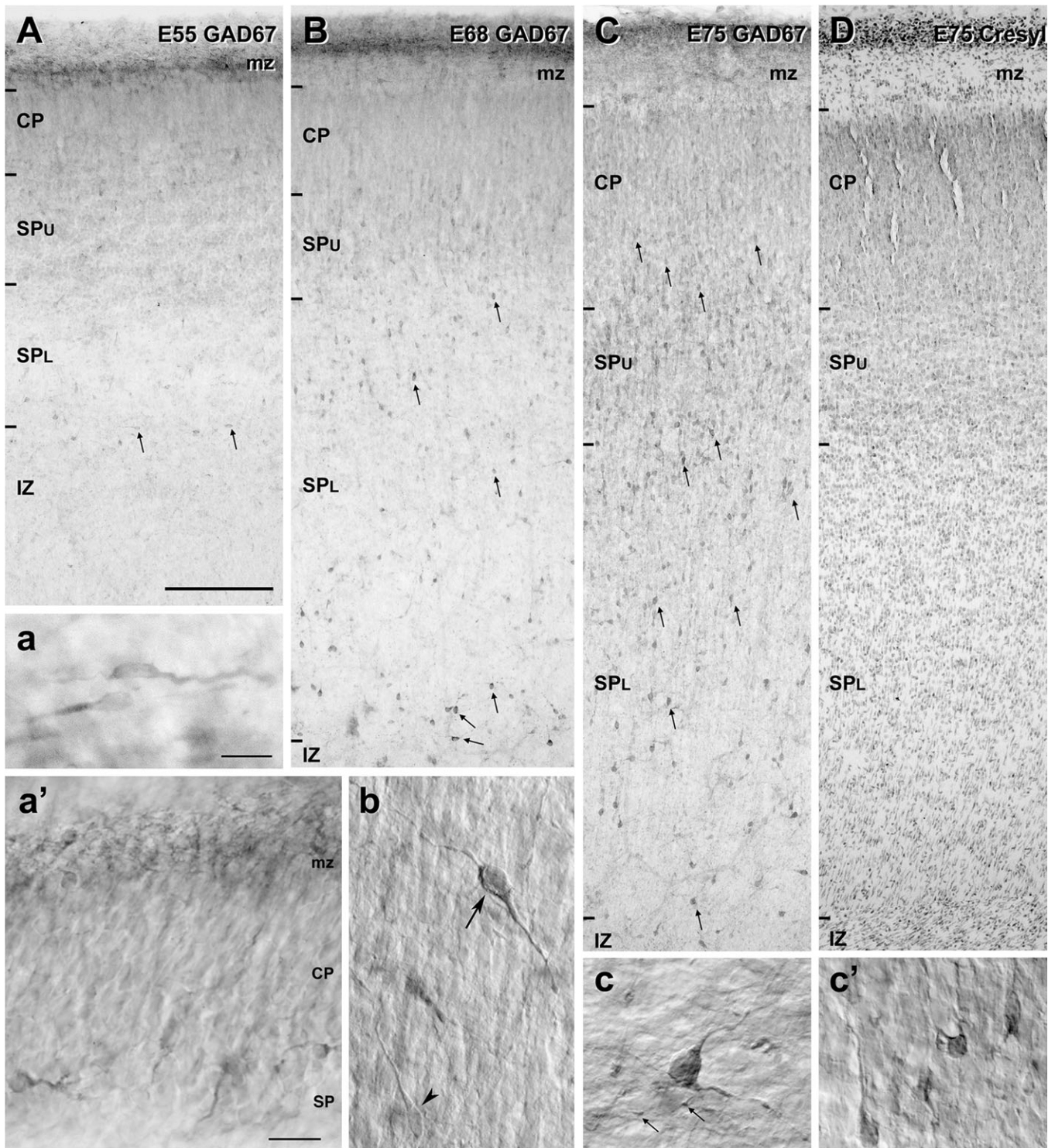
Figure 7. GAD67 labeling patterns in coronal sections of monkey fetal telencephalon at E55 (*A*, *a*, *a'*), E68 (*B*, *b*) and E75 (*C*, *c*, *c'*). (*A*) At E55 as at earlier stage of cortical development, GAD67-containing neurons were mainly present in the MZ and below the CP in the upper part of the subplate (SPu). In the MZ these neurons were surrounded by dense plexus of stained fibers as illustrated at higher magnification (inset *a'*). Some neurons were also observed in the IZ (arrows). Many of them in this layer displayed a unipolar migratory-like morphology (inset a). (*B*) At E68 a general increased density of GAD67-labeled neurons was observed but this increase was particularly striking in the lower part of the subplate (SPL) (arrows). Inset b: In this later layer, many GAD67-labeled cells corresponded to morphologically differentiated neurons, that is, neurons with strong labeling of the cell body and proximal dendrites (arrow). These neurons contrast with migratory-like postmitotic neurons lightly labeled for GAD67 (arrowhead). (*C*) At E75 the thickness of the SP and the density of GAD67-containing neurons further increased. More GAD67-labeled neurons were also observed in the MZ and the IZ. Some GAD67-containing neurons were now present in the CP (arrows). Higher magnification illustrating morphologically differentiated neurons and axon terminals labeled for GAD67 in the SP (inset c) and in the CP (inset *c'*). (*D*) At E75 Adjacent section stained for cresyl violet showed that the SP is at this age up to 3 times thicker than the CP. Scale bars = 200 μm (*A–D*), 50 μm (*a'*, b, c, *c'*), 25 μm (*a*).

Zecevic, 2003a) or whether as in rodents (Anderson et al. 1999, 2002; Lavdas et al. 1999; Nery et al. 2002; Xu et al. 2003, 2004; Wonders and Anderson 2006) they are produced almost exclusively by the proliferative regions of the ventral telencephalon. The present findings strongly support that in the cynomolgus monkey cortical GABAergic neurons are generated not only in the proliferative region of the ventral telencephalon, the GE and septal region but also in the VZ/SVZ of the dorsal telencephalon (prospective cortex). These data thus extend to a nonhuman primate, the previous demonstration of two distinct sites of origin for neocortical GABAergic neurons reported in human (Letinic et al. 2002).

Two main other features are reported for the first time. Neurogenesis of GABAergic neurons which is induced very early during monkey brain development (i.e., at the beginning of the cortical neurogenesis, Rakic 2002), in both ventral and dorsal telencephalon occurs however with different temporal profiles, starting first in the ventral telencephalon and then in the proliferative regions of the dorsal telencephalon. Secondly, the present data further suggest that cortical GABAergic neurons from dorsal and ventral origins display different laminar distribution. Finally, we discuss the differential regulation of the two GABA synthesizing enzymes (GAD65 and GAD67) during GABAergic neurons differentiation.

Considerations about Developmental Stage and Neural Lineages of the Different Populations of Mash1- and GAD-Containing Cells

The role of the bHLH transcription factor Mash1 in the early specification of GABAergic neurons has been demonstrated in several studies (Guillemot and Joyner 1993; Porteus et al. 1994; Casarosa et al. 1999; Guillemot 1999; Horton et al. 1999; Fode et al. 2000; Bertrand et al. 2002; Schuurmans and Guillemot 2002; Nakada et al. 2004) and many studies in the rat and human have reported the presence of GABA in Mash1-containing progenitors (Fode et al. 2000; Letinic et al. 2002). In this study, we demonstrate, that the vast majority of these Mash1-containing progenitors observed in the proliferative zones of the ventral and dorsal telencephalon coexpress GAD65, one of the synthesizing enzymes for GABA, thus confirming the GABAergic phenotype of these cells. Our results further confirm that these GAD65-containing cells labeled for Mash1 observed in the proliferative zones of the ventral telencephalon from E47 on, and in the dorsal telencephalon from E64 on, display features of progenitors cells including mitotic properties characterized by their immunoreactivity for Ki67.

The data obtained in the GE at E47–E55, further indicate a spatial gradient of expression for Mash1 and GAD65 within the ventral telencephalon that suggests a sequential regulation in the expression of these two proteins during neuronal differentiation. Early progenitors in the VZ and deep SVZ of the GE express high level of Mash1 whereas only some of them express GAD65. The intensity of labeling for Mash1 strongly decreases in the SVZ, but the vast majority of these progenitors express GAD65. When these GAD65-containing progenitors become migrating cells and leave the GE, they do not express detectable level of Mash1. Furthermore, these migrating-like GAD65-containing cells are not labeled for Ki67 and therefore correspond to early postmitotic neurons. These converging data demonstrate that progenitors of GABAergic neurons are labeled for Mash1, whereas postmitotic migrating GAD65-

containing neurons do not express Mash1. These results confirm and further extend to nonhuman primates previously reported data obtained in human fetuses (Letinic et al. 2002).

The transcription factor Mash1 has been shown to be associated also to specification of a nonneuronal cell type, the oligodendrocyte (Nieto et al. 2001; Parras et al. 2004, 2007). Our data showing the presence of cells double-labeled for both Mash1 and PDGFR-α support these results. These Mash1/PDGFR-α cells that were observed first in the GE at E47–E55 and then in the SVZ/IZ of the dorsal telencephalon from E64 on, are in keeping with previous studies that report oligodendrocyte genesis in both ventral and dorsal telencephalon in rodents (Kessaris et al. 2006) and in humans (Ulfig et al. 2002; Rakic and Zecevic 2003b; Jakovcevski and Zecevic 2005). In addition, our results clearly illustrate, that at these developmental stages, Mash1/GAD65-containing progenitors are more numerous than Mash1/PDGFR-α progenitors in the GE as well as in the SVZ/IZ of the dorsal telencephalon. These two populations of progenitors likely provide respectively migrating-like GAD65-containing neurons and PDGFR-α–containing oligodendrocytes.

In the Cynomolgus Monkey Cortical GABA Neurons are Generated in the Proliferative Regions of both Ventral and Dorsal Telencephalon

In macaque from at least E47 on, a contingent of cortical GABAergic neurons is produced in the GE. At E47–E55, in addition to progenitor-like cells containing Mash1 and GAD65 located in the proliferative zone (SVZ and VZ) of GE, our data clearly illustrate a large contingent of GAD65-containing cells with clear morphological features of postmitotic migrating neurons leaving the GE and displaying a main tangential orientation in the upper SVZ and lower IZ of the dorsal telencephalic wall. Such migratory pathways of GABAergic neurons from the GE to the prospective cortical region are similar to those previously reported in rodents (DeDiego et al. 1994; Tamamaki et al. 1997; Anderson et al. 2001; Marin and Rubenstein 2001, 2003; Jimenez et al. 2002; Nadarajah and Parnavelas 2002; Polleux et al. 2002) and were also described in human fetuses (Letinic et al. 2002).

In addition to this GE origin, our data demonstrate that cortical GABAergic neurons are also produced in the proliferative zones of the dorsal telencephalon. Indeed from E64 on, that is, 2 weeks later than in the GE, many Mash1-containing progenitors that do coexpress GAD65 are now present in the SVZ and VZ of the entire dorsal telencephalon. These double-labeled cells display features of proliferative cells and these neuronal progenitors are clearly morphologically different from adjacent postmitotic GAD65-containing migrating neurons. The latter as mentioned before are not labeled for Mash1 and display clear leading processes. Many of these migrating neurons display nontangential orientation as opposed to the tangentially oriented migrating neurons observed at earlier stages (E47–E55). The multidirectional orientations of migrating-like GAD65 neurons at E64–E75 and the presence of GAD65-containing progenitors in the VZ and SVZ of the dorsal telencephalon strongly support that many migrating GAD65-containing neurons originate from precursors of GABAergic neurons in the prospective cortex.

Furthermore, our results strongly support the view that the progenitors of GABAergic neurons observed from E64 on in the proliferative zones of the prospective neocortex are generated locally rather than to migrate from the GE to the proliferative

zones of the dorsal telencephalon. We observed at E47–E55, an expression of Mash1 in the VZ/SVZ of the most dorsal part of the dorsal telencephalon whereas no Mash1-containing cells were observed in the dorsal telencephalic wall close to the GE, arguing against a migration of these progenitors from the GE to the dorsal telencephalon. At these earlier ages, the Mash1-containing cells in the dorsal telencephalon do not contain GAD65 and their GABAergic phenotype could be discussed. We cannot completely exclude that the Mash1-containing cells could generate other types of cells than GABAergic neurons such as oligodendrocytes but these cells do not contain PDGFR-α. It is thus likely that this expression of Mash1 in the dorsal telencephalon at E47–E55 reflects the beginning of GABAergic neurogenesis in this region since at E64 the vast majority of Mash1-containing cells in the VZ/SVZ of the entire dorsal telencephalon express GAD65, whereas only a few Mash1-containing cells express PDGFR-α and these oligodendrocyte progenitors cells are mainly located in the SVZ/IZ.

Temporal Differences in the Generation and Laminar Targets of Cortical GABAergic Neurons

Our data further demonstrate that induction of neurogenesis for GABA neurons occurs earlier in the GE and septal proliferative regions than in the proliferative zones of the prospective neocortex. GABAergic neurons progenitors containing Mash1 and GAD65 are present as early as E47 in the GE and only twenty days later in the proliferative zone of the dorsal telencephalon. In addition, the data obtained also suggest that GABAergic neurons observed in the MZ and SP are generated in the subcortical proliferative region such as the GE whereas those in the CP are likely generated in both subcortical and cortical proliferative zones. Indeed, as early as E47, morphologically differentiated GAD67-containing neurons were observed first in the MZ and the SP just below the CP. A similar pattern of early generated GABAergic neurons was described in human fetuses (Zecevic and Milosevic 1997; Meyer et al. 2000). Their number increases in the SP with the development of this layer between E47 and E68. The fact that GAD67-containing neurons in the MZ and SP began to morphologically differentiate at a period during which GABAergic progenitors are mainly observed in the ventral telencephalon and the routes of migrating GABA neurons are mainly tangential strongly favors their generation in the GE. The role of such early generated GABAergic neurons in the MZ and SP has still to be demonstrated, however it has been shown that SP neurons (Hanganu et al. 2002) as well as early generated neurons in the hippocampal MZ are functional and play a crucial role in early generated network activity (Khazipov et al., 2001). From E68–E75 on GABAergic neurons integrate the CP following an inside-out gradient. Their number increases during a period where progenitors of GABAergic neurons are also massively produced in the proliferative regions of the entire dorsal telencephalon suggesting that in primates, a large proportion of cortical GABA neurons could also originate from the pool generated in proliferative cortical regions.

Preferential Expression of the Two Synthesizing Enzymes for GABA (GAD65 and GAD67) during Neuronal Maturation

The present data suggest that the two isoforms of GAD are differently regulated at the protein level during development of GABAergic neurons. GAD65 is preferentially expressed in progenitors and early postmitotic migrating neurons, whereas GAD67 is preferentially expressed in GABA neurons that start to morphologically differentiate. Thus, Mash1-containing progenitors do contain GAD65 whereas few of these cells are detectable with GAD67 antibodies. In addition many neurons with profile of tangentially migrating neurons were labeled for GAD65 as previously reported in the rat (Del Rio et al. 1992) and human (Meyer et al. 2000) but at a much lower extent for GAD67. In contrast many differentiated GABAergic neurons were labeled for GAD67. All these data suggest the presence of the two isoforms in all prospective and mature GABA neurons. Their differential regulation at the protein level during development could serve different function, but this has to be demonstrated.

Conclusion

In conclusion, in monkeys as in humans, cortical GABAergic neurons are generated in the proliferative cortical regions of the dorsal telencephalon, in addition to the proliferative regions of the ventral telencephalon. This neurogenesis of GABAergic neurons occurs very early during brain development in both ventral and dorsal telencephalon but with distinct temporal profiles. It starts at the beginning of the main neurogenesis period in the ventral telencephalon and only 2 weeks later in the dorsal telencephalon. Strong arguments favor a direct induction of this later event in the prospective neocortex rather than to result from migration of progenitors from the proliferative region of the ventral telencephalon. The dorsal telencephalic origin of neocortical GABAergic neurons is therefore not human-specific and appears as a former event in the ascent of evolution. This mechanism could take place through mammalian evolution in order to provide GABA neurons to an expending neocortex.

Notes

Conflict of Interest: None declared.

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References

Anderson SA, Eisenstat DD, Shi L, Rubenstein JL. 1997. Interneuron migration from basal forebrain to neocortex: dependence on Dlx genes. Science. 17:474–476.

Anderson SA, Kaznowski CE, Horn C, Rubenstein JL, McConnell SK. 2002. Distinct origins of neocortical projection neurons and interneurons in vivo. Cereb Cortex. 12:702–709.

Anderson SA, Marin O, Horn C, Jennings K, Rubenstein JL. 2001. Distinct cortical migrations from the medial and lateral ganglionic eminences. Development. 128:353–363.

Anderson S, Mione M, Yun K, Rubenstein JL. 1999. Differential origins of neocortical projection and local circuit neurons: role of Dlx genes in neocortical interneuronogenesis. Cereb Cortex. 9:646–654.

Anderson SA, Qiu M, Bulfone A, Eisenstat DD, Meneses J, Pedersen R, Rubenstein JL. 1997. Mutations of the homeobox genes Dlx-1 and Dlx-2 disrupt the striatal subventricular zone and differentiation of late born striatal neurons. Neuron. 19:27–37.

Arcelli P, Frassoni C, Regondi MC, De Biasi S, Spreafico R. 1997. GABAergic neurons in mammalian thalamus: a marker of thalamic complexity? Brain Res Bull. 42:27–37.

Ball DW, Azzoli CG, Baylin SB, Chi D, Dou S, Donis-Keller H, Cumaraswamy A, Borges M, Nelkin BD. 1993. Identification of a human achaete-scute homolog highly expressed in neuroendocrine tumors. Proc Natl Acad Sci USA. 90:5648-5652.

Bertrand N, Castro DS, Guillemot F. 2002. Proneural genes and the specification of neural cell types. Nat Rev Neurosci. 3:517-530.

Casarosa S, Fode C, Guillemot F. 1999. Mash1 regulates neurogenesis in the ventral telencephalon. Development. 126:525-534.

Chang YC, Gottlieb DI. 1988. Characterization of the proteins purified with monoclonal antibodies to glutamic acid decarboxylase. J Neurosci. 8:2123-2130.

Corbin JG, Nery S, Fishell G. 2001. Telencephalic cells take a tangent: non-radial migration in the mammalian forebrain. Nat Neurosci. 4:1177-1182.

De Carlos JA, Lopez-Mascaraque L, Valverde F. 1996. Dynamics of cell migration from the lateral ganglionic eminence in the rat. J Neurosci. 16:6146-6156.

DeDiego I, Smith-Fernandez A, Fairen A. 1994. Cortical cells that migrate beyond area boundaries: characterization of an early neuronal population in the lower intermediate zone of prenatal rats. Eur J Neurosci. 6:983-997.

DeFelipe J. 2002. Cortical interneurons: from Cajal to 2001. Prog Brain Res. 136:215-238.

DeFelipe J. 2004. Cortical microanatomy and human brain disorders: epilepsy. Cortex. 40:232-233.

Del Rio JA, Soriano E, Ferrer I. 1992. Development of GABA-immunoreactivity in the neocortex of the mouse. J Comp Neurol. 326:501-526.

Esclapez M, Petanjek Z, Ben-Ari Y, Berger B. 2004. Origin of cortical GABAergic neurons in cynomolgus monkey. Society for Neuroscience. 34th Annual Meeting, San Diego (CA), 23-27.10.2004. Program No. 493.11.

Esclapez M, Tillakaratne NJ, Tobin AJ, Houser CR. 1993. Comparative localization of mRNAs encoding two forms of glutamic acid decarboxylase with nonradioactive in situ hybridization methods. J Comp Neurol. 331:339-362.

Esclapez M, Tillakaratne NJ, Kaufman DL, Tobin AJ, Houser CR. 1994. Comparative localization of two forms of glutamic acid decarboxylase and their mRNAs in rat brain supports the concept of functional differences between the forms. J Neurosci. 14:1834-1855.

Fode C, Ma Q, Casarosa S, Ang SL, Anderson DJ, Guillemot F. 2000. A role for neural determination genes in specifying the dorsoventral identity of telencephalic neurons. Genes Dev. 14:67-80.

Gabbott PL, Bacon SJ. 1996. Local circuit neurons in the medial prefrontal cortex (areas 24a,b,c, 25 and 32) in the monkey II: Quantitative areal and laminar distributions. J Comp Neurol. 364:609-636.

Gabbott PL, Dickie BG, Vaid RR, Headlam AJ, Bacon SJ. 1997. Local-circuit neurones in the medial prefrontal cortex (areas 25, 32 and 24b) in the rat: morphology and quantitative distribution. J Comp Neurol. 377:465-499.

Guillemot F. 1999. Vertebrate bHLH genes and the determination of neuronal fates. Exp Cell Res. 253:357-364.

Guillemot F, Joyner AL. 1993. Dynamic expression of the murine achaete-scute homologue Mash-1 in the developing nervous system. Mech Dev. 42:171-185.

Hanganu IL, Kilb W, Luhmann HJ. 2002. Functional synaptic onto subplate neurons in neonatal rat somatosensory cortex. J Neurosci. 22:7165-7176.

Hendrickson AE, Tillakaratne NJ, Mehra RD, Esclapez M, Erickson A, Vician L, Tobin AJ. 1994. Differential localization of two glutamic acid decarboxylases (GAD65 and GAD67) in adult monkey visual cortex. J Comp Neurol. 343:566-581.

Hendry SH, Schwark HD, Jones EG, Yan J. 1987. Numbers and proportions of GABA-immunoreactive neurons in different areas of monkey cerebral cortex. J Neurosci. 7:1503-1519.

Horton S, Meredith A, Richardson JA, Johnson JE. 1999. Correct coordination of neuronal differentiation events in ventral forebrain requires the bHLH factor MASH1. Mol Cell Neurosci. 14:355-369.

Houser CR. 1999. Neuronal loss and synaptic reorganization in temporal lobe epilepsy. Adv Neurol. 79:743-761.

Houser CR, Vaughn JE, Barber RP, Roberts E. 1980. GABA neurons are the major cell type of the nucleus reticularis thalami. Brain Res. 200:341-354.

Houser CR, Vaughn JE, Hendry SHC, Jones EG, Peters A. 1984. GABA neurons in the cerebral cortex. In: Jones EGPA, editor. Cerebral cortex, Vol. 2. Functional properties of cortical cells. New York: Plenum. p. 63-89.

Jakovcevski I, Zecevic N. 2005. Olig transcription factors are expressed in oligodendrocyte and neuronal cells in human fetal CNS. J Neurosci. 25:10064-10073.

Jimenez D, Lopez-Mascaraque LM, Valverde F, De Carlos JA. 2002. Tangential migration in neocortical development. Dev Biol. 244:155-169.

Jones EG. 1993. GABAergic neurons and their role in cortical plasticity in primates. Cereb Cortex. 3:361-372.

Kaufman DL, Houser CR, Tobin AJ. 1991. Two forms of the gamma-aminobutyric acid synthetic enzyme glutamate decarboxylase have distinct intraneuronal distributions and cofactor interactions. J Neurochem. 56:720-723.

Kessaris N, Fogarty M, Iannarelli P, Grist M, Wegner M, Richardson WD. 2006. Competing waves of oligodendrocytes in the forebrain and postnatal elimination of an embryonic lineage. Nat Neurosci. 9:173-179.

Khazipov R, Esclapez M, Caillard O, Bernard C, Khalilov I, Tyzio R, Hirsch J, Dzhala V, Berger B, Ben-Ari Y. 2001. Early development of neuronal activity in the primate hippocampus in utero. J Neurosci. 21:9770-9781.

Kornack DR. 2000. Neurogenesis and the evolution of cortical diversity: mode, tempo, and partitioning during development and persistence in adulthood. Brain Behav Evol. 55:336-344.

Lavdas AA, Grigoriou M, Pachnis V, Parnavelas JG. 1999. The medial ganglionic eminence gives rise to a population of early neurons in the developing cerebral cortex. J Neurosci. 19:7881-7888.

Letinic K, Kostovic I. 1997. Transient fetal structure, the gangliothalamic body, connects telencephalic germinal zone with all thalamic regions in the developing human brain. J Comp Neurol. 384:373-395.

Letinic K, Rakic P. 2001. Telencephalic origin of human thalamic GABAergic neurons. Nat Neurosci. 4:931-936.

Letinic K, Zoncu R, Rakic P. 2002. Origin of GABAergic neurons in the human neocortex. Nature. 417:645-649.

Levitt P. 2005a. Developmental neurobiology and clinical disorders: lost in translation? Neuron. 46:407-412.

Levitt P. 2005b. Disruption of interneuron development. Epilepsia. 46:22-28.

Levitt P, Eagleson KL, Powell EM. 2004. Regulation of neocortical interneuron development and the implications for neurodevelopmental disorders. Trends Neurosci. 27:400-406.

Lewis DA. 2000. GABAergic local circuit neurons and prefrontal cortical dysfunction in schizophrenia. Brain Res Brain Res Rev. 31:270-276.

Lewis DA, Hashimoto T, Volk DW. 2005. Cortical inhibitory neurons and schizophrenia. Nat Rev Neurosci. 6:312-324.

Lu QR, Yuk D, Alberta JA, Zhu Z, Pawlitzky I, Chan J, McMahon AP, Stiles CD, Rowitch DH. 2000. Sonic hedgehog-regulated oligodendrocyte lineage genes encoding bHLH proteins in the mammalian central nervous system. Neuron. 25:317-329.

Marin O, Rubenstein JL. 2001. A long, remarkable journey: tangential migration in the telencephalon. Nat Rev Neurosci. 2:780-790.

Marin O, Rubenstein JL. 2003. Cell migration in the forebrain. Annu Rev Neurosci. 26:441-483.

Meyer G, Schaaps JP, Moreau L, Goffinet AM. 2000. Embryonic and early fetal development of the human neocortex. J Neurosci. 20:1858-1868.

Nadarajah B, Alifragis P, Wong RO, Parnavelas JG. 2002. Ventricle-directed migration in the developing cerebral cortex. Nat Neurosci. 5:218-224.

Nadarajah B, Alifragis P, Wong RO, Parnavelas JG. 2003. Neuronal migration in the developing cerebral cortex: observations based on real-time imaging. Cereb Cortex. 13:607-611.

Nadarajah B, Parnavelas JG. 2002. Modes of neuronal migration in the developing cerebral cortex. Nat Rev Neurosci. 3:423-432.

Nakada Y, Hunsaker TL, Henke RM, Johson JE. 2004. Distinct domains within Mash1 and Math1 are required for function in neuronal differentiation versus neuronal cell type-specification. Development. 131:1319-1390.

Nery S, Fishell G, Corbin JG. 2002. The caudal ganglionic eminence is a source of distinct cortical and subcortical cell populations. Nat Neurosci. 5:1279-1287.

Nieto M, Schuurmans C, Britz O, Guillemot F. 2001. Neural bHLH genes control the neuronal versus glial fate decision in cortical progenitors. Neuron. 29:401-413.

Parnavelas JG. 2000. The origin and migration of cortical neurones: new vistas. Trends Neurosci. 23:126-131.

Parnavelas JG. 2002. The origin of cortical neurons. Braz J Med Biol Res. 35:1423-1429.

Parnavelas JG, Alifragis P, Nadarajah B. 2002. The origin and migration of cortical neurons. Prog Brain Res. 136:73-80.

Parnavelas JG, Anderson SA, Lavdas AA, Grigoriou M, Pachnis V, Rubenstein JL. 2000. The contribution of the ganglionic eminence to the neuronal cell types of the cerebral cortex. Novartis Found Symp. 228:129-139.

Parras CM, Galli R, Britz O, Soares S, Galichet C, Battiste J, Johnson JE, Nakafuku M, Vescovi A, Guillemot F. 2004. Mash1 specifies neurons and oligodendrocytes in the postnatal brain. EMBO J. 23:4495-4505.

Parras CM, Hunt C, Sugimori M, Nakafuku M, Rowitch D, Guillemot F. 2007. The proneural gene Mash1 Specifies an Early Population of Telencephalic Oligodendrocytes. J Neurosci. 27:4233-4242.

Pleasure SJ, Anderson S, Hevner R, Bagri A, Marin O, Lowenstein DH, Rubenstein JL. 2000. Cell migration from the ganglionic eminences is required for the development of hippocampal GABAergic interneurons. Neuron. 28:727-740.

Polleux F, Whitford KL, Dijkhuizen PA, Vitalis T, Ghosh A. 2002. Control of cortical interneuron migration by neurotrophins and P13-kinase signaling. Development. 129:3147-3160.

Porteus MH, Bulfone A, Liu JK, Puelles L, Lo LC, Rubenstein JL. 1994. DLX-2, MASH-1, and MAP-2 expression and bromodeoxyuridine incorporation define molecularly distinct cell populations in the embryonic mouse forebrain. J Neurosci. 14:6370-6383.

Powell EM, Campbell DB, Stanwood GD, Davis C, Noebels JL, Levitt P. 2003. Genetic disruption of cortical interneuron development causes region- and GABA cell type-specific deficits, epilepsy, and behavioral dysfunction. J Neurosci. 23:622-631.

Rakic P. 1995. A small step for the cell, a giant leap for mankind: a hypothesis of neocortical expansion during evolution. Trends Neurosci. 18:383-388.

Rakic P. 2000. Molecular and cellular mechanisms of neuronal migration: relevance to cortical epilepsies. Adv Neurol. 84:1-14.

Rakic P. 2002. Pre- and post-developmental neurogenesis in primates. Clin Neurosci Res. 2:29-39.

Rakic S, Zecevic N. 2003a. Emerging complexity of layer I in human cerebral cortex. Cereb Cortex. 13:1072-1083.

Rakic S, Zecevic N. 2003b. Early Oligodendrocyte progenitor cells in the human fetal telencephalon. GLIA. 41:117-127.

Schuurmans C, Guillemot F. 2002. Molecular mechanisms underlying cell fate specification in the developing telencephalon. Curr Opin Neurobiol. 12:26-34.

Sidman RL, Rakic P. 1973. Neuronal migration, with special reference to developing human brain: a review. Brain Res. 62:1-35.

Skaggs WE, McNaughton BL, Permenter M, Archibeque M, Vogt J, Amaral DG, Barnes CA. 2007. EEG sharp waves and sparse ensemble unit activity in the macaque hippocampus. J Neurophysiol. 98:898-910.

Sloviter RS, Dichter MA, Rachinsky TL, Dean E, Goodman JH, Sollas AL, Martin DL. 1996. Basal expression and induction of glutamate decarboxylase and GABA in excitatory granula cells of the rat and monkey hippocampal dentate gyrus. J Comp Neurol. 373:593-618.

Smith Y, Seguela P, Parent A. 1987. Distribution of GABA-immunoreactive neurons in the thalamus of the squirrel monkey (Saimiri sciureus). Neuroscience. 22:579-591.

Somogyi P, Klausberger T. 2005. Defined types of cortical interneurone structure space and spike timing in the hippocampus. J Physiol. 562:9-26.

Sussel L, Marin O, Kimura S, Rubenstein JL. 1999. Loss of Nkx2.1 homeobox gene function results in a ventral to dorsal molecular respecification within the basal telencephalon: evidence for a transformation of the pallidum into the striatum. Development. 126:3359-3370.

Tamamaki N, Fujimori KE, Takauji R. 1997. Origin and route of tangentially migrating neurons in the developing neocortical intermediate zone. J Neurosci. 17:8313-8323.

Tamamaki N, Sugimoto Y, Tanaka K, Takauji R. 1999. Cell migration from the ganglionic eminence to the neocortex investigated by labeling nuclei with UV irradiation via a fiber-optic cable. Neurosci Res. 35:241-251.

Ulfig N, Briese M, Bohl J. 2002. Expression of oligo dendrocyte-specific protein (OSP)/claudin-11 in the human fetal forebrain. Neuroembryology. 1:48-53.

Uylings HB, van Eden CG. 1990. Qualitative and quantitative comparison of the prefrontal cortex in rat and in primates, including humans. Prog Brain Res. 85:31-62.

Van Eden CG, Mrzljak L, Voorn P, Uylings HB. 1989. Prenatal development of GABA-ergic neurons in the neocortex of the rat. J Comp Neurol. 289:213-227.

Wang XJ, Tegner J, Constantinidis C, Goldman-Rakic PS. 2004. Division of labor among distinct subtypes of inhibitory neurons in a cortical microcircuit of working memory. Proc Natl Acad Sci USA. 101:1368-1373.

Wonders C, Anderson SA. 2006. The origin and specification of cortical interneurons. Nat Rev Neurosci. 7:687-696.

Xu Q, Cobos I, De La Cruz E, Rubenstein JL, Anderson SA. 2004. Origins of cortical interneuron subtypes. J Neurosci. 24:2612-2622.

Xu Q, de la Cruz E, Anderson SA. 2003. Cortical interneuron fate determination: diverse sources for distinct subtypes? Cereb Cortex. 13:670-676.

Zecevic N, Milosevic A. 1997. Initial development of gamma-aminobutyric acid immunoreactivity in the human cerebral cortex. J Comp Neurol. 380:495-506.

Zecevic N, Rakic P. 2001. Development of layer I neurons in the primate cerebral cortex. J Neurosci. 21:5607-5619.