Nuclear Receptor COUP-TFII-Expressing Neocortical Interneurons Are Derived from the Medial and Lateral/Caudal Ganglionic Eminence and Define Specific Subsets of Mature Interneurons

Yuqun Cai,1 Qiangqiang Zhang,1 Congmin Wang,1 Yue Zhang,1 Tong Ma,1 Xing Zhou,1 Miao Tian,1 John L.R. Rubenstein,2 and Zhengang Yang1*

1Institutes of Brain Science and State Key Laboratory of Medical Neurobiology, Fudan University, Shanghai, 200032, China
2Department of Psychiatry, Nina Ireland Laboratory of Developmental Neurobiology, University of California San Francisco, San Francisco, California 94158, USA

ABSTRACT

Neocortical GABAergic interneurons in rodents originate from subpallial progenitor zones. The majority of mouse neocortical interneurons are derived from the medial and caudal ganglionic eminences (MGE and CGE, respectively) and the preoptic area (POA). It is controversial whether the lateral ganglionic eminence (LGE) also generates neocortical interneurons. Previously it was shown that the transcription factor COUP-TFII is expressed in the CGE; here we show that COUP-TFII is also expressed in the dorsal MGE, dorsal LGE (dMGE and dLGE, respectively), and POA. In the adult neocortex, COUP-TFII+/somatostatin (SOM)+ interneurons are mainly located in layer V. Using a genetic fate-mapping approach (Shh-Cre and Nkx2.1-Cre), we demonstrate that the POA and ventral MGE do not give rise to COUP-TFII+ neocortical interneurons, suggesting that the dMGE is the source of COUP-TFII+/SOM+ neocortical interneurons. We also observed a migratory stream of COUP-TFII+/Sp8+ cells emanating from the dLGE to the neocortex mainly through the subventricular zone at later embryonic stages. Slice culture experiments in which dLGE progenitors were labeled with BrdU provided additional evidence that the dLGE generates neocortical interneurons. While earlier-born dMGE-derived COUP-TFII+ interneurons occupy cortical layer V, later-born dLGE- and CGE-derived COUP-TFII+ ones preferentially occupy superficial cortical layers. A similar laminar distribution was observed following neonatal transplantation of embryonic day (E)14.5 dMGE and E15.5 dLGE. These results provide novel information about interneuron fate and position from spatially and temporally distinct origins in the ganglionic eminences. J. Comp. Neurol. 521:479–497, 2013.

© 2012 Wiley Periodicals, Inc.

INDEXING TERMS: COUP-TFII; ganglionic eminence; interneurons; neocortical development; neural progenitor cells; Sp8

About 80% of neurons in the neocortex are excitatory glutamatergic projection neurons and 20% are inhibitory GABAergic interneurons. Unlike projection neurons that originate in the ventricular zone (VZ) of the embryonic neocortex, gamma-aminobutyric acid (GABA)ergic neurons in rodents derive from the ganglionic eminences of the ventral telencephalon and tangentially migrate into the neocortex (Marin and Rubenstein, 2001; Metin et al., 2006; Wonders and Anderson, 2006; Batista-Brito and Fishell, 2009; Gelman et al., 2012). Increasing evidence suggests that defects in neocortical interneurons contribute to human neurodevelopmental disorders such as epilepsy, autism, and schizophrenia (Lewis et al., 2005; Marin, 2012). Moreover, recent studies using interneuron transplants have suggested the possibility of a cell-based therapy to modulate aberrant brain activity in the

Grant sponsor: National Basic Research Program of China; Grant numbers: 2011CB504400 and 2010CB945500; Grant sponsor: National Natural Science Foundation of China; Grant numbers: 30970949, 30990261, 31028009 and 31121061; Grant sponsor: Shanghai Shuguang Project; Grant number: 09SG05.

The first two authors contributed equally to this work.

*Correspondence to: Zhengang Yang, Ph.D., Institutes of Brain Science and State Key Laboratory of Medical Neurobiology, Fudan University, 138 Yi Xue Yuan Road, Shanghai 200032, China. E-mail: yangz@fudan.edu.cn

Received April 5, 2012; Revised June 5, 2012; Accepted July 6, 2012
DOI 10.1002/cne.23186
Published online July 13, 2012 in Wiley Online Library (wileonlinelibrary.com)
treatment of neurological disorders characterized by an imbalance between excitation and inhibition, such as seizure disorders (Alvarez-Dolado et al., 2006; Baraban et al., 2009; Waldau et al., 2010; Zipancic et al., 2010) and Parkinson’s disease (Martinez-Cerdeno et al., 2010). The temporal and spatial origins of interneuron subclasses have been intensively investigated. The majority of mouse neocortical interneurons are derived from the medial and caudal ganglionic eminence (MGE and CGE, respectively) (Sussel et al., 1999; Lavdas et al., 1999; Wichterle et al., 2001; Nery et al., 2002; Xu et al., 2004; Butt et al., 2005; Cobos et al., 2005; Fogarty et al., 2007; Miyoshi et al., 2007, 2010; Rubin et al., 2010; Vucurovic et al., 2010) and the preoptic area (POA) (Gelman et al., 2009, 2011). Although several studies already provided some evidence that the lateral ganglionic eminence (LGE) may contribute to at least some interneurons for the neocortex (de Carlos et al., 1996; Tamamaki et al., 1997; Anderson et al., 1997, 2001; Jimenez et al., 2002; Rubin et al., 2010; Ma et al., 2011; Wu et al., 2011; Riccio et al., 2012), other studies showed that the CGE and POA generate nearly all neocortical interneurons outside of the MGE (Lee et al., 2010; Gelman et al., 2011; Rudy et al., 2011). Thus, it still remains to be proven whether the LGE produces neocortical interneurons.

In the present study we show that at later embryonic stages interneurons migrating into the cortical subventricular zone (SVZ) from the dorsal LGE and dorsal CGE (dLGE and dCGE, respectively) express COUP-TFII (chicken ovalbumin upstream promoter-transcriptional factor II; also known as Nr2f2) and Sp8. Slice culture experiments in which progenitors in the dLGE were labeled with bromodeoxyuridine (BrdU) provided evidence that the dLGE generates neocortical interneurons. While earlier-born dorsal MGE (dMGE)-derived COUP-TFII+ interneurons preferentially occupy cortical layer V, later-born dLGE/dCGE-derived COUP-TFII+ ones preferentially occupy superficial cortical layers. A similar laminar distribution was observed following neonatal transplantation of embryonic day (E)14.5 dMGE and E15.5 dLGE. These results provide novel information about interneuron fate and position from spatially and temporally distinct origins in the ganglionic eminences.

MATERIALS AND METHODS

Animals

CD1 mice and Wistar rats were purchased from Shanghai SLAC Laboratory Animal. Enhanced green fluorescent protein (EGFP)-expressing transgenic mouse (Okabe et al., 1997), Shh-Cre (Harfe et al., 2004), Nkx2.1-Cre (Xu et al., 2008), and Rosa26-yellow fluorescent protein (Rosa-YFP) (Srinivas et al., 2001) were obtained from the Jackson Laboratory (Bar Harbor, ME). All lines were maintained in a mixed genetic background of C57BL/6J and CD1. The day of vaginal plug detection was designated E0.5 and the day of birth was designated as postnatal day 0 (P0). All experiments were performed in accordance with institutional guidelines.

BrdU labeling

For BrdU birth dating, timed pregnant female mice (E10.5, E11.5, E12.5, E13.5, E14.5, E15.5, E16.5, E17.5, and E18.5) and P0 mice received a single intraperitoneal injection of BrdU (50 mg/kg, body weight, Sigma, St. Louis, MO).

Slice culture

Pregnant CD1 mice at 14.5 days gestation were killed by cervical dislocation. The embryos were removed quickly into ice-cold Hank’s buffered salt solution (HBSS) (Invitrogen, La Jolla, CA, 14175-095). The brains were dissected and then embedded in 4% low-melting-point agarose (Fisher Scientific, Pittsburgh, PA, BP165-25) prepared using artificial cerebrospinal fluid (ACSF) (KCl, 2.5 mM; NaH2PO4·2H2O, 1.09 mM; NaHCO3, 25 mM; CaCl2, 125 mM; MgCl2·6H2O, 1 mM; D-glucose, 2.5 mM; ascorbic acid, 1.3 mM). Using vibroslicer (Vibratome Series 3000), 300-μm coronal slices were obtained. Only the slices from the anterior part of the forebrain which contains the LGE and/or MGE were used for the culture and those slices containing CGE were excluded. Slices were then rinsed three times and transferred on Millicell-CM inserts (Millipore, Bedford, MA, PICMORG-50) in brain slice culture medium which contains Dulbecco’s modified Eagle’s medium (DMEM)/F12 (1:1) (Invitrogen, 10565-018) supplemented with 25% newborn calf serum (Invitrogen, 16010-142), 1% N2 (Invitrogen, 17502-048), 2% B27 (Invitrogen, 17504-044), 1 mM N-acetylcysteine (NAC, Invitrogen, A9165-5G), and 1% penicillin/streptomycin (Invitrogen). Slices were incubated at 37°C in 5% CO2 for 12 hours and BrdU (1 mg/ml, Sigma) was added and maintained for 24 hours. After removing BrdU, the slices were then fixed using 4% paraformaldehyde (PFA) and cut into 60-μm sections for immunostaining.

Cell transplantation

E14.5 dMGE and E15.5 dLGE were dissected from EGFP-expressing transgenic mouse (Okabe et al., 1997) in ice-cold HBSS. The dLGE and dMGE were dissociated in the medium. The dissociation medium containing DMEM/F12, dispase II (Roche, Nutley, NJ, 10 mg/ml) and DNase I (Invitrogen, 6 μl) was prewarmed in the incubator. The tissue tubes were placed on a rotator for 30 minutes at a low speed (12 rpm) at 37°C. After dissociation the tissues were settled for 1 minute, rinsed using resuspending medium containing DMEM/F12 and 1 mM
NAC, pelleted by centrifugation at 200g for 5 minutes, resuspended, and triturated 10–20 times using a 1 ml pipette, centrifuged at 300g for 5 minutes, and resuspended in 100 μl medium. The density of the isolated cell solutions was about 1–5 × 10^4 cells/μl and were maintained on ice during the transplantation. Cell suspension was loaded into beveled glass micropipettes (≈50 μm diameter). Micropipettes were mounted on a stereotactic apparatus. P1–3 CD1 mice were anesthetized on ice for 3 minutes until pedal reflex was abolished. Cells were bilaterally injected into the neocortex at two different sites (1 × 10^5 cells/site) per hemisphere according to the following coordinates: (2 mm anterior [A], ±3 mm lateral [L], 0.5 mm deep [D], −2 mm A, ±3 mm L, 0.5 mm D). Grafted mice were returned to the dam and analyzed after 4 weeks.

Immunohistochemistry

Postnatal brains were fixed by intracardiac perfusion with 0.9% saline followed by 4% PFA, then postfixed overnight at 4°C in the same fixative. Embryonic brains were dissected and fixed overnight in 4% PFA at 4°C. Brains were cryoprotected for at least 24 hours in 30% sucrose in 0.1M phosphate-buffered (PB, pH 7.4) and then frozen in embedding medium (O.C.T., Sakura Finetek, Torrance, CA) on a dry ice/ethanol slush and stored at −80°C. Immunohistochemistry staining was performed on 30–60-μm brain sections. Primary antibodies, listed in Table 1, were incubated overnight. For BrdU immunostaining sections were pretreated with 2 N HCl for 1 hour at room temperature (RT).

Secondary antibodies against the appropriate species were incubated for 2 hours at RT (all from Jackson Laboratory, Bar Harbor, ME, 1/200). No signal above background was obtained when the primary antibodies were replaced with preimmune sera. All sections were counterstained with DAPI (Sigma, 400 ng/ml, 3 minutes).

Antibody characterization

Please see Table 1 for a list of all antibodies used in this study.

Rat monoclonal anti-BrdU, clone BU1/75 ICR1, reacts with BrdU in single-stranded DNA, BrdU attached to protein carrier or free BrdU (manufacturer’s technical information). No staining was observed in cases in which animals were not infused with BrdU.

Rabbit polyclonal anti-CR recognizes a band of 31 kDa on western blot of rat brain (manufacturer’s technical information). The staining that we obtained in the neocortex was identical to that described previously (Xu et al., 2008).

Mouse anti-COUP-TFII antibody has been systematically tested in a knockout mouse (Qin et al., 2007). The staining pattern that we obtained in the developing mouse telencephalon was identical to that described previously (Tripodi et al., 2004; Kanatani et al., 2008).

The specificity of chicken polyclonal anti-GFP antibody was confirmed by immunohistochemistry using transgenic mice expressing the GFP gene product (manufacturer’s data sheet).

Rabbit polyclonal anti-Nkx2.1 antibody recognizes an Nkx2.1 band at 38 KD from a human lung carcinoma cell

### TABLE 1.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Immunogen</th>
<th>Manufacturer, species, mono- vs. polyclonal, catalog number</th>
<th>Dilution used</th>
</tr>
</thead>
<tbody>
<tr>
<td>BrdU</td>
<td>BrdU</td>
<td>Accurate Chemical (Westbury, NY), rat monoclonal, #OBT0030S</td>
<td>1:500</td>
</tr>
<tr>
<td>Calretinin (CR)</td>
<td>Recombinant rat CR</td>
<td>Chemicon (Temecula, CA), rabbit antiserum, #AB2504</td>
<td>1:3,000</td>
</tr>
<tr>
<td>COUP-TF II</td>
<td>Human COUP TFII amino acids 43-64</td>
<td>Perseus Proteomics (Tokyo, Japan), mouse monoclonal, #PP-H7147-00</td>
<td>1:400</td>
</tr>
<tr>
<td>GFP</td>
<td>Purified recombinant GFP</td>
<td>Aves Labs (Tigard, OR), chicken polyclonal, #GFP-1020</td>
<td>1:1,000</td>
</tr>
<tr>
<td>Nkx2.1</td>
<td>Amino acids 1-190 of human TTF-1</td>
<td>Santa Cruz (Santa Cruz, CA), rabbit polyclonal, #13040</td>
<td>1:500</td>
</tr>
<tr>
<td>PH3</td>
<td>KLH-conjugated peptide corresponding to amino acids 7-20 of human histone H3</td>
<td>Sigma-Aldrich (St. Louis, MO), rabbit polyclonal, #H0412</td>
<td>1:400</td>
</tr>
<tr>
<td>Parvalbumin (PV)</td>
<td>PV purified from rat muscle PV</td>
<td>Swant (Bellinzona, Switzerland), rabbit polyclonal, #PV25</td>
<td>1:1,000</td>
</tr>
<tr>
<td>Somatostatin (SOM)</td>
<td>Synthetic peptide mapping amino acids 84-102 of human SOM</td>
<td>Santa Cruz (Santa Cruz, CA), rabbit polyclonal, #SC-7819</td>
<td>1:100</td>
</tr>
<tr>
<td>Sox6</td>
<td>KLH-conjugated peptide corresponding to residues 800 to the C-terminus of mouse Sox6</td>
<td>Abcam (Cambridge, UK), rabbit polyclonal, #ab-30455</td>
<td>1:4,000</td>
</tr>
<tr>
<td>Sp8</td>
<td>Peptide: amino acids 491-508 of human Sp8</td>
<td>Santa Cruz (Santa Cruz, CA), goat polyclonal, #SC-104461</td>
<td>1:500</td>
</tr>
</tbody>
</table>
line (A549) (manufacturer’s data sheet). The staining pattern that we obtained in the MGE and POA was identical to that described previously (Xu et al., 2008).

Rabbit polyclonal anti-PH3 antibody recognizes histone H3 phosphorylated on Ser10 (17 kDa). This histone H3 sequence is identical in many species and is highly conserved. This antibody recognizes mitotic cells in the M phase of the cell cycle. The staining that we obtained was identical to that described previously (Ma et al., 2011).

Rabbit polyclonal anti-PV antibody labels a subpopulation of neurons in the normal brain with high efficiency, but does not stain the brain of parvalbumin knockout mice (by manufacturer). The stain pattern in the mouse brain was identical to that described previously (Xu et al., 2008).

Goat polyclonal anti-SOM antibody identified a human recombinant SOM fusion protein by western blotting (manufacturer’s technical information). The labeling pattern in the present study was similar to the immunostaining pattern reported previously (Xu et al., 2008).

Rabbit polyclonal anti-Sox6 antibody has been tested in immunohistochemistry in wildtype postnatal mouse brain sections and showed specific staining. No immunoreactivity was observed in control samples of postnatal brain from a Sox6 knockout mouse (manufacturer’s information).

Goat polyclonal anti-Sp8 detects a single 50-kDa band by western blotting. No immunoreactivity was observed in OB sections of Dlx5/6-Cre; Sp8flox/flox mice (Li et al., 2011).

Confocal imaging and quantification

All images presented in the study were acquired using the Olympus FV1000 confocal microscope system. The following filter sets with the indicated wavelengths (in nm) for the excitation laser line and emission filters: DAPI, 405/(LP 420); Cy2, 488/(505-530); Cy3, 543/(560-615); Cy5, 633/(LP 650). Confocal Z sectioning was done at 0.5-3.0-μm intervals if necessary. Images were acquired and a Z-stack was reconstructed using the Olympus FV10-ASW software. Adobe Photoshop CS2 (Adobe Systems, San Jose, CA) was then used to crop images uniformly and apply identical brightness/contrast adjustments.

For quantification of BrdU+/COUP-TFII+ neocortical interneurons from P28 mice, three sections (n = 2 mice per group) spanning primary somatosensory or motor cortex were selected. Cortical layers were delineated by DAPI-stained nucleus. Quantification data are expressed as the mean ± standard error of the mean (SEM).

For quantification of GFP+/COUP-TFII+, interneurons and GFP+/Sp8+ interneurons in the P30 neocortex after E14.5 dMGE or E15.5 dLGE were transplanted into the P1–3 neocortex, 6–9 sections at 480-μm intervals were examined per brain.

RESULTS

COUP-TFII is expressed in the dMGE, POA, CGE, and dLGE

We recently provided evidence that the transcription factor Sp8 is a marker for a subpopulation of neocortical interneurons that are derived from the dCGE, and possibly the dLGE, but not from the MGE or POA (Ma et al., 2011). Moreover, we also found that about one-third of COUP-TFII+ neocortical interneurons express Sox6 (Ma et al., 2011), suggesting that this subset of COUP-TFII+ interneurons are derived from the Nkx2.1-expressing domain (Azim et al., 2009; Batista-Brito et al., 2009).

Here we systematically analyzed the expression of COUP-TFII, in conjunction with Nkx2.1 and Sp8, in the developing mouse telencephalon at E13.5 and E16.5. Previous studies reported COUP-TFII subpallial expression at E13.5 in the CGE, and at the LGE-MGE boundary, but that it was largely excluded from the dLGE (Butt et al., 2008; Kanatani et al., 2008). Consistent with these observations, at E13.5 we found many COUP-TFII+/Nkx2.1+ cells in the dMGE and a large number of COUP-TFII+ cells in the CGE (Fig. 1). We also found a large number of COUP-TFII+/Nkx2.1+ cells in the POA at E13.5, but no detectable COUP-TFII+ cells in the dLGE (Fig. 1). However, by E16.5, surprisingly, COUP-TFII was expressed in the dLGE, dMGE, and CGE (Fig. 2). In the dMGE, we observed COUP-TFII+ cells in the VZ and SVZ (Fig. 1D,F). By contrast, in the dLGE, COUP-TFII+ cells were mainly in the SVZ, with increasing expression from anterior to posterior (Fig. 2B,F). The majority of COUP-TFII+ cells in the dLGE and CGE also expressed Sp8 (Fig. 2) (Ma et al., 2011). In the pallium, COUP-TFII expression was present in Cajal-Retzius cells (Fig. 1B,C) (Tri podi et al., 2004), choroid plexus (Fig. 1C), and ventral pallial structures, particularly in the amygdala (Fig. 2I) (Tang et al., 2012).

Proliferation analysis, using the M-phase marker PH3, found COUP-TFII+/PH3+ cells in the VZ of the dMGE at E13.5 (data not shown). However, at E16.5 no COUP-TFII+/PH3+ cells were found in the SVZ of the dLGE, although some dividing cells, such as Sp8+ cells, were found in this same region (data not shown) (Waclaw et al., 2006; Ma et al., 2011; Wu et al., 2011; Riccio et al., 2012).

At E16.5 there was a migratory stream from the dLGE and CGE to the neocortex (Fig. 2) (Rubin et al., 2010). The majority of cells in the stream were COUP-TFII+/Sp8+, but some cells expressing only COUP-TFII+ or Sp8+ were also found (Fig. 2E). This migration primarily invaded the neocortex through the SVZ (Fig. 2).
Rat brains at E13.5 and E16 were extremely similar to mouse brains with regard to the expression pattern of COUP-TFII and the migration of COUP-TFII+ and Sp8+ cells from the dLGE and CGE to the neocortex (Fig. 3). Taken together, these data provide strong evidence that the CGE is not the only source of COUP-TFII+ neocortical interneurons (Kanatani et al., 2008; Miyoshi et al., 2010), and that the MGE, POA, and dLGE may be additional sources.

COUP-TFII+/SOM+ interneurons mainly occupy neocortical layer V

Previously, it was reported that COUP-TFII+/CR+/SOM+ interneurons are in both superficial layers and deeper layers of the neocortex (Kanatani et al., 2008). Our recent study found that the majority of COUP-TFII+/Sp8+ neocortical interneurons are in superficial layers, whereas COUP-TFII+/Sox6+ neocortical interneurons are mainly in deeper layers (Ma et al., 2011).

Figure 1. Nuclear receptor COUP-TFII expression in the mouse dMGE, POA, and CGE. A–C: COUP-TFII/Nkx2.1 double-immunostained E13.5 mouse brain sections revealed COUP-TFII expression in the dMGE, POA, and CGE, but not in the dLGE. GP, globus pallidus. D–G: Higher magnification of boxed areas in (A,B). The LGE–MGE boundary is indicated by the dashed lines. Note COUP-TFII expression in Cajal-Retzius cells (arrows in B,C) and choroid plexus (arrowheads in C). Scale bars = 200 μm in A–C; 100 μm in D–G. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
Here, we found that COUP-TFII+ /SOM+ interneurons are mainly in layer V of the motor, somatosensory, and visual cortex (Fig. 4A,F). Occasionally, a very small number of COUP-TFII+/SOM+ interneurons in layer IV and VI was found, but none of them were in superficial layers (Fig. 4B–E,G). Furthermore, we observed no CR+/SOM+/COUP-TFII+ neocortical interneurons (Fig. 4H–K). Thus, the vast majority of COUP-TFII+ interneurons in layer V expressed SOM (Fig. 4) and Sox6 (Ma et al., 2011), which represent descendents of Nkx2.1+ progenitors. By contrast, in superficial cortical layers COUP-TFII appears to be a marker for dLGE (see below), and dCGE-

![Image of Figure 2](wileyonlinelibrary.com)
derived neocortical interneurons (Kanatani et al., 2008; Miyoshi et al., 2010; Ma et al., 2011).

Although both SOM$^+$ and PV$^+$ neocortical interneurons are derived from Nkx2-1$^+$ progenitors, we did not observe COUP-TFIIfi$^+$/PV$^+$ interneurons in the neocortex. This is consistent with previous observations (Kanatani et al., 2008). Thus, COUP-TFIIfi expression in cortical interneurons in Nkx2-1-lineage specifically marks SOM$^+$ interneurons. These expression patterns were maintained in the neocortex of aged mice (>14 months, data not shown).

We also examined the distributions of COUP-TFIIfi$^+$ neocortical interneurons in P0 mice by performing COUP-TFIIfi/Sp8/Sox6 triple-immunostaining. Previous studies
suggested that MGE and CGE-derived interneurons show a similar layer distribution in the neonatal cortex, but then sort into distinct laminar patterns at later stages (Miyoshi and Fishell, 2011). Consistent with this, we found that Sp8+ cells do not appear to exhibit spatial bias at P0 (Fig. 5C), although Sp8+ interneurons preferentially occupy superficial cortical layers in the adult brain (Ma et al., 2011). However, the majority of COUP-TFII+/Sox6+ cells (>90%) already occupied layer V at P0 (Fig. 5). Compared to other Sox6+ cells in the neocortex, these cells were heavily labeled with Sox6 and had a large soma (Fig. 5). Thus, COUP-TFII+/Sox6+ neocortical interneurons show layer specificity in the P0 neocortex.

Figure 4. Overall distribution of COUP-TFII+/SOM+ neocortical interneurons in young adult mice (P28). A,F: COUP-TFII+/SOM+ interneurons mainly occupied layer V of the motor (A), somatosensory (A), and visual cortex (F). B–E,G: Higher magnification of boxed areas in (A,F). H–K: Many SOM+/CR+ (arrows) but not COUP-TFII+/SOM+/CR+ interneurons were observed in the neocortex. Scale bars = 200 μm in A,F; 50 μm in B–E,G–K. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
COUP-TFIÎ± neocortical interneurons in superficial and deeper layers are generated at different developmental times

To determine the birth date of COUP-TFIÎ± interneurons in superficial layers (layers I and II/III) and layer V, we intraperitoneally injected BrdU once into timed pregnant mice and P0 mice and then analyzed at P28. BrdU/COUP-TFIÎ± double-immunostaining revealed that, in both the motor and somatosensory cortex, the majority of COUP-TFIÎ± interneurons in layer V were born between E11.5 and E13.5, with a peak at E12.5 (Fig. 6). By contrast, the majority of COUP-TFIÎ± interneurons in superficial layers were born between E13.5 and E15.5, with a peak at E14.5 (Fig. 6). This is consistent with previous observations that the majority of MGE-derived neocortical interneurons were born earlier than CGE-derived populations (Miyoshi et al., 2007, 2010; Ma et al., 2011).

dMGE but not POA generates COUP-TFIÎ±/SOMÎ± layer V neocortical interneurons

Recent studies show that the POA is the source for ~10% of neocortical interneurons (Gelman et al., 2009, 2011). Because COUP-TFIÎ± is expressed in the POA, we tested whether POA generates some COUP-TFIÎ± neocortical interneurons. Sonic hedgehog (Shh) is expressed in progenitors of the ventral MGE and POA (Flames et al., 2007; Flandin et al., 2010, 2011; Gelman et al., 2011). To determine whether they give rise to COUP-TFIÎ± neocortical interneurons we thus crossed Shh-Cre mice (Harfe et al., 2004) to a Cre-reporter line (Rosa-YFP) (Srinivas et al., 2001). By E11.5, in Shh-Cre; Rosa-YFP mice, YFPÎ± cells (detected using antibodies against GFP) had migrated from the POA and ventral MGE into the LGE (Fig. 7A–C). Virtually all of these cells expressed Sox6 (Fig. 7D,G–I). However, none of them expressed COUP-TFIÎ± or Sp8 (Fig. 7E,F). By adulthood, the neocortex of Shh-Cre; Rosa-YFP mice had very few YFPÎ± cells, indicating that POA does not generate COUP-TFIÎ± neocortical interneurons.

Figure 5. Overall distribution of COUP-TFIÎ±, Sox6Î±, and Sp8Î± cells in the neocortex of P0 mice. A–D: While Sp8Î± cells did not appear to exhibit spatial bias, most dMGE-derived COUP-TFIÎ±/Sox6Î± interneurons occupied the deeper layers at P0. Scale bars = 200 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
interneurons, whereas the majority of YFP+ cells were pyramidal neurons (Fig. 7J), a result consistent with recent studies (Flandin et al., 2010; Harwell et al., 2012). No YFP+ / COUP-TFII+ cells in the neocortex were found (Fig. 7J–L). These results indicate that the ventral MGE and the POA is not a significant source for COUP-TFII+ cortical interneurons. This is consistent with evidence that the POA produces a minority of the SOM+ neocortical interneurons (less than 1% of total cortical interneurons) (Gelman et al., 2011).

By contrast with the Shh-Cre results, the adult neocortex of Nkx2.1-Cre; Rosa-YFP mice had YFP+ / COUP-TFII+ / SOM+ cells; these were mainly in layer V (Fig. 8). Taken together, our results provide strong evidence that the dMGE is a major source of COUP-TFII+ / SOM+ neocortical interneurons.

Slice culture experiments provide evidence that the dLGE generates Sp8+ / COUP-TFII+ neocortical interneurons

To more directly test whether the dLGE can generate a subpopulation of neocortical interneurons, we used an in vitro slice culture assay. Coronal 300-μm slices from E14.5 mouse forebrain, which contained the LGE and MGE, but not the CGE, were cultured for 12 hours, followed by exposure BrdU for 24 hours (to label proliferating cells). The slices were then fixed for immunostaining (Fig. 9A). Our recent study provided evidence that Sp8 is expressed in neocortical interneurons derived from the dLGE and CGE, but not from the MGE or POA (Ma et al., 2011). As anticipated, many BrdU+/Sp8+ cells were observed in the neocortex (Fig. 9B–E), consistent with the idea that the dLGE may generate a subpopulation of neocortical interneurons. However, since these slices do not contain the rostral migratory stream (RMS), dLGE-derived Sp8+ interneurons that are destined for the olfactory bulb (OB) may ectopically migrate into the neocortex. However, some cells that migrated into the cortex were BrdU+/Sp8+/COUP-TFII+ (Fig. 9F–I); we suggest that these are dLGE-derived cortical interneurons. It is unlikely that these are misrouted OB interneurons, as there are very few Sp8+/COUP-TFII+ cells in the neonatal and adult OB (data not shown). In addition, there were
BrdU+/COUP-TFII+ cells in the neocortex (Fig. 9F–I); they could be derived from either the dMGE or vLGE (the LGE–MGE boundary).

Layering of dMGE- and dLGE-derived cortical interneurons is intrinsically programmed

The results in Figure 6 show that earlier-born dMGE-derived COUP-TFII+ cortical interneurons preferentially occupy cortical layer V, while later-born dLGE- and CGE-derived COUP-TFII+ ones preferentially occupy superficial cortical layers. To test whether the layering of dMGE- and dLGE-derived cortical interneurons is intrinsically programmed, we performed in vivo transplantation studies. Because COUP-TFII is expressed in a domain spanning the vLGE and dMGE, we separated these domains by dissection from E14.5 EGFP-expressing transgenic mouse

Figure 7. The POA does not generate COUP-TFII+ neocortical interneurons. A–C: In E11.5 Shh-Cre; Rosa-YFP mice, YFP+ cells (detected using antibodies against GFP) that migrated from the POA and ventral MGE into the neocortex were observed. D–F: Higher magnification of boxed areas in (A–C) showing YFP/Sox6, YFP/COUP-TFII, and YFP/Sp8 double immunostaining. G–I: Virtually all YFP+ cells expressed Sox6. J–L: No YFP+/COUP-TFII+ cells were found in the neocortex of adult Shh-Cre; Rosa-YFP mice. Scale bars = 100 μm in A–C; 50 μm in D–F,J–L; 20 μm in G–I. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
embryos. Single-cell suspensions from E14.5 dMGE and E15.5 dLGE were each transplanted into P1–3 mouse neocortex. Twenty-eight days after transplantation, the E14.5 dMGE donor cells yielded more GFP+/COUP-TFII+ interneurons in layer V compared to superficial layers (66% vs. 34%, n = 3) (Fig. 10A–E). By contrast, the E15.5 dLGE donor cells yielded more GFP+/Sp8+ interneurons in superficial layers (layers I–III) compared to deeper layers (layer IV–VI) (69% vs. 31%, n = 5) (Fig. 10F). Thus, we observed similar laminar distribution patterns of COUP-TFII+ and Sp8+ interneurons in both the normal adult neocortex, and in the adult cortex following transplantation of E14.5 dMGE and E15.5 dLGE, respectively.

Figure 8. COUP-TFII+/SOM+ neocortical interneurons are derived from the Nkx2.1-lineage. A–D: In adult Nkx2.1-Cre; Rosa-YFP mice, YFP+ cells (detected using antibodies against GFP) that expressed COUP-TFII and SOM were mainly located in neocortical layer V. E–H: Higher magnification of YFP+/COUP-TFII+/SOM+ cells (arrows). Scale bars = 50 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
DISCUSSION

The majority of mouse neocortical interneurons are derived from the MGE, CGE, and POA (Anderson et al., 1997; Lavdas et al., 1999; Sussel et al., 1999; Wichterle et al., 2001; Nery et al., 2002; Xu et al., 2004, 2010; Butt et al., 2005; Cobos et al., 2005; Fogarty et al., 2007; Miyoshi et al., 2007, 2010; Gelman et al., 2009, 2011; Vucurovic et al., 2010; Lee et al., 2010; Rubin et al., 2010). Here we provide evidence that the dLGE is also a source of neocortical interneurons (Fig. 11). We also show that COUP-TFII is expressed in at least two distinct sets of neocortical interneurons: “early” born dMGE-derived interneurons that settle in cortical layer V; these are largely SOM+, and do not express PV. “Late” born dLGE- and dCGE-derived COUP-TFII+ interneurons mainly settle in superficial cortical layers (Fig. 11E); these largely express Sp8 (Ma et al., 2011). These distinct laminar patterns were also observed following heterochronic transplantation experiments using E14.5 dMGE and E15.5 dLGE, providing evidence for cell autonomous mechanisms that regulate their migration to the correct laminar position.

Origin of neocortical interneurons

Although it is suggested that primate pallial (neocortical) VZ and SVZ generate some GABAergic interneurons for the neocortex (Letinic et al., 2002; Jones, 2009; Yu and Zecevic, 2011; Jakovcevski et al., 2011), at least in rodents, most if not all GABAergic neocortical interneurons are produced in the subpallial ganglionic eminences and POA (Marin and Rubenstein, 2001; Metin et al., 2006; Wonders and Anderson, 2006; Batista-Brito and Fishell, 2009; Gelman et al., 2012). It is well recognized that neocortical interneurons are derived from the MGE (Lavdas et al., 1999; Sussel et al., 1999; Pleasure et al., 2000; Wichterle et al., 2001; Nery et al., 2002; Valcanis and Tan, 2003; Lopez-Bendito et al., 2004; Xu et al., 2004; Butt et al., 2005; Fogarty et al., 2007; Rubin et al., 2010), CGE (Nery et al., 2002; Xu et al., 2004; Lee et al., 2010; Miyoshi et al., 2010), and POA (Gelman et al., 2009, 2011). While previous analyses have provided evidence that the LGE may generate cortical interneurons (Anderson et al., 2001), this idea remains controversial, particularly because there are no molecular markers or genetic tools that unequivocally distinguish LGE- and CGE-derived cells.

Figure 9. The dLGE generates a subpopulation of neocortical interneurons. A: Schematic diagram of the experimental design. Coronal 300-μm slices from E14.5 mouse forebrain, which contained the LGE and MGE, but not the CGE, were cultured for 12 hours, followed by exposure BrdU for 24 hours (to label proliferating cells). The slices were then fixed for BrdU/COUP-TFII/Sp8 triple-immunostaining. B–E: Many BrdU+/Sp8+ cells (arrowheads) were found in the neocortex. F–I: BrdU+/Sp8+/COUP-TFII+ cells (arrows) and BrdU+/COUP-TFII+ cells (arrowhead) were also found in the neocortex. Scale bar = 10 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
COUP-TFII expression has been reported to specifically mark CGE-derived cortical interneurons (Kanatani et al., 2008; Willi-Monnerat et al., 2008; Miyoshi et al., 2010); however, our data shows that this gene is also expressed in progenitor zones of the dLGE, dMGE, and POA, raising the possibility that COUP-TFII is also expressed in mature interneurons derived from these domains. Because the POA and ventral MGE do not appear to give rise to COUP-TFII+ neocortical interneurons (using Shh-Cre fate mapping), fate mapping using Nkx2.1-Cre further suggest that COUP-TFII+/SOM+ interneurons in cortical layer V are derived from the dMGE (Fig. 11E).

Sp8 is expressed in the dLGE and dCGE, but not in the MGE (Fig. 11A–C) (Ma et al., 2011). Sp8+ cells in the dLGE and CGE either migrate into the OB via the RMS (Doetsch and Alvarez-Buylla, 1996; Waclaw et al., 2006; Long et al., 2007; Liu et al., 2009; Li et al., 2011) or migrate into the neocortex (Ma et al., 2011). Sp8+/COUP-TFII+ cells found in the dLGE strongly argue against the possibility that the COUP-TFII+ cells in the dLGE are dMGE-derived COUP-TFII+ cells that migrate through the LGE on route to the cortex.

Previous studies suggest that CGE-derived cells do not migrate through the LGE into the neocortex (Yozu et al., 2005; Kanatani et al., 2008). Because very few Sp8+/COUP-TFII+ cells were found in the OB, we propose that these cells in the dLGE mainly migrate into the cortex. Indeed, consistent with previous studies suggesting that the LGE generates at least some of neocortical interneurons (de Carlos et al., 1996; Tamamaki et al., 1997; Anderson et al., 1997, 2001; Jimenez et al., 2002; Rubin et al., 2010; Wu et al., 2011; Ma et al., 2011; Riccio et al., 2012), we observed a migratory stream from the dLGE to the neocortex. Slice culture experiments in this work also

![Figure 10.](image_url)
support the hypothesis that the dLGE is a source of interneurons for the neocortex.

Our data raise another question: is the CGE a distinct subpallial structure or just the caudal extension of the LGE and MGE (Flames et al., 2007)? The CGE is named on the basis of morphological criteria (Nery et al., 2002; Flames et al., 2007), but currently there are no defined molecular markers that label the CGE and not the LGE (Flames et al., 2007; Long et al., 2009a,b). Some transcription factors, such as COUP-TFI, may be initially expressed in the CGE, but later are also expressed in the dLGE. At the anterior level of dLGE of rodent brains, the vast majority of dLGE cells migrate via the RMS into the OB (Yun et al., 2003; Stenman et al., 2003; Waclaw et al., 2006). At posterior levels of the dLGE and CGE, however, these progenitor zones appear to largely generate interneurons that migrate to the cortex (Fig. 11D), although some may be migrating to the OB as well. In contrast to the mouse, the dLGE of the fetal human brain appears to generate more cells that migrate into the neocortex than into the OB (Ma and Yang, unpubl. obs.). Thus, our data support the notion that the CGE represents the caudal end of the dLGE and dMGE progenitor domains (Flames et al., 2007). On the other hand, it is worth noting that if there is a vLGE component of the CGE, it does not produce cells expressing striatal markers, as the vLGE does (Long et al., 2009b). Thus, either there is no vLGE equivalent in the CGE, or the fates of its derivatives are different.

Figure 11. COUP-TFI+ neocortical interneurons are derived from the dMGE and dLGE/CGE. A–C: Whole-mount Sp8/Nkx2.1 double-immunostaining on E13.5, E15.5, and E16.5 mouse brains. D: A model of migration of dLGE- and CGE-derived neocortical interneurons. In the rostral levels of dLGE, the vast majority of cells (i.e., Sp8+ cells) are destined for the olfactory bulb (OB) via the rostral migratory stream (RMS) (white); while in the caudal telencephalon, the majority of cells (i.e., COUP-TFI+ and Sp8+ cells) in the CGE are destined for the neocortex (black). Note that there are no anatomical landmarks to clearly discriminate the CGE from the dLGE. E: Schema of distribution of dMGE-, dLGE/CGE-derived COUP-TFI+ neocortical interneurons. Scale bars = 200 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
Sp8+ interneurons in all layers and COUP-TFII+ interneurons in superficial layers mark dLGE- and CGE-derived neocortical interneurons

PV and SOM are expressed in MGE-and POA-derived cortical interneurons (Wichterle et al., 2001; Nery et al., 2002; Valcanis and Tan, 2003; Lopez-Bendito et al., 2004; Xu et al., 2004; Butt et al., 2005; Fogarty et al., 2007; Gelman et al., 2011). These interneurons also can be identified by transcription factors Lhx6 and Sox6 (Cobos et al., 2005; Du et al., 2008; Azim et al., 2009; Batista-Brito et al., 2009).

We recently demonstrated that the transcription factor Sp8 labels interneurons that are derived from outside of the MGE and POA, which account for about 20% of neocortical interneurons (Ma et al., 2011). Although COUP-TFII+ dMGE-derived neocortical interneurons (COUP-TFII+/SOM+/Sox6+) are mainly in layer V, COUP-TFII also labels a subpopulation of interneurons in superficial cortical layers that do not express Sp8. Therefore, we propose that Sp8+ interneurons in all layers, and COUP-TFII+ interneurons in superficial layers, can represent the majority of dLGE- and CGE-derived neocortical interneurons.

Neocortical interneurons are generated from spatially and temporally defined stem/progenitor domains of the ganglionic eminences

Like the spinal cord, the subpallial germinal region is also composed of molecularly distinct domains that give rise to distinct types of interneurons at different times in development. For instance, different interneuron subtypes in different OB layers are generated from different stem/progenitor domains at different stages (De Marchis et al., 2007; Long et al., 2007; Merkle et al., 2007; Young et al., 2007; Batista-Brito et al., 2008; Li et al., 2011). MGE-derived neocortical interneurons (PV+ and SOM+) are generally born around 2 days earlier than dLGE- and CGE-derived ones; the latter interneurons mainly occupy superficial cortical layers (Miyoshi et al., 2007, 2010; Lee et al., 2010; Vucurovic et al., 2010; Ma et al., 2011).

There is evidence that the dMEG, which expresses Nkx6.2, preferentially generates SOM+/CR+ neocortical interneurons (Flames et al., 2007; Fogarty et al., 2007; Sousa et al., 2009; Inan et al., 2012). Like Nkx6.2, COUP-TFII is also expressed at the boundary of the LGE and MGE. Indeed, in our study only SOM+/COUP-TFII+, but no PV+/COUP-TFII+ interneurons were found in the neocortex, consistent with possibility that COUP-TFII+ cells in the dMGE have the propensity to generate SOM+ neurons. However, we found that none of SOM+/COUP-TFII+ interneurons expressed CR. Thus, although most SOM+/CR+ and SOM+/COUP-TFII+ are generated from the dMGE, these two cell types are derived from the different progenitor pools. Furthermore, we found that SOM+/COUP-TFII+ neocortical interneurons that are derived from the dMGE preferentially occupy cortical layer V (Fig. 11E). In summary, our data provide evidence that intrinsic properties of interneuron precursors, and their derived interneurons, contribute to the specificity of their molecular phenotype and laminar positioning. These features of interneuron development may contribute to the observation that clonally related neocortical interneurons are organized in spatially correlated clusters (Brown et al., 2011).

ACKNOWLEDGMENT

We thank Dr. Guoqi Zhu and Yuying Huang for technical assistance with slice cultures.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ROLE OF AUTHORS


LITERATURE CITED


Youth M, Tabata H, Nakajima K. 2005. The caudal migratory stream: a novel migratory stream of interneurons derived...