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ORIGINAL ARTICLE

Subtype-Specific Genes that Characterize Subpopulations of Callosal Projection Neurons in Mouse Identify Molecularly Homologous Populations in Macaque Cortex

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Abstract

Callosal projection neurons (CPN) interconnect the neocortical hemispheres via the corpus callosum and are implicated in associative integration of multimodal information. CPN have undergone differential evolutionary elaboration, leading to increased diversity of cortical neurons—and more extensive and varied connections in neocortical gray and white matter—in primates compared with rodents. In mouse, distinct sets of genes are enriched in discrete subpopulations of CPN, indicating the molecular diversity of rodent CPN. Elements of rodent CPN functional and organizational diversity might thus be present in the further elaborated primate cortex. We address the hypothesis that genes controlling mouse CPN subtype diversity might reflect molecular patterns shared among mammals that arose prior to the divergence of rodents and primates. We find that, while early expression of the examined CPN-enriched genes, and postmigratory expression of these CPN-enriched genes in deep layers are highly conserved (e.g., *Ptn*, *Nnmt*, *Cited2*, *Dkk3*), in contrast, the examined genes expressed by superficial layer CPN show more variable levels of conservation (e.g., *EphA3*, *Chn2*). These results suggest that there has been evolutionarily differential retraction and elaboration of superficial layer CPN subpopulations between mouse and macaque, with independent derivation of novel populations in primates. Together, these data inform future studies regarding CPN subpopulations that are unique to primates and rodents, and indicate putative evolutionary relationships.

Key words: corpus callosum, development, evolution, primate, rodent

Introduction

The neocortex is the seat of complex cognitive, perceptive, and motor function in mammals. As such, it has undergone dramatic expansion throughout mammalian evolution, with increased neocortical size and complexity in primates not found in rodents (Rakic 2009). Because a fossil record of soft tissues, including the nervous system, is lacking (de Sousa and Wood 2007), the field must rely on the identification of conserved features across contemporary species to determine those that likely arose from common ancestors.

Since the divergence of mammalian ancestors from the sauropsid ancestors of reptiles and birds over 315 million years ago, the cortex has undergone considerable expansion, with a substantial, disproportionate increase in neuronal numbers in the superficial cortical layers II–IV among mammalian species (Reiner 1991, 1993; Marín-Padilla 1992; Aboitiz et al. 2003), with the primate

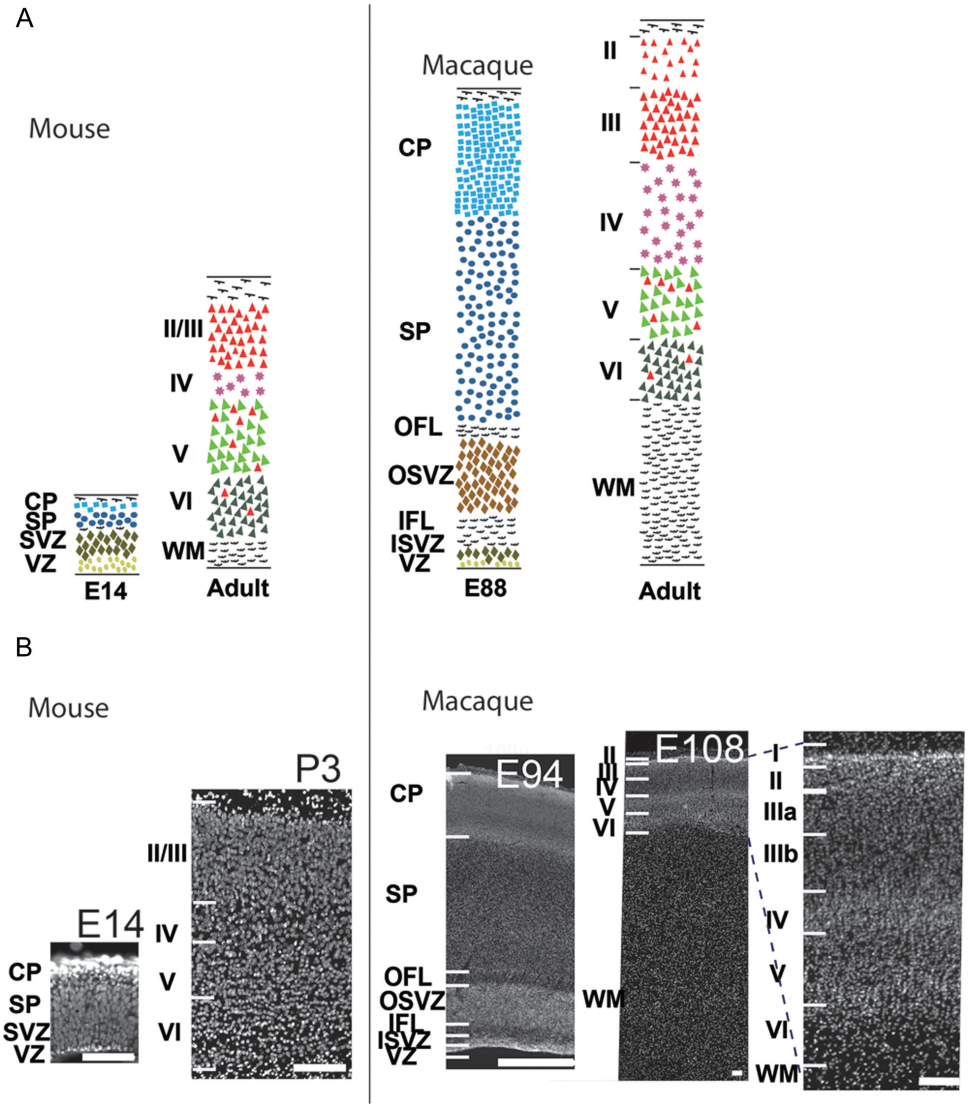

Figure 1. Schematic representation and cytoarchitectural view of mouse and macaque somatosensory cortex. (*A*) Schematic representation of developing and adult mouse and macaque cortex in S1, drawn to a common internal scale. (*B*) DAPI nuclear stain at mid-corticogenesis (E14/ E94) and after neuronal migration (P3/ E108) in macaque to show cytoarchitectural layers in S1. Scale bars: *B*, 100 μm. E, embryonic day; P, postnatal day; VZ, ventricular zone; SVZ, subventricular zone; SP, subplate; ISVZ, inner subventricular zone; IFL, inner fiber layer; OSVZ, outer subventricular zone; OFL, outer fiber layer; CP, cortical plate; S1, primary somatosensory area; roman numerals indicate cortical layers. *A*, adapted from (Fame et al. 2011).

cortex exhibiting greater expansion than rodents (Herculano-Houzel 2012). In terms of neuron number, computational analyses estimate that mice have about 100 times fewer brain neurons than monkeys, and that monkeys, in turn, have about 10–15 times fewer brain neurons than humans, with a 15-fold increase in brain size from mouse to human using Jerison's encephalization quotient (Jerison 1973; Braitenberg 2001; Fish et al. 2008; Mota and Herculano-Houzel 2012). Investigation of the similarities and differences among these cortices continues to be an active area of research.

The corpus callosum (CC) and callosal projection neurons (CPN) are found exclusively in placental mammals (eutheria) (Aboitiz and Montiel 2003; Mihrshahi 2006). CPN interconnect the 2 cortical hemispheres, and, with the exception of primate area VI (Dehay et al. 1988; Chalupa et al. 1989), the expansion of superficial cortical layers correlates reasonably well with that of CPN, which are mostly located in superficial cortical layers (~90% in primates; ~80% in rodents) (Isseroff et al. 1984; Meissirel et al. 1991). Primate superficial layers comprise discrete layers II and III, containing multiple distinct sublayers that are easily distinguished at the histological level, while rodents and other small-brained mammals have less cytoarchitectural specification in these layers and rodent layer II/III is histologically a single, indivisible layer (Lund 1973; Rakic and Kornack 2001; Smart et al. 2002) (Fig. 1*A*,*B*).

With delineation of layers II, IIIa, and IIIb in primate cerebral cortex, it is possible to address distinct cell types that populate these neuroarchitecturally distinct laminar units. Compared with rodents, primates exhibit expansion of cortical superficial layers, as well as expansion of cortical and interhemispheric white matter, raising the possibility that expansion of superficial layers might be, at least in part, due to increased CPN populations. However, while CPN in mice are evenly distributed throughout layer II/III (Figs 1*A* and 6), essentially all superficial layer primate CPN in sensorimotor cortex are restricted to layer III, and more specifically, are concentrated in deeper layer III (IIIb) (Jones and Wise 1977; Killackey et al. 1983; Killackey and Chalupa 1986; Manzoni et al. 1986; Bullier et al. 1990; Meissirel et al. 1991).

These data suggest either that mouse layers II/III contain a mixture of all of these neuron types that segregated following expansion in primate cortex, and/or that new neuron types have emerged to populate the expanded regions of primate cortex.

Although routine histology in mouse fails to reveal the cytoarchitectural diversity evident in primate cortex, recently published data on the molecular diversity of rodent CPN identify diverse neuronal subtypes that group into sublayers of the superficial cortical layers (Molyneaux et al. 2009). These data suggest the possibility that similar neuronal diversity might exist in both rodents and primates. This hypothesis is further supported by the fact that, while in monkey, feedforward and feedback associative pathways are overwhelmingly segregated to layers IIIb, and IIIa, respectively; they are dispersed in a "salt-and-pepper" fashion in mouse superficial layers (Markov et al. 2014). Newly identified molecular controls over CPN subtype diversity in mice might reflect evolutionarily older, shared molecular expression and function, not revealed by routine histology. To investigate this hypothesis, we took advantage of the already existing knowledge of cell-type-specific gene expression in bona fide mouse CPN, previously identified via retrograde labeling (Molyneaux et al. 2009). We compared mouse expression patterns of representative examples of these same genes, with those of macaque at similar developmental stages.

The enlargement of the neocortex and its superficial layers follows the emergence and expansion of a specialized precursor pool: the subventricular zone (SVZ) (Smart et al. 2002; Lukaszewicz et al. 2005; Kriegstein et al. 2006). Basal progenitors of the SVZ transiently express Tbr2, which is a critical regulator of neurogenesis from the SVZ (Arnold et al. 2008) and Tbr2 lineage tracing in mouse reveals that basal progenitors can contribute to all cortical layers. Cellular and molecular evidence supporting the hypothesis that the basal progenitors of the SVZ, $Cux2^+$ ventricular zone/SVZ progenitors, and the progenitors of the outer SVZ, preferentially contribute to superficial layers has been provided by a number of studies in multiple species (Tarabykin et al. 2001; Smart et al. 2002; Noctor et al. 2004; Zimmer et al. 2004; Lukaszewicz et al. 2005; Wu et al. 2005; Dehay and Kennedy 2007; Kowalczyk et al. 2009; Fietz et al. 2010; Franco et al. 2012; Reillo and Borrell 2012; Betizeau et al. 2013; Arcila et al. 2014; Gil-Sanz et al. 2014, 2015; Vasistha et al. 2014).

Unlike many genome-wide, layer-specific, microdissection-based, or regional-specific gene expression analyses in primate and mouse (Donoghue and Rakic 1999a, 1999b; Yoneshima et al. 2006; Johnson et al. 2009; Wang et al. 2009; Belgard et al. 2011; Ip et al. 2011; Bernard et al. 2012; Sorensen et al. 2015), the present investigation follows the cell-type-driven logic of previous studies (Hevner et al. 2003; Arlotta et al. 2005; Molyneaux et al. 2009) to address the intersection of laminar molecular diversity within a specific population of neocortical projection neurons—CPN—between mouse and primate during cortical development. Here, we report that many of the molecular controls acting to establish CPN diversity exhibit a conserved laminar expression between mouse and macaque during corticogenesis, but with some notable distinctions, predominantly with respect to the superficial layer CPN.

Materials and Methods

In Situ Hybridization Candidate Selection and Probe Design

Gene candidates for this study are expressed by purified bona fide CPN in developing mouse (validated by CPN subtype-specific neuronal FACS purification and Affymetrix microarray analysis in Molyneaux et al. (2009). Due to limited macaque tissue, we were not able to examine the entire set of genes reported in Molyneaux, et al.; rather we selected representative examples from each laminar division grouping (also considering robust expression in mouse).

All macaque in situ hybridization probes were designed to closely match reported homologous regions of corresponding mouse mRNA (Molyneaux et al. 2009). Optimized primers were chosen to amplify between 400 and 900 bp of this conserved region (Supplementary Table 1). Since the macaque genome is not fully annotated, not all of the sequences could be obtained from verified sequences; so some were obtained from NCBI-predicted homologs or whole-chromosome shotgun sequencing data. The in situ hybridization probes for E14 mouse were those designed by GenePaint.org, and were, therefore, not identical to those in Molyneaux et al. (2009), nor are they necessarily homologous to the regions selected for the macaque probes developed specifically for this study.

RNA Extraction, and First-Strand cDNA Synthesis and Library Construction

Total RNA was extracted from frozen E85 macaque cortical tissue, following the product insert instructions for organic RNA extraction with TRIzol® Reagent (Invitrogen Life Sciences). First-strand cDNA was synthesized using Oligo(dT) primers (25 ng/μL) and total RNA (0.5 ng/μL), following the product insert instructions for Superscript II® reverse transcriptase (Ambion Life Sciences). To remove RNA complementary to the cDNA, *Escherichia coli* RNase H (0.1 U/μL) was added and incubated at 37°C for 20 min. This single-stranded DNA was then used as template for PCR (sequences of all primers used are listed in Supplementary Table 1), and cloned into TOPO II® (Invitrogen Life Sciences) bacterial vectors using appropriate restriction enzymes (New England Biolabs).

Animals

Mice were bred on a pure C57BL/6 background. The day of vaginal plug detection was designated as E0.5. The day of birth was designated as P0. For embryonic tissue collection, timed pregnant females were anesthetized with a lethal dose of Avertin, and embryos were removed from the uterine horns. Embryos and postnatal pups (P6 and younger) were lethally anesthetized by hypothermia. Postnatal pups (P7 and older) were anesthetized with a lethal dose of Avertin. All mouse studies were approved by the Harvard University IACUC, and were performed in accordance with institutional and federal guidelines.

Fetuses from timed-pregnant cynomolgus monkeys (Macaca fascicularis, gestation period 165 days) were delivered by caesarian section and lethally anesthetized (via intraperitoneal injection of Sodium Pentobarbital 60 mg/kg) as previously described (Lukaszewicz et al. 2005). All macaque experiments were in compliance with national and European laws as well as with institutional guidelines concerning animal experimentation. Macaque surgical procedures were in accordance with European requirements 2010/63/UE. The protocol C2EA42-12-11-0402-003 has been reviewed and approved by the Animal Care and Use Committee CELYNE (C2EA #42).

In Situ Hybridization

Nonradioactive colorimetric in situ hybridization was performed using probes labeled with digoxigenin-labeled uridine

triphosphate. Sense probes were used as negative controls in all experiments. Each mouse age represents 3 individual brains with 2 technical replicates to control for variability. Due to limited tissue, E94 macaque data (roughly equivalent to E14 in mouse) represents a single individual brain with 2–3 technical replicates to control for variability, as noted in the figure legend. In addition, 2 developing macaque brains were analyzed at E108 ($N = 3$ technical replicates) and E113 ($N = 2$ technical replicates), a developmental stage roughly equivalent to P3 in mouse. Results were reported if expression patterns were consistent between repeats and across individuals within each stage, even if observed expression levels varied somewhat between individuals.

For in situ hybridization, 25-μm cryosections of fixed [4% paraformaldehyde (PFA)], cryoprotected (30% sucrose) E94 and E108/113 macaque forebrains were mounted on superfrost plus slides® (Fisher Scientific) and postfixed in 4% PFA in phosphate-buffered saline (PBS) for 10 min, rinsed in PBS for 3 min, permeabilized in 0.3% Triton X-100 (Sigma) followed by radioimmunoprecipitation assay (RIPA) cell lysis buffer [150 mM sodium chloride, 1% Triton X-100, 1% deoxicholic acid sodium salt, 0.1% sodium dodesyl sulfate, 50 mM Tris-HCl, pH 7.5, 2 mM ethylenediaminetetraacetic acid (EDTA)], re-fixed in 4% PFA, acetylated for 15 min in 0.1 M triethanolamine/0.4% HCl/0.25% acetic anhydride (Sigma), and prehybridized for 1 h in 65°C hybridization buffer (50% formamide, 5× saline sodium citrate buffer (SSC), 5× Denhardt's solution [1 μg/mL Ficoll 400, 1 μg/mL polyvinylpyrrolidone, 1 μg/mL bovine serum albumin], 500 μg/mL sheared salmon sperm DNA, 250 μg/mL yeast RNA). Slides were incubated overnight (14–20 h) at 65°C in 2-μg/mL dig-labeled probe in hybridization buffer coverslipped with GeneFrame® adhesive spacers (Thermo Scientific) in a well-humidified oven. Slides were then subjected to stringency washes in 2× SSC/50% formamide/0.1% Tween-20 at 65°C for 1 h each. Sections were then rinsed in maleic acid buffer containing Tween 20 (MABT) (0.9 M maleic acid [Sigma], 0.1 M NaCl [Sigma], 0.0005% Tween 20 [Sigma], 0.175 M NaOH [Sigma]) at room temperature (RT), blocked in 10% goat serum in MABT, and incubated overnight in goat alkaline phosphatase-conjugated anti-dig (1:1000, Roche) primary antibody in block, rinsed with MABT, followed by a 30-min wash in alkaline phosphatase reaction buffer (100 mM Tris pH 9.5, 50 mM $MgCl_2$, 100 mM NaCl, 0.1% Tween-20). The alkaline phosphatase reaction was developed with nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate in phosphatase reaction buffer, changing to a fresh solution every 1–4 h at RT or every 6–9 h at 4°C. When the reaction was judged complete (48–100 h), tissue was rinsed in 0.1% Tween-20 in PBS, postfixed in 4% PFA for 30 min, counterstained for 1 min in 1:10 000 4′,6-diamidino-2-phenylindole (DAPI), and rinsed in 0.033 M phosphate buffer (27 mM dibasic sodium phosphate, 6.3 mM monobasic sodium phosphate). Slides were coverslipped with Fluromount® (Sigma), dried, and edges were protected with clear nail polish.

For E108/E113 tissue, an additional permeabilization step with proteinase K (Sigma) treatment (10 μg/mL enzyme in 0.005 M EDTA, 0.05 M Tris, pH 8.0) for 10 min at RT was added after the RIPA permeabilization.

All E14 mouse in situ hybridization images reported from GenePaint.org are from the C57/Bl6 strain, except for *Cited2*, which is only available from the Naval Medical Research Institute strain.

Immunocytochemistry

Immunostaining was performed largely as described elsewhere (Molyneaux et al. 2009). However, all immunocytochemical reactions were performed on 25-μm cryosections of fixed (4% PFA), cryoprotected (30% sucrose) E94 or E108/113 macaque forebrain mounted on superfrost plus slides® (Fisher Scientific). Sections were brought to RT, postfixed in 4% PFA in PBS (10 min), rinsed in PBS (3 min), and stained. Antigen retrieval in 0.1 M citric acid (pH = 6.0) for 10 min at 95–98°C was used for CAV1 and LMO4 staining. Primary antibodies were used as follows: mouse-anti-CAV1, 1:500 (Cell Signaling #3238), goat-anti-LMO4, 1:200 (Santa Cruz Biotech SC-11122), rabbit-anti-Nectin-3, 1:500 (Abcam ab63931).

Microscopy and Image Analysis

Images were acquired using a Nikon E90i microscope, equipped with a 1.5 megapixel cooled CCD digital camera (Andor Technology, Dublin, Northern Ireland), a 5 megapixel color CCD digital camera (Nikon Instruments, Melville, NY, USA), and Elements acquisition software (Nikon Instruments). All analysis was performed in primary somatosensory area (S1) unless otherwise noted. Images were processed using Adobe Photoshop.

Results

In Situ Hybridization Protocol Detects mRNA in Macaque Tissue

Because even subtle tissue preparation and worksite setup differences can influence the outcome of in situ hybridization results, and particularly because the primate tissue was prepared in 1 laboratory, and mouse tissue preparation and analysis of both primate and mouse were performed in the other, we chose a control probe (*Fezf2*) that is known to have a laminar expression in both mouse and primate, and that is highly conserved in layer V (high expression) and layer VI (low expression) (Molyneaux et al. 2005; Kwan et al. 2008; Ip et al. 2011; HK Padmanabhan, US Sohur, JD Macklis, unpublished observations). In mouse, *Fezf2* is expressed at high levels by corticospinal motor neurons (CSMN) and related subcerebral projection neurons (SCPN), and at lower levels by corticothalamic projection neurons (and other related corticofugal neurons). *Fezf2* is necessary and sufficient (within cortical context) to produce CSMN/SCPN, but is excluded from CPN. We confirmed that the macaque tissue, handling, and protocols provided accurate *Fezf2* expression results.

In E94 macaque (late-corticogenesis), *Fezf2* is expressed in the cortical plate (CP) (Fig. 2*A*). At E108/113, when most neocortical projection neurons have largely completed their migration to their final laminar positions (Rakic 1995), expression of *Fezf2* is high in layer V, and low in layer VI (Fig. 2*B*). This is similar to *Fezf2* expression in mouse layer V CSMN/SCPN (high level) and layer VI CThPN (low level) (Arlotta et al. 2005; Chen, Rasin et al. 2005; Chen, Schaevitz et al. 2005; Molyneaux et al. 2005; Rouaux and Arlotta 2010). These expression patterns in macaque confirm previous findings (Kwan et al. 2008; Ip et al. 2011) and validate the experimental strategy employed by the present study for comparing macaque and mouse gene expression.

Genes Expressed by CPN Early in Mouse Development Are Similarly Expressed in Macaque

Because some of the mouse CPN-subtype-specific genes show high expression levels early in development, and because early gene expression is more likely conserved between species than late gene expression (Donoghue and Rakic 1999a, 1999b), we examined expression of 4 genes in E94 macaque cortex that are

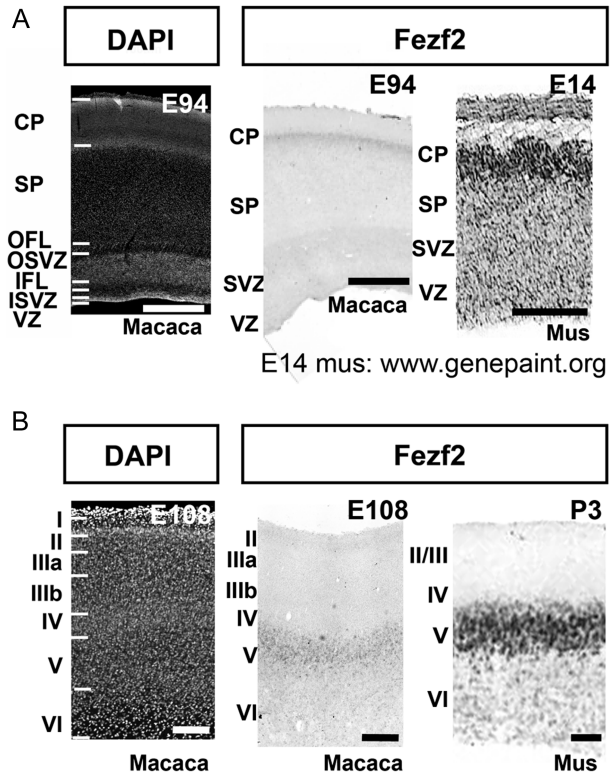

Figure 2. *Fezf2* is detectable in macaque tissue using current in situ hybridization approach. (*A*) DAPI nuclear stain in E94 macaque for reference, followed by in situ hybridization for *Fezf2* at E94 in macaque and E14 in mouse, showing early similarities in expression between mouse and macaque. (*B*) DAPI nuclear stain in E108 macaque for reference, followed by in situ hybridization for *Fezf2* at E108 in macaque and P3 in mouse, confirming early similarities in expression between mouse and macaque, and validating the approach employed in these analyses. $N = 3$ individual mice, $N = 2$ technical replicates each. $N = 1$ macaque, $N = 2$ technical replicates. Scale bars: (*A*,*B*) 100 μm. E, embryonic day; P, postnatal day; VZ, ventricular zone; SVZ, subventricular zone; IZ, intermediate zone; CP, cortical plate; S1, primary somatosensory area; Roman numerals indicate cortical layers. E14 mouse in situ from GenePaint digital expression atlas www.genepaint.org.

expressed in early mouse CPN development. Strikingly, the subset of genes examined that are expressed in the SVZ exhibit conserved expression in E14 mouse and E94 macaque, including the high expression of *Ptn* (Fig. 3A,*B*). This is particularly pertinent with respect to CPN given the hypothesis of SVZ origin of superficial layer neurons (see Introduction). In addition, the postmitotic, CP gene expression of *Ptn* is also conserved, as is that of *Lmo4* and *Inhba* (Fig. 3A–*F*). Low-level *Inhba* expression extends into progenitors in macaque, but not in mouse. Additionally, *Cited2* expression, present in both the progenitor and postmitotic regions, is also highly conserved between E14 mouse and E94 macaque (Fig. 4*G*,*H*). Together, these data suggest that in both species' populations of progenitors and immature postmitotic neurons are largely similar in terms of early gene expression at the ages examined.

The above data add to a growing body of work revealing similarities and differences between rodents and primates in progenitors and early postmitotic neurons (Donoghue and Rakic 1999a, 1999b; Johnson et al. 2009; Ip et al. 2011; Bernard et al. 2012). The present results, focused on laminar similarities between mouse and primate during corticogenesis, point to a general conservation of expression in the 2 species.

In the macaque E94 stage examined, only a fraction of CPN have terminated their migration, and the progenitors that produce the majority of the CPN population are still proliferating. To refine our investigation of the degree of gene expression conservation of CPN between primates and rodents, we investigated a later stage of corticogenesis (P3 in mouse and E108/113 in macaque). The similarities in laminar gene expression in both progenitor and postmitotic zones (Fig. 3) motivate further investigations (addressed below) into later similarities or divergences of gene expression.

Postmigration CPN Gene Expression Suggests Homologous CPN Populations in Mouse and Macaque

Multiple molecular subpopulations of CPN have been identified in mouse by examining unique sublaminar expression patterns of novel genes expressed by bona fide CPN during corticogenesis, after most neurons have migrated to their final laminar positions (Molyneaux et al. 2009). Therefore, we examined expression of 4 genes in E108/113 macaque cortex that are expressed in mouse CPN development at P3. Strikingly, the subset of genes examined that are expressed in both deep and superficial layers are largely conserved in laminar expression between mouse and macaque at these ages, including *Cited2*, *Chn2*, *EphA3*, and *Limch1* (Fig. 4A–*L*).

Cited2 is expressed in P3 mouse and E108/113 macaque in both superficial and deep layers (Fig. 4A–*C*). *Chn2* is expressed in the deeper part of mouse layer II/III, and appears more highly expressed in layer III in macaque somatosensory cortex (Fig. 4*D*–*F*). Interestingly, *Chn2* is not expressed throughout macaque layer III at E108/113, but only in the more superficial part of this layer. This difference suggests that *Chn2*-expressing neurons in macaque at this age are more segregated than their P3 mouse counterparts. Even if they are fated to be overtaken by later-generated neurons, they comprise a more restricted sublaminar population in macaque than in mouse. If *Chn2*-expressing cells remain in the superficial portion of layer III and are not completely overtaken by later-born cells, they might include populations of local associative neurons, or even a subpopulation of dual CPN/intrahemispheric association neurons, since most CPN in macaque S1 are in deeper portions of layer III (Jones and Wise 1977; Killackey et al. 1983; Manzoni et al. 1986; Meissirel et al. 1991), though this would need to be verified by combining gene expression analysis with tract-tracing studies of connectivity.

EphA3$^+$ neurons are restricted to the most superficial portion of layer II/III in mouse at P3, while they are more widely distributed throughout these layers in macaque at E108/113 (Fig. 4*G*–*I*; Donoghue and Rakic 1999b). *Limch1* shows a complementary species-specific expression pattern, with its expression restricted to the most superficial cells of layer II in E108/113 macaque, but more broadly throughout layer II/III in P3 mouse (Fig. 4*J*–*L*). These examples indicate that mouse CPN-enriched genes are also expressed in the CP of the developing macaque at E108/113, where they show a laminar-specific pattern in agreement with the known distribution of CPN in primates, but with notable differences between mouse and macaque sublaminar expression.

Differential Developmental Regulation of CPN Genes Between Mouse and Macaque

We observed that CPN-expressed genes display varying levels of conservation over developmental time. *Dkk3* exhibits strong conservation of early SVZ/CP expression and later layer VI cortical expression (Fig. 5A,*B*). Analogously, *Nnmt* exhibits strong conservation of early SVZ/CP expression, and *Nnmt* is expressed later

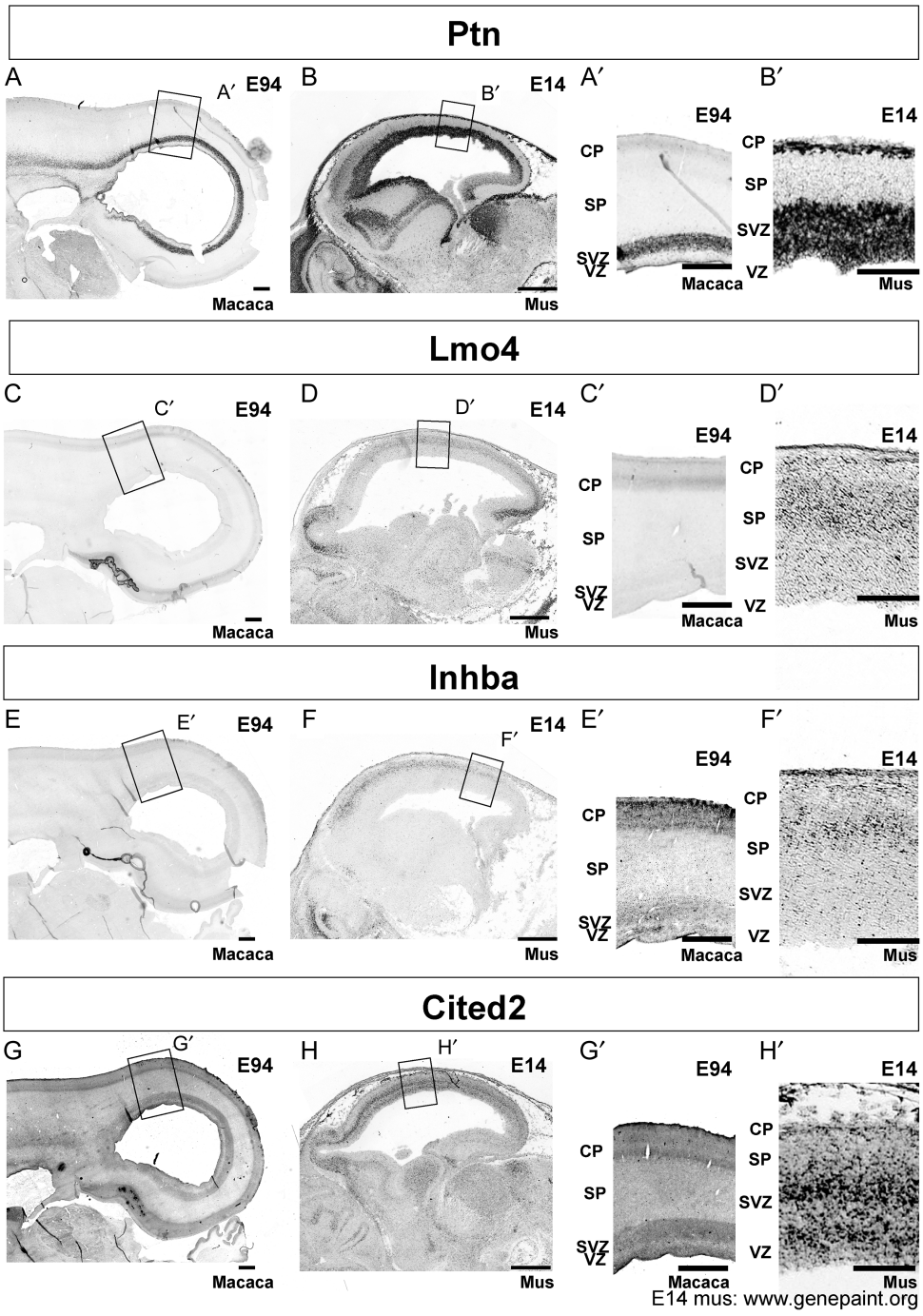

Figure 3. At mid-corticogenesis (E14 and E94), early-expressed CPN genes are largely similarly expressed in mouse and macaque. (*A–H*) in situ hybridization at E94 in macaque (*A*,*A*′,*C*,*C*′,*E*,*E*′,*G*,*G*′) and E14 in mouse (*B*,*B*′,*D*,*D*′,*F*,*F*′,*H*,*H*′) for early expressed CPN genes (*Ptn*, *Lmo4*, *Inhba*, *Cited2*) shows early similarities in expression between mouse and macaque. Low-level *Inhba* expression extends into progenitors in macaque, but not in mouse. *N* = 1 macaque, *N* = 3 technical replicates. Scale bar: 100 μm. E, embryonic day; P, postnatal day; VZ, ventricular zone; SVZ, subventricular zone; IZ, intermediate zone; CP, cortical plate; S1, primary somatosensory area; Roman numerals indicate cortical layers. E14 mouse in situ from GenePaint digital expression atlas www.genepaint.org.

exclusively in the superficial portion of layer II, in both species (Fig. 5*A*,*B*). *Inhba* exhibits strong conservation of early SVZ/CP expression, but later is expressed in layer II in E108/113 macaque, while it is expressed more broadly throughout layer II/III in P3 mouse (Fig. 5*C*).

Other genes, while present during development in both mouse and macaque cortex, do not show complete spatial or temporal conservation. A notable exception to the largely conserved early expression of developmentally regulated neocortical control genes is that of the cell adhesion and guidance molecule *Plexin-D1*. By E14 in mouse, *Plexin-D1* is strongly expressed by immature CP neurons as they begin to extend their axons. However, no *Plexin-D1* expression is observed in E94 macaque CP (Fig. 5*D*). Postmigratory expression of *Plexin-D1* is conserved between mouse and macaque in layer V (Fig. 5*D*). This lack of *Plexin-D1* expression in early macaque cortex does not appear to drive differential

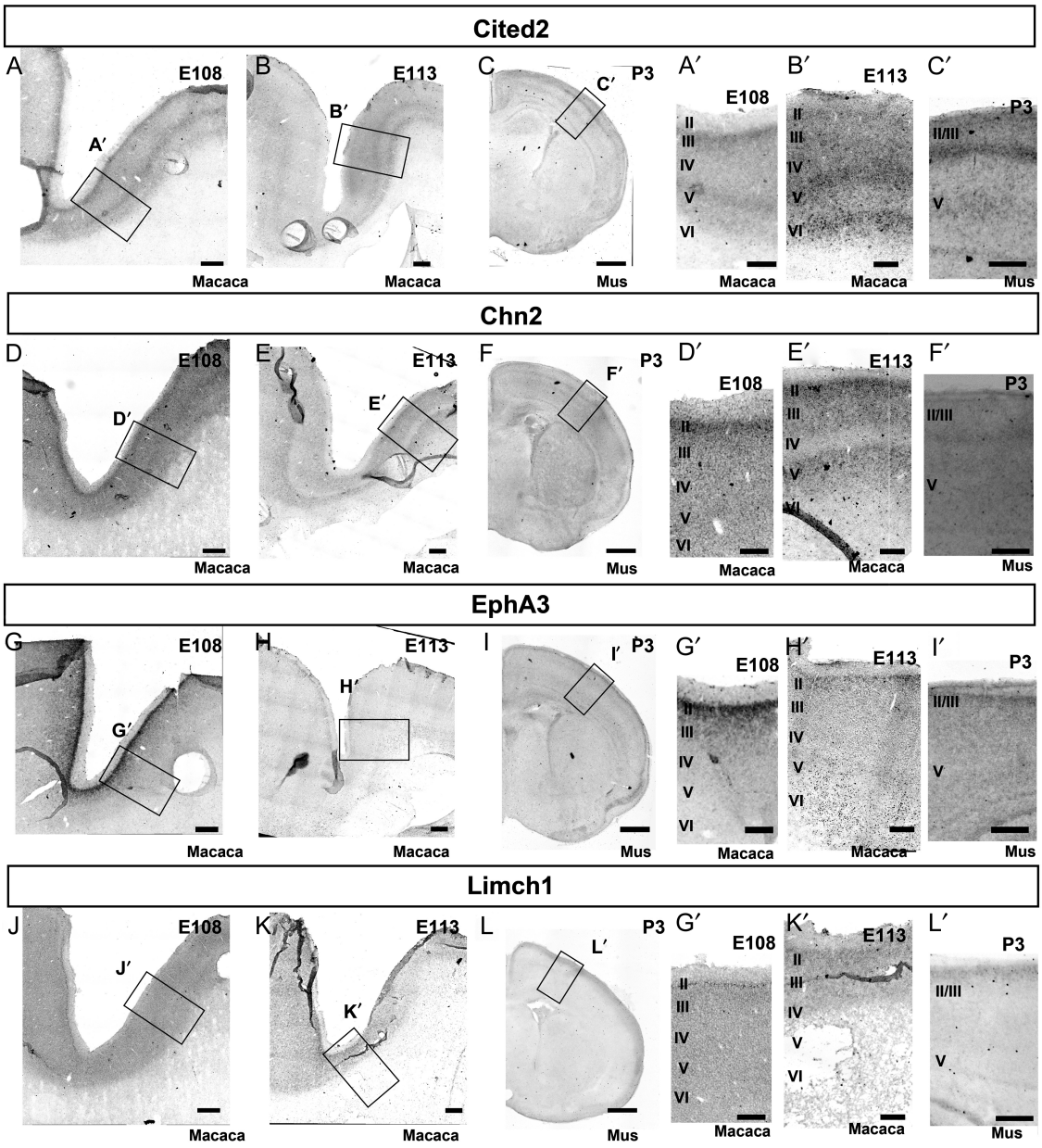

Figure 4. At P3 in mouse, and at E108 and E113 in macaque, CPN genes reveal related populations in mouse and macaque superficial (*Cited2*, *Chn2*, *Epha3*, *Limch1*) and deep (*Cited2*) cortical layers. (*A–L*) In situ hybridization at E108in macaque (*A*,*D*,*G*,*J*), at E113 in macaque (*B*,*E*,*H*,*K*), and at P3 in mouse (*C*,*F*,*I*,*L*), for *Cited2* in layers II/III and V; and (*A–C*), *Chn2* (*D–F*), *Epha3* (*G–I*), and *Limch1* in superficial layers (*J–L*) showing molecular similarities between cellular populations in mouse and macaque cortex. (*A′–L′*) detailed insets. *N* = 3 individual mice, *N* = 2 technical replicates each. *N* = 1 E108 macaque, *N* = 3 technical replicates. *N*= 1 E113 macaque, *N* = 2 technical replicates. Scale bars: (*A–L*) 100 μm; (*A′*,*B′*,*D′*,*E′*,*G′*,*H′ J′*,*K′*) 200 μm macaque; (*C′*,*F′*,*I′*,*L′*) 100 μm mouse. E, embryonic day; VZ, ventricular zone; SVZ, subventricular zone; IZ, intermediate zone; CP, cortical plate.

expression of *Plexin-D1* later in immature migrating neurons. Conversely, absence of *Gfra2* expression in early CP is conserved between mouse and macaque. Interestingly, in addition to its conserved expression in layers Va and VI in P3 mouse and E108/113 macaque, *Gfra2* is also expressed at E108/113 by superficial layer neurons in macaque, suggesting a potentially novel neuronal subpopulation of superficial layer macaque CPN not present in mice (Fig. 5*E*).

A significant proportion of CPN (~10% in primates; ~20% in rodents) reside in deep layers and CPN are an appropriate deep layer population to study in order to better understand evolutionary diversity in deep layers of the cortex. Strikingly, all of the P3 deep layer mouse CPN genes examined (*Cited2* (Fig. 4A–C), *Dkk3*, *Plexin-D1*, and *Gfra2* (Fig. 5*B*,*D*)) are conserved in their deep layer expression in E108/113 macaque, suggesting that deep layer CPN could be evolutionarily relatively consistent between rodent and primate at these stages. Together, these data suggest that at E108/113, while expanded superficial layer CPN populations likely have refined and elaborated in primates, deep layer, evolutionarily older CPN have remained largely consistent molecularly, and therefore potentially also functionally.

The selected subset of superficial layer CPN genes (*Chn2*, *Limch1*, *EphA3* (Fig. 4*D–L*), *Nnmt*, and *Inhba* (Fig. 5*B*,*C*)) reveals populations of neurons that, while present in both rodent and

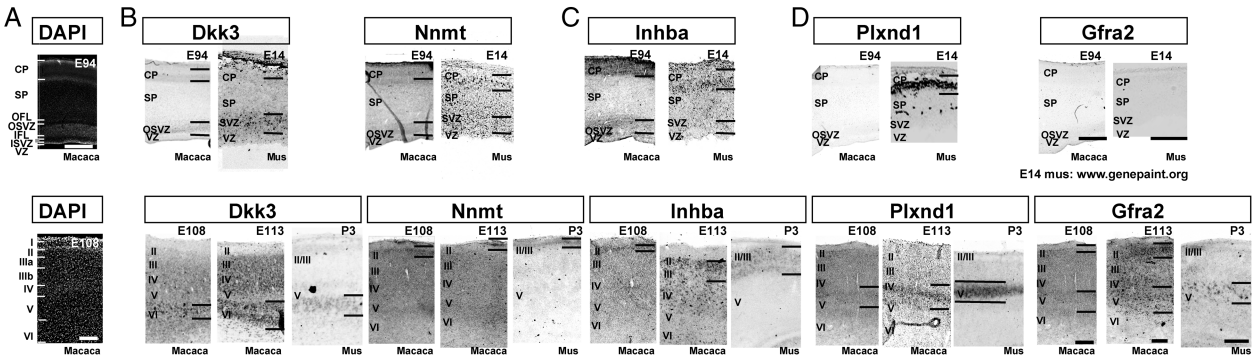

Figure 5. Some CPN genes have largely analogous developmental expression patterns in mouse and macaque, but with some divergence. (*A*) DAPI nuclear stain in E94 and E108 macaque for reference, horizontal bars indicate the extent of each named layer and are used to indicate dominant expression patterns in the following panels. (*B*) In situ hybridization at E94 in macaque and E14 in mouse for CPN genes with strong conservation of expression early in SVZ and CPN, as well as later at E108 and E113 (*Dkk3*, *Nnmt*) (*C*) in situ hybridization at E94 in macaque and E14 in mouse for *Inhba* shows early similarities in expression between mouse and macaque and more restricted expression later at E108 and E113. (*D*) In situ hybridization at E94 in macaque and E14 in mouse for *Plxnd1* reveal divergence in early expression, but conservation later at E108 and E113 in macaque and P3 in mouse. (*E*) In situ hybridization for *Gfra2* reveals that, while early expression is conserved, at later times gene expression in deep layers is conserved with additional expanded gene expression into layer II. $N = 3$ individual mice, $N = 2$ technical replicates each. $N = 1$ E108 macaque, $N = 3$ technical replicates. $N = 1$ E113 macaque, $N = 2$ technical replicates. Scale bars: 200 μm macaque; 100 μm mouse. E, embryonic day; P, postnatal day; VZ, ventricular zone; SVZ, subventricular zone; SP, subplate; ISVZ, inner subventricular zone; IFL, inner fiber layer; OSVZ, outer subventricular zone; OFL, outer fiber layer; CP, cortical plate; S1, primary somatosensory area; roman numerals indicate cortical layers. Horizontal gray bars indicate layers of dominant gene expression.

macaque, are differentially elaborated. While the most superficial portions of layer II/III do not contain the dominant proportion of mouse CPN, extremely superficial layer II/III CPN subpopulations do exist in P3 mouse cortex (Molyneaux et al. 2009); Fig. 6). Similarly, while layer II in macaque does not contain the dominant population of CPN at E108 (Killackey and Chalupa 1986; Dehay et al. 1988; Bullier et al. 1990; Meissirel et al. 1991), there is a small CPN population present in somatosensory cortex layer II at E108 (Killackey and Chalupa 1986). While *Limch1* and other layer II CPN genes are likely not exclusive to CPN, and are probably also expressed by intrahemispheric association neurons, *Limch1* is expressed by CPN and uniquely in mouse superficial layer II/III at P3 (Fig. 6*B*). Well-defined *Limch1* expression in macaque layer II (Fig. 4*J*–*K*) might correspond to the small number of layer II CPN present in macaque at E108.

Subcellular and Functional Areal Localization of CPN-Expressed Proteins Suggest Related Functions for Conserved Genes Expressed by CPN Populations

The above analyses reveal both substantial molecular conservation and some divergence between populations of CPN in mouse and macaque in distinct laminar locations within somatosensory areas at 2 developmental times. While comparative genomics has revealed more changes in regulatory than in coding regions (Stern and Orgogozo 2008), it remains unknown whether genes expressed by mouse and macaque CPN have shared functionality within these neurons. As a second assessment of potentially conserved gene function (after temporal- and laminar-specific expression, as described above), we investigated whether proteins developmentally expressed in macaque cortex share areal and/or subcellular localization with those expressed by mouse CPN.

LMO4 is differentially expressed in particular neocortical areas in mouse (Arlotta et al. 2005; Sun et al. 2005; Joshi et al. 2008; Huang et al. 2009; Cederquist et al. 2013). We investigated whether this is a shared feature between mouse and macaque, even though it might be modified by divergence in the complex process of cortical arealization between rodents and primates (Rakic 1988; Donoghue and Rakic 1999a, 1999b). Strikingly, neocortical areal restriction of LMO4 is conserved (Fig. 7*A*, Supplementary Fig. 1*A*). In E108 macaque, as in P3 mouse, LMO4 expression in motor cortex is throughout all neocortical layers, instead of the more restricted expression found in somatosensory cortex.

To compare subcellular localization of subtype-specific proteins, we selected 3 with distinct subcellular localization in mouse CPN: 1) Nectin-3, with axonal white matter localization in superficial layer CPN axons in the CC early in development (see Molyneaux et al. 2009); 2) CAV1, with cell body and membrane/neurite localization in a restricted subpopulation of CPN (Gaillard et al. 2001; Boulware et al. 2007; Molyneaux et al. 2009); and 3) LMO4, with nuclear localization in deep layer CPN of somatosensory cortex (Molnár and Cheung 2006; Huang et al. 2009; Cederquist et al. 2013). At E94, Nectin-3 is localized specifically in white matter tracts in developing macaque cortex (Fig. 7*B*, Supplementary Fig. 1*B*). There is minimal localization in the cell-dense CP, higher localization throughout the cell-sparse subplate, and very high, fiber-enriched localization in both the outer and inner fiber layers. Similarly, CAVI and LMO4 subcellular localizations are also highly conserved in both species. In E108 macaque, CAV1 is localized in neuronal cell membranes and neurites, as well as developing blood vessels (Fig. 7*C*, Supplementary Fig. 1*C*) (Gaillard et al. 2001; Sbaa et al. 2006; Boulware et al. 2007; Molyneaux et al. 2009), while LMO4 is localized in nuclei of deep layer somatosensory neocortical neurons (Fig. 7*D*, Supplementary Fig. 1*C*), as in P3 mouse (Arlotta et al. 2005; Molnár and Cheung 2006; Huang et al. 2009; Cederquist et al. 2013). Together, these data suggest that gene product function could be shared by Nectin-3, CAV1, and LMO4 in early cortical development of rodents and primates, based on conserved protein subcellular localization and areal-specific expression between the mouse and macaque brains, motivating future functional analyses of these proteins in neocortical development. Conserved subcellular localization for these gene products within shared neuronal populations adds further support to the hypothesis that not only did rodents and primates likely arise from shared ancestors with specific genetic controls over complex cortical neuronal populations already present, but also supports the hypothesis that gene products have similar functions. If further functional analyses validate conserved function, it would

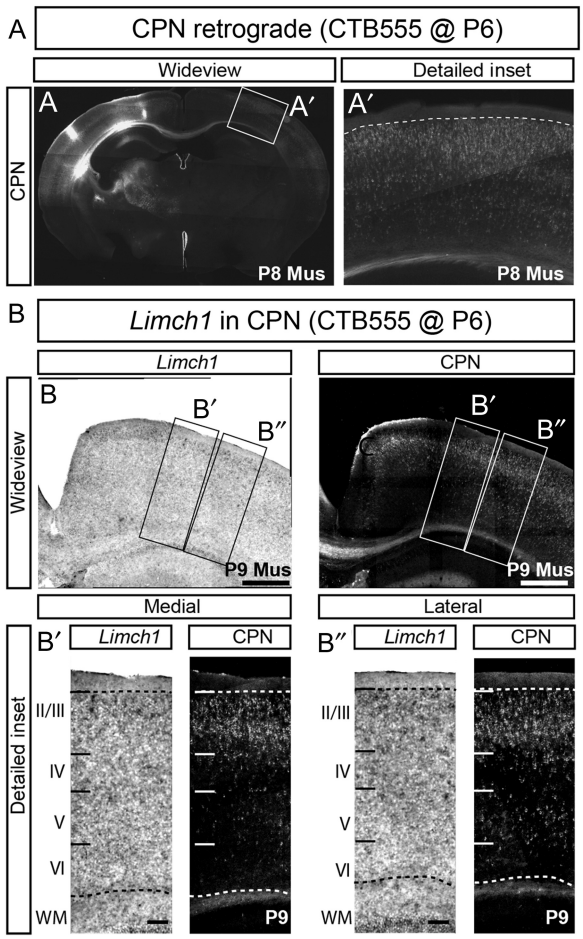

Figure 6. While superficial layer II/III does not contain the dominant proportion of CPN in mouse, superficial layer II/III CPN subpopulations do exist in P3 mouse cortex and express superficial layer II/III CPN genes, including *Limch1*. (*A*) Retrograde labeling in mouse using cholera toxin subunit B (CTB) reveals CPN cell bodies in cortical layer II/III at P8, including superficial portions of layer II/III. (*B*) In situ *hybridization* combined with retrograde labeling using CTB reveals CPN cell bodies in superficial portions of layer II/III whose localization overlaps with expression of the CPN gene *Limch1* in mouse, suggesting that the layer II expression of *Limch1* in Macaque (Fig. 4) might include CPN within that layer. (*B′*) Detailed medial inset from (*B*). (*B″*) Detailed lateral inset from (*B*). Scale bars: (*B*) 500 μm; (*B′*,*B″*) 100 μm. CPN, callosal projection neurons; CTB, B subunit of cholera toxin, P, postnatal day.

suggest applicability of rodent studies of these proteins' functions to understanding human development and disease.

Discussion

It is interesting to speculate that the presence of a molecularly diverse population of CPN in the superficial layers of the rodent cortex (Molyneaux et al. 2009) is inherited from a common ancestor of rodents and primates that had already undergone early stages of the evolutionary expansion of these layers and conspicuously developed further complexity during subsequent evolution of the primate cortex. The present study begins to investigate this hypothesized evolutionary trajectory by analyzing comparative expression in primate of a representative selection of genes expressed by bona fide CPN in mouse. Genes that identify neurons located at distinct positions within mouse layer II/III support the hypothesis that specialization of distinct populations of CPN within the superficial layers are at least partially molecularly conserved in E14 and P3 rodent; and E94 and E108/113 primate cortex (Table 1, Supplementary Fig. 2), and possibly also with respect to connectivity and function. The work presented here directly investigates within the mouse and macaque cortex the expression of genes initially identified in distinct populations of mouse CPN, to provide insight into cortical evolution and elaboration of distinct populations of neocortical neurons. Enlargement of the superficial layers during cortical evolution is accompanied by the emergence and expansion of the SVZ (see Introduction), and the concomitant appearance of diverse progenitors with complex lineages largely fated to produce neurons of the superficial layers (Smart and McSherry 1982; Martinez-Cerdeno et al. 2006; Molyneaux et al. 2007; Woodworth et al. 2012; Betizeau et al. 2013; Custo-Grieg et al. 2013; Dehay et al. 2015).

While rodents and primates both contain basally-located progenitors in the SVZ, the type, function, and specific localization of these progenitors differ significantly between mouse and macaque. The vast majority (~90%) of mouse intermediate SVZ progenitors are not polarized and are similar to the progenitors of the inner SVZ (ISVZ) in primate; they express *Tbr2*, but downregulate *Pax6* (Fish et al. 2008; Fietz et al. 2010; LaMonica et al. 2012; Betizeau et al. 2013). A distinct, polarized, radial basal progenitor type is highly expanded in primate outer subventricular zone (OSVZ) and these progenitors are more similar to radial glial cells, both molecularly and morphologically (Smart et al. 2002; Lukaszewicz et al. 2005; Fish et al. 2008; Fietz et al. 2010; Hansen et al. 2010; Betizeau et al. 2013). Such progenitors constitute only a minute proportion of mouse progenitors, and these are not restricted to a specific zone. Polarization in these progenitors allows for more nonconsuming, nonterminal divisions (Fish et al. 2008; LaMonica et al. 2012; Betizeau et al. 2013). Importantly, these primate OSVZ progenitors exhibit stage-specific cell cycle dynamics, with a reduction of cell cycle duration in later stages of corticogenesis when neurons destined for the superficial layers are being generated. This suggests that the molecular and cellular properties of these progenitors contribute to increased superficial layer complexity and expansion in primates in comparison to rodents (Betizeau et al. 2013; Arcila et al. 2014; Dehay et al. 2015). Further, the radial glial-like progenitors of the OSVZ undergo both symmetric, self-renewing divisions, as well as asymmetric, neurogenic self-renewing divisions, and their cellular dynamics in primates vary at distinct developmental stages (Fietz et al. 2010; Hansen et al. 2010; Lui Jan et al. 2011; Betizeau et al. 2013). This capacity of OSVZ progenitors to undergo self-renewing asymmetric divisions to generate progenitors that can further proliferate greatly enhances neuronal output, and might have been an important evolutionary step in the expansion of the cortex, especially superficial layers (Dehay et al. 2015).

We find that many of the early-expressed CPN genes in rodents and primates are highly consistent in laminar expression (e.g., *Ptn*, *Inhba* (Fig. 3), and *Dkk3*, *Nnmt*, (Fig. 5)), but none are overtly restricted to the OSVZ, which reaches its maximum expansion in the primates. In addition, *Epha3*, expression in the CP has been shown previously to have similar CP expression in macaque earlier at E65, but restricted to caudal cortex (Donoghue and Rakic 1999a, 1999b). Taken together, conservation of these genes' expression might reflect a more ancient evolutionary origin of CPN than previously identified (at least during CPN generation), but also allows for the possibility of additional molecular complexity in primate progenitor domains than is observed in mouse (Arcila et al. 2014).

Because superficial neocortical layers contain the overwhelming majority of CPN, we hypothesized that some

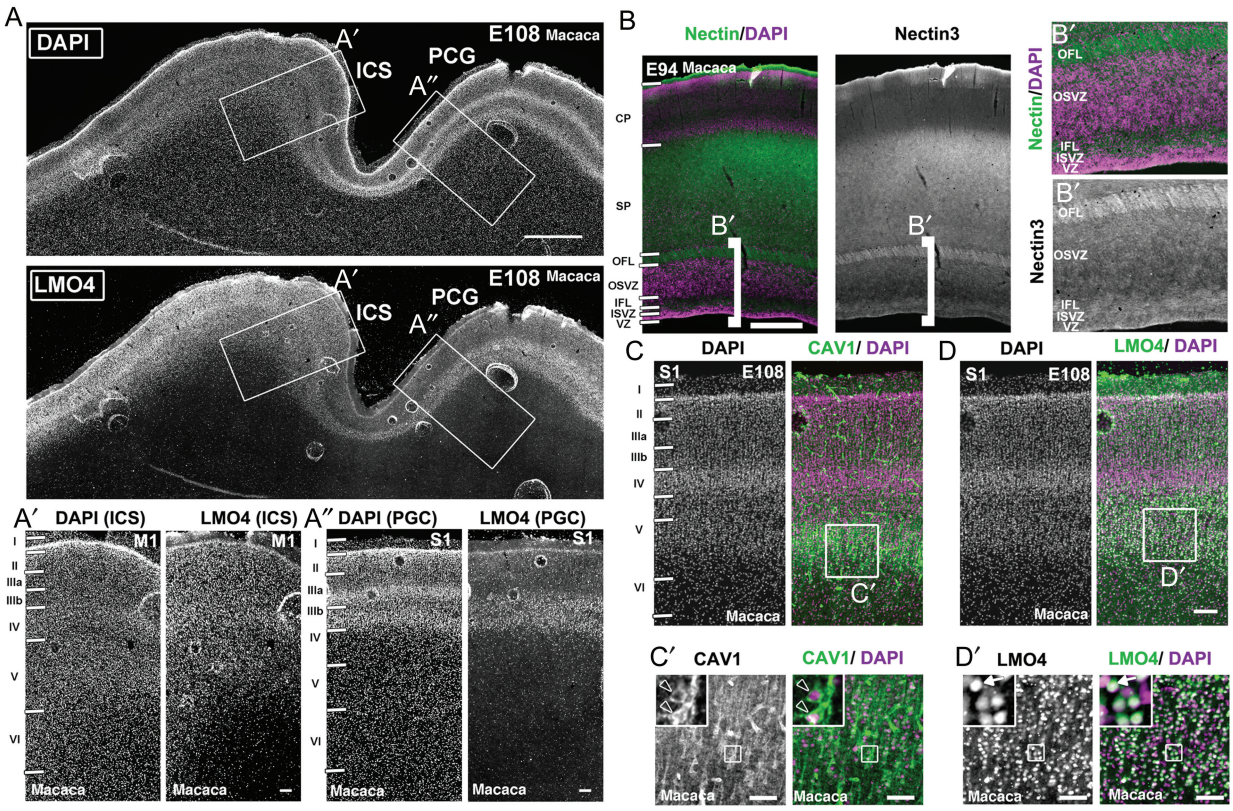

Figure 7. Functional areal and subcellular localization of CPN-expressed proteins suggests related functions in rodents and primates for conserved genes expressed by CPN populations. (*A*,*A*′,*A*″) Areal restriction of LMO4 is conserved between mouse and macaque. LMO4 expression in motor cortex extends throughout all neocortical layers, and more restricted expression limited to deep layers is observed in somatosensory cortex. (*B*,*B*′,*C*,*C*′,*D*,*D*′) Three proteins with distinct subcellular localization in mouse CPN were selected for study of subcellular localization in macaque: 1) Nectin-3, with axonal white matter localization by superficial layer CPN axons in the CC; 2) CAV1, with cell body and neurite localization by a subpopulation of deep layer CPN; and 3) LMO4, with nuclear localization in deep layer CPN of somatosensory cortex. (*B*,*B*′) At E94, Nectin-3 is localized specifically in white matter tracts in developing macaque cortex. There is low-level localization in the cell-dense cortical plate, higher level localization throughout the cell-sparse subplate, and very high-level fiber-localization in both the outer and inner fibrous layers. (*C*,*C*′) CAV1 is localized to neuronal cell membranes and neurites, as well as to developing blood vessels, as is seen in mouse ((Gaillard et al. 2001; Boulware et al. 2007; Molyneaux et al. 2009), and (*D*,*D*′) LMO4 shows nuclear localization in deep layer somatosensory neocortical neurons, as has been shown in mouse. Empty arrowheads indicate membranous localization, and filled arrows indicate nuclear localization. Scale bars: (*A*) 1 mm; and (*A*′,*A*″) 100 μm; (*B*–*D*) 100 μm; (*C*′,*D*′) 50 μm. E, embryonic day; P, postnatal day; ICS, incipient central sulcus; PCG, postcentral gyrus; S1, primary somatosensory area; M1, primary motor area; VZ, ventricular zone; SVZ, subventricular zone; SP, subplate; ISVZ, inner subventricular zone; IFL, inner fiber layer; OSVZ, outer subventricular zone; OFL, outer fiber layer; CP, cortical plate; roman numerals indicate cortical layers.

Table 1 Summary of laminar localization of CPN-enriched genes in mouse and macaque

A	E94 (Macaque)	E14 (Mouse)
CP	*Ptn*, *Lmo4*, *Inhba*, *Dkk3*, *Nnmt*	*Ptn*, *Lmo4*, *Inhba*, *Dkk3*, *Nnmt*, *Plxnd1*
SP		
SVZ	*Ptn*, *Dkk3*, *Nnmt*, *Inhba*	*Ptn*, *Dkk3*, *Nnmt*
VZ		
B	**E108/113 (Macaque)**	**P3 (Mouse)**
II (II/III sup)	*Limch1*, *Nnmt*, *Epha3*, *Inhba*, *Gfra2*, *Chn2*	*Limch1*, *Nnmt*, *Epha3*, *Inhba*
III (II/III deep)	*Cited2*, *Epha3*	*Cited2*, *Inhba*, *Chn2*
IV		
V	*Cited2*, *Dkk3*, *Plxnd1*, *Gfra2*	*Cited2*, *Dkk3*, *Plxnd1*, *Gfra2*
IV		

Early (A: E94 Macaque; E14 Mouse) and late (B: E108/113 Macaque; P3 Mouse) laminar localization of gene expression examined in this study. Black-colored gene names show similar expression at these times between the 2 species. Red-colored gene names show differential expression at these times between the 2 species.
E, embryonic day; P, postnatal day; VZ, ventricular zone; SVZ, subventricular zone; IZ, intermediate zone; CP, cortical plate; S1, primary somatosensory area; Roman numerals indicate cortical layers.

molecular controls over CPN development that segregate superficial layers in mice might reflect a common origin for CPN in the expanded superficial layers identified in primates. Some gene expression patterns identified in mouse at P3, including those of *Nnmt* and *Chn2*, very closely match those of E108/113 macaque. However, while mouse CPN molecular diversity suggests correlates to some more elaborated populations of primate CPN, the mouse repertoire does not appear to contain all of the primate molecular diversity, and has likely independently acquired unique populations critical for rodent cortical function. For example, while both *EphA3* and *Limch1* are expressed in rodent and macaque superficial layers, they are expressed by differentially expanded populations. This suggests that some CPN populations common to mouse and macaque were additionally elaborated in primates, whereas others were preferentially elaborated in rodents. These discrete populations might reflect functional processing differences that distinguish mouse and macaque, and might provide insight into CPN functions that are more dominant in human integrative function and cognition.

Compared with superficial layers, deep neocortical layers contain a high proportion of CPN, but have undergone less extensive expansion over mammalian evolution. Therefore, we hypothesized that most of the newly identified molecular controls over deep layer CPN development in mouse might likely be conserved in macaque. Indeed, all of the deep layer CPN gene expression patterns investigated are conserved between P3 mouse and E108/113 macaque (*Cited2*, *Dkk3*, *Plexin-D1*, and *Gfra2*), supporting the hypothesis that deep layer CPN are likely relatively evolutionarily consistent between rodent and primate during corticogenesis. A more systematic comparative study would be needed to verify function, but these data suggest that, while the expanded superficial layer CPN populations have likely refined and elaborated in primates, deep layer, evolutionarily older CPN have remained largely consistent molecularly, and therefore potentially also functionally.

Unexpectedly, *Gfra2*, expressed exclusively in layer Va and VI CPN in P3 mouse, is, in addition to deep layer expression, also expressed strongly by superficial layer neurons in E108/113 macaque, suggesting a potentially entirely new population of CPN that acquired genetic controls first employed by deep layer CPN. During development, deep layer CPN cross the midline prior to superficial layer CPN, and are related molecularly and by birthdate to corticofugal neurons, leading to the hypothesis that deep layer CPN were modified from existing corticofugal neuronal populations to become the first to cross the midline early in evolution (Lai et al. 2008; Azim et al. 2009; Fame et al. 2011). These results suggest the possibility that similar molecular pathways existing in basal populations could evolve for new and/or expanded functions in the *Gfra2*-expressing population of superficial layer neurons in E108/113 macaque. In this case, superficial layer CPN in E108/113 macaque might be derived from neurons forming very different connections, even from a subpopulation of corticofugal neurons. Data showing that genes (e.g., *Gfra2*) preferentially expressed in deep layers in the mouse exhibit an additional superficial layer expression in the macaque confirm and extend a large scale comparative study between mouse and human cortex (Zeng et al. 2012), reporting a shift in gene expression from layer V in mouse to layer III in human cortex; hypothesized to contribute to differences in cortical function across species. Without functional data, divergence could also reflect passive deregulation of gene expression, while conservation argues strongly for conserved function. However, for divergently expressed genes such as early *Plexin-D1*, which has been shown to be critical for neocortical development (Bribian et al. 2014), passive deregulation is unlikely. Our current study further motivates functional analysis of the other divergent factors during corticogenesis.

In addition to CPN, both superficial and deep neocortical layers contain other populations of neurons. Deep layers predominantly contain corticofugal neurons. Superficial layers, also contain populations of noncallosal pyramidal neurons. In addition, all layers contain inhibitory interneurons, which could share a subset of gene expression with nearby excitatory neurons with common generation time and laminar/sublaminar location. While the genes examined here in macaque were identified in a comparative microarray analysis in mice designed to identify genes more highly expressed by CPN than by CSMN and other SCPN in mice (also compared with corticothalamic projection neurons; MJ Galazo et al., unpublished), many are likely not exclusive to CPN. However, many of these genes are expressed quite specifically by mouse CPN compared with other cortical neuron subtypes (Molyneaux et al. 2009). In the macaque, we still lack an understanding of the connectivity of the neurons expressing these genes. It could be enlightening in the future to examine the gene expression of CPN identified by retrograde labeling during macaque corticogenesis, with the provision that the gene expression level is sufficient at developmental stages when CPN retrograde labeling is practical (around E120– E130).

Complementary to comparative gene expression results in radially expanded neocortical neuronal populations, we also investigated the subcellular localization of CPN-expressed gene products in mouse and macaque to gain insight into potentially conserved gene function. We found that protein subcellular localization for Nectin-3, CAV1, and LMO4 is conserved between mouse and macaque, with Nectin-3 in axonal white matter, CAV1 in cell membranes and apical dendrites, and LMO4 in nuclei. Additionally, differential expression in distinct cortical areas is also conserved for LMO4. While conserved areal expression reflects conserved gene regulatory regions, identification of conserved protein localization additionally suggests conserved gene product function, which remains to be functionally investigated.

While differential segregation of gene expression has occurred in rodent and primate cortices, both contain CPN, and, therefore, it is likely that conserved gene expression at least partially maps onto conserved CPN populations. However, in some species of marsupials, in which distinct layers II and III are observed without CPN, evolutionary relationships between cortical neuron subtypes are less easily derived. Marsupials have fewer neurons compared with their rodent counterparts, but they do have a distinct SVZ with basal progenitors expressing conserved genetic factors, which appears later in cortical development, perhaps contributing to the smaller overall cortex (Cheung et al. 2010). Determining whether genes that identify CPN subpopulations in placental mammals are expressed in marsupial cortex might clarify how superficial layers without CPN developed from a common ancestor. Therefore, understanding relationships between gene expression in particular CPN subpopulations in mouse and macaque, and comparing with large cortices in marsupials, might provide evolutionary insight into the origins of marsupial connectivity, and cortical expansion; and generation of neuronal diversity across all mammalian species. The sauropsid cortex contains only 3 layers, which, share features with multiple neuronal populations in mammals (Reiner 1991; Suzuki et al. 2012; Belgard et al. 2013). Further investigation of subtype-specific gene expression in those sauropsid cortical populations most closely related to CPN, in particular those genes with conserved expression in mouse and macaque, could reveal additional evolutionary relationship among these neuronal populations.

The data presented here provide the first comparison of the molecular identity of CPN subpopulations in rodent and primate. Such comparative information will likely be important for defining the extent to which rodent research can address the complex integrative functions of primate cortex. In the present study, the laminar distribution of recently identified mouse CPN genes in macaque at 2 developmental stages reveals the comparative homology of molecularly defined subpopulations that had not yet been previously described at histological, morphological, or anatomical levels. It is likely that distinct combinations of molecular developmental controls define key aspects of CPN diversity in both species—subtype-specific differentiation, axon collateralization, synaptic connectivity, and physiologic function—underlying their central roles in interhemispheric connectivity. Together, these data provide a foundation of evolutionary relationships that will inform future studies about those complex subpopulations of primate CPN that can be studied with molecular and genetic approaches in mouse and those that are unique to primate.

Authors' Contributions

R.M.F. and J.D.M. initiated the investigation. R.M.F. performed laboratory experimental work and imaging. R.M.F., C.D., H.K., and J.D.M. planned all experiments. C.D. and H.K. performed macaque tissue harvesting and preparation. R.M.F., C.D., H.K., and J.D.M. interpreted data and wrote the manuscript.

Supplementary Material

Supplementary material can be found at: http://www.cercor.oxfordjournals.org/.

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Notes

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