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Spatiotemporal Fate Map of Neurogenin1 (Neurog1) Lineages in the Mouse Central Nervous System

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Abstract

Neurog1 (Ngn1, Neurod3, neurogenin1) is a basic helix-loop-helix (bHLH) transcription factor essential for neuronal differentiation and subtype specification during embryogenesis. Due to the transient expression of Neurog1 and extensive migration of neuronal precursors, it has been challenging to understand the full complement of Neurog1 lineage cells throughout the central nervous system (CNS). Here we labeled and followed Neurog1 lineages using inducible Cre-flox recombination systems with *Neurog1-Cre* and *Neurog1-CreER^{T2}* BAC (bacterial artificial chromosome) transgenic mice. Neurog1 lineage cells are restricted to neuronal fates and contribute to diverse but discrete populations in each brain region. In the forebrain, Neurog1 lineages include mitral cells and glutamatergic interneurons in the olfactory bulb, pyramidal and granule neurons in the hippocampus, and pyramidal cells in the cortex. In addition, most of the thalamus, but not the hypothalamus, arises from Neurog1 progenitors. Although Neurog1 lineages are largely restricted to glutamatergic neurons, there are multiple exceptions including Purkinje cells and other GABAergic neurons in the cerebellum. This study provides the first overview of the spatiotemporal fate map of Neurog1 lineages in the CNS.

Indexing Terms

bHLH transcription factor; neural progenitor; brain development; genetic fate mapping; Ngn1

The central nervous system (CNS) comprises diverse types of neurons with elaborate connectivity required for its functions. As a first step in constructing functional neural circuitry, it is essential to generate the correct numbers of neurons with the correct identity in a temporally and spatially defined order. During embryogenesis, neural basic helix-loop-helix (bHLH) transcription factors such as Atoh1 (previously Math1), Neurog1 (previously Ngn1, neurogenin1, Neurod3), and Ascl1 (previously Mash1) are important regulators of these processes and are expressed in discrete, largely nonoverlapping progenitor domains (Lee, 1997; Gowan et al., 2001). This small set of bHLH factors regulates neuronal

differentiation and subtype specification for diverse populations of neurons throughout the central and peripheral nervous systems (for review, see Bertrand et al., 2002). In this study we used in vivo genetic fate mapping to define the regions in the brain that are derived from Neurog1 progenitor cells.

During embryogenesis, Neurog1 is present in progenitor cells in the ventricular zones within the dorsal telencephalon, diencephalon, mesencephalon, hindbrain, and the spinal neural tube as well as in the peripheral nervous system including the olfactory epithelium, and the cranial and spinal sensory ganglia (Ma et al., 1996, 1997; Cau et al., 1997; Perez et al., 1999; Fode et al., 2000; Gowan et al., 2001; Koundakjian et al., 2007). Overexpression studies using *Xenopus* embryos, primary mouse cortical progenitors, or mouse embryonic carcinoma P19 cells demonstrate that Neurog1 is sufficient to induce neuronal differentiation (Ma et al., 1996; Farah et al., 2000; Sun et al., 2001). Indeed, in primary cultures of cortical progenitors Neurog1 induced neuronal and suppressed glial differentiation (Sun et al., 2001). Furthermore, a role for Neurog1 in neuronal subtype specification was seen in overexpression in the chick neural crest that biased the cells to a sensory neuron fate, while expression in the dorsal neural tube biased cells to dI2 interneurons (Perez et al., 1999; Gowan et al., 2001). Loss of function studies using Neurog1 null mice showed that Neurog1 is required for the formation of sensory neurons in the olfactory epithelium and proximal cranial ganglia (Ma et al., 1998; Cau et al., 2002). Neurog1 is also required for sensory neurons in the dorsal root ganglia, dI2 interneurons in the dorsal neural tube, and excitatory neurons in the cerebral cortex when deleted in combination with the related factor Neurog2 (Ma et al., 1999; Fode et al., 2000; Gowan et al., 2001). Taken together, Neurog1 functions as a cell fate determination factor, regulating neuronal differentiation and subtype specification.

Neurog1 is largely restricted to proliferating cells, and as the cells move out of the ventricular zone and differentiate, Neurog1 expression disappears. Thus, despite some Neurog1 lineages being defined, such as those mentioned above in the loss of function studies, a fate map of Neurog1 derived lineages, particularly in the brain, is not complete. Using *Neurog1-Cre* and *Neurog1-CreER^{T2}* transgenic mice combined with Cre recombinase reporter mouse strains, we identify the neural cells derived from Neurog1-expressing progenitors from different embryonic germinal zones at different embryonic stages. First, Neurog1 lineage cells contribute almost exclusively to the neuronal rather than oligodendrocyte or astrocyte lineages. Second, they contribute to defined populations in many CNS regions such as mitral cells and glutamatergic interneurons in the olfactory bulb, pyramidal neurons in the cortex and hippocampus, and most thalamic neurons projecting to the cortex. Although most Neurog1 lineage neurons are glutamatergic, there are exceptions, such as Purkinje cells and interneurons in the cerebellum that are GABAergic. These results support the idea that Neurog1 functions as a neuronal differentiation factor in all its lineages, whereas its function in subtype specification varies depending on the developmental contexts. This study correlates the temporal and spatial origin of Neurog1 progenitors and their final identities throughout the brain.

Materials and Methods

Transgenic mice and tamoxifen injection

Neurog1-Cre (*NI⁴⁵⁷-Cre*), *Neurog1-CreER^{T2}* (*Tg(Neurog1-cre/ESR1)1Good*), *R26R^{lacZ}* (*Gt(ROSA)26Sor^{tm1Sor}*), *Tau^{mGFP-nlacZ}* and *Z/EG* (*Tg(CAG-Bgeo/GFP)21Lbe*) mice have been previously described (Soriano, 1999; Novak et al., 2000; Hippenmeyer et al., 2005; Koundakjian et al., 2007; Quinones et al., 2010). Briefly, *Neurog1-Cre* and *Neurog1-CreER^{T2}* mice are transgenic mice containing a modified bacterial artificial chromosome (BAC) (RP23 457E22) where Cre or CreER^{T2} precisely replaces the *Neurog1* coding region, respectively (Koundakjian et al., 2007; Quinones et al., 2010). The *Neurog1-Cre* mice were generated using the entire modified BAC clone, whereas *Neurog1-CreER^{T2}* mice were generated with a NotI fragment from the modified BAC (see Fig. 1). *R26R^{lacZ}*, *Tau^{mGFP-nlacZ}*, and *Z/EG* mice are reporter mice that express β -galactosidase, membrane-targeted GFP-IRES-nuclear-localized β -galactosidase, or EGFP, respectively, after Cre recombination (Soriano, 1999; Novak et al., 2000; Hippenmeyer et al., 2005). *R26R^{lacZ}* and *Z/EG* mice use the Rosa26 locus or chick beta-actin promoter for broad expression, while *Tau^{mGFP-nlacZ}* is largely restricted to neurons. All animal work was approved by UT Southwestern's Institutional Animal Care and Use Committee.

Tamoxifen induction of Cre recombinase was accomplished by intraperitoneal injection of pregnant dams at noon of a given day postcoitum (dpc) with 50–75 mg tamoxifen per kg mouse (Sigma, St. Louis, MO; T55648) in sunflower oil. Embryonic or adult brains were harvested at the times specified after tamoxifen treatment.

Tissue preparation and X-gal staining

Whole embryos (embryonic day [E]9.5–13.5) were fixed by immersion in 4% formaldehyde for 1–2 hours at room temperature. Postnatal day (P)30 adult brains were dissected from the skull after animals were anesthetized with Avertin and perfused with 4% formaldehyde transcardially. The brains were postfixed with 2% or 4% formaldehyde overnight at 4°C, rinsed in phosphate buffer, cryo-protected with 30% sucrose overnight, and sectioned sagittally or coronally at 50–100 μ m.

For X-gal staining, whole embryos or adult brain sections mounted on slides were incubated overnight in X-gal staining solution (1 mg/mL 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside [X-gal], 5 mM K₃Fe(CN)₆, 5 mM K₄Fe(CN)₆, 2 mM MgCl₂ in phosphate-buffered saline [PBS] and 0.02% NP-40). Adult brain sections were counterstained using Nuclear Fast Red. X-gal-stained whole embryos or tissue sections were photographed using an Olympus SZX12 or Zeiss Discovery V12 microscope.

Immunofluorescence

For immunofluorescence staining, free-floating sections or sections mounted on slides were incubated in the appropriate dilution of primary antibody in PBS pH 7.4 with 3% donkey (or goat) serum/0.2% NP-40 (or 0.2% Triton X-100), followed by appropriate secondary antibody conjugated with Alexa Fluor 488, 568, or 594 (Molecular Probes, Eugene, OR). Antibodies were used as markers for specific cell types and all staining patterns observed

with these antibodies were consistent with previously reported patterns of cellular morphology and distributions of these proteins throughout the brain. All have met the requirements for use in *Journal of Comparative Neurology* publications. NeuroTrace Fluorescent Nissl Stain was used to counterstain neurons (1:200, Molecular Probes). Confocal imaging was carried out on a Zeiss LSM510. Images were stored as tiff files and imported to Adobe Photoshop CS2 (San Jose, CA) to crop and adjust size of images for figure composites generated in Adobe Illustrator CS2. Any adjustments to brightness or contrast were applied equally across the entire image.

Antibody characteristics

Please see Table 1 for a list of all antibodies used. β -Galactosidase (Abcam, Cambridge, MA; Ab9361), β -galactosidase (Biogenesis, Brentwood, NH; 4600-1409), GFP (Aves Lab, Tigard, OR; GFP-1020), and GFP (Molecular Probes, A6455) antibody specificity in each case is concluded from the absence of signal in nontransgenic animals. Anti-glutamine synthetase (Chemicon/Millipore, Bedford, MA; MAB302) reacts with a single 45-kDa band on western blots of retinal tissue (Chang et al., 2007). Here the morphology of the cells and the cellular localization to the cytoplasm was characteristic of astrocyte specificity in the mouse brain (Cai et al., 2007). Anti-NeuN (Chemicon; MAB377) specificity was determined by the staining pattern/morphology in cerebellum where immunoreactivity is seen as nuclear staining in neurons in the granular layer. Anti-Olig2 (Chemicon; AB9610) specificity was determined by the absence of staining in tissue lacking oligodendrocytes, and the presence in a characteristic nuclear localization pattern in tissues containing oligodendrocytes (Cai et al., 2007). Anti-Pax2 (Invitrogen, La Jolla, CA; 71-6000) recognizes both Pax2a (minor band at 48 kDa) and Pax2b (major band at 46 kDa) as seen in western blots of mouse embryonic kidney (Dressler and Douglass, 1992). In the cerebellum, Pax2 is nuclear as expected for a transcription factor and is characteristically present in GABAergic interneurons. For anti-Tbr2 (gift from R. Hevner, Seattle Children's Research Institute) Western blotting of mouse brain homogenates shows a major 73 kDa band, matching the predicted molecular weight of Tbr2 and immunostaining is nuclear localized in the characteristic Tbr2 pattern in cortex (Quinn et al., 2007).

In situ hybridization

In situ hybridization for detecting mRNA was performed based on standard protocols (Birren et al., 1993). Briefly, digoxigenin (DIG)-labeled antisense RNA probes were made using T7 or T3 RNA polymerase and dNTP/DIG-UTP, DNase-treated, and purified using Roche Quick Spin (TE) G-50 Sephadex columns. Cryosections were prefixed 10 minutes with 4% paraformaldehyde in PBS, treated with 5 μ g/mL Proteinase K for 15 minutes, postfixed for 10 minutes, acetylated with acetic anhydride in triethanolamine, and prehybridized for 1–4 hours at 65°C. Hybridization of DIG-labeled riboprobes (2.5 μ g/mL) was performed overnight at 65°C. Sections were washed with 2 $\times$ SSC at 65°C for 15 minutes, treated with 10 μ g/mL RNase A and 1 U/mL RNase T1 in 2 $\times$ SSC at 37°C for 30–45 minutes, washed with 0.2 $\times$ SSC at 65°C for 1 hour, blocked with 10% sheep serum in PBS/2 mg/mL bovine serum albumin (BSA), 0.1% Triton X-100 (PBT). Sections were then incubated with anti-digoxigenin AP antibody (Roche, Nutley, NJ) in PBT overnight at 4°C, and incubated in NBT/BCIP (Roche) at room temperature until a blue precipitate was

detected. A more detailed procedure is available upon request. Digoxigenin-labeled riboprobes were generated from plasmids containing the entire coding region of mouse *Neurog1* (750 bp) or a 1-kb EcoR1/XhoI fragment from pCre-ERT2 corresponding to *Cre* (gift from A. Joyner). The images were photographed using a Zeiss Discovery V12 microscope and incorporated into the figure in Adobe Illustrator.

Neuroanatomical analyses

Nomenclature of anatomical structures for the adult brain was assigned using *The Mouse Brain in Stereotaxic Coordinates* (Paxinos and Franklin, 2001) as a reference guide. For analysis of the embryonic brain, *The Atlas of Prenatal Rat Brain Development* (Altman and Bayer, 1995) and *Chemoarchitectonic Atlas of the Developing Mouse Brain* (Jacobowitz and Abbott, 1997) were used as guides.

Results

Neurog1-Cre and Neurog1-CreER^{T2} transgenic lines to fate map Neurog1 lineage cells

In order to trace the spatiotemporal fate map of Neurog1 lineage cells in brain, we used two BAC transgenic mice expressing Cre recombinase under the control of *Neurog1* regulatory regions (Fig. 1). The *Neurog1-Cre* (also known as *NI⁴⁵⁷-Cre*) transgenic line expresses constitutively active Cre recombinase mimicking the *Neurog1* expression pattern (Raft et al., 2007; Quinones et al., 2010). In transgenic mice containing both *Neurog1-Cre* and Cre reporter transgenes such as *R26R^{lacZ}* (Soriano, 1999), Cre recombination permanently removes the stop sequence, allowing reporter gene expression in the Neurog1 progenitor cell and all subsequent progeny. The cumulative representation of *Neurog1* lineage cells is visualized by reporter gene expression. In contrast, the *Neurog1-CreER^{T2}* transgenic line expresses an “inducible” Cre recombinase mimicking the *Neurog1* expression pattern (Koundakjian et al., 2007). In this case, Cre recombinase is activated only upon the treatment of the mice with tamoxifen; the activation of Cre recombination starts a few hours after tamoxifen administration and persists ≈24 hours. Thus, progenitor cells expressing Neurog1 only within a given time window (specific embryonic stage) will be labeled in this paradigm.

To verify that *Cre* is expressed in these transgenic models in the same pattern as *Neurog1*, we used mRNA in situ hybridization with *Cre* and *Neurog1* probes in transgenic embryos at E11.5 and E14.5. The *Cre* mRNA in situ pattern recapitulates *Neurog1* mRNA in situ pattern in both *Neurog1-Cre* and *Neurog1-CreER^{T2}* embryos (Fig. 1B-I and data not shown) (Koundakjian et al., 2007; Quinones et al., 2010). Expression of *Cre* is restricted to the ventricular zones in *Neurog1*-specific domains throughout all regions of the embryonic neural anlage. Thus, these two transgenic lines can be used to examine the dynamics of *Neurog1* lineage development throughout embryogenesis and to assess their fates in the mature brain.

Neurog1 lineage cells identify discrete populations of neurons, but not glia, throughout the brain

Neurog1-Cre;R26R^{lacZ/+} mouse brains were harvested at P30. X-gal-stained parasagittal sections demonstrate that *Neurog1* progenitors give rise to many neurons in specific subdomains in every major brain region (Fig. 2F). In the forebrain, many X-gal-stained cells are present densely in all layers of cerebral cortex, hippocampus, and thalamus, whereas scattered X-gal-stained cells are in striatum and hypothalamus. Both the mitral cell layer and the glomerular layer in the olfactory bulb contain X-gal-stained *Neurog1* lineage cells (Fig. 3M). In the midbrain and hindbrain, X-gal-stained cells are present densely in the superior and inferior colliculi, while less dense but discrete populations are in the ventral midbrain, pons, and medulla, and the Purkinje cell layer and granule cell layer in the cerebellum. Brain regions notably lacking contribution from *Neurog1* lineages include granule cell layers in the olfactory bulb, the hypothalamus, subcortical domains such as striatum or basal ganglia, and granule cells in the cerebellum. These same *Neurog1* lineage domains were seen in P30 brains of *Neurog1-Cre;Tau^{mGFP-nlacZ/+}*, which uses a different Cre reporter mouse strain (Supporting Fig. 1).

All the subregions that lack or have minimal contribution from *Neurog1* lineages are regions that arise largely from *Ascl1* or *Atoh1*-expressing progenitors. For example, in the cerebellum the granule cells arise from *Atoh1*-expressing progenitor cells, while the *Neurog1* lineage includes some Purkinje cells and other minor populations of GABAergic neurons but not the granule cells (Machold and Fishell, 2005; Wang et al., 2005; Lundell et al., 2009). On the other hand, some *Ascl1* lineages comprise neurons in the basal ganglia, hypothalamus, and granule cells in the olfactory bulb, lineages largely lacking cells from *Neurog1* progenitor cells (Kim et al., 2008). This is consistent with embryonic expression studies that demonstrate these bHLH factors are in nonoverlapping populations of progenitor cells throughout the developing embryo (Ma et al., 1997; Fode et al., 2000; Gowan et al., 2001).

It has been shown that progenitor populations expressing the bHLH factors *Ascl1* or *Olig2* can give rise to both neurons and oligodendrocytes (Lu et al., 2002; Zhou and Anderson, 2002; Parras et al., 2004; Masahira et al., 2006; Battiste et al., 2007; Kim et al., 2007, 2008). To determine if *Neurog1*-expressing populations also had this characteristic, we examined the identity of *Neurog1* lineage cells in the brain with markers specific to the neuron (NeuN), oligodendrocyte (*Olig2*), or astrocyte (glutamine synthetase) in the adult brain. Cells in the *Neurog1* lineage were identified exclusively as neurons, not oligodendrocytes or astrocytes as assessed in multiple brain regions including the cortex, thalamus, midbrain, and cerebellum (Fig. 2G–I; Supporting Fig. 2). This result is consistent with previous studies in vitro where overexpression of *Neurog1* induced cortical or spinal cord progenitors to differentiate exclusively to neurons while repressing glial fates (Sun et al., 2001; Sugimori et al., 2007).

Temporal dynamics of *Neurog1* generated neuronal lineages

To fate-map specific cohorts of *Neurog1* lineage cells generated at specific embryonic stages, we utilized *Neurog1-CreER^{T2};R26R^{lacZ/+}* mice. Brains from P30 mice that received

tamoxifen at different times in utero were harvested, sectioned, and X-gal-stained. An overview of para-sagittal sections of each brain set illustrates distinct but overlapping populations of *Neurog1* lineage cells in each brain region depending on the embryonic stage when they received tamoxifen (E10.5, E11.5, E12.5, E14.5, and E17.5) (Fig. 2A–E).

With tamoxifen administration at E10.5, *Neurog1* lineage cells contribute to most brain subregions, although they contribute more strongly to certain subregions such as the pons, medulla, and superior colliculus compared to the cells labeled at later embryonic stages (Fig. 2A). These results are consistent with birthdating studies for these regions (Altman and Bayer, 1981b, 1987) suggesting cells expressing *Neurog1* are or will shortly be undergoing differentiation. With tamoxifen at E11.5 or E12.5, although *Neurog1* lineages continue to contribute to most brain subregions, there are some notable dynamics. Over these developmental times the contribution of *Neurog1* lineage cells to the cortex and thalamus increases as the contribution of cells to the pons and medulla is diminished (Fig. 2B,C). In the dorsal midbrain, *Neurog1* lineage cells populate less in the superior colliculus but more in the inferior colliculus at these later stages of tamoxifen administration. With tamoxifen at E14.5, *Neurog1* lineage cells continue to populate the olfactory bulb, the cortex, and hippocampus while contribution to the thalamus, dorsal midbrain, and cerebellum dramatically decreases (Fig. 2D). And finally, tamoxifen administration at E17.5 demonstrates that by this embryonic stage only sparse and scattered cells in the cortex, hippocampus, and cerebellum are derived from *Neurog1* lineage cells (Fig. 2E). Generally, the temporal dynamics of the *Neurog1* lineage contributions from different embryonic stages are consistent with previous birthdating analyses using ³H-thymidine or BrdU, implicating that *Neurog1* is present in cells initiating differentiation on all these brain regions (Altman and Bayer, 1981a,b, 1985a,b, 1987, 1988, 1989).

Overall, the cumulative cell lineages summed up for the different stages of tamoxifen injection in the *Neurog1-CreER^{T2};R26R^{lacZ/+}* mice are comparable with the lineages detected from the constitutive Cre in the *Neurog1-Cre;R26R^{lacZ/+}* mice. One possible exception is the labeling seen in some GABAergic (Pax2⁺) interneurons detected within the granule cell layer of the cerebellum from tamoxifen injection at E17.5 (Fig. 5E) that are only rarely detected with the constitutive Cre (Lundell et al., 2009; data not shown). A more detailed examination of the contribution of *Neurog1* lineage cells to the major brain regions is provided in the following sections.

Neurog1 progenitors generate glutamatergic neurons in the olfactory bulb, the cerebral cortex, and the hippocampus

The mammalian telencephalon gives rise to diverse forebrain structures such as the olfactory bulb, cerebral cortex, basal ganglia, and septal nuclei (Marin and Rubenstein, 2001; Molyneaux et al., 2007). Neural bHLH transcription factors *Neurog1/Neurog2* are expressed dorsally, while another bHLH factor, *Ascl1*, is found ventrally in the telencephalon, and each bHLH factor regulates distinct neuronal identities arising from these domains (Fode et al., 2000). Generally, *Neurog1* and *Neurog2* specify glutamatergic neurons while *Ascl1* specifies GABAergic or cholinergic neurons (Horton et al., 1999; Casarosa et al., 1999; Fode et al., 2000; Schuurmans and Guillemot, 2002). In the cortex of *Neurog1* and *Neurog2*

double knockout mouse embryos, the generation of pyramidal neurons in the deep layers is greatly decreased (Schuurmans et al., 2004). Instead, expression of ventral telencephalic markers such as *Ascl1* and the GABAergic type neurons derived from *Ascl1* lineages is increased (Fode et al., 2000; Schuurmans et al., 2004). These studies demonstrate that *Neurog1* promotes dorsally derived pyramidal neuron identity but suppresses the ventral derived neuronal phenotypes, illustrating the importance of these factors to generating the correct balance of excitatory and inhibitory neurons in the cortex. In the following sections we identify the fate of the *Neurog1* progenitors in the olfactory bulb, the cortex, and the hippocampus. Consistent with the earlier findings described above, the *Neurog1* lineage neurons appear to be restricted to glutamatergic neurons. Furthermore, adult brain regions arising from ventral telencephalon structures such as striatum are lacking *Neurog1* lineage as predicted from embryonic expression.

Olfactory bulb

The olfactory bulb is made up of glutamatergic projection neurons in the mitral cell layers, GABAergic interneurons in the granule cell layers, and small numbers of excitatory and inhibitory interneurons in the glomerular layer (Hinds, 1968). To determine which if any of these neurons arise from *Neurog1* progenitor cells, P30 brains were harvested from *Neurog1-Cre;R26R^{lacZ/+}* animals and the olfactory bulbs examined for X-gal staining (Fig. 3M). Two regions were enriched for X-gal-stained cells and include cells in the mitral layer and the glomerular layer. To define the temporal characteristics of how these neurons arise from *Neurog1* progenitors, P30 brains were harvested from *Neurog1-CreERT²;R26R^{lacZ/+}* animals that had received tamoxifen as embryos at E10.5, E12.5, E14.5, or E17.5 (Figs. 3A–L, 2E). Neurons in the mitral cell layer arise from progenitors expressing *Neurog1* from E10.5 to E12.5, whereas the neurons in the glomerular cell layer arise from *Neurog1* expressing progenitors at E14.5. The glomerular cell layer is where GABAergic periglomerular neurons make synapses, but using immunofluorescence the β -gal⁺ cells did not coexpress any of the markers for these GABAergic neurons such as calbindin, calretinin, or tyrosine hydroxylase (data not shown). Rather, these cells coexpress *Tbr2*, a marker for glutamatergic juxtglomerular neurons including short-axon cells in this region (Fig. 3Q–Q'') (Brill et al., 2009). Taken together, *Neurog1* progenitor cells give rise to glutamatergic neurons in the mitral cell layer followed by glutamatergic neurons in the glomerular cell layer in the olfactory bulb.

Cerebral cortex

The cerebral cortex develops in an inside-out laminar pattern (McConnell, 1995). Thus, early-born neurons are located in the deep layers, whereas late-born neurons are located in the superficial layers of cortex. *Neurog1* progenitors originate in the ventricular zone in the dorsal telencephalon and migrate radially to contribute neurons in the cortex (Fode et al., 2000; Schuurmans et al., 2004). We show that *Neurog1-Cre;R26R^{lacZ/+}* P30 brains contain “excitatory” *Neurog1* lineage neurons in all layers of the cortex (Fig. 3M–P,R–V). In *Neurog1-CreERT²;R26R^{lacZ/+}* P30 brains with tamoxifen administration at E10.5, marked cells have mostly settled in layer VI, but occasionally radial columns of cells (see inset of Fig. 3A) are found in more superficial layers as well (Fig. 3B–D,R). This suggests that a small number of *Neurog1* progenitors labeled at E10.5 continue to proliferate to generate the

radial clones of neurons, whereas most E10.5 Neurog1 progenitors become postmitotic within a short timeframe and differentiate into layer VI neurons. With tamoxifen at E12.5, the cortex shows an increased number of Neurog1 lineage neurons in the deep layers V and VI (Fig. 3F–H,S). A few scattered cells were also found in more superficial layers. In addition, significant numbers of Neurog1 lineage cells were detected in the piriform cortex (Pir) and amygdala (Amyg) when tamoxifen was administered at these early stages (E10.5 or E12.5) (Fig. 3B–C,G–H). With tamoxifen injection at E14.5, Neurog1 lineage neurons appear to be fated specifically to layer IV (Fig. 3J–L,T). No labeled cells were found in the piriform cortex and amygdala. When tamoxifen was administered at E16.5, Neurog1 lineage neurons populate more superficial layers such as layer II/III (data not shown). The contribution of Neurog1 lineage cells to the neocortex after E17.5 is minor but when detected were found scattered but mostly in superficial layers such as layer II/III (Figs. 2E inset 1, 3U). Therefore, the temporal fate mapping indicates that Neurog1 lineage neurons in the cortex follow an inside-out laminar pattern, and demonstrates that except for a minor population of progenitors at the earliest stages of cortical development, Neurog1 is present in cells that will shortly become postmitotic and differentiate into layer-specific neurons.

Hippocampus

Neurog1 lineage cells also contribute neurons to all regions of the hippocampus. Neurog1 lineage cells from E10.5 to E15.5 contribute to pyramidal neurons in CA1, 2, and 3 and also granule neurons in the dentate gyrus (Fig. 3C,G,K, insets; Supporting Fig. 1D'). In E17.5 tamoxifen-treated animals, Neurog1 lineage cells continue to populate the dentate gyrus but have little contribution to the pyramidal layers in CA1–3 (Fig. 2E inset 2). Interestingly, Ascl1 lineages derived from late embryonic stages such as E14.5 also contribute pyramidal neurons and granule neurons in the hippocampus (Kim et al., 2008). It remains to be determined whether these hippocampal neurons derived from Neurog1 or Ascl1 are identical or whether they are distinct. If overlapping, it would be one of the few examples where Ascl1 and Neurog1 are found in the same lineage.

Neurog1 progenitors from the diencephalon contribute to almost the entire thalamus but only few cells in the hypothalamus

Neurog1 is expressed in the progenitor domain in the diencephalon where cells contribute to thalamic nuclei that project to the cortex (Vue et al., 2007). In *Neurog1-Cre; R26R^{lacZ/+}*, with the constitutive Cre, Neurog1 lineage cells from diencephalon heavily populate almost all thalamic and habenular nuclei, but in the hypothalamus only a few cells in limited nuclei are detected (Figs. 2F, 3N–P). To determine how Neurog1 lineage cells contribute to different thalamic nuclei over time, we analyzed the P30 brains of *Neurog1-CreERT2; R26R^{lacZ/+}* transgenic mice after tamoxifen administration at E10.5, E12.5, E14.5, or E17.5. With tamoxifen at E10.5, Neurog1-derived populations span the entire thalamus but are enriched in the more lateral regions such as the dorsal lateral geniculate nucleus (DLG) and the ventral posterior nucleus (VP) (Fig. 3C). Within the posterior thalamus, the epithalamus, Neurog1 lineage cells were also found heavily in the lateral habenular nucleus (LHb). With tamoxifen administration at E12.5, lineage marked cells disappear from the lateral aspects of thalamus but remain medially, specifically in the posterior thalamic groups such as lateral posterior thalamic nuclei (LP) and ventral medial thalamic nuclei (VM) (Fig.

3G). With tamoxifen administration at E14.5, Neurog1 lineage cells are largely absent from thalamus except for the posterior thalamic nuclear groups (Po) (Fig. 3K). In addition, the labeled cells have moved from the lateral habenular nucleus to the medial habenular nucleus (MHb). Therefore, this temporal fate mapping shows Neurog1 progenitors contributing to almost the entire thalamus, with the more anterior lateral regions being generated before the posterior lateral regions followed by anterior medial and finally the posterior medial. This temporal pattern follows birthdating known for these brain regions (Altman and Bayer, 1988, 1989), again supporting the interpretation that Neurog1 progenitors are transitioning from diencephalic ventricular zones to postmitotic differentiating neurons. It is of note that *Ascl1* lineages do not contribute to these Neurog1 derived nuclei but to pretectal thalamic neurons or a cluster of cells lateral to the habenular that lack contribution from Neurog1 lineages, consistent with the nonoverlapping expression of *Ascl1* and Neurog1 in thalamic progenitor domains (Vue et al., 2007).

Neurog1 lineage cells give rise to neurons in superior and inferior colliculi sequentially

The dorsal mesencephalon gives rise to the superior and inferior colliculi, multilayered structures where neurogenesis contributes to each layer in an inside-outside manner (Altman and Bayer, 1981a,b). Although the superior and inferior colliculi are the brain subregions receiving one of the heaviest contributions from Neurog1 progenitors (Figs. 3P, 4F), the functions or the lineage development of Neurog1 in the dorsal mesencephalon remain less well characterized. A recent study showed that Neurog1 is specifically expressed in glutamatergic neuronal progenitors and is important for their specification (Nakatani et al., 2007). Here we assessed the temporal generation pattern of Neurog1 lineages focusing on cellular lamination, deep versus superficial layers of colliculi.

In the dorsal midbrain of *Neurog1-CreERT²;R26R^{lacZ/+}* P30 animals tamoxifen treated at E10.5, Neurog1 lineage cells are largely located in the intermediate or deep layers superior colliculus (InG or DpG, respectively) (Figs. 3D, 4A). More ventrally, the labeled cells are seen throughout the deep mesencephalic nucleus (DpMe) (Fig. 3D). With tamoxifen at E11.5, Neurog1 lineage cells start to appear in the inferior colliculus, with fewer cells maintained in the superior colliculus (Fig. 4B). With tamoxifen at E12.5, Neurog1 lineage cells still contribute to both the superior and inferior colliculi; however, in the superior colliculus there is a dorsal shift with labeled cells found more heavily in the superficial layer (SuG) rather than the intermediate or deep layer (InG or DpG) (Figs. 3H, 4C). At this time there is also a decrease in the number of labeled cells in the deep mesencephalic nucleus (DpMe) (Fig. 3H). By E14.5 tamoxifen administration, only the most superficial tip of the inferior colliculus has contribution from the Neurog1 lineage cells, and this is further decreased with tamoxifen administered at E17.5 (Figs. 3L, 4D,E inset). Thus, in the midbrain, the Neurog1 lineage follows previous birthdating studies ³H-thymidine where the dorsal midbrain generally follows an inside to outside pattern over time, and the superior colliculus is generated prior to the inferior colliculus (Altman and Bayer, 1981a,b).

Distinct Neurog1 lineages in cerebellum and brainstem nuclei

Glutamatergic and GABAergic neurons in the cerebellum are generated from two discrete germinal zones: the upper rhombic lip and the ventricular zone, respectively (Hatten et al.,

1997; Wang and Zoghbi, 2001). The bHLH factor *Atoh1* is expressed in the upper rhombic lip and is essential for generating glutamatergic neurons including granule neurons and deep cerebellar nuclei neurons (Ben-Arie et al., 1997; Machold and Fishell, 2005; Wang et al., 2005). In contrast, *Ascl1* and *Ptf1a* are expressed in the ventricular zone, giving rise to Purkinje cells and other GABAergic interneurons in the cerebellum, and they play essential roles in their generation (Hoshino et al., 2005; Hori et al., 2008; Kim et al., 2008; Grimaldi et al., 2009). Since *Neurog1* is known for its function in excitatory neuron specification in most brain regions, it is surprising that it is found in a subregion of the ventricular zone that will give rise to cerebellar neurons (Zordan et al., 2008; Lundell et al., 2009). A recent report using BAC transgenic *Neurog1-GFP* or *Neurog1-Cre* showed that *Neurog1* lineage cells contributed to Purkinje neurons and other GABAergic interneurons in the cerebellum (Lundell et al., 2009).

We confirm this earlier report and demonstrate that *Neurog1* lineage cells are located in a mosaic pattern in the Purkinje cell layer of *Neurog1-Cre;R26R^{lacZ/+}* cerebellum at P30 (Fig. 5F,F'). The distinct Purkinje cell morphology is clear in GFP⁺ cells using a different Cre reporter line, Z/EG, where GFP is expressed after Cre recombinase activity (Fig. 5F, inset). To investigate the temporal generation of these *Neurog1* lineage Purkinje cells, tamoxifen was administrated to *Neurog1-Cre^{ERT2};R26R^{lacZ/+}* at different embryonic stages and X-gal-stained P30 brains were analyzed. *Neurog1* progenitors from E10.5–E12.5 are fated to become Purkinje cells, with E11.5 being the highest contributor, and cells labeled at E12.5 are preferentially found in the rostral cerebellar lobes (Fig. 5A–C). *Neurog1*-derived cells labeled at later stages such as E14.5 or E17.5 no longer contribute to the Purkinje cell population, but rather the β -gal⁺ cells are now located in the granule cell layer (GCL) (Fig. 5D,E). Since most of the β -gal⁺ cells co-express Pax2, a GABAergic interneuronal marker, these cells are Golgi or Lugaro interneurons (Maricich and Herrup, 1999), and are not excitatory granule cells (Fig. 5E', inset).

Surprisingly, the *Neurog1* lineages in the cerebellum appear to be restricted to GABAergic neurons, in contrast to other brain regions where *Neurog1* progenitors are exclusively glutamatergic. In the cerebellum, all GABAergic neurons require the bHLH factor *Ptf1a*. However, it is clear that at least subpopulations of Purkinje cells are arising from *Ascl1* and/or *Neurog1*-expressing progenitor cells (Kim et al., 2008; Zordan et al., 2008; Lundell et al., 2009). *Ascl1* and *Neurog1* are expressed in the ventricular zone of cerebellum primordia with only a partial overlap (Zordan et al., 2008). Purkinje cells from *Ascl1* and *Neurog1* lineages are generated around E11.5–12.5, consistent with birthdating studies (Miale and Sidman, 1961; Inouye and Murakami, 1980). Since both *Neurog1* and *Ascl1* lineages appear to contribute to only subpopulations of Purkinje cells, it would be of interest to determine if these are distinct subpopulations, and if so, whether Purkinje cells derived from distinct bHLH lineages display different anatomical or functional properties.

The lower rhombic lip (LRL) in the hindbrain germinal zone is responsible for diverse sets of brainstem nuclei comprising the precerebellar system (Rodriguez and Dymecki, 2000). Different bHLH transcription factors such as *Atoh1*, *Ascl1*, and *Ptf1a* are expressed in discrete progenitor domains along the dorsal-ventral axis of the LRL similar to those of the spinal neural tube (Landsberg et al., 2005; Sieber et al., 2007; Yamada et al., 2007).

Progenitors defined by one or more bHLH factors give rise to distinct brainstem nuclei (Wang et al., 2005; Landsberg et al., 2005; Yamada et al., 2007; Kim et al., 2008). Atoh1 lineage cells contribute to all mossy fiber precerebellar nuclei (the reticulotegmental, pontine gray, lateral reticular, and external cuneate nuclei) projecting to granule neurons, whereas Ptf1a lineage cells contribute to neurons in the inferior olive nucleus that send climbing fibers to connect to Purkinje cells (Hoshino et al., 2005; Wang et al., 2005; Yamada et al., 2007). Ascl1 lineages labeled by the BAC transgenic *Ascl1-CreERTTM* line populate the trigeminal sensory nuclear complex such as primary sensory nucleus, pericellular reticular nucleus and the gigantocellular nucleus (Kim et al., 2008).

Neurog1 is predicted to be in brainstem nuclei since it is expressed in discrete dorsal and ventral progenitor domains of the lower rhombic lip (Landsberg et al., 2005; Yamada et al., 2007). Using *Neurog1-CreERT²;R26R^{lacZ/+}*, we show that Neurog1 lineage cells in the brainstem largely arise in early embryonic stages (E10.5–E12.5) (Fig. 2A–C, 5G,H; data not shown). E10.5 Neurog1 lineage cells label discrete brainstem nuclei such as the cochlear nuclei, lateral lemniscus nuclei, vestibular nuclei, reticular nuclei (intermediate, lateral, gigantocellular), and pontine nuclei. Interestingly, many Neurog1 derived brainstem lineages are the components of the auditory/vestibular system. As Neurog1 also contributes to auditory and vestibular sensory ganglia (Koundakjian et al., 2007), it would be of interest to determine if Neurog1 governs the development of multiple components in this pathway.

Discussion

This study provides an overview of the fate of *Neurog1*-expressing progenitors throughout the brain. To identify cells in the adult brain arising from *Neurog1*-expressing progenitors it was necessary to use genetic approaches since *Neurog1* expression is transient and is absent in mature neurons. To interpret in vivo fate-map studies using Cre recombinase paradigms as used here, it is important to note that this strategy is not 100% efficient and it depends on the level of Cre expression and efficiency of recombination of the Cre-reporter alleles. Therefore, at any given time only a subset of a given population will be labeled, and those with lowest Neurog1 (Cre) may not be efficiently detected. However, the matched expression patterns of *Neurog1* and *Cre* in the transgenic models used here (Fig. 1), and in accordance with previous studies for several lineages, strongly support the validity of using these models for identifying *Neurog1* lineages throughout the brain.

There are multiple general principles that arise from combining the results here with previous literature on Neurog1 expression and function. 1) Neurog1 exclusively marks neuronal restricted progenitor cells in the brain. 2) Neurog1 is present in progenitor cells as they are progressing from mitotic to postmitotic states. 3) Neurog1 neuronal lineages are often restricted to glutamatergic types particularly in the rostral CNS, but there are plenty of exceptions in more caudal regions where GABAergic neurons are generated in the lineage. And 4) Neurog1 lineages are nonoverlapping with lineages derived from Ascl1 and Atoh1-expressing progenitors with a few notable exceptions.

The finding that Neurog1 marks neuronal restricted progenitor cells is not unexpected from Neurog1 functional studies, but it is in contrast with recent fate-mapping studies with

another neural bHLH factor *Ascl1*. In similar in vivo genetic fate-mapping studies with *Ascl1*, it was found that *Ascl1* is also in neuronal lineage restricted progenitors in the neural tube, but later it is present in progenitors that will give rise to oligodendrocytes (Parras et al., 2004; Battiste et al., 2007; Kim et al., 2008). Indeed, forced expression of *Ascl1* in neural stem cells promoted neurons and oligodendrocytes (Sugimori et al., 2007) while loss of *Ascl1* function disrupts neurons and oligodendrocyte development (Casarosa et al., 1999; Horton et al., 1999; Parras et al., 2004; Sugimori et al., 2008). In contrast, forced expression of *Neurog1* in cortical progenitor cells or in neural stem cells induced neuronal differentiation while suppressing glial differentiation, consistent with *Neurog1* lineage restriction to neurons (Sun et al., 2001; Sugimori et al., 2007). Restriction of *Neurog1*-derived cells to neuronal lineages is more similar to that seen with *Atoh1*-expressing progenitors in the CNS, which are also restricted to neuronal over glial fates, possibly reflecting a closer evolutionary relationship between *Neurog1* and *Atoh1* compared to *Ascl1* (Bertrand et al., 2002).

The second principle highlighted with these studies is that *Neurog1* is present in progenitor cells as they are progressing from mitotic to postmitotic states. This is inferred from the observation that in every brain region the pattern of neurons labeled with the lineage marker X-gal after tamoxifen administration at a specific embryonic stage follows the same pattern of neurons known through birthdating studies with ^3H -thymidine (Altman and Bayer, 1981a,b, 1985a, 1987, 1988). Thus, these observations using temporal control of the genetic fate-mapping paradigm support the definition of *Neurog1* as an inducer of neuronal differentiation. An exception to this concept is seen in the cortex with tamoxifen injections at E10.5. In this case, there are apparent clones that are labeled with columns of cells from lower layers to the upper layers of the cortex (see Fig. 3B, inset). This implies that, at least at this early stage, *Neurog1* is present in progenitor cells that undergo multiple rounds of the cell cycle before differentiating.

One early characteristic of the *Neurog1* lineage was that it was found in progenitors to glutamatergic neurons (Fode et al., 2000; Schuurmans et al., 2004). The fate-mapping studies here confirm this bias for *Neurog1* lineages derived from progenitors in the telencephalon and diencephalon. However, in more caudal lineages, such as those arising from hindbrain and spinal cord, some GABAergic cell types are clearly in the *Neurog1* lineage. A clear demonstration of this is in the cerebellum, where the *Neurog1* lineage contributes to two GABAergic cell populations with some Purkinje neurons and some Golgi or Lugaro neurons arising from *Neurog1* progenitors. It will be important in future studies to determine the downstream targets of *Neurog1* transcription activity in glutamatergic neurons versus nonglutamatergic neurons to understand what role this essential factor is playing in neuronal subtype specification.

Previous studies with detailed examination of *Neurog1* expression compared to that of other bHLH factors have shown that in multiple regions of the developing nervous system, *Neurog1* is nonoverlapping with *Ascl1* and *Atoh1* (Sommer et al., 1996; Gowan et al., 2001; Landsberg et al., 2005; Nakatani et al., 2007; Zordan et al., 2008). These distinct expression patterns support the idea that discrete bHLH transcription factor codes determine diverse neuronal cell types in the CNS. The nonoverlap in lineages from *Neurog1*, *Ascl1*, and *Atoh1*

holds true for many of the *Neurog1* lineages using the genetic fate-mapping paradigm (Machold and Fishell, 2005; Wang et al., 2005; Vue et al., 2007; Kim et al., 2008; Quinones et al., 2010). However, there are some regions that contain neurons from both *Ascl1* and *Neurog1* lineages, although with this analysis it cannot be determined whether *Ascl1* and *Neurog1* are marking the same neurons or distinct subpopulations of neurons. One of the regions with a contribution from both *Neurog1* and *Ascl1* lineages are the excitatory pyramidal and granule neurons in the hippocampal formation. Hippocampus is formed from progenitors located in the medial wall of dorsal telencephalon (Altman and Bayer, 1990; Tole and Grove, 2001). A detailed examination of *Neurog1* and *Ascl1* expression in the hippocampal neuroepithelium has not been reported, so it remains unclear if they are marking different lineages, or are possibly expressed sequentially in the same lineage. The latter relationship has been determined for *Ascl1* and *Neurog1* in the development of the olfactory epithelium (Cau et al., 2002). A second neuronal population with contributions from both lineages is the Purkinje cell layer in the cerebellum. In the lineage studies, *Ascl1* and *Neurog1* fate mapping only labels subsets of the Purkinje neurons but it has not been determined if they are overlapping. If they are different subsets of neurons in the hippocampus and cerebellum, they may define functionally distinct populations. For reference, a summary of the comparison between *Neurog1* and *Ascl1* lineage cells contributing to each CNS structure is provided in Table 2.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Transgenic mouse strains expressing Cre or inducible Cre recombinase in the *Neurog1* pattern. **A:** Diagram of two transgenic mouse models that were generated with BAC-containing *Neurog1* regulatory sequences. *Neurog1-Cre* (*N1*⁴⁵⁷-*Cre*) replaces the *Neurog1* coding sequence with Cre, whereas *Neurog1-CreERT2* replaces the *Neurog1* coding sequence with inducible Cre (*CreERT2*). When crossed with a Cre reporter strain such as *R26R*^{lacZ/+}, *Neurog1-Cre* and *Neurog1-CreERT2* reveal *Neurog1* lineages in a constitutive or tamoxifen-inducible manner, respectively. **B–I:** In situ hybridization of *Neurog1* and Cre mRNA in the ventricular zones of E11.5 dorsal telencephalon (B,F), E14.5 dorsal telencephalon (C,G), cerebellum anlage (D,H), and olfactory epithelium (E,I) of *Neurog1-CreERT2* embryos. Arrowheads indicate areas of high *Neurog1* or Cre expression. Dashed line in (B,F) indicates similar boundaries of expression for *Neurog1* and Cre in the telencephalon. Scale bar = 200 μm for B–I.

Figure 2.

Temporal specific fate maps of the *Neurog1* lineages in brain. Sagittal views of X-gal staining of *Neurog1-CreER^{T2};R26R^{lacZ/+}* brains at P30 that were treated with tamoxifen (TM) at embryonic stages indicated (A–E) and *Neurog1-Cre;R26R^{lacZ/+}* (F).

Insets in E show magnified views of the scattered X-gal-stained cells in the cerebral cortex (1), dentate gyrus of hippocampus (2), and cerebellum (3). G–I: Immunofluorescence for β-galactosidase-labeled cells (green) with the neuronal marker NeuN (G, red), the oligodendrocyte marker Olig2 (H, red), and the astrocyte marker glutamine synthetase (I, red) in the cortex of *Neurog1-Cre;R26R^{lacZ/+}*. Cb, cerebellum; Ctx, cortex; Hp, hippocampus; Hyp, hypothalamus; IC, inferior colliculus; OB, olfactory bulb; Pn, Pons; SC, superior colliculus; Str, striatum; Th, thalamus. Scale bars = 1,000 μm in A–F; 20 μm in G–I.

Figure 3.

Temporal specific fate maps of the *Neurog1* lineages in the forebrain. X-gal staining of *Neurog1-CreERT2;R26R^{lacZ/+}* brains exposed to tamoxifen at E10.5 (A–D), E12.5 (E–H), and E14.5 (I–L) or *Neurog1-Cre;R26R^{lacZ/+}* (M–P) and harvested at P30. A, E, I, M insets: Magnified views to show *Neurog1* lineage cells in the olfactory bulb. Q–Q'': Colocalization of β-galactosidase (green) and excitatory inter-neuron marker *Tbr2* (red) in the glomerular layer of the olfactory bulb of *Neurog1-CreERT2;R26R^{lacZ/+}* treated tamoxifen at E14.5. R–V: β-Galactosidase-labeled neurons contribute to specific layers of the primary somatosensory cortex of *Neurog1-CreERT2;R26R^{lacZ/+}* treated with tamoxifen at specified stages (R–U) or *Neurog1-Cre;R26R^{lacZ/+}* (V) on the basis of Nissl-stained sections (W). Amyg, amygdala; Ctx, cortex; DLG, dorsal lateral geniculate nucleus; DpG, deep gray layer of superior colliculus; DpMe, deep mesencephalic nucleus; Gl, glomerular layer; HP, hippocampus; Hyp, hypothalamus; InG, intermediate gray layer of superior colliculus; LHb, lateral habenular nucleus; LM, the nuclei of lateral lemniscus; LP, lateral posterior thalamic nucleus; MHb, medial habenular nucleus; Mi, mitral cell layer; OB, olfactory bulb; Pir, piriform cortex; Pn, pons; Po, posterior thalamic nuclear group; SC, superior colliculus; Str, striatum; SuG, superficial gray layer of superior colliculus; Th, thalamus; VM, ventromedial thalamic nucleus; VP, ventral posterior thalamic nucleus. Scale bars = 1,000 μm in A–P; 20 μm in Q–Q''; 100 μm in R–W.

Figure 4.

Temporal specific fate maps of the *Neurog1* lineages in the dorsal midbrain. **A–F**: Sagittal views of β -galactosidase⁺ *Neurog1* lineage contribution to superior and inferior colliculi in P30 brains of *Neurog1-CreERT2;R26R^{lacZ}/+* when tamoxifen was administered at E10.5 (A), E11.5 (B), E12.5 (C), E14.5 (D), E17.5 (E), or *Neurog1-Cre;R26R^{lacZ}/+* (F). Arrow in E (also inset) indicates few labeled cells in the inferior colliculus. DpG, deep gray layer of superior colliculus; IC, inferior colliculus; InG, intermediate gray layer of superior colliculus; SC, superior colliculus; SuG, superior gray layer of superior colliculus. Scale bar = 200 μ m.

Figure 5.

Neurog1 progenitors contribute to Purkinje cells and interneurons in the cerebellum and discrete brainstem nuclei. **A–F**: β -Galactosidase-labeled Purkinje cells in P30 *Neurog1-CreERT2*; *R26R^{lacZ}/+* when tamoxifen is administered at early stages such as E10.5 (**A–A'**), E11.5 (**B–B'**), E12.5 (**C–C'**). In contrast, when tamoxifen was administered at later stages such as E14.5 (**D–D'**) or E17.5 (**E–E'**), β -galactosidase-labeled GABAergic interneurons are found in the molecular layer. Inset in **E'** shows localization of β -gal and GABAergic neuronal marker Pax2 in the molecular layer. (**F–F'**) β -galactosidase in cerebellum of *Neurog1-Cre*; *R26R^{lacZ}/+* labels Purkinje cells. Inset in **F** shows EGFP⁺ *Neurog1* lineage Purkinje cells in P30 *Neurog1-Cre*; *Z/EG* brain. Coronal (**G**) or sagittal (**H**) sections of P30 *Neurog1-CreERT2*; *R26R^{lacZ}/+* brain exposed to tamoxifen at E10.5 show β -galactosidase-labeled brainstem nuclei. DC, dorsal cochlear nucleus; GCL, granule cell layer; Gi, gigantocellular reticular nucleus; IRt, intermediate reticular nucleus; LRt, lateral reticular nucleus; Pn, pontine nuclei.

Scale bars = 500 μ m for **A–F**; 100 μ m for **A'–F'**; 20 μ m for inset of **E'**.

Table 1
Antibody Characteristics

Antibody	Immunogen	Manufacturer, catalog #, (lot #), species, dilution
Beta(β)-galactosidase	Purified full length native protein <i>E. coli</i>	Abcam, Ab9361 (540230), chick polyclonal, 1:2,000
Beta(β)-galactosidase	Purified full length native protein <i>E. coli</i>	Biogenesis, 4600-1409 (5252KCL), goat polyclonal, 1:500
Green fluorescent protein (GFP)	Purified recombinant green fluorescent protein	Aves lab, GFP-1020 (1229FP08), chick polyclonal, 1:500
Green fluorescent protein (GFP)	GFP isolated directly from <i>Aequorea victoria</i>	Molecular probes, A6455 (685223), rabbit polyclonal, 1:500
Glutamine synthetase	Glutamine synthetase purified from sheep brain	Chemicon/Millipore, MAB302 (LV1501431), mouse monoclonal, 1:500
NeuN	Purified cell nuclei from mouse brain	Chemicon, MAB377 (LV1573084), mouse monoclonal, 1:1,000
Olig2	Recombinant mouse Olig2	Chemicon, AB9610 (0605030911), rabbit polyclonal, 1:2,000
Pax2	GST-Pax2 murine (aa188-385)	Invitrogen, 71-6000, rabbit polyclonal, 1:200
Tbr2	peptide sequence EYSKDTSKGMG AYYAFYTSP from mouse Tbr2	R. Hevner, rabbit polyclonal, 1:2,000

Table 2
Neuronal Derivatives of Neurog1 or Ascl1 Lineages in CNS

Anatomical structure	Neurog1 lineage	Ascl1 lineage	References
Forebrain			
Olfactory bulb	Mitral cells glutamatergic neurons	GABAergic interneurons	This study, Parras, 2004; Long, 2007; Kim, 2008
Cortex	Pyramidal neurons	GABAergic interneurons	This study, Fode, 2000
Ventral limbic system	Amygdala (subtypes?)	Cholinergic, calretinin neurons	This study, Marin, 2000
Hippocampus	Pyramidal & granule neurons	Pyramidal and granule neurons	This study, Pleasure, 2000; Kim, 2007
Thalamus	Thalamic nuclei	Epi-, Pre-, Reticular thalamic nuclei	This study, Vue, 2007; Kim, 2008
Hypothalamus	ND	POMC neurons	McNay, 2006
Midbrain			
Superior colliculus & Inferior colliculus	Glutamatergic neurons	Glutamatergic & GABAergic neurons	This study, Miyoshi, 2004; Nakatani, 2007; Kim 2008
Trigeminal sensory system	ND	Mesencephalic trigeminal nucleus	Kim, 2008
Hindbrain			
Cerebellum	Golgi or Lugaro cells, Purkinje cells	Deep cerebellar nuclei interneurons, Purkinje cells	This study, Kim, 2008; Zordan, 2008
Pons/medulla	Cochlear, vestibular, & reticular nuclei	Spinal trigeminal nucleus (Sp5), associated nuclei (pontine reticular, parvicellular reticular, gigantocellular reticular)	This study, Kim, 2008
Spinal cord	dI2, dI6, V0-2 neurons	dI3-5, V2, dILA and dILB neurons	Gowan, 2001; Helms, 2005; Li, 2005; Wildner, 2006; Quinones, 2010
Neuromodulatory systems	ND	Noradrenergic (Locus coeruleus) Serotonergic (Dorsal raphe nucleus)	Hirsch, 1998; Kim, 2008 Pattyn, 2004

* Gi, gigantocellular reticular nucleus; PCrTA, parvicellular reticular nucleus; Pr, pontine reticular nucleus.