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Developmental controls are re-expressed during induction of neurogenesis in the neocortex of young adult mice

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Whether induction of low-level neurogenesis in normally non-neurogenic regions of the adult brain mimics aspects of developmental neurogenesis is currently unknown. Previously, we and others identified that biophysically induced, neuron subtype-specific apoptosis in mouse neocortex results in induction of neurogenesis of limited numbers of subtype-appropriate projection neurons with axonal projections to either thalamus or spinal cord, depending on the neuron subtype activated to undergo targeted apoptosis. Here, we test the hypothesis that developmental genes from embryonic corticogenesis are re-activated, and that some of these genes might underlie induction of low-level adult neocortical neurogenesis. We directly investigated this hypothesis via microarray analysis of microdissected regions of young adult mouse neocortex undergoing biophysically activated targeted apoptosis of neocortical callosal projection neurons. We compared the microarray results identifying differentially expressed genes with public databases of embryonic developmental genes. We find that, following activation of subtype-specific neuronal apoptosis, three distinct sets of normal developmental genes are selectively re-expressed in neocortical regions of induced neurogenesis in young adult mice: (1) genes expressed by subsets of progenitors and immature neurons in the developing ventricular and/or subventricular zones; (2) genes normally expressed by developmental radial glial progenitors; and (3) genes involved in synaptogenesis. Together with previous results, the data indicate that at least some developmental molecular controls over embryonic neurogenesis can be re-activated in the setting of induction of neurogenesis in the young adult neocortex, and suggest that some of these activate and initiate adult neuronal differentiation from endogenous progenitor populations. Understanding molecular mechanisms contributing to induced adult neurogenesis might enable directed CNS repair.

Keywords: induced adult neocortical neurogenesis, developmental molecular controls, cortex

INTRODUCTION

There is increasing evidence that elements of the molecular controls over developmental neurogenesis during brain formation also control homologous aspects of constitutive neurogenesis in the hippocampal dentate gyrus and olfactory bulb (Magavi et al., 2005; Sohur et al., 2006; Steele et al., 2006; Alvarez-Buylla et al., 2008; Suh et al., 2009; Ma et al., 2010; Kempermann, 2011), but whether the same is true of induction of neurogenesis in normally non-neurogenic regions is unknown. Substantial progress has recently been made in understanding molecular controls over corticogenesis and neocortical neuron subtype-specific neurogenesis during development (Molyneaux et al., 2007; Leone et al., 2008; Shoemaker and Arlotta, 2010; Fame et al., 2011; MacDonald et al., 2012). It is increasingly being identified that there is remarkable heterogeneity and diversity of partially fate-restricted progenitors in the developing CNS (Chambers et al., 2001; Hack et al., 2005; Kohwi et al., 2005; Molyneaux et al., 2005; Wu et al., 2005; Gal et al., 2006; Costa et al., 2007; Merkle et al., 2007; Mizutani et al., 2007; Lai et al., 2008; Lledo et al., 2008; Azim et al., 2009a) and that there are lineage- and subtype-specific molecular controls over the specification, differentiation, and ultimate function of broad classes and distinct subtypes of cortical projection neurons (Arlotta et al., 2005; Chen et al., 2005; Molyneaux et al., 2005, 2007; Ozdinler and Macklis, 2006; Sohur et al., 2006; Alcamo et al., 2008; Britanova et al., 2008; Joshi et al., 2008; Lai et al., 2008; Azim et al., 2009a,b; Bedogni et al., 2010; Tomassy et al., 2010; Han et al., 2011; McKenna et al., 2011). Collectively, these studies are beginning to identify the complex molecular controls over developmental neurogenesis within the mammalian neocortex.

In contrast, almost nothing is understood about molecular controls that might be mechanistically involved in activation and/or regulation of induced neurogenesis in the adult mammalian neocortex (Wang et al., 1998; Magavi et al., 2000; Chen et al., 2004; Brill et al., 2009). Based on the similarities and conserved mechanisms identified by many groups between developmental and adult neurogenesis in the hippocampal dentate gyrus and olfactory bulb, we hypothesized that there might potentially

be recapitulation of at least some molecular controls over normal embryonic neocortical neurogenesis during induced adult neurogenesis. We directly investigated this hypothesis via microarray analysis of microdissected regions of young adult mouse neocortex undergoing biophysically activated targeted apoptosis of neocortical callosal projection neurons (CPN; Macklis, 1993; Sheen and Macklis, 1995; Hernit-Grant and Macklis, 1996; Wang et al., 1998; Leavitt et al., 1999; Magavi et al., 2000; Scharff et al., 2000; Fricker-Gates et al., 2002; Chen et al., 2004). We targeted CPN as the most abundant projection neuron class maximally enabling optimal gene expression analysis. These conditions were previously identified to result in induction of low-level neurogenesis in the neocortex of young adult mice (∼100 newborn neurons/mm^3, 2 weeks after induction) by our lab and others (Magavi et al., 2000; Chen et al., 2004; Brill et al., 2009) and in the zebra finch forebrain song system (Scharff et al., 2000). Other groups have reported complementary results in other normally non-neurogenic regions – e.g., the hippocampus outside of the dentate gyrus (Nakatomi et al., 2002) and striatum (Arvidsson et al., 2002; Parent et al., 2002) – following controlled hypoxia to produce selective vulnerability and apoptosis in targeted neuron subtypes. We compared the microarray results identifying differentially expressed genes with public databases of embryonic developmental genes. Our experiments enable direct investigation of potential re-expression of molecular controls from embryonic neurogenesis in the setting of induction of adult neocortical neurogenesis.

MATERIALS AND METHODS

ANIMALS AND EXPERIMENTAL DESIGN

All animal studies were approved by the Massachusetts General Hospital Institutional Animal Care and Use Committee, and performed in accordance with institutional and federal guidelines. We focused on the sensorimotor cortex (in the area ∼+1 mm anterior to −1.5 mm posterior from bregma – exact start location depended on blood vessel anatomy), and we biophysically targeted CPN, cortical pyramidal neurons that connect the two cerebral hemispheres, by unilateral injection of chlorin e_6-conjugated fluorescent latex nanospheres between postnatal days 1 and 3 inbred C57/Bl6 mice of either sex (P1–P3; day of birth P0) based on established protocols (e.g., Magavi et al., 2000). After 8 weeks, subtype-specific targeted apoptosis of contralateral CPN was initiated in the experimental mice by near-infrared laser activation, as previously described (**Figures 1A,B**). Six-hundred seventy nanometers laser energy was applied to the contralateral sensorimotor cortex through an ∼2-mm diameter craniotomy directly contralateral to the initial nanosphere injection site, to induce synchronous apoptotic degeneration of nanosphere-containing CPN (Macklis, 1993; Sheen and Macklis, 1995; Magavi et al., 2000; Chen et al., 2004). Control mice were injected under the same surgical conditions at the same neonatal age, except with targeting nanospheres that were *not* conjugated with chlorin e_6; allowed to survive for the same period of time until 8 weeks of age; then treated with the same procedural, anesthetic, and surgical conditions for a photo-exposure step (including use of the same fiberoptic, timing of procedure, and laser light exposure).

PREPARATION OF TISSUE AND RNA EXTRACTION AND HYBRIDIZATION

Based on previous experiments where we had determined when maximum induced transcriptional activity occurs *in situ* after initiation of biophysical degradation (e.g., Wang et al., 1998), 8 days after chlorin e_6-mediated CPN apoptosis, mice were deeply anesthetized, the craniotomy site was exposed, and a 2-mm × 2-mm × 0.5-mm sample (enriching for layers II/III, and thus excluding the VZ/subventricular zones, SVZ) of cortex was microdissected from the center of each of the regions of targeted apoptosis (**Figure 1C**). Subsequently, mice were euthanized by additional anesthesia. For each of three biological replicates, microdissected samples from eight experimental neocortical hemispheres were collected and pooled, and compared with matched samples pooled from eight control mice (total of 24 experimental and 24 control mice). Each sample was placed in RNA*later* (Ambion, Inc.) immediately after microdissection, and stored at −80˚C.

RNA was extracted using the StrataPrep Total RNA Mini Kit (Stratagene, La Jolla, CA, USA), and RNA quality was assessed using a bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). RNA (10 μg of RNA per biological replicate) was hybridized to Affymetrix GeneChip Murine Genome U74 Version 2 [MGU74Av2; contains probes for more than 12,400 transcripts, coding for 7,000 mouse genes and 5,000 expressed sequence tags (ESTs)] according to the manufacturer's protocol (Affymetrix; Santa Clara, CA, USA) and as previously described (**Figure 1D**; Arlotta et al., 2005).

TRANSCRIPTOME ANALYSIS OF REGIONS UNDERGOING INDUCED NEUROGENESIS IN THE YOUNG ADULT MOUSE

We combined two statistical approaches, and integrated the results to optimize rigor, and raise confidence in gene expression changes that were identified.

Statistical analysis of microarrays

To identify genes that are differentially expressed in regions undergoing induced adult neurogenesis with very high confidence, we used two different approaches to analyze the Affymetrix data. In the first, we applied the robust multi-array average (RMA) function within Bioconductor (Irizarry et al., 2003), and the "Error Model" method within Rosetta Resolver (version 5.0, Rosetta Biosoftware, Seattle, WA, USA). Statistical significance of gene expression differences between control and experimental tissue expression was determined using statistical analysis of microarrays (SAM; Tusher et al., 2001). We used a P-value of <0.05 as a filter for differentially expressed genes.

Multivariate analysis of variance (linear modeling; MANOVA)

To even more stringently analyze the results, we subjected the six .CEL files to GC-content-based robust multi-array average (GCRMA) normalization (Irizarry et al., 2003). Expression levels were log (base 2) transformed. The number of genes was reduced from 12,488 to 4,349 by requiring a "presence call" for at least 50% in a group of samples classified by experimental treatment. All calculations were done using R and Bioconductor computational tools (Gentleman, 2005). To identify differentially expressed genes between groups of samples, we applied the multivariate analysis of variance approach (also known as linear modeling; MANOVA)

to fit gene expression levels (log2 transformed) according to the defined groups of samples and Bayesian posterior error analysis (Smyth, 2004). Genes that exhibited a P-value ≤ 0.05 and fold change greater than 1.5 were considered significantly differentially expressed.

COMPARING THE INDUCED ADULT NEUROGENESIS DATASET WITH DEVELOPING NEOCORTEX TRANSCRIPTOME DATA

Gene expression in neural progenitors from developing mouse cortex has been profiled by other groups (Pinto et al., 2008; Sanosaka et al., 2008). The deposited .CEL files of the Affymetrix raw data from these publications were accessed at the National Center for Biotechnology Information's Gene Expression Omnibus (GEO)[1].

Genes differentially expressed between early- and mid-neocortical neurogenesis

The Nakashima laboratory previously compared expression profiling in neural stem/progenitor cell cultures ("NSC") of the telencephalon from mouse embryonic day (E) 11.5 (early neocortical neurogenesis) and E14.5 (mid-neocortical neurogenesis; Sanosaka et al., 2008; GEO Accession Number: GSE10796).

Genes differentially expressed in radial glia subtypes

Gotz and colleagues used a mouse line that expresses green fluorescent protein (GFP) driven by a human glial fibrillary acidic protein (*hGFAP*) promoter to profile gene expression by radial glial subtypes (Pinto et al., 2008; GEO Accession Number: GSE8034). At E14.5, during the mid-neurogenic phase in the neocortex, essentially all GFAP positive cells are radial glia. Two subtypes of radial glia were isolated in the cortical prominin positive (CD133+; ventricular neural cell precursors) population by FACS: those that expressed high levels of GFAP (i.e., CD133 + GFAP^{hi}), and those that expressed low levels of GFAP (CD133 + GFAP^{low}). Because these experiments investigated gene expression in high-level GFAP-expressing cells, we added *Gfap* to this dataset as a "differentially" expressed gene.

A gene list for each individual dataset was generated using MANOVA/linear modeling analysis approach.

IN SITU HYBRIDIZATION IMAGES

From the analysis above, we searched established databases of gene expression to identify representative patterns of expression for target genes, primarily using the Eurexpress/Genepaint consortium[2] (Visel et al., 2004). Sagittal E14.5 mouse *in situ* hybridization images are shown in **Figure 2**: *Cry2* (T50260); *Litaf* (T31645); *Myo10* (T36658); *Nelf* (T5943).

RESULTS

We identified genes differentially expressed in regions of induced neurogenesis in the neocortex of young adult mice. Specifically, we find that regions of young adult neocortex undergoing induction of neurogenesis differentially express genes that are active during normal development in neural precursors and radial glia, and during synaptogenesis. These data suggest that some normal neocortical developmental molecular controls are re-activated during induced young adult neocortical neurogenesis.

We identified differentially expressed genes by comparative transcriptional profiling and analysis between microdissected regions undergoing induction of young adult cortical neurogenesis and matched control regions. The two analysis and statistical approaches employed both indicated that genes previously identified as specifically expressed during normal forebrain development

[1]http://www.ncbi.nlm.nih.gov/gds

[2]http://www.eurexpress.org/ee/intro.html

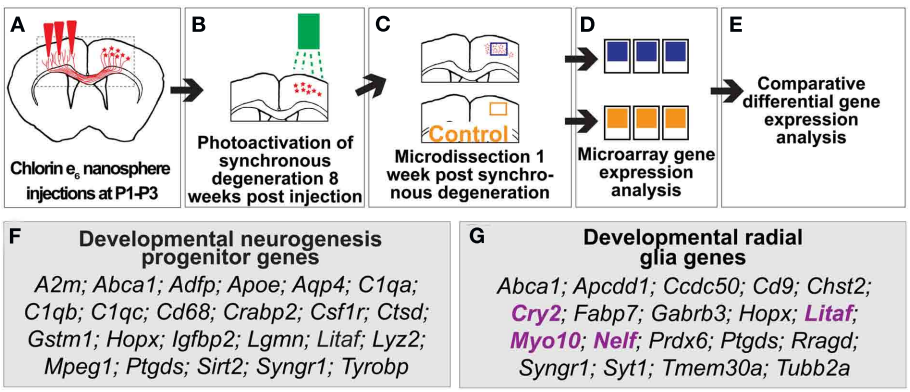

FIGURE 1 | Genes involved in developmental neocortical neurogenesis are differentially expressed in regions of induced young adult neocortical neurogenesis. (A–E) Approach to identify differentially expressed genes in regions of induced young adult neurogenesis. **(A)** Coronal representation of a mouse brain, indicating site of injection (red arrows) of chlorin e_6-conjugated nanospheres into mice at postnatal day 1–3 (P1–P3; day of birth P0) to label callosal projection neurons (CPN; red somas in right cortex). Dashed box represents the area of focus in **(B,C)**. **(B)** Photoactivation of chlorin e_6-conjugated nanospheres within the lysosomes of CPN to induce apoptotic CPN degeneration at 8 weeks of age. **(C,D)** Microdissection and microarray comparative differential gene expression analysis of regions of induced young adult neurogenesis and control tissue. **(E)** 107 genes were identified as differentially expressed in regions of induced young adult neurogenesis. **(F)** Genes differentially expressed in regions of induced young adult neurogenesis in common with genes expressed in mid-neurogenesis in neural progenitor cell culture. **(G)** Genes expressed in regions of induced young adult neurogenesis in common with genes expressed by developmental radial glia. The genes indicated in purple are those shown in **Figure 2**.

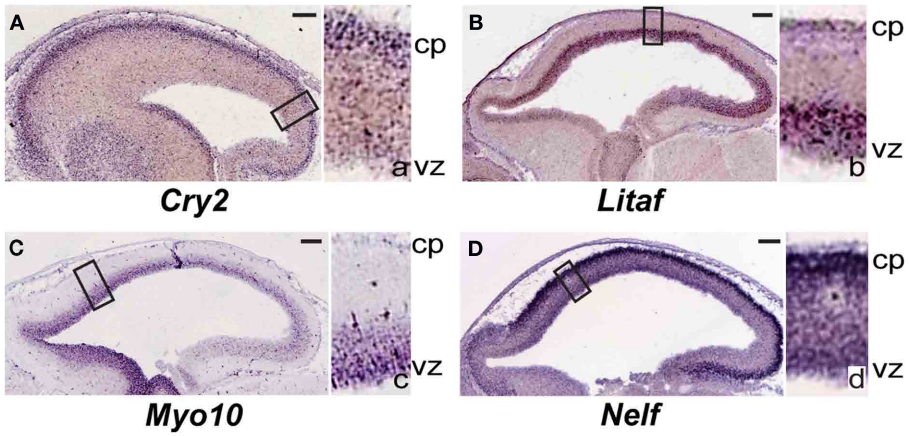

FIGURE 2 | Genes differentially re-expressed in regions of induced young adult neurogenesis previously not specifically identified to be expressed during neocortical development. (A–D) Sagittal sections of *in situ* hybridization of embryonic day (E) 14.5 mouse obtained from the publicly available Genepaint/Eurexpress consortium (data presented with permission). Insets to the right in each panel are higher magnification views of the boxed areas in the main images; these are denoted by the corresponding lower-case letter. **(A)** *Cry2* is predominantly expressed in the developing cortical plate. *Litaf* **(B)** and *Myo10* **(C)** are expressed strongly in the ventricular zone. **(D)** *Nelf* is highly expressed in the developing cortical plate, and also diffusely throughout the thickness of the cortex. cp, cortical plate; vz, ventricular zone. Scale bars, 100 μm. See text for details.

are re-activated during induced young adult neocortical neurogenesis. These include genes previously identified as expressed by developmental neural progenitors (Sanosaka et al., 2008) and developmental radial glia (Pinto et al., 2008). We then accessed publicly available *in situ* hybridization expression databases to identify which of these genes are regionally expressed in the germinal zone (VZ, SVZ) of the developing neocortex.

DIFFERENTIALLY EXPRESSED GENES IN REGIONS UNDERGOING INDUCED YOUNG ADULT NEOCORTICAL NEUROGENESIS

The SAM method identified 83 genes differentially expressed in regions of induced young adult neocortical neurogenesis, while the MANOVA method identified 55 differentially expressed genes; 31 genes were common to both analyses. Together, the results of both transcriptome analyses identified 107 genes as differentially expressed (either over- or under-expressed) in regions of induced young adult neocortical neurogenesis (**Figure 1E**; **Table A1** in Appendix). These genes likely have relatively high degrees of differential expression that can be detected even from heterogeneous tissue. This very focused set of differentially expressed genes reinforces the specificity of gene activation in this system, and suggests that many are likely critical in induced young adult neurogenesis.

GENES DIFFERENTIALLY EXPRESSED IN REGIONS OF INDUCED YOUNG ADULT NEOCORTICAL NEUROGENESIS IN COMMON WITH MURINE DEVELOPING NEOCORTICAL TRANSCRIPTOME DATABASES

Radial glial genes are expressed in regions of induced young adult neurogenesis

We first analyzed the previously published datasets for $CD133+GFAP^{hi}$ vs. $CD133+GFAP^{low}$ murine radial glia at E14.5 (Pinto et al., 2008) and identified over 1,200 genes differentially expressed between these populations (data not shown). Nineteen genes were common between those differentially expressed in regions of induced young adult neocortical neurogenesis and this radial glia dataset (**Figure 1G**); this represents ∼ 20% of the differentially expressed genes in regions of induced adult neocortical neurogenesis. Two well-known radial glia genes stand out: *Fabp7* (also known as *Blbp*; Rousselot et al., 1997; Hartfuss et al., 2001; Zimmerman and Veerkamp, 2002; Anthony et al., 2004; Arai et al., 2005) and *Gfap* (Leavitt et al., 1999; Shapiro et al., 2005; Emsley and Macklis, 2006; Chojnacki et al., 2009; **Table 1**). These results reinforce that radial glial-like progenitors likely contribute to induced neocortical neurogenesis.

Table 1 | Genes differentially expressed in regions of induced young adult neurogenesis that are associated with and expressed by developmental radial glia.

Gene	Description	Fold change	*P*-value
Fabp7	Lipid transporter	1.53	0.02
Gfap	Cytoskeletal protein	3.93	2.98×10^{-3}

Developmental synaptogenic genes are expressed in regions of induced young adult neurogenesis

Sanosaka et al. (2008) compared neural progenitor cell cultures generated from mouse telencephalon at E11.5 vs. E14.5. Our analysis of their raw Affymetrix dataset revealed over 1,300 genes as differentially expressed between these two types of cultures (data not shown). Twenty-three of the differentially expressed genes in regions of induced young adult neocortical neurogenesis are common to this developmental progenitor dataset (**Figure 1F**): ∼23% of our dataset of differentially expressed genes in regions of induced young adult neocortical neurogenesis. Of these, it is interesting to note that several genes are known to be involved in synaptogenesis and/or maintenance of synapses, e.g., *Abca1* (Hirsch-Reinshagen et al., 2004; Kim et al., 2008; Karasinska et al., 2009); *Apoe* (Masliah et al., 1995; Hirsch-Reinshagen et al., 2004; Wahrle et al., 2004); *C1qa*, *C1qb*, and *C1qc* (Stevens et al.,

2007); *Ctsd* (Mariani et al., 2006; Partanen et al., 2008); *Syngr1* (Belizaire et al., 2004; Verma et al., 2004), and *Tyrobp* (a.k.a. *Dap12/Karap*; Roumier et al., 2004; Nataf et al., 2005; **Table 2**). These results strongly suggest that induced young adult neurogenesis involves active plastic changes of synapse formation, pruning, and/or reorganization.

IDENTIFICATION OF GENES DIFFERENTIALLY EXPRESSED IN REGIONS OF INDUCED YOUNG ADULT NEUROGENESIS THAT HAVE NOT PREVIOUSLY BEEN STUDIED IN NEOCORTICAL DEVELOPMENT, BUT ARE EXPRESSED IN DEVELOPING CORTICAL VENTRICULAR ZONE AND CORTICAL PLATE

From our dataset of 107 genes, we identified genes (e.g., *Cry2*, *Litaf*, *Myo10*, and *Nelf*; **Table 3**) previously not studied in the neocortex, that are interestingly expressed regionally in the cortex at E14.5 (**Figures 2A–D**). *Litaf* and *Myo10* are expressed in the ventricular zone, and *Cry2* and *Nelf* are expressed in the cortical plate. These results suggest homology of potential molecular mechanisms between developmental neocortical neurogenesis and induced young adult neocortical neurogenesis.

DISCUSSION

In this report, we provide evidence at the level of microarray analysis that at least some developmental molecular controls over developmental neurogenesis are re-activated in the setting of induction of neurogenesis in the young adult neocortex. Although mouse strain differences in adult neurogenesis are present in details of timing and magnitude of response (Schauwecker, 2006), the results in our report are generalizable. Specifically, we find at the microarray level that genes normally expressed in the developing ventricular and/or SVZ, radial glial progenitors, and genes involved in synaptogenesis are selectively re-expressed at the microarray level in neocortical regions of induced young adult neurogenesis. In addition, we identify differential expression of several genes not previously identified as expressed in the developing neocortex.

Table 2 | Genes differentially expressed in regions of induced young adult neurogenesis known to be involved in synaptic development, function, and plasticity.

Gene	Significance	Fold change	*P*-value
Abca1	Lipid transporter	3.40	1.04×10^{-3}
Apoe	Lipid transporter	1.20	4.00×10^{-4}
C1qa	Cell–cell signaling	2.77	0.01
C1qb	Cell–cell signaling	2.48	1.50×10^{-3}
C1qc	Cell–cell signaling	2.23	0.01
Ctsd	Protease	1.62	0.03
Syngr1	Presynaptic vesicle integral membrane protein	2.71	3.30×10^{-4}
Tyrobp	Transmembrane signal transducing peptide	2.39	4.84×10^{-4}

Table 3 | Genes differentially expressed in regions of induced young adult neurogenesis not previously studied in the neocortex, but expressed in the developmental neocortical ventricular zone, subventricular zone, and cortical plate.

Gene	Significance	Fold change	*P*-value
Cry2	Lipid metabolism	2.24	3.27×10^{-3}
Litaf	Transcription factor	1.57	0.01
Myo10	Cytoskeletal protein	1.96	3.11×10^{-3}
Nelf	Axon guidance	3.85	0.03

NINETEEN GENES CONNECTED TO RADIAL GLIA AND RADIAL GLIAL PROGENITOR FUNCTION ARE DIFFERENTIALLY EXPRESSED BY MICROARRAY ANALYSIS IN REGIONS OF INDUCED YOUNG ADULT NEOCORTICAL NEUROGENESIS

Radial glia are critical components of the developing cerebral cortex, both as cellular scaffolds along which newly born projection/pyramidal neurons migrate to their final positions, and as neural progenitors during later stages of cortical development (Hartfuss et al., 2001; Noctor et al., 2001; Gotz et al., 2002) and in other regions of the telencephalon such as the ganglionic eminences (Anthony et al., 2004; Chojnacki et al., 2009). Our analysis identified the re-expression of multiple genes associated and functionally connected with radial glial/progenitor and neuron migration roles in cortical development.

For example, fatty acid binding protein 7 (*Fabp7*)/brain lipid binding protein (*Blbp*; Zimmerman and Veerkamp, 2002) and glial acidic fibrillary protein (*Gfap*) were both identified as differentially expressed in regions activated to undergo induced neurogenesis. *Fabp7*/*Blbp* is expressed during development exclusively by radial glia and astrocytes (Anthony et al., 2004). It has been proposed that *Fabp7*/*Blbp* has a role in adult neurogenesis, since it is heavily expressed in the adult canary brain, which exhibits high levels of neurogenesis (Rousselot et al., 1997). Prior work from our laboratory (Hernit-Grant and Macklis, 1996) identified that stellate astroglia partially de-differentiate into ∼150 μm long radial glia in the setting of targeted apoptosis and migration of immature neurons, potentially a cellular population re-expressing *Fabp7*/*Blbp*. Expression by activated radial glial-like progenitors is another possibility. GFAP is a major intermediate filament protein expressed by radial glia and astroglia; it is used as a marker for astroglia during development and when activated. It is also expressed in the adult SVZ where GFAP-expressing "SVZ astrocytes" are multipotent neural progenitors (Doetsch et al., 1999; Alvarez-Buylla et al., 2001; Seri et al., 2001; Chojnacki et al., 2009). Upregulation of *Gfap* might represent activation or proliferation of radial glia or radial glia-like neural progenitors in regions of induced adult neurogenesis. Our previous work (e.g., Macklis, 1993) indicates that *Gfap* expression is unlikely to be due to gliosis in this biophysically activated targeted apoptosis leading to induction of neurogenesis. Also of note, the U74Av2 Affymetrix microarray we utilized does not contain the probe sets for *Glast*, another important marker for radial glia; in this microarray analysis, it would have been interesting to investigate its expression in the experimental tissue.

INCREASED EXPRESSION BY MICROARRAY ANALYSIS OF SYNAPSE-RELATED GENES IN REGIONS OF INDUCED YOUNG ADULT NEUROGENESIS

Our analysis also identified the increased expression of multiple genes associated with formation and maintenance of synapses during initial cortical development. Development and

stabilization of new synapses is predicted in regions of induced adult neurogenesis, and is consistent with much prior work in the field (Magavi et al., 2000, 2005; Scharff et al., 2000; Fricker-Gates et al., 2002). As examples, we highlight three differentially expressed genes/gene groups involved in synapse development and maintenance.

Barres and colleagues identified that *C1q*, in addition to its known role in the immune complement cascade, is also important in eliminating and shaping synapses (Stevens et al., 2007). All of the three components of *C1q* (*C1qa, C1qb, C1qc*) are differentially over-expressed in regions of induced adult neurogenesis (**Figure 1F**).

Synaptotagmin 1 (*Syt1*) is also differentially over-expressed in regions of induced young adult neurogenesis. It is a member of the synaptotagmin family of integral membrane proteins that are located on synaptic vesicles, and is important for neurotransmitter release. These data are consistent with the generation of new synapses onto and by newly incorporated neurons.

Tyrobp (*Karap/Dap-12*), a gene related to the CD3ζ signaling polypeptide associated with the T-cell receptor, is also differentially expressed in regions of induced young adult neurogenesis. *Tyrobp* is critical to synaptic function through its action in microglia, in which it is expressed (Roumier et al., 2004). Mice deficient in *Tyrobp* have decreased postsynaptic AMPA receptor *GluR2* expression, with substantial effects on synaptic plasticity (Roumier et al., 2004; Nataf et al., 2005). *Tyrobp* differential expression is consistent with new synapse formation, establishment, and plasticity in the setting of induced adult neocortical neurogenesis.

Taken together, the differential over-expression of several synapse-related genes reinforces the interpretation that synaptogenesis is ongoing in the setting of induced adult neurogenesis. Both formation of synapses onto newborn neurons, and formation of new synapses by those neurons, might jointly account for the increase in synaptogenic genes following induction of adult neurogenesis.

GENES DIFFERENTIALLY RE-EXPRESSED BY MICROARRAY ANALYSIS IN REGIONS OF INDUCED YOUNG ADULT NEUROGENESIS PREVIOUSLY NOT SPECIFICALLY IDENTIFIED TO BE EXPRESSED DURING NEOCORTICAL DEVELOPMENT

Other genes identified as differentially over-expressed in regions activated to undergo induced neocortical neurogenesis were previously not recognized to be expressed in the developing cortex. We searched gene expression databases and the primary literature regarding these genes in other systems or CNS regions, revealing their expression in progenitors and developing neurons of the cortical plate. Their regional and temporal expression during corticogenesis, combined with their differential over-expression in the setting of induced young adult neocortical neurogenesis, suggest the re-expression of these genes as functional in cortical development and both developmental and adult neurogenesis. Four examples of such genes are *Cry2*, *Litaf*, *Myo10,* and *Nelf*. Identification of these genes as both highly expressed during initial corticogenesis in developing VZ/SVZ and cortical plate, and during induction of adult neocortical neurogenesis, suggests functional linkage between developmental and induced cortical neurogenesis.

Cryptochrome 2 (Cry2)

Cry2 has been described as a circadian clock gene (Ishida, 2007), but we identify it from the publicly available Genepaint/Eurexpress consortium to be expressed strongly in the cortical plate at E14.5 (**Figure 2A**). This suggests potential function for *Cry2* during developmental corticogenesis, and during induced cortical neurogenesis.

Lipopolysaccharide-induced TNF factor (Litaf)

Litaf is expressed strongly in the neocortical germinal zone at E14.5 (**Figure 2B**), again suggesting function in developmental and young adult cortical neurogenesis, in addition to previously identified function in other systems. *Litaf* mutations result in the peripheral neuropathy syndrome Charcot–Marie–Tooth (CMT)1c, which accounts for between 1 and 2% of all CMT1 (Street et al., 2003; Bird, 2010). The biological function of lipopolysaccharide-induced TNF factor (LITAF) is not well understood. It was originally cloned as a transcription factor modulating the tumor necrosis factor-α (*TNF*α) gene (Polyak et al., 1997; Myokai et al., 1999), but it has also been found to encode a lysosomal protein (Moriwaki et al., 2001). *Litaf* has not been investigated regarding potential function in cortical development. It would be of interest to determine whether *Litaf* has a critical functional role during developmental corticogenesis, and, if so, whether it functions through the TNF signaling pathway, as a lysosomal protein, or by another mechanism.

Myosin 10 (Myo10)

Myo10 was also identified as differentially over-expressed in the setting of induction of young adult neocortical neurogenesis. *Myo10* is a member of the myosin family of molecular motors. We identified from Genepaint/Eurexpress that it exhibits strong expression in the neocortical germinal zone (**Figure 2C**). Recently, a shorter version of *Myo10* that does not contain the stereotypical "head" that allows these proteins to act as molecular motors was shown to be expressed by CNS neurons during development (Sousa et al., 2006). It would be of interest to investigate the specific isoform(s) expressed in the developmental germinal zone to gain insight into potential function during developmental neurogenesis.

Nasal embryonic LHRH factor (Nelf)

We also identified *Nelf* as differentially over-expressed in the setting of young adult neocortical neurogenesis, and from Genepaint/Eurexpress as being strongly expressed in the cortical plate (**Figure 2D**). *Nelf* is known as a guidance molecule previously reported to be responsible for axonal outgrowth of olfactory neurons (McTavish et al., 2007). It would be of interest to investigate potential function(s) during developmental and induced adult neurogenesis.

MOLECULAR CONTROLS AND MECHANISMS EXPRESSED DURING DEVELOPMENTAL CORTICOGENESIS ARE RE-EXPRESSED BY MICROARRAY ANALYSIS IN REGIONS OF INDUCED YOUNG ADULT NEUROGENESIS

Taken together, these experiments and analysis demonstrate that multiple molecular controls and likely functional mechanisms

active during normal developmental neurogenesis are re-expressed during induced neurogenesis in the neocortex. Genes implicated in developmental neocortical neurogenesis, synaptogenesis, and radial glial progenitor and migrational function are re-expressed with induction of young adult neocortical neurogenesis.

Finally, it is important to note that non-mutually exclusive possibilities exist that together might contribute to the induction of neurogenesis of neurons ultimately residing in neocortex in experiments parallel to those reported here. Progenitors (quite possibly distinct subsets of the much broader set of SVZ progenitors) appear to be activated and mobilized from the young adult SVZ (as reported in Magavi et al., 2000). Additionally, populations of intra-parenchymal progenitors that have been identified by multiple groups might contribute through more local activation and differentiation (indirect evidence suggesting this possibility in addition to SVZ activation was also presented in Magavi et al., 2000). The targeted neurons themselves, of course, will alter their gene expression. Beyond these populations, there are other populations of glia and other cells that can regulate their gene expression in the microenvironment.

Future studies could elucidate function(s) and mechanism(s) of these genes, proteins, and pathways. It appears likely that fundamental mechanisms are shared during neurogenesis in the developing and adult CNS.

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APPENDIX

Table A1 | Genes differentially expressed in regions of induced young adult neurogenesis.

Full name	Gene symbol	Fold change	*P*-value	Affymetrix ID	Accession number
A					
Apolipoprotein E	*Apoe*	1.20	4.00E-04	95356_at	D00466
Aquaporin 4	*Aqp4*	2.23	4.58E-03	102703_s_at	U48398
Basigin	*Bsg*	1.17	1.90E-03	101078_at	Y16258
Calcium/calmodulin-dependent protein kinase II alpha	*Camk2a*	7.21	4.71E-03	93659_at	X14836
Calcium/calmodulin-dependent protein kinase II, delta	*Camk2d*	2.18	9.50E-03	93214_at	AF059029
Coiled-coil domain containing 50	*Ccdc50*	−1.90	4.88E-03	104056_at	AI573367
CD63 molecule	*Cd63*	1.27	2.00E-05	160493_at	D16432
Carbohydrate sulfotransferase 2	*Chst2*	2.07	8.08E-03	97110_at	AW121776
Coatomer protein complex, subunit beta 1	*Copb1*	−1.36	8.40E-04	94992_at	AI840667
Cellular retinoic acid binding protein II	*Crabp2*	−2.27	2.14E-03	100127_at	M35523
Cryptochrome 2 (photolyase-like)	*Cry2*	2.24	3.27E-03	97724_at	AB003433
Casein kinase 1, delta	*Csnk1d*	1.84	9.48E-03	97263_s_at	AI846289
Cathepsin L	*Ctsl*	1.36	7.50E-03	101963_at	X06086
ELOVL family member 5, elongation of long chain fatty acids (yeast)	*Elovl5*	1.25	5.10E-03	93496_at	AI852098
Epidermal growth factor receptor pathway substrate 15	*Eps15*	1.98	3.55E-03	104006_at	L21768
FBJ osteosarcoma oncogene	*Fos*	−1.79	6.13E-03	160901_at	V00727
Ferritin light chain 1	*Ftl1*	1.42	1.53E-03	99872_s_at	L39879
Glutathione S-transferase, mu 1	*Gstm1*	−1.35	5.34E-03	93543_f_at	J03952
General transcription factor II H, polypeptide 1	*Gtf2h1*	1.17	1.04E-03	94811_s_at	AJ002366
Histocompatibility 2, D region	*H2-d1*	1.75	3.80E-04	101886_f_at	X52490
MHC class I like protein GS10	*H2-gs10*	1.69	7.04E-03	98438_f_at	X16202
Histocompatibility 2, K1, K region	*H2-k1*	3.82	5.15E-03	93120_f_at	V00746
Histocompatibility 2, Q region locus 2	*H2-q2*	1.82	2.00E-04	102161_f_at	X58609
Histocompatibility 2, T region locus 23	*H2-t23*	1.44	1.73E-03	98472_at	Y00629
HOP homeobox	*Hopx*	1.37	5.46E-03	96672_at	AW123564
Importin 8	*Ipo8*	−1.28	8.00E-03	104163_at	AA711002
Lysosomal multispanning membrane protein 5	*Laptm5*	2.12	9.52E-03	161819_f_at	AV356071
Lectin, galactose binding, soluble 1	*Lgals1*	1.24	6.75E-03	99669_at	X15986
Legumain	*Lgmn*	1.26	1.40E-03	93261_at	AJ000990
Myelin basic protein	*Mbp*	1.28	2.34E-03	96311_at	M11533
Myelin-associated oligodendrocytic basic protein	*Mobp*	3.45	6.54E-03	99048_g_at	U81317
PERP, TP53 apoptosis effector	*Perp*	−2.75	2.76E-03	97825_at	AI854029
Peroxiredoxin 6	*Prdx6*	1.49	1.11E-03	100332_s_at	AF093853
RAB11a, member RAS oncogene family	*Rab11a*	1.14	4.99E-03	92854_at	D50500
RAN GTPase activating protein 1	*Rangap1*	1.67	5.74E-03	98603_s_at	U20857
ribosomal protein, large, P1	*Rplp1*	−1.48	1.39E-03	161480_i_at	AV055186
Ribosomal protein S27	*Rps27*	−1.13	6.40E-03	96300_f_at	AI854238
Ras-related GTP binding D	*Rragd*	1.85	8.99E-03	93614_at	AA600647
S100 calcium binding protein A6	*S100a6*	1.27	8.40E-04	92770_at	X66449
Serine incorporator 3	*Serinc3*	1.15	6.79E-03	100151_at	L29441
Sirtuin 2 (silent mating type information regulation 2, homolog) 2 (*S. cerevisiae*)	*Sirt2*	1.34	7.29E-03	95502_at	AI840267
Small nucleolar RNA host gene 11	*Snhg11*	−1.93	7.29E-03	97752_at	AI854265
Synaptogyrin 1	*Syngr1*	2.71	3.30E-04	102221_at	AJ002306
Synaptotagmin I	*Syt1*	2.62	5.94E-03	93005_at	D37792

(Continued)

Table A1 | Continued

Full name	Gene symbol	Fold change	*P*-value	Affymetrix ID	Accession number
Transgelin	*Tagln*	−1.50	5.70E-03	93541_at	Z68618
Thrombospondin 1	*Thbs1*	−2.21	5.79E-03	160469_at	M62470
Transmembrane protein 30A	*Tmem30a*	1.52	1.43E-03	95613_at	AW122573
Trafficking protein, kinesin binding 2	*Trak2*	2.01	1.64E-03	97111_at	AA290180
Tubulin, beta 2	*Tubb2a*	1.19	4.09E-03	94835_f_at	M28739
Tubulin, beta 5	*Tubb5*	1.13	7.32E-03	94788_f_at	X04663
Tubulin, beta 6	*Tubb6*	1.20	7.60E-03	160461_f_at	AW215736
Zinc finger, X-linked, duplicated A	*Zxda*	−2.16	7.66E-03	104191_at	AI322972
B					
Alpha-2-macroglobulin	*A2m*	2.22	0.01	104486_at	AI850558
ADAM metallopeptidase domain 10	*Adam10*	1.86	0.01	100751_at	AF011379
Adipose differentiation-related protein	*Adfp*	1.53	0.02	98589_at	M93275
Adenomatosis polyposis coli down-regulated 1	*Apcdd1*	1.54	6.53E-04	96132_at	AB023957
Baculoviral IAP repeat-containing 6 (Apollon)	*Birc6*	−1.52	0.02	102754_at	Y17267
Complement component 4A (Rodgers blood group); complement component 4B (Childo blood group)	*C4a; C4b*	2.85	0.03	103033_at	X06454
Caspase 9, apoptosis-related cysteine peptidase	*Casp9*	2.29	0.02	100368_at	AB019601
CD68 molecule	*Cd68*	2.49	0.02	103016_s_at	X68273
Colony stimulating factor 1 receptor	*Csf1r*	1.79	0.04	104354_at	X06368
CUG triplet repeat, RNA binding protein 1	*Cugbp1*	2.67	0.05	93630_at	X61451
Ecotropic viral integration site 2A	*Evi2a*	2	0.01	98026_g_at	M34896
H2-K2 histocompatibility 2, K region locus 2	*H2-k2*	1.74	0.01	93714_f_at	AI117211
Histocompatibility 2, Q region locus 7	*H2-q7*	1.72	0.04	98438_f_at	X16202
Major histocompatibility complex, class I, C	*Hla-c*	1.9	0.00	101886_f_at	X52490
3-Hydroxy-3-methylglutaryl-coenzyme A reductase	*Hmgcr*	1.63	0.02	99425_at	X07888
Lipopolysaccharide-induced TNF factor	*Litaf*	1.57	0.01	93753_at	AI852632
Mitogen-activated protein kinase kinase kinase 7	*Map3k7*	1.55	0.04	160854_at	D76446
Matrix Gla protein	*Mgp*	−1.75	0.02	93866_s_at	D00613
Osteoglycin	*Ogn*	−1.54	2.68E-03	160877_at	AA647799
RAB34, member RAS oncogene family	*Rab34*	−1.61	0.03	160317_at	AI835712
Ribose 5-phosphate isomerase A (ribose 5-phosphate epimerase)	*Rpia*	−1.79	0.04	103322_at	L35034
Solute carrier family 5 (sodium-dependent vitamin transporter), member 6	*Slc5a6*	−1.69	0.01	104200_at	AW048729
TAP binding protein (tapasin)	*Tapbp*	1.55	0.01	100154_at	AI836367
Trans-Golgi network protein; trans-Golgi network protein 2	*Tgoln1; Tgoln2*	1.68	0.04	93881_i_at	D50032

(Continued)

Table A1 | Continued

Full name	Gene symbol	SAM		MANOVA		Affymetrix ID	Accession number
		Fold change	*P*-value	Fold change	*P*-value		
C							
ATP-binding cassette, sub-family A (ABC1), member 1	*Abca1*	3.40	1.04E-03	2.83	0.02	97198_at	X75926
Beta 2 microglobulin	*B2m*	1.46	2.00E-05	1.55	0.02	93088_at	X01838
Brain derived neurotrophic factor	*Bdnf*	−1.41	3.00E-04	−1.60	1.42E-03	102727_at	X55573
Complement component 1, q subcomponent, A	*C1qA*	2.93	1.06E-08	2.77	0.01	98562_at	X58861
Complement component 1, q subcomponent, B	*C1qB*	2.33	1.23E-09	2.48	1.50E-03	96020_at	M22531
Complement component 1, q subcomponent, C	*C1qC*	2.05	8.15E-06	2.23	0.01	92223_at	X66295
CD53 molecule	*Cd53*	2.25	1.00E-05	1.67	0.01	94939_at	X97227
CD9 molecule	*Cd9*	1.65	2.20E-04	1.64	0.01	95661_at	L08115
Cathepsin D	*Ctsd*	1.53	2.34E-03	1.62	0.03	93810_at	X68378
Cathepsin S	*Ctss*	1.87	4.35E-15	2.10	4.80E-04	98543_at	AJ223208
Cathepsin Z	*Ctsz*	1.60	1.08E-03	1.75	0.01	92633_at	AJ242663
Cytochrome b-245, alpha polypeptide	*Cyba*	1.78	1.65E-03	2.37	4.02E-03	100059_at	M31775
Emerin (Emery–Dreifuss muscular dystrophy)	*Emd*	−1.26	7.98E-03	−1.68	0.04	103420_at	U79753
Fatty acid binding protein 7, brain	*Fabp7*	1.51	2.30E-04	1.53	0.01	98967_at	U04827
Fibromodulin	*Fmod*	−5.00	6.00E-04	−2.13	0.03	99152_at	X94998
Gamma-aminobutyric acid (GABA-A) receptor, subunit beta 3	*Gabrb3*	3.29	4.19E-03	2.25	0.02	99897_at	U14420
Glial fibrillary acidic protein	*Gfap*	3.93	2.98E-03	2.39	0.04	94144_g_at; 94143_at	X02801
Gap junction membrane channel protein beta 2	*Gjb2*	−1.74	6.24E-03	−1.74	0.01	98423_at	M81445
Insulin-like growth factor binding protein 2	*Igfbp2*	−1.29	2.80E-03	−1.54	0.03	98627_at	X81580
Lymphocyte cytosolic protein 1 (L-plastin)	*Lcp1*	1.43	7.85E-03	1.99	2.74E-03	94278_at	D37837
Lymphocyte antigen 86	*Ly86*	3.01	2.00E-05	3.88	6.71E-04	94425_at	AB007599
Lysozyme 1; lysozyme 2	*Lyz1; Lyz2*	7.32	4.15E-03	5.92	4.22E-03	101753_s_at	X51547
Myelin-associated glycoprotein	*Mag*	1.55	1.60E-03	1.66	0.01	102405_at	M31811
Macrophage expressed gene 1	*Mpeg1*	1.89	2.19E-03	2.17	0.02	99071_at	L20315
Myosin X	*Myo10*	1.96	3.11E-03	1.60	6.04E-03	100923_at	AJ249706
Nasal embryonic LHRH factor	*Nelf*	3.09	8.30E-04	3.85	0.03	99557_at	AI849565
Proenkephalin 1	*Penk1*	−1.39	5.90E-03	−1.56	0.02	94516_f_at	M55181
Prostaglandin D2 synthase (brain)	*Ptgds*	−2.54	5.73E-06	−3.06	0.01	92546_r_at	AB006361
Serine (or cysteine) peptidase inhibitor, clade A, member 3N	*Serpina3n*	4.94	5.50E-04	3.32	0.03	104374_at	M64086
Splicing factor, arginine/serine-rich 1 (ASF/SF2)	*Sfrs1*	2.32	1.82E-07	2.13	2.01E-04	160539_at	X66091
TYRO protein tyrosine kinase binding protein	*Tyrobp*	1.93	1.78E-06	2.39	4.84E-04	100397_at	AF024637

(A) Genes identified by statistical analysis of microarrays (SAM), but not by multivariate analysis of variance (also known as linear modeling; MANOVA). (B) Genes identified by MANOVA, but not by SAM. (C) Genes identified by both SAM and MANOVA (Source: .CEL files from this work will be deposited at NCBI's GEO).