Expression of Nuclear Factor One A and -B in the Olfactory Bulb

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ABSTRACT

The nuclear factor one (NFI) family of transcription factors consists of four members in vertebrates, NFIA, NFIB, NFIC, and NFIX, which share a highly conserved N-terminal DNA-binding domain. *NFI* genes are widely expressed in the developing mouse brain, and mouse mutants lacking *NFIA*, *NFIB*, or *NFIX* exhibit developmental deficits in several areas, including the cortex, hippocampus, pons, and cerebellum. Here we analyzed the expression of NFIA and NFIB in the developing and adult olfactory bulb (OB), rostral migratory stream (RMS), and subventricular zone (SVZ). We found that NFIA and NFIB are expressed within these regions during embryonic and postnatal development and in the adult. Immunohistochemical analysis using cell-type-specific markers revealed that migrating neuroblasts in the adult brain express NFI transcription factors, as do astrocytes within the RMS and progenitor cells within the SVZ. Moreover, astrocytes within the OB express NFIA, whereas mitral cells within the OB express NFIB. Taken together these data show that *NFIA* and *NFIB* are expressed in both the developing and the adult OB and in the RMS and SVZ, indicative of a regulatory role for these transcription factors in the development of this facet of the olfactory system. J. Comp. Neurol. 520:3135–3149, 2012.

INDEXING TERMS: rostral migratory stream; olfactory bulb; neuroblast; subventricular zone

The olfactory bulb (OB) is a distinctly laminated structure in the central nervous system. In rodents, olfactory neurons are derived both prenatally and postnatally from different populations of progenitor cells. The mitral and tufted cells, the principal projection neurons of the OB, derive from the ventricular zone of the OB prenatally (Bayer, 1983; Hinds, 1968a), whereas most of the OB interneurons arise postnatally from the subventricular zone (SVZ) lining the lateral ventricles of the forebrain (Hinds, 1968b; Luskin, 1993). The SVZ is one of the few structures in the mammalian forebrain in which continuous neurogenesis takes place throughout life (Altman, 1969; Bayer, 1983; Brunjes and Frazier, 1986). In the adult rodent brain, neural progenitor cells within the SVZ continually give rise to neuroblasts (Lois and Alvarez-Buylla, 1994; Luskin, 1993) that migrate from the SVZ along a stereotypical route known as the rostral migratory stream (RMS) toward the OB (Ihrie and Alvarez-Buylla, 2011). The migration of neuroblasts along the RMS occurs through specialized glial structures known as glial tubes (Lois et al., 1996; Peretto et al., 1997). Once they reach the OB, SVZ-derived neuroblasts differentiate into interneurons, primarily granule and periglomerular cells (Alvarez-Buylla et al., 2000; Lois and Alvarez-Buylla, 1994; Luskin, 1993). Different molecules and transcription factors have been shown to participate in the

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TABLE 1.

Summary of Primary Antibody Dilutions Used for Immunohistochemistry (IHC) or Immunofluorescence (IF) in This Study

Antibody	Host	Source, catalog No.	Antigen	Dilution
NFIA	Rabbit polyclonal	Active Motif, 29311004	Amino acid residues 478–492 of human NFIA	1/50,000 (IHC) 1/1,000 (IF)
NFIB	Rabbit polyclonal	Active Motif, 25111004	Amino acid residues 402–415 of human NFIB	1/25,000 (IHC) 1/1,000 (IF)
GFAP	Mouse monoclonal	Millipore, MAB360	Purified GFAP from porcine spinal cord	1/1,000 (IF)
Tuj1	Mouse monoclonal	R&D Systems, MAB1195	Rat brain microtubules	1/1,000 (IF)

generation and migration of SVZ-derived neuroblasts (Anton et al., 2004; Conover et al., 2000; Garzotto et al., 2008; Hu et al., 1996; Menezes et al., 2002; Ono et al., 1994; Wu et al., 1999), implying multifactorial control of this process.

One suite of proteins that may contribute to development of the olfactory system is the nuclear factor one (NFI) family of transcription factors. NFI proteins have been shown to play a role in regulating gene transcription as both repressors and activators (Mason et al., 2009; Piper et al., 2007), and NFIA and NFIB are dynamically expressed in the developing and adult cerebral cortex and hippocampus (Plachez et al., 2008). Furthermore, transcripts of the *NFI* genes have previously been identified in the olfactory epithelium (Baumeister et al., 1999), and, at a mechanistic level, recent studies have shown that these molecules contribute to many facets of neural development, including the regulation of progenitor cell self-renewal (Namihira et al., 2009; Piper et al., 2010), gliogenesis (Barry et al., 2008; Deneen et al., 2006), and neuronal differentiation and migration (Kilpatrick et al., 2010). Because the development of the olfactory system incorporates all of these processes, here we sought to characterize the expression of NFIA and NFIB proteins in the SVZ, RMS, and OB of the developing and adult mouse brain. Our data demonstrate that NFIA and NFIB are dynamically expressed within the developing OB and are highly expressed within the SVZ and RMS of the adult brain. The expression pattern of these genes suggests that *NFIA* and *NFIB* play a role during OB development as well as during SVZ progenitor cell differentiation and neuroblast migration within the adult brain.

MATERIALS AND METHODS

Animals

Embryos were obtained from time-mated wild-type C57BL/6J mice under approval from the institutional animal ethics committee. The observation of a copulatory plug was taken as embryonic day (E) 0, and the day of birth was designated postnatal day (P) 0. Tissues were collected at E15, E18, P0, P7, and P14 as well as from adult mice. Adult transgenic mice expressing green fluorescent protein (GFP) under control of the glutamic acid decarboxylase 67 (GAD67) promoter were also used in this study (Tamamaki et al., 2003). In these mice, in which GFP has been knocked in to the GAD67 locus, expression of GFP has previously been shown to colocalize with GAD67 expression (Tamamaki et al., 2003). We also used transgenic mice expressing GFP under control of the doublecortin (DCX) promoter (Walker et al., 2007). These transgenic mice (DCX-GFP/bacterial artificial chromosome [BAC]) were originally obtained from the Mutant Mouse Regional Resource Center, The Gene Expression Nervous System Atlas BAC transgenic project (Gong et al., 2002). The pattern of GFP expression in these mice matches previously reported expression of DCX (Gleeson et al., 1999). Both the GAD67-GFP and the DCX-GFP mice were bred at The University of Queensland under approval from the institutional animal ethics committee.

Antibody characterization

Antibodies, sources, and the concentrations at which they were used are listed in Table 1.

NFIA

The anti-NFIA antibody specifically detects a single band at 57.6 kDa on Western blots of nuclear extracts derived from rat liver (manufacturer's information) and JEG-3 cells (Plachez et al., 2008). The signal corresponding to NFIA can be eliminated by addition of the immunizing peptide (manufacturer's information), demonstrating the specificity of this antibody. We have also previously demonstrated the lack of cross-reactivity between the different anti-NFI antibodies via Western blot (Plachez et al., 2008). Finally, the anti-NFIA antibody has also been shown to be specific via immunohistochemistry in wild-type and *NFIA*$^{-/-}$ cortical tissue (Plachez et al., 2008).

NFIB

The anti-NFIB antibody specifically detects a single band at 49.1 kDa on Western blots of JEG-3 cells (Plachez et al., 2008). We have previously demonstrated the lack of cross-reactivity between the different anti-NFI antibodies via Western blot (Plachez et al., 2008). The anti-NFIB

antibody has also been shown to be specific via immunohistochemistry in wild-type and *NFIB*$^{-/-}$ cortical tissue (Plachez et al., 2008).

Glial fibrillary acidic protein (GFAP)

The mouse monoclonal anti-GFAP antibody detects a band at ~51 kDa on Western blots of extracts from the human glioma cell line U33CG/343MG (manufacturer's information). This antibody has been used previously to detect GFAP-expressing cells (Piskuric et al., 2011), and the pattern of GFAP reactivity observed within the subventricular zone matches previous descriptions (Liu et al., 2006).

Neuron-specific class IIIβ-tubulin (Tuj1)

The mouse monoclonal anti-Tuj1 antibody has previously been used to detect postmitotic neurons within the central nervous system (Casanovas et al., 2008). The anti-Tuj1 antibody is produced from the mouse hybridoma clone Tuj1, which was derived from a mouse immunized with microtubules isolated from rat brain. The antibody recognizes the mammalian Tuj1, but not other β-tubulin isotypes, on Western blots (manufacturer's information). We used both the laminar position and the expression of Tuj1 by large cells with abundant cytoplasm as described previously (Leo et al., 2000) to identify mitral cells within the olfactory bulb and to differentiate them from tufted cells, which are smaller and are scattered throughout the external plexiform layer (Nicoll, 1970).

Immunohistochemistry

To obtain embryonic tissue, dams were anesthetized with sodium pentobarbital (Abbott Laboratories, North Chicago, IL), and embryos were transcardially perfused with saline, followed by 4% paraformaldehyde in 1× phosphate-buffered saline (PBS; pH 7.0). Older animals (from P0 to adult) were anesthetized and transcardially perfused with saline, followed by 4% paraformaldehyde in 1× PBS. Brains were removed from the skull and sectioned coronally or sagittally at 50 μm on a vibratome. Immunohistochemical analysis of floating sections was performed by using the chromogen 3,3′-diaminobenzidine (DAB) as described previously (Piper et al., 2009b, 2011). Biotin-conjugated goat anti-rabbit IgG (BA-1000; Vector Laboratories, Burlingame, CA) secondary antibodies were used for chromogenic immunohistochemistry. To perform immunofluorescence labelling, sections were incubated overnight with the primary antibody at 4°C. They were then washed and incubated in secondary antibody, before being washed again and counterstained with 4′,6-diamidino-2-phenylindole (DAPI). The secondary antibodies used in this study were goat anti-rabbit IgG AlexaFluor594 and goat anti-mouse IgG AlexaFluor488 (both 1/1,000; Invitrogen, Carlsbad, CA). Sections were then mounted in 50% glycerol diluted in 1× PBS.

For all immunohistochemical and immunofluorescence analyses, at least three brains were analyzed. Sections labelled with DAB were imaged using an upright microscope (Zeiss upright Axio-Imager Z1; Zeiss, Goettingen, Germany) fitted with an Axio-Cam HRc camera. Sections labelled with fluorescent antibodies were imaged with a confocal microscope (Zeiss LSM 510 Meta) using Zen software (Zeiss). The images presented are 2 μm optical sections of the labelled tissue. Figures were cropped for presentation in Adobe Photoshop (San Jose, CA).

Bioinformatic promoter screen

To obtain an NFI binding site motif, data from a recent study identifying NFI binding sites in vivo using chromatin immunoprecipitation sequencing (ChIP-seq; Pjanic et al., 2011) were analyzed. NFI peaks were called using ChIP-Peak (Schmid and Bucher, 2010) with the parameters: Server-resident SGA file: mm9/nf1_wt.sga; strand: any; centering: 75 bp; repeat masker: checked; window width: 300 bp; vicinity range: 300 bp; peak threshold: 8; count cutoff: 1; refine peak positions: checked. The NFI motif was created by running MEME (Bailey et al., 2009) on the sequence of 600 of the 708 peak regions declared by ChIP-Peak. The 600 regions were each trimmed to 100 base pairs in width and chosen randomly from among the 708. MEME was run with parameters: -dna -minw 6 -maxw 30 -revcomp. Potential NFI binding sites were then identified in the promoter region of candidate genes using FIMO (Grant et al., 2011). The candidate genes were selected on the basis of previously reported roles in regulating neuroblast migration within the rostral migratory stream and included *DCX* (Belvindrah et al., 2011; Koizumi et al., 2006), *SLIT1* (Kaneko et al., 2010), *β8 integrin* (Mobley and McCarty, 2011), *NCAM* (Chazal et al., 2000), and *CDK5* (Hirota et al., 2007). The promoter sequence of each candidate gene, which we defined as the region from −2,000 to +199 base pairs relative to the transcription start site (TSS), was downloaded from the UCSC Genome Browser (mm9 July 2007; Fujita et al., 2011). FIMO was run on the promoter sequence of each candidate gene using a zero-order background generated on all mouse promoter regions and a pseudocount of 0.1. All potential binding sites with $P \leq 10^{-3}$ were reported.

RESULTS

NFI expression in the developing and adult OB

The expression of NFIA and NFIB in the developing OB was assessed using immunohistochemistry with specific

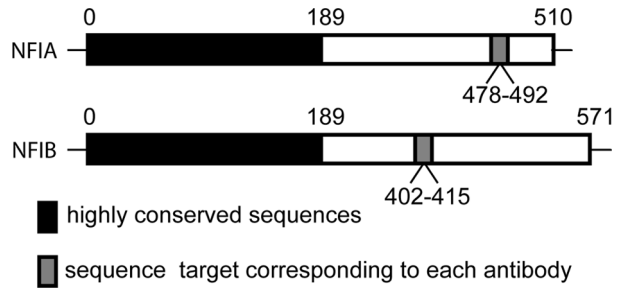


Figure 1. NFIA and NFIB antibody specificity. NFIA and NFIB share a highly conserved sequence in their N-terminal domains (black rectangles). The C-terminal regions of NFIA and NFIB (white rectangles) are less well conserved. The anti-NFIA and anti-NFIB antibodies were specifically designed to recognize targets in the nonoverlapping C-terminal regions (gray rectangles) to avoid cross-reactivity among the antibodies directed against the different family members.

anti-NFIA and anti-NFIB antibodies (Fig. 1). We have previously tested the specificity of these antibodies and shown that neither exhibits cross-reactivity with other NFI family members (Plachez et al., 2008). NFIA was first detected within cells in the olfactory ventricle and the olfactory nerve layer of the OB at E15 (Fig. 2A). At E18, NFIA expression was present in the olfactory nerve layer, the granular cell layer, and the olfactory ventricle (Fig. 2B). At P7, NFIA was expressed by cells within all layers of the OB (Fig. 2C,D). In the adult OB, NFIA was expressed by scattered cells within the internal plexiform layer, the external plexiform layer, the glomerular cell layer, the granular cell layer and the olfactory nerve layer (Fig. 2E,F).

To determine which cells in the adult OB express NFIA, we first performed immunofluorescence labelling of OB sections taken from transgenic mice expressing GFP under control of the GAD67 promoter (GAD67-GFP; Tamamaki et al., 2003). GAD67 is a key enzyme used during the production of γ-aminobutyric acid (GABA), which mediates fast synaptic inhibitory neurotransmission (Martin and Rimvall, 1993). In the OB, granule cells and periglomerular cells are the principal GABAergic interneurons. Confocal microscopic analysis of OB sections from GAD67-GFP mice indicated that NFIA was not expressed by GFP-positive cells, suggesting that NFIA is not expressed by mature, GAD67-expressing interneurons within the adult OB (Fig. 3A–D). Moreover, when we analyzed expression of NFIA and Tuj1, a microtubule marker specific for mature neuronal populations such as OB mitral cells (Leo et al., 2000), we observed that Tuj1-positive mitral cells did not express NFIA (Fig. 3E–H). Finally, given the role of NFIA in astrocytogenesis (Deneen et al., 2006; Namihira et al., 2009), we investigated whether the cells expressing NFIA in the adult OB were

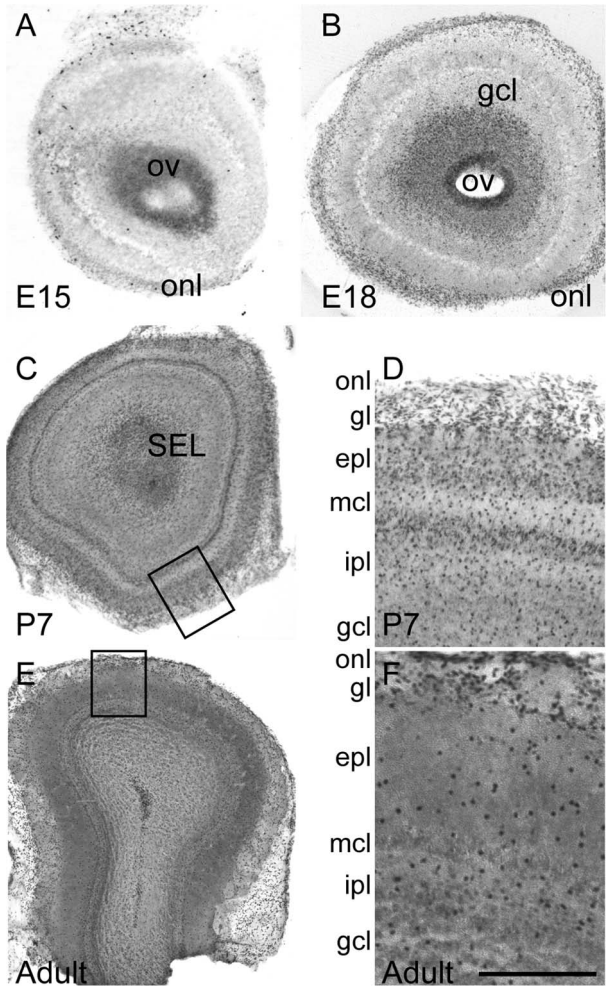


Figure 2. NFIA expression in the developing and adult OB. Coronal sections of murine embryonic (A,B), postnatal (C,D), and adult (E,F) olfactory bulbs, showing expression of NFIA. **A**: At E15, NFIA expression was observed within the olfactory nerve layer and the olfactory ventricle. **B**: At E18, NFIA expression was present in the olfactory nerve layer, the granular cell layer, and the olfactory ventricle. **C**: Expression of NFIA in the OB at P7 was observed within the subependymal layer as well as within the laminae of the OB. **D**: Higher magnification view of the boxed region in C, revealing expression of NFIA within all layers of the OB. **E**: Expression of NFIA within the adult OB. **F**: Higher magnification view of the boxed region in E, showing expression of NFIA by scattered cells within the internal plexiform layer, the external plexiform layer, and the glomerular cell layer. onl, Olfactory nerve layer; gl, glomerular cell layer; epl, external plexiform layer; mcl, mitral cell layer; ipl, internal plexiform cell layer; gcl, granular cell layer; ov, olfactory ventricle; SEL, subependymal layer. Scale bar = 100 μm in F; 400 μm for A; 500 μm for B; 650 μm for C; 150 μm for D; 800 μm for E.

astrocytes. With a anti-GFAP antibody, we demonstrated that the NFIA-positive cells within the internal plexiform layer were surrounded by GFAP-positive fibers, indicating that astrocytes within the OB probably express NFIA (Fig. 3I–L). However, the scattered NFIA-positive cells

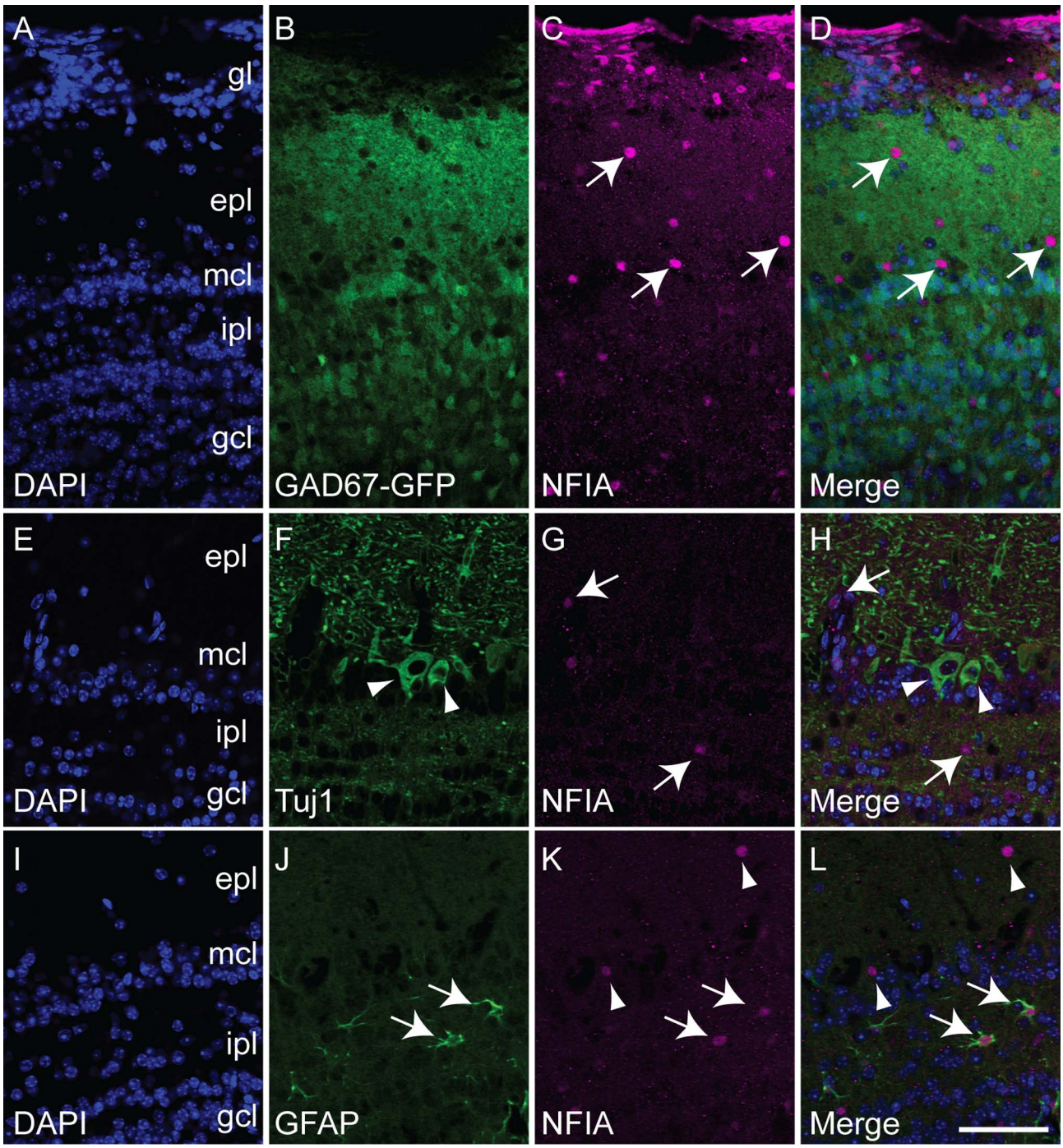


Figure 3. Cell-type-specific expression of NFIA within the adult OB. Coronal sections through the OB of adult GAD67-GFP (**A–D**) and wild-type (**E–L**) mice. Coimmunofluorescence labelling and confocal microscopy was used to determine cell-type-specific expression of NFIA within the adult OB. Cell nuclei were labelled with DAPI (A,E,I). Expression of NFIA within the adult OB (arrows in C,D) did not coincide with GAD67-GFP expression (B; merged image in D). Moreover, expression of NFIA (arrows in G,H) was not coincident with Tuj1-expressing mitral cells within the OB (arrowheads in F,H). GFAP-expressing astrocytic fibers within the internal plexiform layer (arrows in J,L) surrounded NFIA-expressing nuclei (arrows in K,L), suggesting that OB astrocytes express NFIA. Some NFIA-expressing cells within the mitral cell layer and external plexiform layer were not GFAP-expressing astrocytes (arrowheads in K,L). gl, Glomerular cell layer; epl, external plexiform layer; mcl, mitral cell layer; ipl, internal plexiform cell layer; gcl, granular cell layer. Scale bar = 50 μm.

within the external plexiform and mitral cell layers were not GFAP-positive astrocytes, suggesting that these cells form a distinct population, perhaps oligodendrocytes.

We next analyzed expression of NFIB within the developing and adult OB. In contrast to NFIA expression, NFIB expression was not detected in the OB at E15 (Fig. 4A).

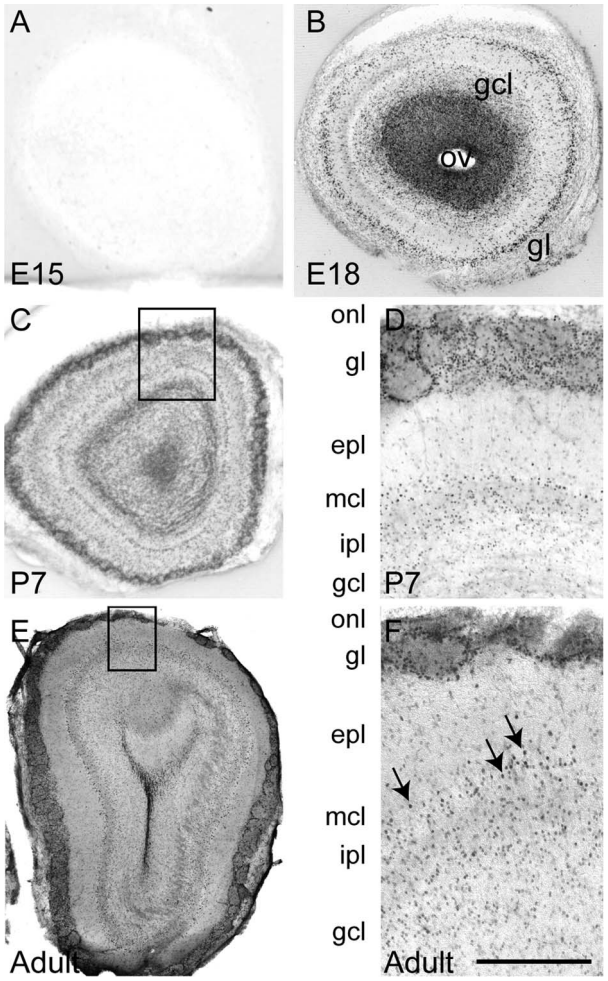


Figure 4. NFIB expression in the developing and adult OB. Coronal sections of embryonic (A,B), postnatal (C,D), and adult (E,F) olfactory bulbs, showing expression of NFIB. **A**: At E15, NFIB was not expressed within the OB. **B**: By E18, expression of NFIB was evident within the OB, being observed within the olfactory ventricle, granular cell layer, and glomerular cell layer. **C,D**: Expression NFIB in the OB at P7 was observed within all of the laminae of the OB. D is a higher magnification view of the boxed region in C. **E**: Expression of NFIB within the adult OB. **F**: Higher magnification view of the boxed region in E. Expression of NFIB in the adult OB appeared low, although cells within the mitral cell layer (arrows in F) and glomerular cell layer expressed NFIB. onl, Olfactory nerve layer; gl, glomerular cell layer; epl, external plexiform layer; mcl, mitral cell layer; ipl, internal plexiform cell layer; gcl, granular cell layer; ov, olfactory ventricle. Scale bar = 100 μm in F; 400 μm for A; 500 μm for B; 650 μm for C; 150 μm for D; 800 μm for E.

However, by E18, NFIB was expressed in the olfactory ventricle, the granular cell layer, and the glomerular cell layer (Fig. 4B). At P7 (Fig. 4C,D), expression of NFIB was detected within cells in all layers of the OB, albeit at a low level. In the adult OB, expression of NFIB was observed in the glomerular cell layer and within the mitral cell layer, as well as by scattered cells within the internal plexiform layer and the granular cell layer (Fig. 4E,F). To characterize NFIB expression within the OB further, we assessed expression of NFIB in OB sections from GAD67-GFP mice. GAD67-expressing interneurons within the adult OB did not express NFIB (Fig. 5A–D). However, in contrast to the case for NFIA (Fig. 3), we confirmed that some cells in the mitral cell layer were NFIB positive (Fig. 5A–D). This was further demonstrated by coimmunolabelling of wild-type OB sections with anti-NFIB and anti-Tuj1 antibodies, with confocal analysis revealing that mitral cells do indeed express NFIB (Fig. 5E–H). Finally, we showed that GFAP-positive astrocytes within the adult OB do not express NFIB (Fig. 5I–L). Collectively, these data demonstrate that NFIA and NFIB are expressed in distinct cell subtypes within the OB, indicative of divergent roles for these transcription factors within this structure.

NFI expression in the developing and adult RMS and SVZ

Olfactory interneurons are continuously generated throughout life (Graziadei and Monti-Graziadei, 1978a,b). These cells are derived from progenitor cells located within the SVZ lining the walls of the lateral ventricles (Ihrie and Alvarez-Buylla, 2011). These progenitor cells give rise to neuroblasts that migrate anteriorly toward the OB along the RMS. Our previous studies have demonstrated that NFI proteins play a key role in regulating the differentiation of progenitor cells in the developing hippocampus (Barry et al., 2008) and cortex (Piper et al., 2010; Shu et al., 2003) and that neural progenitor cells within the embryonic dorsal telencephalon, which ultimately give rise to SVZ progenitors, express both NFIA and NFIB (Plachez et al., 2008). Therefore, we next investigated the expression of NFIA and NFIB within the developing and adult SVZ and RMS.

Analysis of sagittal sections of the brain at P0 indicated that NFIA was broadly expressed within the cortex and hippocampus but was also expressed by cells within the SVZ (arrowheads in Fig. 6A), and by cells within the emerging RMS (arrows in Fig. 6A). By P7, expression of NFIA within the cortex had become more restricted. Expression of NFIA was still, however, observed within the SVZ and RMS (Fig. 6B). This expression pattern was recapitulated at P14 (Fig. 6C). Finally, coronal sections of the adult brain revealed expression of NFIA by cells within the SVZ (Fig. 6D,E). We next performed coimmunofluorescence labelling, followed by confocal microscopy, to determine which cells within the adult SVZ and RMS express NFIA. Within the SVZ, colabelling of sections with antibodies specific for NFIA and GFAP (which is expressed by SVZ progenitor cells), indicated that some NFIA-expressing cells were surrounded by GFAP-positive fibers, indicating that at least a proportion of GFAP-

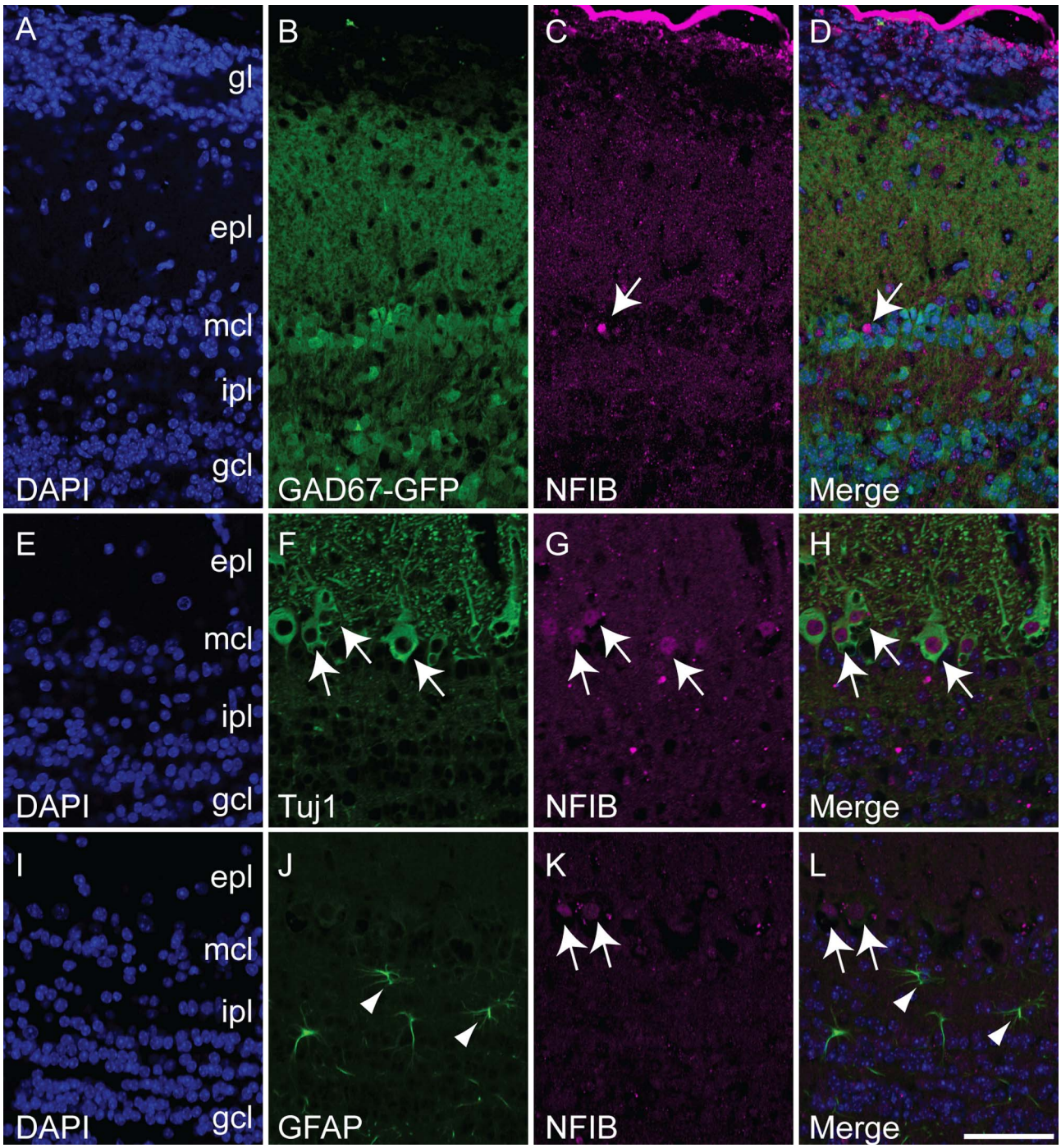


Figure 5. Cell-type-specific expression of NFIB within the adult OB. Coronal sections through the OB of adult GAD67-GFP (**A–D**) and wild-type (**E–L**) mice. Coimmunofluorescence labelling and confocal microscopy was used to determine cell-type-specific expression of NFIB within the adult OB. Cell nuclei were labelled with DAPI (A,E,I). Expression of NFIB within the adult OB (arrow in C,D) was observed within the mitral cell layer but did not coincide with GAD67-GFP expression (B; merged image in D). Analysis of Tuj1 expression revealed that mitral cells do indeed express NFIB (arrows in F–H). However, NFIB-expressing cells (arrows in K,L) were distinct from those expressing GFAP (arrowheads in J,L), indicating that astrocytes do not express this transcription factor within the adult OB. gl, Glomerular cell layer; epl, external plexiform layer; mcl, mitral cell layer; ipl, internal plexiform cell layer; gcl, granular cell layer. Scale bar = 50 μm.

positive cells within this neurogenic niche express this transcription factor (Fig. 7A–C). However, because NFIA was extensively expressed within the SVZ, we also analyzed the expression of DCX, a microtubule-associated protein expressed by neuroblasts within the SVZ and RMS (Nacher et al., 2001). With a DCX-GFP transgenic line (Walker et al., 2007), we demonstrated that all GFP-positive cells within the SVZ also expressed NFIA (Fig.

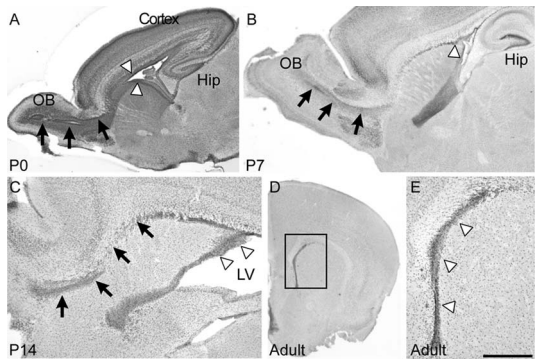


Figure 6. NFIA is expressed in the developing and adult RMS and SVZ. Sagittal (A–C) and coronal (D,E) sections of postnatal (A–C) and adult (D,E) brains, showing expression of NFIA. **A**: At P0, NFIA was widely expressed within the brain, including within the cortex and hippocampus. Expression of NFIA was also observed within the SVZ (arrowheads in A) and the emerging RMS (arrows in A). **B**: At P7, expression of NFIA within the cortex had declined, but expression was still observed within the hippocampus, SVZ (arrowhead in B), and RMS (arrows in B). **C**: At P14, NFIA expression was found in the SVZ of the lateral ventricle (arrowheads in C) and the RMS (arrows in C). **D**: Expression of NFIA within the adult SVZ. **E**: Higher magnification view of the boxed region in D, showing expression of NFIA by cells within the SVZ (arrowheads in E). OB, olfactory bulb; Hip, hippocampus; LV lateral ventricle. Scale bar = 500 μm in E; 800 μm for A–C; 1,250 μm for D.

7D–G), indicating that, within the SVZ, neuroblasts as well as progenitor cells express NFIA.

Within the adult RMS, neuroblasts continue to express DCX and also begin to express the enzymes central to GABA synthesis, such as GAD67. With the GAD67-GFP line, we demonstrated that GFP-positive neuroblasts within the adult RMS continue to express NFIA (Fig. 8A–D). This was supported by analysis of the DCX-GFP line, which revealed that all GFP-positive neuroblasts within the RMS expressed NFIA (Fig. 8E–H). However, in both the GAD67-GFP and the DCX-GFP lines, cells expressing NFIA, but not GFP, were observed, suggesting that another population of cells within the RMS also expressed this transcription factor. We postulated that these NFIA-expressing cells could be astrocytes, because astrocytes are abundant in the RMS, where they form a network of processes referred to as glial tubes (Peretto et al., 1997) that facilitate the chain migration of neuroblasts toward the OB (Alvarez-Buylla and Garcia-Verdugo, 2002; Doetsch et al., 1999). Double immunostaining of sections of the adult brain at the level of the RMS with anti-GFAP and anti-NFIA antibodies indicated that a proportion of GFAP-positive astrocytes within the RMS also expressed NFIA (Fig. 8I–L). Collectively, these data

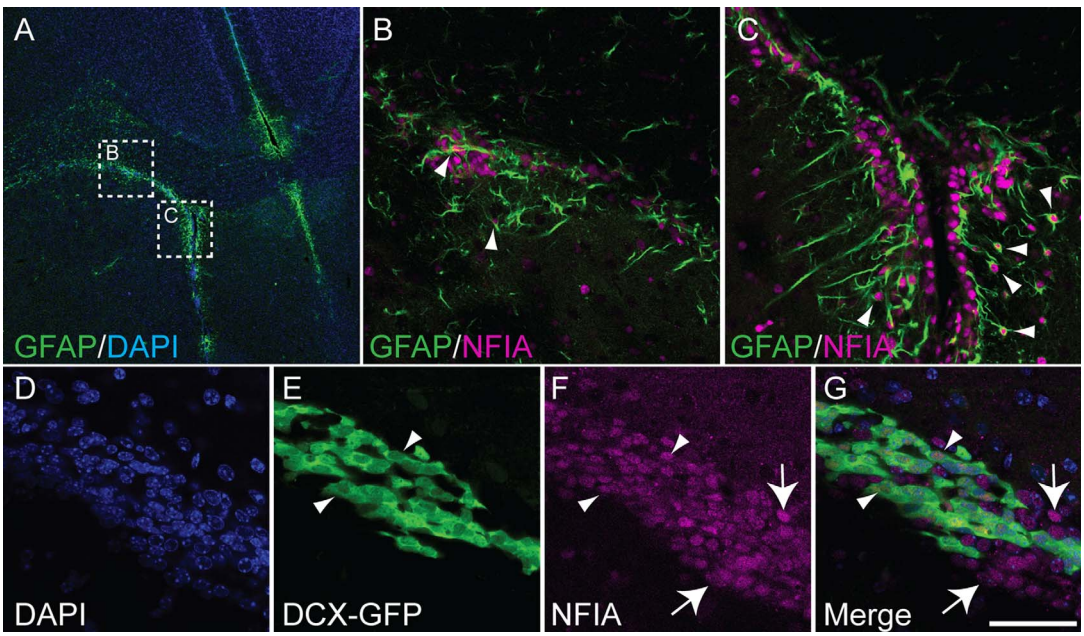


Figure 7. Cellular expression of NFIA within the adult SVZ. Coronal sections through the SVZ of adult wild-type (A–C) and DCX-GFP (D–G) mice. Coimmunofluorescence labelling and confocal microscopy was used to determine cell-type-specific expression of NFIA within the adult SVZ. **A**: Low-magnification image of the SVZ, showing expression of the nuclear marker DAPI (blue) and GFAP (green). The boxed regions in A are shown at higher magnification in B,C. **B,C**: NFIA (magenta) expression was observed by cells lining the lateral ventricles. Furthermore, some NFIA-expressing cells were surrounded by GFAP-positive fibers (green), indicating that these may be neural progenitor cells (arrowheads in B,C). **D–F** show the expression of DAPI (D), DCX-GFP (E), and NFIA (F) within the SVZ. The merged image is shown in **G**. All DCX-GFP-expressing neuroblasts within the SVZ express NFIA (arrowheads in E–G). However, some SVZ cells express NFIA but not DCX-GFP (arrows in F,G). Scale bar = 50 μm in G (applies to D–G); 500 μm for A; 150 μm for B,C.

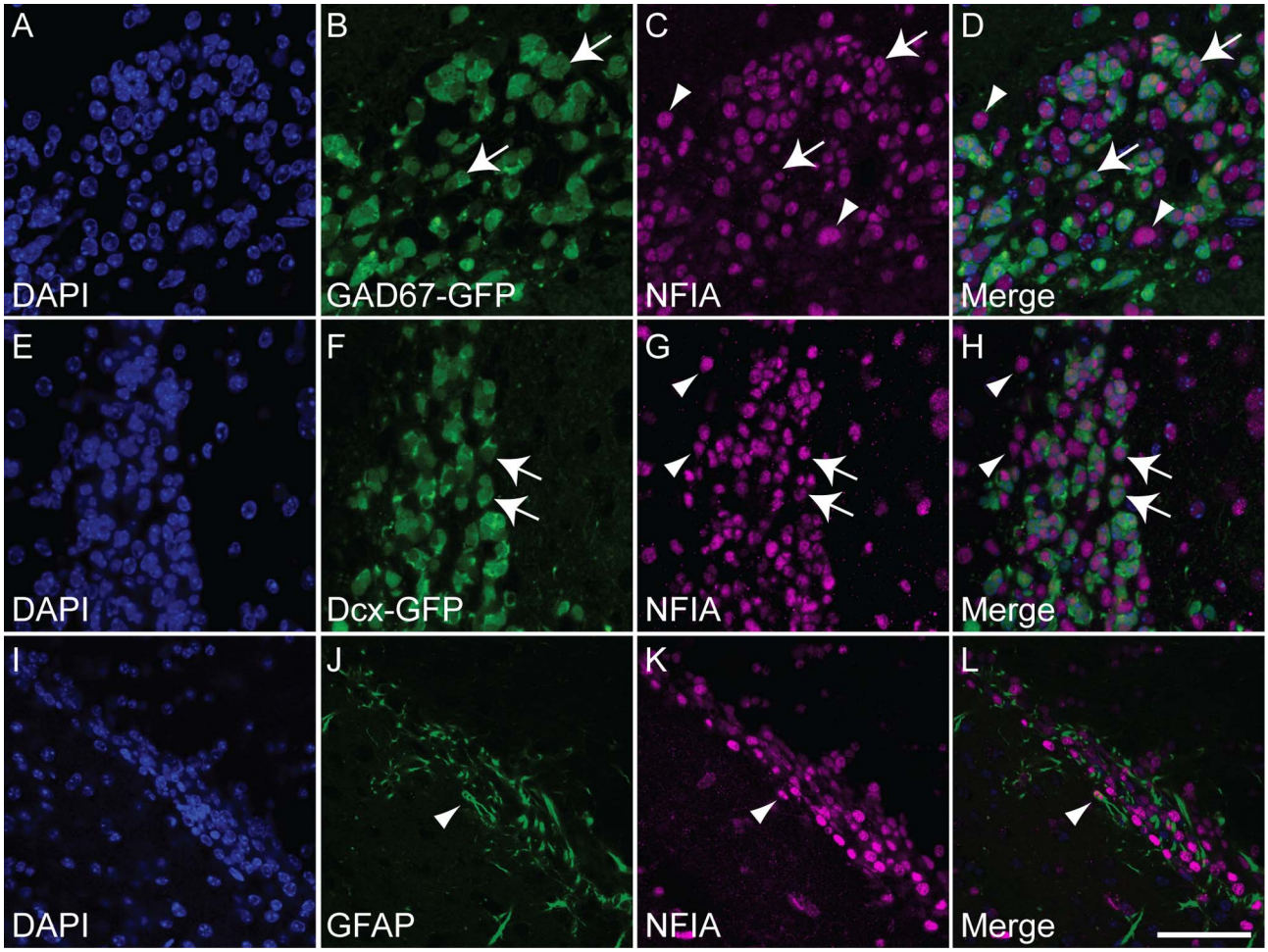


Figure 8. Cell-type-specific expression of NFIA within the adult RMS. Coronal sections through the RMS of adult GAD67-GFP (A–D), DCX-GFP (E–H), and wild-type (I–L) mice. Coimmunofluorescence labelling and confocal microscopy was used to determine cell-type-specific expression of NFIA within the adult RMS. Cell nuclei were labelled with DAPI (A,E,I). **A–D**: GAD67-GFP-positive cells within the RMS were seen to express NFIA (arrows in B–D). Some NFIA-expressing cells, however, were GFP negative (arrowheads in C,D). **E–H**: DCX-GFP-expressing neuroblasts within the RMS were also shown to express NFIA (arrows in F–H), but, again, some NFIA-expressing cells within the RMS were GFP negative (arrowheads in G,H). **I–L**: Some NFIA-positive cells within the RMS were also shown to be GFAP-expressing astrocytes (arrowheads in J–L). Scale bar = 50 μm.

suggest that both astrocytes and neuroblasts within the RMS express NFIA.

We have previously shown that NFIB is strongly expressed within the late embryonic and postnatal forebrain (Plachez et al., 2008). Here we extended these findings by demonstrating expression of NFIB within the SVZ at P0 (Fig. 9A). By P7, NFIB expression was also observed in cells of the RMS (Fig. 9B,C), combined with ongoing expression in the SVZ (Fig. 9B). Expression of NFIB within the SVZ and RMS was also detected at P14 and within the adult brain (Fig. 9D–F). Coimmunofluorescence labelling of adult SVZ sections with anti-NFIB and anti-GFAP antibodies revealed that NFIB is strongly expressed by cells lining the lateral ventricles and that some of these cells are surrounded by GFAP-positive fibers, suggesting that progenitor cells within the adult SVZ neurogenic niche express NFIB (Fig. 10A–C). Furthermore, analysis of DCX-GFP expression within the adult SVZ showed that all GFP-positive cells within the SVZ were expressing NFIB (Fig. 10D–F). This suggests that both NFIA and NFIB are expressed by neuroblasts within the adult SVZ.

Finally, we analyzed expression of NFIB within the adult RMS in conjunction with cell-type-specific markers. Expression of NFIB within the RMS was low, indicating that neuroblasts within the RMS may begin to downregulate expression of this transcription factor. Nevertheless, significant proportions of GAD67-GFP-expressing neuroblasts (Fig. 11A–D) and DCX-GFP-expressing neuroblasts (Fig. 11E–H) were shown to express NFIB within the adult

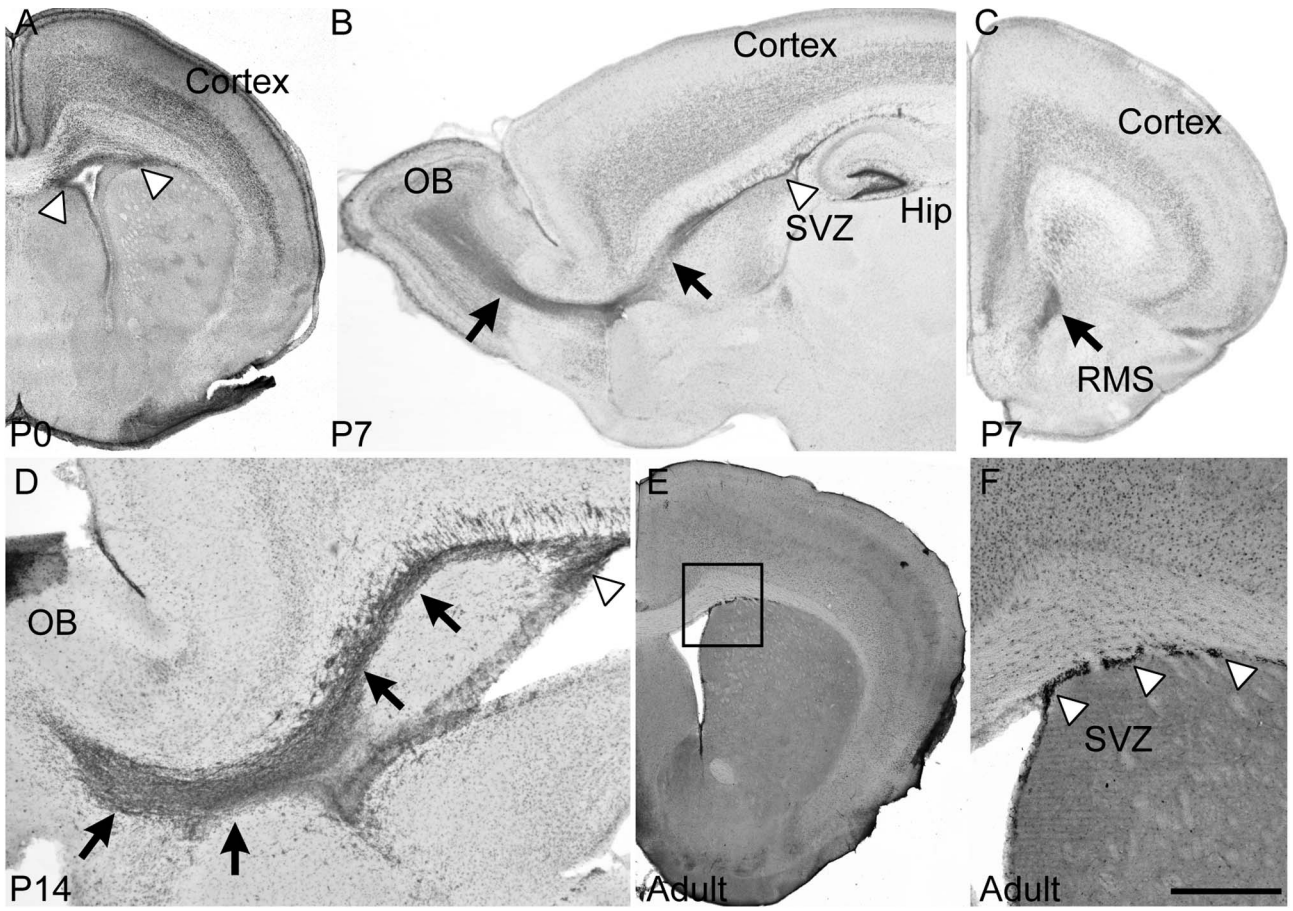


Figure 9. NFIB is expressed in the developing and adult RMS and SVZ. Sagittal (B,D) and coronal (A,C,E,F) sections of postnatal (A–D) and adult (E,F) brains, showing expression of NFIB. **A**: At P0, NFIB was expressed within the cortex and SVZ (arrowheads in A). **B**: At P7, NFIB expression was detected within the hippocampus, SVZ (arrowhead in B), and RMS (arrows in B). **C**: Coronal section through a P7 brain, showing that cells within the RMS (arrow in C) express NFIB. **D**: At P14, NFIB expression was found in the SVZ of the lateral ventricle (arrowhead in D) and the RMS (arrows in D). **E**: Expression of NFIB within the adult SVZ. **F**: Higher magnification view of the boxed region in E, showing expression of NFIB by cells within the SVZ (arrowheads in F). OB, olfactory bulb; Hip, hippocampus; RMS, rostral migratory stream; SVZ, subventricular zone. Scale bar = 500 μm in F; 800 μm for A,B,D; 350 μm for C; 1,250 μm for E.

RMS. Colabelling of the RMS with antibodies against NFIB and GFAP also revealed that some NFIB-expressing cells within the RMS were probably astrocytes, insofar as they were surrounded by GFAP-positive fibers (Fig. 11I–L). Taken together, these data indicate that both NFIA and NFIB are broadly expressed within the adult SVZ and RMS.

DISCUSSION

This study examined the expression and distribution of NFI proteins in the developing and adult OB, RMS, and SVZ. Our data show that NFIA and NFIB proteins are present during embryonic and postnatal development of these regions, as well as within the adult brain, with NFIA and NFIB displaying overlapping but distinct expression patterns. NFIA expression was initiated within the OB at E15, whereas NFIB expression was not observed until E18. Postnatally, both transcription factors were broadly expressed within the OB, but these expression patterns became far more restricted within the adult OB. We have previously shown that NFIA and NFIB proteins are expressed by cortical neuronal and glial cells (Plachez et al., 2008) and by cortical progenitor cells in vivo (Barry et al., 2008; Piper et al., 2010). We therefore examined which cellular subtypes within the adult SVZ, RMS, and OB expressed NFIA and NFIB.

Within the adult SVZ, both NFIA and NFIB were broadly expressed, with GFAP-positive cells and DCX-GFP-positive neuroblasts exhibiting expression of these transcription factors. This expression pattern suggests that the *NFI* genes may be playing a role in regulating the differentiation of progenitor cells and maturation and/or the migration of neuroblasts within this neurogenic niche. In

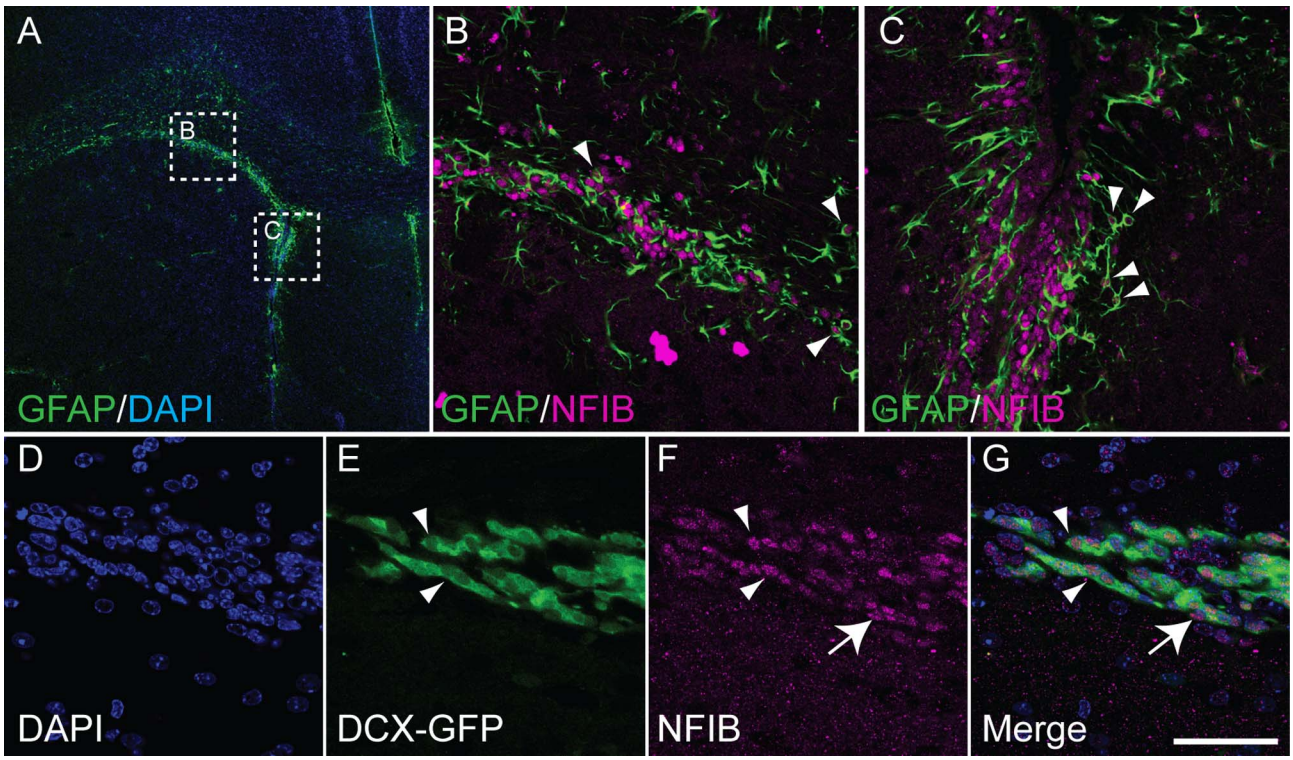


Figure 10. Cellular expression of NFIB within the adult SVZ. Coronal sections through the SVZ of adult wild-type (A–C) and DCX-GFP (D–G) mice. Coimmunofluorescence labelling and confocal microscopy was used to determine cell-type-specific expression of NFIB within the adult SVZ. **A**: Low-magnification image of the SVZ, showing expression of the nuclear marker DAPI (blue) and GFAP (green). The boxed regions in A are shown at higher magnification in B,C. **B,C**: NFIB (magenta) expression was observed in cells lining the lateral ventricles. Moreover, a proportion of NFIB-expressing cells appear to be surrounded by GFAP-positive fibers (green), indicating that these may be neural progenitor cells (arrowheads in B,C). **D–F** show expression of DAPI (D), DCX-GFP (E), and NFIB (F) within the SVZ. The merged image is shown in **G**. DCX-GFP-expressing neuroblasts within the SVZ express NFIB (arrowheads in E–G). However, some SVZ cells express NFIB but not DCX-GFP (arrows in F,G). Scale bar = 50 μm in G (applies to D–G); 500 μm for A; 150 μm for B,C.

support of this, postnatal *NFIX*$^{-/-}$ mice exhibit a significant expansion of proliferative cells within the SVZ, indicative of excessive neural progenitor cell proliferation or of defects in neuroblast migration (Campbell et al., 2008). How *NFI* genes act to modulate progenitor cell activity within the SVZ is at this stage unclear, although recent reports suggesting that *NFIA* regulates progenitor cell differentiation within the neocortex via interaction with the Notch pathway (Namihira et al., 2009; Piper et al., 2010) may be pertinent, in that Notch signaling has been shown to be involved in SVZ progenitor cell self-renewal (Imayoshi et al., 2010). Currently, analysis of the role of NFIA and NFIB in SVZ neurogenesis is hampered by the fact that both *NFIA* and *NFIB* knockout mice die perinatally from kidney and lung defects, respectively (Lu et al., 2007; Steele-Perkins et al., 2005). In future, the development of conditional *NFI* alleles will allow the role of these genes in regulating adult neurogenesis to be studied in depth.

Both NFIA and NFIB were also expressed within the postnatal and adult RMS. GFAP-expressing astrocytes around the RMS express NFIA and NFIB. This finding is consistent with previous reports showing that NFI proteins regulate *GFAP* expression in vitro (Brun et al., 2009; Cebolla and Vallejo, 2006; Piper et al., 2011) and are critical for gliogenesis in vivo (Deneen et al., 2006; Piper et al., 2009a; Shu et al., 2003). Moreover, neuroblasts within the RMS were also shown to express NFIA and NFIB. This finding is interesting in that it reveals that NFI transcription factors play a much broader role during development than driving gliogenesis. Indeed, within the murine cerebellum, NFI proteins have been shown to regulate a number of different genes, including *Tag1*, *N-cadherin*, *GABRA6*, and *ephrin B1*, genes that play a vital role in the differentiation and subsequent migration of cerebellar granule neurons within the postnatal cerebellum (Wang et al., 2004, 2007, 2010). Our findings suggest that NFI proteins may regulate genes necessary for neuroblast migration. In an effort to identify potential NFI target genes, we performed an in silico bioinformatic screen of candidate genes known to be involved in neuroblast migration, namely, *DCX* (Belvindrah et al., 2011; Koizumi

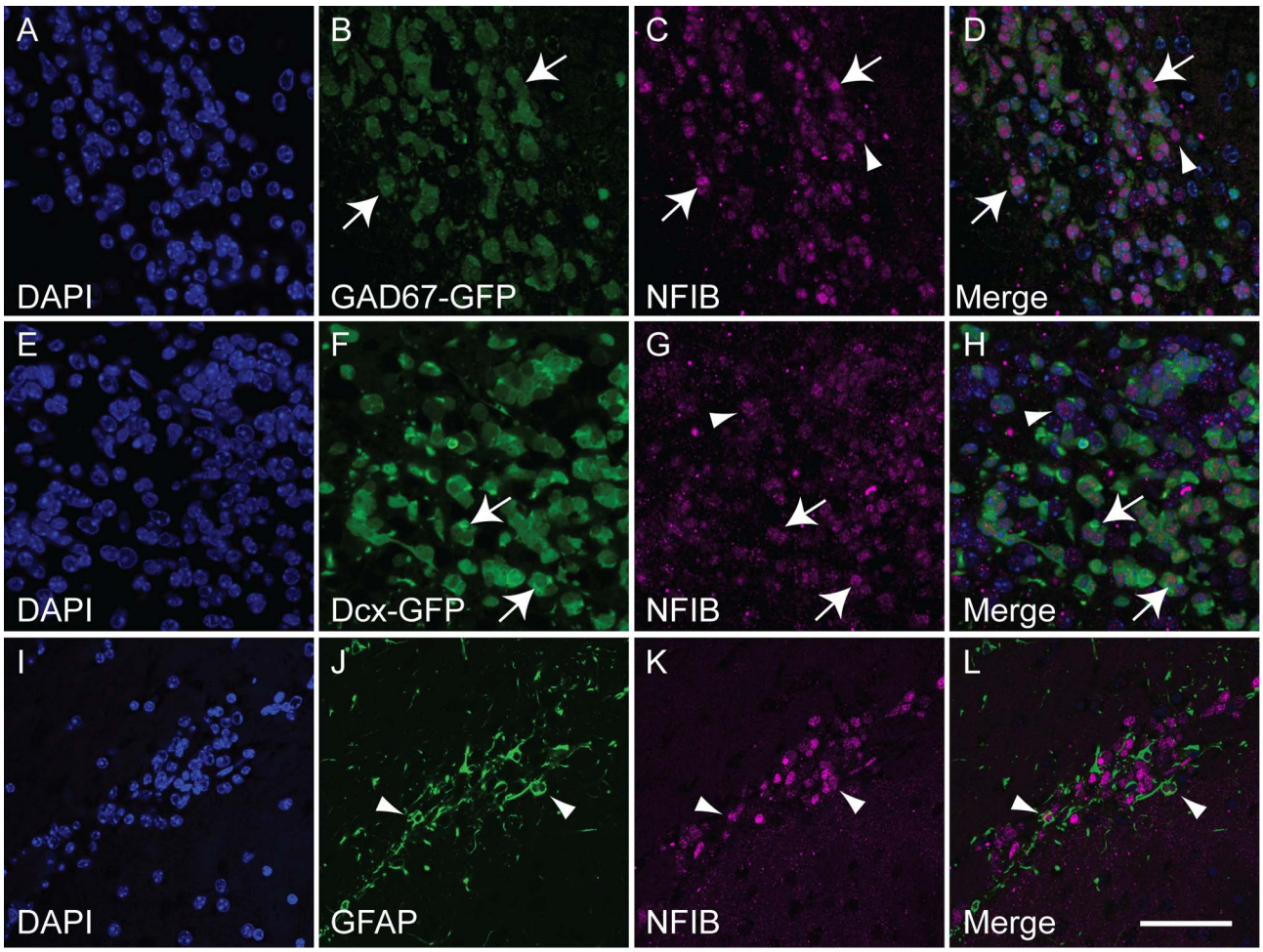
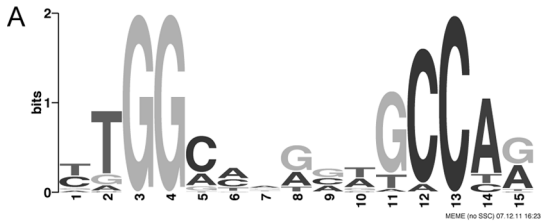


Figure 11. Cell-type-specific expression of NFIB within the adult RMS. Coronal sections through the RMS of adult GAD67-GFP (A–D), DCX-GFP (E–H), and wild-type (I–L) mice. Coimmunofluorescence labelling and confocal microscopy were used to determine cell-type-specific expression of NFIB within the adult RMS. Cell nuclei were labelled with DAPI (A,E,I). **A–D**: Many GAD67-GFP-positive cells within the RMS were seen to express low levels of NFIB (arrows in B–D). Within the RMS, there were also NFIB-positive cells that did not express GFP (arrowheads in C,D). **E–H**: DCX-GFP-expressing neuroblasts within the RMS were also shown to express low levels of NFIB (arrows in F–H). Furthermore, some NFIB-positive cells within the RMS were GFP negative (arrowheads in G,H). **I–L**: Some NFIB-expressing cells within the RMS were also shown to be GFAP-expressing astrocytes (arrowheads in J–L). Scale bar = 50 μm.

et al., 2006), *SLIT1* (Kaneko et al., 2010), *β8 integrin* (Mobley and McCarty, 2011), *NCAM* (Chazal et al., 2000), and *CDK5* (Hirota et al., 2007). This screen employed an NFI motif DNA-binding site that was derived from a recent report identifying NFI binding sites in vivo using chromatin immunoprecipitation sequencing (Pjanic et al., 2011). We scanned the 2.2-kb promoter region around the transcription start site of these candidate genes to identify potential NFI binding sites (see Materials and Methods). Each of the candidate genes investigated possessed multiple putative NFI binding sites within the promoter region, suggesting that NFI transcription factors may regulate multiple aspects of neuroblast migration (Fig. 12). The *DCX* promoter contained nine potential NFI binding sites (Fig. 12), and, furthermore, a recent microarray analysis of brains from P16 *NFIA*$^{-/-}$ mice found *DCX* expression to be significantly upregulated in the absence of this transcription factor (Wong et al., 2007). The *DCX* gene encodes a microtubule-associated protein that modulates cytoskeletal stability (Gleeson et al., 1999) and is crucial for normal neuroblast migration along the RMS in vivo (Koizumi et al., 2006). This being the case, the presence of many putative NFI binding sites within the *DCX* promoter indicates that the NFI proteins may influence cytoskeletal dynamics within migrating neuroblasts through transcriptional regulation of *DCX*.

NFIA and NFIB were also expressed within the adult OB, but in distinct patterns. The OB is composed primarily of excitatory glutamatergic neurons (mitral and tufted cells) and inhibitory GABAergic interneurons (granule and



B

Gene	Position relative to TSS	Site strand	Site *p*-value	Site sequence
CDK5	-280	+	$3.1 \cdot 10^{-5}$	GAGGAAAGAAGCCAG
CDK5	-50	+	$9.6 \cdot 10^{-5}$	CAGGCCAGCCTCCAG
CDK5	-1180	-	$3.4 \cdot 10^{-4}$	TGGGCAGCGAGACAG
CDK5	-1044	-	$4.7 \cdot 10^{-4}$	GAGGCCAGCTGACTG
CDK5	-489	+	$5.6 \cdot 10^{-4}$	GTGGCCAAGACCCTG
CDK5	-1375	-	$8.9 \cdot 10^{-4}$	ACGGCCTAGATCCCG
CDK5	-1025	+	$9.6 \cdot 10^{-4}$	CTGGGGGACACCCAG
CDK5	-1134	-	$9.9 \cdot 10^{-4}$	AAGGACACACGCCAG
DCX	-1253	+	$8.5 \cdot 10^{-5}$	TTGGAGAAGTGCCAC
DCX	-1011	+	$1.1 \cdot 10^{-4}$	TTGGGTATATGCCCA
DCX	-1779	+	$3.0 \cdot 10^{-4}$	TTGGAGCTCAGACAA
DCX	-1012	-	$4.9 \cdot 10^{-4}$	CTGGGCATATACCCA
DCX	-1253	-	$6.4 \cdot 10^{-4}$	GTGGCACTTCTCCAA
DCX	-1745	-	$7.9 \cdot 10^{-4}$	GTGGATAGTTGCCTT
DCX	-62	-	$8.5 \cdot 10^{-4}$	AAGGGATTCAGCCCA
DCX	-1089	+	$8.5 \cdot 10^{-4}$	TGGGCCTAGAGACCT
DCX	-1011	-	$8.9 \cdot 10^{-4}$	TGGGCATATACCCAA
ITGB8	-1474	+	$6.0 \cdot 10^{-5}$	GTGGCCAGACGCCTG
ITGB8	-244	+	$2.3 \cdot 10^{-4}$	TGGGTCTGATGCCTG
ITGB8	-1316	+	$8.9 \cdot 10^{-4}$	TGGGGAGGTTTCCTA
ITGB8	-1660	-	$9.6 \cdot 10^{-4}$	CGGGAGAGGAGACAC
NCAM1	-309	+	$7.9 \cdot 10^{-5}$	TTGGCTCAGGGACAA
NCAM1	162	-	$9.0 \cdot 10^{-5}$	CTGGGATTCTGCCCA
NCAM1	163	+	$2.5 \cdot 10^{-4}$	GTGGGCAGAATCCCA
NCAM1	162	+	$2.7 \cdot 10^{-4}$	TGGGCAGAATCCCAG
NCAM1	-618	+	$2.7 \cdot 10^{-4}$	CTGGGAGACAGACTG
NCAM1	-1696	-	$6.4 \cdot 10^{-4}$	CAGGGAACCTTCCAG
SLIT1	97	+	$3.6 \cdot 10^{-5}$	TGGGGTTGGTGCCAG
SLIT1	-368	+	$2.9 \cdot 10^{-4}$	AGGGAAAACAACCAA
SLIT1	-1851	-	$7.2 \cdot 10^{-4}$	CCGGCAGTCATCCAT
SLIT1	-538	-	$8.9 \cdot 10^{-4}$	ATGGTCAAGAGTCAA
SLIT1	-1663	-	$9.2 \cdot 10^{-4}$	GGGGCTTACAGACTG

Figure 12. In silico screen of potential NFI binding sites within the promoter regions of genes implicated in neuroblast migration. **A**: The sequence logo for the NFI binding motif used in this study. **B**: Potential NFI binding sites reported by FIMO in the promoters (defined as −2,000 to +199 base pairs relative to the respective transcription start site [TSS]) of *DCX*, *SLIT1*, *β8 integrin* (*ITGB8*), *NCAM*, and *CDK5*. We report the position in bases of each potential site relative to the TSS, the strand of the potential site, the *P* value of the motif match, and the site sequence.

periglomerular neurons). Studies have shown that GABAergic granule and periglomerular neurons (tyrosine hydroxylase-expressing, calbindin-expressing, and calretinin-expressing neurons) are the two main products of postnatal/adult neurogenesis (Hack et al., 2005; Vergano-Vera et al., 2006). However, despite their expression within migrating neuroblasts, we did not observe expression of either NFIA or NFIB within GAD67-positive cells within the adult OB. This suggests that these transcription factors are downregulated as neuroblasts mature into interneurons within the OB. How this occurs is unclear, because the transcriptional control of the *NFI* genes is poorly understood. Developmentally, genes such as *Pax6*, *Neurogenin 2*, and *Emx2* have been implicated upstream of *NFI* genes (Gangemi et al., 2006; Holm et al., 2007; Mattar et al., 2004), but how *NFI* genes are regulated within the adult brain is not known. We did, however, reveal that NFIB was expressed by mitral cells within the adult OB. The expression of NFIB by mitral cells in the OB, which provides the major output projection of the bulb, parallels the expression of this protein within the layer V corticospinal neurons within the cortex, which also express this protein (Plachez et al., 2008), indicating that NFIB expression may be central to the development of projection neurons from the OB and cortex. Furthermore, NFIA was expressed by OB astrocytes. These results imply divergent roles for NFIA and NFIB within the adult OB, and collectively our data reveal dynamic expression patterns of NFIA and NFIB in the developing and adult OB, RMS, and SVZ.

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