Published in final edited form as:

Neuroscience. 2007 August 24; 148(2): 385–399.

Disruption of *Foxg1* expression by knock-in of Cre Recombinase: Effects on the development of the mouse telencephalon

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Abstract

The Cre/*loxP* system is used routinely to manipulate gene expression in the mouse nervous system. In order to delete genes specifically from the telencephalon, the *Foxg1-cre* line was created previously by replacing the intron-less *Foxg1* coding region with *cre*, resulting in a *Foxg1* heterozygous mouse. As the telencephalon of heterozygous *Foxg1* mice was reported to be normal, this genotype often has been used as the control in subsequent analyses. Here we describe substantial disruption of forebrain development of heterozygous mice in the *Foxg1-cre* line, maintained on the C57BL/6J background. High resolution magnetic resonance microscopy reveals a significant reduction in the volume of the neocortex, hippocampus and striatum. The alteration in the neocortex results, in part, from a decrease in its tangential dimension, although gross patterning of the cortical sheet appears normal. This decrease is observed in three different *Foxg1* heterozygous mouse lines, independent of the method of achieving deletion of the *Foxg1* gene. Although *Foxg1* is not expressed in the diencephalon, three-dimensional magnetic resonance microscopy revealed that thalamic volume in the adult is reduced. In contrast, at postnatal day 4, thalamic volume is normal, suggesting that interactions between cortex and dorsal thalamus postnatally produce the final adult thalamic phenotype. In the *Foxg1-cre* line maintained on the C57BL/6J background, the radial domain of the cerebral cortex also is disrupted substantially, particularly in supragranular layers. However, neither *Foxg1* heterozygous mice of the *Foxg1-tet* line, nor those of the *Foxg1-lacZ* and *Foxg1-cre* lines maintained on a mixed background, displayed a reduced cortical thickness. Thus Cre recombinase contributes to the radial phenotype, although only in the context of the congenic C57BL/6J background. These observations highlight an important role for *Foxg1* in cortical development, reveal noteworthy complexity in the invocation of specific mechanisms underlying phenotypes expressed following genetic manipulations and stress the importance of including *appropriate* controls of all genotypes.

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Section Editor: John Rubenstein

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Keywords

cortical development; supragranular layers; magnetic resonance microscopy; thalamus; patterning; telencephalon

Development of the cerebral cortex relies on extracellular cues and intrinsic signals that instruct the proliferation, differentiation and migration of neuronal precursors. The duration and location of these cues are critical to producing the final cortical architecture (Fukuchi-Shimogori and Grove, 2001, Parnavelas et al., 2002, Rakic, 2002, Shimogori et al., 2004, Rash and Grove, 2006, Storm et al., 2006). The advent of methods that allow for spatially- and temporally-restricted inactivation of genes, including the Cre/*loxP* system, has allowed significant advances in identifying the roles of many of these cues in specific events during cortical development [for example (Gorski et al., 2003, Machon et al., 2003, Fuccillo et al., 2004, Hanashima et al., 2004, Mizutani and Saito, 2005, Britz et al., 2006, Kessaris et al., 2006)]. These techniques, however, introduce potential confounds that need to be considered during the design of experiments and interpretation of data. For example, several *in vitro* studies have described effects of Cre recombinase, including growth inhibition and death, in mammalian cells lacking exogenous *loxP* sites (Loonstra et al., 2001, Pfeifer et al., 2001, Silver and Livingston, 2001). A recent study extended these observations to the mouse central nervous system, where it was reported that high levels of Cre recombinase in the nucleus of neural progenitors lead to impaired proliferation and increased cell death, resulting in hydrocephaly (Forni et al., 2006). Similar effects of Cre recombinase at low doses, however, have not been reported *in vivo*, although it is unclear whether subtle phenotypes have gone unnoticed.

The *Foxg1-cre* line, generated by replacing the intron-less *Foxg1* coding region with *cre*, was designed to permit the deletion of genes specifically from the telencephalon (Hebert and McConnell, 2000). The resulting *Foxg1-cre/+* mouse, which is heterozygous for *Foxg1*, usually has been considered the appropriate control when examining the biological effects of conditionally deleting the gene of interest. This is because several studies have indicated that mice with a single allele of *Foxg1*, including the *Foxg1-cre* line, have an ostensibly normal cortex (Xuan et al., 1995, Dou et al., 1999, Hebert and McConnell, 2000, Hanashima et al., 2002, Hanashima et al., 2004), although recently, microcephaly and impaired neurogenesis in the hippocampus have been reported in a *Foxg1* heterozygous mouse (Shen et al., 2006b). The present study describes the disruption of forebrain development in the *Foxg1-cre/+* mouse in the context of a congenic C57BL/6J strain.

Methods

Animals

C57BL/6J mice were purchased from the Jackson Laboratory (Bar Harbor, ME). *Foxg1-cre/+* mice were a generous gift from Dr J. Hebert, Albert Einstein College of Medicine. Animals were provided free access to food and water and housed in a 12 hour light:dark cycle. All research procedures using mice were approved by the Institutional Animal Care and Use Committee at Vanderbilt University and conformed to NIH guidelines.

All mice used in this study were generated by heterozygous $\times$ wild type matings of the original *Foxg1-cre* mixed background line that we had backcrossed on the C57BL/6J strain for 8 to 9 generations, the point at which the Jackson Laboratory considers a strain to have reached incipient congenic status (Silver, 1995). Noon on the day a plug was detected was considered embryonic day (E) 0.5 and the day of birth was designated postnatal day (P) 0. The stage of the embryos was confirmed using crown rump length and external physical features (Kaufmann, 1992). Mice were genotyped from trunk (embryonic) or tail (postnatal) DNA via

PCR with primers to *cre*: 5'CACCCTGTTACGTATAGCCG 3' and 5' GAGTCATCCTTAGCGCCGTA 3'. The PCR protocol used was the following: after an initial denaturation step of 5 min at 94°C, samples were submitted to 35 cycles of denaturation (94°C, 45 sec) / annealing (55°C, 30 sec) / elongation (72°C, 1min), followed by a final elongation step of 7 min at 72°C. The presence of a PCR product of 320bp indicates that the mouse is *Foxg1-cre*/+.

In addition, fixed brains or Nissl-stained sections of both wild type and *Foxg1* heterozygous mice from three additional *Foxg1* knock-in lines were kindly provided: adult *Foxg1-tet*, C57BL/6 background (Dr S. Anderson, Weill Medical College of Cornell, Nissl-stained sections), P8 *Foxg1-lacZ*, CBA × C57BL/6 mixed background (Dr D. Price, University of Edinburgh, fixed brains) and P8 *Foxg1-cre*, CBA × C57BL/6 mixed background (Dr D. Price, fixed brains).

Northern hybridization

Total RNA was isolated from the cerebral cortex of P4 mice using the Trizol method according to the manufacturer's protocol (Invitrogen, Carlsbad, CA). Two litters were used, with the RNA from an individual animal considered a single sample. Following the initial extraction, RNA aliquots were further purified using RNeasy Mini spin columns (Qiagen, Valencia, CA). Ten µg total RNA was run on a 1.5% agarose/formaldehyde gel, with the samples from a single litter run on the same gel. The electrophoresed RNA was transferred to Nytran membrane (Schleicher & Schuell, Keene, NH) via overnight capillary transfer. Northern hybridization was performed as previously described (Pimenta et al., 1996), with the blot first probed with a 1.4kb cDNA that recognizes the 3'UTR of mouse *Foxg1* (kind gift from Dr S. Anderson), then stripped and probed with a mouse cDNA for the coding sequence of cyclophilin. At each probing, the blot was exposed to autoradiographic film (Eastman Kodak Corp., Rochester, NY) to detect signal. The film was scanned using ScanWizard5 (Version 5.0, Microtek International, Inc) and analyzed using the public domain program ImageJ, version 1.33. Loading errors were corrected by normalizing the values for *Foxg1* to those for *cyclophilin*.

Imaging

Adult (3 heterozygous and 3 wild type) and P4 (4 heterozygous and 4 wild type) male mice were deeply anesthetized with sodium pentobarbital (60 mg/kg i.p.) and perfused transcardially with 4% paraformaldehyde in phosphate buffered saline (PBS, pH 7.2). The brains were dissected and postfixed overnight at 4°C.

Adult—Adult brains were subjected to magnetic resonance microscopy (MRM) at the Center for Small Animal Imaging at Vanderbilt University. Whole brains were immersed in > 20 ml of PBS doped with Magnevist (Berlex Inc, Montville, NJ) at a ratio of 33:1. Brains were immersed for no less than 1 week prior to imaging and stored at 4 °C during that time. All magnetic resonance images were acquired at 400 MHz using a 9.4T/21 cm horizontal bore magnet equipped with imaging gradients capable of 40 G/cm and operated by a VNMRJ console (Varian Inc, Palo Alto, CA). Mouse brains were loaded into a 10 mm glass NMR tube with a sufficient volume of doped PBS to cover the brain to prevent drying during the image acquisition. The NMR tube was then placed in a home-built, 15-mm diameter loop-gap resonator and loaded into the center of the magnet for image acquisition.

Image data were acquired using a 3D spin-echo pulse sequence with the following parameters: TE/TR = 7.5 ms/50 ms, 24 averaged excitations, 100 kHz acquisition bandwidth, and 256 × 160 × 160 sampled values over a 19.2 × 12.8 × 12.8 mm³ volume. Total acquisition time was approximately 8.5 hours. Data were zero-padded to a size of 480 × 320 × 320 and Fourier transformed to create 3D image volume with an isotropic resolution of 40 µm. Each volume

was then visually rotated about each dimension using a bilinear interpolation algorithm in order to approximately match the orientation of the image data to a standard mouse brain atlas (Paxinos and Franklin, 2001). Finally, for each orientation a 2D image was generated for each slice and contrast-enhanced using an adaptive histogram equalization algorithm in MATLAB (The Mathworks; Natick, MA).

P4—P4 brains were imaged at the Pittsburgh NMR Center for Biomedical Research at Carnegie Mellon University. Each brain was immersed in PBS, sealed in a 10-mm diameter plastic syringe, and stored at 4 °C. For imaging, each sample was aligned in a 10-mm diameter birdcage RF resonator then inserted into an 11.7 T, 89-mm vertical-bore, Bruker AVANCE micro-imaging system equipped with imaging gradients capable of 100 G/cm operated by a ParaVision 3.1 console (Bruker BioSpin, Billerica, MA). A diffusion-weighted 3D-Fourier transform spin echo pulse sequence was used with settings TR/TE = 19/1000 ms, and a spectral width of 50 kHz. During each scan sample temperature was regulated at 14 °C. Diffusion gradients were applied along the anterior – posterior direction with $\delta = 5$ ms, $\Delta = 15$ ms, and a gradient strength of 30 G/cm. This diffusion-weighted imaging sequence provides improved gray/white matter contrast in these early-aged premyelinated brains compared to standard T_2 - or T_2^* -weighted imaging methods (Wimberger et al., 1995). MRM data sets were acquired with a $256 \times 128 \times 128$ matrix size and zero-filled yielding a final resolution of $33 \mu\text{m} \times 66 \mu\text{m} \times 66 \mu\text{m}$. The total imaging time per brain was approximately 19 hours using four signal averages.

3D MRM data sets were imported into the software package Amira (Mercury Computer Systems, Chelmsford, MA). Data were then rotated along axial, sagittal, and coronal axes to perfect symmetry. Image stacks cutting through the newly symmetrical axial and sagittal planes were exported for volume analysis. To calculate the new slice thickness and voxel size that resulted from axis rotation and subsequent image exportation, voxel dimensions were recalibrated using Amira's internal measurement tools. Images were then exported in lossless TIFF format for region-of-interest analysis.

***In situ* hybridization**

Two probes were used in the present study: *Brn2* (probe template generated by PCR amplification of a 277bp fragment corresponding to nucleotides 857-1134 of the mouse *brn-2* cDNA [GenBank NM_008899]) and *Tbr1* (264 bp template generously provided by J. Rubenstein, UCSF, (Puelles et al., 2000)). Mice were analyzed at E16.5, with 3 wild type and 3 heterozygous mice examined for each probe. The tissue processing, probe synthesis and hybridization protocols were as described previously (Bonnin et al., 2006). Briefly, the brains were immersion fixed overnight in 4% paraformaldehyde in PBS, then equilibrated in sequential, 12-hour changes of 10, 20 and 30% sucrose in PBS. The entire telencephalon was cut on a cryostat, in the sagittal plane, in five series of 25 μm thick sections that were collected on Superfrost Plus glass slides (Fisher, Pittsburgh, PA). In each hybridization run, an entire series from a wild type and mutant brain were processed simultaneously. Digoxigenin (DIG)-UTP labeled riboprobes were transcribed with T7 or T3 polymerase from the partial cDNA clone of *Brn2* and *Tbr1*, according to the manufacturers recommendations (Digoxigenin Labeling Kit, Roche). All sections were dehydrated through an ethanol series, cleared with xylene and mounted with DPX (Fisher).

Immunocytochemistry and histochemistry

Six antibodies were used in the present study: rabbit anti-serotonin transporter (SERT, 1:1000, Calbiochem/EMD Biosciences, La Jolla, CA), mouse anti-GAD-67 (1:1000, Chemicon, Temecula, CA), goat anti-Brn1 (1:200, Santa Cruz Biotechnology, Santa Cruz, CA), rabbit anti-FoxP2 (1:1000, Abcam Inc, Cambridge, MA), rabbit anti-Tbr1 (1:500, Abcam Inc) and

rabbit anti-Cux1 (1:1000, Santa Cruz Biotechnology). In addition, some sections were processed for cytochrome oxidase (CO) histochemistry or cresyl violet (CV). In each staining run, sections from heterozygous mutants and wild type controls were processed together.

Male mice were analyzed at P4 (Brn1, FoxP2, Tbr1 and Cux1), P8 (SERT) and >P60 (adult; GAD-67, CO and cresyl violet), with at least 3 heterozygous and 3 wild type animals analyzed at each age for each antibody/stain. Mice were perfused as described for imaging. One set of brains was postfixed overnight at 4°C, then equilibrated in sequential, 12-hour changes of 10, 20 and 30% sucrose in PBS. Coronal sections through the telencephalon were cut in five series with a sliding microtome at 40 µm and collected in tissue freezing medium (P8 and adult), or cut in five series on a cryostat at 25 µm and collected on Superfrost glass slides (P4). In a second set of brains, the cerebral hemispheres were separated from the rest of the brain, flattened overnight in 30% sucrose between two glass slides, sectioned tangentially on a sliding microtome at 50µm and collected as a single series in tissue freezing medium.

The fixed P8 *Foxg1-lacZ* and *Foxg1-cre* (CBA × C57BL/6 mixed background) wild type and heterozygous mutant brains were cut in the coronal plane as described above and wild type and mutant sections were processed together for CV.

Immunocytochemistry—Sections were processed as described previously (Eagleson et al., 2005). Briefly, free floating sections were incubated in primary antibody (anti-GAD67 or -SERT) for 72 hours at 4°C, followed by biotin-SP-conjugated donkey anti-rabbit or anti-mouse IgG (1:1000, Jackson ImmunoResearch, West Grove, PA) for one hour at room temperature and processed by the ABC histochemical method (Vector, Burlingame, CA). The antibody labeling was visualized by treating the sections for 4 minutes at room temperature in 0.5% 3'-diaminobenzidine (DAB) with 0.05% H₂O₂. The sections were washed, mounted onto gelatin-subbed slides, dehydrated with alcohols, cleared with CitriSolve (Fisher) and coverslipped in DPX. Cryostat sections were incubated in primary antibody (anti-Brn1, -FoxP2, -Tbr1 or -Cux1) overnight at room temperature, followed by biotin-SP-conjugated anti-goat or anti-rabbit IgG (1:1000, Jackson ImmunoResearch) for 1 hour at room temperature and avidin-Cy³ (1:1000, Jackson ImmunoResearch) for 1 hour at room temperature. Sections were coverslipped in propyl-gallate.

CO histochemistry—Adult tangential and coronal sections were reacted for CO. Sections were first rinsed with PBS, then incubated in the dark at 37°C in 0.1M phosphate buffer containing 10% sucrose, 0.05% cytochrome C, 0.037% catalase and 0.025% DAB. The reaction was stopped when the barrels in somatosensory cortex were clearly visible (approximately 1-2 hours). After rinsing in PBS, sections were mounted onto gelatin-subbed slides, dehydrated with alcohols, cleared with CitriSolve and coverslipped in DPX.

Morphometric Analyses

Grayscale images of histological sections were acquired with a Zeiss AxioCam HRc camera (Zeiss, Jena, Germany) that was mounted onto a Zeiss axioplan 2 microscope, Zeiss Axiovision 4.1 software was used. Macroscopic images of the whole brain were acquired with a Zeiss AxioCam MRc camera that was mounted onto a Leica MZ FLIII stereoscope (Leica Microsystems, Wetzlar, Germany) running MRGrab 1.0 software (Zeiss). Both the optical micrographs and the MRM images were imported into the public domain program ImageJ, version 1.33, for morphometric analysis. Figures were prepared using these images in Adobe Photoshop 7.0 (Adobe Systems Incorporated, San Jose, CA). All images received only minor, required adjustments of contrast and brightness.

The dimensions of the dorsal surface of the cerebral hemispheres were determined from macroscopic images of fixed whole brains from male mice at P4 and P60. In the anterior-

posterior domain, two measurements were made: medially along the longitudinal fissure, and more laterally from the frontal to the occipital pole. In the medial-lateral domain, the hemisphere was measured at its widest extent from the most lateral point on the right side to that on the left side.

The volume of selected structures in the forebrain was determined from MRM images from male mice at P4 and P60. Each region of interest was outlined in serial sections through the prosencephalon, in either the coronal (neocortex, hippocampus and striatum) or sagittal (thalamus) plane. Digital segmentation was performed manually, relying on contrast provided by the diffusion weighting in the scans to delineate structure boundaries. The volume in each slice was determined by multiplying the area of the structure by the slice thickness, with the sum of all volumes representing the total volume of the structure.

Neocortical areas were measured in tangentially sectioned hemispheres of P8 SERT-reacted and adult CO-reacted cortices. Only those sections in which the entire posteromedial barrel subfield (PMBSF) was present were analyzed. In addition, the area of the PMBSF and of primary visual cortex (V1) was measured at P8; representative sections with the clearest outline of these cortical areas were chosen for analysis from each hemisphere.

The thickness of the supragranular, infragranular and granular layers, as well as total cortical thickness, were measured in coronal sections at P8 and in the adult. Because the mutant brains were smaller in both the anterior-posterior and medial-lateral planes, the same stereotaxic location (cross-referenced from bregma) does not represent the same cortical area in the two genotypes. Thus, we took advantage of the ability to identify primary sensory areas using SERT immunoreactivity (P8) and CO histochemistry (adult). Measures were then taken along a line perpendicular to the pial surface at the level corresponding to the center of the PMBSF.

Cell Counts

Cells were counted in the adult S1 cortex and the ventrobasal complex of the thalamus from images acquired under brightfield illumination with a Zeiss AxoCam HRc camera in Axiovision 4.1 software. In the barrel fields of parietal cortex, profiles of GAD-67 immunoreactive cells, or cell nuclei in CV-stained sections, were counted at three levels, spaced at 500 μm intervals. At each level, two 440 μm strips of cortex were counted. The first strip extended from the marginal zone/layer II boundary to a line drawn through the center of the barrels (superficial) and the second from that line to the gray/white matter interface (deep). Within the thalamus, the number of cell nuclei in CV-stained sections contained within the anatomical boundaries of the ventrobasal complex was counted at two levels, spaced 500 μm apart. For both regions, only profiles that were completely in focus were counted. In addition, only those nuclei in which a dark nucleolus on a lightly stained nucleus could be visualized were counted. The average number of cells at each level was obtained from bilateral counts in a single section and the estimated number of cells calculated according to Abercrombie's formula (Abercrombie, 1946). To determine the average profile size, the diameter of the immunoreactive soma (GAD-67) or cell nuclei (CV) was measured using Image J. For the cortical counts, the number of cells at each level was summed to give a single value for each animal.

Statistical Analyses

For all analyses, data are provided as means $\pm$ SEM. A two-tailed *t*-test was used to determine significance, with the alpha level set at 0.05. For the analyses of cortical and laminar thickness, and of the dimensions of the cerebral hemispheres, a Bonferroni correction for multiple comparisons was applied. All analyses were implemented in Statview, Version 5.0.1 (SAS Institute, Inc., Cary, NC).

Results

The *Foxg1-cre* line was created by replacing the intron-less *Foxg1* coding region with *cre* (Hebert and McConnell, 2000). This insertion produces *Foxg1* haploinsufficiency, with a $54.0 \pm 6.0\%$ decrease in *Foxg1* expression in the cerebral cortex at P4, as assessed by Northern blot (Figure 1; $n=6$ *Foxg1-cre/+* and $n=4$ wild type mice from 2 litters). This is consistent with a previous report that the level of Foxg1 protein is reduced in heterozygous *Foxg1* mice as early as E12.5 (Hanashima et al., 2002).

The cerebral hemispheres of *Foxg1-cre/+* mice are smaller

Compared to wild type littermates, adult male *Foxg1-cre/+* mice exhibit a significant reduction in the length of the medio-lateral (-19.5% ; Figure 2E, $p = 0.0002$) and midline anterior-posterior (-25.7% ; Figure 2E, $p = 0.0142$) axes of the cerebral hemispheres, thus exposing the superior colliculi when viewed dorsally (Figure 2A, B). However, more laterally, the difference in the anterior-posterior axis between the two genotypes is less than at the midline (-9.2% ; Figure 2E, $p = 0.0090$), creating a distortion in the shape of the cerebral hemisphere in *Foxg1-cre/+* mice. The reduction in the dimensions of the cerebral hemispheres is already apparent at P4 (Figure 2C, D, F). This phenotype also was observed consistently in three other mouse lines: heterozygous mice in the *Foxg1-cre* and *Foxg1-lacZ* lines, maintained on a mixed CBA $\times$ C57BL/6 background (Table 1), and adult Foxg1 haploinsufficient mice of the *Foxg1-tetracycline transactivator* (*Foxg1-tet*) line maintained on a C57BL/6J background (Shen et al., 2006b). Together, these data indicate that, independent of the method of achieving deletion of the *Foxg1* gene, reduced levels of *Foxg1* underlie the reduction in the tangential extent of the cerebral hemispheres (Table 2).

To assess whether the alteration in Foxg1 function disrupts the species-specific patterning of the cortical sheet, we examined SERT- and CO-stained tangential sections through flattened cerebral hemispheres at P8 and in the adult, respectively (Figure 3). As predicted from our previous observations, the area of the cortical sheet is significantly reduced at both P8 (Table 3) and in the adult ($39.74 \pm 0.57 \text{ mm}^2 \text{ wt}$; $29.75 \pm 1.67 \text{ mm}^2 \text{ Foxg1-cre/+}$, $p = 0.0013$). The barrel fields, in particular the PMBSF that represents the large whiskers on the face, can be observed clearly and appear normally organized in both SERT- and CO-labeled *Foxg1-cre/+* mutant cortices (Figure 3), although some barrels, such as those in row A of the PMBSF, are fused. However, it is readily apparent that the size of the barrel field representation, as well as individual barrels, is smaller in mutants than in wild type mice. Although other primary sensory areas, in particular V1, can be observed in CO material, they are more easily identified using SERT immunoreactivity and thus we quantified the area of V1 and PMBSF at P8. The area of both V1 and PMBSF are decreased in the mutant cortex, although not to the same extent (Table 3). Thus, when corrected for the reduced size of the flattened hemisphere, there is a more pronounced effect rostrally in the PMBSF, which occupies a smaller proportion of the mutant cortical sheet, than in V1, which occupies a larger proportion (Table 3).

Other Forebrain Defects

To probe for potentially broader alterations in the prosencephalon of *Foxg1-cre/+* mice, high resolution MRM images were obtained in intact adult male brains of both genotypes (Figure 4A-D) and the volume of select telencephalic structures that express *Foxg1* determined (Table 4). The volume of the *Foxg1-cre/+* prosencephalon is significantly reduced (-28.3% ; Table 4, Figure 4E), reflecting alterations in the volume of several telencephalic structures, including the cerebral cortex (-40.7% ; Table 4, Figure 4F), hippocampus (-18.6% ; Table 4, Figure 4G) and striatum (-29.7% ; Table 4, Figure 4H). Interestingly, each structure within the telencephalon is affected to a different extent, demonstrating that there is not a proportional scaling down of the telencephalon in the *Foxg1-cre/+* mouse.

Foxg1 is not expressed in the thalamus at any stage of development. Nonetheless, it we hypothesized that there may be a thalamic phenotype because 1) cre-mediated recombination has been observed in reporter mouse lines crossed to *Foxg1-cre* mice maintained on a C57BL/6 background (Hebert and McConnell, 2000), and 2) alterations in the cortex could result in adaptive changes in the thalamus because of the important developmental interactions between these structures. We therefore determined the volume of the thalamus in adult male mice from sagittal MRM images (Figure 5A-D). The volume of the *Foxg1-cre/+* thalamus is significantly reduced (-21.6%, Table 4 and Figure 5E). Cell counts in Nissl-stained sections through the ventrobasal complex (VB) of the thalamus revealed a significant reduction in the total number of cells in this structure (Figure 5F; -20.8%, $p = 0.0396$, level 1; -28.3%, $p = 0.0428$, level 2). Consistent with the decreased thalamic volume observed in MRM images, there was a reduction in the area of the VB at both levels (Figure 5G; -19.4%, $p = 0.0136$, level 1; -27.6%, $p = 0.0151$, level 2), such that the density of cells was comparable in wild type and *Foxg1-cre/+* mice (Figure 5H; -3.9%, $p = 0.7271$, level 1; 0.01%, $p = 0.9978$, level 2). Interestingly, in contrast to the adult, the thalamic volume is not significantly different between the two genotypes in P4 male mice (Figures 5I-L; $7.35 \pm 0.13 \text{ mm}^3$ wt; $7.56 \pm 0.16 \text{ mm}^3$ *Foxg1-cre/+*; $p = 0.3335$; Figure 5H). However, even at this early stage, there is already a significant difference in the volume of the neocortex ($26.83 \pm 1.13 \text{ mm}^3$ wt; $20.89 \pm 0.67 \text{ mm}^3$ *Foxg1-cre/+*; $p = 0.004$; Figure 5I).

Laminar defects in *Foxg1-cre/+* cerebral cortices

We noted in our MRM images that there appeared to be a marked diminution in cortical thickness throughout the rostro-caudal axis (Figure 4). However, as noted in the methods, because the mutant brains are smaller in both the anterior-posterior and medial-lateral axes, it can not be assumed that the same stereotaxic location would represent the same cortical area in the two genotypes. Thus, we took advantage of the ability to identify primary sensory areas using CO-stained tissue in the adult to measure cortical thickness in the somatosensory area containing the barrel fields (Figure 6A, B). Total cortical thickness is reduced significantly in the mutant compared to the wild type (-20.1%; Figure 6C, $p = 0.0109$). We next compared separately the supragranular (I-III), granular (IV) and infragranular (V-VI) layers, with intense CO staining defining layer IV. The thickness of the supragranular layers is significantly reduced (-41.4%; Figure 6C, $p = 0.0028$), whereas that of the granular and infragranular layers is not significantly altered, in the mutant compared to wild type (Figure 6C). Adjacent CV-stained sections from adult brains confirmed that the appearance of superficial layers is most altered. In particular, large and medium sized pyramidal cells normally present in layers II and III are reduced (Figure 6D, E). Cell counts confirmed this observation, revealing a significant reduction in the total number of cells (-29.1%; Figure 6E, $p = 0.0114$), accounted for by a significant reduction of those in superficial (-44.4%; Figure 6E, $p = 0.0042$), but not deep (-13.3%; Figure 6E, $p = 0.0856$), layers. This phenotype contrasts with that observed in the *Foxg1-tet* haploinsufficient mouse, also maintained on the C57BL/6 background, in which there is no change in the thickness of the cortex (Shen et al., 2006b). We further examined histological material from these mice, and found that the thickness of superficial layers appears normal (data not shown). This indicates that the expression of *cre* on the congenic C57BL/6 background contributes to the alteration in the radial domain. In addition, the significant reduction of cells in the superficial, but not deep, layers suggests that Cre recombinase has a bigger impact on later generated neurons.

Foxg1, and thus *cre*, is also expressed subcortically within the telencephalon, including the medial and caudal ganglionic eminences that give rise to cortical interneurons. Cortical interneurons are generated in a similar inside-outside pattern as projection neurons, and cells from both populations born on the same day typically are located in the same layer (Anderson et al., 2002, Valcanis and Tan, 2003). To determine if there is a similar differential impact on

later generated interneurons in the *Foxg1-cre/+* line, we counted the number of GAD-67 immunoreactive cells in sections adjacent to the CV stained sections analyzed above. There was a significant reduction in the total number of GAD-67-positive cells in the mutant cortex (-20.8%; Figure 6I, $p = 0.0043$), including a significant reduction in those present in the superficial layers (excluding the marginal zone, -41.4%; Figure 6I, $p = 0.0043$). However, there was no difference in the number of earlier-generated interneurons located in the marginal zone (-4.4%; Figure 6I, $p = 0.2962$) or deep cortical layers (-12.9%; Figure 6I, $p = 0.1576$).

In vivo and *in vitro* studies indicate that the adverse effects of Cre recombinase in the absence of exogenous *loxP* sites occurs predominantly in proliferating, rather than postmitotic, cells (Loonstra et al., 2001, Forni et al., 2006), suggesting that the disruption in the radial domain that we observe in the adult occurred earlier in development. We therefore performed *in situ* hybridization (E16.5) or immunocytochemistry (P4), using markers that identified specific layers in the developing cortex, to examine potential laminar defects of *Foxg1-Cre/+* mice during late corticogenesis and early postnatal development. *Tbr1* is highly expressed in preplate and layer VI neurons soon after they differentiate (Bulfone et al., 1995, Hevner et al., 2001). There is a thickening of a zone of *Tbr1* staining at E16.5 in *Foxg1-Cre/+* mice (Figure 7A, B), suggesting an abnormal representation of subplate and layer VI neurons in the cortex of heterozygous mice at this time. *Brn-2* marks layer II-III neurons (Sugitani et al., 2002). *In situ* hybridization demonstrated that the superficial layer of *Brn-2* staining in wild type mice is considerably narrowed at E16.5 in *Foxg1-Cre/+* mice (Figure 7C, D). In addition, in contrast to the *Tbr1* staining, there is a shortening of the anterior-posterior extent of the *Brn-2* staining at this age, perhaps reflecting a more pronounced effect on later-generated neurons. By P4, *Tbr1* and *FoxP2* immunoreactivity at P4 reveal no significant difference in deep layer neurons (Figure 7E, F, I, J), whereas *Brn1* and *Cux1* immunoreactivity indicate a significant reduction in supragranular neurons (Figure 7G, H, K, L). Consistent with this, measurements of cortical thickness at P8, using SERT-immunoreactivity to identify the barrel fields (Figure 7M, N,O), demonstrate a reduced cortical thickness (-10.5%, $p = 0.0141$) with preferential attenuation of the supragranular layers (-31.6%, $p = 0.0009$). In contrast, there is no alteration in the thickness of the P8 cerebral cortex in *Foxg1* haploinsufficient mice in the *Foxg1-lacZ* ($1082 \pm 26 \mu\text{m}$ wt; $1075 \pm 46 \mu\text{m}$ *Foxg1-cre/+*, $p = 0.8744$) and *Foxg1-cre* ($952 \pm 66 \mu\text{m}$ wt; $939 \pm 14 \mu\text{m}$ *Foxg1-cre/+*, $p = 0.4862$) lines maintained on a CBA $\times$ C57BL/6 mixed background. This suggests that the radial phenotype is only observed in the *Foxg1-cre* line in the context of the congenic C57BL/6 strain, but not when the CBA strain is a component of the background (Table 2).

Supragranular layers of the cortex project primarily to other cortical areas, including the homologous region in the contralateral hemisphere. Thus, a reduction in commissural projections would be expected in the *Foxg1-cre/+* telencephalon. We therefore measured the cross-sectional area of the corpus callosum in midsagittal MRM images from adult male mice (Figure 5 A,C). There is a 44.9% reduction in the area of this structure ($1.07 \pm 0.02 \text{ mm}^2$ wt; $0.59 \pm 0.04 \text{ mm}^2$ *Foxg1-cre/+*, $p < 0.0001$), indicating that interhemispheric connections are disrupted in the *Foxg1-cre/+* mouse. In contrast, there is no significant difference in the cross-sectional area of the anterior commissure in the midsagittal plane ($0.12 \pm 0.01 \text{ mm}^2$ wt; $0.11 \pm 0.01 \text{ mm}^2$ *Foxg1-cre/+*; Figure 5A,C).

Discussion

Over the past two decades, there have been rapid advances in techniques that manipulate the spatial and temporal activation/inactivation of specific genes in the mouse [reviewed in (Mallo, 2006, Miyoshi and Fishell, 2006)]. Each technique, however, introduces one or more variables that are not always considered carefully when analyzing the phenotypic outcomes of such genetic manipulations. The phenotype of the *Foxg1-cre/+* mouse that we describe here

highlights several of these confounds, namely the effects of halopinsufficiency, Cre recombinase activity in the absence of exogenous *loxP* sites and background strain, all of which may influence the interpretation of the specific mechanisms that contribute to the final mature neuroanatomical and functional phenotype.

***Foxg1* haploinsufficiency and cortical development**

Previous studies have reported that cortical development is normal in *Foxg1* heterozygous mice (Xuan et al., 1995, Dou et al., 1999, Hebert and McConnell, 2000, Hanashima et al., 2002, Shen et al., 2006b). Two recent studies, however, have ascribed disruption in telencephalic development to *Foxg1* haploinsufficiency. The first reported telencephalic hypoplasia in hypomorphic and null *fgf8* mice, which likely is due, in part, to alterations in *Foxg1* expression (Storm et al., 2006). In the second, cerebral microcephaly and impaired postnatal hippocampal neurogenesis were demonstrated in adult *Foxg1* haploinsufficient mice (Shen et al., 2006b). The results we describe here are consistent with these latter studies: namely, a reduction in *Foxg1* levels *in vivo* results in a smaller dorsal pallium, which is scaled down in the tangential dimension (Table 2). Our analyses additionally reveal that all primary sensory areas are present, but suggest that the regulation of specific levels of *Foxg1* expression may be a key modulator of the relative size of functional areas in the cerebral cortex in the tangential domain. A similar dependence has been reported for *Emx2*, where the size of primary sensory and motor areas was influenced by altered levels of the transcription factor (Hamasaki et al., 2004). The suggestion that *Foxg1* may reduce *Emx2* expression levels (Muzio and Mallamaci, 2005) is consistent with our finding in the *Foxg1* haploinsufficient mouse of a disproportionate increase in V1 and decrease in PMSBF, similar to the phenotype observed when *Emx2* is overexpressed in cortical progenitors. However, in contrast to *Emx2*-overexpressing mice, the cortical sheet is smaller in mice with reduced levels of *Foxg1*. Thus, the dysmorphologies we observed may be due to regional differences in the effects of *Foxg1* on early proliferative behavior and hence expansion rates of the number of radial units that comprise the cortex in the tangential domain. Regardless of the specific mechanism underlying the effect of reduced levels of *Foxg1* on cortical development, these observations, together with those from the *Foxg1-lacZ* and *Foxg1-tet* lines (Shen et al., 2006a), demonstrate that heterozygosity at the *Foxg1* locus contributes to the tangential phenotype (Table 2).

Effects of Cre recombinase

The Cre/*loxP* system has been used *in vivo* and *in vitro* to activate and inactivate genes in mammalian cells. The efficiency of recombination at the *loxP* sites in the targeted cells is a key consideration when determining the utility of this system. The strategies used to enhance efficiency have included increasing the copy number of *cre*, for example, using transgenic mice, in which multiple copies of the *cre* transgene were inserted into the genome, or including a nuclear localization signal (NLS) to promote the accumulation Cre recombinase in the nucleus. However, as originally described in bacteria and yeast (Sauer, 1992, Sauer, 1996), there are pseudo *loxP* sites present in the mammalian genome that are able to serve as substrates for Cre recombinase (Thyagarajan et al., 2000), resulting in unwanted chromosome rearrangements and occasionally genomic instability, particularly when high levels of the recombinase are present.

The undesired effects of Cre recombinase in mammalian cells were first noted *in vitro*. In primary cultures of mouse embryonic fibroblasts, in the absence of exogenous *loxP* sites, the introduction of the *cre* gene via a viral vector resulted in recombination events, growth arrest and, often, increased cell death (Loonstra et al., 2001). Similar effects have been noted in a variety of mammalian cell lines (Pfeifer et al., 2001, Silver and Livingston, 2001, Baba et al., 2005). More detailed analysis indicated that proliferating, rather than postmitotic, cells were vulnerable to the effects of Cre recombinase, as cryptic recombination events were only

observed in mitotically-active cells (Loonstra et al., 2001). There have been two reports of overt pathology in *cre*-expressing transgenic mice, where high levels of Cre resulted in male infertility when expressed in postmeiotic spermatids (Schmidt et al., 2000) and hydrocephaly when expressed in neuronal progenitors (Forni et al., 2006). In the latter study, the genotoxic effects required the levels of Cre recombinase to reach a critical, high threshold in the cell nucleus, as had been reported previously *in vitro* (Baba et al., 2005). Thus, hydrocephalus was observed in homozygous, but not hemizygous, mice in a transgenic line, in which multiple copies of the *cre* transgene had been inserted into unknown locations in the genome (Forni et al., 2006). In contrast, the present study utilized a *Foxg1-cre* line that has only a single copy of the *cre* gene and effects of Cre recombinase at low doses *in vivo* have not been reported previously. This may reflect the importance of genetic modifiers in the expression of a Cre recombinase phenotype (see below) or the expression of subtle phenotypes that may have gone unnoticed in other *cre* lines.

It should be noted here that the *Emx1-cre* line, which also has a single copy of the *cre* gene, exhibits no overt cerebral cortical phenotype [(Gorski et al., 2002) and Judson, Eagleson and Levitt, unpublished observations], even though it is maintained on the identical C57BL/6 background as the one used here (Table 2). An important difference between the two lines is the presence of a nuclear localization signal in the *Foxg1-cre* line (Hebert and McConnell, 2000), which is absent in the *Emx1-cre* line (Gorski et al., 2002), likely resulting in higher levels of Cre recombinase in the nucleus. As noted above, there is evidence *in vivo* and *in vitro* that the accumulation of Cre recombinase specifically in the nucleus, rather than the absolute levels in the cell, underlies the DNA damage and growth inhibition observed in the absence of exogenous *loxP* sites (Baba et al., 2005, Forni et al., 2006). Thus, because of the time required to achieve threshold levels of nuclear Cre recombinase, overt abnormalities may not be apparent until several days after *cre* is first expressed. This may underlie the more potent effect, in the *Foxg1-cre/+* mouse, of *cre* expression on progenitor cells later in development when supragranular neurons are produced. The results of the present study highlight that, in those cases where *cre*-expressing mice with no exogenous *loxP* sites have a phenotype different from that of wild type, any additional phenotype that results from the Cre-mediated mutation is a product of an interaction between two distinct mutations.

The influence of genetic background on the expression of phenotypes

Our observation that the radial phenotype was expressed on the congenic C57BL/6J, but not the mixed CBA $\times$ C57BL/6J, background (Table 2), suggests that genetic modifiers may modulate the extent of phenotypic disruption due to Cre recombinase in the absence of exogenous *loxP* sites. This possibility was proposed by Hebert and McConnell (2000) to explain the differences in the pattern of recombination noted on different background strains when *Foxg1-cre* mice were crossed with reporter lines. They suggested that modifier loci could influence the expression of the *Foxg1-cre* allele or modulate the activity of the Cre recombinase itself (Hebert and McConnell, 2000). Genetic background has long been known to influence the behavioral phenotype of mutant mice (Shanks and Anisman, 1988, Gerlai, 1996, Logue et al., 1997, Bouwknecht and Paylor, 2002, Schauwecker, 2002, McKhann et al., 2003, Sik et al., 2003, Waddell et al., 2004), but fewer studies have focused on the brain-based mechanisms for such strain dependence. Brain (Bilovocky et al., 2003) and limb (Murcia et al., 2004) phenotypes caused by mutation of the *Engrailed-1* (*en-1*) gene are highly dependent upon genetic background, as are the neural phenotypes produced by mutations of *p75NGF* (Greferath et al., 2000), *Unc5c* (Burgess et al., 2006), *fmr-1* (Paradee et al., 1999, Dobkin et al., 2000, Ivanko and Greenough, 2002, Mineur et al., 2002, Errijgers and Kooy, 2004) and *Pax2* (Schwarz et al., 1997). Herrup and colleagues have identified one significant modifier and several other possible loci that may be responsible for trait differences in limb and cerebellum specification in the *en-1* knockout (Murcia et al., 2004, Murcia et al., 2006), and a recent report

identified a major modifier locus that influences phrenic motor axon guidance in the absence of *Unc5c* (Burgess et al., 2006). Similar modifier mechanisms for other genes remain to be determined. It is important to note the data presented here show the *Foxg1-cre* mouse has a robust altered phenotype in the radial dimension of the cerebral cortex on the congenic C57BL/6J background, but no differences are observed when the CBA background is a component of the mouse strain (Table 2). In contrast, the reduction in the tangential domain, which is not modulated by the presence of Cre recombinase, is observed independent of whether the CBA background is present (Table 2).

Alterations in structures that do not express *Foxg1*

A particularly interesting observation in the present study was the reduction in thalamic volume in the adult, although *Foxg1* is not expressed in this structure. This may be due to the expression of Cre recombinase in the thalamus of *Foxg1-cre* mice maintained on the C57BL/6 background (Hebert and McConnell, 2000), or to secondary interactions that may regulate adaptive responses of the thalamus and cortex when one structure is perturbed developmentally. We favor the second possibility as, in contrast to the cortex, the thalamic phenotype is not apparent during the early postnatal period. *In vivo* and *in vitro* studies indicate that, in rodents, embryonic dorsal thalamic neurons do not require trophic support from the cortex (Lotto et al., 2001). In contrast, based on lesion studies, early postnatal neurons exhibit an exquisite dependence on their cortical target (Haun and Cunningham, 1987; Eagleson et al., 1990). Moreover, in *Pax6* null mice, in which thalamocortical axons fail to reach the cortex, there is an increase in cell death in the dorsal thalamus starting at birth (Lotto et al., 2001). Thus, although their axons reach the cortex during embryonic development, it appears that dorsal thalamic neurons become dependent on target-derived factors postnatally. Given the early development of normal patterns of primary sensory areas, demarked by CO and SERT staining, it appears that in the *Foxg1-cre/+* mouse, an initially normal-sized thalamus projects to the correct cortical fields. This occurs, however, even though the target field is smaller at the earliest time we measured (P4). The subsequent reduction in thalamic volume, due at least in part to an increase in cell death of thalamic neurons, may reflect an important mechanism for size matching of these forebrain structures that initially develop independently (Miyashita-Lin et al., 1999; Nakagawa et al., 1999).

The present study reveals the complexity of the mechanisms that underlie the expression of the adult forebrain phenotype of the *Foxg1-cre/+* mouse maintained on the C57BL/6J background. Cre recombinase, *Foxg1* haploinsufficiency, and background strain contribute to the final phenotype, with Cre recombinase and background strain modulating the radial phenotype, and *Foxg1* haploinsufficiency influencing the tangential phenotype (Table 2). These data clearly indicate the need for very careful evaluation of any new or modified conditional genetic system for perturbing gene expression.

Acknowledgements

We are grateful to Dr David Price and Martine Manuel for supplying fixed brains from the *Foxg1-lacZ* and *Foxg1-cre* lines on the mixed background, and Drs Stewart Anderson and Lijian Shen for supplying Nissl-stained material from the *Foxg1-tet* line. We thank Dr Ken Catania for advice and assistance with the flattened cortical preparation and analysis, and Paula Woods, Donte Smith and Frank Liu for assistance in maintaining the mouse colony and genotyping. We also thank Drs Elizabeth Hammock, Philip Ebert and Gregg Stanwood for their helpful comments on the manuscript. This study was supported by NIMH grant 67842 (PL), NICHD P30 grant 15052 and NCRR 1S10 RR17799. One of us (ETA) acknowledges support from NIH grants R01-EB4155, P41-EB001977, and P50-ES012359.

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Abbreviations

CO	cytochrome oxidase
CV	cresyl violet
E	embryonic day
GAD-67	glutamic acid decarboxylase-67
MRM	magnetic resonance microscopy
P	postnatal day
PMBSF	posteromedial barrel subfield
SERT	serotonin transporter
tet	tetracycline transactivator
V1	primary visual cortex

Figure 1. *Foxg1* expression is reduced in the *Foxg1-cre/+* cortex. Northern blot analysis of *Foxg1* mRNA expression in P4 cerebral cortex of wild type and *Foxg1-cre/+* mice. Cyclophilin probe was used as a control for mRNA integrity and loading.

Figure 2.

The cerebral hemispheres are reduced in the *Foxg1-cre/+* mouse. Photomicrographs of the dorsal view of wild type and mutant brains at P60 (A,B) and P4 (C,D). The *Foxg1-cre/+* cerebral hemispheres are visibly reduced in size compared with wild type littermates at both ages while the cerebellum and brainstem appear unaffected. (E, F) Quantitative analysis reveals that both the anterior-posterior (A-P) and medial-lateral (M-L) dimensions of the cerebral hemispheres are affected. Black histograms: wild type, grey histograms: *Foxg1-cre/+*. CH: cerebral hemispheres, IC: inferior colliculus, SC: superior colliculus, lat: lateral, med: medial. Asterisks – significantly different from wild type, $p < 0.05$. Scale bar in D = 5 mm for all micrographs.

Figure 3.

A-D: Low power photomicrographs of tangential sections through cortical layer IV stained at P8 for serotonin transporter (SERT; A, B) or in the adult for cytochrome oxidase (CO; C, D). (E-H) Higher magnification photomicrographs of S1, including the postero-medial barrel subfield (PMBSF). Note that the cortical sheet is smaller in *Foxg1-cre/+* (B, D) compared to wild type (A, C) mice at both ages. SERT- and CO-immunoreactivity show that primary sensory cortices (V1, A1 and S1, including the PMBSF), although smaller, are located in their normal position in the cortical sheet. Scale bar, 1.6mm (A-D) and 500µm (E-H).

Figure 4.

Upper panels: representative coronal slices from MRM data sets at two rostral-caudal levels in wild type (A,C) and *Foxg1-cre/+* (B,D) P60 male mice. The boundaries of the structures analyzed are outlined: neocortex (green), striatum (blue) and hippocampus (red). Scale bar = 2 mm. Lower panels: distribution plots of prosencephalon (E), neocortex (F), hippocampus (G) and striatum (H) volumes across the rostral-caudal axis in wild type (solid line) and *Foxg1-cre/+* (dashed line) P60 male mice. Zero represents the first, most rostral, coronal slice in which the structure can be visualized. Note that the total prosencephalon volume, as well as the volume of each individual structure, is smaller in the heterozygous mouse.

Figure 5.

Upper panels: sagittal slices from MRM data sets at two medial-lateral levels in wild type (A,B) and *Foxg1-cre/+* (C,D) P60 male mice. The boundaries of the structures analyzed are outlined: thalamus (red), corpus callosum (green) and anterior commissure (blue). Scale bar = 2.5 mm. (E) Distribution plot of thalamic volume across the medial-lateral axis in wild type (solid line) and *Foxg1-cre/+* (dashed line) P60 male mice. Zero represents the midsagittal section. Middle panels: Quantification of (F) total number of cells in the ventrobasal complex (VB) of the thalamus at two rostro-caudal levels spaced 500 μm apart, (G) the area of the VB at the same two levels and (H) the density of cells in the VB in wild type (black histograms) and *Foxg1-cre/+* (grey histograms) P60 male mice. Asterisks – significantly different from wild type, $p < 0.05$. Note that there is a reduction in the number of cells and the area of VB, but the density of cells is the same, in the mutant mouse. Lower panels: sagittal slices from MRM data sets in wild type (I) and *Foxg1-cre/+* (J) P4 male mice. The boundary of the thalamus (red) is outlined. Scale bar = 2 mm. Distribution plot of thalamic volume (K) across the medial-lateral axis and

neocortical volume (L) across the rostral-caudal axis in wild type (solid line) and *Foxg1-cre/+* (dashed line) P4 male mice. Zero represents the midsagittal (K) or most rostral (L) section. Note that the thalamus is smaller in the adult heterozygous mouse, but is the same as wild type at P4, even though the neocortex is already reduced in volume at the earlier age.

Figure 6.

The superficial layers of cortex are preferentially affected in the adult *Foxg1-cre/+* mouse. Left panels: photomicrographs of coronal sections through the barrel fields of somatosensory of adult male wild type (A, D, G) and *Foxg1-cre/+* (B, E, H) mice, illustrating CO (A, B), Nissl (D, E) and GAD-67 (G, H) staining. Scale bar = 200 μ m. Right panels: Quantification of (C) total cortical thickness, as well as that of the supragranular (SG), granular (G) and infragranular (IG) layers, (F) total number of cells in superficial (super) and deep layers, and (I) number of GABAergic interneurons in the marginal zone (MZ), superficial and deep layers, in somatosensory cortex in wild type (black histograms) and *Foxg1-cre/+* (grey histograms) mice. Asterisks – significantly different from wild type, $p < 0.05$. Note that the total cortical thickness

is reduced and can be attributed to a significant attenuation of the supragranular layers. Similarly, the number of total cells and of GAD-67 positive cells is preferentially loss in the superficial layers.

Figure 7.

The attenuation of the superficial layers of cortex is observed throughout development in the *Foxg1-cre/+* mouse. Photomicrographs illustrating sagittal (A-D) and coronal (E-N) sections through wild type (A,C,E,G,I,K,M) and *Foxg1-cre/+* (B,D,F,H,J,L,N) brains at three stages of development. At E16.5 (A-D), *in situ* hybridization reveals an increase in *Tbr1* (deep layer marker) and a reduction in *Brn2* (upper layer marker) in the mutant cortex during late corticogenesis. This results in fewer *Brn1*- (G,H) and *Cux1*- (K,L) immunoreactive neurons in the superficial layers, but comparable numbers of *Tbr1*- (E,F) and *FoxP2* (I,J) neurons in deep layers, of the *Foxg1-cre/+* cortex at P4. At P8, SERT immunoreactivity (M,N) permits the ready identification of the thalamic recipient granular (G) layer. (O) Quantification of

cortical thickness confirms the attenuation in the thickness of supragranular layers (SG), but relatively normal infragranular layers (IG), in somatosensory cortex in wild type (black histograms) and *Foxg1-cre/+* (grey histograms) mice. Asterisks – significantly different from wild type, $p < 0.05$.

Dimensions of the cerebral hemispheres (mm) in P8 *Foxg1* heterozygous mice maintained on a mixed CBA $\times$ C57BL/6 background

Table 1					
Line	Dimension	Wild type Mean $\pm$ SEM	<i>Foxg1</i> ^{+/+} Mean $\pm$ SEM	Difference (%)	<i>p</i>
Foxg1-lacZ	Midline anterior-posterior	5.88 $\pm$ 0.08	4.55 $\pm$ 0.10	-22.7	<0.0001
	Lateral anterior-posterior	8.76 $\pm$ 0.07	7.60 $\pm$ 0.12	-13.3	0.0002
	Medial-lateral	11.05 $\pm$ 0.02	10.28 $\pm$ 0.09	-6.9	0.0013
Foxg1-cre	Midline anterior-posterior	6.06 $\pm$ 0.08	4.31 $\pm$ 0.14	-28.9	<0.0001
	Lateral anterior-posterior	8.25 $\pm$ 0.13	7.46 $\pm$ 0.04	-9.3	0.0006
	Medial-lateral	11.01 $\pm$ 0.15	10.15 $\pm$ 0.15	-7.8	0.0032

Table 2
Forebrain phenotypes of Foxg1 heterozygous and Emx1-cre mouse lines

Line	Background	Tangential	Radial	Other
Foxg1-cre	C57Bl6	Reduced with anterior regions more affected ^a	Reduced, upper layers preferentially affected ^a	Thalamic volume reduced in adult with decreased neuron number ^a
Foxg1-cre	CBA/C57Bl6	Reduced ^a	Normal ^a	Postnatal neurogenesis in hippocampus impaired ^b
Foxg1-tet	C57Bl6	Reduced ^b	Normal ^b	
Foxg1-lacZ	CBA/C57Bl6	Reduced ^a	Normal ^a	
Emx1-cre	C57Bl6	Normal ^c	Normal ^c	

^a Current paper

^b (Shen et al., 2006b)

^c (Gorski et al., 2002)

Table 3
Cortical area (mm²) and ratio comparisons between P8 male wild type and *Foxg1-cre*+/+ mice

	Region	Wild type Mean ± SEM	<i>Foxg1</i> +/+ Mean ± SEM	Difference (%)	<i>p</i>
Area	Total cortex	28.78 ± 0.39	20.96 ± 0.47	-27.2	<0.0001
	PMBSF	1.36 ± 0.01	0.81 ± 0.01	-40.4	<0.0001
	V1	2.31 ± 0.05	1.82 ± 0.01	-21.2	0.0006
Ratio (×100)	PMBSF/total	4.71 ± 0.09	3.90 ± 0.10	-17.1	0.0016
	V1/total	8.04 ± 0.16	8.67 ± 0.16	+7.8	0.0423

Table 4
Volume (mm³) comparisons between P60 male wild type and *Foxg1-cre*+/+ mice

Structure	Wild type Mean $\pm$ SEM	<i>Foxg1</i> +/+ Mean $\pm$ SEM	<i>p</i>
Prosencephalon	313.25 $\pm$ 9.15	224.46 $\pm$ 3.01	0.0008
Neocortex	86.19 $\pm$ 1.41	51.13 $\pm$ 2.69	0.0003
Hippocampus	18.37 $\pm$ 0.17	15.09 $\pm$ 0.23	0.0003
Striatum	18.53 $\pm$ 0.02	13.03 $\pm$ 0.27	<0.0001
Thalamus	15.78 $\pm$ 0.07	12.37 $\pm$ 0.13	<0.0001