Development/Plasticity/Repair

Molecular Mechanisms Controlling the Migration of Striatal Interneurons

Verona Villar-Cerviño,[1] Caroline Kappeler,[1] Sandrina Nóbrega-Pereira,[1] Mark Henkemeyer,[2] Luciano Rago,[1] M. Angela Nieto,[1] and Oscar Marín[1,3]

[1]Instituto de Neurociencias, Consejo Superior de Investigaciones Científicas & Universidad Miguel Hernández, 03550 Sant Joan d'Alacant, Spain, [2]Department of Developmental Biology and Kent Waldrep Foundation Center for Basic Research on Nerve Growth and Regeneration, University of Texas Southwestern Medical Center, Dallas, Texas 75390-9133, and [3]MRC Centre for Developmental Neurobiology, King's College London, Guy's Campus, London SE1 1UL, United Kingdom

In the developing telencephalon, the medial ganglionic eminence (MGE) generates many cortical and virtually all striatal interneurons. While the molecular mechanisms controlling the migration of interneurons to the cortex have been extensively studied, very little is known about the nature of the signals that guide interneurons to the striatum. Here we report that the allocation of MGE-derived interneurons in the developing striatum of the mouse relies on a combination of chemoattractive and chemorepulsive activities. Specifically, interneurons migrate toward the striatum in response to Nrg1/ErbB4 chemoattraction, and avoid migrating into the adjacent cortical territories by a repulsive activity mediated by EphB/ephrinB signaling. Our results also suggest that the responsiveness of MGE-derived striatal interneurons to these cues is at least in part controlled by the postmitotic activity of the transcription factor Nkx2-1. This study therefore reveals parallel mechanisms for the migration of MGE-derived interneurons to the striatum and the cerebral cortex.

Key words: **Eph; ErbB4; GABAergic; interneuron; migration; striatum**

Introduction

During the development of the telencephalon, the medial ganglionic eminence (MGE) gives rise to several populations of GABAergic neurons, including cortical and striatal interneurons (Lavdas et al., 1999; Sussel et al., 1999; Marin et al., 2000; Wichterle et al., 2001; Xu et al., 2008). Cortical interneurons exist in a large variety of classes, some of which are reminiscent to those found in the striatum (DeFelipe et al., 2013). For example, parvalbumin-expressing fast-spiking interneurons are present in both the cortex and the striatum (Kawaguchi, 1993, 1995). Since interneuron specialization might directly depend on developmental programs that are specific to target territories (Kepecs and Fishell, 2014), understanding the mechanisms sorting GABAergic interneurons to the striatum and cortex may shed light on this process.

The migration of cortical interneurons has been extensively studied over the years (for review, see Marín, 2013). Multiple lines of evidence support the notion that the guidance of cortical interneurons involves the simultaneous activity of chemoattractive factors released by intermediate targets or by the cortex, and chemorepulsive molecules produced by territories that cortical interneurons must avoid, such as the striatum and the preoptic area (Marín et al., 2003; Wichterle et al., 2003). Moreover, many of the attractive and repulsive molecules involved in the guidance of cortical interneurons have been identified (Marín et al., 2001; Powell et al., 2001; Flames et al., 2004; Pozas and Ibañez, 2005; Zimmer et al., 2008; Hernández-Miranda et al., 2011).

In contrast to our extensive knowledge of the mechanisms underlying the guidance of cortical interneurons, our understanding of the events controlling the migration of striatal interneurons is very limited. We previously showed that Nkx2-1, a transcription factor required for the specification of MGE progenitor cells (Sussel et al., 1999; Butt et al., 2008), is also involved in the migration of striatal interneurons. Virtually all MGE progenitor cells express Nkx2-1, but its expression is differentially regulated in striatal and cortical interneurons. While most MGE-derived striatal interneurons maintain Nkx2-1 postmitotically, expression of this gene is downregulated in cortical interneurons (Marín et al., 2000). Forced expression of Nkx2-1 in the MGE enhances interneuron migration to the striatum and blocks interneuron migration to the cortex (Nóbrega-Pereira et al., 2008). Nkx2-1 mediates this process by repressing the expression of class III Semaphorin receptors, which during normal development prevent cortical interneurons from invading this territory (Marín et al., 2001). However, the observation that Nkx2-1-expressing interneurons accumulate in the striatum rather than simply passing through this structure on their way to the cortex suggests that

Received Oct. 18, 2014; revised March 30, 2015; accepted April 10, 2015.

Author contributions: V.V.-C., C.K., S.N.-P., L.R., M.A.N., and O.M. designed research; V.V.-C., C.K., S.N.-P., M.H., and L.R. performed research; M.H., M.A.N., and O.M. contributed unpublished reagents/analytic tools; V.V.-C., C.K., and S.N.-P. analyzed data; V.V.-C. and O.M. wrote the paper.

This work was supported by grants from the National Institutes of Health (5R01 MH066332) to M.H., and from the Spanish Ministry of Economy (SAF2011-28845) and CONSOLIDER (CSD2007-00023) to O.M. We thank A. Casillas and T. Gil for excellent technical assistance, and members of the Marín and Rico labs for stimulating discussions.

The authors declare no competing financial interests.

Correspondence should be addressed to Oscar Marín, MRC Centre for Developmental Neurobiology, King's College London, Guy's Campus, London SE1 1UL, United Kingdom. E-mail: oscar.marin@kcl.ac.uk.

S. Nóbrega-Pereira's present address: Instituto de Medicina Molecular, Universidade de Lisboa, Portugal.

DOI:10.1523/JNEUROSCI.4317-14.2015

 0270-6474/15/358718-12$15.00/0

other mechanisms control the confinement of interneurons into the striatum.

In this study, we explored additional mechanisms controlling the migration of striatal interneurons. We found that striatal interneurons express ErbB4 and rely on this signaling system to migrate to the developing striatum. In addition, striatal interneurons are actively repelled by the cerebral cortex, a process that is mediated by Eph/ephrin signaling. These results reveal that, similar to cortical interneurons, MGE-derived striatal interneurons depend on both target chemoattraction and off-target chemorepulsion to reach their final destination.

Materials and Methods

Mouse lines. Wild-type mice and ubiquitously expressing GFP transgenic mice (Hadjantonakis et al., 2002) maintained in a CD1 background were used for expression analysis and tissue culture experiments. *HER4^{heart}* transgenic mice (Tidcombe et al., 2003), which express a human *ErbB4* (*HER4*) cDNA under the control of the cardiac-specific α-HMC (myosin heavy chain) promoter, were mated to *ErbB4* heterozygous mice (Gassmann et al., 1995) to generate *ErbB4$^{+/+}$;HER4^{heart}* and *ErbB4$^{-/-}$;HER4^{heart}* littermate mice, maintained in a mixed C57BL/6–129/SvJ-CBA background. *EphB1* and *EphB3* mutant mice were maintained in a mixed 129-CD1 background, *TRE-Bi-SG-T* mice in a mixed CD1-129-C57BL/6 background, and *EphB3-rtTA* in a CD1 background. For the expression of tdTomato in *TRE-Bi-SG-T;EphB3-rtTA* mice, pregnant females were treated with 3 mg/ml doxycycline in 5% sucrose water and food (Harlan Laboratories Teklad Diets) for 4 consecutive days before being killed. All control animals used in our experiments were obtained from the same familial genetic background as the corresponding mutant mice. The day of vaginal plug was considered to be E0.5. Mice were kept at Instituto de Neurociencias in accordance with Spanish and European Union regulations.

In vitro *focal electroporation.* A cDNA encoding *Nkx2-1* (accession number NM_009385) was used. All constructs (*Gfp*, *Nkx2-1*, and *Nkx2-1-IRES-Gfp*) were cloned in a *pCAGGS* chicken β-actin promoter expression vector. Expression vectors were electroporated at a concentration of 1 μg/μl and mixed in a 0.9/1.5 ratio when *Nkx2-1* and *Gfp* were coelectroporated. Expression vectors were pressure injected focally into the MGE and embryonic slice cultures were electroporated as previously described (Flames et al., 2004).

Slice and explants culture. E13.5 organotypic coronal slice cultures from wild-type or GFP-expressing transgenic embryos were obtained as described previously (Anderson et al., 1997). Slice transplantation was performed immediately after the preparation of the organotypic slices, and the slices returned to the incubator for the appropriate time. MGE explants were dissected from organotypic slices after electroporation and placed in the cortex of host slices in transplantation experiments. For gene-expression analyses, MGE explants were dissected 36 h after electroporation. For immunofluorescence and migratory performance, MGE explants were cultured on 3D Matrigel matrices.

In situ *hybridization and immunohistochemistry.* For *in situ* hybridization, brains were fixed overnight in 4% PFA in PBS. Twenty micrometer frozen sections and 250 μm dehydrated organotypic slices were hybridized with digoxigenin-labeled probes as described previously (Flames et al., 2007). Immunohistochemistry was performed on the following: 20 μm cryostat sections, 60 μm vibratome resectioned organotypic slices, and MGE explants in Matrigel pads. Slices, explants, and embryos were fixed in 4% PFA at 4°C from 2 to 6 h. The following primary antibodies were used: chicken anti-GFP (1/1000; Aves Labs), rabbit anti-Nkx2-1 (1/2000; BioPAT), rabbit anti-PH3 (1/200; Millipore), and rabbit anti-ErbB4 (1/300; a gift from Dr. Cary Lai). The following secondary antibodies were used: goat anti-chicken 488, donkey anti-rabbit 555, donkey anti-rabbit 488 (Invitrogen), and donkey anti-rabbit Cy3-conjugated Fab fragment (The Jackson Laboratory). For ErbB4/Nkx2-1 double stainings, sections were first processed for the rabbit anti-ErbB4 immunofluorescence using anti-rabbit Cy3-conjugated Fab fragment as a secondary antibody, fixed in 4% PFA-4% sucrose for 10 min, and then processed for rabbit anti-Nkx2-1 immunohistochemistry. DAPI (Sigma) was used for fluorescent nuclear counterstaining.

Quantification. For the quantification of interneurons in E15.5 control and *ErbB4$^{-/-}$;HER4^{heart}* mutant brains, the outline of the striatum at rostral and caudal levels was delineated in 20-μm-thick sections, Nkx2-1-expressing cells were counted for three different brains from each genotype, and cell densities (number of cells/mm^2) were calculated. Proliferative cells were quantified in the MGE of four different control and *ErbB4$^{-/-}$;HER4^{heart}* mutant embryos at E13.5 on 20-μm-thick sections stained with antibodies against PH3. In the case of control and *EphB* mutants, cell density was calculated in a fixed volume in 60-μm-thick vibratome sections of six different brains per genotype. Three different striatal levels (at the anterior commissure and 120 μm rostral and caudal to this region) were analyzed. In slice overexpression experiments, 60 μm vibratome resectioned organotypic slices were used to count the number of GFP-expressing cells in a fixed volume of the striatum for control and *ErbB4$^{-/-}$;HER4^{heart}* mutant slices. For the quantification of MGE^{GFP} transplants into the cortex and cortex/striatum, the percentage of Nkx2-1/GFP-expressing cells was counted for a fixed volume of tissue adjacent to the ectopic MGE explant. The number of GFP-expressing cells was counted in the volume of cortical tissue immediately adjacent to the explant.

Phylogenetic footprinting analysis. In silico analysis of the 5′ flanking region of the *Mus musculus EphB1* and *EphB3* locus (*EphB1* accession number NM_173447 and *EphB3* accession number NM_010143) was performed using ECR browser (Ovcharenko et al., 2004). Five different putative *EphB1* (regions 1–5) and two putative *EphB3* (regions 1 and 2) regulatory regions containing Nkx2-1 binding sequences were found upstream of the transcription initiation sites.

Chromatin Immunoprecipitation assay. Chromatin immunoprecipitation (ChIP) assay was performed using Magna ChIP G Tissue Kit (Millipore), according to manufacturer's instructions, except for the tissue lysis step. Briefly, we dissected the subpallium of E13.5–E14.5 mouse embryos and cross-linked with 1% PFA 15 min at room temperature. The tissue was then lysed 40 min in SDS lysis buffer (1% SDS, 10 mM EDTA, and 50 mM Tris, pH 8.0) and sonicated in dilution buffer (0.01% SDS; 1.2 mM EDTA; 16.7 mM Tris, pH 8.0; 1.1% Triton X-100; and 167 mM NaCl) on ice (Bioruptor; Diagenode; 200 W potency, 40/20 s on/off cycle for 5 min). After sonication, we obtained chromatin fragments between 100 and 400 bp lengths. Chromatin was immunoprecipitated with 5 μg of rabbit anti-Nkx2-1 (BioPAT) and rabbit anti-IgG antibodies. Immunoprecipitated DNA sequences were analyzed by PCR using the following primer pairs: EphB1_region1 forward: GTGTTCGCTGAAAAAGCTGC, EphB1_region1 reverse: TCTTGTGAGCTTTCCCTGTCCAG; EphB1_region2 forward: TGGTCCTCCAGACAGGAGA, EphB1_region2 reverse: GCCGTCTGCTTTTTGAACACT; EphB1_region3 forward: CTGGGCAATGCAATACCAAAT, EphB1_region3 reverse: TTCAGTTACCCACCCCAGGA; EphB1_region4 forward: GAGGAGGCCTGTGAGTTCAA, EphB1_region4 reverse: AGTTCTGCTAGGAACGCGTG; EphB1_region5 forward: TGGTTTCCCGTGGCATCTAC, EphB1_region5 reverse: CTGATGGCCTGCCAA; EphB3_region1 forward: CTAGGCCCTGGCTCTCCCTA, EphB3_region1 reverse: CAGGCAGACTCAAAGGTCCC; EphB3_region2 forward: AGGGGAGAAAGGCAAGAAGC, EphB3_region2 reverse: AATAGCCACGGCCAGCTG. For ChIP primer design, potential Nkx2–1-binding sites were identified within evolutionary conserved regions of *EphB1* and *EphB3* promoters. Relative intensities of PCR products were analyzed with an ImageJ gel plugin and normalized against the input band.

Luciferase assay. HEK293 cells were cultured in DMEM supplemented with 10% FCS, 10 U/ml penicillin, and 10 μg/ml streptomycin. For luciferase assays, 1 × 105 cells/well were seeded and transfected with 100 ng of the promoter fragments cloned in pGL3P vector, 50 ng of the pRL-Tk control vector, and 200 ng of the pCAG-*Nkx2-1-IRES-Gfp* plasmid using Lipofectamine 2000, according to the manufacturer's protocol. After 48 h, the cells were washed once in PBS and lysed using the Passive Lysis Buffer (Promega). Mutant forms were generated by deleting the consensus CTCTCAAG site in the *EphB1_reg3*, the consensus CACAGGA site in the *EphB1_reg4*, and the consensus AGCCCAAGA (del1) and CTGCCAAG (del2) sites in the *EphB3_reg1–2*. Luciferase enzymatic activity was analyzed using a Sirius Luminometer (Berthold).

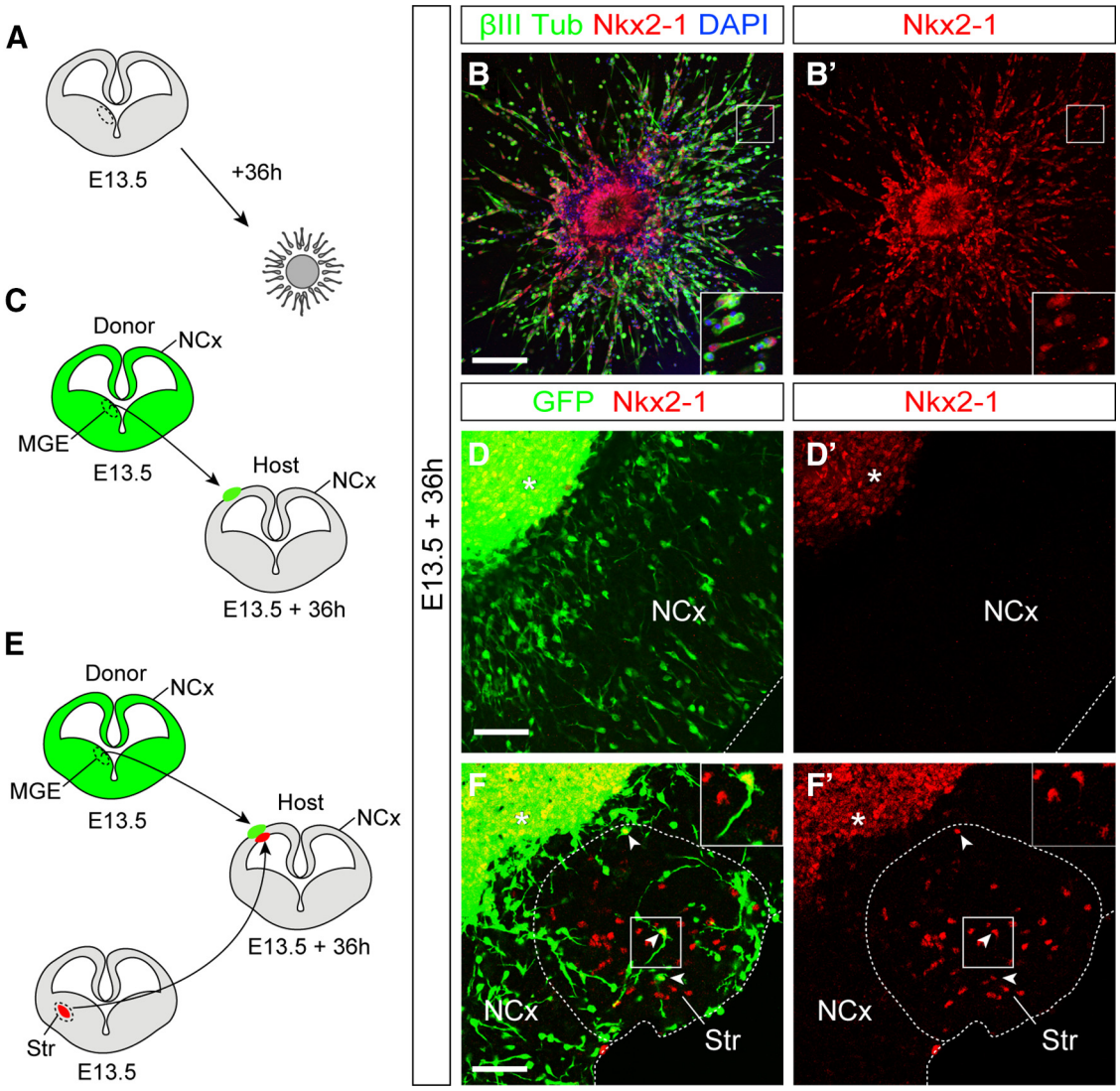

Figure 1. Striatal interneurons are not able to migrate into the cortex. ***A***, ***C***, ***E***, Schematic of slice transplantation paradigms, in which a piece of the MGE (ectopic MGE or MGE ^{ect}) was placed in Matrigel (***A***) or in the developing neocortex (NCx) with or without an additional piece of striatum (Str; ***C***, ***E***, respectively). ***B***, ***B′***, Neurons derived from E13.5 MGE explants cultured in Matrigel. ***D***, ***D′***, MGE ^{ect}-derived GFP migrating cells in the cortex of E13.5 wild-type host slices are Nkx2-1 negative. ***F***, ***F′***, MGE ^{ect}-derived GFP migrating cells only colabel for Nkx2-1 (arrowheads) in the striatal explants and not in the cortex of E13.5 wild-type host slices. Dotted lines indicate the limits of the grafted striatal explant. Asterisks indicate the MGE ^{ect}. Scale bars: 100 μm.

Quantitative PCR. Total RNA from MGE cells electroporated either with *pCAGIG* or *pCAGIG* + *Nkx2-1* plasmids from three different experiments was extracted with mirVana miRNA isolation kit (Ambion, Life Technologies), according to the manufacturer's instructions. For cDNA synthesis, the Maxima First Strand cDNA synthesis kit for RT-qPCR (Thermo Scientific) was used following the manufacturer's instructions. Quantitative PCR was performed in a Step One Plus real-time PCR unit (Applied Biosystems) using Fast SYBR Green master mix (Applied Biosystems) and the following primer pairs: EphB1 forward: GGGATTGTCATGTGGGAAGT; EphB1 reverse: AGCCGGTAATCCTGCTCAAT; EphB3 forward: GGGTTTGTCTTCATGGTGGT; EphB3 reverse: AACTTTCATCCCAGGAGCAAT. Each independent sample was assayed in triplicate, and gene expression levels were normalized using TATA box binding protein.

Results

Striatal interneurons are attracted to the striatum and repelled by the cortex

To explore whether the cerebral cortex and/or the striatum influence the migration of striatal interneurons, we designed transplantation assays using small MGE explants. We first characterized the MGE explants in culture to verify that the dissected embryonic regions were able to produce both striatal and cortical interneurons. To this end, we cultured E13.5 MGE explants in Matrigel for 36 h and analyzed the fraction of putative cortical and striatal migrating interneurons based on the postmitotic expression of Nkx2-1 (Fig. 1*A*). We have previously shown that interneurons migrating toward the cortex downregulate the expression of Nkx2-1 within a few hours *in vivo* (Nóbrega-Pereira et al., 2008), so we reasoned that neurons expressing Nkx2-1 after 36 h in culture are putative striatal interneurons. We found that more than half of the neurons derived from E13.5 MGE explants retain expression of Nkx2-1 (n = 11 explants; Tuj1+/Nkx2-1+ cells: 64.34 ± 3.4%, average ± SEM; Fig. 1*B*,*B′*), suggesting that both striatal and cortical interneurons are generated in these experiments.

We next transplanted small E13.5 MGE explants from GFP-expressing embryos (MGE ^{GFP}) into the cortex of telencephalic slices obtained from isochronic wild-type mouse embryos (Fig. 1*C*). After 36 h in culture, many MGE-derived GFP-expressing

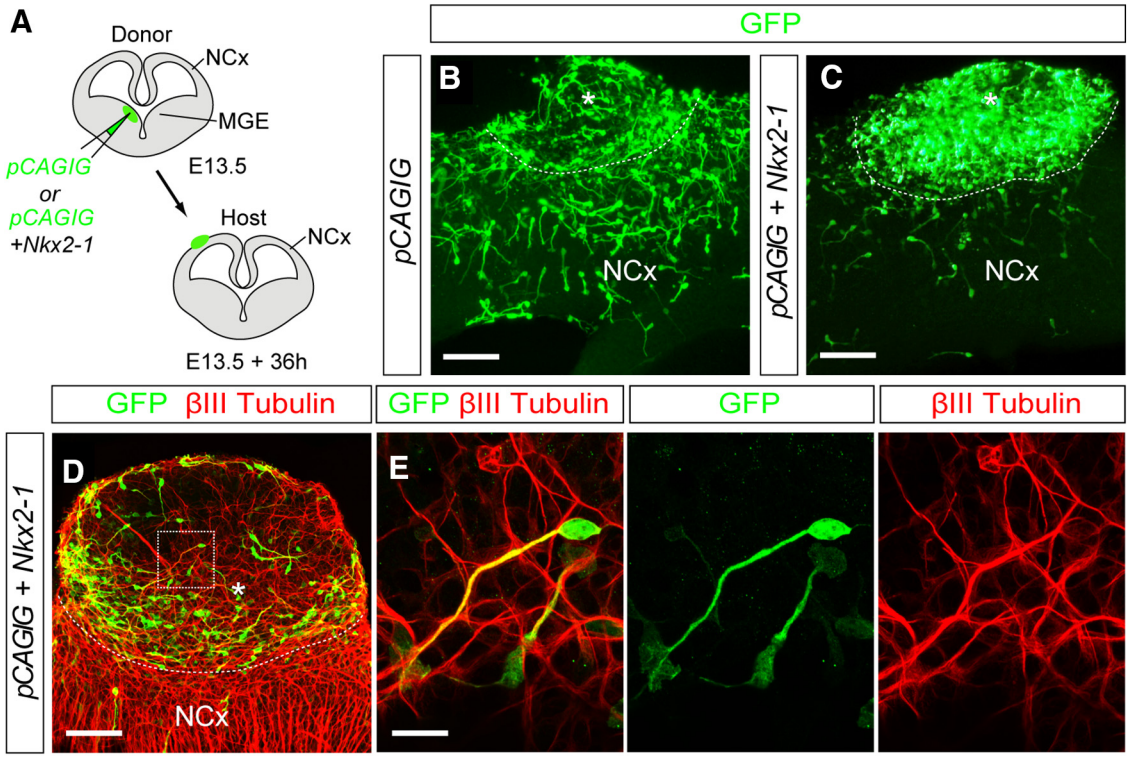

Figure 2. Striatal interneurons are attracted to the striatum and repelled by the cortex. ***A***, Schematic of slice transplantation paradigms, in which a piece of the MGE was placed in the developing neocortex (NCx) after electroporation with *Gfp* or with *Gfp* and *Nkx2-1*. ***B***, ***C***, Migration of MGE ^{ect}-derived cells electroporated with *Gfp* (***B***) or with *Gfp* and *Nkx2-1* (***C***). ***D***, ***E***, Most electroporated cells that remain in the transplants are neurons. Scale bars: ***B–D***, 100 μm; ***E***, 25 μm.

cells were found throughout the host cortex (Fig. 1*D*). However, virtually none of the GFP cells that invaded the cortex expressed Nkx2-1 ($n = 5$; GFP+/Nkx2-1+ cells: 1.2 ± 0.5%, average ± SEM; Fig. 1*D*,*D*′). These findings suggested two possibilities: (1) the cortex contains an activity that represses the expression of Nkx2-1 and/or (2) the cortex contains an activity that repels striatal interneurons. To address these possibilities, we repeated the previous experiments after placing a small piece of striatal tissue in the cortex of host slices (Fig. 1*E*). Under these circumstances, we observed that GFP+ MGE-derived cells left the explants and invaded the ectopic striatum and the adjacent cortex (Fig. 1*F*). Interestingly, the fraction of GFP+/Nkx2-1+ cells increased prominently within the striatum (Fig. 1*F*,*F*′; $n = 8$; GFP+/Nkx2-1+ cells: 24.0 ± 6.2%, average ± SEM). These results indicate that striatal interneurons, identified by the postmitotic expression of Nkx2-1, can migrate in close proximity to cortical tissue without invading it, but they are preferentially attracted to the striatum.

The previous experiments also suggested that the cortex might contain a nonpermissive or repulsive activity for striatal interneurons. To directly test this hypothesis, we focally electroporated a plasmid encoding for *Nkx2-1* in the MGE of E13.5 organotypic slices and then placed small MGE-electroporated transplants in the cortex (Fig. 2*A*). In control experiments, many GFP-expressing cells migrated out of the MGE explant and colonized the entire developing cortex (Fig. 2*B*). In contrast, forced expression of *Nkx2-1* prevented MGE-derived cells from invading the cortex Fig. 2*C*; $n = 7$; GFP+ cells in cortex: 23.6 ± 1.4 (GFP) and 6.6 ± 0.9 (GFP/Nkx2-1), average ± SEM; $p < 0.001$, *t* test]. To verify that forced expression of *Nkx2-1* does not prevent the generation of striatal interneurons we analyzed the expression of class III β-tubulin (Tuj1) in Nkx2-1+ neurons. We found that the vast majority of *Nkx2-1* overexpressing cells are neurons and have the characteristic morphology of migrating interneurons ($n = 5$; Nkx2-1+/Tuj1+ cells: 90.57 ± 2.08%, average ± SEM; Fig. 2*D*,*E*). These results demonstrate that putative striatal interneurons are generated in MGE explants but fail to invade the cortex, which suggests that this transcription factor renders MGE-derived cells responsive to a repulsive or nonpermissive activity present in the developing cortex. Altogether, our experiments revealed that the striatum and the cortex might contain chemoattractive and chemorepulsive activities for striatal interneurons, respectively.

ErbB4 signaling is required for the migration of interneurons to the striatum

CRD-Nrg1, the membrane-bound isoform of Nrg1, is strongly expressed in the developing striatum at the time of cortical and striatal interneuron migration (Flames et al., 2004; Fig. 3*A*,*A*′). This suggests that, in addition to regulating the migration of cortical interneurons through the LGE corridor, Nrg1 may also guide MGE-derived striatal interneurons to their target. To test this hypothesis, we first analyzed the expression of the Nrg1 receptor ErbB4 in migrating striatal interneurons. At E13.5, *ErbB4* transcripts were found in cells migrating to the cortex and in the developing striatum (Fig. 3*B*,*B*′). Because *ErbB4* expression in the striatum may correspond to cells passing through this structure rather than immature striatal interneurons, we examined the expression of Nkx2-1 in migrating MGE-derived cells. Double labeling stainings demonstrated that striatal interneurons, identified by postmitotic expression of Nkx2-1, also express the ErbB4 receptor (Fig. 3*C*,*C*′). Consistent with this observation, we found that ErbB4 is expressed in many parvalbumin-expressing (PV+) and a few somatostatin (SST+) interneurons in the postnatal

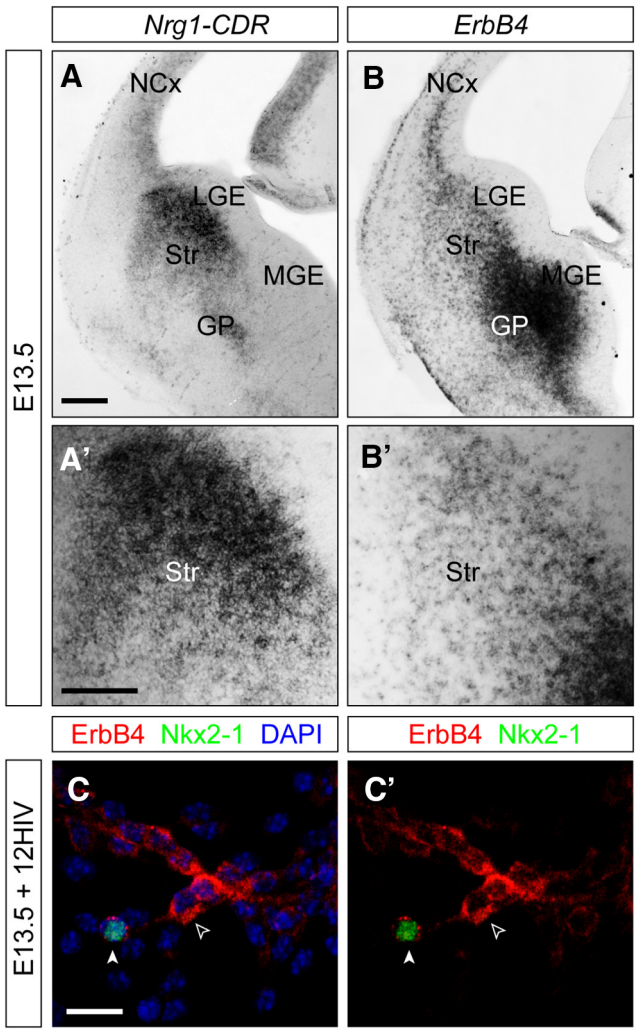

Figure 3. The ErbB4 receptor is expressed in migrating MGE-derived cortical and striatal interneurons. ***A–B′***, Coronal sections through the telencephalon of E13.5 embryos depicting *Nrg1-CRD* (***A***, ***A′***) and *ErbB4* (***B***, ***B′***) mRNA expression in the developing striatum. ***C***, ***C′***, MGE-derived cells stained with DAPI and immunohistochemistry against ErbB4 and Nkx2-1 after 12 h in culture. Most ErbB4+ cells do not express Nkx2-1, but some prospective striatal interneurons do (***C′***, open and solid arrowheads, respectively). GP, globus pallidus; NCx, neocortex; Str, striatum. Scale bars: ***A***, ***B′***, 100 μm; ***C***, ***C′***, 25 μm.

cortex ($n = 5$; PV+/ErbB4+ cells: 61.5 ± 1.56%; SST+/ErbB4+ cells: 9.75 ± 1.38%; average ± SEM).

Expression of ErbB4 in migrating striatal interneurons is consistent with the hypothesis that these neurons rely on Nrg1/ErbB4 signaling to target the striatum. To directly test this hypothesis, we asked whether ErbB4 signaling is required for interneurons to migrate to the striatum. Because the proportion of MGE-derived cells that migrate to the striatum is very low compared with the number of cortical interneurons under normal circumstances, we artificially increased the number of interneurons migrating to the striatum by forcing Nkx2-1 expression in all MGE-derived cells (Nóbrega-Pereira et al., 2008), and asked whether the accumulation of interneurons in the striatum requires ErbB4 function. To this end, we focally electroporated a plasmid encoding *Nkx2-1-IRES-Gfp* in the MGE of embryonic organotypic slices obtained from control or $ErbB4^{-/-}$;$HER4^{heart}$ mutant embryos (Fig. 4*A*). In these mice, expression of human *ErbB4* (*HER4*) under a cardiac-specific myosin promoter avoids the embryonic lethality caused by loss of *ErbB4* function in the myocardium (Tidcombe et al., 2003). Consistent with previous results (Nóbrega-Pereira et al., 2008), we observed that most *Nkx2–1*-expressing MGE-derived cells accumulate in the developing striatum in wild-types slices (Fig. 4*B*). In $ErbB4^{-/-}$;$HER4^{heart}$ organotypic slices, *Nkx2–1*-expressing cells were still largely confined to the subpallium. However, compared with controls, very few neurons reached the striatum and instead accumulated throughout the MGE subventricular zone and mantle [Fig. 4*C*,*D*; $n = 4$ slices; GFP-expressing cells in the striatum: 58.88 ± 2.12 cells (control) and 21.50 ± 3.00 cells (mutant), average ± SEM; $^{***}p < 0.001$, *t* test]. To discard the possibility that *ErbB4* function is required for the movement of MGE-derived interneurons and not for their guidance, we cultured wild-type and $ErbB4^{-/-}$;$HER4^{heart}$ MGE explants that were previously electroporated with *Nkx2-1-IRES-Gfp*. We found that MGE-derived *Nkx2–1*-expressing cells lacking *ErbB4* migrate similar distances than control cells [Fig. 4*E*,*F*; $n = 4$ explants for each condition; mean distance: 325.75 ± 46.97 μm (control) and 331.7 ± 34 μm (mutant), average ± SEM], suggesting that loss of *ErbB4* function does not impair cell migration. Finally, we analyzed the distribution of interneurons in the striatum of $ErbB4^{-/-}$;$HER4^{heart}$ mutant embryos. We found that the striatum of E15.5 $ErbB4^{-/-}$;$HER4^{heart}$ mutant embryos contained fewer Nkx2-1-expressing neurons than controls [Fig. 4*G–I*; $n = 3$; *Nkx2–1*-overexpressing cells in the striatum: 771.46 ± 45.05 cells/mm^2 (control) and 643.49 ± 76.77 cells/mm^2 (mutant), average ± SEM; $^{*}p < 0.05$, *t* test]. These changes are likely due to defects in the migration of striatal interneurons and not in their generation, because the density of proliferating cells in the MGE at E13.5 is similar between controls and $ErbB4^{-/-}$;$HER4^{heart}$ mutant embryos [$n = 4$; PH3+ cells: 962.25 ± 23.66 cells/mm^2 (control) and 937.25 ± 70.12 cells/mm^2 (mutant), average ± SEM; $p = 0.689$, *t* test; Rakiæ et al., 2015]. These results suggest that the correct allocation of MGE-derived striatal interneurons partly relies on ErbB4 signaling through a mechanism likely to involve CRD-Nrg1-mediated chemoattraction.

Eph/ephrin signaling mediates the cortical repulsion of striatal interneurons

The cerebral cortex contains high levels of expression of secretable forms of Nrg1, such as Ig-Nrg1, which contribute to direct cortical interneurons to their final target (Flames et al., 2004). Since striatal interneurons also express ErbB4 receptors, additional mechanisms might exist to prevent striatal interneurons from populating the cortex. Eph receptor tyrosine kinase and their ephrin ligands are membrane-bound or transmembrane proteins that mediate cell repulsion in a large variety of biological contexts, and are known to be expressed in the developing telencephalon (Passante et al., 2008; Zimmer et al., 2011). For instance, the EphB receptors EphB1 and EphB3 are expressed both in the MGE and the striatum (Fig. 5*A–B′*), while their ligands ephrinB1 and ephrinB2 are highly expressed in the neocortex (Fig. 5*C–D′*). In particular, ephrinB2 is expressed at high levels in the cortex and in the progenitor regions of the lateral ganglionic eminence (LGE), but it is absent or expressed at comparatively very low levels in the striatal mantle (Fig. 5*D′*). These observations prompted us to consider these molecules as candidates to mediate the cortical repulsion of striatal interneurons. To test this hypothesis, we first examined whether EphB receptors are indeed specifically expressed in the membrane of migrating striatal interneurons. To this end, we performed surface labeling of living interneurons by incubating MGE explants with soluble Fc-tagged ephrinB2 ectodomains and then identified striatal interneurons

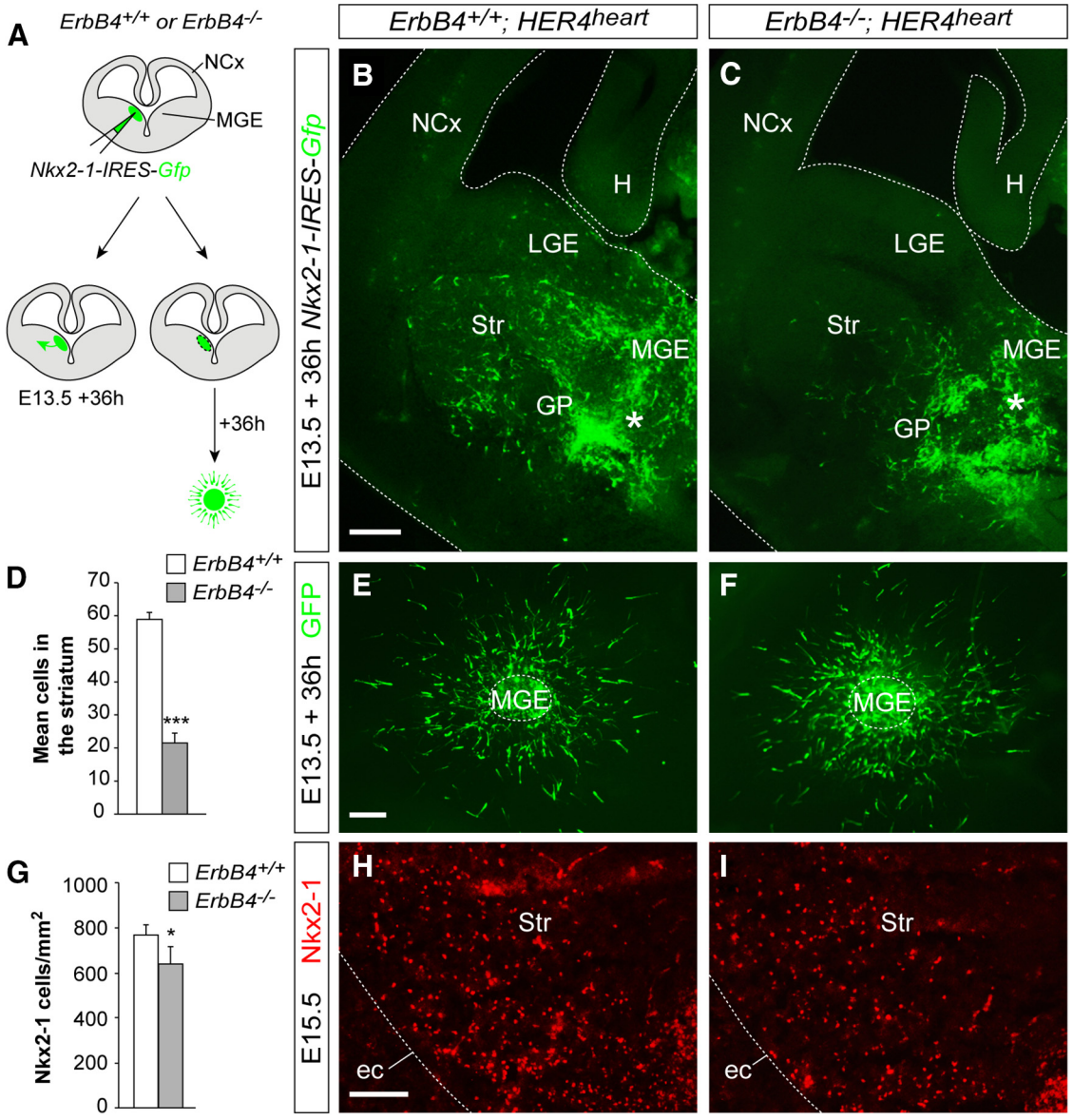

Figure 4. *ErbB4* is required for the migration of MGE-derived interneurons to the striatum (Str). ***A***, Schematic diagram of the focal electroporation experiment. ***B***, ***C***, Migration of MGE-derived cells overexpressing *Nkx2-1-IRES-Gfp* in E13.5 slices from control (***B***) and *ErbB4*$^{-/-}$;*HER4*^{heart} mutant (***C***) embryos. Dotted lines indicate the limits of the slices. ***D***, Quantification of the number of GFP+ (Nkx2–1-overexpressing) neurons in the striatum of E13.5 slices from control and *ErbB4*$^{-/-}$;*HER4*^{heart} mutant embryos. Graphs show average ± SEM. ***E***, ***F***, Migration of neurons overexpressing *Nkx2-1-IRES-Gfp* from E13.5 explants from control (***E***) and *ErbB4*$^{-/-}$;*HER4*^{heart} mutant (***F***) embryos. ***G***, Quantification of the number of Nkx2-1-expressing cells in the caudal striatum of E15.5 control and *ErbB4*$^{-/-}$ *HER4*^{heart} mutant embryos. Graphs show average ± SEM. ***H***, ***I***, Expression of Nkx2-1 in the striatum of E15.5 *ErbB4*$^{+/+}$;*HER4*^{heart} (***H***) and *ErbB4*$^{-/-}$;*HER4*^{heart} mutant (***I***) embryos. The asterisks indicate the electroporation sites. Dotted lines indicate the external capsule (ec). GP, globus pallidus; H, hippocampus; NCx, neocortex. Scale bars: ***B–D***, ***F***, 200 μm; ***H***, ***D***, 100 μm.

by the expression of Nkx2-1. Using this approach, we detected EphB receptors throughout the membrane of migrating striatal interneurons (Fig. 5*E*). To extend these observations, we took advantage of TRE-Bi-SG-T reporter mice that express tdTomato under the control of the bidirectional tet-responsive promoter (Li et al., 2010). When these mice are bred with a strain that expresses the reverse tetracycline-controlled transactivator protein (rtTA) under the control of the EphB3 promoter (EphB3-rtTA), the expression of the reporter protein tdTomato can be elicited by the tetracycline analog doxycycline (Villasenor et al., 2012). We then analyzed the expression of tdTomato in the striatum of E15.5 embryos that were previously treated with doxycycline. Despite the scarcity in the induction of tdTomato expression (highly dependent on tamoxifen dosage), we consistently observed scattered tdTomato+ cells (i.e., EphB3+ cells) throughout the embryonic striatum that also express Nkx2-1 (Fig. 5*F*). These experiments revealed that migrating striatal interneurons express EphB receptors.

We next wondered whether Nkx2-1 might directly regulate the expression of EphB receptors in developing striatal interneurons. To begin testing this hypothesis, we examined whether Nkx2-1 directly binds to *EphB* promoter regions. To this end, we first performed a phylogenetic footprinting analysis of the 20 kb sequence upstream of the *EphB1* and *EphB3* transcription initiation sites. This analysis revealed five putative regulatory regions containing Nkx2-1 binding sequences within the promoter regions of *EphB1* and *EphB3*, respectively. ChIP analysis demonstrated that Nkx2-1 binds to all the regions that we analyzed,

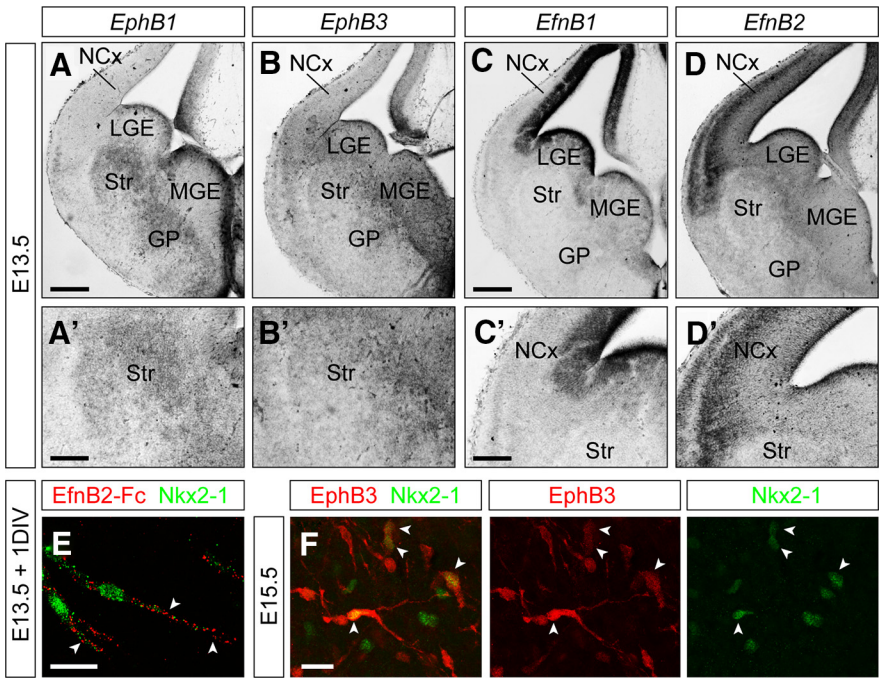

Figure 5. Ephrins are expressed in the developing cortex and Eph receptors are expressed by striatal interneurons. ***A–D′***, Coronal sections through the telencephalon of an E13.5 mouse embryos showing the expression of *EphB1*, *EphB3*, *ephrinB1*, and *ephrinB2* mRNA. ***E***, Distribution of EphB receptors in the membrane of Nkx2-1-expressing interneurons in culture revealed by the selective binding of ephrinB2-Fc fragments. Arrowheads point to ephrinB2-Fc clusters. ***F***, Coronal section through the striatum (Str) of an E15.5 EphB3-rtTA;TRE-Bi-SG-T mouse embryo showing tdTomato expression (EphB3) and Nkx2-1 immunohistochemistry. Arrowheads point to double-labeled cells. GP, globus pallidus; NCx, neocortex. Scale bars: ***A–D***, 200 μm; ***A′–D′***, 100 μm; ***E***, ***F***, 25 μm.

although with very high affinity to *EphB1_ region 3*, *EphB1_region 4*, and *EphB3_region 2* [Fig. 6*A–C*; $n = 3$; relative intensity of PCR band normalized against the input band: 0.77 ± 0.06 (Nkx2-1) and 0.11 ± 0.01 (Rb IgG) for *EphB1_region 4*, 0.78 ± 0.08 (Nkx2-1) and 0.16 ± 0.02 (Rb IgG) for *EphB1_region 4*, 0.87 ± 0.17 (Nkx2-1) and 0.07 ± 0.05 (Rb IgG) for *EphB3_region 2*, average ± SEM; $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$, *t* test]. To further confirm that Nkx2-1 can activate the expression of *EphB1* and *EphB3* we then performed luciferase assays using the promoter regions that appeared more significant in ChIP assays (Fig. 6*D*). For each of these regions, we performed parallel experiments in which we compared the effect of Nkx2-1 on luciferase activity using the wild-type *Nkx2-1* consensus binding sites and equivalent constructions in which these regions were specifically deleted. We found a significant decrease in the luciferase activity in the presence of Nkx2-1 when consensus binding sites were deleted for both EphB1 and EphB3 [Fig. 6*D*; $n = 5$; relative luciferase activity: 0.95 ± 0.11 (*EphB1_region 3_wt*), 1.04 ± 0.13 (*EphB1_region 3_del1*), 0.92 ± 0.05 (*EphB1_region 4_wt*), 0.46 ± 0.03 (*EphB1_region 4_del1*), 0.98 ± 0.04 (*EphB3_region 1–2_wt*), 0.59 ± 0.09 (*EphB3_region 1–2_del1*), 0.98 ± 0.04 (*EphB3_region 1–2_wt*), and 0.51 ± 0.13 (*EphB3_region 1–2_del2*), average ± SEM; $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$, *t* test].

The previous results are consistent with the hypothesis that Nkx2-1 enhances the expression of *EphB1* and *EphB3* in striatal interneurons. To extend these observations, we isolated RNA from MGEs electroporated with *pCAGIG* or *pCAGIG+Nkx2-1* and we performed quantitative PCR. In these experiments, we confirmed a significant increase in the expression of *EphB1* in Nkx2-1 overexpressing cells [$n = 3$; mRNA fold change: 1.05 ± 0.04 (control), 2.21 ± 0.35 (Nkx2-1), average ± SEM; $^{*}p < 0.05$, *t* test], but not for *EphB3* (Fig. 6*E*). This later result might be a consequence of the timing of the experiment or the sensitivity of our assay for this particular receptor. However, our collective results suggest that Nkx2-1 directly regulates the expression of EphB receptors in striatal interneurons, which reinforce the view that EphB/ephrinB interactions control their migration.

The expression patterns of ephrinB and EphB proteins in the developing telencephalon are consistent with the idea that neocortical ephrins repel striatal interneurons, preventing their entry into the cortex. To directly test this hypothesis, we placed small *Nkx2-1*-electroporated MGE transplants into the cortex of E13.5 organotypic slices and cultured them in medium containing control Fc fragments or several combinations of Eph-Fc reagents (Fig. 7*A*). Eph-Fc and ephrin-Fc fragments have been previously shown to block Eph/ephrin signaling by interfering with the ability of Eph receptors to cluster appropriately (Davis et al., 1994; Stein et al., 1998). After 24 h in culture, we observed that simultaneously blocking A and B ephrin/Eph signaling in slices dramatically increases the number of putative striatal interneurons that colonize the neocortex compared with controls [Fig. 7*B–D*,*G*; $n = 33$ slices (control hFc), 23 slices (EphA3/B1-Fc), and 39 slices (ephrinA1/B1-Fc) from six different experiments; density of *Nkx2-1*-overexpressing cells in the neocortex: 106 ± 13.6 cells/mm^2 (control hFc), 656 ± 51.3 cells/mm^2 (EphA3/B1-Fc), and 716 ± 62 cells/mm^2 (ephrinA1/B1-Fc), $F = 32.125$, average ± SEM; $p = 0.0001$, one-way ANOVA, $^{***}p < 0.001$ with Bonferroni *post hoc* test]. Analysis of experiments in which we only added ephrinA-Fc or ephrinB-Fc fragments revealed that blocking either one of these two signaling pathways is sufficient to enhance the migration of putative striatal interneurons into the cortex, with the inhibition of EphB function having a most prominent effect in this process [Fig. 7*E–G*; $n = 33$ slices (control hFc), 23 slices (EphA3/B1-Fc), 39 slices (ephrinA1/B1-Fc), 34 slices (ephrinA1-Fc), and 61 slices (ephrinB1-Fc) from six different experiments; density of *Nkx2–1*-overexpressing cells in the neocortex: 106 ± 13.6 cells/mm^2 (control hFc), 345 ± 32.5 cells/mm^2 (ephrinA1-Fc), and 427 ± 29.8 cells/mm^2 (ephrinB1-Fc), average ± SEM; $F = 32.125$, $p = 0.0001$, one-way ANOVA, $^{***}p < 0.001$ with Bonferroni *post hoc* test].

To confirm the involvement of Eph/ephrin signaling in the migration of striatal interneurons, we performed additional transplantation experiments using wild-type and mutant mice. In brief, we focally electroporated a plasmid encoding *Nkx2-1* in the MGE of organotypic slices obtained from E13.5 control, *EphB3*$^{-/-}$, and *EphB1*$^{-/-}$;*EphB3*$^{-/-}$ embryos, and transplanted the electroporated MGE regions in the neocortex of isochronic wild-type organotypic slices (Fig. 8*A*). After 24 h *in vitro*, we observed a minor increase in the number of *EphB1*$^{-/-}$ interneurons that were able to invade the cortex compared with control. This effect was much more significant when interneurons lacked both EphB1 and EphB3 receptors

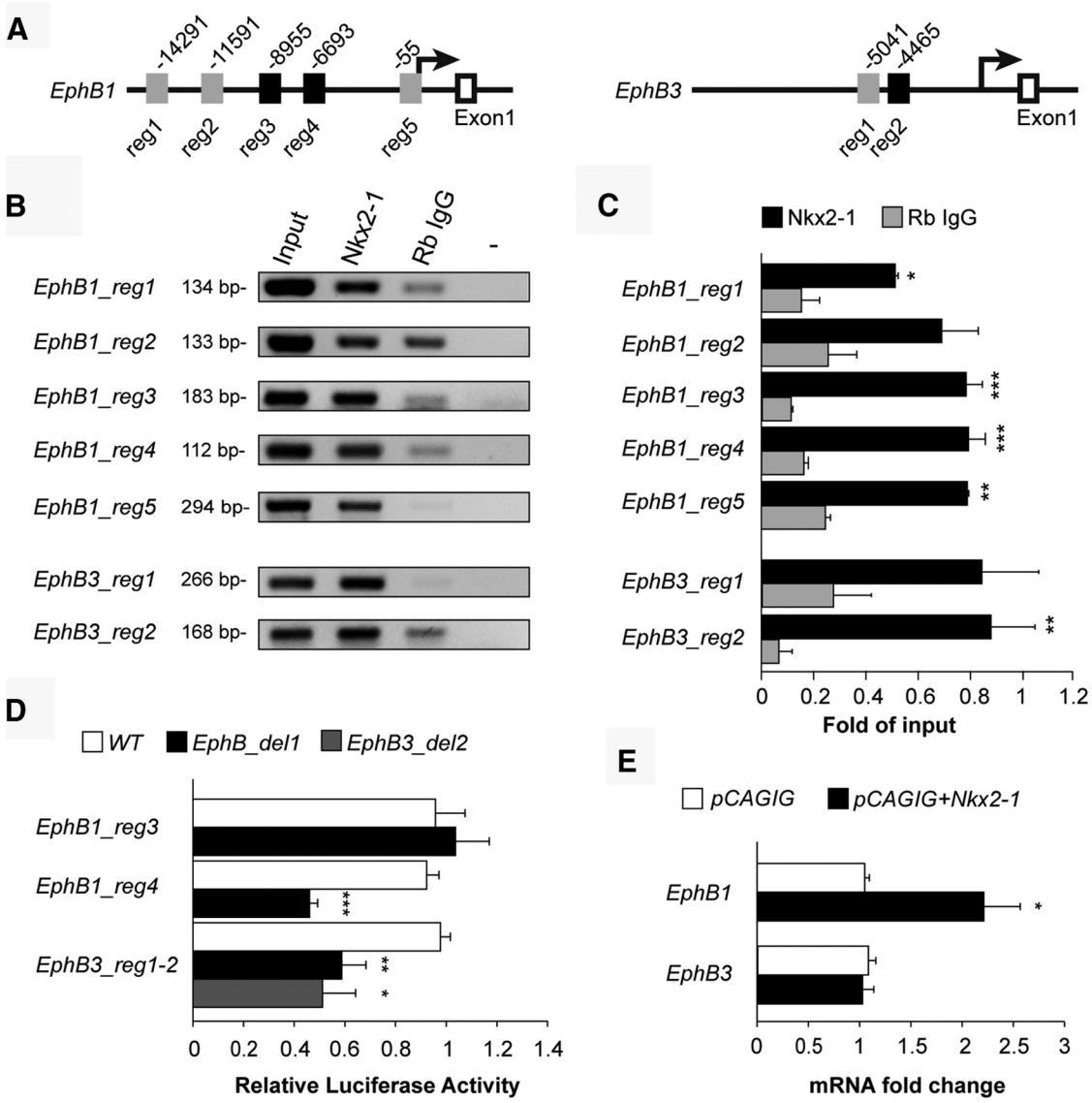

Figure 6. Nkx2-1 binds to both *EphB1* and *EphB3* promoters *in vivo* and drives their expression. ***A***, Schematic drawing of Nkx2-1 binding sites (gray and black boxes) in the *EphB1* and *EphB3* loci. ***B***, ChIP assays of E13.5 subpallium using a polyclonal antibody against Nkx2-1 and a nonspecific rabbit anti-IgG (RbIgG). Input chromatin represents 1% of the total chromatin. Negative (−, all reagents except DNA) controls were included in each run. ***C***, Quantification of the intensity of each PCR band obtained from the ChIP assays and normalized against the input band. Graphs show average ± SEM. ***D***, Quantification of the relative luciferase activity generated by the wild-type *EphB1_reg3*, *EphB1_reg4*, or *EphB3_reg1–2* promoter regions compared with the luciferase activity generated by the same promoter regions but with a deletion of the Nkx2-1 consensus binding sites. Graphs show average ± SEM. ***E***, Quantitative PCR analysis for *EphB1* and *EphB3* in cells obtained from MGEs electroporated with *pCAGIG* or *pCAGIG* + *Nkx2-1*. Graphs show average ± SEM.

[Fig. 8*B–E*; $n = 30$ slices (+/+), 8 slices ($EphB1^{-/-}$), and 10 slices ($EphB1^{-/-};EphB3^{-/-}$) from four different experiments; density of *Nkx2–1*-overexpressing cells in the neocortex: 33.7 ± 7.70 cells/mm^2 (+/+), 77.6 ± 2.37 cells/mm^2 ($EphB1^{-/-}$), and 293 ± 84.9 cells/mm^2 ($EphB1^{-/-};EphB3^{-/-}$), average ± SEM; $F = 15.632$, $p = 0.0001$, one-way ANOVA, $^{***}p < 0.001$ with Bonferroni *post hoc* test].

Our previous experiments demonstrated that EphB1 and EphB3 are required for the repulsion of striatal interneuron by the cortex. We therefore reasoned that mouse mutants lacking these receptors might have decreased numbers of striatal interneurons. To test this hypothesis, we quantified the density of Nkx2-1-expressing neurons in the striatum of E15.5 control, $EphB3^{-/-}$, or $EphB1^{-/-};EphB3^{-/-}$ mouse embryos. We found a significant decrease in the number of striatal interneurons in $EphB1^{-/-};EphB3^{-/-}$ embryos compared with control and $EphB3^{-/-}$ embryos [Fig. 8*F–I*; $n = 6$ brains; density of *Nkx2–1*-expressing cells in the striatum: 3507 ± 125 cells/mm^2 (+/+), 3908 ± 143 cells/mm^2 ($EphB3^{-/-}$), and 2959 ± 126 cells/mm^2 ($EphB1^{-/-};EphB3^{-/-}$), average ± SEM; $F = 13.085$, $p = 0.001$one-way ANOVA, $^{*}p < 0.05$ with Bonferroni *post hoc* test], suggesting that EphB1 and EphB3 are important for the correct migration of striatal interneurons. While some of these defects might be due to differences in proliferation dynamics between controls and mutants [$n = 4$; PH3+ cells: 3400 ± 200 cells/mm^2 (control), and 1700 ± 30 cells/mm^2 (mutants), average ± SEM; $p = 0.01$, *t* test] we observed that MGE explants obtained from control and mutants generate similar numbers of interneurons [$n = 9$; Tuj1+ cells: 0.0375 ± 0.0062 cells (control), and 0.0453 ± 0.0042 cells (mutants), average ± SEM; $p = 0.31$, *t* test]. In summary, our experiments suggest a role for Eph/ephrin signaling in preventing the migration of striatal interneurons into the developing cortex.

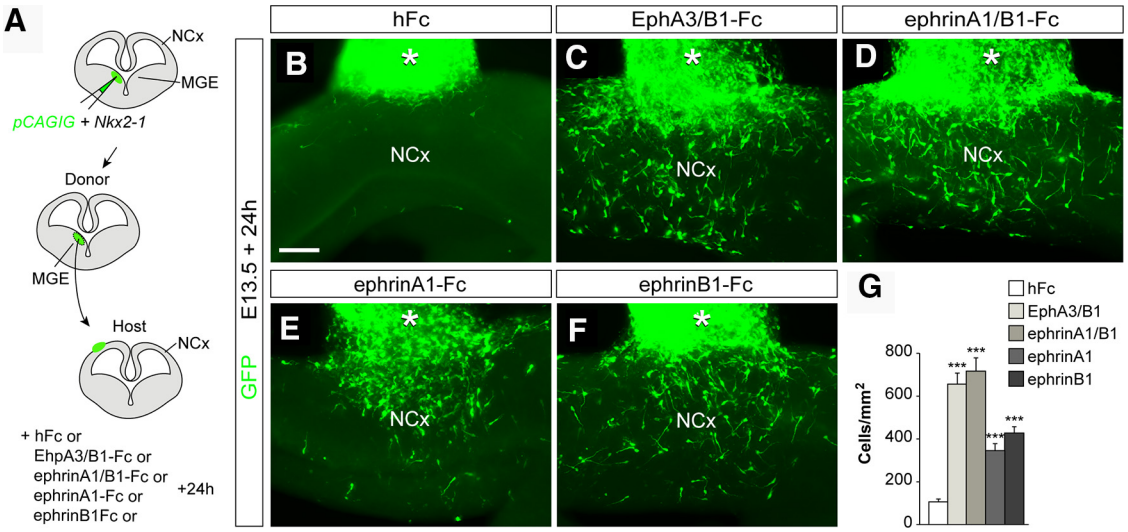

Figure 7. Blocking Eph/ephrin signaling enhances cortical invasion by striatal interneurons. ***A***, Schematic diagram of the focal electroporation and transplantation experiment. ***B–F***, Cortical invasion by Nkx2-1 overexpressing interneurons after treatment with hFc (***B***), a mix of EphA3-Fc and EphB1-Fc (***C***), a mix of ephrinA1-Fc and ephrinB1-Fc (***D***), ephrinA1-Fc (***E***), or ephrinB1-Fc fragments (***F***). ***G***, Quantification of the density of Nkx2-1 overexpressing interneurons that invaded the neocortex (NCx) after treatment with different Fc fragments. Asterisks indicate MGE transplants. Graphs show average ± SEM. Scale bar, 100 μm.

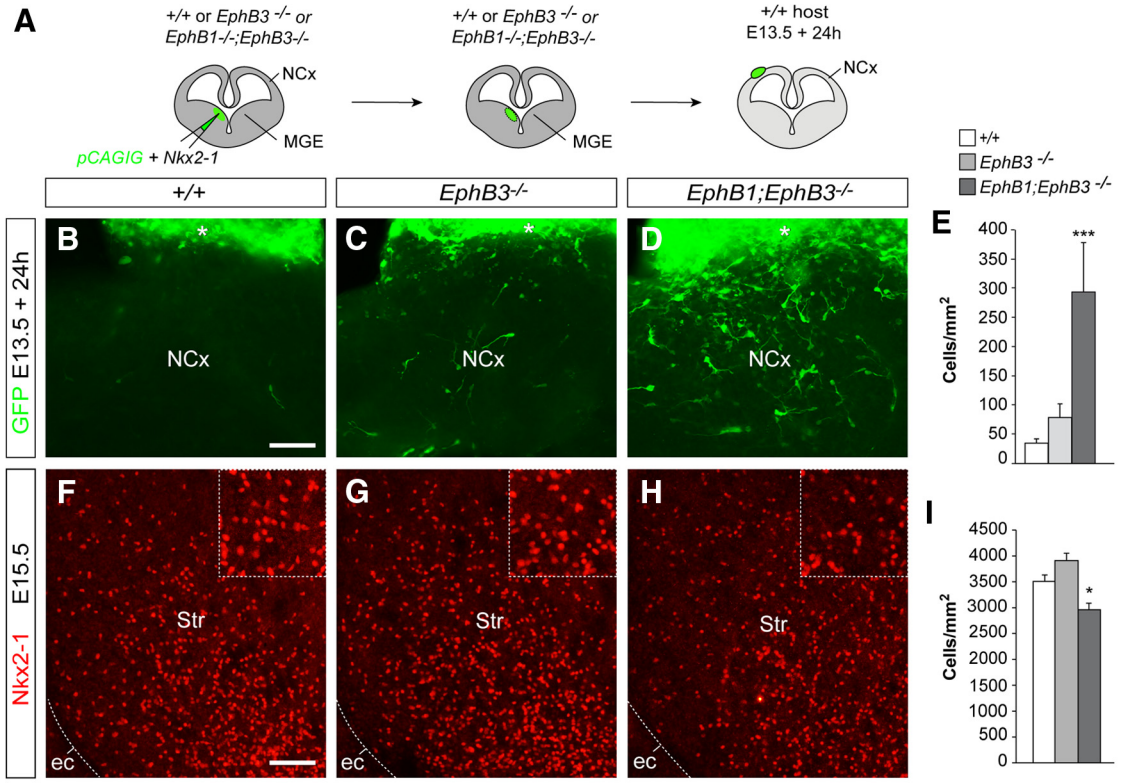

Figure 8. Loss of EphB receptors enhances cortical invasion by striatal interneurons *in vitro* and reduces the number of striatal interneurons *in vivo*. ***A***, Schematic diagram of the focal electroporation and transplantation experiments. ***B–D***, Cortical invasion of interneurons from +/+ (***B***), *EphB3*$^{-/-}$ mutant (***C***), and *EphB1*$^{-/-}$;*EphB3*$^{-/-}$ mutant (***D***) MGE transplants electroporated with *Nkx2-1-IRES-Gfp*. ***E***, Quantification of the density of +/+, *EphB3*$^{-/-}$, and *EphB1*$^{-/-}$;*EphB3*$^{-/-}$ mutant Nkx2-1 overexpressing interneurons that invaded the neocortex (NCx). Graphs show average ± SEM. ***F–H***, Coronal sections through the telencephalon of E15.5 +/+ (***F***), *EphB3*$^{-/-}$ (***G***), and *EphB1*$^{-/-}$;*EphB3*$^{-/-}$ mutant (***H***) embryos showing Nkx2-1 protein expression. ***I***, Quantification of the number of Nkx2-1-expressing cells in the striatum of E15.5 +/+, *EphB3*$^{-/-}$, and *EphB1*$^{-/-}$;*EphB3*$^{-/-}$ mutant embryos. Asterisks indicate MGE transplants. Graphs show average ± SEM. ec, external capsule, Str, striatum. Scale bar, 100 μm.

Discussion

Our results reveal that the migration of striatal interneurons is regulated by the combination of chemoattractive and chemorepulsive signals (Fig. 9), a picture that is largely reminiscent of that observed for cortical interneurons (Marín, 2013). Both classes of MGE-derived interneurons express ErbB4 and are attracted to sources of Nrg1 in the striatum and cortex, respectively. Consequently, the sorting of these populations into their respective territories largely

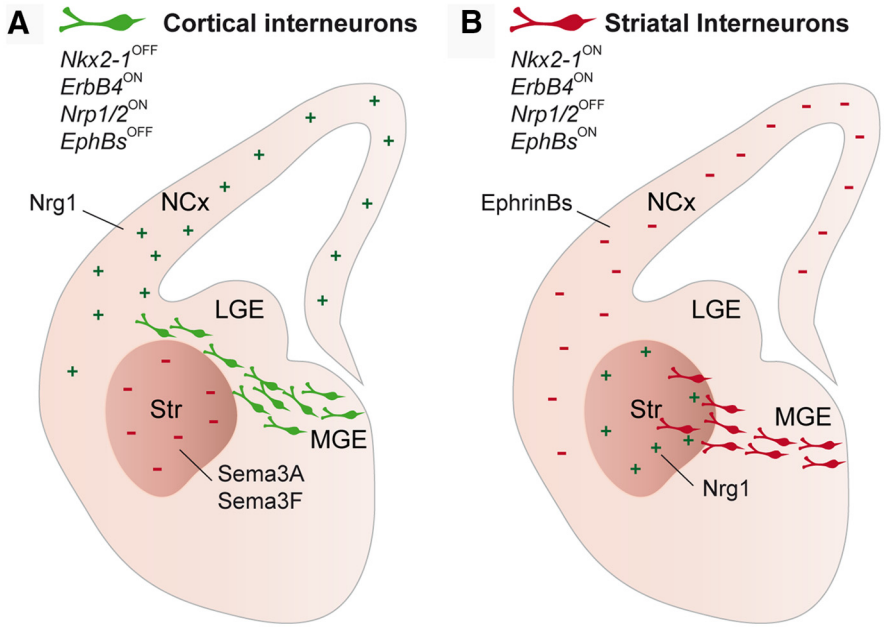

Figure 9. Striatal interneurons are repelled by the cortex and attracted by the striatum. ***A***, ***B***, Schematic drawings of transversal hemisections through the developing telencephalon summarizing the chemorepellent and chemoattractive cues that drive interneuron migration to the cortex (***A***) or the striatum (***B***). NCx, neocortex; Str, striatum.

relies on the ability of chemorepulsive signals to prevent entry into inappropriate locations. Cortical interneurons actively avoid the striatum during their migration because of the expression of class III semaphorins
in this territory (Marín et al., 2001; Hernández-Miranda et al., 2011). In turn, the present results suggest that striatal interneurons avoid entering the cortex because of the high levels of ephrinB molecules in this region.

The migration of MGE-derived striatal cells partially relies on ErbB4/Nrg1 interactions. This reveals that both striatal and cortical interneurons are able to respond to Nrg1 signals, although it is unclear whether they use similar signaling pathways. Interestingly, recent work has shown that MGE-derived interneurons express different isoforms of ErbB4 during the migration through the telencephalon (Rakiæ et al., 2015). In brief, there are two alternatively spliced ErbB4 isoforms that differ in their cytoplasmic domain and have distinct signaling properties, Cyt1 and Cyt2. Both receptors activate the MAPK pathway, but only the Cyt1 isoform activates PI3K/Akt signaling (Elenius et al., 1997, 1999). Of note, MGE-derived interneurons migrating through the subpallium express Cyt2, but only interneurons reaching the cortex express Cyt1 (Rakiæ et al., 2015). While this may just indicate different maturational stages of the same population of neurons, it is also conceivable that striatal and cortical interneurons contain different receptor dimers, and this might contribute to provide certain specificity in their response to different Neuregulin isoforms. In addition, it is worth noting that only some of the MGE-derived, Nkx2-1-expressing cells seem to express ErbB4, which suggests that additional chemoattractive cues might contribute to the migration and final allocation of interneurons into the striatum. ErbB4 is expressed primarily in parvalbumin-expressing interneurons in the postnatal striatum (Yau et al., 2003; Fox and Kornblum, 2005), so perhaps this is the main population of striatal interneurons that depends on ErbB4 function.

ErbB4 receptor is required for the migration of both MGE-derived cortical and striatal interneurons, which raises the possibility that the transcription factor Nkx2-1 controls its expression in these cells. Previous studies have shown that Nkx2-1 binds and activates *in vitro* the promoter of ErbB2, a closely related member of the ErbB family of receptors (Lee et al., 2001). Consistent with this possibility, we observed that electroporation of Nkx2-1 in organotypic slices leads to the induction of ErbB4 expression in MGE-derived cells but not in LGE-derived cells (data not shown), which suggest that additional factors might be required for the induction of ErbB4 in MGE-derived interneurons. One of these factors is Lhx6, a direct target of Nkx2-1 that is required to maintain the expression of ErbB4 in interneurons migrating to the cortex (Du et al., 2008; Zhao et al., 2008), in which the expression of Nkx2-1 is downregulated (Nóbrega-Pereira et al., 2008).

Our study demonstrates that striatal interneurons express EphB receptors and that these molecules mediate their avoidance of cortical territories in a process that is in part mediated by ephrinB molecules. Consistently, the developing cortex expresses several ephrinB molecules, most notably ephrinB1 and ephrinB2. Interestingly, our experiments indicate that blocking ephrinA molecules in the cortex also promotes the invasion of striatal interneurons, which suggest that ephrinA signaling might also be involved in this process. Eph/ephrin interactions have been previously shown to mediate chemorepulsive interactions for cortical neurons. For example, it has been suggested that the avoidance of the striatum by cortical interneurons is in part mediated by EphA/ephrinA interactions (Rudolph et al., 2010). More recently, we have identified a role for EphB receptors as mediators of contact repulsion among Cajal–Retzius cells (Villar-Cerviño et al., 2013). So, EphB/ephrin interactions seem to play a major role in neuronal migration in the developing telencephalon.

Our results reinforce the view that regulation of Nkx2-1 expression in postmitotic migrating cells is a key step in the sorting of striatal and cortical interneurons (Nóbrega-Pereira et al., 2008). Although the progenitor cells of both striatal and cortical interneurons express Nkx2-1 (Sussel et al., 1999), the postmitotic expression of Nkx2-1 distinguishes striatal (Nkx2-1^{on}) and cortical (Nkx2-1^{off}) interneurons derived from the MGE (Marin et

al., 2000). Some of the downstream targets of Nkx2-1, such as Lhx6, continue to be expressed by cortical interneurons after Nkx2-1 expression has been downregulated in cortical interneurons (Liodis et al., 2007; Du et al., 2008; Zhao et al., 2008). This suggests that the regulation of the migration of striatal and cortical interneurons to their respective territories is under the direct control of Nkx2-1, and not by factors acting downstream of it. Indeed, postmitotic expression of Nkx2-1 is sufficient to simultaneously render striatal interneurons insensitive to chemorepulsive signals present in the striatum (class III Semaphorins) and sensitive to chemorepulsive signals found in the cortex (ephrinBs). Nkx2-1 performs this function by directly binding the promoter regions of the genes encoding Neuropilins (Nóbrega-Pereira et al., 2008) and EphB receptors (this study).

The mechanisms that control the postmitotic expression of Nkx2-1 in MGE-derived interneurons and, by extension, the sorting of striatal and cortical interneurons, remain largely unknown. The expression of Nkx2-1 is repressed by factors that are normally expressed in the cortex, such as Wnt molecules (Backman et al., 2005), and this may contribute to explaining the rapid downregulation of Nkx2-1 in MGE-derived cells transplanted to the cortex. However, it is likely that additional, perhaps intrinsic, mechanisms control the expression of this transcription factor. Indeed, striatal and cortical interneurons seem to derive from largely distinct lineages of progenitor cells located in adjacent territories of the basal telencephalon (Flames et al., 2007; Flandin et al., 2010).

In sum, our results shed light on the molecular mechanisms regulating the migration of striatal interneurons. Together, with previous work, these observations reveal a prominent parallelism in the mechanisms controlling the sorting of striatal and cortical interneurons in the developing telencephalon.

References

Anderson SA, Eisenstat DD, Shi L, Rubenstein JL (1997) Interneuron migration from basal forebrain to neocortex: dependence on Dlx genes. Science 278:474–476. CrossRef Medline

Backman M, Machon O, Mygland L, van den Bout CJ, Zhong W, Taketo MM, Krauss S (2005) Effects of canonical Wnt signaling on dorso-ventral specification of the mouse telencephalon. Dev Biol 279:155–168. CrossRef Medline

Butt SJ, Sousa VH, Fuccillo MV, Hjerling-Leffler J, Miyoshi G, Kimura S, Fishell G (2008) The requirement of Nkx2-1 in the temporal specification of cortical interneuron subtypes. Neuron 59:722–732. CrossRef Medline

Davis S, Gale NW, Aldrich TH, Maisonpierre PC, Lhotak V, Pawson T, Goldfarb M, Yancopoulos GD (1994) Ligands for EPH-related receptor tyrosine kinases that require membrane attachment or clustering for activity. Science 266:816–819. CrossRef Medline

DeFelipe J, López-Cruz PL, Benavides-Piccione R, Bielza C, Larrañaga P, Anderson S, Burkhalter A, Cauli B, Fairén A, Feldmeyer D, Fishell G, Fitzpatrick D, Freund TF, González-Burgos G, Hestrin S, Hill S, Hof PR, Huang J, Jones EG, Kawaguchi, Y, et al. (2013) New insights into the classification and nomenclature of cortical GABAergic interneurons. Nat Rev Neurosci 14:202–216. CrossRef Medline

Du T, Xu Q, Ocbina PJ, Anderson SA (2008) NKX2.1 specifies cortical interneuron fate by activating Lhx6. Development 135:1559–1567. CrossRef Medline

Elenius K, Corfas G, Paul S, Choi CJ, Rio C, Plowman GD, Klagsbrun M (1997) A novel juxtamembrane domain isoform of HER4/ErbB4. Isoform-specific tissue distribution and differential processing in response to phorbol ester. J Biol Chem 272:26761–26768. CrossRef Medline

Elenius K, Choi CJ, Paul S, Santiestevan E, Nishi E, Klagsbrun M (1999) Characterization of a naturally occurring ErbB4 isoform that does not bind or activate phosphatidyl inositol 3-kinase. Oncogene 18:2607–2615. CrossRef Medline

Flames N, Long JE, Garratt AN, Fischer TM, Gassmann M, Birchmeier C, Lai C, Rubenstein JL, Marín O (2004) Short- and long-range attraction of cortical GABAergic interneurons by neuregulin-1. Neuron 44:251–261. CrossRef Medline

Flames N, Pla R, Gelman DM, Rubenstein JL, Puelles L, Marín O (2007) Delineation of multiple subpallial progenitor domains by the combinatorial expression of transcriptional codes. J Neurosci 27:9682–9695. CrossRef Medline

Flandin P, Kimura S, Rubenstein JL (2010) The progenitor zone of the ventral medial ganglionic eminence requires Nkx2-1 to generate most of the globus pallidus but few neocortical interneurons. J Neurosci 30:2812–2823. CrossRef Medline

Fox IJ, Kornblum HI (2005) Developmental profile of ErbB receptors in murine central nervous system: implications for functional interactions. J Neurosci Res 79:584–597. CrossRef Medline

Gassmann M, Casagranda F, Orioli D, Simon H, Lai C, Klein R, Lemke G (1995) Aberrant neural and cardiac development in mice lacking the ErbB4 neuregulin receptor. Nature 378:390–394. CrossRef Medline

Hadjantonakis AK, Macmaster S, Nagy A (2002) Embryonic stem cells and mice expressing different GFP variants for multiple noninvasive reporter usage within a single animal. BMC Biotechnol 2:11. CrossRef Medline

Hernández-Miranda LR, Cariboni A, Faux C, Ruhrberg C, Cho JH, Cloutier JF, Eickholt BJ, Parnavelas JG, Andrews WD (2011) Robo1 regulates semaphorin signaling to guide the migration of cortical interneurons through the ventral forebrain. J Neurosci 31:6174–6187. CrossRef Medline

Kawaguchi Y (1993) Physiological, morphological, and histochemical characterization of three classes of interneurons in rat neostriatum. J Neurosci 13:4908–4923. Medline

Kawaguchi Y (1995) Physiological subgroups of nonpyramidal cells with specific morphological characteristics in layer II/III of rat frontal cortex. J Neurosci 15:2638–2655. Medline

Kepecs A, Fishell G (2014) Interneuron cell types are fit to function. Nature 505:318–326. CrossRef Medline

Lavdas AA, Grigoriou M, Pachnis V, Parnavelas JG (1999) The medial ganglionic eminence gives rise to a population of early neurons in the developing cerebral cortex. J Neurosci 19:7881–7888. Medline

Lee BJ, Cho GJ, Norgren RB, Junier MP, Hill DF, Tapia V, Costa ME, Ojeda SR (2001) TTF-1, a homeodomain gene required for diencephalic morphogenesis, is postnatally expressed in the neuroendocrine brain in a developmentally regulated and cell-specific fashion. Mol Cell Neurosci 17:107–126. CrossRef Medline

Li L, Tasic B, Micheva KD, Ivanov VM, Spletter ML, Smith SJ, Luo L (2010) Visualizing the distribution of synapses from individual neurons in the mouse brain. PLoS One 5:e11503. CrossRef Medline

Liodis P, Denaxa M, Grigoriou M, Akufo-Addo C, Yanagawa Y, Pachnis V (2007) Lhx6 activity is required for the normal migration and specification of cortical interneuron subtypes. J Neurosci 27:3078–3089. CrossRef Medline

Marín O (2013) Cellular and molecular mechanisms controlling the migration of neocortical interneurons. Eur J Neurosci 38:2019–2029. CrossRef Medline

Marín O, Anderson SA, Rubenstein JL (2000) Origin and molecular specification of striatal interneurons. J Neurosci 20:6063–6076. Medline

Marín O, Yaron A, Bagri A, Tessier-Lavigne M, Rubenstein JL (2001) Sorting of striatal and cortical interneurons regulated by semaphorin/neuropilin interactions. Science 293:872–875. CrossRef Medline

Marín O, Plump AS, Flames N, Sánchez-Camacho C, Tessier-Lavigne M, Rubenstein JL (2003) Directional guidance of interneuron migration to the cerebral cortex relies on subcortical Slit1/2-independent repulsion and cortical attraction. Development 130:1889–1901. CrossRef Medline

Nóbrega-Pereira S, Kessaris N, Du T, Kimura S, Anderson SA, Marín O (2008) Postmitotic Nkx2-1 controls the migration of telencephalic interneurons by direct repression of guidance receptors. Neuron 59:733–745. CrossRef Medline

Ovcharenko I, Nobrega MA, Loots GG, Stubbs L (2004) ECR Browser: a tool for visualizing and accessing data from comparisons of multiple vertebrate genomes. Nucleic Acids Res 32:W280–286. CrossRef Medline

Passante L, Gaspard N, Degraeve M, Frisén J, Kullander K, De Maertelaer V, Vanderhaeghen P (2008) Temporal regulation of ephrin/Eph signalling

is required for the spatial patterning of the mammalian striatum. Development 135:3281–3290. CrossRef Medline

Powell EM, Mars WM, Levitt P (2001) Hepatocyte growth factor/scatter factor is a motogen for interneurons migrating from the ventral to dorsal telencephalon. Neuron 30:79 – 89. CrossRef Medline

Pozas E, Ibáñez CF (2005) GDNF and GFRalpha1 promote differentiation and tangential migration of cortical GABAergic neurons. Neuron 45:701–713. CrossRef Medline

Rakiæ S, Kanatani S, Hunt D, Faux C, Cariboni A, Chiara F, Khan S, Wansbury O, Howard B, Nakajima K, Nikoliæ M, Parnavelas JG (2015) Cdk5 Phosphorylation of ErbB4 is Required for Tangential Migration of Cortical Interneurons. Cereb Cortex 25:991–1003. CrossRef Medline

Rudolph J, Zimmer G, Steinecke A, Barchmann S, Bolz J (2010) Ephrins guide migrating cortical interneurons in the basal telencephalon. Cell Adh Migr 4:400 – 408. CrossRef Medline

Stein E, Lane AA, Cerretti DP, Schoecklmann HO, Schroff AD, Van Etten RL, Daniel TO (1998) Eph receptors discriminate specific ligand oligomers to determine alternative signaling complexes, attachment, and assembly responses. Genes Dev 12:667– 678. CrossRef Medline

Sussel L, Marin O, Kimura S, Rubenstein JL (1999) Loss of Nkx2.1 homeobox gene function results in a ventral to dorsal molecular respecification within the basal telencephalon: evidence for a transformation of the pallidum into the striatum. Development 126:3359–3370. Medline

Tidcombe H, Jackson-Fisher A, Mathers K, Stern DF, Gassmann M, Golding JP (2003) Neural and mammary gland defects in ErbB4 knockout mice genetically rescued from embryonic lethality. Proc Natl Acad Sci U S A 100:8281– 8286. CrossRef Medline

Villar-Cerviño V, Molano-Mazón M, Catchpole T, Valdeolmillos M, Henkemeyer M, Martínez LM, Borrell V, Marín O (2013) Contact repulsion controls the dispersion and final distribution of Cajal-Retzius cells. Neuron 77:457– 471. CrossRef Medline

Villasenor A, Marty-Santos L, Dravis C, Fletcher P, Henkemeyer M, Cleaver O (2012) EphB3 marks delaminating endocrine progenitor cells in the developing pancreas. Dev Dyn 241:1008–1019. CrossRef Medline

Wichterle H, Turnbull DH, Nery S, Fishell G, Alvarez-Buylla A (2001) In utero fate mapping reveals distinct migratory pathways and fates of neurons born in the mammalian basal forebrain. Development 128:3759–3771. Medline

Wichterle H, Alvarez-Dolado M, Erskine L, Alvarez-Buylla A (2003) Permissive corridor and diffusible gradients direct medial ganglionic eminence cell migration to the neocortex. Proc Natl Acad Sci U S A 100:727–732. CrossRef Medline

Xu Q, Tam M, Anderson SA (2008) Fate mapping Nkx2.1-lineage cells in the mouse telencephalon. J Comp Neurol 506:16–29. CrossRef Medline

Yau HJ, Wang HF, Lai C, Liu FC (2003) Neural development of the neuregulin receptor ErbB4 in the cerebral cortex and the hippocampus: preferential expression by interneurons tangentially migrating from the ganglionic eminences. Cereb Cortex 13:252–264. CrossRef Medline

Zhao Y, Flandin P, Long JE, Cuesta MD, Westphal H, Rubenstein JL (2008) Distinct molecular pathways for development of telencephalic interneuron subtypes revealed through analysis of Lhx6 mutants. J Comp Neurol 510:79–99. CrossRef Medline

Zimmer G, Garcez P, Rudolph J, Niehage R, Weth F, Lent R, Bolz J (2008) Ephrin-A5 acts as a repulsive cue for migrating cortical interneurons. Eur J Neurosci 28:62–73. CrossRef Medline

Zimmer G, Rudolph J, Landmann J, Gerstmann K, Steinecke A, Gampe C, Bolz J (2011) Bidirectional ephrinB3/EphA4 signaling mediates the segregation of medial ganglionic eminence- and preoptic area-derived interneurons in the deep and superficial migratory stream. J Neurosci 31:18364–18380. CrossRef Medline