

EGTA, and 0.1% mercaptoethanol. The proteins were 80 to 95% pure, as estimated by analysis of silver-stained gels. InsP₆ and Ins(1,3,4,5,6)P₅ were from Calbiochem and Sigma. InsS₂ was from Sigma. Ins(1,2,3,4,6)P₅ was synthesized by G. W. Mayr. In Fig. 2E, clonal insulin-producing RINm5F cells were incubated for 5 days in 60-mm culture dishes in RPMI 1640 medium supplemented with FBS (10%). The medium was quickly removed and cells were washed three times in phosphate-buffered saline, scraped off plates in 0.4 ml of 20 mM tris-HCl buffer (pH 7.4) containing 1 mM EDTA and 2 mM dithiothreitol (DTT), and disrupted in a Polytron (Kinematica, Lucerne, Switzerland) homogenizer. Phosphoprotein phosphatase activity was determined as described (75). Assays (80-μl total volume) contained 50 mM tris-HCl (pH 7.4), 0.5 mM DTT, 1 mM EDTA, 30 μg of BSA, 1.6 μM [³²P]phosphohistone, and the appropriate concentrations of inositol polyphosphates or derivatives. After 10 min of incubation at 30°C, 0.1 ml of 14% TCA was added, and the mixture was centrifuged for 5 min at 12,000g. The radioactivity was determined in a 0.1-ml portion of the supernatant.

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21. The clonal insulin-secreting cell lines RINm5F and RIN14B were cultured in RPMI 1640 medium for 24 hours, after which the cells were washed with a solution containing 138 mM choline chloride, 5.6 mM KCl, 1.2 mM MgCl₂, 10 mM CaCl₂, 10 mM tetraethylammonium chloride, and 5 mM Hepes (pH 7.4) and then placed in this medium. The pipette solution contained 150 mM *N*-methyl-D-glucamine, 110 mM HCl, 1 mM MgCl₂, 2 mM CaCl₂, 10 mM EGTA, 3 mM Mg-ATP, and 5 mM Hepes (pH 7.15). All experiments were performed at room temperature (22° to 24°C). The whole-cell configuration of the patch-clamp technique [O. P. Hamill, A. Marty, E. Neher, B. Sakmann, F. J. Sigworth, *Pfluegers Arch. Eur. J. Physiol.* **391**, 85 (1981)] was used with an Axopatch 200 patch-clamp amplifier (Axon Instruments). Voltage steps were generated, digitized, and stored with the program pClamp (Axon Instruments) and Labmaster ADC (Scientific Solutions, Solon, OH). The current responses were filtered at 1 kHz with a Bessel filter (-3-dB point).
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Interneuron Migration from Basal Forebrain to Neocortex: Dependence on *Dlx* Genes

S. A. Anderson, D. D. Eisenstat, L. Shi, J. L. R. Rubenstein*

Although previous analyses indicate that neocortical neurons originate from the cortical proliferative zone, evidence suggests that a subpopulation of neocortical interneurons originates within the subcortical telencephalon. For example, γ -aminobutyric acid (GABA)-expressing cells migrate in vitro from the subcortical telencephalon into the neocortex. The number of GABA-expressing cells in neocortical slices is reduced by separating the neocortex from the subcortical telencephalon. Finally, mice lacking the homeodomain proteins DLX-1 and DLX-2 show no detectable cell migration from the subcortical telencephalon to the neocortex and also have few GABA-expressing cells in the neocortex.

The primary subdivisions of the forebrain, including the neocortex and the basal ganglia, have distinct molecular and cellular properties (1, 2). Previous evidence suggests that these subdivisions develop from separate proliferative zones that do not intermix (3). Here we show that cell migration occurs between the primordia of the basal ganglia and the cerebral cortex. Our results suggest that many neocortical interneurons are generated by the proliferative zone of the basal ganglia.

Neocortical neurons include two types: the excitatory pyramidal neurons and the inhibitory (GABA-containing) interneurons. During development, neocortical neurons were thought to derive from the proliferative zone of the neocortical primordium. However, studies of neuronal migration in vitro indicate that cells migrate from the lateral ganglionic eminence (LGE) (4), which is the primordium of the striatum (5), into the neocortex. Other evidence suggests that these cells might be interneurons. For example, clonally related GABA-containing cells tend to be more dispersed across the neocortex than are clones of pyramidal neurons (6); there are GABA-containing cells in the intermediate zone (IZ) at the transition between the LGE and the neocortex, which have a morphology of tangentially migrating cells (7); and interneurons migrate tangentially from the subventricular zone

(SVZ) near the cortical-striatal junction into the olfactory bulb (8).

To investigate the migration of subcortically derived cells into the neocortex, we used a slice culture preparation (9). Crystals of 1,1'-dihexadecyl-3,3,3'-tetramethylindocarbocyanine perchlorate (DiI) were placed into the LGE of embryonic day 12.5 (E12.5) mice; after 36 hours in culture, many labeled cells were detected in the neocortex (Fig. 1A). This migration begins on about E12.5, as only a few labeled cells reached the cortex from E11.5 slices that were grown in culture for 36 hours (10). Many of the DiI-containing cells in the neocortex look like tangentially migrating cells, with leading processes tipped by growth cones and a trailing process (Fig. 1, A and F).

Calbindin is present in cells resembling the tangentially oriented GABA-containing cells that are found in the IZ of the developing neocortex (7, 11). To determine whether cells migrating from the LGE into the neocortex express calbindin or GABA, DiI was inserted into the LGE of slices from E12.5 to E14.5 mice; the slices were then incubated for 30 hours and resectioned. GABA (Fig. 1, B through E) or calbindin (Fig. 1, G through J) immunofluorescence was present in about 20% of DiI-labeled neocortical cells (12).

To provide additional evidence for the migration of GABA- and calbindin-expressing cells from the subcortical telencephalon to the neocortex, we made slice cultures that were transected at the cortical/subcortical angle on one side. After 40 hours in vitro, the neocortical IZ on the transected sides had about 10 times fewer GABA- (13, 14) and calbindin-expressing cells (Fig. 2, A through C) than on the

Nina Ireland Laboratory of Developmental Neurobiology, Center for Neurobiology and Psychiatry, Department of Psychiatry and Programs in Neuroscience, Developmental Biology, and Biomedical Sciences, 401 Parnassus Avenue, University of California at San Francisco, CA 94143-0984, USA.

*To whom correspondence should be addressed. E-mail: jllr@cgl.ucsf.edu

intact sides. To study the molecular regulation of this process, we focused on the transcription factors *Dlx-1* and *Dlx-2*, which are homeobox-containing genes with virtually identical patterns of expression in the developing forebrain (15). Although their expression in the telencephalon is initially restricted to the proliferative zone of the basal ganglia primordia (16), by E13.5 they are expressed in the neocortex (10, 16). Like GABA and calbindin, DLX-1 expression is reduced in the neocortex of transected slice cultures (Fig. 2, D through F). Double labeling for DLX-1 and either GABA or calbindin revealed coexpression in tangentially oriented IZ cells (Fig. 2, G and H), which suggests that *Dlx* genes could be required for cell migration from the subcortical telencephalon to the neocortex.

Analysis of mice with a mutation in both *Dlx-1* and *Dlx-2* (*Dlx-1/2*) also suggests that these genes are required for the subcortical-to-cortical migration. Homozygous *Dlx-1/2* mutants have abnormal migration out of the LGE, resulting in an accumulation of partially differentiated neurons in the LGE, hypoplasia of the striatum, and a loss of normal olfactory bulb interneurons (9). These findings suggest that migration from the LGE to the neocortex might also be affected in the *Dlx-1/2* mutants. In the slice culture preparation, there is no detectable migration of cells from the LGE to the neocortex in E12.5 or E15.5 mutant embryos (Fig. 3).

Because migration from the LGE to the neocortex is not detectable in the *Dlx-1/2*

mutants, we predicted a reduction of GABA- and calbindin-expressing cells in their neocortexes. Indeed, early in corticogenesis and at the day of birth (P0) (when the mutants die), the number of neocortical GABA- (17) and calbindin-expressing cells (Fig. 4, A through D) is greatly reduced in the *Dlx-1/2* mutants. The expression of the synthesizing enzyme for GABA, glutamic acid decarboxylase (GAD), was also reduced in the neocortex of these mutants (Fig. 4, E and F). The reduction of interneuron markers was present throughout the neocortex and the hippocampal CA fields. However, not all cortical areas were affected by the mutation, as the GAD immunoreactivity of the paleocortex marginal zone (MZ) appeared normal (arrow in Fig. 4F).

Although the number of GABA-reactive cells in layer I of the *Dlx-1/2* mutants was reduced (17), Cajal-Retzius neurons, which facilitate radial migration within the developing neocortex, were present, as indicated by the expression of reelin and calretinin (10, 18). The analysis of cortical lamination in the *Dlx-1/2* mutants by Nissl stain and bromodeoxyuridine birth-dating (10), as well as the appearance of calbindin-reactive pyramidal neurons in layer V (Fig. 4D), suggest that the radial migration of neocortical projection neurons is unaffected in these mutants.

In summary, we have provided evidence for the migration of GABA-expressing cells from a subcortical source (the LGE) to the neocortex (19, 20). Because this migration is absent from the *Dlx-1/2* mutants, which

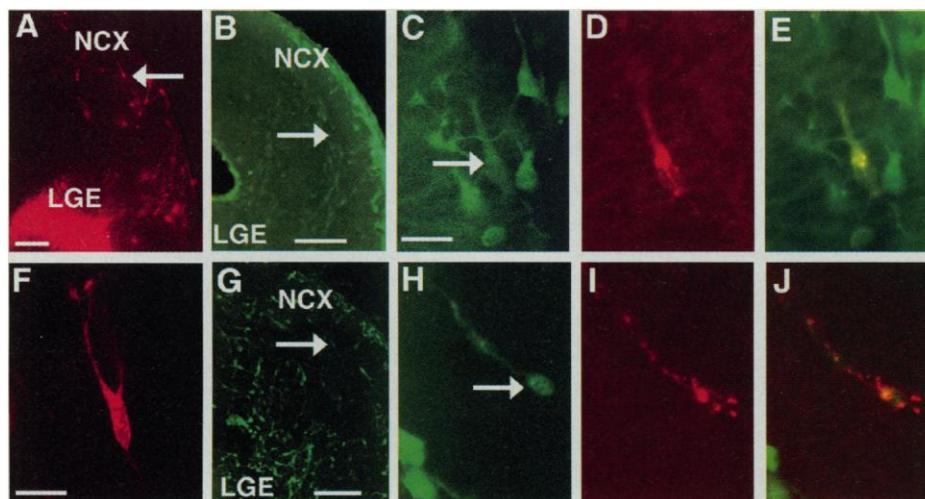


Fig. 1. Cell migration from the LGE to the neocortex (NCX) in slice cultures. (A) This slice was prepared from an E12.5 embryo and cultured for 36 hours. Many Dil-labeled cells are present in the NCX. The arrow indicates the cell shown in (F). Analysis of GABA- (B through E) and calbindin- (G through J) expressing cells in slice cultures. Slices were prepared at E12.5 with Dil placed in the LGE (12). (B and G) Low-power views of GABA (B) and calbindin (G) immunofluorescence. The arrows indicate the positions of the cells shown in (C) and (H) [(C), GABA; (H), calbindin]; these cells also contain Dil [(D) and (I)]. [(E) and (J)] Double exposures show colabeling with immunofluorescence and Dil [(E), GABA; (J), calbindin]. Scale bars, 100 μ m; except in (C) and (F), where bar = 15 μ m.



Fig. 2. Calbindin (A through C), and DLX-1 (D through F) immunoreactivity in E12.5 slices cultured after transection between the NCX and LGE on the right side. Slices were then resectioned at 10 μ m and processed for immunohistochemistry (13). (A) After 40 hours in culture, calbindin-positive cells are reduced in the transected (C) as compared with the intact (B) side of the slice. Boxed areas in (A) are shown at higher magnification in (B) and (C). (D) DLX-1 expression after 60 hours in culture; the expression in the LGE is maintained (arrow), and DLX-1 positive cells are detectable in the NCX of the intact side but not on the transected side (F). Boxed areas in (D) are shown at higher magnification in (E) and (F). (G and H) DLX-1 (dark nuclear stain) is coexpressed with GABA (G) and calbindin (H) (brown cytoplasmic stain) in the neocortical primordium of the E13.5 embryo. Arrowheads, double-labeled cells; black arrows, DLX-1 only; white arrows, calbindin (G) or GABA (H) only. Scale bars in (A) and (D), 200 μ m; in (G), 15 μ m.

Fig. 3. Comparison of cell migration out of the LGE in slice cultures from wild-type (A and C) and *Dlx-1/2* mutants (B and D). Slices were cultured for 36 hours. At E12.5 [(A) and (B)], cells in the wild-type slice (A) have migrated from the LGE into the NCX; this migration is absent in the mutant slice (B). [(C) and (D)] Slices from E15.5 animals. The Dil was photoconverted in diaminobenzidine. As at the earlier age, little or no migration into the neocortex occurred in the *Dlx-1/2* mutant slice [(D)]. v, lateral ventricle. Scale bar, 200 μ m.

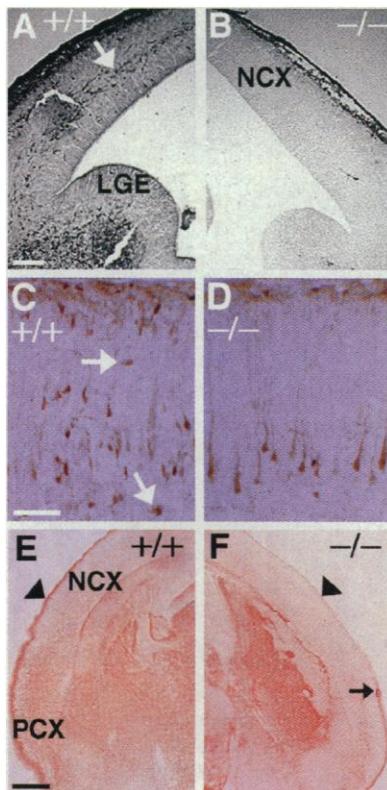
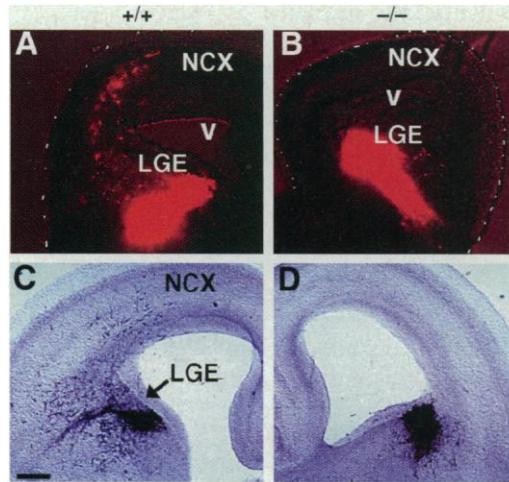


Fig. 4. Analysis of the developing NCX in the *Dlx-1/2* mutants. Immunohistochemistry for calbindin (A through D) in coronal sections. (A) At E14.5, tangentially oriented cells are present in the IZ (arrow) of the wild-type NCX. (B) The number of these cells is markedly reduced in the mutant. [(C) and (D)] At P0, calbindin-expressing pyramidal neurons are present in layer V of the mutant neocortex (D), but fewer calbindin-positive interneurons [arrows in (C)] are detectable. (E and F) At P0, GAD immunoreactivity in layer I (arrowheads) is reduced in the mutant NCX (F). This reduction occurs abruptly in the region (arrow) between the NCX and paleocortex (PCX). Scale bars, 100 μ m in (A), 50 μ m in (C), and 500 μ m in (E).

also lack most GABA-expressing cells in the olfactory bulb (9), we propose that the subcortical SVZ produces interneuron precursors for both the neocortex and the olfactory bulb (21). The residual presence of neocortical GABA-expressing cells in the mutants suggests that neocortical interneurons are derived from multiple spatially distinct sources.

Although we have previously proposed that the LGE and neocortex are independent compartmentlike structures (1), the present study demonstrates that a more complex situation exists, in which there is cell mixing between the mantle zones of these domains. We suggest that the ventricular zones (VZs) of the neocortex and LGE correspond to compartments, where the regional identity of precursor cells are specified and there is clonal restriction of precursor cells to one compartment. As cells mature and leave the VZ, specific lineages (such as interneurons and projection neurons) follow distinct differentiation programs, which include tangential migration of some interneurons to different forebrain regions.

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12. To combine cell migration experiments with immunofluorescence, plastic strips (~200 to 400 μ m in diameter) coated with Cell Tracker Dil (Molecular Probes) were placed into the LGE of E12.5 to E14.5 slices. After incubation, fixation, and vibratome resectioning (into sections 40 to 50 μ m thick), immunofluorescence was performed with the use of fluorescein-conjugated secondary antibodies (Vector). One or two cells per hemisphere (about 20%) were clearly double-labeled with Dil and with either GABA or calbindin. However, because of methodological constraints, we believe that this underestimates the percentage of Dil-labeled cells expressing GABA or calbindin. Although markers for pyramidal neurons appear grossly normal in the *Dlx-1/2* mutant neocortex (see text), it is possible that some of the cells migrating from the subcortical telencephalon to the neocortex could be pyramidal neuron precursors.
13. Immunohistochemical methods were performed as described (9). Primary antibody sources and dilutions were as follows: GABA polyclonal, Sigma, 1:5000; GAD polyclonal, Chemicon, 1:2000; calbindin polyclonal, Swiss Antibodies, 1:5000. The DLX-1 polyclonal antibody (diluted 1:20) was generated in our laboratory.
14. Counts (made with an ocular grid and expressed per square millimeter \pm SD) of GABA-positive cells in the transected slice experiment were as follows: intact side IZ, 780 \pm 139; transected side IZ, 9 \pm 19.5; intact side marginal zone (MZ), 561 \pm 141; transected side MZ, 117 \pm 65. MZ counts were complicated by background along the section edge.
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17. At E14.5, counts (per square millimeter \pm SD) of GABA-positive cells in the neocortices of sections from wild-type mice versus *Dlx-1/2* mutants were as follows: MZ, 827 \pm 138 versus 151 \pm 91.7; IZ, 788 \pm 161 versus 136 \pm 43. At P0, counts of GABA-reactive neurons in mutant versus wild-type somatosensory cortices at P0 were as follows: MZ, 1342 \pm 178 versus 363 \pm 87; cortical plate, 627 \pm 75 versus 168 \pm 42; layers V/VI, 829 \pm 63 versus 216 \pm 32 cells.
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19. The medial ganglionic eminence may also be a source of cells migrating to the neocortex.
20. These results may explain how transplanted embryonic striatal neurons are able to incorporate and differentiate in the neocortex [G. Fishell, *Development* **121**, 803 (1995); O. Brustle, U. Maskos, R. D. McKay, *Neuron* **15**, 1275 (1995)].
21. This possibility is supported by the finding that cells sharing the same progenitor can be found in the olfactory bulb and the neocortex [C. Walsh and C. L. Cepko, *Nature* **362**, 632 (1993)].
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