connected to a frame grabbing board (Scion LC3) in a Macintosh computer. Frames were acquired at ~30 ms/frame and analyzed with NIH-image.

32. Injections of fluorescent latex microspheres (Lumat) were made into the superior colliculus, 2 to 4 mm before the experiment. Injection coordinates were tailored to the age.
33. We report SD in all measurements.
34. Every trigger-follower pair was recorded with bicistron-filled electrodes to recover morphologies following standard procedures (26). Neurons were reconstructed with a ~x0/100 NA objective, and contacts were identified with a ~x100/1.3 NA objective.
35. Supplementary information is available at www.nature.com/cgi/full/293/5317/868/DC1.
36. To maximize followers’ detection, we used ACSF containing 126 mM NaCl, 3 mM KCl, 26 mM NaHCO3, 1 mM NaH2PO4, 2 mM CaCl2, 1 to 2 mM MgSO4, 10 mM dextrose, 5 mM nicotinate, 0.5 mM 4-aminoypyridine (4AP), and 0.5 mM bicuculline. Followers can also be detected with normal or Mg-free ACSF (26). Experiments were done at 27°C. One to four followers were observed per trigger neuron, and followers chosen for dual recordings were reliably detected optically in 70 ± 38% of subsequent trials. Whole-cell recordings were performed with 6 to 9 meqammon pipettes, filled with 130 mM K-methylsulfonyloctanoate, 17 mM MgCl2, 10 mM KCl, 10 mM HEPES, 5 mM NaCl, 2.5 mM Mg-adenosine triphosphate, 0.3 mM Na-guanosine triphosphate, and 0.0 to 0.05 mM fura-2 pentapotassium salt (Molecular Probes). Dual recordings were made with an Axopatch 200B (Axon Instruments) and a BVC-700 (Dagan Instruments) amplifier and digitized with an A/D board (Instrutech) with Igor (Wavemetrics). For optical probing, a stereotyped spike train was used (10 pulses adapting over 200 ms in blue in Fig. 1D).
37. For each pixel, we defined the fluorescence change over time as \( \Delta F/F = (F - F_0)/F_0 \), expressed in %, where \( F_0 \) is the baseline intensity and \( F \) is fluorescence at the beginning of each trial. We tailored the imaging and analysis protocols to the kinetics of somatic calcium signals. To detect followers online, we computed a \( \Delta F/F \) movie and adjusted the look-up table so that pixels recording calcium increases appeared lighter over a black background (Fig. 1D).
39. For each reconstructed neuron, we measured 18 morphological variables describing soma size and shape, dendrite number and total length, circular distribution of dendrites (vector average), size and shape of the dendritic autorization (tile), distribution of dendrites along the radial axis, and depth of axonal ramification (Table 1) (35). The positional data were not included in the cluster analysis. From these variables, we extracted the five principal components for follower clustering (Fig. 1B; eigenvalues > 1; Statistica) that accounted for most of the observed variance (89%); only the 11 standardized variables that made up the significant principal coordinates (absolute value > 0.7) were used. For large interneuron clustering (Fig. 2B; eigenvalues > 1), all variables were included and weighted as described below (44). Cluster analyses were performed with Ward’s method (Statistica) and squared Euclidian distances.
40. Spikes half-widths were averaged across at least 10 spikes, in each cell, and means were averaged across cells in a class.
41. To measure facilitation and depression, we averaged responses to pairs of spikes (80-ms interval) over at least 10 trials and computed the mean changes in averaged EPSP peak amplitudes across cells in a class. In some instances, three to four spikes were presented at 80-ms intervals.
42. Responses in all interneurons were blindly classified as LTS or FS on the basis of standard criteria (M. Beierlein, personal communication).
43. For random sampling of interneurons, layer 5 neurons without apical dendrites were patched. Physiological responses to 800-ms current steps were examined to further confirm the nonpyramidal identity of recorded neurons.
44. To compare follower interneurons (n = 10) with the random sample (n = 48), we measured 19 variables from all cells. Our null hypothesis, that trigger axons contact interneurons randomly, was tested by comparing the original distribution of follower types with the density distribution of each randomly selected interneuron type in the neuropil. To achieve this, we weighted each randomly selected neuron on the basis of the length of its dendrites projected onto a plane, normalized to the cell with the longest dendrites. Weights were assigned on an integer scale from 1 to 10 (10 being the longest), and the MANOVA was significant both with and without a single outlier whose dendritic lengths exceeded the mean by 4 SD (upper left cell in Web fig. 2A) (35); all remaining weighted neurons’ dendritic lengths were within 2 SD of the mean.
46. To correlate the axonal and dendritic arborizations, we constructed pairs of normalized probability density contour plots, using a 40-μm region surrounding each process, and calculated the linear correlation between plots (Matlab).
49. We thank S. Ciboni, Z. Peterlin, G. Tamás, and A. Tisza for help and members of the laboratory for comments. This work was funded by the National Eye Institute, the National Institute of Mental Health, and the National Institute of Neurological Disorders and Stroke.
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Sorting of Striatal and Cortical Interneurons Regulated by Semaphorin-Neuropilin Interactions
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Most striatal and cortical interneurons arise from the basal telencephalon, later segregating to their respective targets. Here, we show that migrating cortical interneurons avoid entering the striatum because of a chemorepulsive signal composed at least in part of semaphorin 3A and semaphorin 3F. Migrating interneurons expressing neuropilins, receptors for semaphorins, are directed to the cortex; those lacking them go to the striatum. Loss of neuropilin function increases the number of interneurons that migrate into the striatum. These observations reveal a mechanism by which neuropilins mediate sorting of distinct neuronal populations into different brain structures, and provide evidence that, in addition to guiding axons, these receptors also control neuronal migration in the central nervous system.

Most striatal and cortical interneurons derive from a distant region in the basal telencephalon, the medial ganglionic eminence (MGE) (1–3). During development, interneurons migrate tangentially along stereotypical pathways to reach the stratum and the cortex, but the mechanisms that regulate their segregation into these two telenephalic subdivisions are not known.

To study the telenephalic migration of telenephalic interneurons, we used slice cultures (4) and transplanted portions of the MGE from green fluorescent protein (GFP)-expressing transgenic mice (5) into host slices obtained from wild-type littermate embryos (Fig. 1). In agreement with previous reports (1–4), this assay consistently labeled a large number of migrating neurons whose transmission is mediated by γ-aminobutyric acid (GABA) (6). GFP-expressing cells followed two major routes in the basal telencephalon. Early migrations occurred superficial to the stratum (embryonic day 12 (E12); Fig. 1, A to DJ), whereas later migrations (E13.5 and older) occurred primarily deep to the stratum (Fig. 1, E to H). Cells migrating toward the cortex seemed to avoid the stratum (Fig. 1, A to H), raising the possibility that cortical interneurons might be instructed to avoid the stratum to promote their migration into cortical territories. To test this hypothesis, we transplanted striatal tissue into the cortex (Fig. 11). GFP-expressing cells migrating from the MGE avoided the ectopic stratum (Fig. 1, J and K), but migrated normally when a piece of piriform cortex was transplanted into the same location (Fig. 1, K to M).

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These results suggest that an extracellular signal present in the developing striatum repels cells migrating to the cortex.

Class 3 semaphorin proteins are chemorepellents for growing axons (8, 9), allowing them to avoid specific regions and channeling them into appropriate locations in fiber tracts (10). The similarity of this mechanism to our previous observations prompted us to investigate the role of semaphorins in directing interneuron migrations. We found that during the period of interneuron migration (E12 to E16), semaphorins 3A and 3F (Sema3A and Sema3F) are expressed in the striatum but are excluded from regions surrounding it (Fig. 2, A, B, E, and F). We next examined the expression of the high-affinity semaphorin receptors neuropilin1 and neuropilin2, which are required for mediating the repulsive effect of class 3 semaphorins on axons (8, 9). During the period of interneuron migration, neuropilin1 and neuropilin2 are expressed in the basal telencephalon in a pattern complementary to that of Sema3A and Sema3F—that is, in cells located either superficial or deep to the striatum but excluded from striatal cells (Fig. 2, C, D, H, and I). Comparison of this pattern with the pathways followed by migrating interneurons suggested that migrating cells might express these receptors. To test this hypothesis, we examined the expression of neuropilin1 and neuropilin2 in GFP-expressing cells or in cells containing GABA or calbindin (4). The distribution of neuropilin1 was analyzed using affinity-purified antibodies. To determine the distribution of cells expressing neuropilin2, we took advantage of the expression of β-galactosidase from the neuropilin2 locus in mice with a targeted mutation for this gene (11). The pattern of expression of semaphorin proteins and their neuropilin receptors is consistent with a model in which cortical interneurons express neuropilin1 and/or neuropilin2 while migrating to the cortex and are repelled by striatal cells expressing Sema3A and Sema3F. To test this model, we placed aggregates of semaphorin-expressing COS cells (Sema3A, Sema3F, or both) (11, 12) at the corticostriatal boundary in slice cultures and studied the migration of 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI)-labeled cells from the MGE (Fig. 3A). As a control, aggregates of GFP-expressing COS cells were placed on the opposite side of the slices (Fig. 3A). Cell aggregates expressing both Sema3A and Sema3F blocked the migration of interneurons into the dorsal cortex (Fig. 3, B to D, F, and G). In contrast, cell aggregates expressing exclusively Sema3A (which operates via neuropilin1 receptors) or Sema3F (which operates via neuropilin2 receptors) (8, 9) only partially arrested the migration of cortical interneurons (Fig. 3, E and G). Moreover, whereas migrating cells orient what appears to be their leading process toward the cortex when they approach the control aggregates (Fig. 3, H and J), most cells that approached the Sema3A/3F-expressing aggregates turn their processes away from them (Fig. 3, I and J).

We next performed loss-of-function experiments, first analyzing the migration of telencephalic interneurons in neuropilin2 mutants (11). To avoid the possible redundant functions of neuropilin1 and neuropilin2 in tangential migrations at later embryonic stages, we analyzed the pattern of tangential migration in slices derived from E12 brains. At this age, neuropilin2, but not neuropilin1, is expressed in cells tangentially migrating into the piriform cortex (Fig. 2D), suggesting that loss of neuropilin2 function at this stage should not be compensated by neuropilin1. Some of these ectopias expressed neuropeptide Y (NPY) but did not express neuropilin1 (11, 12), suggesting that at least a large subpopulation of NPY interneurons exclusively express neuropilin2.

The pattern of expression of semaphorin proteins and their neuropilin receptors is consistent with a model in which cortical interneurons express neuropilin1 and/or neuropilin2 while migrating to the cortex and are repelled by striatal cells expressing Sema3A and Sema3F. To test this model, we placed aggregates of semaphorin-expressing COS cells (Sema3A, Sema3F, or both) (11, 12) at the corticostriatal boundary in slice cultures and studied the migration of 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI)-labeled cells from the MGE (Fig. 3A). As a control, aggregates of GFP-expressing COS cells were placed on the opposite side of the slices (Fig. 3A). Cell aggregates expressing both Sema3A and Sema3F blocked the migration of interneurons into the dorsal cortex (Fig. 3, B to D, F, and G). In contrast, cell aggregates expressing exclusively Sema3A (which operates via neuropilin1 receptors) or Sema3F (which operates via neuropilin2 receptors) (8, 9) only partially arrested the migration of cortical interneurons (Fig. 3, E and G). Moreover, whereas migrating cells orient what appears to be

**Fig. 1.** Cortical interneurons avoid the striatum in their migration to the cortex. (A to H) Transplantation paradigm to analyze the migration of GFP + cells in E12 (A) and E13.5 (E) living coronal slices (B and F) or after immunohistochemistry against GFP (C and G). Arrowheads, migrating cells; asterisks, striatum (Str); dotted outlines, grafts. Scale bars, 200 μm. Schematic representations of cell migration routes are shown for E12 (D) and E13.5 (H). AEP, anterior entopeduncular region; MGE, medial ganglionic eminence; NCx, neocortex; PCx, piriform cortex. (I to M) Transplantation paradigm to analyze the migration of cortical interneurons into an ectopic striatum (eStr) (I) or piriform cortex (ePCx) (L). Dashed lines in (J) and (M), ectopic tissue; scale bar, 100 μm. Numbers of cells migrating into ectopic tissue (n = 12) are quantified in (K); histograms show averages ± SE. χ² test, *P < 0.0001.
pilin1 signaling. For this set of experiments, we adapted an electroporation method to transfect a Gfp expression vector into the MGE in slice cultures (Fig. 4A) (12). In control experiments, most GFP-expressing cells migrated superficial to the striatum on their way to the cortex (Fig. 4, B and C). In contrast, in slices from neuropilin2 mutants, most GFP-expressing cells migrated directly into the striatum (Fig. 4, D and E). These results suggest that loss of neuropilin2 signaling may result in an imbalance in the number of interneurons in the striatum or cortex. Consistent with this, the developing striatum of neuropilin2 mutants contained numerous ectopic neuropilin2-expressing cells, identified by ex-
expression of β-galactosidase from the neuropilin2 locus (Fig. 4, F and G) (12), and adult mice lacking neuropilin2 had about twice as many striatal NPY-expressing interneurons as did controls (Fig. 4, H to J) (12, 14). Thus, neuropilin2 appears to be required in vivo for sorting of migrating cortical and striatal interneurons to their correct destination.

Neuropilin1 mutant mice die by E13.5 (15), precluding the analysis of interneuron migration in these animals. To circumvent this problem, we expressed a dominant-negative form of neuropilin1 (Nrp1dn) in migrating neurons (12, 16). Coelectroporation of Gfp and Nrp1dn (17) into the MGE in slice cultures resulted in a drastic reduction in the numbers of neurons migrating into the cortex (Fig. 4, K to O) (12, 18). To eliminate the possibility that expression of Nrp1dn simply prevents the normal migration of MGE-derived cells, we coelectroporated Gfp and Nrp1dn into the MGE and cultured it in matrigel (BD Biosciences). Migration of GFP/Nrp1dn-expressing cells (17) was indistinguishable from that observed in cells electroporated with Gfp alone (12), suggesting that expression of Nrp1dn does not nonspecifically impair cell migration. Thus, signaling through neuropilin1 receptors appears also to be required for proper segregation of cortical and striatal interneurons.

Our results indicate that neuropilin receptors are required for the sorting of striatal and cortical interneurons. MGE-derived interneurons directed toward the cortex express semaphorins (neuropilin1, neuropilin2, or both) as they migrate. Striatal cells express Sema3A and Sema3F, which presumably contribute to creating an exclusion zone for interneurons migrating to the cortex, channeling them into adjacent paths. In the absence of loss-of-function data for Sema3A and Sema3F, we cannot exclude the possibility that additional neuropilin ligands expressed in the striatum also contribute to this repulsive activity. Finally, MGE-derived interneurons migrating into the striatum either never express neuropilins or down-regulate their expression before entering the striatum. We suggest that the final destination of tangentially migrating interneurons (striatum or cortex) is determined by expression of neuropilin1 and neuropilin2.

Our results implicate neuropilin receptors and their semaphorin ligands in the control of neuronal migration in the central nervous system (CNS), a role previously proposed for other axon guidance systems, such as the netrin-1/DCC and Slit/Robo (19) systems. In vitro experiments suggest that Sem3A may also pattern neural crest migration (20). We suggest that neuropilins and semaphorins sort and channel different populations of migratory neurons into distinct paths. The creation of selective exclusion zones for subpopulations of migrating neurons may represent a general role for neuropilins and semaphorins in the formation of functional boundaries between different neuronal populations during development of the CNS.

References and Notes
7. Regions ventral to the MGE (the anterior entopeduncular region and the preoptic area) also appear to contribute to this early and superficial migration to the cortex.
12. Supplementary figures and details of experimental procedures are available at Science Online at www.sciencemag.org/cgi/content/full/293/5531/872-D1C1.
14. Approximately 30% of the cases (4 of 14).
17. Analysis of the expression of GFP and Nrp1dn in slice cultures (n = 5) demonstrates extensive (~95%) coelectroporation of both plasmids.
18. Numbers of labeled cells per cortex were 60.4 ± 2.9 (average ± SE) in control slices, 12.9 ± 3.2 in GFP + Nrp1dn slices. \( x^2 \) test, \( P < 0.001 \) (n = 14).
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Fig. 4. Loss of neuropilin function perturbs the migration of cortical interneurons. (A) Experimental paradigm. (B and D) Migration of cells electroporated with a Gfp expression vector (\( n = 8/8 \)). (C and E) Schematic representation of migratory routes. (F and G) X-Gal staining of coronal sections through the telencephalon (\( n = 6/6 \)). (H and I) NPY immunohistochemistry. (J) Quantification of the number of NPY+ cells in the striatum of Nrp2+/- and Nrp2-/- mice. \( x^2 \) test, *\( P < 0.001 \) (n = 4). (K) Experimental paradigm. (L and N) Migration of cells electroporated with a Gfp expression vector alone (L) or with Gfp and Nrp1dn expression vectors (N). (M and O) Schematic representation of migratory routes. Dotted lines, slice outline; P/Sp, pallial-subpallial boundary; Pd, pallidum. Scale bar, 200 μm.