Ezh2 Orchestrates Topographic Migration and Connectivity of Mouse Precerebellar Neurons

Thomas Di Meglio et al.

*Science* **339**, 204 (2013);

DOI: 10.1126/science.1229326

---

If you wish to distribute this article to others, you can order high-quality copies for your colleagues, clients, or customers by clicking here.

Permission to republish or repurpose articles or portions of articles can be obtained by following the guidelines here.

The following resources related to this article are available online at www.sciencemag.org (this information is current as of September 8, 2013):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:

http://www.sciencemag.org/content/339/6116/204.full.html

Supporting Online Material can be found at:

http://www.sciencemag.org/content/suppl/2013/01/10/339.6116.204.DC1.html

This article cites 36 articles, 9 of which can be accessed free:

http://www.sciencemag.org/content/339/6116/204.full.html#ref-list-1

This article has been cited by 1 articles hosted by HighWire Press; see:

http://www.sciencemag.org/content/339/6116/204.full.html#related-urls

This article appears in the following subject collections:

Neuroscience

http://www.sciencemag.org/cgi/collection/neuroscience
Ezh2 Orchestrates Topographic Migration and Connectivity of Mouse Precerebellar Neurons

Thomas Di Meglio,1* Claudius F. Kratochwil,1,2* Nathalie Vilain,1 Alberto Loche,1,2 Antonio Vitobello,1,2 Keisuke Yonehara,3 Steven M. Hrycaj,3 Botond Roska,1,2 Antoine H. F. M. Peters,1,2 Anne Eichmann,3,4 Deneen Wellik,5 Sebastien Ducret,1 Filippo M. Rijli1,2†

We investigated the role of histone methyltransferase Ezh2 in tangential migration of mouse precerebellar pontine nuclei, the main relay between neocortex and cerebellum. By counteracting the sonic hedgehog pathway, Ezh2 represses Netrin1 in dorsal hindbrain, which allows normal pontine neuron migration. In Ezh2 mutants, ectopic Netrin1 derepression results in abnormal migration and supernumerary nuclei integrating in brain circuitry. Moreover, intrinsic topographic organization of pontine nuclei according to rostrocaudal progenitor origin is maintained throughout migration and correlates with patterned cortical input. Ezh2 maintains spatially restricted Hox expression, which, in turn, regulates differential expression of the repulsive receptor Unc5b in migrating neurons; together, they generate subsets with distinct responsiveness to environmental Netrin1. Thus, Ezh2-dependent epigenetic regulation of intrinsic and extrinsic transcriptional programs controls topographic neuronal guidance and connectivity in the cortico-ponto-cerebellar pathway.

We thank the authors thank C. Reisenman, R. Alarcón, A. Dacks, G. Davidson, and J. Bronstein for advice and assistance; and M. Dickinson and H. Bradshaw for manuscript comments. This work was supported by NSF grants IOS 0822709 to J.A.R. and CHE 0216226 to L.A., and NIH grant R01-DC-02751 to J.G.H.

Acknowledgments: The authors thank C. Reisenman, R. Alarcón, A. Dacks, G. Davidson, and J. Bronstein for advice and assistance; and M. Dickinson and H. Bradshaw for manuscript comments. This work was supported by NSF grants IOS 0822709 to J.A.R. and CHE 0216226 to L.A., and NIH grant R01-DC-02751 to J.G.H.

Supplementary Materials www.sciencemag.org/cgi/content/full/science.1225483/DC1 Materials and Methods

Fig. 1. Ezh2 non–cell autonomous role in pontine neuron tangential migration. (A, B, E, F, and G) Migratory phenotypes in control (A) and (B) and r5-6::Cre;Ezh2fl/fl mutants (E) to (G). Barh1/t in situ hybridization in E14.5 whole-mount (A) and (E), E16.5 (B) and (F), and E18.5 (G) sagittal sections. Pontine gray and reticulotegmental [arrowheads (G)] nuclei (PNs) are duplicated (PN9s). (C and H) Tracings from P7 cortex (rabies-ΔG-eGFP) and cerebellum (rabies-ΔG-mCherry) in controls (C) and r5-6::Cre;Ezh2fl/fl mutants (H). PNs and PN9s are connected to cortex and cerebellum. (D, J, and K) Barhl1/Netrin1 expression in E14.5 control (D), r5-6::Cre;Ezh2fl/fl (J), rSpast::Cre;Ezh2fl/fl;Shhfl/+(K), and rSpast::Cre;Ezh2fl/fl;Shhhfl/+ (J) coronal sections. Ectopic Ntn1 [arrowheads: (I) and (K)] and PN9 ectopic migration [arrow and white arrowhead, respectively: (I) and (K)] are partially rescued (J).

References (22–36)

Published online 6 December 2012; 10.1126/science.1225483

1 Fédéric Miescher Institute for Biomedical Research, Maulbeerstrasse 66, 4058 Basel, Switzerland. 2University of Basel, 4056 Basel, Switzerland. 3Yale Cardiovascular Research Center, Department of Internal Medicine, Yale University School of Medicine, New Haven, CT 06511–6664, USA. 4CRIB (Centre Interdisciplinaire de Recherche en Biologie), Inserm U1050, 11 Place Marcelin Berthelot 75005 Paris, France. 5Department of Cell and Developmental Biology, University of Michigan, Ann Arbor, MI 48109–2200, USA.

*These authors contributed equally to this work. †To whom correspondence should be addressed. E-mail: filippo.rijli@fmi.ch
and r5, which do not contribute to the pontine migratory stream (3), resulted in small ectopic PNs in posterior r5 (PNs) (fig. S5), which supports an Ezh2 non–cell autonomous role. Deletion in r5 and r6 (r5–6:Cre;Ezh2fl/fl) resulted in a more prominent phenotype. A neuronal subset split from the migratory stream, turned ventrally, and generated an ectopic duplication of PNs (PNs) (fig. 1, E to G). PNs were nonrecombined Ezh2+/+ H3K27me3+ located within the r5- and r6-derived territory mostly devoid of H3K27me3 (fig. S2), which confirmed Ezh2’s non–cell autonomous function.

To assess whether PN’s integrated cortico-cerebellar connectivity, we carried out cortex-to-PN and cerebellum-to-PN tracings. We injected viral constructs expressing green fluorescent protein (GFP) (rabies-ΔG-GFP) and/or mCherry (rabies-ΔG-mCherry) in postnatal day 2 (P2) wild type and r5–6:Cre;Ezh2fl/fl and Krox20::Cre;Ezh2fl/fl mutants. At P7, PNs and PNs’s triggered collateralization of corticospinal axons and innervated the cerebellum (fig. 1, C and H, and fig. S6).

To evaluate Ezh2 cell-autonomous function, we used the Wnt1::Cre deleter (10). Ezh2 transcripts and H3K27me3 were selectively deleted from lower rhombic lip and migratory stream in Wnt1::Cre;Ezh2fl/fl mutants (figs. S2 and S7). Nonetheless, the mutation was not sufficient to induce ectopic posterior pontine neuron migration (fig. 2, J and M, and figs. S5 and S7). The most severe phenotype was observed in Hoxa2::Cre;Ezh2fl/fl mutants, where Ezh2 was inactivated in both AES neurons and their migratory environment, i.e., throughout r3- to r6- and dorsal r7- to r8-derived structures including the lower rhombic lip (figs. S2 and S3). The whole AES did not migrate anterior to r6 and settled into a single posterior ectopic nucleus (PNs) (fig. S5). Thus, Ezh2 has a non–cell autonomous role in AES migration, which is enhanced by a cell-autonomous function in progenitors and migrating neurons.

Netrin1 (Netn1) and Slit1-3 are attractive or repulsive secreted cues that influence pontine neuron migration (6, 7). Slit1-3 expression was not altered in embryonic day 14.5 (E14.5) r5–6:Cre;Ezh2fl/fl mutants (fig. S8). In contrast, Netn1, normally expressed in floor-plate and ventral ventricular progenitors (fig. 1D and fig. S5) (11), was ectopically expressed in dorsal progenitors and the mantle layer medi ally to the AES in r5–6:Cre;Ezh2fl/fl, Krox20::Cre;Ezh2fl/fl, Hoxa2::Cre;Ezh2fl/fl, and r7post::Cre;Ezh2fl/fl mutants (fig. 11 and fig. S5). Additional deletion of Shh was sufficient to prevent strong ectopic Netn1 activation in dorsal progenitors of E12.5 r7post::Cre;Ezh2fl/fl conditional mutants (fig. S5). At E14.5, ectopic Netn1 was almost undetectable and the Ezh2 knockout was partially rescued (fig. 1, J and K, and fig. S5). Therefore, Ezh2 is required to restrict Netn1 expression to ventral progenitors by silencing Netn1 in the dorsal neural tube. However, Ezh2 deletion is not sufficient to ectopically induce Netn1, which additionally requires Shh signaling from the floor plate. Thus, in the dorsal neural tube, Ezh2-mediated epigenetic repression of Netn1 may normally counteract Shh-mediated activation.

Ectopic and/or increased environmental Netn1 levels may trigger premature migration toward the midline. In E14.5 r5–6:Cre;Ezh2fl/fl mutants, only a subset of pontine neurons split from the stream and entered the alternative ventral migratory pathway at the level of the ectopic Netn1+ domain (fig. 11 and fig. S5), which suggested that AES neurons may display intrinsic differential responsiveness to Netn1 signaling. To map the contributions of r6 (r6RLp) or r7 and r8 (r7–8RLp) lower rhombic lip–derived neuronal progenies into the pontine stream and nuclei (fig. 2, B, C, and H, and fig. S3), we crossed floxed reporter lines to r5–6:Cre or r7post::Cre (Cre is expressed up to the r6–7 boundary) (fig. S3H) in which Cre is down-regulated before AES migration (fig. S4). r6RLp mapping was confirmed by the tamoxifen-inducible MafB::CreERT2 transgenic line, whose reporter expression pattern is restricted to r5 and r6, similar to that in r5–6:Cre (10) (fig. S3). To trace the whole precerebellar lower rhombic lip progeny (r6–8RLp), we used r7post::Cre (figs. S3A and S4).

r6–8RLp contributed to the whole PNs (fig. S3), whereas r6RLp mapped to the most anterior (arrow in fig. 2H and fig. S3), and r7–8RLp filled the remaining posterior portions of PNs (fig. S3). This topographic organization of pontine neuronal subsets directly correlated with their relative position within the migratory stream. Namely, r6RLp mapped to the dorsalmost AES, whereas r7–8RLp contributed to the remaining portion ventrally to r6RLp (fig. 2, B and C). Thus, the precerebellar lower rhombic lip is rostrally mapped onto the AES dorsoventral axis (fig. 3E and fig. S1J) and, in turn, onto the PN rostrocaudal axis (fig. S1K), with neuronal subsets maintaining their relative position throughout migration and settling.

Next, we investigated molecular correlates of this intrinsic cellular regionalization and asked whether Hox paralog groups (PG) 2 to 5 maintain their spatially restricted progenitor expression patterns in pontine migratory stream and nuclei (fig. 2). Indeed, Hox PG2 (Hoxa2/Hoxb2) and PG3 (Hoxa3/Hoxb3), expressed in the whole precerebellar rhombic lip, were correspondingly maintained throughout the pontine migratory stream and nuclei (6) (fig. S1). Hoxb4 is normally expressed up to the r6–7 boundary, whereas the Hox PG5 rostral expression limit is posterior to PG4 genes (2). In the AES and PNs, Hoxb4+ neurons extended just ventrally and posteriorly, respectively, to r6RLp (fig. 2, C and D, and fig. S1L), whereas Hoxa5 and Hoxb5 transcripts and Hoxa5 protein mapped to the ventromost migratory stream and posteriormost PNs, respectively (fig. 2, A, F, H and I, and figs. S1 and S2). Simultaneous detection of Hoxa5 and ZsGreen in r5–6:Cre;R26R::ZsGreen specimens demonstrated rostralcaudal segregation of r6RLp and Hoxa5+ neurons within the PNs (fig. 2H). To permanently label Hoxa5-expressing neurons, we generated a transgenic line in which Cre was inserted in-frame at the Hoxa5 locus (Hoxa5::Cre) (10) (fig. S3). Hoxa5::Cre-expressing neurons segregated to the ventromost AES and posteriormost PNs, faithfully overlapping endogenous Hoxa5 distribution (fig. 2, D, E, F and G, and figs. S1F, S3, and S4). Thus, pontine neuron subsets of distinct rostralcaudal origin maintain their relative topographic positions and Hox codes throughout migration and settling within the target nucleus (fig. 4A and fig. S1).
Is Ezh2 required to maintain Hox nested expression in migrating pontine neurons and PNs? In E14.5 Wnt1::Cre;Ezh2<sup>−/−</sup> and Hoxa2::Cre;Ezh2<sup>−/−</sup> mutants, Hoxb4, Hoxa5, and Hoxb5 were ectopically expressed within the anterior lower rhombic lip and spread ventrodorsally throughout the pontine migratory stream (Fig. 2, L and M, and figs. S2 and S7). Thus, by preventing Hox<sub>PG4</sub> and PG5 expression in anterior precerebellar rhombic lip and migrational neuronal progeny, Ezh2-mediated repression contributes to the maintenance of molecular heterogeneity in the migratory stream. This, in turn, may underlie intrinsic differential response of migrating neuron subsets to environmental Ntn1.

Netrin-mediated attraction is counteracted by Unc5 repulsive receptors (12), and Unc5c inactivation results in variable ectopic migration of AES. Unc5c/eGFP strongly reduces at E18.5 ectopically migrating PN<sup>−</sup> neurons (K), as compared with EP of eGFP (J), and partially rescues the phenotype. ML, ventral midline. (L to Q) In E13.5 wild type, EP of Ntn1 results in posterior ectopic pontine neuron migration at E17.5, phenocopying r5-6::Cre;Ezh2<sup>−/−</sup> mutants (arrowheads (L)). Although EP at E13.5 of eGFP (M) or Unc5c/eGFP constructs has no apparent effect on migration at E18.5 (N), EP of Unc5b/eGFP results in anterior ectopic migration and/or dorsal-lateral arrest (arrowheads (O)). Immunostaining on sagittal sections shows that anterior ectopic GFP+/Unc5b<sup>−</sup> electroporated cells are Hoxa5-negative (P); Hoxa5<sup>+</sup> cells are normally restricted in posterior PN (Q).

### Fig. 3. Ezh2- and Hox-dependent regulation of Unc5b in pontine neuron migration. (A and B) X-gal (green) and Pax6 (red) costainings of E14.5 Unc5b<sup>Gal/−</sup> heterozygotes (A) and Unc5b<sup>−/−</sup> homozygotes (B) showing X-gal–stained cell distribution in AES (arrowheads). (C to E) Unc5b<sup>−/−</sup>/Barhl1<sup>−/−</sup> (C) and Hoxa5<sup>−/−</sup>/Barhl1<sup>−/−</sup> (D) in situ hybridization in E14.5 AES showing complementary dorsoventral expression of Unc5b and Hoxa5 (arrowheads) and summary (E). (F and G) In r5-6::Cre;Ezh2<sup>−/−</sup> mutants, PN<sup>−</sup> migrating neurons are Unc5b-negative (F) and Hoxa5<sup>−/−</sup>/Barhl1<sup>−/−</sup> (G) (arrowheads). (H and I) In E14.5 Hoxa5<sup>−/−</sup>/Hoxb5<sup>−/−</sup>/Hoxc5<sup>−/−</sup> AES, Unc5b is up-regulated ventrally (l), whereas Hoxb4 and Pax6 are normally expressed (H). (J and K) In utero EP in E14.5 r5-6::Cre;Ezh2<sup>−/−</sup> mutants of Unc5b/Unc5c/eGFP strongly reduces at E18.5 ectopically migrating PN<sup>−</sup> neurons (K), as compared with EP of eGFP (J), and partially rescues the phenotype. ML, ventral midline. (L to Q) In E13.5 wild type, EP of Ntn1 results in posterior ectopic pontine neuron migration at E17.5, phenocopying r5-6::Cre;Ezh2<sup>−/−</sup> mutants (arrowheads (L)). Although EP at E13.5 of eGFP (M) or Unc5c/eGFP constructs has no apparent effect on migration at E18.5 (N), EP of Unc5b/eGFP results in anterior ectopic migration and/or dorsal-lateral arrest (arrowheads (O)). Immunostaining on sagittal sections shows that anterior ectopic GFP+/Unc5b<sup>−</sup> electroporated cells are Hoxa5-negative (P); Hoxa5<sup>+</sup> cells are normally restricted in posterior PN (Q).

### Fig. 4. PN regionalization and patterned cortical input. (A, B, D, E, and F), Hox expression summary in migrating pontine neurons of control (A) and r5-6::Cre;Ezh2<sup>−/−</sup> mutants (D). Barhl1/Hoxb5 in situ hybridization on E17.5 sagittal sections (B), (E), and (F). In r5-6::Cre;Ezh2<sup>−/−</sup> mutants (E) and (F), Hoxb<sup>−</sup> neurons spread throughout the rostrocaudal extent of the ectopic nuclei (PNs) [arrowheads (F)], whereas, in PNs, they are normally posteriorly restricted as in control [arrowheads: (B) and (E)]. Barhl1/Hoxb<sup>−</sup> viruses injected in control visual/medioposterior cortex (MPC) and SSC anterogradely trace fibers into anterior (green, *) and posterior (red, arrow) PNs (C), respectively. In r5-6::Cre;Ezh2<sup>−/−</sup> mutants (G), PN<sup>−</sup> lacks innervation by MPC, whereas it is innervated by SSC (arrow). (H) Ezh2- and Hox-dependent genetic circuitry of intrinsic and extrinsic Unc5b/Ntn1 regulation.
neurons (13). Unc5c is expressed in lower rhombic lip progenitors, down-regulated in migrating neurons, and reactivated upon approaching the midline (fig. S9) (13). Thus, Unc5c is unlikely to confer a dorsoventrally biased response of the AES to Ntn1. Unc5b has been involved in vascular development (14), though a role in neuronal development was not explored. We found a dorsoventral high-to-low density of cells expressing Unc5b (Fig. 3C and fig. S9) and β-galactosidase activity in Unc5b/GalGal"fetuses (Fig. 3A). Unc5b expression was, in turn, down-regulated when the migratory stream turned toward the midline (fig. S9C). Dorsoventral Unc5b transcript distribution in the migratory stream anti-correlated with Hox PG5 expression (Fig. 3, C and D). In E14.5 Hoxa5+/−;Hoxb5+/−;Hoxc5+/− mutant embryos, Unc5b was up-regulated in ventral Hoxb4/Pax6 AES neurons (Fig. 3, H and I). Thus, Hox PG5 normally represses Unc5b in ventral AES neurons originating from posterior precerebellar lower rhombic lip.

In E14.5 Unc5b/GalGal" null mutants, β-galactosidase−cells partially lost their normal dorsolateral restriction and spread into ventral AES (Fig. 3B). Thus, Unc5b contributes to maintaining topographical organization of dorsal AES subsets. In E16.5 Unc5c+− fetuses, dorsal Unc5b-expressing AES neurons maintained their normal migratory path, whereas ectopic neurons were Hox PG5− and mainly Unc5b-negative (fig. S9). Therefore, in the absence of Unc5c, ventral Unc5b-negative AES neurons become more sensitive to Ntn1-mediated attraction than dorsal Unc5b-expressing neurons. Similarly, in r5-6−:Cre;Ezh2+/− mutants, Unc5b-expressing neurons remain dorsal and pursue their normal migration, whereas Hox PG5+/Unc5b-negative neurons are preferentially influenced by Ntn1 up-regulation and ectopically attracted to the midline (Fig. 3, F and G, and fig. S2). Moreover, in Hox2a−:Cre;Ezh2+/− mutants, in which all pontine neurons are Ezh2−/−Mik2Tmem3 expressing and migrate through an environment ectopically expressing Ntn1 (fig. S5), all migrating neurons are prematurely attracted to an ectopic posterior midline position, are Hox PG5−, and down-regulate Unc5b (figs. S2 and S7).

Next, Unc5b/Gal expression by in utero electroporation (EP) of E14.5 lower rhombic lip progenitors was sufficient to cell-autonomously rescue the PN− phenotype in r5-6−:Cre;Ezh2+/− mutant embryos [enhanced GFP (+eGFP)] neuron quantification in PN's compared with PNs: eGFP (n = 5) 33.79% ± 0.1060; eGFP/Unc5b/S (n = 5) 0.64% ± 0.0044; P = 0.00011] (Fig. 3, J and K), which demonstrated that elevating Unc5c receptor levels counteracts increased Ntn1-mediated attraction. Ntn1 overexpression by EP in E13.5 wild-type fetuses induced ectopic posterior migration of AES neurons (Fig. 3L), partially phenocopying the r5-6−:Cre;Ezh2+/− mutant phenotype and showing that increasing Ntn1 is sufficient to cause ectopic ventral migration of neuronal subsets.

Furthermore, although overexpression of Unc5c in E13.5 wild-type fetuses had no apparent effect on AES migration (Fig. 3, M and N), Unc5b EP triggered ectopic anterior migration and/or a block in dorsal position of Hoxa5-negative pontine neuron subsets (Fig. 3, O to Q). Therefore, maintaining constitutively high Unc5b levels in migrating neurons prevents or delays turning toward the midline; the latter results in ectopic anterior migration. Conditional Hoxa2 overexpression in rhombic lip derivatives by mating Wnt1::Cre with a ROSA26::lox-stop-loxHoxa2 internal ribosome entry site (IRES)-eGFP (Wnt1::Cre;R26R;Hoxa2) allele (10) also resulted in anterior ectopic migration generating rostrally elongated PNs, which maintained high Unc5b expression, unlike in control mice (fig. S9). Therefore, although Hox PG5 are involved in negatively regulating Unc5b in the ventral migratory stream, Unc5b expression in dorsal AES may be under Hox PG2-positive regulation and generates differential responses to environmental Ntn1.

Finally, we investigated whether the PN and PN− patterning differences in r5-6−:Cre;Ezh2+/− mutants result in distinct cortical inputs. In P7 Pept2−:Cre;R26R;Tomato animals (10) expressing Cre in medioposterior (including visual) cortex (MPC), tdTomato+ axons projected onto the rostral PN, in agreement with (15), including the r6RL neuronal subset (Fig. S6). Projection of rabbits-ΔG-GFP and rabbits-ΔG-mCherry into visual and/or MPC and medial somatosensory cortex (SSC) resulted in rostral GFP+ and caudal mCherry+ animal inputs onto the PNs, respectively (Fig. 4C). In r5-6−:Cre;Ezh2+/− mutants, PN was targeted both by visual or MPC-derived GFP (rostally) and SSC-derived mCherry (caudally) axons, whereas PN− was innervated by SSC-derived though not visual/MPC-derived axons (Fig. 4G), correlating with their posterior Hox PG5+ profile (Fig. 4, A, B, and D to F).

During radial migration, correlation to rostrocaudal position of origin is maintained through interaction with glial progenitors (16). How long-range tangentially migrating neurons (17) maintain information about their origin is less well understood. We show that the topographic migratory program of r6− to r8-derived pontine neurons is largely established in progenitor pools according to rostrocaudal origin and maintained in migrating neurons. We found similar organizational principles during lateral reticular nucleus migration (fig. S10). Moreover, the r2 to r5 rhombic lip also gives rise to neurons that migrate tangentially along a short dorsoventral extramural path and populate distinct brainstem cochlear nuclei with a rostrocaudal topography (3). On its caudoral route, the precerebellar stream migrates ventrally to the cochlear stream (3), although they do not mix despite close cellular proximity, which suggests that rhombomere-specific programs may control appropriate precerebellar neuron position during tangential migration. Indeed, we show that the topography of r6 versus r7 versus r8 origin is preserved throughout migration, mapped along the dorsoventral axis of the pontine stream, and eventually within rostrocaudal subregions of the PNs, correlating with patterned cortical input. The transcriptional regulation of this tangential migratory program is epigenetically maintained (Fig. 4H). Ezh2-mediated repression maintains dorsoventrially restricted environmental distribution of attractive and/or repulsive cues, such as Ntn1, and an intrinsically heterogeneous Hox transcriptional program in the migratory stream, in turn, provides neuronal subsets with distinct Unc5b-dependent responses to environmental Ntn1, and thus contributes to maintaining the neuronal position during migration.

References and Notes
10. Materials and methods are available as supplementary materials on Science Online.
12. K. Hong et al., Cell 97, 927 (1999).

Acknowledgments: We thank K. Balint, F. Boukhtouch, Y.-Y. Lee, C.-Y. LIang, D. Kraus, T. Mathivet, F. Santagati, J. F. Spetz, and A. Yallowitz for technical support and discussion. We are grateful to M. Tessier-Lavigne, S. H. Orkin, E. Callaway, V. Castellani, P. Molehn, O. Naby, and J. Haigh for gifts of mouse lines, probes, or reagents. The mouse transgenic lines r5-6−:Cre, Hoxa2-Cre, cRtpost-Cre, r7post-Cre, MafB-CreERT2, Hoxa5-Cre, ROSA26::lox-stop-loxHoxa2-IREs-EGFP generated in this research and their respective DNA constructs are available from F.M.U.R. under a material transfer agreement with the Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland. The SAD G-eGFP and SAD G-mCherry G−deleted rabies viruses used in this research are available from E. M. Callaway under a material transfer agreement with Salk Institute for Biological Studies, La Jolla, CA, USA. T.D. is the recipient of a European Molecular Biology Organization Long-Term Fellowship. Work in F.M.U.R.'s laboratory is supported by the Swiss National Science Foundation (Sinergia CRSI3_127440), Association pour la Recherche sur la Scérose en Plaques, and the Novartis Research Foundation.

Supplementary Materials
www.sciencemag.org/cgi/content/full/339/6116/204/DC1
Materials and Methods
Figs. S1 to S10
References (10–37)
27 August 2012; accepted 7 November 2012
10.1126/science.1229326