Motor training compensates for cerebellar dysfunctions caused by oligodendrocyte ablation

Ludovic Collin, Alessandro Usiello, Eric Erbs, Carole Mathis*, and Emiliana Borrelli†

Institut de Génétique et de Biologie Moléculaire et Cellulaire, Department of Molecular Neurobiology, Centre National de la Recherche Scientifique/Institut National de la Santé et de la Recherche Médicale/Université Louis Pasteur, 1 Rue Laurent Fries, 67404 Illkirch Cedex, Communauté Urbaine de Strasbourg, France

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The role played by oligodendrocytes (OLs), the myelinating cells of the CNS, during brain development has not been fully explored. We have addressed this question by inducing a temporal and reversible ablation of OLs on postnatal CNS development. OL ablation in newborn mice leads to a profound alteration in the structure of the cerebellar cortex, which can be progressively rescued by newly generated cells, leading to a delayed myelination. Nevertheless, the temporal shift of the OL proliferation and myelinating program cannot completely compensate for developmental defects, resulting in impaired motor functions in the adult. Strikingly, we show that, despite these abnormalities, epigenetic factors, such as motor training, are able to fully rescue cerebellar-directed motor skills.

The CNS is composed of neurons and glial cells. Among glial cells, astrocytes and oligodendrocytes (OLs) possess pleiotropic functions. Unexpectedly, these cells have been recently reported to participate in key events such as axonal organization (1), survival (2), and synaptogenesis (3, 4), indicating that their function is still far from being completely known.

OLs are devoted to myelin sheath formation around most axons in higher vertebrates. Defective myelination has been largely documented in human diseases and in animal models (5), caused by genetic or still unidentified origins. Myelination is nevertheless a specialized function of mature OLs, leaving the role of these cells, during development, still only partially explored.

To analyze the impact of OL ablation on the development and functional organization of the CNS, we used myelin basic protein (MBP)-thymidine kinase (TK) transgenic mice (1, 6). In these transgenics, temporal OL killing is achieved through the expression of the herpes virus 1 TK gene under the control of the MBP gene promoter (6). TK toxicity is induced by systemic administration of the nucleoside analog 1-(2-deoxy-2-fluoro- β -D-arabinofuranosyl)-5-iodouracil (FIAU) only in dividing OLs (7). The inducibility of this system (1, 6, 8) enabled us to analyze the eventual recovery of OLs and myelin after the arrest of FIAU-induced toxicity in MBP-TK mice. We chose the cerebellum for our analyses, because its development is coincident with the bulk of OL proliferation and myelination. We report that OL ablation during the first 6 postnatal days (p1–p6) is sufficient to heavily alter the organization of the cerebellar cortex and its final size in adult mice. Importantly, once FIAU treatment has ceased, proliferation of OLs and myelination is resumed. This process leads to a delayed myelination program, causing a recovery of OLs and myelin content. Interestingly, by performing behavioral analyses we were able to follow the rescue of motor skills, which were severely affected by the loss of OLs. We found that the motor phenotype induced by OL ablation can be only partially rescued by delayed OL proliferation and myelination. Strikingly, we show that to obtain full motor rescue animals need to be submitted to intensive motor training. Thus, training is a crucial therapeutic factor for the complete restoration of motor coordination upon a demyelination event followed by delayed myelination.

Methods

Animals. WT and MBP-TK siblings, in a C57/Bl6 background, were treated daily with FIAU (40 mg/kg s.c.) from p1 to p6 (6).

Immunofluorescence and Quantifications. After deep anesthesia, mice were perfused transcardially with 4% paraformaldehyde in PBS. Brains were postfixed, and 100- μ m vibratome sections were made. Sections were blocked in 5% normal goat serum in PBS, 0.1–0.3% Triton X-100. The antibodies used were: mouse anti-MBP (67–74) (1:1,000), anti-NeuN (1:200), and guinea pig anti-GLAST (1:4,000) (Chemicon), mouse anti-glial fibrillary acidic protein (GFAP) (1:800, ICN), mouse anti-synaptobrevin-2 (VAMP-2) (1:1,000, Synaptic Systems, Göttingen, Germany), and rabbit anti-calbindin D-28k (1:1,000, Swant, Bellinzona, Switzerland). Fluorescent secondary antibodies used were: goat anti-mouse, goat anti-rabbit IgG conjugated with Alexa Fluor 350, 488, or 594 (1:800, Molecular Probes), and goat anti-guinea pig conjugated to Cy3 (1:200, Chemicon). Nuclei were stained with 4',6-diamidino-2-phenylindole. Immunolabeled sections were examined with a Zeiss Axiophot or a confocal microscope (DMRE, Leica, Heidelberg, Germany). Controls were always performed by omitting primary antibodies. At least three different animals per genotype and time point were analyzed, and experiments were repeated three times. Quantification of TK-positive cells was performed on 10- μ m serial sections from six different animals in folia V of the cerebellar vermal region of treated and untreated MBP-TK mice. Number of cells per field of view (FOV) have been reported (FOV = 0.141 mm²). Cerebellar surfaces were calculated by an in-house-developed software. For this calculation, 10 sections (10 μ m) of WT (n = 3) and MBP-TK (n = 3) treated cerebella were used.

Fluorescence Quantification. Quantification of VAMP-2 immunostainings of vesicular presynaptic compartments was accomplished by using an unbiased counting method. An in-house-developed software was used to estimate the intensity of VAMP-2 expression by determining the corresponding number of pixels in samples from the upper part of the molecular layer (ML) of WT and MBP-TK cerebella (2,162 μ m²). A minimum of three independent areas was analyzed for each animal (n = 3).

Immunoblot Analysis. Cerebella from WT and MBP-TK mice (p21 and p60) were rapidly dissected and homogenized in RIPA

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Abbreviations: OL, oligodendrocyte; MBP, myelin basic protein; TK, thymidine kinase; FIAU, 1-(2-deoxy-2-fluoro- β -D-arabinofuranosyl)-5-iodouracil; p(n), postnatal day; GFAP, glial fibrillary acidic protein; PC, Purkinje cell; BG, Bergmann glia; IGL, inner granular layer; ML, molecular layer.

*Present address: Department of Developmental Neurobiology, The Rockefeller University, 1230 York Avenue, New York, NY 10021.

†To whom correspondence should be addressed. E-mail: eb@igbmc.u-strasbg.fr.

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buffer (150 mM NaCl/50 mM Tris, pH 8/1% Nonidet P-40/0.5% deoxycholate/0.1% SDS/5 μ g/ml PMSF/1 mM NaF/1 mM Na_2VO_4 /1 μ g/ml leupeptin/1 μ g/ml aprotinin). Fifteen micrograms of proteins extracts was separated by SDS/PAGE and transferred to nitrocellulose membranes. Membranes were blocked in 5% skim milk in 1 $\times$ PBS/0.1% Tween 20 and incubated overnight at 4°C with primary antibodies. Membranes were then incubated and developed as described (1). Mouse anti-MBP (antibody reference number 67–74) (1:5,000) and mouse anti-calbindin D-28k (1:3,000, Sigma) antibodies were used. To verify for equal protein loading, membranes were stripped and incubated with rabbit anti- β -tubulin (1:1,000, Sigma).

Northern Blot and *in Situ* Hybridization. Five micrograms of total cerebellar RNAs from p1–p6 treated WT and MBP-TK mice was analyzed on p21, p29, p35, p45, and p60 ($n = 3$ for each time point and genotype) by Northern blot. Filters were hybridized with ^{32}P -labeled mouse MBP and proteolipid protein probes. A glucose-6-phosphate dehydrogenase probe was used as an internal control of loading quantities. Quantifications were performed by densitometric scanning of autoradiograms with a Fuji Bio-imaging Analyzer BAS 2000.

In situ hybridizations were performed as described (6). Analyses were repeated on three different animals.

Behavioral Analysis. Rotarod. Mice were habituated to a rotating drum (3-cm diameter) (Bioseb LE8200, Chaville, France) before the test and then given three trials of 5 min each at 30-min intertrial rest intervals. Afterward, mice were placed on the rod under constant acceleration (4–40 rpm in 5 min), and fall latency was measured over the three trials with the best performance taken for statistical analysis. Results were analyzed by ANOVA with repeated measures followed by the appropriate post hoc comparisons.

Footprint analysis. Hind paws were dipped into black ink, and animals were allowed to walk through a dark $30 \times 10 \times 7$ -cm tunnel. Footprint patterns made on white paper lining the tunnel floor were collected. Step length was calculated by measuring the number of steps used to traverse the tunnel (9).

Open field. Animals were analyzed for 10 min in a squared arena ($60 \times 60 \times 40$ cm) placed in a soundproof room under 150 lux. Motor activity was automatically evaluated (View Point, Lyon, France) as distance traveled.

Electron microscopy. Mice were perfused with 4% paraformaldehyde, 3% glutaraldehyde in phosphate buffer (pH 7.4, 0.1 M). Samples of the vermal ML were postfixed for 1 h in 1% osmium tetroxide in phosphate buffer, dehydrated in serial ethanol solutions, and embedded in an araldite-epon at 60°C for 2 days. Ultrathin sections (50 nm) were observed on a Philips C12–208 electron microscope.

Results

Inducible and Reversible Cerebellar Dysmyelination. Cerebellum development occurs postnatally and coincides with the bulk of OL proliferation and myelination (10–13). We have explored the consequence of delayed myelination after OL ablation on cerebellar development and function by treating animals with FIAU during the first postnatal week.

TK expression in MBP-TK mice is restricted to oligodendrocytes and was never detected in other cerebellar cell types (see Fig. 5, which is published as supporting information on the PNAS web site).

After a FIAU administration (40 mg/kg s.c.) schedule from p1 to p6, only rare TK-positive OLs and myelinated fibers were found in cerebella folia of 6-day-old MBP-TK mice in comparison to untreated animals (Fig. 1*a*). However, despite an OL ablation close to 100% in p6 mice (Fig. 1*b*), only 50% reduction

Fig. 1. OL ablation and regeneration in FIAU-treated MBP-TK mice. (*a*) Immunofluorescent analysis of MBP-positive cerebellar fibers in MBP-TK untreated and p1–p6 FIAU-treated mice. (Scale bar: 42 μ m.) (*b*) Quantification of herpes virus 1-TK (HSV1-TK)-positive OLs in the cerebella of untreated (■, $n = 6$) versus treated (□, $n = 6$) mice. Values are mean $\pm$ SEM (***, $P < 0.0001$, Student's *t* test). (*c*) MBP mRNA expression during recovery in MBP-TK treated mice. WT values were arbitrarily taken as 100%. (*d*) Western blot analyses of MBP levels. Genotype and treatment are indicated. (*e*) MBP *in situ* hybridization of p60 FIAU-treated WT and MBP-TK sagittal brain sections. (Scale bar: 0.4 cm.) (*f*) Quantification of the vermal cerebellar surface of p21 and p60 treated mice of both genotypes. Values are mean $\pm$ SEM (***, Student's *t* test; $P < 0.001$).

in the expression of myelin markers was observed in p21 MBP-TK treated mice (Fig. 1*c*). p1–p6-treated mice survived, indicating that in contrast to FIAU chronically treated mice (6, 13) they recover from the induced damage. Thus, we analyzed OL recovery and myelination both at the anatomical and functional levels *in vivo*, after the induction of dysmyelination. RNA extracted from the cerebella of treated p1–6 MBP-TK and WT littermates was analyzed and quantified for MBP expression at 21, 29, 35, 45, and 60 days after the arrest of FIAU treatment (Fig. 1*c*). Interestingly, these analyses showed a gradual and progressive increase of MBP expression levels, which by day 45 were completely rescued to WT levels (Fig. 1*c*). The increase in MBP mRNA was paralleled by that of MBPs (Fig. 1*d*). *In situ* hybridization analyses (Fig. 1*e*) in which 60-day-old WT and MBP-TK treated mice were compared, showed a recovery of MBP expression throughout the brain of transgenic animals. This finding strongly indicates a recovery of myelinating OLs despite the temporal delay imposed by OL ablation. Nevertheless, these analyses showed a reduction of cerebellum size in MBP-TK mice compared to WT (Fig. 1*e* and *f**), indicating that OL ablation impairs cerebellum growth.

Myelin Deficit Induces Cerebellar Abnormalities. Both myelination and cerebellum development are normally completed by p21 in the mouse. Purkinje cell (PC) axons are myelinated (14) and together with afferent pontine and precerebellar myelinated fibers (15) form the white matter tract (WMT). Double immunofluorescence experiments performed in the cerebellum vermal region of treated MBP-TK and WT mice, at 21 and 60 days, showed a defined and compact MBP-positive WMT in WT

Fig. 2. Immunofluorescence analyses of the cerebellar cortex of WT and MBP-TK treated mice at p21 and p60. (a–c) Cerebellar folia V from WT and p21 and p60 MBP-TK treated mice were analyzed by using antibodies directed against MBP (green) and calbindin D28-k (red). (d–f) GFAP-positive cells (green) in the same regions and genotypes, as indicated. Nuclei were counterstained by using 4',6-diamidino-2-phenylindole (blue). Arrows indicate the BG radial processes crossing the ML. (d'–f') Shown are larger magnifications of GFAP-positive BG processes (green) unwrapping calbindin-positive PCs bodies (blue). (g–i) Double immunostaining using anti-calbindin D28-k (blue) and neuronal nuclei (NeuN, green) antibodies to label PC and granule neurons, respectively. Note the disorganized IGL at p21 in treated transgenics (h) and the partial reorganization at p60 (i) after recovery. [Scale bars: 173.5 μ m (a–c); 33 μ m (d–f); 20 μ m (d'–f'); 86.75 μ m (g–i).]

treated cerebella (Fig. 2a) delimited by the monolayer of calbindin-positive PCs. No age-dependent difference (p21 = p60) in the staining patterns of these antibodies was observed in WT mice (data not shown). In contrast, a thin and loosened WMT was observed in MBP-TK mice at p21 (Fig. 2b) as was a greatly disorganized PC layer. The disorganization of the PC layer was not caused by a reduction in the number of these cells (data not shown). However, by p60 in MBP-TK treated cerebella, a larger recovery of myelinated fibers is observed (Fig. 2c). The WMT becomes thicker and more compact as compared to p21 MBP-TK cerebella. Nevertheless, the recovery of myelinated fibers was not followed by a complete reorganization of the PC layer. PCs remain misaligned with a poorly developed dendritic tree, evoking failures of other cerebellar components. PC dendrites synapse with granule neurons parallel fibers, whose extensions are concomitant to granule cells migration on Bergmann glia (BG) scaffolds (16). BG also provide trophic factors and an adhesive substrate for PC dendritic trees (17) and play a substantial role in synapse integrity (3, 4, 18).

The analysis of BG cells and granule neurons was performed by using antibodies raised against GFAP and NeuN, respectively.

At p21, the BG palisade, observed in the WT (Fig. 2d), appears strongly affected, and GFAP-positive processes are randomly oriented in MBP-TK cerebella (Fig. 2e). Nevertheless, astroglial processes remain closely associated with PC bodies, as in WT cerebella (Fig. 2e'–f'). NeuN-positive granule cells, forming a compact inner granular layer (IGL) in WT mice (Fig. 2g), are sparse in MBP-TK cerebella (Fig. 2h). By p60, a partial reorganization of the BG and granule cells is observed in MBP-TK cerebella (Fig. 2f and i). At this time point, astroglial processes extend radially through the ML, and a recompaction of granule neurons in the IGL is observed (Fig. 2f and i). Nonetheless, the reorganization is not complete (Fig. 2f and i); a proportion of granule cells are still located in the ML (Fig. 2i), and the boundary between the ML and the IGL is still disordered.

This finding suggests that delayed OL proliferation and myelination strongly impairs the normal development of the cerebellar cortex.

Motor Coordination Is Strongly Affected by Dysmyelination. Default in myelination (5, 14) and mutations affecting cerebellar cell development (11, 19) lead to motor deficits and motor learning

Fig. 3. Behavioral analyses of MBP-TK treated mice during recovery. (a) Footprint patterns of WT and p21 and p60 MBP-TK treated mice. Mutant mice at p21 ($n = 12$) present an aberrant gait with respect to WT ($n = 11$) mice (step size: MBP-TK = 3.21 ± 0.09 cm; WT = 4.56 ± 0.22 ; $P < 0.0001$). Conversely, at p60 MBP-TK mice ($n = 9$) recovered a normal gait WT ($n = 14$) (step size: MBP-TK = 4.38 ± 0.13 cm; WT = 4.65 ± 0.11 ; $P = 0.1436$). (b) Locomotor activity of WT and MBP-TK treated mice was measured at p25 (WT $n = 7$, MBP-TK $n = 7$) and p70 (WT $n = 10$, MBP-TK $n = 11$) in an open field. Values are mean $\pm$ SEM. ***, $P < 0.0001$ versus WT. (c) MBP-TK ($\square$, $n = 21$) and WT ($\blacksquare$, $n = 11$) treated groups were analyzed on the rotarod during recovery from p21 to p60. ANOVA with repeated measures analyses using as dependent variable the fall latency (sec) indicated a significant effect of the genotype ($F_{1,140} = 76.846$; $P < 0.0001$) and a significant genotype $\times$ age (repeated measures) interaction ($F_{4,140} = 7.481$; $P < 0.001$). (d) The performance of MBP-TK treated mice on the rotarod was measured after five consecutive training sessions (from p60 to p65) in trained (already tested from p21 to p60) and untrained (never exposed to the test) animals. ANOVAs revealed a significant effect of training ($F_{1,46} = 44.314$; $P < 0.0001$) and a significant training $\times$ genotype interaction ($F_{1,46} = 10.160$; $P = 0.0024$). WT (filled columns) untrained, $n = 14$; trained, $n = 11$; MBP-TK (empty columns) untrained, $n = 11$; trained, $n = 21$.

impairment. MBP-TK mice were runts with respect to WT treated siblings (Fig. 1 *e* and *f*) with a statistically significant reduction of body weight (data not shown). At p21, MBP-TK mice presented a severe motor phenotype characterized by tremors and tonic seizures. Indeed, comparison of the hind footprint pattern of p21 WT and MBP-TK mice revealed a severe ataxic gait in the latter (Fig. 3*a*). Interestingly, by p60, concomitant with myelination the gait of MBP-TK mice is restored (Fig. 3*a*), and tremors and seizures disappeared. Motor performance of 25- and 70-day-old WT and MBP-TK treated mice was also evaluated in the open field. Statistical analyses showed a significant genotype $\times$ age interaction ($F_{1,36} = 13.618$; $P < 0.001$). Post hoc comparisons indicated a significant difference between WT and MBP-TK mice at p25 ($P < 0.001$) (Fig. 3*b*). Conversely, by p70, the performance of MBP-TK mice in this test was undistinguishable from that of WT littermates (Fig. 3*b*). Thus, despite cerebellar structural abnormalities, myelin recovery is sufficient to rescue ambulation in transgenic mice.

Because of the known role of the cerebellum in motor

coordination, the same groups of MBP-TK and WT mice were also tested on the rotarod (Fig. 3*c*) during recovery, after their performance at 21, 29, 35, 45, and 60 days. A significant effect of the genotype ($F_{1,140} = 76.846$; $P < 0.0001$) and genotype $\times$ age interaction ($F_{4,140} = 7.481$; $P < 0.001$) were found. The best MBP-TK performance was observed at p60, although even on this day, it was significantly different from that of WT mice ($P < 0.05$ Fisher's probable least-squares difference post hoc comparison). Therefore, delayed myelination is not sufficient to completely restore motor coordination.

Motor Training Rescues Movement Coordination by Enhancing Cerebellar Synapse Formation. The partial and gradual rescue observed on the rotarod after repeated sessions suggested that motor training might have been beneficial to improve motor coordination (20). Thus, we submitted the same groups to additional consecutive days of training on the rotarod. Strikingly, following this regimen for only an additional 5 days, trained MBP-TK mice were undistinguishable from WT mice (Fig. 3*d*). To support the role of motor training in rescuing the MBP-TK mice performance, completely naive groups of treated WT and MBP-TK mice of the same age (p65) were analyzed in parallel. Importantly, untrained MBP-TK treated mice compared to their WT controls or trained animals of the same genotype and treatment showed a dramatic impairment in performing the test (Fig. 3*d*). This difference was not caused by muscle weakness in MBP-TK mice, with or without training, as estimated by the similar performance in the grip strength test (data not shown). These findings highlight the central function of training to rescue behavioral defects related to a delayed myelination and cerebellar disorganization.

The remarkable motor performance of trained MBP-TK mice versus untrained animals strongly suggested that exercise stimulates functional recovery, by very likely establishing newly made synaptic contacts (20–25) or by increasing synapse efficiency. Immunofluorescence analyses, using synaptic markers, of the cerebella cortex of 65-day-old trained versus untrained animals supported this hypothesis. In particular, we used antibodies directed against synaptobrevin-2/VAMP-2, a marker of synaptic vesicles in the presynaptic compartments (26), and the glutamate transporter GLAST (27), whose expression level is regulated by PC synapse formation (17, 28) (Fig. 4).

The cerebellar ML is composed of an intricate series of connections between PC dendrites, granule axons, and interneurons. Synaptobrevin-2 and GLAST strongly label the cerebellar ML, because this area is very rich in synapses. The intensity of staining in WT mice trained or untrained was similar (Fig. 4 *a*, *e*, and *d* and data not shown). Importantly, a clear induction of synaptobrevin-2 and GLAST immunoreactivity is observed in the ML of trained MBP-TK mice (Fig. 4 *c*, *g*, and *d*) in comparison to that of untrained mice (Fig. 4 *b* and *f*). Interestingly, qualitative analyses at the electron microscopy level showed the presence of higher vesicular contents in synapses found in the ML of trained, with respect to untrained, MBP-TK mice (Fig. 4 *h–j*).

Discussion

Myelin degeneration and OL dysfunction lead to devastating neurological diseases, such as multiple sclerosis and leukodystrophies, in humans. In recent years, the importance of glial cells has been further amplified by their role in neuronal development and functionality (3, 4, 18).

Taking advantage of the TK system (1, 6, 8), we could follow the cerebellum recovery after ceasing the FIAU-induced toxicity in OLs from p1 to p6 after birth. Analyses of recovering MBP-TK mice from p21 to p60 showed recuperation, by p45, of OLs and myelin markers to WT levels. This finding indicates that, although delayed in the time of appearance, the newly

Fig. 4. Training increases synapse formation in the ML of MBP-TK mice. (a–c) Double immunostaining of PC-positive calbindin D28-k (red) and vesicular presynaptic VAMP2-positive compartments (green) in the ML of treated WT (a), untrained MBP-TK (b), and trained MBP-TK (c) mice. (d) Motor training specifically increases VAMP-2 expression as quantified in trained (c and d) versus untrained (b and d) MBP-TK mice. (e–g) Calbindin D28-k (blue) and GLAST immunostaining (red) of PC- and BG-positive cells in the ML of WT (e), untrained MBP-TK (f), and trained MBP-TK (g) mice. (h–j) Electron microscopy shows the increase and clustering of synaptic vesicles (arrow and *insets*) in trained MBP-TK mice (j) as compared to untrained animals (i) of the same genotype and treatment, as indicated. [Scale bars: 22.25 μ m (a–c); 96 μ m (e–g); 285 nm (h–j).]

generated OLs are able to repopulate and myelinate the cerebellum, illustrating a surprising plasticity of this tissue. This event is concomitant to a partial remodeling of the cerebellar cortex. In particular, we observed that the PCs reacquired the alignment into a monolayer and the IGL became redefined and more compact. Nevertheless, the size of the cerebella of MBP-TK mice remained smaller with respect to WT animals even after recovery. This finding indicates that OL ablation during the first postnatal week has a permanent impact on the normal development of the cerebellum. Quantifications of the size and

number of OLs, in the cerebella of transgenic animals after recovery with respect to WT, showed a 40% size reduction accompanied by a 50% decrease of the number of OLs in cerebellar folia. Granule cells loss also contributed to the smaller cerebellar size, very likely caused by the inability of these cells to migrate into the IGL (29), because of the disordered structure of the BG scaffolds.

Interestingly, the motor dysfunctions observed in treated animals at p21 involving the posture, gait, and ambulation is slowly rescued, and by p60 transgenic mice are undistinguishable

from WT littermates. However, whereas ambulation and horizontal locomotion are completely recovered by p60, motor coordination, although improved with time, never reaches WT levels. This finding suggests that the partial amelioration of the cerebellar structure, observed at the anatomical level, is not sufficient to obtain a full recovery of all motor deficits, which indicates that the recovery of OL and myelin content is not solely sufficient to rehabilitate all functions, at least the most complex. Cerebellum is a primary site of motor learning (30, 31). In particular, synaptic plasticity, occurring at PC and parallel fiber synapses, has been postulated as the putative cellular substrate for the storage of memory traces for motor learning in this structure (23, 24). Motor learning significantly induces synaptogenesis and cerebellar activity (20, 23). Interestingly, we show that motor training is able to eliminate the deficit in motor coordination still observed after recovery in MBP-TK mice. This finding indicates that a reduction of OLs and delayed myelination does not affect functional potentials such as the learning of motor tasks. Importantly, the improvement of motor skills that follows motor training does not depend on an increased proliferation of cells in the cerebellar cortex between p60 and p65 in trained MBP-TK mice (data not shown). Rather, motor training is paralleled by an increase of synaptic markers and vesicles

between BG, PCs, and parallel fibers (21, 25) in the ML of the transgenic treated cerebella. Taken together, these findings show that OLs and myelin are an absolute requirement for the development of the cerebellar cortex during the first postnatal week. More importantly, we also show that spontaneously occurring delayed myelination is able to partially rescue the structure of the cerebellar cortex and the motor phenotype associated with myelin loss. Nevertheless, to obtain full recovery of the system epigenetic factors, such as those represented by motor training, are needed. These findings underlie the importance of strictly controlled therapeutical care in demyelinating diseases such as leukodystrophies in humans.

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