Rapid production of new oligodendrocytes is required in the earliest stages of motor-skill learning

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We identified mRNA encoding the ecto-enzyme Enpp6 as a marker of newly forming oligodendrocytes, and used Enpp6 in situ hybridization to track oligodendrocyte differentiation in adult mice as they learned a motor skill (running on a wheel with unevenly spaced rungs). Within just 2.5 h of exposure to the complex wheel, production of Enpp6-expressing immature oligodendrocytes was accelerated in subcortical white matter; within 4 h, it was accelerated in motor cortex. Conditional deletion of myelin regulatory factor (Myrf) in oligodendrocyte precursors blocked formation of new Enpp6+ oligodendrocytes and impaired learning within the same ~2–3 h time frame. This very early requirement for oligodendrocytes suggests a direct and active role in learning, closely linked to synaptic strengthening. Running performance of normal mice continued to improve over the following week accompanied by secondary waves of oligodendrocyte precursor proliferation and differentiation. We concluded that new oligodendrocytes contribute to both early and late stages of motor skill learning.

In the vertebrate central nervous system (CNS) many axons are ensheathed by myelin, tight spiral wraps of plasma membrane made by oligodendrocytes. Myelin greatly increases the speed of propagation of action potentials, permitting rapid information transfer over long distances and allowing the evolution of larger animals with bigger, more powerful brains. Approximately 5% of all neural cells in the rodent and human brain are oligodendrocyte precursors (OPs). These glial precursors generate the majority of myelinating oligodendrocytes during the early postnatal period (the first ~10 weeks in mice and 5–10 years in humans)1,2, but continue to generate oligodendrocytes and myelin at a declining rate subsequently2–9.

Differentiation of OPs into oligodendrocytes depends on the transcription factor Myrf10,11. Myrf is not expressed in cycling OPs but is first transcribed in differentiating oligodendrocytes, in which it is required for activation of many downstream genes including those encoding myelin structural proteins10–12.

We previously investigated the function of adult-born oligodendrocytes in mice by inactivating Myrf conditionally in OPs, using tamoxifen-inducible CreERT2 under transcriptional control of platelet-derived growth factor receptor-alpha (Pdgfra-CreERT2) to recombine and delete a loxP-flanked allele of Myrf (P-Myrf−/− mice)13. This dramatically reduced new oligodendrocyte production from their precursors without affecting preformed oligodendrocytes or myelin and prevented mice from mastering a new motor skill (running on a 'complex wheel' with irregular rung spacing). We concluded that development of new oligodendrocytes during adulthood is required for motor learning13. However, the precise role of new oligodendrocytes in the learning mechanism remains unclear. They might be needed in a purely permissive role; for example, to repair myelin that is lost or damaged in use, so that the underlying neural circuitry remains competent for learning. Alternatively, they might be involved more directly. For example, they might improve conduction by synthesizing myelin, by inducing sodium channels to cluster at 'pre-nodes' before myelination14 or by transferring substrates for energy production (lactate and pyruvate) into axons15,16. Any or all of these mechanisms might improve the performance of new circuits while preserving them for future use.

A key component of learning at the subcellular level is synaptic modification17–20. This can occur very rapidly; there are dynamic changes to the number and size of dendritic spines (sites of synaptic contact) on pyramidal neurons in the mouse motor cortex within one-and-a-half hours of initiating fine-motor training21. This is much faster than previously reported responses of oligodendrocyte lineage cells to novel experience11, or to other physiological or artificial stimuli18,13,22,23, which have been reported to occur over days to weeks. This might suggest that oligodendrocytes act far downstream of synaptic change or in an entirely separate pathway. However, our knowledge of how oligodendrocyte lineage cells change in response to novel experience is still rudimentary, and more work is required before we can understand their role in neural plasticity.

To help elucidate the contribution of oligodendrocytes to motor learning, we examined the time course of learning and the accompanying cellular events at higher temporal resolution than has been done before. We analyzed complex wheel-running data for P-Myrf−/− mice

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and their P-Myrf\textsuperscript{−/−} littermates and discovered that the performance of the two groups diverged very early, within 2–3 h of first introducing mice to the wheel. This result implies that oligodendrocyte differentiation is required at a very early stage of motor-skill learning, close to the point at which synaptic change occurs\textsuperscript{21}, suggesting that oligodendrocytes and myelin have a more active role in learning and memory than might have been imagined previously.

To look for direct evidence of early involvement of oligodendrocyte lineage cells, we analyzed OP proliferation and differentiation in the motor cortex and subcortical white matter of wild-type mice during the early stages of learning. Using a new molecular marker Enpp6 (a choline-specific ecto-nucleotide pyrophosphatase/phosphodiesterase)\textsuperscript{24–26} that is preferentially expressed in early differentiating oligodendrocytes (ref. 27 and this work), we detected accelerated differentiation of OPs into newly forming oligodendrocytes after just 2.5 h self-training on the complex wheel. This early accelerated differentiation of OPs into new oligodendrocytes that participate in and are required for optimal learning. Plotting average speed over successive 2-h (rather than 12-h) intervals showed that for both P-Myrf\textsuperscript{−/−} and control groups most improvement during the first night occurred within the first 4 h, after which running performance leveled off (Fig. 1b). At the beginning of the second night, following 12 h of daytime inactivity and sleep, performance was immediately better than it had been at any time in the previous 24 h (Fig. 1b). This mirrored the sleep-dependent ‘consolidation’ that is observed in humans learning a motor task\textsuperscript{29}. On subsequent nights, performance improved incrementally from one night to the next, beginning at a level close to the previous night’s peak and improving further for a few hours before tailing off (Fig. 1b and Supplementary Fig. 1).

Plotting average running speed over 20-min intervals revealed that performance of P-Myrf\textsuperscript{−/−} mice was already impaired relative to their P-Myrf\textsuperscript{−/−} littermates within the first 2–3 h of the first night (Fig. 1c). This early requirement for Myrf presumably reflects differentiation of Pdgfra-expressing OPs into new oligodendrocytes; it cannot reflect modification or adaptation of preexisting oligodendrocytes or myelin because differentiated oligodendrocytes do not express Pdgfra-Cre\textsuperscript{ERT2} and therefore do not delete Myrf\textsuperscript{loxP}–expressing OPs lacking Myrf do not differentiate properly but arrest and die before the expression of myelin structural genes\textsuperscript{10,11,13}. 

![Figure 1](image)

**Figure 1** Time course of motor skill learning in mice and the requirement for Myrf. (a) Average speeds on complex wheel (pictured) during successive 24-h intervals of P-Myrf\textsuperscript{−/−} mice (n = 32; 17 male) and their P-Myrf\textsuperscript{−/−} littermates (n = 36, 20 male). (b) Average speeds during successive 2-h intervals during the first 3 d of the experiment. (c) Average speeds in 20-min intervals during the first 4 h of the first night. Data were analyzed by two-way ANOVA with Bonferroni’s post hoc tests. Error bars indicate s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 10\textsuperscript{−3}, *****P < 10\textsuperscript{−4}. In a, night 1: P = 0.09, t = 2.5; night 2: P = 0.01, t = 3.3; night 3: P = 0.004, t = 3.5; night 4: P = 0.0009, t = 3.9; night 5: P = 0.0001, t = 4.6; night 7: P = 10\textsuperscript{−4}, t = 4.7; night 8: P = 0.001, t = 3.8. F\textsubscript{1,497} = 109.9 and degrees of freedom (d.f.) = 497 throughout. In b, night 1: 2 h, t = 1.81; 4 h, P = 0.03, t = 2.8; 6 h, P = 0.009, t = 3.2; 8 h, P = 0.004, t = 3.4; 10 h, P = 0.002, t = 3.7; 12 h, P = 0.004, t = 3.5. F\textsubscript{1,396} = 56.2. night 2: 2 h, P = 0.007, t = 3.3; 4 h, P = 0.003, t = 3.5; 6 h, P = 0.063, t = 2.6; 8 h, P = 0.015, t = 3.0; 10 h, P = 0.11, t = 2.4; 12 h, P = 0.03, t = 2.8. F\textsubscript{1,396} = 51.1; night 3: 2 h, P = 0.002, t = 3.6; 4 h, P = 0.004, t = 3.4; 6 h, P = 0.02, t = 2.9; 8 h, P = 0.072, t = 2.53; 10 h, P = 0.00, t = 1.084; 12 h, P = 0.24, t = 2.06. F\textsubscript{1,396} = 40.79. d.f. = 396 throughout. In c, 20 min: P > 0.99, t = 0.69; 40 min: P > 0.99, t = 0.10; 60 min: P > 0.99, t = 0.13; 80 min: P > 0.99, t = 0.81; 100 min: P > 0.99, t = 1.41; 120 min: P = 0.30, t = 2.4; 140 min: P = 0.022, t = 3.12; 160 min: P = 0.10, t = 1.83; 180 min: P = 0.83, t = 2.63; 200 min: P = 0.14, t = 2.63; 220 min: P = 0.017, t = 3.21; 240 min: P = 0.095. F\textsubscript{1,792} = 42.32 and d.f. = 792 throughout.
Motor training accelerates OP differentiation

This rapid improvement in motor performance far preceded the running-induced OP proliferation that we had detected at 2−4 d in our previous study.13 However, in that study we had administered the nucleotide analog 5-ethyl-2'-deoxyuridine (EdU) from the time mice first encountered the wheel, so we could follow the fates only of those cells that entered S phase after that point. That could have limited our ability to detect the earliest cellular responses. Therefore, in new experiments we prelabeled wild-type mice with EdU for 10 d, from P75 to P85, before introducing the wheel (Fig. 2a). This was sufficient to label ~20% of OPs in the cortical gray matter and ~75% of OPs in the subcortical white matter (data not shown). We then followed the fates of those EdU-labeled cells in mice that had self-trained on the wheel for 48 h (P85−P87), by immunolabeling for Pdgfra to identify OPs and with monoclonal CC1 for oligodendrocytes. The majority of EdU+ cells in both gray and white matter was also Sox10+ (96.7 ± 0.6%); >1,000 cells counted in more than three sections from each of 3 mice, Fig. 2b), so EdU labeling can be used as a proxy oligodendrocyte lineage marker in these experiments. We observed a decrease in the

Figure 2  Oligodendrocyte dynamics during motor-skill learning. (a) Experimental design: all mice (approximately equal numbers of male and female) were given tamoxifen by gavage on 4 successive days (P60 to P63 inclusive), and then EdU was administered in the drinking water for 10 d (P75 to P84) before transferring the mice to cages equipped with a complex wheel (CW) for up to 8 d. (b) Double immunolabeling for Sox10 (left) and CC1 (right) combined with EdU detection (red) on a section of subcortical white matter of wild-type mice housed with a wheel for 8 d (‘8-d runners’). ~97% of EdU+ cells were also Sox10+ oligodendrocyte lineage cells. There was a mixture of CC1-negative presumptive OPs (arrowheads) and CC1+ newly formed oligodendrocytes (arrows). Images are representative of >3 similar experiments. Scale bar, 40 µm. (c,d) Numbers of newly generated (EdU+) oligodendrocyte lineage cells at different developmental stages in 2-d runners vs. control littermates, housed without a wheel (nonrunners) in motor cortex (c) and underlying white matter (WM) (d). (e,f) Production of (EdU+ CC1+) new myelinating oligodendrocytes in motor cortex (e) and subcortical white matter (f) of runners vs. nonrunners. Number of new oligodendrocytes was strongly reduced in P-Myrf−/− mice, both runners and nonrunners, compared to wild-type mice (nonrunner 8 d, motor cortex: \( P = 0.00091, t = −4.71, d.f. = 9; \) sub-cortical white matter: \( P < 10^{-5}, t = −15.43, d.f. = 10, \) n = 6 mice each group; 8 d (WT), n = 6 nonrunners). Error bars, s.e.m.; * \( P < 0.01, \) ** \( P < 0.001, \) *** \( P < 0.0001, \) two-tailed unpaired t-test. In c, CC1+, \( P = 0.81, t = −0.25; \) Pdgfra+, \( P = 0.012, t = 3.1; \) Pdgfra− CC1+, \( P = 0.012, t = −3.1, d.f. = 10, n = 6 mice. In d, CC1+, \( P = 0.90, t = −0.13; \) Pdgfra+, \( P = 0.014, t = 2.94; \) Pdgfra− CC1+, \( P = 0.012, t = 3.07; \) d.f. = 10. n = 6 mice. In e, 2 d, \( P = 0.90, t = −0.13, d.f. = 10, n = 6 mice each group; 4 d, \( P = 0.027, t = −2.90, d.f. = 6, n = 4 mice each group; 6 d, \( P = 0.0039, t = −3.73, d.f. = 10, n = 6 mice each group; 8 d (WT), \( P = 0.0006, t = −5.0, d.f. = 10, n = 6 mice each group; 8 d (Myrf−/−), \( P = 0.18, t = −1.47, d.f. = 8, n = 6 mice each group. In f, 2 d, \( P = 0.61, t = −0.53, d.f. = 10; n = 6 mice each group; 4 d, \( P = 0.004, t = −4.8, d.f. = 6, n = 4 mice each group; 6 d, \( P = 0.0027, t = −3.54, d.f. = 10, n = 6 mice each group; 8 d (wild type), \( P = 0.0007, t = −4.82, d.f. = 10, n = 6 mice each group; 8 d (Myrf−/−), \( P = 0.61, t = −0.53, d.f. = 8, n = 5 mice each group.

Figure 3  Enpp6 marks newly forming oligodendrocytes. (a) Enpp6 RNA-seq data adapted from ref. 27, http://web.stanford.edu/group/barres_lab/brain_rnaseq.html. OLs, oligodendrocytes; FPKM, fragments per kilobase of transcript per million mapped reads. Error bars, s.e.m. (b) ISH for Mbp transcripts on sections of P90 mouse motor cortex. Arrowheads indicate small cell bodies of mature oligodendrocytes; arrows indicate larger process-bearing ‘spidery’ cells, resembling ‘premyelinating’ oligodendrocytes described previously.30,31 (c) Double ISH for Mbp and Enpp6 showing that the spidery Mbp+ cells (arrows) are also Enpp6+. Insets, magnified images of right-most Enpp6+ cell. (d,e) ISH for Enpp6 in forebrain sections of wild type (d) and P-Myrf−/− (e) mice. Boxed regions are magnified on the right. SCWM, subcortical white matter. Images are representative of >3 similar experiments. Scale bars, 50 µm (b, c, d right and e right) and 200 µm (d left and e left).
number density (cells per mm²) of EdU−Pdgfra+ OPs in mice housed with a wheel ('runners') relative to littermates housed without a wheel ('nonrunners') both in the motor cortex (7.3 ± 0.6 cells/mm² vs. 10.4 ± 0.7 in runners vs. nonrunners; \( P = 0.01 \), two-tailed unpaired \( t \)-test) and in subcortical white matter (71.2 ± 6.2 vs. 91.9 ± 4.0 cells/mm²; \( P = 0.02 \); ≥3 sections analyzed from each of 6 mice) (Fig. 2c,d). This was accompanied by a reciprocal increase in the number density of EdU−Sox10− Pdgfra−CC1− cells, that is, newly differentiating oligodendrocytes that had lost Pdgfra but not yet acquired CC1 immunoreactivity (motor cortex: 4.1 ± 0.5 vs. 2.3 ± 0.2 cells/mm² in runners vs. nonrunners (\( P = 0.01 \)); subcortical white matter: 73.5 ± 8.9 vs. 51.0 ± 5.5 cells/mm² (\( P = 0.03 \)) (Fig. 2c,d)). These data demonstrate that novel experience with the complex wheel stimulated differentiation of OPs that had replicated their DNA in the 10 d before introduction to the wheel. At that time point (2 d with the wheel) there was no difference in the density of EdU+CC1+ oligodendrocytes in runners vs. nonrunners (Fig. 2c–e), but a significant increase in density of those cells developed in runners between 2 d and 4 d, both in the motor cortex and subcortical white matter, and the increases persisted beyond 8 d (Fig. 2f). Numbers of EdU+CC1+ newly formed oligodendrocytes decreased in P-Myrf−/− mice (\( P < 0.10 ^{−4} \)), as expected (Fig. 2f). There was no increase in oligodendrocyte production in the optic nerves of runners vs. nonrunners (Fig. 2g), demonstrating regional specificity of the response.

**Enpp6, a new marker of newly forming oligodendrocytes**

The cellular responses to wheel running after 2 d were still much delayed relative to the 2–3 h required to register an improvement in running performance (Fig. 1c). Nevertheless, the results described above drew our attention to the potential role of OP differentiation in early stages of learning. To facilitate study of early OP differentiation, we scanned existing expression data-bases for developmental-stage-specific markers and identified a gene, *Enpp6*, that is expressed highly in newly forming oligodendrocytes and at a much lower level in more mature myelinating oligodendrocytes, but not at all in OPs, neurons, astrocytes or vascular endothelial cells27 (Fig. 3a). *In situ* hybridization (ISH) for *Enpp6* labeled cells in both the gray and white matter of the P60 mouse brain (Supplementary Fig. 2). We detected two populations of *Enpp6*-positive cells; the majority was small, weakly labeled cell bodies but a minor subpopulation had larger cell bodies that were more intensely labeled (Fig. 3b and Supplementary Fig. 2). This expression pattern is consistent with the interpretation that the numerous weakly labeled cells (*Enpp6low*) are mature oligodendrocytes, whereas the less abundant strongly labeled cells (*Enpp6high*) were newly differentiating, in keeping with the high-throughput RNA sequencing (RNA-seq) data27.

By appropriately adjusting ISH conditions, we filtered out the weakly labeled cells and visualized only the strongly labeled, putative newly differentiating oligodendrocytes (Supplementary Fig. 2b). These *Enpp6high* cells did not express Pdgfra, so they were not OPs, but they all had Sox10 and/or Olig2 immunoreactivity (Supplementary Figs. 2c and 3, and Supplementary Table 1). Most but not all of them also labeled with CCI (Supplementary Fig. 3 and Supplementary Table 1), and they all expressed *Mbp* mRNA (Fig. 3b,c and Supplementary Table 1), which identified them as oligodendrocytes. At P90 the *Enpp6highMbp* cells had a distinctive ‘spider’ morphology with multiple radial processes (Fig. 3b,c). All *Enpp6high* cells were *Mbp* spindly cells and vice versa (Fig. 3c and Supplementary Table 1) and comprised < 5% of all *Mbp* oligodendrocytes at P90. The *Enpp6highMbp* spindly cells were practically absent from *P-Myrf−/−* brains (Fig. 3d,e), confirming that they were newly forming oligodendrocytes12. A morphological subclass of oligodendrocyte resembling the *Enpp6highMbp* cells and described as ‘premyelinating’ has been visualized previously in rat and human brains, by immunolabeling for the myelin Proteolipid protein (Plp, DM20 isoform)29,31.

We found that in P10 cerebral cortex, ~45% (66/146) of *Enpp6highMbp* cells also expressed the myelin structural proteins
Accelerated production of Enpp6+ cells during training

We used ISH for Enpp6 mRNA to detect newly differentiating oligodendrocytes in mice that were learning to run on the complex wheel (Fig. 6a–i). Within just 2.5 h, we detected an increase in the number of Enpp6high cells in the subcortical white matter of runners vs. nonrunners (35.9 ± 2.0 cells/mm² vs. 28.4 ± 1.7 cells/mm²; P = 0.003; ≥23 sections from each of 8 mice) (Fig. 6k). This increase in cell number occurred in the same time frame as the earliest improvement in running performance that develops during sleep or inactivity (Fig. 1c) might be related to ongoing oligodendrocyte generation; it has been reported that OP proliferation and differentiation is circadian, with S-phase entry occurring preferentially during the day and M phase and oligodendrocyte differentiation at night.32,33 Production of Enpp6high cells was dramatically reduced in P-Myrf-/- mice relative to wild-type mice both in nonrunners and 12-h runners (Fig. 6c.f–l–k), as expected. We still observed increased numbers of newly formed Enpp6high oligodendrocytes in 8-d runners, though in reduced numbers compared to earlier time points (Fig. 6j,k).

Motor learning, not exercise, stimulates oligodendrogenesis

 Increased oligodendrocyte differentiation might conceivably have been part of a systemic response to exercise, rather than to motor learning per se. To disentangle these effects, we compared two cohorts of wild-type mice, one of which self-trained on the complex wheel for 8 d, rested for 2 weeks, and then was reintroduced to the complex wheel for 24 h. The other cohort we introduced to the complex wheel once only, for 24 h (Fig. 7a). The average running speed and distance traveled over 24 h of the latter group (here referred to as ‘first-timers’) was much less than that for the second-timers that had already mastered the wheel 2 weeks previously (Fig. 7b,c). Despite this, the Enpp6high number density was increased in motor cortex and underlying white matter only in the first-timers (Fig. 7d,e). This experiment dissociates running speed (physical exercise) from novel running experience (learning) and demonstrated that the rate of production of new oligodendrocytes increased only during the primary learning event.
Figure 6 Rapid increase in Enpp6<sup>hi</sup> newly forming oligodendrocytes in response to motor-skill learning. (a–l) ISH for Enpp6 in sections of nonrunner, runner (24 h with the complex wheel) and P-Myrf<sup>−/−</sup> (nonrunner) subcortical white matter (a–f) or motor cortex (g–l). Magnification of boxed areas in a–c is shown in d–f, respectively. Scale bars, 200 µm (a–c) and 100 µm (d–l). Arrows, examples of Enpp6<sup>hi</sup> cells. Dashed line, boundary between gray and white matter (WM). Images are representative of >3 similar experiments. (j, k) Quantification of Enpp6<sup>hi</sup> cells in wild-type or P-Myrf<sup>−/−</sup> mice housed with or without a complex wheel for indicated durations. Numbers of Enpp6<sup>hi</sup> cells were greatly decreased in P-Myrf<sup>−/−</sup> compared to wild-type mice, (nonrunner 12 h, motor cortex: P < 10<sup>−5</sup>, t = −13.37, d.f. = 12; subcortical white matter: P < 10<sup>−5</sup>, t = −11.28, d.f. = 12; n = 10 for wild-type mice, n = 4 for Myrt<sup>−/−</sup> mice).

Error bars, s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001, comparing runners with nonrunners using two-tailed unpaired t-test. WM, white matter. In j, 2.5 h, P = 0.43, t = −0.81, d.f. = 15, n = 8 mice for nonrunners, n = 9 mice for runners; 4 h, P = 9 × 10<sup>−5</sup>, t = −5.024, d.f. = 18, n = 10 mice each group; 12 h (wild type), P < 10<sup>−5</sup>, t = −8.50, d.f. = 18, n = 10 mice each group; 24 h, P = 0.00019, t = −6.46, d.f. = 8, n = 5 mice each group; 48 h, P = 0.0038, t = 4.03, d.f. = 9, n = 4 mice for nonrunners, n = 6 for runners; 8 d, P = 0.0013, t = −4.42, d.f. = 10, n = 6 mice each group; 12 h (Myrt<sup>−/−</sup>), P = 0.049, t = −2.39, d.f. = 5, n = 4 mice for nonrunners, n = 3 mice for runners. In k, 2.5 h, P = 0.0026, t = −3.50, d.f. = 18, n = 10 mice each group; 12 h (wild type), P < 10<sup>−5</sup>, t = −6.20, d.f. = 18, n = 10 mice each group; 24 h, P = 0.001, t = −5.06, d.f. = 8, n = 5 mice each group; 48 h, P = 0.0038, t = 4.03, d.f. = 8, n = 4 mice for nonrunners, n = 6 for runners; 8 d, P = 2 × 10<sup>−5</sup>, t = −5.71, d.f. = 10, n = 6 mice each group; 12 h (Myrt<sup>−/−</sup>), P = 0.013, t = −3.77, d.f. = 5, n = 4 mice for nonrunners, n = 3 mice for runners.

Moreover, increased production of Enpp6<sup>hi</sup> newly differentiating oligodendrocytes was induced in motor cortex but not visual cortex of the first-timers in this experiment (Fig. 7), demonstrating regional specificity of the early learning response.

Discussion

We presented evidence that oligodendrocyte development was required for motor learning in adult mice, within the first few hours of their being introduced to the complex running wheel. The performance of P-Myrf<sup>−/−</sup> and P-Myrf<sup>−/−</sup> groups both started out at baseline, but mice in the two groups improved at different rates over the first 2–3 h, before equilibrating at different levels. This observation reinforced our conclusion that the two groups learned at different rates, rather than possessing inherently different physical abilities, as the physical effort required for the initial low-speed wheel-turning was unlikely to be limiting. At the cellular level, the primary response was accelerated production of Enpp6 and Mbp—expressing early differentiating (spindly) oligodendrocytes, which we detected within the first 2.5 h (Supplementary Fig. 4). This might underestimate the rapidity of the response, as Enpp6 does not mark the very earliest differentiating

Figure 7 Increased production of Enpp6<sup>+</sup> newly formed oligodendrocytes was a response to motor learning, not physical exercise. (a) Experimental design, in which one group of mice (2nd) self-trained on the complex wheel (CW) for 1 week, rested for 2 weeks, and then was reintroduced to the wheel along with a separate group that was introduced for the first time (1st). After 24 h (P85–P86) both groups of mice (and parallel groups of nonrunners) were analyzed by ISH for Enpp6. (b) Average speeds (m/min) of first-timers (1st) and second-timers (2nd) during the 48 h P85–P86. (c) Distances run by 1st and 2nd during P85–P86. (d–e) Number densities (cells per mm<sup>2</sup>) of Enpp6<sup>hi</sup> newly formed oligodendrocytes in 1st and 2nd after running on the complex wheel for 48 h (P85–P86), versus age-matched nonrunner controls, in motor cortex (d) and subcortical white matter (e). (f) New Enpp6<sup>hi</sup> oligodendrocyte production in the visual cortex in 1st versus age-matched nonrunner controls, n = 4 mice in each group. Error bars, s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001, comparing runners with nonrunners using two-tailed unpaired t-test.

In b, P = 10<sup>−5</sup>, t = −11.72, d.f. = 6. In c, P = 0.00035, t = −7.23, d.f. = 6. In d, 1st, P = 0.00033, t = −7.29, d.f. = 6; 2nd, P = 0.38, t = −0.95, d.f. = 6. In e, 1st, P = 0.0021, t = −5.16, d.f. = 6; 2nd, P = 0.34, t = −1.03, d.f. = 6. In f, P = 0.80, t = 0.27, d.f. = 6.
cells; there was still a gap between downregulation of Pdgfra and appearance of Enpp6 (Supplementary Fig. 4). The rapid increase in Enpp6-expressing newly forming oligodendrocytes is likely to result from stimulated differentiation of G1-phase-paused OPs, because at 2 d of training we observed a reduction in the absolute number of OPs (Fig. 2c,d); however, it is possible that increased survival of Enpp6-expressing cells also played a part. Production of Enpp6high newly forming oligodendrocytes peaked around 24 h and declined thereafter, although increased production was still evident after 8 d. We observed that peak of new oligodendrocyte production only during the first encounter with the wheel, not during a subsequent encounter, and only in motor cortex and not visual cortex, suggesting that it is a specific response to motor learning, not exercise per se.

The early surge of OP differentiation was reflected in a reduction at 2 d of running in the number of OPs (prelabeled with EdU) and a corresponding increase in the number of Pdgfra CC1− newly forming oligodendrocytes. This was followed at 2–4 d by accelerated S-phase entry of some of the remaining OPs. This sequence suggests that the S-phase entry observed at 2–4 d was a homeostatic response to the earlier depletion of OPs through differentiation. For example, local depletion of OPs could result in loss of contact inhibition or a local excess (through reduced consumption) of a mitogenic growth factor (for example, Pdgf)12, either or both of which might stimulate proliferation of the remaining OPs in the locality. This spike of OP generation propelled a secondary wave of oligodendrocyte production over the following days to weeks (Figs. 2d and 4g,h and ref. 13). The dynamics of the system are complex, and it seems likely that oligodendrocytes contribute to motor-skill acquisition in different ways at different stages of the process.

Various learning regimens in humans and rodents are associated with changes to the microstructure of gray and white matter, detected by magnetic resonance diffusion tensor imaging. For example, people or rats learning a complex visuomotor skill (for example, juggling or using an abacus for humans, and grasping food pellets for rats) develop altered microstructure in the motor cortex and/or subcortical white matter after training for days to weeks15–18. Changes to gray and white matter microstructure can be detected even within 2 h in people learning to play an action video game. Experience-dependent structural changes to gray and/or white matter could in principle result from synaptogenesis and elaboration of the dendritic arbor17–21, alterations to astrocyte morphology and their interactions with neurons22, changes to the microvasculature23 or altered (adaptive) myelination22,23,42,43. The results of our own present and previous studies support the latter possibility.

Our ability to detect early oligodendrocyte dynamics relied partly on Enpp6, which we characterized as a new marker of early differentiating oligodendrocytes. Enpp6 is likely to become a useful tool for studying oligodendrocyte differentiation in vivo; for example, during remyelination of demyelinated lesions. Enpp6 is considered to be a choline-specific glycerophosphodiester phosphodiesterase, as it can hydrolyze glycerophosphocholine and sphingosylphosphocholine efficiently in vitro24–26. Therefore, it seems likely that Enpp6 has a role in lipid metabolism during formation of the myelin sheath and might be required to initiate myelination rapidly in response to differentiation-inducing signals, including those that operate during motor learning. It will be interesting in future to examine the functional role of Enpp6 and related family members during myelination.

How might newly forming oligodendrocytes contribute to learning? The rapidity of their formation suggests that they work in close partnership with neurons, hand-in-hand with (or close on the heels of) synaptic change, not simply by preserving or ‘ossifying’ new circuits after they have become established. For example, when mice first encountered the complex wheel they tried to develop strategies for coping with unequal rung spacing; this presumably involves exploratory firing of distinct neurons or groups of neurons along the motor pathways. There might be several or many parallel circuits that can generate behavioral outputs within a useful range, some more effective than others. The superior circuit(s) might be activated more frequently than others as the action is rehearsed, and thus become selected and strengthened according to Hebbian principles, both at the level of the synapse and by rapid and selective myelination of the interconnecting axons. It is known that OPs receive synaptic input from axons44, and that oligodendrocyte development and myelination can be regulated by axonal activity in vivo22,23,42–46. Newly differentiating oligodendrocytes might enhance circuit function by initiating myelination and/or clustering of sodium channels and other nodal components before myelination44, by supporting axonal metabolism45,46, or a combination of those effects. This in turn could enhance connectivity at the synaptic level and so on, back and forth. As myelin thickness and compaction increases, the performance of the network and its behavioral outputs would be expected to improve further. Therefore, oligodendrocytes are likely to contribute to learning over an extended period from hours to weeks. Ultimately, myelin protects axons metabolically and physically over the long term, preserving lifelong memories.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

W.D.R. formed the hypotheses and obtained funding. I.A.M. adopted and developed the complex wheel test. B.E. provided Myrfmice, advice and suggestions. W.D.R., I.A.M. and D.O. designed the experiments in Figure 1 and Supplementary Figure 1; D.O. and I.A.M. performed those experiments and DO analyzed the data. W.D.R., H.L. and L.X. designed all the other experiments and L.X. performed them, with assistance from A.S.-W., J.L.W. and A.D.E.H.L. identified Enpp6 and A.F. performed preliminary characterization. H.L. and W.D.R. supervised the work. W.D.R. wrote the paper with input from H.L., L.X. and B.E.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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The complex wheel. We purchased wheel cages (Lafayette Neuroscience) that allow digital recording of wheel rotation speed over time (using an infrared sensor). The complex wheel, in which mice run on a narrow platform that is elevated above a glass bottom, has been described (13). Tomoxifen (Sigma) was dissolved at 40 mg/ml in corn oil by sonication at 21 °C for 1 h. It was administered to mice (both Myf5loxP/loxP and Myf5floxP mice) by oral gavage on four consecutive days ending on P64 (four cohorts) or P94 (one cohort) to induce recombination and deletion of Myf5 in Pdgfra+ OPs (each dose was 300 mg/kg body weight). This generated P-Myf-/- and P-Myf-/- mice for behavioral experiments. Mice were rested 3 weeks between the last tomoxifen dose and being introduced to the complex wheel at P85 or P115 (ref. 13). Wild-type mice were C57B6 (Charles River). All animal experiments were approved by the UCL Ethical Committee and authorized by the Home Office of the UK Government.

Histology and cell counts. Mice were perfusion-fixed with 4% (w/v) paraformaldehyde (PFA; Sigma) in diethylpyrocarbonate (DEPC)-treated phosphate-buffered saline (PBS) at 4 °C. Tissue was cryoprotected in 20% (w/v) sucrose (Sigma) in PBS before freeze-drying. Low-magnification (20× objective) confocal images were collected using a Leica SPE laser-scanning confocal microscope as Z stacks with 1-μm spacing, using standard excitation and emission filters for DAPI, FITC (Alexa Fluor 488), TRITC (Alexa Fluor 568) and Far Red (Alexa Fluor 647). Cells were counted in non-overlapping fields of coronal sections of the corpus callosum or motor cortex, between the dorsolateral corners of the lateral ventricles (six fields per section, three sections from each of three or more mice of a given experimental group).

EdU labeling in vivo. Mice were given 5-ethyl-2'-deoxyuridine (EdU) in their drinking water (0.2 mg/ml) for 10 d from P75-P85. The animals were caged with a complex wheel for different durations, and then were perfusion-fixed and analyzed by immunolabeling floating cryosections (20 μm) with monoclonal CC1, anti-Sox10 and anti-Pdgfra followed by detection of EdU using the Alexa Fluor 555 Click-iT detection kit (Invitrogen).