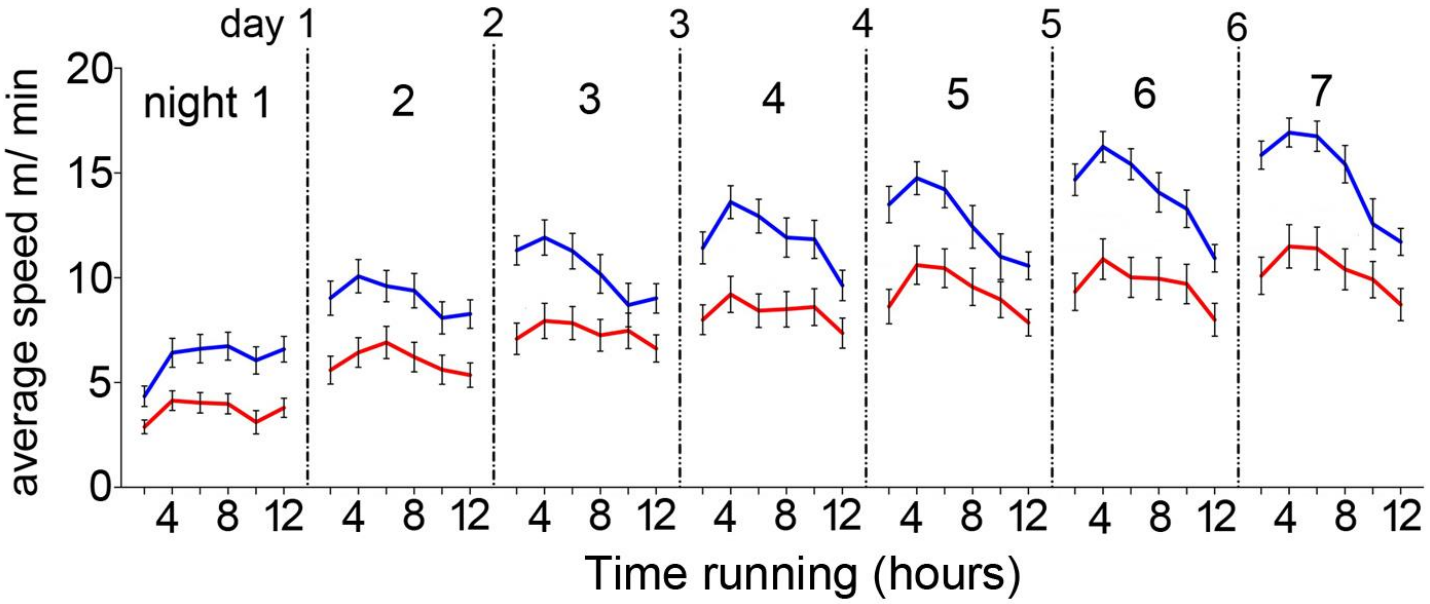
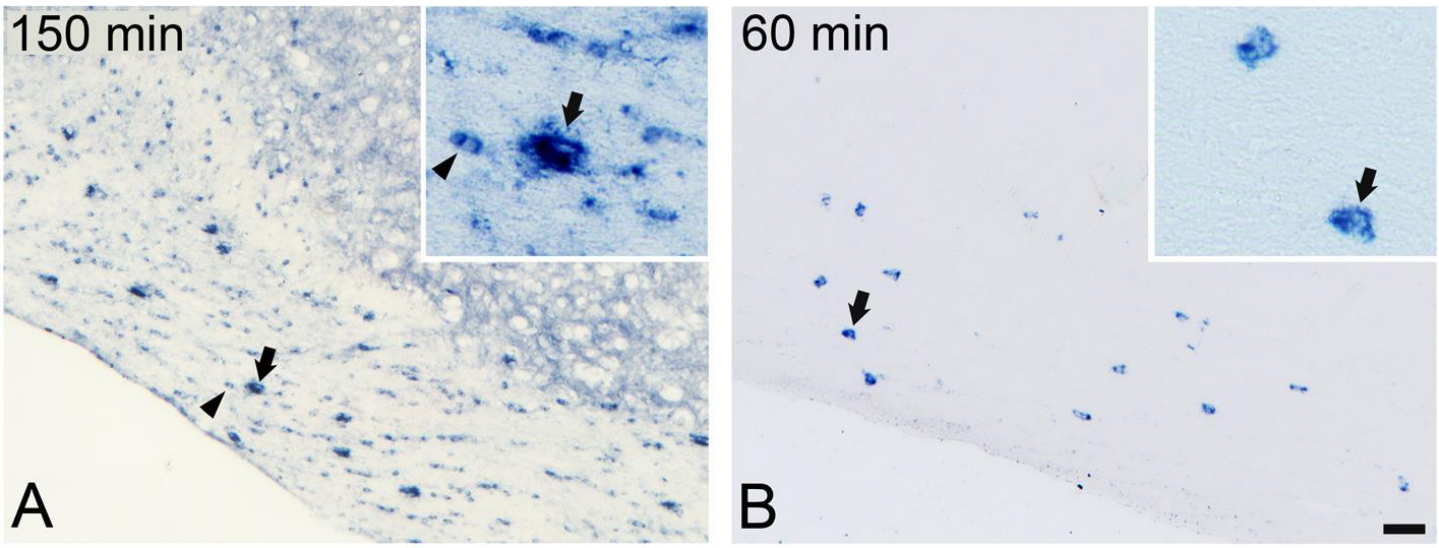


Supplementary Figure 1

Performance of *P-Myrf*$^{-/-}$ versus *P-Myrf*$^{+/-}$ mice on the complex wheel.

Three weeks after tamoxifen administration at P60-64 or P90-P94 (*9*), *P-Myrf*$^{-/-}$ mice and their *P-Myrf*$^{+/-}$ littermates [n=36 (20 males) and 32 (17 males) respectively] were housed singly in cages containing a complex wheel. Average wheel speeds were calculated for each 2-hour time window during the first seven nights (6pm-6am) and plotted as mean ± s.e.m (*P-Myrf*$^{-/-}$, red; *P-Myrf*$^{+/-}$, blue) were analyzed by two-way ANOVA with Bonferroni's post-hoc test. Each night was treated separately for multiple comparisons.
*$p < 0.05$, **$p < 0.01$, ***$p < 10^{-3}$, ****$p < 10^{-4}$

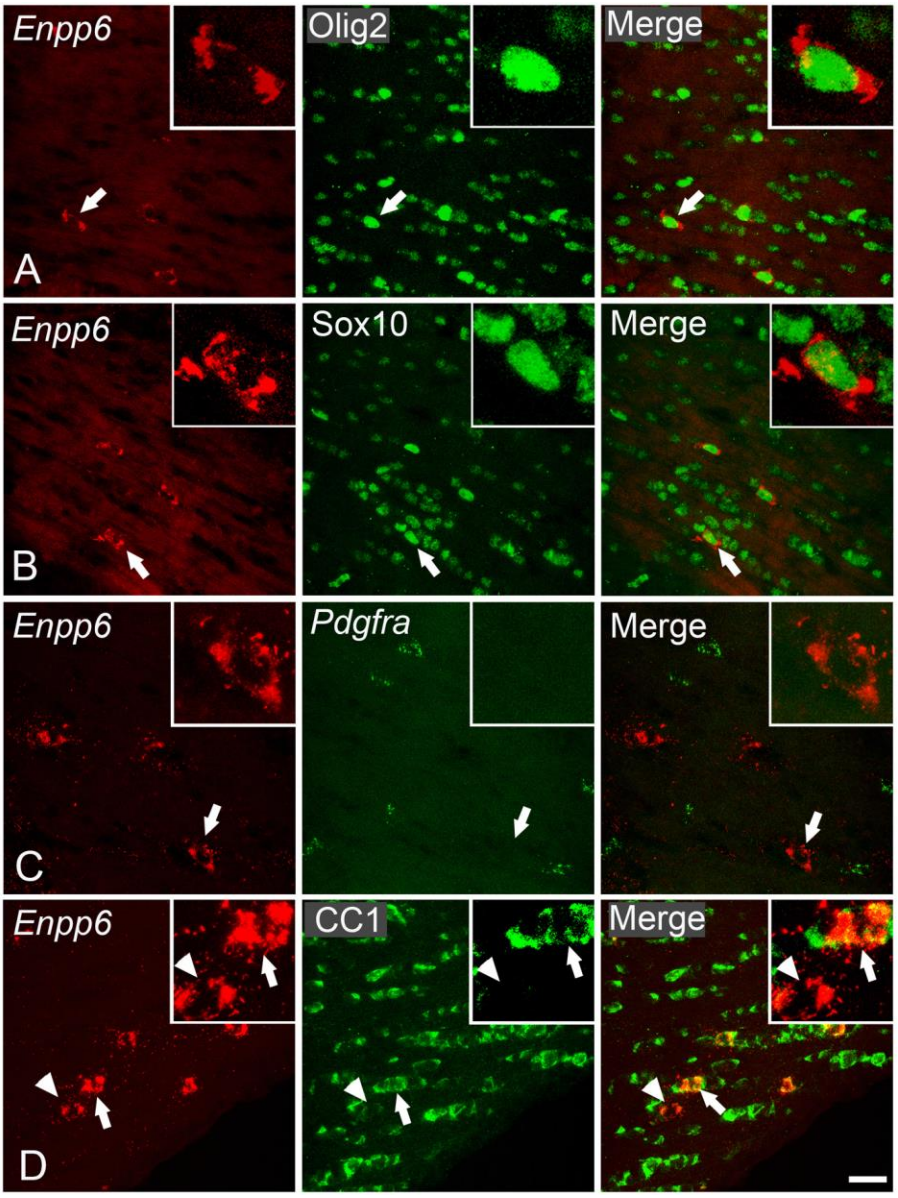
[Night 1: 2 h, p=0.43, t=1.81; 4 h, p=0.029, t=2.83; 6 h, p=0.0089, t=3.20; 8 h, p=0.0044, t=3.40; 10 h, p=0.0017, t=3.66; 12 h, p=0.0036, t=3.46. Night 2: 2 h, p=0.0067, t=3.28; 4 h, p=0.0033, t=3.48; 6 h, p=0.063, t=2.57; 8 h, p=0.016, t=3.03; 10 h, p=0.11, t=2.37; 12 h, p=0.034, t=2.79. Night 3: 2 h, p=0.0019, t=3.63; 4 h, p=0.0041, t=3.43; 6 h, p=0.020, t=2.95; 8 h, p=0.072, t=2.53; 10 h, p>0.99, t=1.05; 12 h, p=0.24, t=2.06. Night 4: 2 h, p=0.020, t=2.96; 4 h, p=0.0010, t=3.80; 6 h, p=0.0007, t=3.90; 8 h, p=0.020, t=2.96; 10 h, p=0.033, t=2.79; 12 h, p=0.30, t=1.96. Night 5: 2 h, p=0.0006, t=3.94; 4 h, p=0.0052, t=3.36; 6 h, p=0.015, t=3.04; 8 h, p=0.13, t=2.32; 10 h, p=0.59, t=1.66; 12 h, p=0.17, t=2.42. Night 6: p<0.0001, t=4.42; 4 h, p<0.0001, t=4.42; 6 h, p<0.0001, t=4.46; 8 h, p=0.0046, t=3.39; 10 h, p=0.020, t=2.96; 12 h, p=0.095, t=2.42. Night 7: 2 h, p<0.0001, t=4.65; 4 h, p<0.0001, t=4.39; 6 h, p=0.0001, t=4.32; 8 h, p=0.0004, t=4.05; 10 h, p=0.20, t=2.13; 12 h, p=0.097, t=2.42. Degrees of freedom=396 throughout.]



Supplementary Figure 2

Two populations of high- and low-expressing *Enpp6*$^{+}$ cells in vivo.

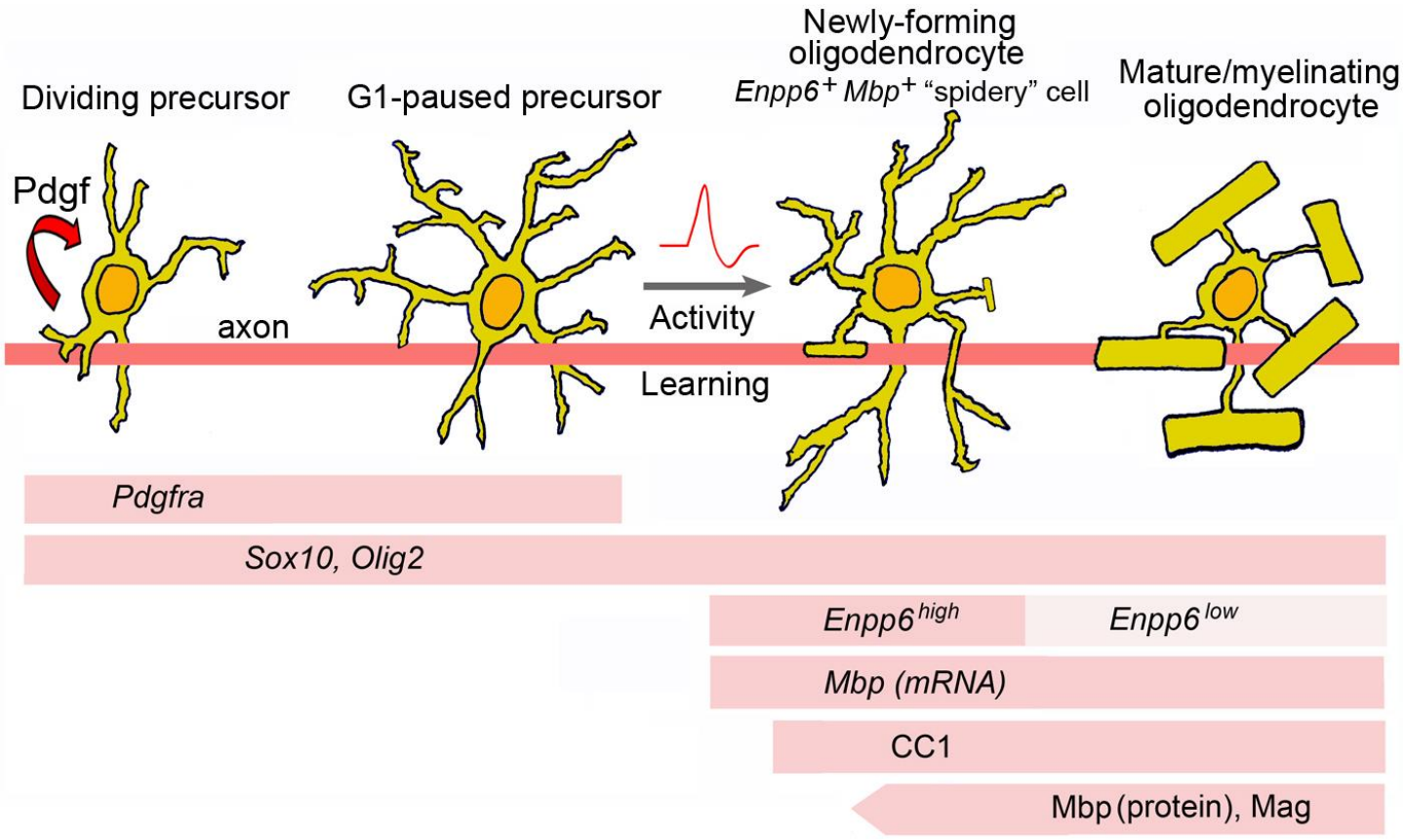
(**a**,**b**) Coronal sections of P60 mouse forebrains were incubated with an *Enpp6* ISH probe and the signal developed using the NBT/BCIP method (Methods). (**a**) At a relatively long development time (150 minutes) two populations of *Enpp6*-positive cells are detected in the subcortical white matter – a few strongly-labeled large cell bodies (arrows) against a background of more numerous, small, weakly-labeled cells (arrowheads), consistent with the RNA-seq data[27] (Fig. 3A) and the likelihood that the strongly-labeled cells are newly-differentiating oligodendrocytes and the weakly-labeled cells more mature, myelinating oligodendrocytes. (**b**) Reducing the development time to 60 minutes allows the strongly-labeled cells to be visualized preferentially (arrows). Images are representative of >3 similar experiments. Scale bar, 50 μm.



Supplementary Figure 3

***Enpp6* expression is oligodendrocyte-specific.**

Forebrain sections of P90 mice (caged without wheels) were analyzed by ISH for *Enpp6* mRNA followed by immuno-fluorescence labeling for stage-specific markers of the oligodendrocyte lineage (Sox10, Olig2, CC1). Alternatively, sections were analyzed by double fluorescence ISH for *Enpp6* and *Pdgfra*. (**a**,**b**) All *Enpp6*$^+$ cells were also Olig2$^+$ and Sox10$^+$. (**c**) No *Enpp6*$^+$ cells were *Pdgfra*$^+$ OPs. (**d**) Most *Enpp6*$^+$ cells were CC1$^+$ mature or maturing oligodendrocytes (Motor cortex, 98.2 ± 1.8%; Subcortical white matter, 86.9% ± 3.8%) (Supplementary Table 1). Images are representative of >3 similar experiments. Scale bar, 25 μm.



Supplementary Figure 4

The role of novel motor activity in stimulating OP differentiation.

OPs are continuously cycling in the young adult CNS in response to mitogenic growth factors such Pdgf. After division one or both daughter OPs can rest in the G1 phase of the cell cycle, sometimes for days or weeks, before either differentiating or entering another division cycle[3,4,7,9,31]. Our data suggest that electrical activity in axon(s) cans stimulate OPs that are paused in G1 to differentiate - losing *Pdgfra* expression and expressing *Enpp6*, *Mbp* and other myelin gene products instead. The newly-differentiating oligodendrocytes have a distinctive spidery morphology in vivo; they remain like this for several days in vivo in rodents[31] before down-regulating *Enpp6* (faint pink line) and assuming the typical morphology of mature myelinating oligodendrocytes. The *Enpp6*^{high} early-differentiating oligodendrocytes and *Enpp6*^{low} myelinating oligodendrocytes probably contribute to improving circuit performance in the early and late stages of motor learning, respectively.

Table S1	*fraction (%) of all Enpp6$^+$ cells that are also:*				
	Olig2$^+$	Sox10$^+$	Pdgfra$^+$	CC1$^+$	*Mbp*$^+$ "spidery"
motor cortex	100	97.3 ± 1.8	0	98.2 ± 1.8	99.4 ± 0.6
sub-cortical WM	100	95.6 ± 2.1	0	86.9 ± 3.8	NA

Supplementary Table 1

Characterization of *Enpp6*$^+$ cells in mouse brain sections.

Cells were counted in sections of mouse forebrain that were subjected to ISH for *Enpp6* followed by immunolabeling for Olig2 or Sox10 (for all oligodendrocyte lineage cells), CC1 (for mature or maturing oligodendrocytes) or by ISH for *Pdgfra* (OPs) (Supplementary Fig. 3). Data from at least 3 sections from each of 3 mice (>1000 cells counted in total). Means ± s.e.m.