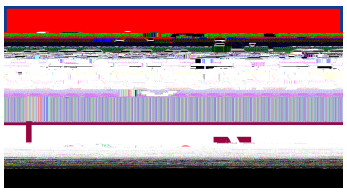

Supplementary Materials for

Motor skills learning requires new central myelination

Ian McKenzie[1, §], David R Ohayon[1, §], Huiliang Li[1]

Materials and Methods

Mouse

Tamoxifen administration

Tamoxifen (Sigma) was dissolved at 40 mg/ml in corn oil by sonicating at 21

Generation of *Sox10-CreER^{T2}* PAC transgenic mice

To generate *Sox10-iCreER^{T2}* PAC transgenic mice, which express iCreER^{T2} in all oligodendrocyte lineage cells, we modified a mouse phage artificial chromosome (PAC) clone (RP21-427F18, UK Human Genome Mapping Project Resource Centre; **Fig. S3**)

http://www.physics.csbsju.edu/stats/KS-test.n.plot_form.html. Performance on the rotarod (time to fall) was compared using a similar approach. Cell counts are displayed as mean ± s.e.m. [(s.d./ (n-1) for n<10]. Single time points were compared by Student's t-test.

Fig. S1. Cre-mediated recombination in

Fig. S2. Inhibition of OL genesis in adult mice.

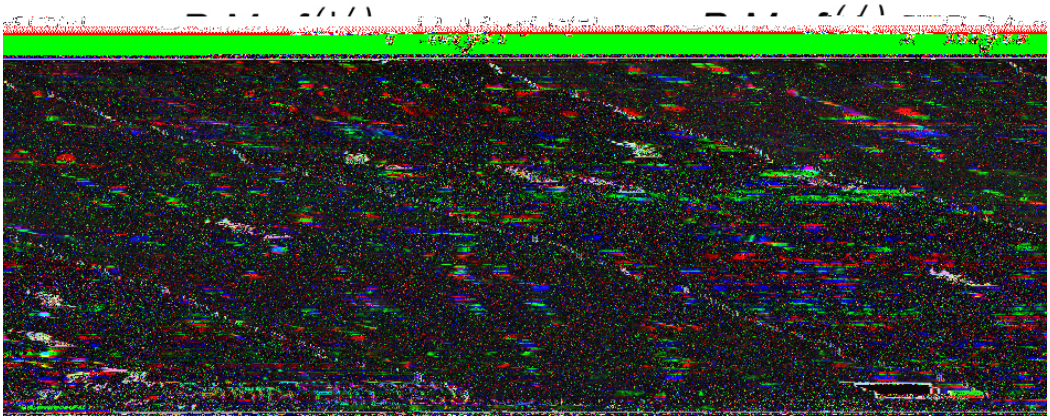

Fig. S2. One month after administering EdU to P60 *P-Myrf*$^{(-/-)}$ mice and their *P-Myrf*$^{+/-)}$ littermates, newly-formed OLs were detected by histochemistry for EdU (red) and CC1 (blue). Many more EdU^{+}, CC1^{+} cells (arrows) were found in *P-Myrf* $^{+/-)}$ than in *P-Myrf*$^{(-/-)}$ corpus callosum (5.7% ± 0.7% versus 0.20% ± 0.07%; means ± s.e.m., 6 fields (>250 cells) counted in each of 3 sections of 3 mice of each genotype). Therefore, production of new OLs was reduced >95% in the *Myrf* null mice relative to heterozygotes, indicating that the recombination efficiency of *Myrf*$^{(flox/flox)}$ was much greater than the ~57% of OPs that recombined the *Rosa-YFP* reporter (see main text) - presumably because of a more favorable configuration of *loxP* sites. Pdgfra^{+} OPs (green) are not EdU-labeled in these images because they would have continued to divide during the month following EdU treatment, diluting EdU below the limit of detection. *Scale bar:* 50 μm

Fig. S3. Generation of transgenic mice.

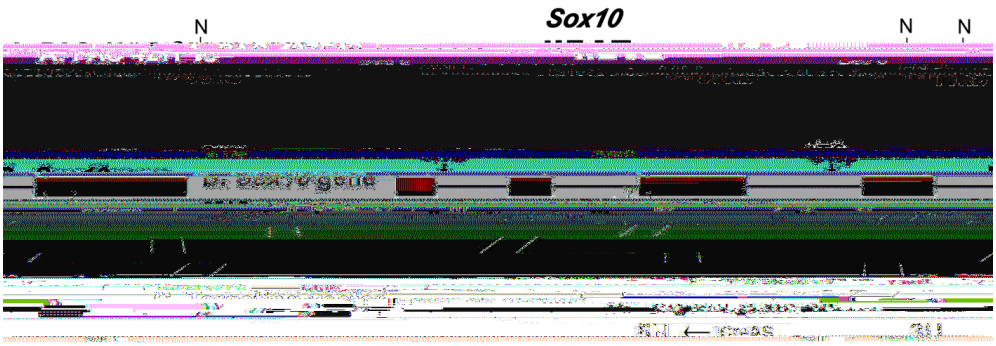

Fig. S3. (A) PAC clone RP21-427F18 spans the *Sox10* gene with upstream and downstream flanking regions; (B) Diagram of the *Sox10* gene locus; (C) The targeting vector used for homologous recombination. N: NotI site; CmR: chloramphenicol resistance cassette; *green chevrons*: *frt* sites; 5H: 5 homology region; 3H: 3 homology region. The positions of genotyping primers (SFAR and iCreAS) are indicated

Fig. S4. Running on CW1 primes mice to run on CW2

Fig. S5. Complex wheel running abilities of females versus males

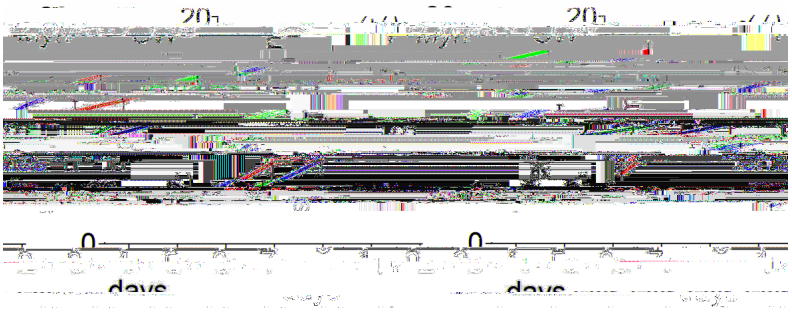

Fig. S5. Female *P-Myrf*$^{(+/-)}$ mice slightly out-performed their male counterparts on the complex wheel after tamoxifen treatment (experimental design as in **Fig. 4B**, main text), although the difference was not statistically significant (e.g. p=0.52 on day 7; ANOVA with Bonferroni's post-hoc test). (Right) A sex difference was not observed with *P-Myrf*$^{(-/-)}$ mice.

Fig. S6. Adult myelination is not required to perform a pre-learned skill

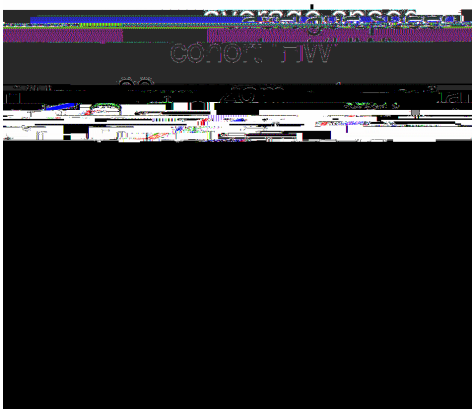

Fig. S6. Experimental design was the same as in **Fig. 4M**. *P-Myrf*$^{(+/-)}$ and *P-Myrf*$^{(-/-)}$ mice (blue and red graphs, respectively; n=5 for both groups) were introduced to the complex wheel for nine days before administering tamoxifen. Only six days are shown because data for days 7-9 were lost due to a power failure. Three weeks post-tamoxifen the mice were re-introduced to the complex wheel. Both before and after tamoxifen there was no difference in the performance of *P-Myrf*$^{(+/-)}$ versus *P-Myrf*$^{(-/-)}$.

Fig. S7. Running on the regular wheel stimulates OP proliferation

Fig. S7. Wild type mice were introduced to the regular wheel on P65 and EdU was administered continuously via the drinking water. The EdU labeling index (fraction of $Pdgfra^{+}$ OPs that was EdU^{+}) was plotted versus time on the wheel. At four days running there was a significant increase in labeling index in runners (red graph) compared to controls without a wheel (blue graph) ($p=0.001$, $n=4$; 2-way ANOVA with Bonferroni's post hoc test).

Movie S1. Demyelination-induced tremor in mice

An *S10-Myrf*$^{(-/-)}$ mouse filmed five weeks post-tamoxifen (administered at P60), displaying general ataxia including tremor and momentary seizures typical of advanced de- or dys-myelination.

Movie S2. First introduction to the complex wheel (wt mouse)

A wild type mouse (C57Bl6/CBA F1 hybrid) was filmed during its first introduction to a complex wheel in low light conditions at the beginning of the dark cycle. The movie plays in slow-motion, one-tenth actual speed.

Movie S3. High-speed running on the complex wheel (wt mouse)

A wild-type mouse after seven days' exposure to a complex wheel (low-light conditions at the beginning of the dark cycle). The movie plays at actual speed, interrupted by freeze-frame sequences to show the foot stepping pattern (ipsilateral fore- and hind-paws grasp the same rungs).

Movie S4. mouse on the complex wheel after training

A *P-Myrf*$^{(-/-)}$ mouse on the complex wheel after seven nights self-training. A slow-motion sequence (one-quarter actual speed) separates two sequences at actual speed. The mouse appears to run less spontaneously than its *P-Myrf*$^{(+/-)}$ littermate (**movie S5**). Although it is difficult to understand the precise problem from movies like this, it seems that placement of the hind paws in particular is less automatic and less accurate.

Movie S5. mouse on the complex wheel after training

A *P-Myrf*$^{(+/-)}$ mouse on the complex wheel after seven nights self-training. A slow-motion sequence (one-quarter actual speed) separates two ac.24 180.0 0 02647do0.2 (e(ua)0.21a) 0.2 (3 0 0