

1 **Supporting Information**

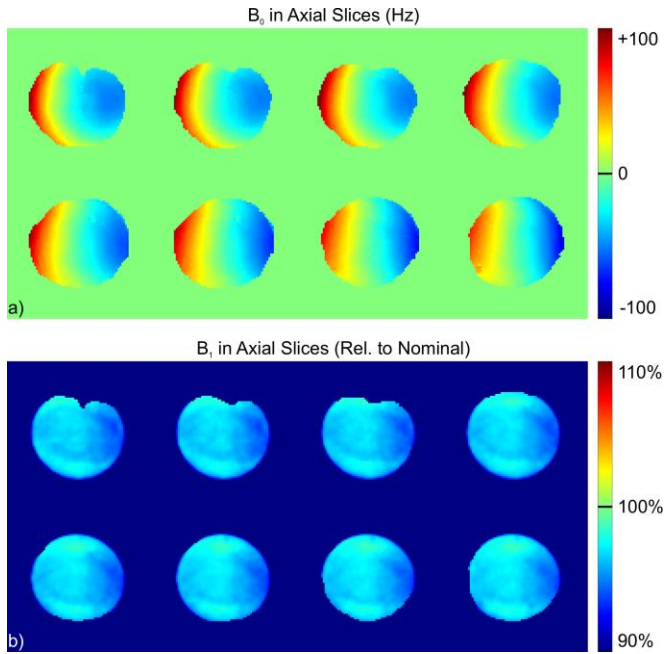
2 **Relaxometry Results**

3 T_1 was 1628 ± 69 ms in unfixed white matter ($n = 14$), 1391 ± 83 ms after 1 day of fixation (a
4 $14.6 \pm 2.2\%$ decrease), and 1338 ± 52 ms after 31 days of fixation (a $17.8 \pm 1.8\%$ decrease). T_2
5 was 63.35 ± 5.68 ms in unfixed white matter, 47.42 ± 4.44 ms after 1 day of fixation (a $24.8 \pm$
6 8.4% decrease), and 48.62 ± 4.34 ms after 31 days of fixation (a $22.8 \pm 9.0\%$ decrease). T_1 was
7 1734 ± 67 ms in unfixed gray matter ($n = 7$), 1504 ± 162 ms after 1 day of fixation (a $13.4 \pm$
8 6.9% decrease), and 1531 ± 171 ms after 31 days of fixation (an $11.9 \pm 7.6\%$ decrease). T_2 was
9 76.80 ± 14.31 ms in unfixed gray matter, 73.70 ± 12.89 ms after 1 day of fixation (a $3.48 \pm$
10 7.28% decrease), and 79.18 ± 12.30 ms after 31 days of fixation (a $3.99 \pm 7.95\%$ increase).

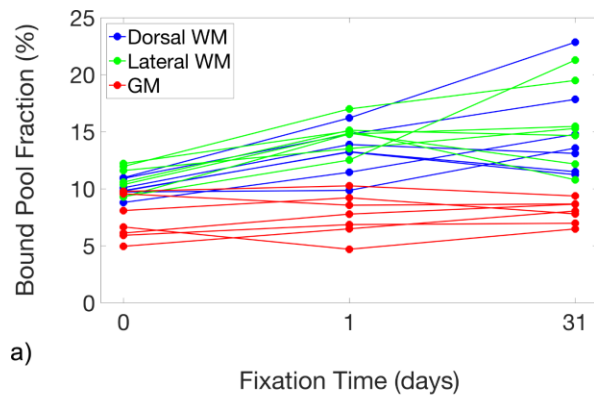
11 **Supporting Information Tables and Figures**

12 Axial maps of the static (B_0) and radiofrequency (B_1) magnetic fields in qMT and MWI scanning
13 sessions are plotted in Supporting Information Figure S1. Measurements of F , MWF, and
14 reference-normalized D_2O -exchanged ZTE intensity within the ROIs are tabulated before
15 (Supporting Information Table S1) and after (Supporting Information Table S2) correction for
16 tissue shrinkage. Exploratory comparisons of shrinkage-corrected measurements before and
17 after fixation are given in Supporting Information Table S3, and shrinkage-corrected
18 measurements are plotted in Supporting Information Figure S2.

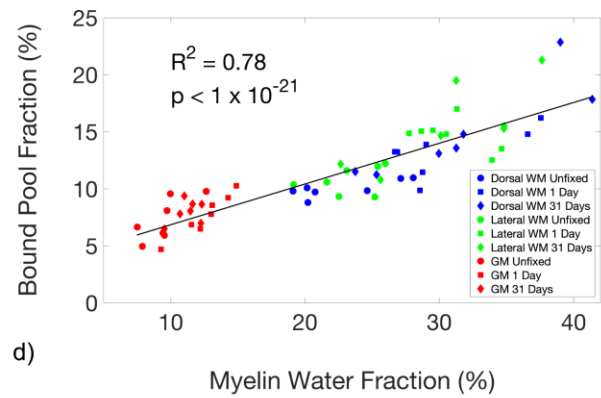
19 **Supporting Information Figures**



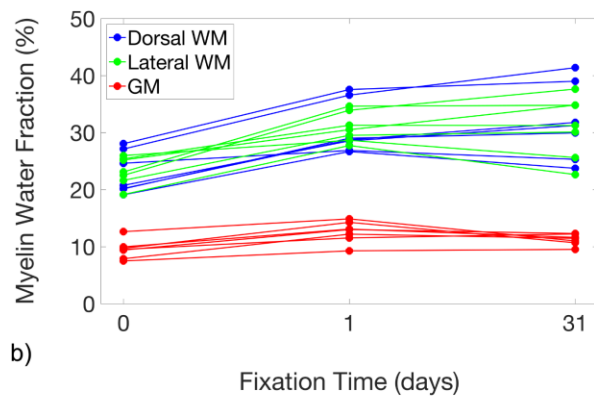
20
21 Supporting Information Figure S1: Axial maps of the static (B_0) (a) and radiofrequency (B_1) (b)
22 magnetic fields in qMT and MWI scanning sessions, averaged across specimens and timepoints
23 ($n = 21$) and masked using the union of the spinal cord masks from each of the 21 imaging
24 sessions. B_0 field maps were acquired after the iterative shimming process converged on stable
25 calculated shim currents.



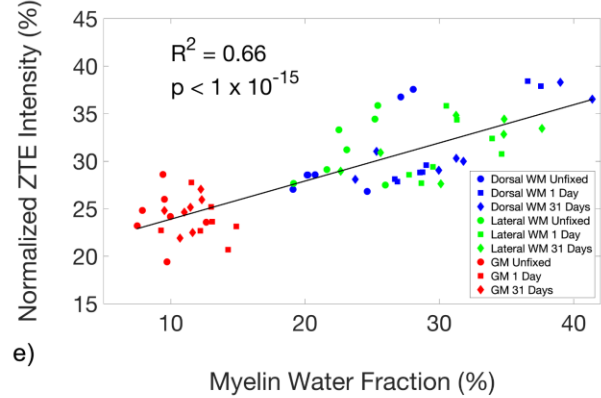
a)



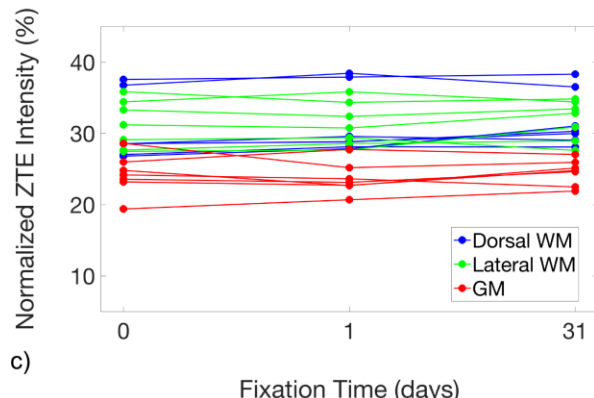
d)



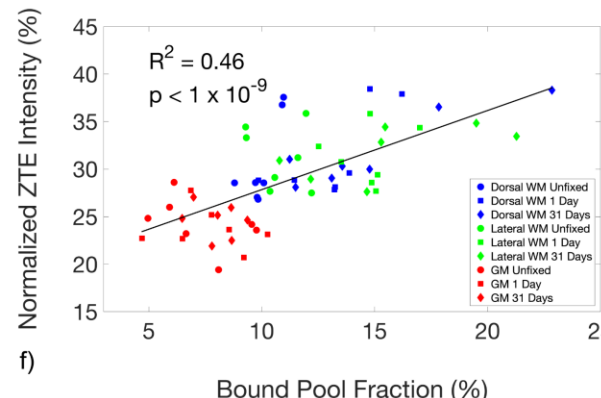
b)



e)



c)



f)

26

27 Supporting Information Figure S2: Line plots showing the changes in the quantitative
 28 magnetization transfer bound pool fraction (a), myelin water fraction (b), and reference-
 29 normalized D₂O-exchanged ZTE signal intensity (c), of unfixed specimens and after 1 day and
 30 31 days of fixation, after correcting for tissue shrinkage due to fixation. Shrinkage correction
 31 removes a large amount of the observed increase in D₂O-exchanged ZTE intensity, but the
 32 inter-measurement correlations (d-f) are not significantly altered.

33 Supporting Information Table S1

Bound Pool Fraction									
ROI	Time (days)	Specimen 2.1	Specimen 3.1	Specimen 3.3	Specimen 4.1	Specimen 4.3	Specimen 5.1	Specimen 5.3	Mean ± SD
Dorsal WM	0	9.84	10.97	10.91	9.72	8.8	10.09	9.8	10.02 ± 0.75
	1	14.01	16.07	14.86	10.35	12.26	14.66	14.11	13.76 ± 1.89
	31	13.18	23.58	16.79	13.84	14.65	13.22	13.93	15.60 ± 3.73
Lateral WM	0	12.21	11.96	9.29	11.6	9.32	10.58	10.37	10.76 ± 1.20
	1	15.96	16.86	14.87	14.19	13.40	15.98	15.83	15.30 ± 1.20
	31	12.66	20.13	14.56	15.60	21.10	14.80	14.75	16.23 ± 3.14
GM	0	9.77	6.12	5.92	6.65	4.96	9.56	8.09	7.30 ± 1.87
	1	10.87	7.71	6.89	4.93	6.95	9.04	9.80	8.03 ± 2.02
	31	10.99	8.93	6.57	6.61	7.98	8.75	9.45	8.47 ± 1.58

Myelin Water Fraction									
ROI	Time (days)	Specimen 2.1	Specimen 3.1	Specimen 3.3	Specimen 4.1	Specimen 4.3	Specimen 5.1	Specimen 5.3	Mean ± SD
Dorsal WM	0	24.63	28.06	27.13	20.75	20.21	20.15	19.11	22.86 ± 3.68
	1	28.49	37.24	36.76	29.96	30.78	30.66	28.41	31.76 ± 3.70
	31	29.70	40.26	38.95	31.89	31.52	30.24	28.77	33.05 ± 4.62
Lateral WM	0	25.98	25.41	25.2	23.11	22.52	21.63	19.15	23.29 ± 2.49
	1	30.38	31.04	30.67	36.34	36.32	31.20	29.53	32.21 ± 2.86
	31	30.07	32.25	32.78	35.50	37.32	30.39	27.45	32.25 ± 3.36
GM	0	12.64	9.42	9.53	7.5	7.89	9.97	9.72	9.52 ± 1.67
	1	15.77	12.90	11.60	9.74	13.06	13.83	15.19	13.16 ± 2.07
	31	12.92	12.71	11.52	9.72	11.37	11.74	12.96	11.85 ± 1.16

ZTE Intensity									
ROI	Time (days)	Specimen 2.2	Specimen 3.2	Specimen 3.4	Specimen 4.2	Specimen 4.4	Specimen 5.2	Specimen 5.4	Mean ± SD
Dorsal WM	0	26.81	37.56	36.76	28.57	28.55	28.55	27.03	30.55 ± 4.58
	1	27.77	39.41	39.03	29.89	29.74	30.69	28.42	32.14 ± 4.94
	31	32.47	41.19	38.68	31.87	31.41	30.41	28.93	33.57 ± 4.55
Lateral WM	0	27.48	35.86	34.42	31.2	33.3	29.11	27.66	31.29 ± 3.35
	1	27.60	35.71	36.39	31.92	33.39	30.50	28.88	32.06 ± 3.32
	31	32.34	37.45	36.45	34.54	35.03	28.91	29.81	33.50 ± 3.26
GM	0	23.57	28.6	25.99	23.2	24.81	24.18	19.4	24.25 ± 2.81
	1	23.06	26.19	28.19	23.57	23.39	24.52	20.91	24.26 ± 2.35
	31	25.78	27.89	28.64	26.09	26.33	23.54	22.57	25.83 ± 2.17

34 Supporting Information Table S1: Measurements of F, MWF, and reference-normalized D₂O-
 35 exchanged ZTE intensity without correction for tissue shrinkage. All values are in percent units.

36 **Supporting Information Table S2**

Bound Pool Fraction (Corrected for Tissue Shrinkage)									
ROI	Time (day)	Specimen 2.1	Specimen 3.1	Specimen 3.3	Specimen 4.1	Specimen 4.3	Specimen 5.1	Specimen 5.3	Mean ± SD
Dorsal WM	0	9.84	10.97	10.91	9.72	8.8	10.09	9.8	10.02 ± 0.75
	1	13.22	16.21	14.79	9.86	11.45	13.88	13.25	13.24 ± 2.09
	31	11.24	22.85	17.84	13.57	14.78	13.10	11.50	14.98 ± 4.12
Lateral WM	0	12.21	11.96	9.29	11.6	9.32	10.58	10.37	10.76 ± 1.20
	1	15.06	17.00	14.80	13.52	12.52	15.13	14.87	14.70 ± 1.40
	31	10.79	19.50	15.47	15.29	21.28	14.66	12.17	15.60 ± 3.73
GM	0	9.77	6.12	5.92	6.65	4.96	9.56	8.09	7.30 ± 1.87
	1	10.26	7.78	6.86	4.70	6.49	8.56	9.21	7.69 ± 1.86
	31	9.37	8.65	6.98	6.48	8.05	8.67	7.80	8.00 ± 1.01

Myelin Water Fraction (Corrected for Tissue Shrinkage)									
ROI	Time (day)	Specimen 2.1	Specimen 3.1	Specimen 3.3	Specimen 4.1	Specimen 4.3	Specimen 5.1	Specimen 5.3	Mean ± SD
Dorsal WM	0	24.63	28.06	27.13	20.75	20.21	20.15	19.11	22.86 ± 3.68
	1	26.88	37.56	36.58	28.56	28.75	29.02	26.69	30.58 ± 4.54
	31	25.32	39.01	41.39	31.26	31.79	29.96	23.74	31.78 ± 6.51
Lateral WM	0	25.98	25.41	25.2	23.11	22.52	21.63	19.15	23.29 ± 2.45
	1	28.66	31.30	30.52	34.64	33.93	29.53	27.74	30.90 ± 2.59
	31	25.63	31.25	34.83	34.80	37.64	30.11	22.65	30.99 ± 5.36
GM	0	12.64	9.42	9.53	7.5	7.89	9.97	9.72	9.52 ± 1.67
	1	14.88	13.01	11.54	9.28	12.20	13.09	14.27	12.61 ± 1.86
	31	11.01	12.31	12.24	9.53	11.47	11.63	10.69	11.27 ± 0.97

ZTE Intensity (Corrected for Tissue Shrinkage)									
ROI	Time (day)	Specimen 2.2	Specimen 3.2	Specimen 3.4	Specimen 4.2	Specimen 4.4	Specimen 5.2	Specimen 5.4	Mean ± SD
Dorsal WM	0	26.81	37.56	36.76	28.57	28.55	28.55	27.03	30.55 ± 4.58
	1	27.85	37.90	38.43	28.81	28.84	29.58	28.12	31.36 ± 4.68
	31	31.03	38.30	36.52	30.30	29.99	29.05	28.09	31.90 ± 3.92
Lateral WM	0	27.48	35.86	34.42	31.2	33.3	29.11	27.66	31.29 ± 3.35
	1	27.68	34.35	35.83	30.76	32.38	29.40	28.57	31.28 ± 3.04
	31	30.90	34.83	34.42	32.83	33.44	27.62	28.94	31.86 ± 2.78
GM	0	23.57	28.6	25.99	23.2	24.81	24.18	19.4	24.25 ± 2.81
	1	23.13	25.19	27.76	22.72	22.68	23.63	20.69	23.69 ± 2.24
	31	24.63	25.94	27.04	24.80	25.14	22.49	21.91	24.56 ± 1.82

37 Supporting Information Table S2: Measurements of F, MWF, and reference-normalized D₂O-
38 exchanged ZTE intensity after correction for tissue shrinkage. All values are in percent units.

39 **Supporting Information Table S3**

Bound Pool Fraction (Corrected for Tissue Shrinkage)			
	0 days vs. 1 day	1 day vs. 31 days	0 days vs. 31 days
Dorsal WM	<i>31.7 ± 14.4% (p = 2x10⁻³)</i>	<i>13.5 ± 24.2% (p = 2x10⁻¹)</i>	<i>48.7 ± 33.6% (p = 1x10⁻²)</i>
Lateral WM	<i>37.4 ± 14.2% (p = 2x10⁻⁴)</i>	<i>7.54 ± 31.8% (p = 6x10⁻¹)</i>	<i>47.7 ± 44.5% (p = 2x10⁻²)</i>
GM	<i>7.53 ± 21.3% (p = 5x10⁻¹)</i>	<i>7.48 ± 18.5% (p = 5x10⁻¹)</i>	<i>14.6 ± 27.5% (p = 3x10⁻¹)</i>
All	<i>25.6 ± 20.9% (p = 2x10⁻⁵)</i>	<i>9.49 ± 14.3% (p = 2x10⁻¹)</i>	<i>37.0 ± 37.7% (p = 4x10⁻⁴)</i>

Myelin Water Fraction (Corrected for Tissue Shrinkage)			
	0 days vs. 1 day	1 day vs. 31 days	0 days vs. 31 days
Dorsal WM	<i>34.5 ± 11.8% (p = 2x10⁻⁴)</i>	<i>3.35 ± 8.91% (p = 3x10⁻¹)</i>	<i>39.3 ± 19.5% (p = 2x10⁻³)</i>
Lateral WM	<i>33.8 ± 15.8% (p = 8x10⁻⁴)</i>	<i>-0.23 ± 11.3% (p = 9x10⁻¹)</i>	<i>33.6 ± 22.5% (p = 8x10⁻³)</i>
GM	<i>33.4 ± 13.8% (p = 3x10⁻⁴)</i>	<i>-9.26 ± 12.5% (p = 9x10⁻²)</i>	<i>20.8 ± 18.6% (p = 4x10⁻²)</i>
All	<i>33.9 ± 13.2% (p = 3x10⁻⁸)</i>	<i>-2.05 ± 11.8% (p = 1)</i>	<i>31.2 ± 20.8% (p = 2x10⁻⁵)</i>

ZTE Intensity (Corrected for Tissue Shrinkage)			
	0 days vs. 1 day	1 day vs. 31 days	0 days vs. 31 days
Dorsal WM	<i>2.69 ± 1.68% (p = 7x10⁻³)</i>	<i>2.10 ± 5.33% (p = 4x10⁻¹)</i>	<i>4.83 ± 5.30% (p = 5x10⁻²)</i>
Lateral WM	<i>0.11 ± 3.07% (p = 1)</i>	<i>2.05 ± 6.03% (p = 4x10⁻¹)</i>	<i>2.11 ± 5.89% (p = 4x10⁻¹)</i>
GM	<i>-1.90 ± 7.00% (p = 4x10⁻¹)</i>	<i>4.00 ± 5.86% (p = 1x10⁻¹)</i>	<i>1.92 ± 7.79% (p = 7x10⁻¹)</i>
All	<i>0.30 ± 4.70% (p = 8x10⁻¹)</i>	<i>2.72 ± 5.53% (p = 6x10⁻²)</i>	<i>2.95 ± 6.24% (p = 5x10⁻²)</i>

40 Supporting Information Table S3: Changes in myelin density measurements between imaging
 41 time points, after correction for tissue shrinkage, expressed as percent change relative to the
 42 pre-fixation measurement, with p-values. Although these statistical tests are exploratory, not
 43 confirmatory, changes with p-values greater than 1.28x10⁻³ are displayed in gray italic text.