Supporting Information

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SI Text

Longer-Lasting Change in Water Diffusion in the Left M1, Right PM, and Left SPL Than in the Left PM and SMA. The length of the time that the rTMS-induced changes in water diffusion were present was quantified as the last time point where the signal intensity of DWI data acquired with a b value of 1,200 s/mm^2 was significantly different to baseline, and the regions were ranked accordingly. The Wilcoxon signed-rank test showed that the left M1, right PM, and left SPL retained the change in water diffusion longer than left PM ($P = 0.02$, $P = 0.006$, and $P = 0.03$, respectively) and the SMA ($P = 0.01$, $P = 0.002$, and $P = 0.01$, respectively). No difference was found between any pairs of regions among the left M1, left SPL, and right PM or between the SMA and left PM ($P > 0.1$).

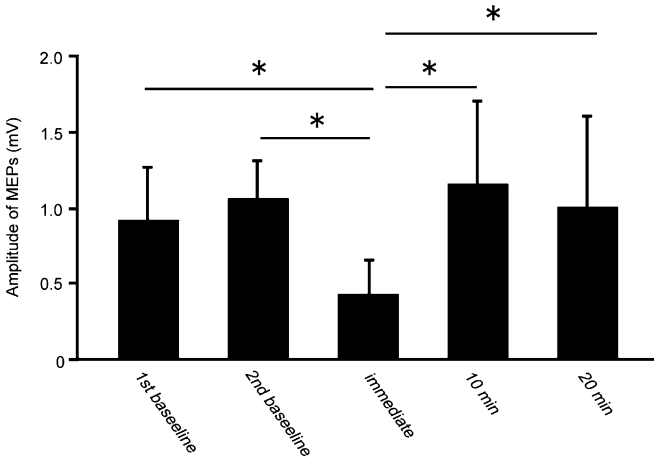

Fig. S1. Change in motor-evoked potential amplitude immediately after the end of repetitive transcranial magnetic stimulation. Motor-evoked potentials (MEPs) were measured to evaluate corticospinal excitability in response to stimulation of the primary motor cortex (M1). Twenty MEPs were recorded at each time point, and the average amplitude was compared across time points using one-way ANOVA followed by paired *t* tests with Bonferroni adjustments. Asterisks indicate significant difference (paired *t* test, $P < 0.05$). One-way ANOVA showed a main effect of time point on MEP amplitude [$F_{(4)} = 5.21$, $P = 0.001$]. Post hoc tests indicated that MEP amplitude was similar at the two baseline time points (multiple comparisons test with Bonferroni adjustments, $P = 0.45$). MEP amplitude was lower immediately after the end of repetitive transcranial magnetic stimulation (rTMS) than at each of the two baselines ($P = 0.013$ and $P = 0.0052$ for first and second baseline, respectively), but had recovered to baseline level by 10 min after rTMS ($P = 0.027$ for 10 min after vs. immediately after rTMS and $P = 0.35$ and $P = 0.42$ for 10 min after vs. first and second baseline, respectively) and 20 min after rTMS ($P = 0.032$ for 20 min after vs. immediately after rTMS and $P = 0.48$ and $P = 0.51$ for 20 min after vs. first and second baseline, respectively). These results show that the excitability of the corticospinal tract decreased immediately after the end of the low-frequency rTMS but recovered to the baseline levels by 10 min after rTMS, providing evidence that our stimulation protocol induced a transient long-term depression (LTD)-like plasticity in the stimulated M1, as reported in previous literature (1). These results do not necessarily suggest that LTD-like plasticity is retained for less time than the increase in water diffusion. Previous studies have indicated comparable retention of the two (2) when plasticity was measured at the intracortical level (1). The relation between long-term potentiation (LTP)/LTD-like plasticity and water diffusion remains unknown in human models, and this issue requires further investigation.

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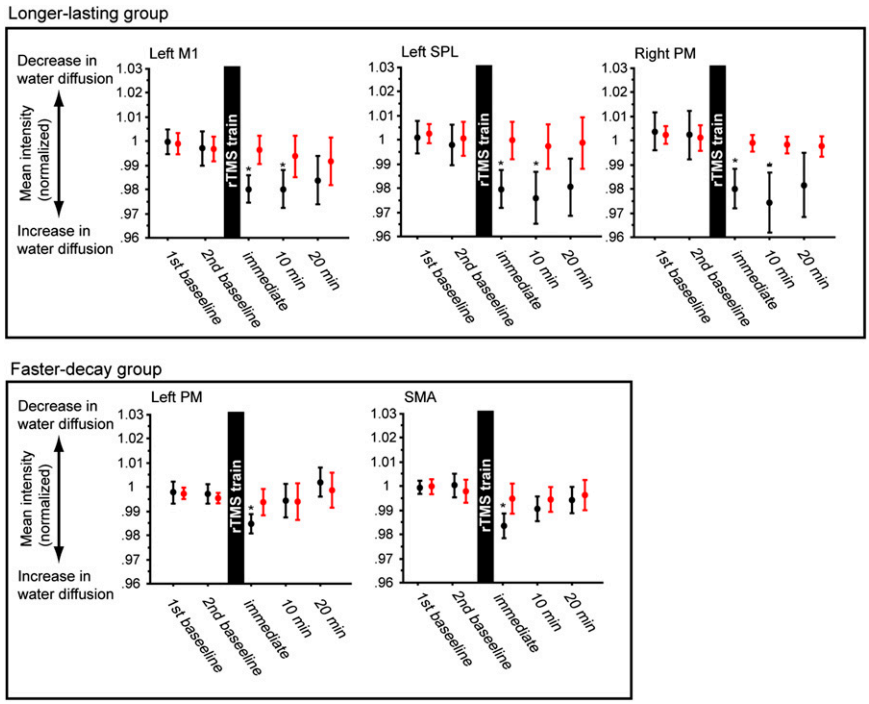

Fig. S2. No significant change in diffusion-weighted MRI signals acquired with a low b value. Voxel-based comparisons across time points were performed on the diffusion-weighted MRI (DWI) data acquired with a low b value using the same procedure described in Fig. 2. There was no significant change across time points for any region, even at the liberal threshold of uncorrected $P = 0.1$. The mean normalized signal intensity is shown for data acquired with high (1,200 mm/s^2; black) and low (300 mm/s^2; red) b values (Fig. 3). Error bars indicate SEM. To examine the difference in DWI data acquired with a high and a low b value (b1200 and b300 data, respectively), a repeated-measures mixed-model ANOVA on mean normalized signal intensity was performed in a factorial design with the factors of b value (b1200 and b300), region [left M1; left superior parietal lobule (SPL); right premotor cortex (PM); left PM; and supplementary motor area (SMA)], and time point (first baseline; second baseline; immediately after rTMS; and 10 min after rTMS). Data collected 20 min after rTMS were not included as they were similar to baseline data for both b values in all regions. The results showed a significant main effect of time point ($F = 22.87$, $P < 0.001$) but no main effect of b value ($F = 0.39$, $P = 0.54$) or region ($F = 1.835$, $P = 0.125$). There was a significant b value × time point interaction ($F = 9.61$, $P < 0.001$), indicating a different time course of signal intensity between data acquired with high and low b values. No other interactions were significant (region × time point, $F = 0.54$, $P = 0.88$; b value × region, $F = 1.97$, $P = 0.11$; b value × region × time point, $F = 0.39$, $P = 0.96$). Post hoc analyses showed that the signal intensity of the b1200 data was higher than that of the b300 data image immediately after rTMS ($P = 0.02$) and 10 min after rTMS ($P = 0.03$), but was similar to that of the b300 data at the two baseline time points ($P = 0.80$ and $P = 0.64$ for first baseline and second baseline, respectively). These results suggest that the b1200 data were more sensitive to rTMS-induced changes in water diffusion than the b300 data.

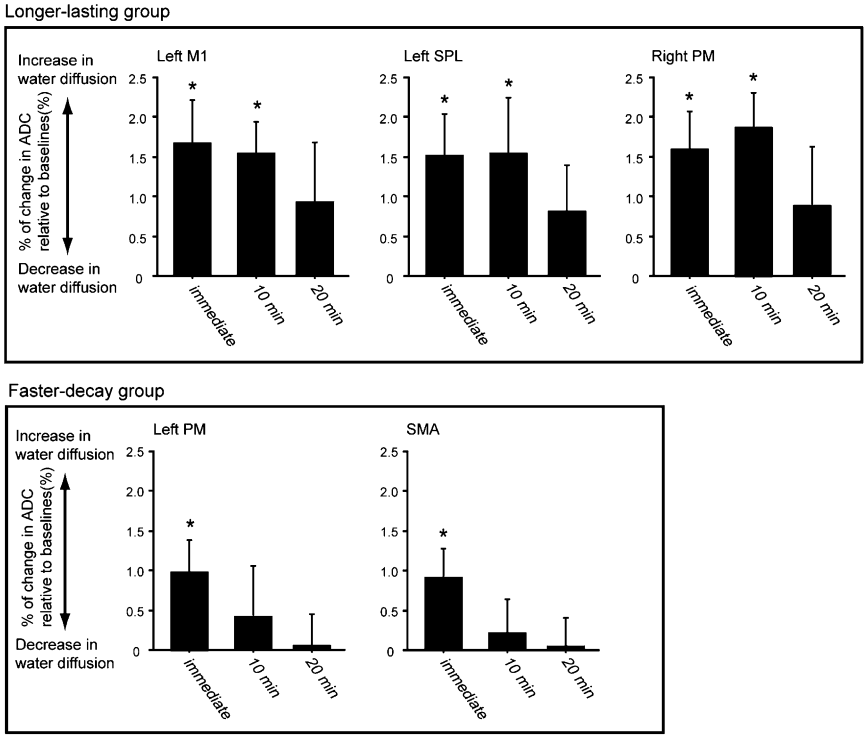

Fig. S3. Significant increase in the apparent diffusion coefficient after the end of rTMS. The apparent diffusion coefficient (ADC) has been used to evaluate intracortical water diffusion in previous studies (1, 2) and was computed using the following equation: $ADC = \ln(S_{300}/S_{1,200})/(1200–300)$, where S_{300} and $S_{1,200}$ are the signal intensity of DWI data obtained with b values of 300 and 1,200 s/mm^2, respectively. The figure shows percent change in ADC at each post-rTMS time point relative to the baseline time point: $[(ADC_{immediate,\ 10\ min,\ or\ 20\ min} - ADC_{baseline})/ADC_{baseline}] \times 100$. At each time point, the ADC was calculated using the mean signal intensity of DWI data obtained with a b value of 1,200 s/mm^2 ($n = 72$ samples per time point) and the mean signal intensity of DWI data obtained with a b value of 300 s/mm^2 ($n = 18$ samples per time point). The baseline ADC was the mean of the ADC in the two baseline sessions. Asterisks indicate that the ratio was different from zero ($P < 0.05$ after Bonferroni-Holm adjustments). The results are consistent with those in Figs. 2 and 3.

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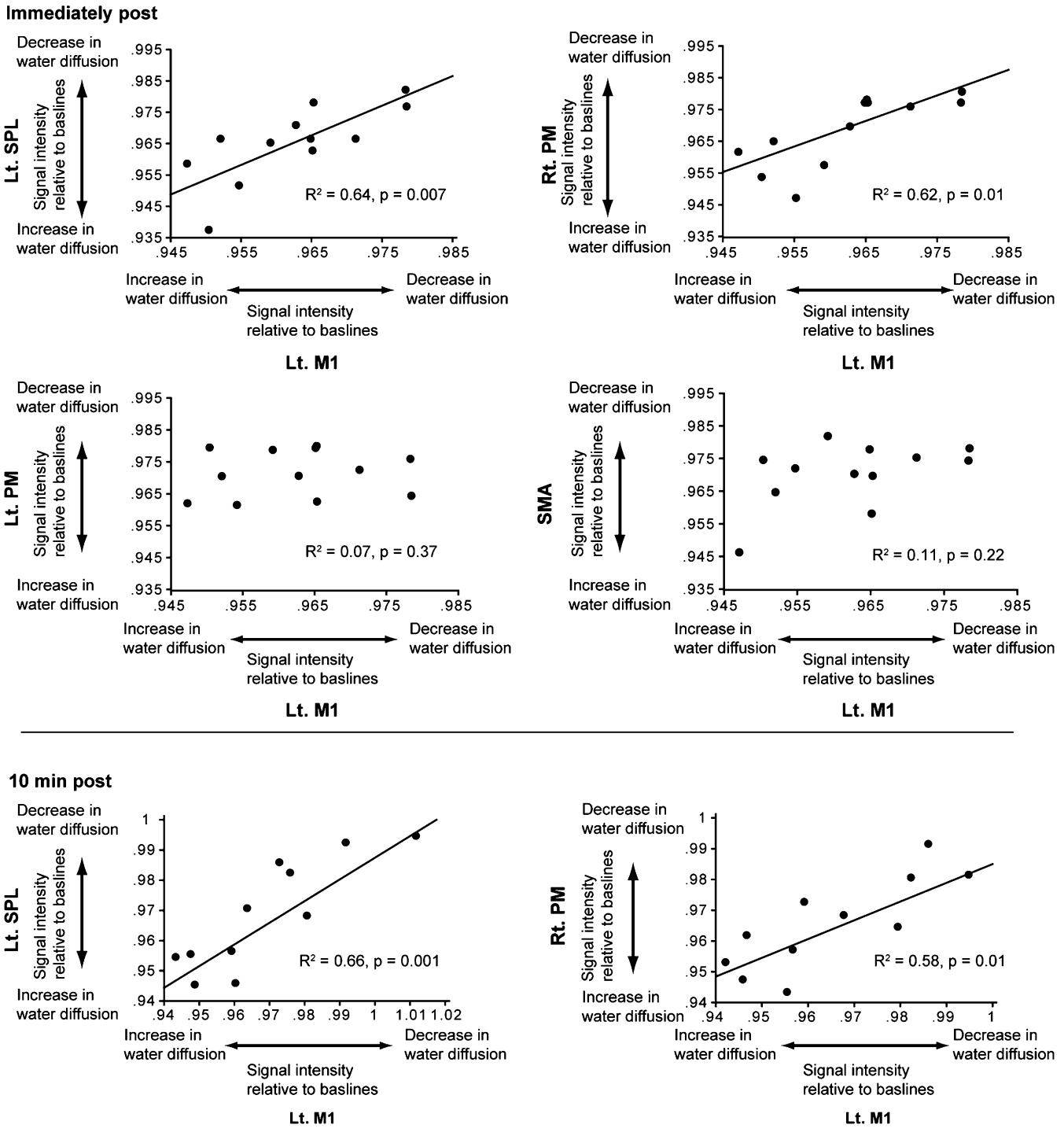

Fig. S4. rTMS-induced change in water diffusion in the left M1 was correlated with the change in water diffusion in the left SPL and right PM. The change in water diffusion was estimated as the ratio of the mean b1200 DWI signal intensity at the immediately or 10 min after rTMS time point and the mean intensity at the two baseline time points. The figure shows the scatter plot of this ratio in the left M1 (*x* axis) and the remote region (*y* axis) at the immediately and 10-min after time points. Each data point represents a single subject. Immediately after the end of rTMS, the increase in water diffusion in M1 was correlated with the increase in water diffusion in the left SPL and right PM but not in the left PM or SMA (*Upper*). Note that decreased signal intensity indicates increased water diffusion. These correlations remained significant at the 10-min after time point (*Lower*). R^2 and *P* values are shown in each regression plot.

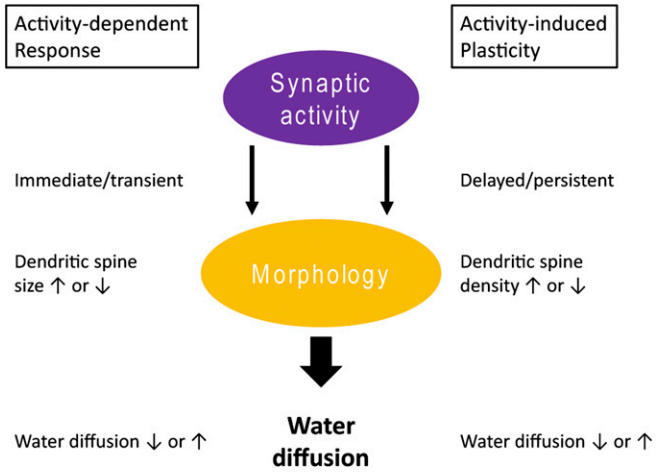

Fig. S5. Possible biological mechanisms underlying changes in intracortical water diffusion. This schema shows the possible biological mechanisms underpinning altered water diffusion in neural tissues. Synaptic activation, which includes intracellular molecular events, induces immediate and transient morphological changes in dendritic spines that occur within seconds to minutes (an activity-dependent response) (1–3) or delayed and persistent changes in dendritic spines that occur within hours to days (activity-induced plasticity) (4–9). Activity-dependent responses alter the size of dendritic spines and activity-induced plasticity modulates the size or density of the dendritic spines, both of which can modulate intracortical water diffusion (10, 11). Note that an increase in size or density would result in decreased water diffusion and a decrease in size or density would result in increased water diffusion. Interregional synchronization in water diffusion might reflect synchronized fluctuation of activity-dependent morphological responses between the regions. Low-frequency rTMS that induces LTD-like plasticity in the stimulated cortex decreases the density of dendritic spines and thus would be expected to increase intracortical water diffusion. Our results indicate that synchronization of basal fluctuations in the morphology of cortical microstructures between stimulated and remote regions might underlie formation of plasticity in remote regions after rTMS.

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