other movement disorders definitively seem caused partly by the clinical similarities of a continuum of involuntary movements including myoclonus, tics, dystonia, and chorea and partly by the lack of reliable diagnostic tests. The identification of a gene, *SGCE*,[5] for MDS and the finding of other loci associated with MDS[6] will make the definitive diagnosis of MDS easier and more exact in the future.

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Repetitive TMS temporarily alters brain diffusion

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Abstract—The authors investigated whether repetitive transcranial magnetic stimulation (rTMS) at 1 Hz (12 minutes; 90% of motor threshold) to the primary motor cortex (M1) leads to changes in diffusion-weighted imaging (DWI). After the rTMS train, there was a temporary small restriction in diffusion within the targeted left M1 that disappeared after 5 minutes. These findings provide a physiologic correlate to the reported behavioral consequences of off-line 1-Hz rTMS and reveal the transitory nature of the effects.

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The so-called "off-line repetitive transcranial magnetic stimulation (rTMS)" design (behavioral measurements before and after a 1-Hz rTMS train applied to a specific brain region) results in temporary effects on the studied cognitive tasks.[1]

Low-frequency (1 Hz) rTMS has been observed to decrease the cortical excitability of the primary motor cortex (M1)[2] and the visual cortex[3] for several minutes after completion of the rTMS train. These inhibitory effects of rTMS have been attributed to the transsynaptic activation of GABAergic, inhibitory interneurons in combination with NMDA-associated postsynaptic modulations,[4] to the recurrent inhibition of the targeted corticomotoneurons through axonal collaterals,[5] or to a long-term depression phenomenon.[4] Although the temporary cortical inhibitory effect of rTMS is sometimes compared with a virtual lesion, no study has directly assessed the severity of such a "virtual lesion" and how it compares with other more permanent lesions such as those seen in regional ischemia. DWI has become a frequently used technique to establish the severity of acute ischemic stroke where restrictions in the diffusivity of water protons have been associated with the development of cytotoxic edema and impairments of Na-K-ATPase. This study was designed to investigate the safety of rTMS and to determine whether behavioral effects after 1-Hz rTMS

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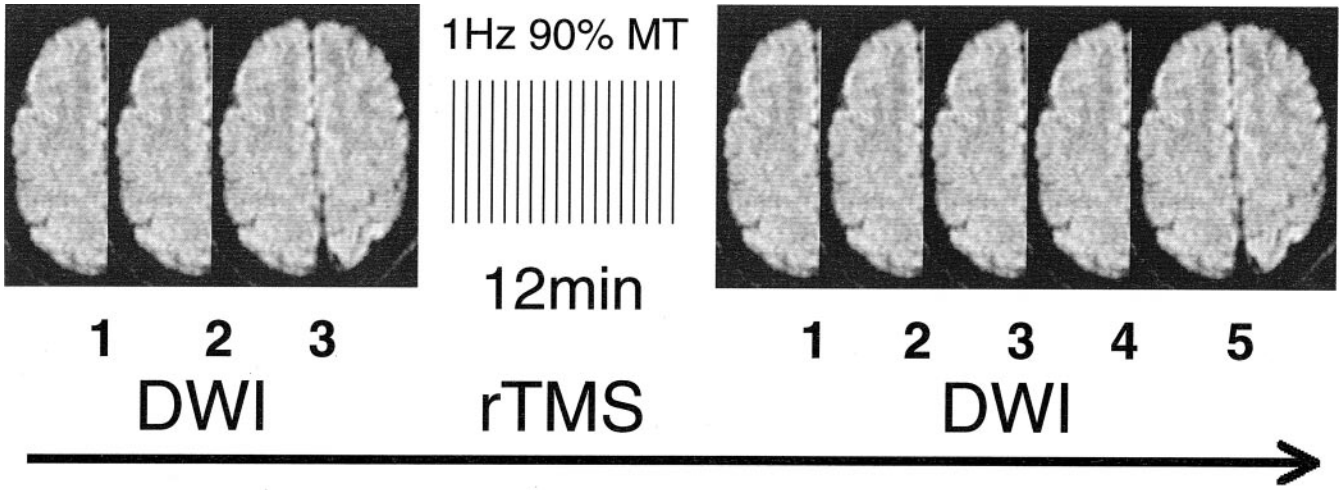

Figure 1. Experimental design: three diffusion-weighted imaging (DWI) MR data sets were acquired before the 12-minute, 1-Hz rTMS train, and five DWI MR data sets were acquired after the train. The 1st diffusion scan was acquired 70 to 90 seconds, the 2nd was acquired 180 to 240 seconds, the 3rd was acquired 300 to 360 seconds, the 4th was acquired around 420 seconds, and the 5th was acquired around 570 seconds after the rTMS train.

may result from changes in tissue diffusion. We investigated whether rTMS at 1 Hz administered for 12 minutes at 90% of motor threshold to left M1 would lead to any changes in the cortical diffusivity of water protons. This would also evaluate the proposed temporal virtual lesion model that rTMS can provide for neuroscience research.

Methods. Eight healthy, right-handed male paid volunteers (median age, 29 years; range, 20 to 38 years) with no history of neurologic or psychiatric illness took part in this study, which was approved by the Institutional Review Board. Subjects gave written, informed consent.

A focal figure-of-eight TMS coil (diameter, 70 mm) connected to a Magstim Super Rapid (The Magstim Company Ltd., UK) stimulator was used. The optimal scalp position from which TMS induced motor evoked potentials (MEPs) of maximal amplitude in the contralateral first dorsal interosseus muscle (FDI) was identified and marked.[5] The motor threshold (MT) was defined as described elsewhere.[5] Subjects were placed in the MRI scanner, and a high-resolution T1-weighted data set (voxel size, 1 mm^3) and three sets of DWIs were acquired (figure 1). The bed of the scanner was then moved outside the bore, and rTMS (1 Hz, 90% MT) was applied for 12 minutes over the previously marked optimal scalp position for activation of the right FDI. All subjects remained in the supine position, and TMS stimulation was done inside the head coil. Immediately after the rTMS train, the bed of the scanner was moved back into the bore, and five more sets of DWIs were acquired during a 15-minute period. The delay of the first DWI after the rTMS train was 70 to 100 seconds (mean, 90 seconds); the delay of the second DWI was 180 to 240 seconds (mean, 210 seconds); the delay of the third DWI was 300 to 360 seconds (mean, 330 seconds); the delay of the fourth DWI was 420 seconds; and the delay of the fifth DWI scan was 570 seconds.

A Siemens Vision (Siemens Erlangen, Germany) 1.5-T EPI MR scanner was used. DWI was performed using a multislice, single-shot, spin-echo EPI sequence (repetition time [TR], 6,000 ms; echo time [TE], 118 ms; matrix size, 128 × 128; field of view, 256 mm; slice thickness, 7 mm without any interslice gap). Each of the 20 axial slices was acquired with b-values of 0 and 1000 s/mm^2. DWIs using the high b-value were acquired applying the diffusion gradients in three orthogonal directions (x, *y*, *z*). A trace image of the diffusion tensor was calculated to minimize the effects of diffusion anisotropy. The calculation of the apparent diffusion coefficient (ADC), the creation of ADC maps, and the determination of relative ADC (rADC) values were done as previously described.[6]

T1-weighted images and DWIs were realigned, coregistered, and normalized using SPM99 (The Welcome Department of Cognitive Neuroscience, London, UK). The accuracy of TMS for locating the primary motor area has been demonstrated.[7] Therefore, regions of interest (ROI) covering the posterior bank of the precentral gyrus (Talairach coordinates, z 35 to 65) were drawn bilaterally on the T1-weighted images. The ROIs were then superimposed on the coregistered and resliced b-0 and b-1000 diffusion

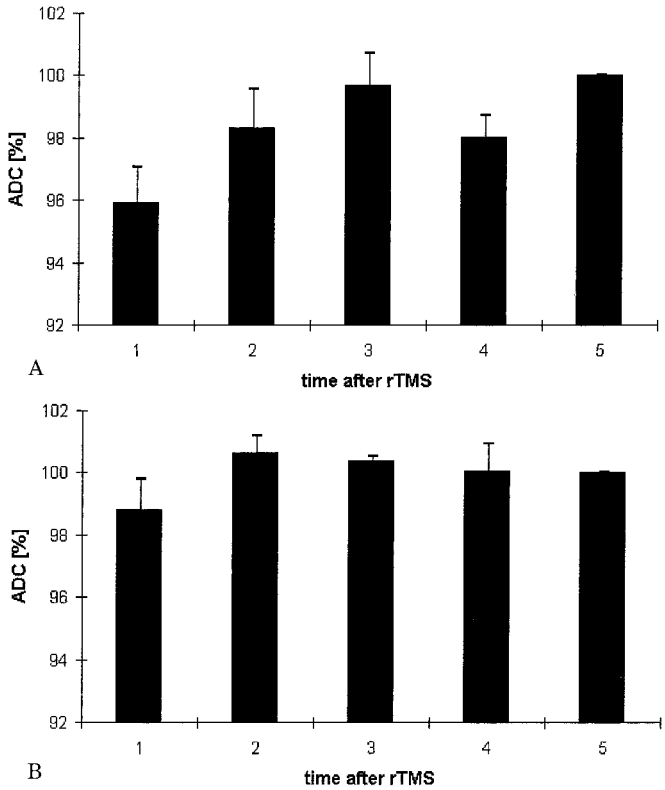

Figure 2. Only the apparent diffusion coefficient changes in the first diffusion-weighted imaging (DWI) in left M1 (A) show a significant difference (p = *0.002) with respect to the reference DWI. (B) In right M1, there is a small but not significant* (p = *0.28) initial decrease.*

images. Raw intensity values were determined on the b-0 and b-max image, and ADC values were calculated off-line for the ROI. Regional ADC values from the three diffusion scans taken before the rTMS and those from the five diffusion scans after the rTMS were normalized to the last DWI of each sequence. ADC variation was expressed in percent over time. Two analyses of variance (ANOVAs) were done. The first was the pre-TMS with ADC as the dependent variable and three time points and two hemispheres as the independent variables. The second ANOVA also had ADC as the dependent variable with five post-TMS time points and the two hemispheres as independent variables.

Results. The two-way ANOVA for the dependent variable ADC pre-rTMS revealed no changes for either factor. The one-way interactions were not significant for the pre-rTMS ADC values.

The two-way ANOVA for the dependent variable ADC post-TMS showed no effect [$F(56,4) = 1.61$, $p = 0.19$]. However, a one-way interaction for the time-course was found [$F(56,4) = 5.45$, $p = 0.0008$; figure 2]. The interhemispheric comparison showed a more pronounced effect of rTMS over the targeted left motor cortex [$F(14,1) = 4.16$, $p = 0.06$]. Newman–Keuls test (corrected for multiple comparisons) was used as a post hoc test and revealed an initial decrease in the ADC in the left [4.1%, $F(14,1) = 14.35$, $p = 0.002$; see figure 2A] but not in the right motor cortex [2.2%, $F(14,1) = 1.28$, $p = 0.28$; see figure 2B] immediately after rTMS. This effect was no longer detectable 5 minutes after rTMS.

Discussion. We demonstrate that a low-frequency rTMS train at sub-MT over M1 leads to a small and short-lived change in ADC in the targeted M1 region. There was also a small nonsignificant trend in the contralateral M1 region. The fast recovery of this effect is remarkably different from the changes seen within minutes after the onset of experimentally induced status epilepticus[8] or in acute ischemic stroke.

A study using conventional MRI sequences showed that a long-lasting, high-frequency rTMS train at high intensities applied to the visual cortex did not lead to any enhancement after application of contrast agent, or to changes in ADC.[9] However, the MRIs in that study were obtained 6 minutes to 6 hours after rTMS was applied, presumably missing the transitory effects of rTMS on cortical tissue.[9]

The minimal and reversible reduction in ADC that was seen in our study may not be severe enough or have a duration that is long enough to cause a problem with protein synthesis or ATP depletion.[10] Furthermore, the association between ADC restrictions and Na-K-ATPase impairments may not be as strong or as linear in the close-to-normal range to suggest that even minimal ADC reductions could indicate a Na-K-ATPase impairment or ATP depletion. The minimal ADC restriction would not suggest energy depletion, but rather, at most, a reduction in ATP with a slowing in the Na-K-ATPase could be proposed, which may be an additional phenomenon leading to the neurophysiologically observable excitability changes.[2-4]

The small temporary restriction of ADC is in agreement with the notion that rTMS may cause impairments in cortical function but not a permanent lesion, and is consistent with the duration of behavioral effects of "off-line rTMS."[1] The current study suggests a possible mechanism of action for "off-line rTMS." However, more studies are required to elucidate the reason why low-frequency rTMS alters proton diffusion.

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