Enhanced brain motor activity in patients with MS after a single dose of 3,4-diaminopyridine

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Abstract—*Background:* 3,4-Diaminopyridine (3,4-DAP), a potassium (K^+) channel blocker, improves fatigue and motor function in multiple sclerosis (MS). Although it was thought to do so by restoring conduction to demyelinated axons, recent experimental data show that aminopyridines administered at clinical doses potentiate synaptic transmission. *Objective:* To investigate motor cerebral activity with fMRI and transcranial magnetic stimulation (TMS) after a single oral dose of 3,4-DAP in patients with MS. *Methods:* Twelve right-handed women (mean ± SD age 40.9 ± 9.3 years) underwent fMRI on two separate occasions (under 3,4-DAP and under placebo) during a simple motor task with the right hand. FMRI data were analyzed with SPM99. After fMRI, patients underwent single-pulse TMS to test motor threshold, amplitude, and latency of motor evoked potentials, central conduction time, and the cortical silent period; paired-pulse TMS to investigate intracortical inhibition (ICI) and intracortical facilitation (ICF); and quantitative electromyography during maximal voluntary contraction. *Results:* FMRI motor-evoked brain activation was greater under 3,4-DAP than under placebo in the ipsilateral sensorimotor cortex and supplementary motor area ($p < 0.05$). 3,4-DAP decreased ICI and increased ICF; central motor conduction time and muscular fatigability did not change. *Conclusion:* 3,4-DAP may modulate brain motor activity in patients with MS, probably by enhancing excitatory synaptic transmission.

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The potassium (K^+) channel–blocking agent 4-aminopyridine (4-AP) and its related compound 3,4-diaminopyridine (DAP) improve fatigue and motor weakness in patients with multiple sclerosis (MS).[1-4] Although early experimental data suggested that APs improve the symptoms of MS by restoring conduction to demyelinated axons,[5] the findings were unreliable because the dose used experimentally was 250 to 1,000 times higher than that used in clinical trials. Recent experimental data have shown that although 4-AP administered at clinical doses fails to restore conduction to demyelinated axons, it potentiates synaptic transmission and increases skeletal muscle twitch tension.[6]

Electrophysiologic studies in patients with MS have also failed to show that APs improve central motor conduction.[3,4] Hence, APs may induce their beneficial effects in MS primarily by not acting on the unmyelinated portion of axons but by modulating synaptic transmission.

Neural activity can be studied in humans in vivo and noninvasively using various techniques including blood oxygen level–dependent (BOLD) fMRI and transcranial magnetic stimulation (TMS). FMRI has been used to investigate brain activation in response to pharmacologic[7] and behavioral[8] interventions. Previous fMRI studies have shown cortical and subcortical changes related to motor tasks in patients with MS with various degrees of clinical disability.[9-11] These changes partly reflect the underlying brain damage, thus providing a rationale for the use of pharmacologic methods to reduce neuronal damage or to enhance brain plasticity in MS.[12] Although fMRI could be a useful tool for evaluating 3,4-DAP-induced cortical and subcortical motor changes, this technique measures neuronal activation only indirectly, providing an index of energy expenditure secondary to excitatory or inhibitory synaptic activity.[13]

Motor cortex excitability can be tested directly by paired-pulse TMS.[14] Paired-pulse stimulation has provided useful information on the effects of various neurotrophic drugs on cortical excitability in healthy subjects[15-17] but not in MS. Despite its strengths, as a method for localizing sources of brain activity and providing information on the overall extension of brain motor networks, TMS is far more limited than fMRI.

In this double-blind, placebo-controlled, cross-over study, we used fMRI and paired-pulse TMS to investigate changes in cerebral motor activation and motor cortex excitability after a single oral dose of 3,4-DAP in 12 patients who were mildly disabled with

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MS. We also evaluated the effects of 3,4-DAP on central motor conduction by single-pulse TMS and on muscular fatigability by quantitative electromyography (EMG).

Subjects and methods. *Subjects.* We studied 12 patients with relapsing–remitting MS[18] who were attending our university outpatient clinic. Inclusion criteria were the following: right-handedness; normal functioning of the right upper limb with no clinically evident motor or sensory impairment; reported fatigue; no concomitant therapy with antidepressant, psychoactive, corticosteroid, or muscle relaxant drugs; no clinical relapses for at least 3 months before study entry; no history of seizures or unexplained loss of consciousness or EEG abnormalities; and no major medical illnesses including renal, hepatic or cardiac disease, or diabetes mellitus.

Disability was evaluated with the Expanded Disability Status Scale (EDSS)[19]; fatigue was scored using the expanded version of the Fatigue Severity Scale (FSS).[20]

All participants gave their written informed consent to the study, and the study procedures were approved by the Ethical Committee of the Department of Neurologic Sciences, University of Rome "La Sapienza."

Study design. All patients were randomly assigned to one of two counterbalanced groups to undergo two combined fMRI and TMS studies 3 days apart. Before each session, patients fasted for at least 3 hours. FMRI started 40 minutes after patients had received 3,4-DAP in a single oral dose of 20 mg (at peak plasma concentrations)[1,21] or placebo. Each patient acted as her own control, and the two groups were counterbalanced to avoid an effect of treatment order. In a cross-over design, six patients underwent the first fMRI 40 minutes after receiving 3,4-DAP. Three days later, they underwent the second fMRI 40 minutes after receiving an identical tablet of placebo. The other six patients underwent the first fMRI study under placebo and the second 3 days later under 3,4-DAP. After each fMRI study, about 60 minutes after oral intake of 3,4-DAP or placebo (the plasma half-life of 3,4-DAP is approximately 3 hours),[1,21] all patients underwent neurophysiologic tests including single-pulse and paired-pulse TMS and quantitative EMG recordings.

Neither the patients nor the investigators were aware of which treatment had been administered. After the experiments were completed, investigators were informed of which group patients belonged to but remained blinded to the treatment used in each group until fMRI and TMS group data were analyzed.

FMRI. Data acquisition. FMRI data were acquired with a 1.5 T magnet scanner (Philips Gyroscan NT 15, Erlangen, Germany) with echo planar capabilities and a head volume radiofrequency coil. Each subject lay supine in the scanner with eyes closed. Head movements were minimized with foam padding and a restraining strap. T2*-weighted echo planar images (64 × 64 matrix over a 24-cm field of view), consisting of 25 consecutive 4-mm-thick axial sections, with repetition time (TR)/echo time (TE) of 3,000/50 milliseconds, a 90° flip angle, and one excitation, were acquired.

During fMRI scanning, patients performed a repetitive index-finger-to-thumb opposition movement with the right hand according to a block design consisting of 12 21-second epochs alternating between rest and activation, yielding a total scan time of 4 minutes 12 seconds. Before image acquisition, subjects were shown how to perform the motor task at a regular rate of 2 Hz. Throughout the fMRI study, an external observer checked that participants did the motor tasks correctly and recorded the rate of hand movement.

Data analysis. FMRI data were analyzed using a dedicated software and general linear model statistics (SPM99, random effect model; Wellcome Department of Cognitive Neuroscience, Institute of Neurology, University College of London, UK).[22] For each study, images were realigned, normalized, and spatially smoothed using a Gaussian kernel of 8 mm. To obtain contrast images for each subject, individual data were modeled using a boxcar design, convolved with the hemodynamic response function chosen to represent the relationship between neuronal activation and blood flow changes.

The contrast images were then used in a second-level analysis to create group brain maps (one-sample *t*-test) for the 12 patients separately for the two conditions (3,4-DAP and placebo). Clusters of voxels (corrected $p < 0.05$) that had a peak Z score of >3.7 were considered to show significant activations. A paired *t*-test was used to assess differences in task-related BOLD responses between 3,4-DAP and placebo. Clusters of voxels (corrected $p < 0.05$) that had a peak Z score of >3.7 were considered to show significant differences.

Finally, two contrast images were created for each patient: One showed 3,4-DAP-related increases (a fixed effects comparison of 3,4-DAP vs placebo sessions), and the other showed 3,4-DAP-related decreases (placebo vs 3,4-DAP). These contrast images were used in a second-level analysis to determine correlations (simple regression) between the changes in fMRI activation and changes in intracortical inhibition (ICI) and intracortical facilitation (ICF) at paired-pulse TMS and the baseline FSS scores. Clusters of voxels (corrected $p < 0.05$) that had a peak Z score of >3.7 were considered to show significant differences.

Within each region of significance, local maxima of signal increase were determined (the voxels of maximum significance), and their location was expressed in terms of x, y, and z coordinates. Montreal Neurologic Institute coordinates were converted to the Talairach space[23] by linear transformation (www.mrc-cbu.cam.ac.uk/Imaging/mnispace.html).

Conventional MRI. Data acquisition and analysis. During the first fMRI study, conventional proton density- and T2-weighted spin echo images (TR/TE 2,000/20, 90 milliseconds, matrix 256 × 256, field of view 24 cm, slice thickness 4 mm, gap 0 mm, 40 slices), and T1-weighted images after the injection of a double dose (0.2 mg/kg) of gadolinium-diethylenetriaminepentaacetic acid (TR/TE 600/15 milliseconds, matrix 256 × 256, field of view 24, slice thickness 4 mm, gap 0, 40 slices) were acquired for all patients.

Hyperintense T2 lesion load was calculated in each patient using the display program Dispunc (Plummer DL, University College of London, UK) with a semiautomated contouring technique.[24]

TMS. Stimulation. TMS was delivered through a magnetic stimulator (Magstim; Magstim Co. Ltd, Whitland, South West Wales, UK) connected to a figure-of-eight coil placed over the motor area of the dominant hemisphere. Single magnetic pulses were delivered to the left motor cortex to find the optimal position for evoking a motor-evoked potential (MEP) in the contralateral first dorsal interosseous (FDI) muscle (target muscle). This position was marked on the scalp and used throughout the experiment. Motor threshold (MTh) was calculated at rest using the lowest intensity able to evoke an MEP of >50 μV in at least 5 of 10 consecutive trials in the FDI muscle. Target muscle relaxation was monitored by means of an EMG acoustic and visual (Tektronic 5103N oscilloscope; Tektronix, Beaverton, OR) feedback.

ICI and ICF were studied using a paired-stimulus paradigm according to a technique previously described.[14] For this purpose, two magnetic stimulators were connected by a Y cable to a figure-of-eight coil positioned over the FDI area of the left hemisphere. The coil was held tangentially to the scalp with the handle pointing back and away from the midline at 45°. The induced current flow was posterior to anterior in the cortex perpendicular to the central sulcus. Paired pulses were randomly delivered at 3- and 10-millisecond interstimulus intervals (ISIs). The intensity of the conditioning stimulus was set at 80% of MTh and the test stimulus at 120% of MTh. Twenty trials were performed for each condition.

TMS was also delivered with the coil positioned at the cervical level to elicit root MEP.

EMG recording. EMG activity was recorded through a pair of surface disk electrodes placed over the right FDI muscle. EMG signals were recorded and filtered with a Digitimer D 160 (bandwidth 20 Hz to 1 kHz, sampling rate 2 kHz), full-wave rectified, when required, with a personal computer through a 1401 plus A/D laboratory interface (Cambridge Electronic Design, UK), and analyzed off-line.

MEPs were recorded at rest. The cortical silent period (CSP) to single-pulse TMS was recorded with the subject maintaining a 50% maximum contraction in the target muscle with the aid of EMG acoustic and visual (Tektronic 5103N oscilloscope) feedback.

Last, a quantitative EMG during a 120-second maximal voluntary isometric contraction of the right FDI muscle was recorded in all the subjects.

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Measurements and analysis. The size of MEP evoked by TMS was measured peak to peak; in the paired-stimuli paradigm, the size of the conditioned MEP was expressed as the percentage of the test MEP. The latency of MEPs was measured from the end of the stimulus artifact and the onset of the MEP. Onset and end latency of the CSP were taken at the end of the preceding MEP and the intersection of the averaged rectified signal, with baseline indicating 80% of the background EMG level; the duration of EMG suppression between these two points was computed automatically.[25] Ten trials were collected and averaged for each condition.

Data from quantitative EMG recordings were measured by dividing the 120-second EMG activity by intervals of 30 seconds.

The central conduction time was calculated as the difference in latency between the scalp MEP and the root MEP. Values of central conduction time from patients were compared with those from 12 age- and sex-matched healthy volunteers (two-sample *t*-test).

A paired *t*-test was used to compare the MTh, the amplitude of the MEP, and the duration of the CSP at single-pulse TMS, ICI, and ICF determined after placebo with the same variables obtained after 3,4-DAP intake. Repeated measures analysis of variance was used to compare quantitative EMG data after 3,4-DAP and placebo intake.

Results. *Clinical and conventional MRI.* There were 12 women aged 26 to 53 years (median 40 years) with a median (range) disease duration of 8 (2 to 22) years, a median (range) EDSS score of 2.5 (1.0 to 3.5), but a mean (SD) FSS score of 4.9 (0.7). None of the 12 patients had major adverse events or suffered seizures after 3,4-DAP intake; six patients reported perioral paresthesias. Eight patients reported a subjective improvement in fatigue after administration of 3,4-DAP compared with placebo.

Patients had a median T2 lesion load of 6.83 mL (range 0.23 to 17.53 mL). None of them had gadolinium-enhancing lesions.

FMRI. Visual inspection indicated that all patients performed the simple motor task in the scanner at a constant frequency during all sessions. No mirror movements were noted during right-hand movement in any of the patients either under placebo or under 3,4-DAP. No significant difference was found in movement rates under 3,4-DAP and under placebo (mean ± SD 1.79 ± 0.4 vs 1.75 ± 0.5 Hz).

During the right-hand motor task under placebo, fMRI showed activation ($Z > 3.7$, $p < 0.05$, corrected at the cluster level) in the bilateral supplementary motor area (SMA; Brodmann area [BA] 6), contralateral sensorimotor cortex (BA 1 to 4), lateral premotor area (BA 6), inferior parietal cortex (BA 40), insula, and basal ganglia, as well as in the lateral premotor area (BA 6), inferior parietal cortex (BA 40), insula and cerebellum of the ipsilateral hemisphere, and vermis (table; figure 1, A1 and B1).

During the same task under 3,4-DAP, patients activated ($Z > 3.7$, $p < 0.05$, corrected at the cluster level) the same brain areas observed under placebo and recruited additional foci located in the ipsilateral sensorimotor cortex (BA 1 to 4) and basal ganglia, bilateral thalamus, and contralateral cerebellum (see the table and figure 1, A2 and B2).

Analysis by paired *t*-test of 3,4-DAP vs the placebo right-hand task BOLD response showed a greater activation under 3,4-DAP than under placebo ($Z > 3.7$, $p < 0.05$, corrected at the cluster level) in the ipsilateral sensorimotor cortex (BA 1 to 4: $x = 28$, $y = -29$, $z = 66$ mm; $x = 36$, $y = -24$, $z = 60$ mm) and SMA (BA 6: $x = 4$, $y = -30$, $z = 66$ mm; $x = 2$, $y = -20$, $z = 66$ mm) (figure 2). No brain foci were more active under placebo than under 3,4-DAP.

Table *FMRI significant activations under placebo and under 3,4-DAP in 12 patients with multiple sclerosis during right-hand movement*

	Placebo		3,4-DAP	
Brain areas	No. of activated voxels	Maximum *Z* value	No. of activated voxels	Maximum *Z* value
Contralateral sensorimotor cortex, BA 1–4	1,476	5.73	1,961	6.29
Contralateral premotor cortex, BA 6	1,084	5.59	1,467	6.29
Contralateral supplementary motor area, BA 6	300	4.35	500	4.77
Contralateral parietal cortex, BA 40	114	3.67	390	5.36
Contralateral insula	496	5.22	668	5.43
Contralateral thalamus	—	—	328	4.38
Contralateral basal ganglia	43	3.56	301	4.22
Contralateral cerebellum	—	—	123	4.56
Ipsilateral sensorimotor cortex, BA 1–4	—	—	49	4.52
Ipsilateral premotor cortex, BA 6	264	4.50	442	4.43
Ipsilateral supplementary motor area, BA 6	126	4.35	369	4.77
Ipsilateral parietal cortex, BA 40	80	3.97	271	4.35
Ipsilateral insula	116	3.71	484	4.67
Ipsilateral thalamus	—	—	107	4.29
Ipsilateral basal ganglia	—	—	67	4.24
Ipsilateral cerebellum	65	5.75	200	5.90
Vermis	597	6.14	1,384	5.65

One-sample *t*-test, SPM99; $Z > 3.7$, $p < 0.05$, corrected at the cluster level.

3,4-DAP = 3,4-diaminopyridine; BA = Brodmann area.

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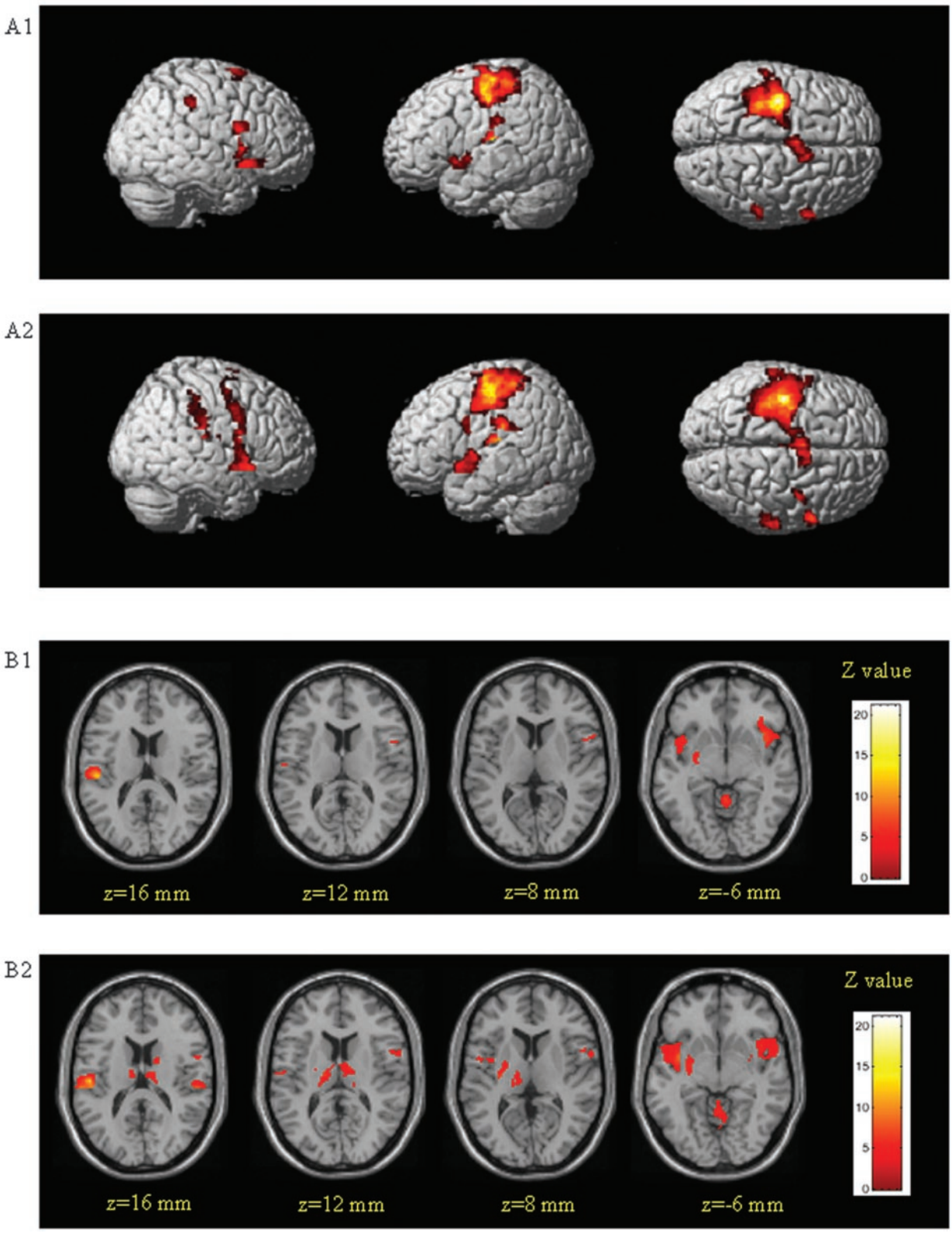

Figure 1. Rendered and axial images (neurologic convention) from SPM99 group maps (one-sample t*-test,* $Z > 3.7$, $p < 0.05$, *corrected at the cluster level), showing significant areas of activation during the right-hand motor task after oral intake of placebo (A1 and B1) and 3,4-diaminopyridine (3,4-DAP) (A2 and B2) in 12 patients with multiple sclerosis. Significant foci of activation under placebo were located in the supplementary motor area, contralateral sensorimotor cortex, lateral premotor area, inferior parietal cortex, insula, and basal ganglia as well as in the lateral premotor area, inferior parietal cortex, insula, and cerebellum of the ipsilateral hemisphere and in the vermis (A1 and B1). After 3,4-DAP, we found activation in the same areas activated under placebo and recruitment of additional foci including the ipsilateral sensorimotor cortex and basal ganglia, the bilateral thalamus, and the contralateral cerebellum (A2 and B2).*

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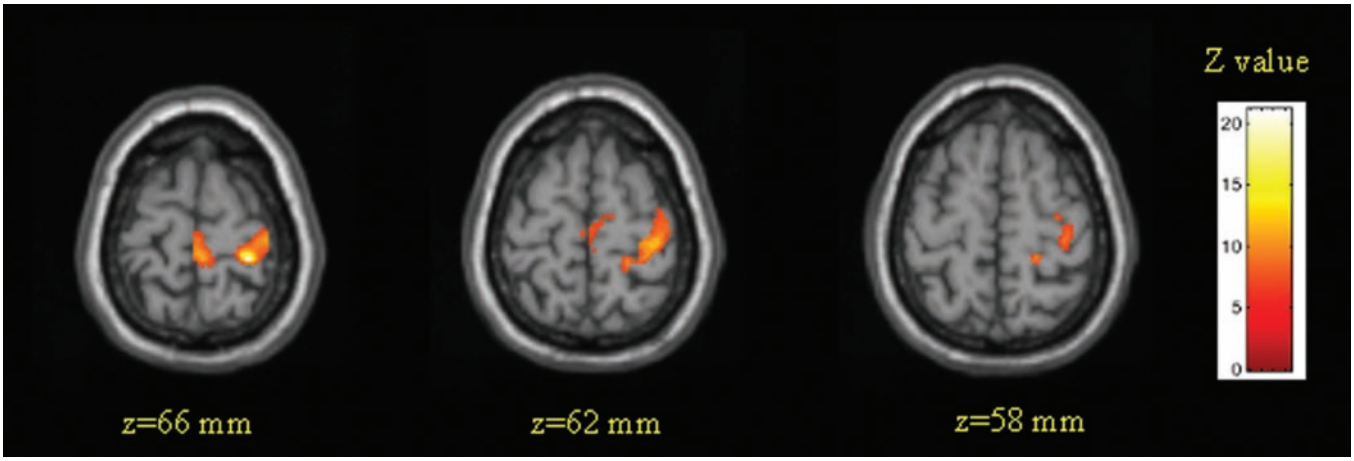

Figure 2. Axial images (neurologic convention) from SPM99 group maps (paired t*-test,* $Z > 3.7$, $p < 0.05$, *corrected at the cluster level), showing areas significantly more active under 3,4-diaminopyridine than under placebo in the 12 patients with multiple sclerosis during a right-hand motor task. Significant foci were activated in the sensorimotor cortex and in the supplementary area of the ipsilateral hemisphere.*

Increase in ICF under 3,4-DAP positively correlated ($Z > 3.7$, $p < 0.05$, corrected at the cluster level) with the extent of activation in the ipsilateral sensorimotor cortex (BA 1 to 4: $x = 52$, $y = -18$, $z = 34$; $x = 40$, $y = -36$, $z = 63$ mm).

Finally, no significant correlation was found between 3,4-DAP-induced changes in fMRI activation and patients' baseline FSS scores.

TMS. None of the TMS test procedures caused adverse events in any of the patients. No significant difference was found in the rest MTh between 3,4-DAP and placebo (mean ± SE 53.4 ± 2.9 vs 51.2 ± 3.6%) in any of the patients. Nor did MEP amplitude and latency of CSP duration at single-pulse TMS differ significantly after drug intake and after placebo (MEP amplitude and latency [placebo vs 3,4-DAP]: 0.76 ± 0.13 vs 0.81 ± 0.12 mV; 22.9 ± 0.7 vs 22.8 ± 0.6 milliseconds; CSP duration [placebo vs 3,4-DAP]: 88 ± 12 vs 98 ± 11 milliseconds).

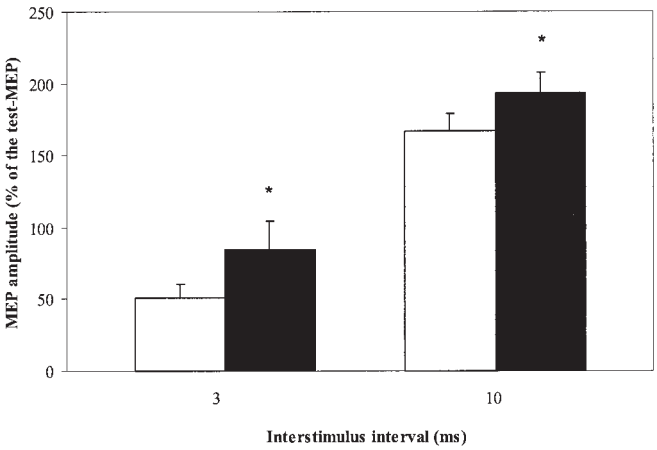

Figure 3. Changes in intracortical excitability tested with paired-pulse transcranial magnetic stimulation after 3,4-diaminopyridine (3,4-DAP; black) and placebo (white) intake in 12 patients with multiple sclerosis. Note the significantly decreased conditioning test motor-evoked potential (MEP) inhibition at the 3-millisecond interstimulus interval (ISI) and the increased conditioning test MEP facilitation at the 10-millisecond ISI induced by 3,4-DAP.
*$p < 0.05$.

Similarly, 3,4-DAP intake did not modify central conduction time (placebo vs 3,4-DAP: 9.1 ± 0.59 vs 8.8 ± 0.48 milliseconds). Patients had higher values of central conduction time than 12 matched control subjects (mean ± SE 5.7 ± 0.14 milliseconds; $p < 0.0001$ by two-sample t-test).

In patients, after placebo intake, the conditioning stimulus delivered at the 3-millisecond ISI inhibited the test MEP (conditioned MEP size, mean ± SE 50.9 ± 9.6% of the test MEP), whereas the conditioning stimulus delivered at the 10-millisecond ISI facilitated the test MEP (conditioned MEP size, mean ± SE: 167.1 ± 12.0% of the test MEP). Administration of 3,4-DAP reduced the inhibitory effect of conditioning stimulation on the test MEP at the 3-millisecond ISI (mean ± SE 84.5 ± 20.4 vs 50.9 ± 9.6%; $p = 0.03$ by paired t-test) and increased the facilitatory effect of conditioning stimulation on the test MEP at the 10-millisecond ISI (mean ± SE 193.0 ± 14.8 vs 167.1 ± 12.0%; $p = 0.01$ by paired t-test) (figure 3).

Quantitative EMG. At quantitative EMG, under both placebo and 3,4-DAP, muscle strength declined during the course of the task (90- to 120- vs 0- to 30-second interval; $F_{3-33} = 7.50$, $p < 0.0006$), but no significant difference was found between the two conditions.

Discussion. In this study, the complementary information we obtained by combining fMRI and TMS provided new evidence that oral administration of 3,4-DAP induces distinct changes in cerebral motor activation and motor cortical excitability in patients with MS. During the course of a right-hand motor task, fMRI showed changes in patterns of brain motor activation, and paired-pulse TMS disclosed specific changes in motor cortex excitability.

FMRI data obtained during right-hand movement under 3,4-DAP showed activation in the same cerebral motor areas activated under placebo and recruitment of additional foci in the ipsilateral sensorimotor cortex (BA 1 to 4) and basal ganglia, bilateral thalamus, and

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contralateral cerebellum. Although these brain areas became hyperactive, patients' rate of right-hand movement remained unchanged. FMRI showed the most intense hyperactivity in the ipsilateral sensorimotor cortex and SMA. Increased activity in the motor areas of the ipsilateral hemisphere has been reported in studies comparing motor activation patterns in patients with MS and healthy control subjects.[9,10] Although the precise function of the ipsilateral motor cortex hyperactivity remains unclear, especially in relation to clinical outcome, findings in patients with stroke suggest that it might be related to loss of transcallosal inhibition.[26] In our study, paired-pulse TMS specified that a single oral dose of 3,4-DAP decreased ICI and increased ICF, thus suggesting an increase in excitability of the contralateral motor cortex. The 3,4-DAP-induced changes in ICF positively correlated with changes in the extent of fMRI activation in the ipsilateral sensorimotor cortex. Hence, the overactivation of the ipsilateral hemisphere may reflect reduced inhibition from the contralateral motor cortex that in turn unmasks or disinhibits activity in the ipsilateral corticospinal tract. A similar change in the balance between motor cortical inhibition and excitation, causing cortical excitability to spread across hemispheric boundaries, has been reported in other conditions characterized by motor cortical hyperexcitability, including patients with cortical myoclonus[27] and healthy subjects undergoing repetitive TMS.[28] We cannot exclude, however, that the increased ipsilateral activity may simply reflect an increased excitability in the ipsilateral hemisphere as well as in the contralateral.

Despite inducing prominent changes in motor cortical excitability, 3,4-DAP intake induced no significant change in motor conduction. This finding confirms previous experimental and electrophysiologic studies showing that 4-AP and its analogues, when administered at the low concentrations used clinically, leave conduction through demyelinated axons appreciably unchanged.[3,4,6] These low concentrations nevertheless effectively potentiate synaptic transmission.[6]

Support for a role of APs in modulating neuronal excitability comes also from biochemical and electrophysiologic data.[29,30] APs may induce neural hyperexcitation by blocking K^+ channels. Blockade of K^+ channels prevents neuronal efflux of K^+, thereby causing depolarization and increased release of several neurotransmitters, including acetylcholine,[31] noradrenaline,[32] dopamine, and serotonin.[33] A nonspecific central stimulant effect induced by APs might explain the increased brain activity we observed on fMRI.

Alternatively, APs could induce neuronal hyperexcitation by increasing the probability of neurotransmitter release. In animal models of epilepsy, 4-AP induces the release of glutamate from synaptic nerve endings, possibly explaining the drug's acute epileptogenic effect.[34] There is evidence from a study using paired-pulse stimulation in hippocampal slices ex vivo that 4-AP induces a permanent facilitation of presynaptic glutamate release.[35] This facilitatory effect seems to be independent from blockade of K^+ channels and reflects 4-AP-induced changes in Ca^{2+} homeostasis in the presynaptic terminals, thus increasing the probability of glutamate release.

Glutamatergic mechanisms may explain why a single dose of 3,4-DAP increased ICF, decreased ICI, and left the CSP unchanged. In the human brain, ICI and ICF obtained with paired-pulse TMS reflect interneural activity in the cortex. ICI is probably a GABAergic effect, especially related to $GABA_A$ receptors,[36] whereas ICF is largely a glutamatergic effect.[17] Both ICI and ICF may be mediated by various neurotrophic agents. Drugs acting on $GABA_A$ receptors mainly mediate enhancement of ICI, but they are involved also in the suppression of ICF, whereas drugs acting on glutamatergic receptors mainly mediate ICF but also influence ICI.[37] The CSP is a pause in the ongoing voluntary EMG activity produced by TMS. The early part of the CSP reflects spinal cord inhibition, whereas the later part originates from cortical mechanisms.[38] Although these mechanisms are not completely understood, the CSP duration gives useful information on motor cortex excitability, probably reflecting $GABA_B$ function.[39] The unchanged CSP, the reduced ICI, and the enhanced ICF we observed in this study suggest that 3,4-DAP through glutamatergic mechanisms acts mainly on the excitatory interneurons of the motor cortex. Previous experimental data have shown that synaptic glutamate release attenuates monosynaptic GABAergic inhibition via an action at kainate receptors.[40]

3,4-DAP may improve symptomatic fatigue in patients with MS.[4] The mechanism underlying this benefit remains unclear because of the wide discrepancy between subjective and objective improvement in this symptom. In our study, eight patients showed a subjective improvement in fatigue after receiving 3,4-DAP. A distinctive finding in this study is that in all the patients with MS, as well as increasing activity in several motor cortical areas, intake of 3,4-DAP increased fMRI activity in the basal ganglia and thalamus. The basal ganglia and thalamus are part of the feedback loops of the limbic system able to modulate cortical motor output.[41] Previous electrophysiologic and functional neuroimaging data have provided converging evidence of a central origin of fatigue in MS, suggesting that MS-related fatigue could be secondary to disruption or dysfunction of corticosubcortical circuits.[42-45] Metabolic changes have been described in the basal ganglia and frontal cortex of MS patients with fatigue,[44] and fMRI data have shown a reduced activation of cortical and subcortical areas devoted to motor planning and execution.[45]

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