Efficient neuroplasticity induction in chronic stroke patients by an associative brain-computer interface

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Mrachacz-Kersting N, Jiang N, Stevenson AJ, Niazi IK, Kostic V, Pavlovic A, Radovanovic S, Djuric-Jovicic M, Agosta F, Dremstrup K, Farina D. Efficient neuroplasticity induction in chronic stroke patients by an associative brain-computer interface. *J Neurophysiol* 115: 1410–1421, 2016. First published December 30, 2015; doi:10.1152/jn.00918.2015.—Brain-computer interfaces (BCIs) have the potential to improve functionality in chronic stoke patients when applied over a large number of sessions. Here we evaluated the effect and the underlying mechanisms of three BCI training sessions in a double-blind sham-controlled design. The applied BCI is based on Hebbian principles of associativity that hypothesize that neural assemblies activated in a correlated manner will strengthen synaptic connections. Twenty-two chronic stroke patients were divided into two training groups. Movement-related cortical potentials (MRCPs) were detected by electroencephalography during repetitions of foot dorsiflexion. Detection triggered a single electrical stimulation of the common peroneal nerve timed so that the resulting afferent volley arrived at the peak negative phase of the MRCP (BCI$_{associative}$ group) or randomly (BCI$_{nonassociative}$ group). Fugl-Meyer motor assessment (FM), 10-m walking speed, foot and hand tapping frequency, diffusion tensor imaging (DTI) data, and the excitability of the corticospinal tract to the target muscle [tibialis anterior (TA)] were quantified. The TA motor evoked potential (MEP) increased significantly after the BCI$_{associative}$ intervention, but not for the BCI$_{nonassociative}$ group. FM scores (0.8 ± 0.46 point difference, $P = 0.01$), foot (but not finger) tapping frequency, and 10-m walking speed improved significantly for the BCI$_{associative}$ group, indicating clinically relevant improvements. Corticospinal tract integrity on DTI did not correlate with clinical or physiological changes. For the BCI as applied here, the precise coupling between the brain command and the afferent signal was imperative for the behavioral, clinical, and neurophysiological changes reported. This association may become the driving principle for the design of BCI rehabilitation in the future. Indeed, no available BCIs can match this degree of functional improvement with such a short intervention.

neuroplasticity; brain-computer interface; associativity; stroke; Hebb

BRAIN-COMPUTER INTERFACES (BCIs) intended for the restoration of lost motor function have been explored only recently. The activation of relevant brain areas generated by a patient's intention to move is detected, interpreted, and used to drive an external device, e.g., electrical stimulator or robot. The hypothesis is that the afferent signal generated artificially induces central nervous system plasticity because it is in causal association with the cortical activity associated with the intention to move. Several research groups have recently provided evidence that this type of BCI leads to functional improvements in upper limb or hand function (Ang et al. 2010; Broetz et al. 2010; Cincotti et al. 2012; Daly et al. 2009; Kasashima-Shindo et al. 2015; Li et al. 2014; Mukaino et al. 2014; Pichiorri et al. 2015; Ramos-Murguialday et al. 2013; Young et al. 2014), although others have found no changes (Ang et al. 2014; Buch et al. 2008) and data are lacking for the use of such a BCI for altering lower limb function (Teo and Chew 2014). These studies report evidence for neuroplasticity, typically inferred from an improved performance of the BCI (Buch et al. 2008; Li et al. 2014), from alterations in the amplitude (Broetz et al. 2010; Cincotti et al. 2012; Li et al. 2014; Pichiorri et al. 2015) or latency (Yilmaz et al. 2013) of the extracted electroencephalographic (EEG) signal, or by functional magnetic resonance imaging (fMRI) (Mukaino et al. 2014; Song et al. 2014; Young et al. 2014). However, there are no reports on the alterations in the excitability of the cortical projections to the target muscle following BCI interventions, although this is a natural consequence of learning new motor skills (Pascual-Leone et al. 1995; Perez et al. 2004). In addition, only a few previous studies have explored the underlying mechanisms of the induced plasticity using fMRI (Ramos-Murguialday et al. 2013) or EEG connectivity (Pichiorri et al. 2015), while no studies have implemented transcranial magnetic stimulation (TMS). According to Hebb, synapses are strengthened through correlated activation of two different inputs to a postsynaptic neuron yet weakened by uncorrelated activity (Hebb 1949). This implies that the sensory signal artificially induced through the BCI must be associated to relevant brain activation with specific timing in order to induce plasticity.

In a recent series of studies, we have introduced an innovative noninvasive BCI designed specifically for neuromodulation (Mrachacz-Kersting et al. 2012a, 2012b; Niazi et al. 2012; Xu et al. 2014) based on the principle of Hebbian associativity.

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In this BCI, the slow component of the scalp EEG, i.e., the movement-related cortical potential (MRCP), extracted with optimized temporal-spatial filtering (Niazi et al. 2011) and manifold learning algorithms (Xu et al. 2014), was used to detect the intent of the user's movement execution. The MRCP is a cortical potential generated when a movement is either imagined or performed and occurs for both self-initiated (Kornhuber and Deecke 1965) and cue-based (Walter et al. 1964) movements. The MRCP is comprised of several well-defined components, linked to specific neurophysiological mechanisms where the peak negativity (PN) is associated to movement execution. Once the MRCP is detected, the artificial activation of somatosensory afferents projecting onto the motor cortex is triggered by means of noninvasive direct nerve stimulation. The signal is timed to arrive at the cortex during the peak negative phase of the MRCP and therefore induces a causal and systematic relationship between the sensory signals arising from muscles involved in the movement and the volitionally generated brain wave during imagination, attempt, or execution of that movement. This timing is critical, as we have shown that neither earlier nor later arrival can induce plasticity (Mrachacz-Kersting et al. 2012a). In healthy subjects, this BCI intervention induces plasticity that is long-lasting and specific to the muscle targeted (Mrachacz-Kersting et al. 2012a; Niazi et al. 2012; Xu et al. 2014). Previous studies have timed the sensory stimulus to arrive at the motor cortex at the precise time that a transcranial magnetic stimulus (TMS) was applied over the motor cortex representation of the target muscle (Stefan et al. 2002). This type of intervention, termed paired associative stimulation (PAS), induces plasticity similar to what we have shown in our BCI$_{associative}$ intervention in healthy participants. Since the PN of the MRCP represents the maximal activation of the motor cortex, our intervention is designed to replace the volley induced by TMS.

In the present proof-of-concept study, we present for the first time the application of this novel associative BCI to chronic stroke patients with motor disability and hypothesize that it would lead to an increased output of the motor cortex to the target muscle. The demonstration of this hypothesis would strongly support the theory that timing is critical in neuromodulatory BCIs and that associativity is the main physiological mechanism underlying the induced plasticity. In an additional control experiment, a control group was exposed to the same protocol, with the exception that the afferents were activated randomly in relation to the user's intent (BCI$_{nonassociative}$ intervention). This is, to our best knowledge, the first systematic study on chronic stroke patients that explicitly explores the associative long-term potentiation theory within a BCI rehabilitation approach that includes patients' volition.

METHODS

Ethical approval. Patient demographical data and baseline clinical evaluation are shown in Table 1. Nineteen male and three female patients (49.57 ± 12.52 yr) underwent neuropsychological assessment, with none meeting DSM-IV criteria for diagnosis of dementia. Inclusion criteria encompassed age over 18 yr having suffered from superior division middle cerebral artery (MCA) stroke 3–24 mo before recruitment and ability to follow commands (no or limited cognitive impairment). Patients were excluded if they presented with concomitant neurological or other severe medical problems, seizure history, cognitive impairments, treatment with drugs that act on

Table 1. *Patient demographic data for main BCI$_{associative}$ experiment and control BCI$_{nonassociative}$ experiment*

Patient No.	Age, yr	Sex	Time After Stroke, mo	MCA Stroke Side	Type of Ischemic Lesion
BCI$_{associative}$ patient group					
1	29	M	10	L	Cortico-subc
2	61	M	24	L	Cortico-subc
3	30	F	23	L	Cortico-subc
4	41	F	23	L	Cortical
5	53	M	11	L	Cortical
6	52	M	24	L	Cortical
7	58	M	9	L	Cortico-subc
8	43	M	19	R	Cortico-subc
9	57	M	12	L	Cortico-subc
10	23	M	12	R	Cortical
11	45	M	9	L	Cortico-subc
12	51	M	14	R	Cortico-subc
13	59	M	10	L	Cortico-subc
Mean	46.31		15.38		
SD	12.51		6.2		
BCI$_{nonassociative}$ patient group					
14	55	M	20	L	Cortico-subc
15	58	M	22	R	Cortico-subc
16	63	F	15	R	Cortico-subc
17	51	M	23	R	Cortico-subc
18	59	M	14	L	Cortico-subc
19	42	M	21	L	Cortico-subc
20	36	M	22	R	Cortico-subc
21	65	M	14	L	Cortico-subc
22	68	M	11	R	Cortico-subc
Mean	55.22		18		
SD	10.63		4.47		

Patient demographic data for the main BCI$_{associative}$ (*patients 1–13*) and control BCI$_{nonassociative}$ (*patients 14–22*) experiments are shown. MCA, middle cerebral artery.

central nervous system, complete paralysis of legs, cardiovascular or respiratory symptoms contraindicative of walking, contraindications to TMS or MRI, and any other significant non-stroke-related impairment affecting walking. Procedures were approved by the Ethics Committee of the Clinical Center of Serbia, and all patients provided their written informed consent.

Movement-related cortical potential. Monopolar EEG signals were recorded by an EEG amplifier (Nicolet 1, VIASYS Healthcare) at 1,024 Hz (0.016–70 Hz). Ag/AgCl scalp electrodes were placed according to the International 10-20 System in positions FP1, F3, F4, Fz, Pz, P3, P4, C3, C4, and Cz. Impedance was maintained below 5 KΩ. Both earlobes were used as a reference, and the ground electrode was placed at the nasion.

Patients were asked to attempt a dorsiflexion of the foot contralateral to the lesion site 30–50 times. The experimental setup is depicted in Fig. 1*B*. A custom-made MATLAB script provided visual information via a screen positioned 2 m in front of the subject on when to mentally prepare, execute, and release the movement (Fig. 1*D*). Patients were instructed to attempt to perform a single dorsiflexion movement as fast as possible when the cursor had reached the upward turn (Fig. 1*D*) and to maintain the new position for 2 s, following which they relaxed again 4–5 s prior to the next cue being provided. Data from recorded EEG trials were used to quantify the time of PN of the MRCPs before proceeding to the intervention described in *Interventions*.

Feature extraction from MRCP. EEG data were divided into epochs of 3 s (from 2 s before to 1 s after the visual cue) for each attempted movement and band-pass filtered from 0.05 Hz to 10 Hz, and subsequently a Laplacian channel (McFarland et al. 1997) was used to enhance the MRCP in each epoch. Next, a window of 500 ms
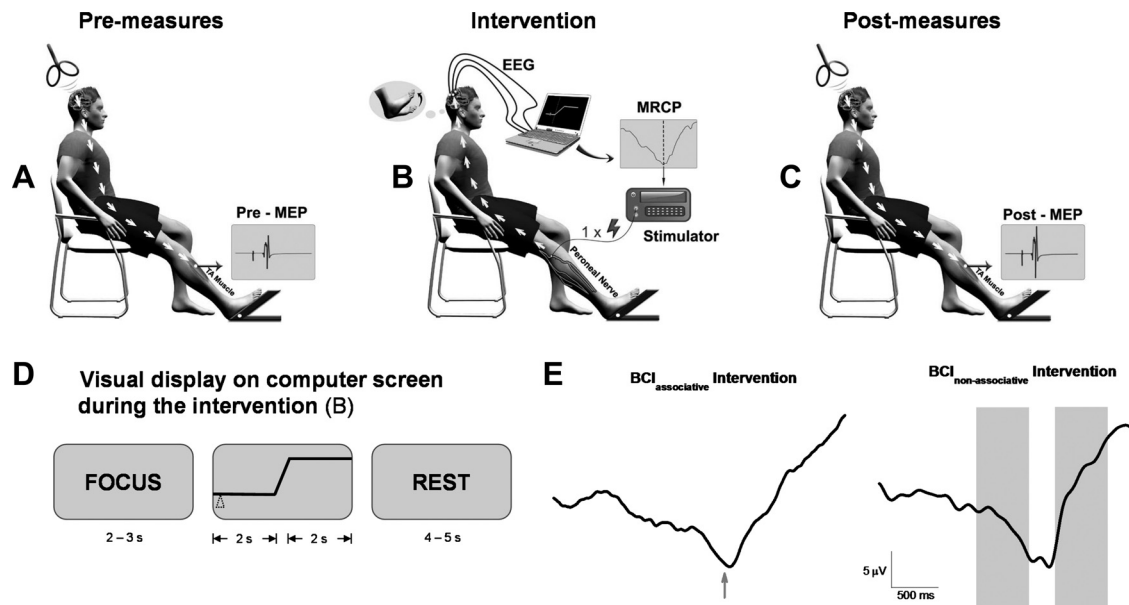
Fig. 1. Schematic of the experimental setup. *A*: preintervention quantification of the excitability of the cortical projections to the target muscle, tibialis anterior (TA), with noninvasive transcranial magnetic stimulation (TMS). Subjects were seated with the TA relaxed while 60 or 72 stimuli at 5 or 6 different intensities were applied. MEP, motor evoked potential. *B*: schematic of the brain-controlled electrical stimulation of the target muscle. Subjects watched a screen placed 2 m in front of them on which a cue provided information on when to attempt the dorsiflexion movement. Relevant brain activity was measured, detected, and converted into an output command for an electrical stimulator. The stimulator applied a single pulse (1-ms duration at an intensity of motor threshold) to the deep branch of the common peroneal nerve. The induced sensory signal produced was timed to arrive at the motor cortex during the time of maximum activation of the motor cortex as seen in the EEG signal for the BCI$_{associative}$ group and randomly for the BCI$_{nonassociative}$ group. MRCP, movement-related cortical potential. Fifty such pairs were performed in 2 sets of 25. *C*: immediately after intervention and 30 min later; measures as for *A*. *D*: the visual display shown to the patients during the intervention. The word "FOCUS" appeared on the screen initially, followed by the schematic of a step function. Subjects were required to start the attempted movement once the moving cursor (triangle) reached the upward slope. The word "REST" appeared last on the screen. *E*: illustration of the timing of the single electrical stimulus in relation to the MRCP during the BCI$_{associative}$ intervention (arrow, time of peak negativity) and during the BCI$_{nonassociative}$ intervention (shaded areas represent time window where the single electrical stimulus was triggered). Data are from 2 different patients of each intervention group.

on either side of task onset was chosen. If any epoch's PN was outside the selected window it was discarded. Epochs with EOG activity exceeding 125 μV were also discarded. Based on these remaining epochs, the mean PN was defined as the time of occurrence of the minimum value of the averaged MRCP in relation to the visual cue. The mean PN was used to calculate the points in time for when to apply the peripheral stimulation in the subsequent intervention session.

Recording and stimulation. Surface electrodes (20 mm Blue Sensor Ag/AgCl, AMBU) were used to record the electromyographic (EMG) activity of the tibialis anterior (TA) muscle of the affected leg. EMG data were sampled at 4 kHz, amplified, and band-pass filtered at 10 Hz to 1 kHz.

A monophasic transcranial magnetic stimulator (Magstim 200, Magstim) with a focal figure of eight double-coned coil (110-mm diameter) was used to apply single pulses (inducing a posterior to anterior directed current in the brain) to elicit a motor evoked potential (MEP) in the TA muscle. MEPs were elicited before, after, and 30 min after the cessation of the intervention (for procedure see *Experimental procedures*) and served as the main outcome measure.

Peripheral nerve stimulation was performed during the interventions only. The deep branch of the common peroneal nerve (CPN—L$_4$ and L$_5$) was stimulated with an external stimulator (Noxitest IES 230, Aalborg, Denmark) with the cathode proximal. A suitable position for the stimulation electrodes (Pals Platinum, Axelgaard Manufacturing) was located where a palpable response was produced in the distal tendon of the TA muscle with no activity from the synergistic peroneal muscles and no activity from the antagonist soleus. This site corresponded to a point just anterior to the level of the caput fibulae. The pulse width was set to 1 ms and the stimulation intensity to motor threshold (MT).

Experimental procedures. Details of the experimental procedures are shown in Fig. 1, *A* and *C*. Subjects were seated with their right and left feet resting on a footplate. The intensity of the magnetic stimulation was set to 50% of stimulator output to find the optimal site for evoking a MEP in the target muscle. In some patients ($n = 3$) this had to be increased to 90% in order to evoke a MEP. Initially we delivered three consecutive stimuli over Cz and proceeded to move in ~1-cm steps laterally. The hot spot was identified as that area where the most consistent MEPs were elicited in the three trials. This position was marked on the patient's head with a felt pen to ensure that the stimuli were consistently delivered over the same area of the motor cortex during the experimental session. Subsequently, the resting MT (RMT), defined as the highest stimulus intensity that in no more than 5 of 10 consecutive stimuli evoked a MEP with amplitude of ~50 μV while the muscle was at rest, was identified. Ten MEPs were subsequently elicited in the resting TA at five TMS intensities: 90%, 100%, 110%, 120%, and 130% of RMT. The stimuli were delivered randomly every 5–7 s. The mean peak-to-peak TA MEP amplitudes were extracted before, after, and 30 min after the cessation of the intervention. In some patients it was not possible to evoke a MEP in the TA at intensities below 90% of the stimulator output. In these cases ($n = 3$) the input-output relation was obtained by stimulating at 87%, 90%, 93%, 95%, and 98% of the stimulator output.

Interventions. The BCI$_{associative}$ intervention protocol ($n = 13$) consisted of a single electrical stimulation (ES) timed so that the artificially generated afferent flow arrived at the PN of the MRCP as outlined in detail in our previous publication (Mrachacz-Kersting et al. 2012a). In that study, we provided conclusive proof that only if the ES was timed so that the resulting afferent inflow would coincide with PN was significant plasticity induced. If it arrived before or after PN, no significant changes were observed (Mrachacz-Kersting et al.

2012a). The timing was calculated according to the following equation: mean PN − 50 ms. The 50 ms represents the mean latency for the afferent inflow resulting from the peripheral stimulus to reach the somatosensory cortex plus a cortical processing delay and is based on previous work (Mrachacz-Kersting et al. 2007). Figure 1*E*, *left*, shows an example from one patient, where the arrow denotes the instant of the ES being triggered with a delay of 50 ms in relation to the PN. A second intervention termed the BCI$_{nonassociative}$ intervention ($n = 9$) served as a control condition. Here, the ES was delivered randomly without any association to the PN of the MRCP. Figure 1*E*, *right*, shows an example from one patient, where the two shaded areas represent the time window of where the ES was triggered in a randomized order across the two windows. A total of 30–50 pairings (every 10–12 s) were applied. Subjects attended three separate testing sessions within 1 wk. A minimum of 24 h elapsed between sessions. Patients were blinded as to the intervention they received, and the two interventions were performed at different time points, with the BCI$_{associative}$ intervention conducted first. A posteriori validation verified that the groups matched (Table 2).

Clinical and behavioral measures. Upon the patients' first and last visit, they were assessed with several clinical scales by a clinician blinded to the protocol: modified Rankin scale score (mRS) (Bonita and Beaglehole 1988), National Institutes of Health Stroke Scale (NIHSS) score (Lyden et al. 2001), Hamilton Depression Rating Scale (HDRS) score (Hamilton 1967), the Lower-Extremity Fugl-Meyer assessment (LE-FM)—motor performance (Fugl-Meyer et al. 1975),

Table 2. *Patient clinical and behavioral data for main BCI$_{associative}$ experiment and control BCI$_{nonassociative}$ experiment*

Patient No.	10-m Walk Test, m/s		mRS		LE-FM		ASS	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
BCI$_{associative}$ patient group								
1	0.99	1.08	2	2	24	26	2	2
2	0.36	0.37	3	3	11	11	5	5
3	1.37	1.39	1	1	32	32	0	0
4	0.88	1.01	2	2	30	30	2	2
5	0.33	0.34	3	3	18	18	4	3
6	0.85	0.98	2	2	25	27	2	2
7	0.65	0.73	2	2	22	24	2	2
8	0.65	0.95	2	1	30	32	0	0
9	0.78	0.79	2	2	31	31	0	0
10	0.93	0.97	1	1	32	32	0	0
11	0.74	0.76	2	2	26	27	2	2
12	0.99	1.09	2	2	26	27	3	3
13	0.39	0.40	3	3	17	17	4	3
Mean	0.76	0.84	2.08	2.00	24.92	25.69	2.00	1.85
SD	0.29	0.31	0.64	0.71	6.49	6.61	1.68	1.52
BCI$_{nonassociative}$ patient group								
14	1.24	1.25	2	2	22	22	2	2
15	1.21							
16	0.98	0.96	1	1	32	23.5	0	0
17	1.09	0.98	2	2	22	22	2	2
18	1.09	1.12	2	2	32	32	0	0
19	1.32	1.29	1	1	27	27	2	2
20	1.80	1.51	1	1	33	33	0	0
21	0.82	0.79	2	2	21	21	2	2
22	0.87	0.84	3	3	22	22	3	3
Mean	1.16	1.09	1.75	1.76	26.44	24.44	1.38	1.39
SD	0.28	023	0.71	0.69	5.21	5.34	1.19	1.17

Patient clinical and behavioral data for the main BCI$_{associative}$ (*patients 1–13*) and control BCI$_{nonassociative}$ (*patients 14–22*) experiments are shown. mRS, modified Rankin scale score; NIHSS, National Institutes of Health Stroke Scale score; LE-FM, Lower-Extremity Fugl-Meyer assessment—motor performance; ASS, Ashworth scale for spasticity of the affected leg.

and the Ashworth scale for spasticity (ASS) of the affected leg (Bohannon and Smith 1987).

All subjects performed the 10-m walk test at their fastest pace. A subgroup of seven patients from the BCI$_{associative}$ group and all patients from the BCI$_{nonassociative}$ group performed two additional motor tasks: index finger and foot tapping of the affected side at the fastest possible pace in the sagittal plane. This was to establish the specificity of the protocol on inducing functional changes. Tapping data were recorded for 14–27 s depending on the patient's ability with a wireless inertial sensor system, as described in Djuric-Jovicic et al. (2011). A high-performance 12-bit digital accelerometer LIS3LV02 (SGS-Thomson Microelectronics), with ±6g range of sensors was modified by displacing one triaxial accelerometer from the inertial unit box. This was placed on the middle phalanges of the hand or on the metatarsal bone of the foot and secured with elastic Velcro bands. The number of finger or foot taps was quantified during the patient-specific time interval.

Magnetic resonance imaging parameters and analysis. Diffusion tensor imaging (DTI) MRI scans were obtained on a 1.5-T Achieva system (Philips) using a pulsed gradient spin-echo single-shot echo-planar sequence (TR = 6,714, TE = 86, flip angle = 90°, matrix size = 112 × 110; 50 axial slices, thickness = 2.6 mm with no gap), with diffusion-encoding gradients applied in 65 noncollinear directions (b factor = 1,000 s/mm², 1 average). These data were collected only for the BCI$_{associative}$ group, as we were interested in quantifying whether DT MRI measures of the corticospinal tracts (CST) and corpus callosum could predict alterations induced by the BCI$_{associative}$ intervention.

Evaluation of DTI data. DT MRI analysis was performed with FSLv4.1.7 (http://www.fmrib.ox.ac.uk/fsl/). Diffusion-weighted images were first corrected for distortions induced by eddy currents and then registered to the non-diffusion-weighted volume (b = 0) with 6 degrees of freedom transformation to correct for head movements. The DT was estimated on a voxel-by-voxel basis with the DTI fit toolbox, part of the FMRIB Diffusion Toolbox within FSL. Maps of mean diffusivity (MD) and fractional anisotropy (FA) were obtained. To transform diffusion data from DT MRI native space to the Montreal Neurological Institute (MNI) space, the following procedure was performed: *1*) FA volumes were aligned to the FA template in MNI space with FMRIB's linear Image Registration Tool (FLIRT) with 12 degrees of freedom, *2*) the nonlinear transformation to FSL's FA template was then computed with FMRIB's nonlinear Image Registration Tool entering the information from the linear transformation and obtaining a coefficient file with both the linear and nonlinear components of the transformation to MNI space, *3*) inverse transformations were calculated from the coefficient file obtained from FNIRT. Seeds for tractography of the CST and corpus callosum were defined in the MNI space on the FA template provided by FSL. Regions of interest (ROIs) were defined manually on sagittal or axial slices based on a priori knowledge of the anatomy of the tracts. Seeds were drawn where these tracts pass through a bottleneck in order to include the highest number of fibers constituting the tract in the starting seed for tractography. Seeds were then transformed back to the native diffusion space, and tractography was performed with a single-seed approach. Masks were used to exclude fibers from neighboring tracts. The seed for the corpus callosum was a sagittal ROI including the four median slices on which the corpus callosum is clearly visible. For the CST, an axial ROI was drawn at the top of the bulbar pyramids of each side and included four slices. Average FA and MD values were obtained from the CST and the corpus callosum. Relative asymmetry indexes for FA (reflecting the preferential directionality of water diffusion along the white matter tracts) were calculated according to the following formula: (FAunaffected − FAaffected)/(FAunaffected + FAaffected). Asymmetry indexes for MD (reflecting the magnitude of diffusion) were calculated according to the following formula: (MDunaffected − MDaffected)/(MDunaffected + MDaffected). Asymmetry indexes of FA and MD values comparing lesional with nonlesional hemispheres were correlated

with motor impairment scores, functional changes, and neurophysiological measures.

Statistical procedures. The main outcome measures were the changes in MEP size as well as all the clinical tests. One-way repeated-measures analysis of variance (ANOVA) with the factor "day" (*day 1*, *day 2*, and *day 3* of the intervention) was used to establish the repeatability of PN, the RMT, and ES intensity. Separate two-way ANOVAs were performed to evaluate the premeasures of TMS-evoked MEPs across all testing days for both groups, with "day" (intervention *day 1*, *day 2*, and *day 3*) and "stimulus intensity" (90%, 100%, 110%, 120%, and 130% RMT) as the repeated-measures factors. Furthermore, separate three-way repeated measures ANOVAs with factors "time" (pre, post 0, and post 30), "day" (intervention *day 1*, *day 2*, and *day 3*), and "stimulus intensity" (90%, 100%, 110%, 120%, and 130% RMT) were used to investigate the effects of the days and interventions on changes in MEP amplitude. Student's *t*-tests were used to compare clinical and behavioral measures before and after all interventions. Correlation coefficients were determined for DT MRI measures (average FA and MD values from CST and corpus callosum) and MEP changes as well as between DT MRI measures and behavioral changes. Statistical significance was set to $P < 0.05$. Tukey's honestly significant difference with Bonferroni correction post hoc tests were administered to determine the locus of the differences. All statistical analyses were conducted with SPSS.

RESULTS

Reliability of MRCP. Patients performed 30–50 attempted movements per session, where an average of 12 ± 3 attempts were rejected because of eyeblinks or movement artifacts. For seven patients we investigated the repeatability of the MRCP across days. The PN occurred at -20.49 ± 77.71 ms in relation to the cue indicating to attempt the dorsiflexion task. The time and standard deviation of the occurrence of the PN for each of the seven subjects across the three sessions were not statistically different [$F_{(6,12)} = 1.76$; $P = 0.29$].

Changes in output properties of motor cortex for BCI$_{associative}$ intervention group. Three patients were excluded from the MEP analysis as it was not possible to record data for all days and time points. The size of the MEP evoked at the highest stimulation intensity before the application of the interventions across all patients attained values of 191 ± 148 μV, 284 ± 275 μV, and 331 ± 351 μV for intervention *days 1–3*, respectively. Two-way ANOVA on the presession measures found no significant interaction between days and stimulation intensity [$F_{(8,80)} = 1.21$, $P = 0.299$]. After pooling the interaction term, "days" was not significant [$F_{(2,20)} = 0.77$, $P = 0.473$], indicating that for all days the experiment started with similar baseline excitability across all subjects (Table 3).

Figure 2*A* shows the averaged MEP data for one subject before, after, and 30 min after each of the 3 days in relation to the TMS intensity; Fig. 2*A*, *inset*, represents the raw MEP at 120% RMT for *day 1* for this subject. Also shown are the respective averaged MRCP signals for this subject at the start of each session (Fig. 2*B*). Figure 3, *A–C*, contains the averaged TA MEP size for all subjects plotted against RMT for each intervention session. Data are expressed as a fraction of the maximum TA MEP prior to the intervention.

In the full-model three-way ANOVA, the three-way interaction was not significant ($P = 0.928$). After pooling the three-way interaction term, there was a significant time × stimulation intensity interaction [$F_{(8,72)} = 4.06$, $P < 0.001$]. Simple main effects post hoc analyses revealed that, at the

Table 3. *Results of separate two-way repeated-measures ANOVAs for each group investigating pretest MEP amplitudes across days*

Effect	F Statistic	df	P Value
BCI$_{associative}$ patient group			
Day	0.78	2,20	0.473
Intensity	**11.56**	**4,40**	**<0.001**
Day × intensity	1.22	8,80	0.299
BCI$_{nonassociative}$ patient group			
Day	0.48	2,14	0.629
Intensity	**6.65**	**4,28**	**0.001**
Day × intensity	0.77	8,56	0.631

Values are results of the separate 2-way repeated-measures ANOVAs for each group investigating the pretest motor evoked potential (MEP) amplitudes across days. The 2 repeated-measures factors were "day" (intervention *day 1*, *day 2*, and *day 3*) and "stimulus intensity" (90%, 100%, 110%, 120%, and 130% of resting motor threshold). All significant results are in bold. Note that the 2-way interaction for both groups was not significant, so the 2-way interaction term was pooled.

90%, 110%, and 130% intensities, MEPs were significantly larger 30 min after the intervention than before the intervention ($P = 0.016$, $P = 0.030$, and $P = 0.022$, respectively). Furthermore, MEPs at the 130% intensity were significantly larger immediately after the intervention compared with before the intervention. The day × time and day × intensity interactions, along with the day main effect, were not significant (all $P > 0.21$).

These analyses demonstrate the effectiveness of the proposed intervention in inducing significant neurophysiological changes. The intervention resulted in a significant increase of the TA MEP size, and this outlasted the intervention time by at least 30 min.

Control experiment: changes in output properties of motor cortex for BCI$_{nonassociative}$ intervention group. The size of the MEP evoked at the highest stimulation intensity before the interventions across all patients attained values of 517 ± 489 μV, 448 ± 247 μV, and 454 ± 320 μV for the BCI$_{nonassociative}$ sessions. There was no significant interaction between days and stimulation intensities [$F_{(8,56)} = 0.77$, $P = 0.631$]. After pooling the interaction terms, "day" was found to be not significant [$F_{(2,14)} = 0.48$, $P = 0.629$], indicating that for all three BCI$_{nonassociative}$ intervention days, the experiment started with similar baseline excitability across subjects (Table 3).

Figure 3, *D–F*, show the averaged TA MEP size before and after the intervention for all subjects plotted against RMT for each intervention session. Data are expressed as a fraction of the maximum TA MEP before the intervention.

In the full-model three-way ANOVA, none of the interaction terms (3-way and 2-way) were significant (all $P > 0.22$). After pooling all interaction terms, the three-way analysis on the main effects found that factors "day" and "time" were not significant (both $P > 0.39$), while the factor "stimulation intensity" [$F_{(4,28)} = 9.14$, $P < 0.001$] was significant. For stimulation intensity, the post hoc comparisons found that the higher stimulation intensity resulted in larger MEP magnitudes than lower stimulation intensities, which was expected. These results indicate that the random stimulation did not have any effect on the TA MEP size following the intervention.
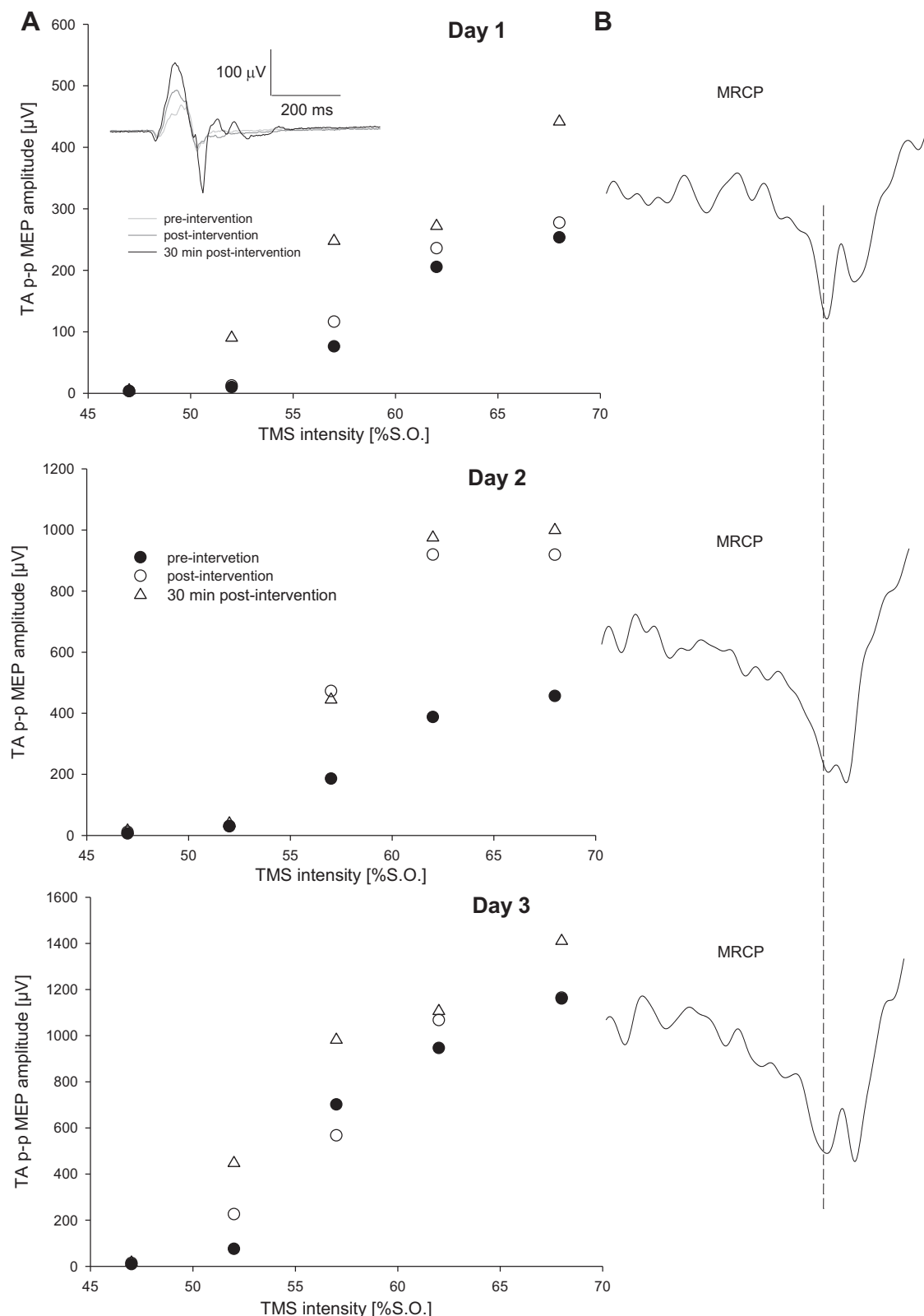
Fig. 2. Changes in motor output after the BCI$_{associative}$ intervention for a single representative participant. *A*: TA MEP recruitment curve before and after the 3 interventions. TA MEP size is expressed as the peak-to-peak amplitude (p-p MEP) and TMS intensity as % of the stimulator output (S.O.). Each data point represents the average of 12 stimuli. *Inset*: representative MEP for this subject for a stimulation intensity of 62% S.O. at *day 1*. *B*: slow MRCP recorded during imaginary movement during the intervention. Data are the average of 30 trials. Vertical dashed line indicates when the cue was presented indicating when the patient should start to attempt the dorsiflexion movement. All data are for *n* = 1.
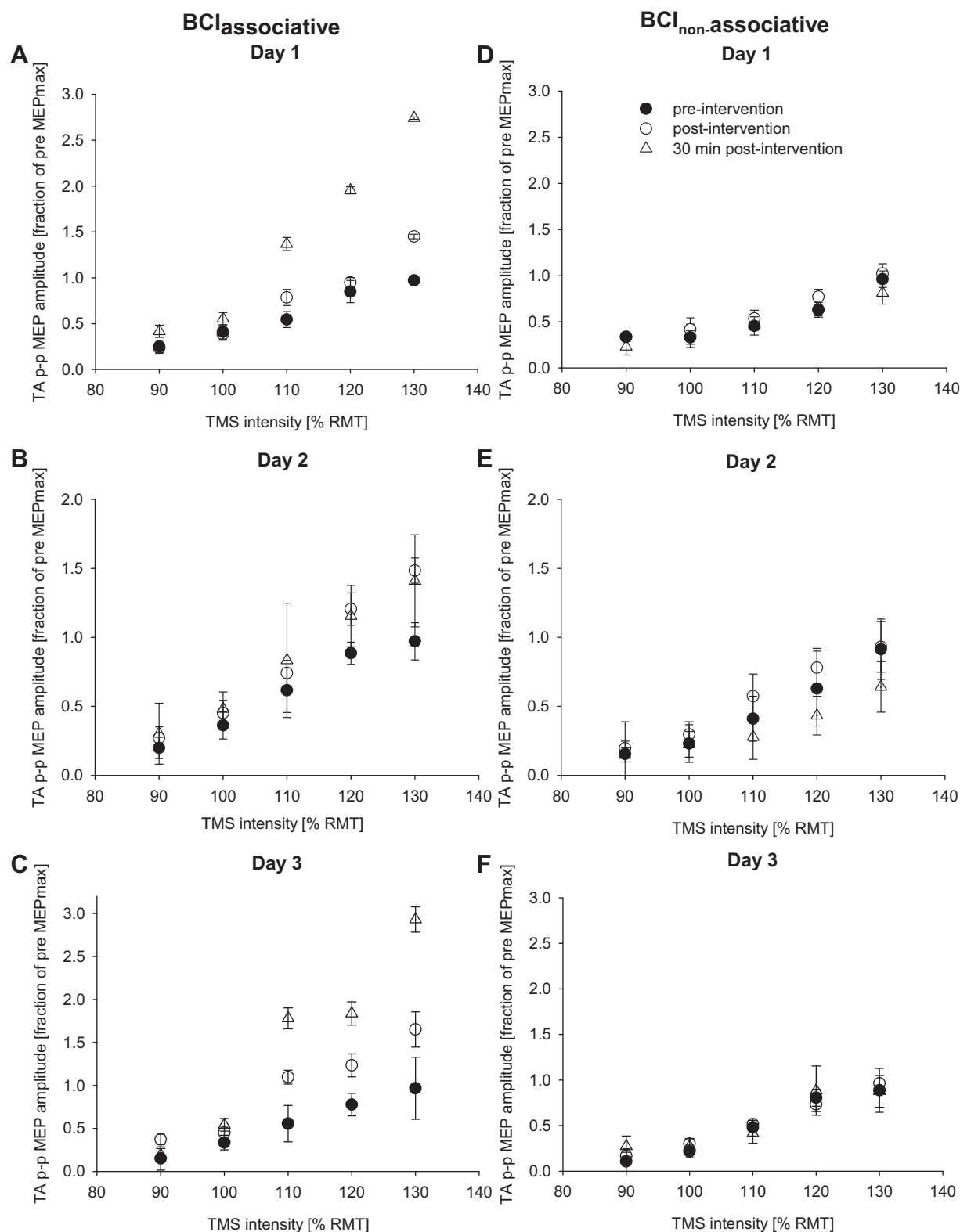
Fig. 3. Input-output properties of the TA MEP before, immediately after, and 30 min after the cessation of the $BCI_{associative}$ (*A–C*) and $BCI_{nonassociative}$ (*D–F*) interventions across all subjects. TA MEP size is expressed as a fraction of the maximum peak-to-peak TA MEP (TA p-p MEP) amplitude before any intervention. Each graph represents a different day of the intervention, and each data point represents the average of 11 subjects (*A–C*) or 8 subjects (*D–F*). Error bars show SE. RMT, resting motor threshold.

Clinical and behavioral measures. Baseline clinical scores for the $BCI_{associative}$ intervention group and for the $BCI_{nonassociative}$ intervention group are shown in Table 2. There were no statistically significant differences between the $BCI_{associative}$ and the $BCI_{nonassociative}$ intervention groups for ASS ($P = 0.47$) and LE-FM ($P = 0.86$) upon enrollment. However, the patients in the $BCI_{associative}$ intervention group were significantly slower in the 10-m walking test [$t_{(19)} = 2.339$, $P = 0.03$].

The clinical assessments were repeated after the 3 days of both interventions. For the BCI_associative intervention group, significant differences were obtained in the 10-m walking test [$t_{(12)} = 3.279$, $P = 0.007$] and the LE-FM scale before and after intervention [$t_{(12)} = -2.993$, $P = 0.011$] as shown in Table 2. No significant changes were detected for mRS ($P = 0.34$) or ASS ($P = 0.186$). For the BCI_nonassociative patients, no significant changes were detected after the intervention for any of the clinical measures (10-m walking test, $P = 0.688$; LE-FM scale, $P = 0.25$; mRS, $P = 0.35$; ASS, $P = 0.977$).

Before the intervention, the BCI_associative patients performed 80 ± 16 taps with the index finger and 70 ± 16 foot taps of the affected side. After the intervention, a statistically significant difference [$t_{(6)} = -3.099$, $P = 0.02$] was observed for the foot tapping task, where tapping frequency increased from 3.43 ± 0.85 Hz before to 4.05 ± 0.52 Hz after. No statistical significance was found ($P = 0.48$) for the index finger tapping task. For the BCI_nonassociative patients, neither foot tapping frequency (pre: 3.29 ± 1.23 Hz, post: 3.16 ± 1.28 Hz; $P = 0.54$) nor index finger tapping frequency (pre: 2.26 ± 1.48 Hz, post: 2.98 ± 2.48 Hz; $P = 0.23$) changed significantly.

Diffusion tensor imaging. These data were only analyzed for the BCI_associative patient group. Four patients were not scanned because of personal reasons. There was a significant decrease of FA values for the CST originating in the lesioned (0.54 ± 0.48) vs. the nonlesioned (0.59 ± 0.03) side [$t_{(8)} = -3.863$, $P = 0.005$]; FA asymmetry = 0.048 ± 0.038. MD values were also significantly different between the two sides [lesioned: 0.85 ± 0.08; nonlesioned: 0.76 ± 0.03; $t_{(8)} = 3.928$, $P = 0.004$]; MD asymmetry = -0.05 ± 0.04. FA and MD values of the corpus callosum were 0.56 ± 0.02 and 0.96 ± 0.07, respectively. No significant correlation was found between FA values with any of the neurophysiological or functional changes induced by the intervention.

DISCUSSION

In this proof-of-concept study, the BCI_associative intervention led to significant neurophysiological and behavioral improvements in all patients investigated after only three sessions. No such alterations were observed for patients engaged in the BCI_nonassociative intervention. Importantly, the effective duration of each BCI_associative intervention session over which the patients were required to be engaged in the task itself was only ~15 min per session. The key feature of our paradigm is that the expected sensory information, triggered by user volition, coincides with the physiologically generated brain activation at the cortical level, satisfying the requirements of the Hebbian associative learning theory (Hebb 1949).

The importance of the precise temporal association between a peripherally generated afferent volley and the activation of the motor cortex was first reported by Stefan and colleagues (Stefan et al. 2002). Their intervention was comprised of a single electrical stimulus delivered to the median nerve that innervates the target muscle abductor pollicis brevis (APB) and a single stimulus applied directly to that area of the motor cortex with projections to the APB with noninvasive TMS. The TMS was triggered 25 ms after the peripheral stimulus, and this represents the delay for the afferent volley to reach the motor cortex. Only with this specific interval of 25 ms between the two stimuli was a significant change in the excitability of

the cortical projections to the APB observed. A similar protocol has been applied to the TA, although the delay between the two stimuli was reported to be longer (50 ms) for any significant induction of plasticity (Mrachacz-Kersting et al. 2007). In the present study, the ES was timed to occur 50 ms before the PN of the MRCP, since the PN is associated to movement execution and may be seen as a more natural activation of the motor cortex compared with TMS. In our past study using this paradigm in healthy participants, we demonstrated that only if the ES was timed so that the generated afferent signal arrived at PN was a significant effect observed. In the present study we thus did not expose our patients to different time intervals between the ES and PN. Instead, the control condition involved the BCI_nonassociative patient group receiving ES timed to occur randomly throughout the MRCP (Fig. 1*E*, *right*). In this way, there was no association between the arrival of the afferent inflow generated by the ES and the PN of the MRCP. It should be noted that the BCI_nonassociative group was tested separately in a control experiment and thus it was not possible to run a statistical analysis with a group factor that might have provided a more robust analysis.

The signal modality (MRCP) used here is natural and physiologically associated with the movement (Kuhtz-Buschbeck et al. 2003; Leifert-Fiebach et al. 2013; Salinet et al. 2012), so no user training was necessary to produce this signal. This is in contrast to other BCIs for neuromodulation in stroke where patients were required to spend several sessions (15 or more, over the duration of months) for learning to produce controllable brain signals (Ang et al. 2009; Buch et al. 2008). Stroke patients have altered temporal as well as spatial MRCPs (Platz et al. 2000; Yilmaz et al. 2013), and it is known that a patient's ability to perform a motor task accurately is significantly enhanced when a cue is provided (Heremans et al. 2009). This was the case in our intervention and may have led to the enhanced effects in the BCI_associative group.

The BCI_associative intervention led to statistically significant improvements in the LE-FM scale and the 10-m walking test in all subjects (Table 2) and in foot tapping frequency in a subgroup of patients, which was not expected. Although from a clinical perspective the improvements are too small to be considered significant, it is important to mention that the patients only performed three intervention sessions and each session comprised only 30–50 pairings. It is likely that the intervention would lead to even greater improvements if applied over a longer duration as has been done for past BCIs in this context (Ramos-Murguialday et al. 2013). The control group taking part in the BCI_nonassociative intervention showed no such improvements, so that the changes in the patients of the BCI_associative group cannot be attributed to an experimental participation effect. The improvements occurred in tasks that were unrelated to the task executed in the BCI intervention. Similar reports of such transfer come from the area of spinal cord plasticity. Wolpaw and colleagues (Chen et al. 2014; Wolpaw 2007) have induced such plasticity by using an operant conditioning of a spinal reflex (the H reflex). Changes in this simple reflex arc are transferred to dynamic tasks in lesioned animals, and most recently similar results have been shown in spinal cord-injured humans (Thompson and Wolpaw 2015).

All patients in the BCI_associative group showed significant increases in the MEP recorded at the TA after the intervention.

The presence of MEPs in the TA in the acute phase following a stroke has been associated to recovery of motor function (Hendricks et al. 2003b). In chronic patients, increases in MEP amplitude over time are an indicator for functional improvements (Piron et al. 2005). In the present study, the changes in MEP size through all three sessions were only weakly correlated with the improvements in function. In light of previous publications, this was unexpected. However, a few recent reports have documented that improvements in motor function are not necessarily mirrored by alterations in the excitability of the cortical projections to the target muscle (Avanzino et al. 2011; Hendricks et al. 2003a). However, it is also important to note that although within each session the sizes of the MEPs were significantly increased for the BCI$_{associative}$ intervention, there was no significant difference between the changes induced across the 3 days and baseline MEP values did not differ between days. This implies that, although a significant within-session effect is present, MEPs do not change across sessions. However, in the present proof-of-concept study patients only partook in a total of three sessions. It is likely that if the intervention was of longer duration, significant changes in MEP size would occur also across sessions.

The MEP amplitudes were significantly larger 30 min after the cessation of the BCI$_{associative}$ intervention compared with immediately before. A similar effect has been reported in previous studies using PAS as the intervention and targeting lower limb muscles (Kumpulainen et al. 2012). This effect is not easily explained on the basis of on current knowledge on the underlying mechanisms following such interventions. It is likely caused by complex interactions between different neuronal populations in the motor cortex of the lesioned hemisphere. However, in the absence of invasive neuronal investigation any hypothesis about the precise nature of this effect remains speculative.

One possible reason for the significant improvements after so few intervention sessions and task repetitions reported here as opposed to other available BCIs may be directly related to the temporal association. After stroke that damages part of the sensory cortex, the intact part significantly enhances its output to both motor cortex and other cortical regions (Brown et al. 2009; Murphy and Corbett 2009). In our BCI intervention, the afferent volley induced by the peripheral nerve stimulus was timed to arrive during the movement execution phase of the MRCP and can thus be viewed as a means to guide the afferent input appropriately to restore the connectivity between the sensory cortex and the motor cortex. In healthy subjects when the timing was altered to either before or after this phase, no plasticity was induced (Mrachacz-Kersting et al. 2012a). This was substantiated by the results from the control BCI$_{nonassociative}$ intervention, where the timing was such that the afferent volley arrived randomized during either the motor preparation phase or the reafferent phase but never during the movement execution phase. Taken together, the results from the BCI$_{associative}$ and the BCI$_{nonassociative}$ interventions underline the importance of the correct sequence of signals for inducing long-lasting plastic changes. The critical importance of the delay between motor command and sensory input shown here for patients and in previous studies for healthy subjects (Mrachacz-Kersting et al. 2012a) may also explain the substantially less efficient outcome of BCI neuromodulation in

stroke patients in previous studies (for review see Silvoni et al. 2011).

Repeated activation of somatosensory afferents projecting onto M1 has a pivotal role in motor skill learning in monkeys (Pavlides et al. 1993). In a recent BCI study of chronic stroke patients, somatosensory feedback triggered from detected brain signals led to functional improvements, while visual feedback induced no such changes (Ono et al. 2014), thus further highlighting its importance. In the present study, the CPN, which is a mixed nerve, was electrically stimulated to generate the afferent volley. Since the intensity of the stimulus was set to MT, we expect that mainly afferents arising from muscle receptors were stimulated and thus provided their input to the sensory cortex. Sensory information arising from muscles contributes substantially (up to 50%) to the activation of muscles by the nervous system (Grey et al. 2004; Mazzaro et al. 2006; Nielsen 2004; Nielsen and Sinkjaer 2002). Sensory information is also modulated by the brain, allowing either large or small influences during a particular movement (Nielsen 2003). For example, during walking, sensory feedback from calf muscles directly activates these muscles during the push-off phase, where a strong contraction is required. However, during the time when the foot has to be placed in the correct position for heel strike, the brain stops the sensory signals from the calf muscles by inhibition of the relevant spinal pathways (Sinkjaer et al. 2000). In this way, these signals will not interfere with the placement of the heel on the ground. The ES was applied at a time when the patient attempted to dorsiflex the foot and was therefore task related and delivered within a meaningful context. However, a dorsiflexion will also impose a lengthening of the antagonist muscles (soleus and the two heads of the gastrocnemius), which would generate afferent signals arising from those muscles that travel toward the brain. Indeed, the effects observed here might be more potent when applied during a walking task at the appropriate time of dorsiflexion (early swing phase) and with relevant additional input from afferents located in the antagonist and also the sole of the foot. In a recent experiment we have demonstrated the feasibility of detecting gait initiation from single-trial MRCPs (Jiang et al. 2014), setting the stage for such experiments.

The peripheral afferent volley induced comprised a single volley set to an intensity at the vicinity of MT. It is currently not known how signals arising from the periphery are attenuated as they are conveyed to the motor cortex. Indeed, functional electrical stimulation (FES) is another therapy that has been used specifically to target the TA muscle after stroke either alone (Burridge et al. 1997; Everaert et al. 2010; Knash et al. 2003; Lyons et al. 2002; Thompson and Stein 2004) or in combination with a BCI (Cincotti et al. 2012; Daly et al. 2009; Li et al. 2014). FES uses a higher-frequency and -intensity stimulation to alter the excitability of the cortical projections to the target muscle than that applied in the present study. The effects of FES are enhanced when the voluntary drive to the TA is augmented such as through an active contraction of the TA (Khaslavskaia and Sinkjaer 2005), suggesting that some form of coincident summation is also involved. It is possible that the effects of our intervention may be further enhanced when the peripheral stimulus is similar to FES in aspects of frequency and stimulus strength. Indeed, with only FES for a duration of 3–12 mo, walking speed was signifi-

cantly increased by 24% in chronic stroke as well as multiple sclerosis patients when the stimulator was in the off position (Everaert et al. 2010).

Residual corticospinal tract integrity and improvement in function. Past studies have suggested that residual corticospinal cord integrity is a predictor for recovery of upper limb function (Stinear et al. 2007; Tombari et al. 2004). In the present study, residual corticospinal integrity was not significantly correlated both to functional improvements and to changes in MEP size, which is in support of such studies targeting lower limb muscles (Dawes et al. 2008). Lower limb muscles have been suggested to be under less cortical control compared with upper limb muscles (Peters 1990). However, one exception is the TA muscle, which requires precise control during foot clearance in the late swing phase of human walking and has been shown to have a strong cortical contribution to its activation (Bawa et al. 2002; Brouwer and Qiao 1995; Petersen et al. 2003). Presence of MEPs in the TA but not in the larger quadriceps muscle group is a predictor of functional recovery (Hendricks et al. 2003b), so it was surprising that DTI data were not well correlated to functional improvements. Possible confounding factors include both the types of patients recruited as well as the low number of patients who took part in the scans.

Conclusions. We have introduced an innovative BCI intervention for stroke rehabilitation, based on Hebbian principles of associativity (Hebb 1949). Different from other BCI protocols reported in the literature, it requires no subject training (all patients were BCI-naive prior to the study); the attempted movement does not need to have residual muscle control, and a small number of repetitions and sessions were sufficient to induce a significant effect. The results presented prove the possibility of using neurofeedback systems, based on BCI concepts, for efficient and targeted induction of plastic changes in the motor cortex in stroke therapy. Because of the efficacy of the intervention and the relatively simple equipment needed, the proposed approach opens a strong perspective for a clinically viable BCI therapy for patient populations to promote functional plasticity and to improve motor function after stroke.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: N.M.-K., N.J., V.K., A.P., S.R., K.D., and D.F. conception and design of research; N.M.-K., I.K.N., A.P., S.R., and M.D.-J. performed experiments; N.M.-K., N.J., A.J.T.S., I.K.N., M.D.-J., and F.A. analyzed data; N.M.-K., N.J., V.K., M.D.-J., F.A., and D.F. interpreted results of experiments; N.M.-K. prepared figures; N.M.-K., N.J., and D.F. drafted manuscript; N.M.-K., N.J., A.J.T.S., V.K., and D.F. edited and revised manuscript; N.M.-K., N.J., A.J.T.S., I.K.N., V.K., A.P., S.R., M.D.-J., F.A., K.D., and D.F. approved final version of manuscript.

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