The effect of type of afferent feedback timed with motor imagery on the induction of cortical plasticity

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Abstract

A peripherally generated afferent volley that arrives at the peak negative (PN) phase during the movement related cortical potential (MRCP) induces significant plasticity at the cortical level in healthy individuals and chronic stroke patients. Transferring this type of associative brain-computer interface (BCI) intervention into the clinical setting requires that the proprioceptive input is comparable to the techniques implemented during the rehabilitation process. These consist mainly of functional electrical stimulation (FES) and passive movement induced by an actuated orthosis. In this study, we compared these two interventions (BCI_{FES} and $BCI_{passive}$) where the afferent input was timed to arrive at the motor cortex during the PN of the MRCP.

Twelve healthy participants attended two experimental sessions. They were asked to perform 30 dorsiflexion movements timed to a cue while continuous electroencephalographic (EEG) data were collected from FP1, Fz, FC1, FC2, C3, Cz, C4, CP1, CP2, and Pz, according to the standard international 10-20 system. MRCPs were extracted and the PN time calculated. Next, participants were asked to imagine the same movement 30 times while either FES (frequency: 20 Hz, intensity: 8-35 mAmp) or a passive ankle movement (amplitude and velocity matched to a normal gait cycle) was applied such that the first afferent inflow would coincide with the PN of the MRCP. The change in the output of the primary motor cortex (M1) was quantified by applying single transcranial magnetic stimuli to the area of M1 controlling the tibialis anterior (TA) muscle and measuring the motor evoked potential (MEP). Spinal changes were assessed pre and post by eliciting the TA stretch reflex.

Both BCI_{FES} and $BCI_{passive}$ led to significant increases in the excitability of the cortical projections to TA ($F_{(2,22)}$ = 4.44, p = 0.024) without any concomitant changes at the spinal level. These effects were still present 30 minutes after the cessation of both interventions. There was no significant main effect of intervention, $F_{(1,11)}$ = 0.38, p = 0.550, indicating that the changes in MEP occurred independently of the type of afferent inflow.

An afferent volley generated from a passive movement or an electrical stimulus arrives at the somatosensory cortex at similar times. It is thus likely that the similar effects observed here are strictly due to the tight coupling in time between the afferent inflow and the PN of the MRCP. This provides further support to the associative nature of the proposed BCI system.

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1. Introduction

Brain-computer-interfaces (BCIs) designed for neuromodulation detect user intent from the brain activation patterns and send a command to an external device that reproduces the intended movement without the conventional routes via nerves and muscles (Wolpaw 2013; Wolpaw and Winter Wolpaw 2012). Since the original report by Daly et al (Daly et al. 2009), demonstrating the effectiveness of a non-invasive BCI intervention in stroke patients, there have been numerous studies conducted in this patient population (for a recent review see (Soekadar et al. 2015)). Here, the BCI may be implemented to either assist movements, thus replacing the lost function through assistive devices (Pfurtscheller et al. 2003; Ethier et al. 2012; Collinger et al. 2013), or conversely to induce neuroplasticity and thus restore normal motor function (Ang et al. 2009; 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; Mrachacz-Kersting et al. 2016; Pichiorri et al. 2015; Ramos-Murguialday et al. 2013; Young et al. 2014). In the latter case, BCIs have to be designed to follow the principles of learning. That is, the control of the external device, irrespective of whether it is an electrical stimulator or a robotic actuator, has to be timed such that the elicited afferent feedback arrives at the primary motor cortex (M1) at the appropriate time.

To date the general consensus is that memory formation and learning follow the principle of associativity first developed by Hebb (Hebb 1949). When a postsynaptic cell fires just prior to receiving a weak presynaptic input, synapses are strengthened. Later animal studies confirmed this theory (Bliss and Lomo 1973; Bliss and Collingridge 1993) that is known as associative long-term potentiation (LTP). In both intact and lesioned participants, a peripheral afferent volley that arrives at the motor cortex when it is depolarized using non-invasive transcranial magnetic stimulation (TMS) induces plasticity. The induced plasticity rapidly evolves, it is long lasting and specific to the target muscle, and it is dependent on both NMDA receptor and calcium channel activation (Stefan et al. 2000; Stefan et al. 2002; Wolters et al. 2003; Stefan et al. 2006; Castel-Lacanal et al. 2007). It thus expresses many of the properties of LTP.

The TMS stimulus may be replaced by a more natural activation of M1 such as due to motor imagination. Motor imagination is associated to movement related cortical potentials (MRCP; (Mrachacz-Kersting et al. 2012) which are slowly developing potentials commencing

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approximately one to two seconds prior to movement execution, with peak negative phase during movement execution (for a review see (Shibasaki and Hallett 2006)). Since it may be detected prior to movement occurrence, it is an ideal signal modality that may be extracted from noninvasive electroencephalography (EEG) to control an external device.

In previous studies, we have demonstrated that this type of intervention induces neuroplasticity at the level of M1 in both healthy and chronic stroke patients (Mrachacz-Kersting et al. 2012; Xu et al. 2014; Mrachacz-Kersting et al. 2016). However, plastic changes were observed only if the artificially generated afferent signal was timed to reach M1 during the peak negative phase of the MRCP. Therefore, we developed methods for online detection of MRCPs that could trigger the induction of afferent feedback in an asynchronous manner (Niazi et al. 2012; Xu et al. 2014) and we termed the resulting intervention associative BCI. Interestingly, in different studies using this concept, we observed that plasticity was induced irrespective of whether the afferent feedback was generated by a single electrical stimulus delivered at an intensity just above motor threshold (Mrachacz-Kersting et al. 2012), or a passive dorsiflexion movement induced by an actuated orthosis (Xu et al. 2014). However, for this BCI to be used within the daily clinical routine, it would be desirable to provide a feedback that is typically used by the existing therapies. These include functional electrical stimulation and passive ankle angle movements provided by a robotic device (for a review see (Laffont et al. 2014)).

In this study, we compared the effects of eliciting afferent feedback either by FES or by an active orthosis on the induced plasticity in an associative BCI. However, to eliminate the confounding effect of variability in MRCP detection accuracy among subjects when comparing the feedback modalities, the timing of PN of the MRCP with respect to a cue was identified from a training set where participants were asked to perform or attempt to perform the motor task and subsequently used for the actual intervention. Therefore, in this study we do not directly test the online detection of MRCP with a BCI during the intervention but we rather focus on the comparison between feedback types. The aim was to quantify plastic changes in healthy participants exposed to an associative intervention when providing either FES or a passive dorsiflexion movement. For this purpose, cortical changes were assessed prior to, immediately following, and 30 minutes after the interventions while spinal changes were quantified with the stretch reflex.

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2. Results

2.1. Reliability of the MRCP

All participant performed 30 dorsiflexion movements prior to the two interventions. An average of 2 ± 3 trials and 2 ± 4 trials were rejected due to eye-blinks or movement artefacts in the BCI_{FES} and $BCI_{passive}$ sessions, respectively. Figure 1 shows the mean PN time (and SE) for each participant for the two BCI sessions while Figure 2 contains single trial MRCPs (and the average MRCP) for one participants for the BCI_{FES} (Figure 2A) and $BCI_{passive}$ (Figure 2B) intervention days. Across all participants, the PN occurred at 15 ± 55 ms (mean and standard deviation) and 13 ± 12 ms (for the BCI_{FES} and $BCI_{passive}$ sessions, respectively) in relation to the cue indicating to perform the movement. The time of the occurrence of the PN between the two sessions was not statistically different ($t_{11} = 0.955$, $p = 0.36$).

2.2. Changes in the output properties of the motor cortex for the BCI_{FES} and $BCI_{passive}$ intervention

The amplitude of the TA MEPs evoked at the highest stimulation intensity prior to the interventions across all participants attained mean ($\pm$ SE) values of 529 ± 133 μV and 512 ± 114 μV for the BCI_{FES} and $BCI_{passive}$ interventions, respectively. The two-way rmANOVA on the pre-intervention measures found no significant interaction between intervention and stimulation intensity, $F_{(1.32,14.53)} = 0.23$, $p = 0.704$. After pooling the interaction term, the main effect of intervention was not significant, $F_{(1,11)} = 0.52$, $p = 0.486$, indicating that the experimental sessions started with a similar baseline excitability across all participants.

Figure 3A and B shows the mean MEP data for one participant prior to, following and 30 minutes after the cessation of the BCI_{FES} (Figure 3A) and $BCI_{passive}$ (Figure 3B) interventions at all intensities tested. For this participant, the maximum MEP evoked at the highest stimulation intensity increased from 714.7 μV (pre) to 829.4 μV (post) to 862.7μV (30 minutes post) for the BCI_{FES} intervention and from 830.6 μV (pre) to 879.9 μV (post) and 1060 μV (30 minutes post) for the $BCI_{passive}$ intervention.

Figure 3C and D show the mean TA MEP amplitudes across all participants immediately following (Figure 3C) and 30 minutes after (Figure3D) the BCI_{FES} and $BCI_{passive}$ interventions for all

stimulation intensities. Data are expressed as a percentage of the corresponding pre-intervention TA MEP amplitudes for all stimulation intensities.

In the full model three-way rmANOVA, the three-way interaction and all two-way interactions were not significant (all p's ≥ 0.097). After pooling the two- and three-way interaction terms, there was a significant main effect of time, $F_{(2,22)}$ = 4.44, p = 0.024. Fisher's least significant difference (LSD) post-hoc analysis revealed that mean (± SD) values TA MEP amplitudes were significantly larger immediately following (280 ± 46 µV) and 30 minutes after (291 ± 47 µV) the BCI interventions compared to pre-intervention MEPs (243 ± 50 µV) regardless of intervention type and stimulation intensity (p's = 0.029 and 0.039, respectively). There was no significant difference between TA MEP amplitudes immediately following and 30 minutes after the BCI interventions (p = 0.490). Furthermore, there was a significant main effect of stimulation intensity, $F_{(1.08,11.92)}$ = 18.02, p = 0.001. Fisher's LSD post-hoc analysis revealed that mean (± SD) values TA MEP amplitudes were significantly larger at stimulation intensities of 140% RMT (536 ± 106 µV) compared to 130% (434 ± 86 µV), 120% (393 ± 58 µV), 110% (201 ± 32 µV), 100% (108 ± 9 µV), and 90% RMT (43 ± 6 µV) regardless of intervention type and stimulation time (all p's ≤ 0.002). In addition, TA MEP amplitudes were also significantly larger at stimulation intensities of: 130% RMT compared to 120%, 110%, 100%, and 90% RMT (all p's ≤ 0.002); 120% RMT compared to 110%, 100%, and 90% RMT (all p's ≤ 0.005); 110% RMT compared to 100% and 90% RMT (both p's ≤ 0.011); and 100% compared to 90% RMT (p < 0.001). There was no significant main effect of intervention, $F_{(1,11)}$ = 0.38, p = 0.550, indicating that the TA MEP changes occurred independently of the type of BCI intervention used.

2.3.   Control experiment: Changes in spinal excitability

Eight participants were exposed to 30 unexpected plantarflexion movements prior to and following the two interventions. These had an amplitude of 4-6 deg and a velocity of 200-300 degs[-1]. An example of one participant for changes in ankle angle (Figure 4A and B upper trace) and the corresponding EMG recording from the TA (Figure 4A and B lower traces) are shown in Figure 4A and B for the BCI$_{passive}$ and the BCI$_{FES}$ intervention respectively. For this participant the stretch reflex was comprised of three distinct peaks with an onset latency of 44 ms and a SLR peak latency of 74 ms. The SLR component of the stretch reflex did not change significantly for either the

BCI$_{passive}$ or the BCI$_{FES}$ intervention (t_7 = -0.267, p = 0.798 and t_7 = 0.355, p = 0.733 for the BCI$_{passive}$ and the BCI$_{FES}$ intervention respectively). Figure 4*C* shows the average size of the SLR prior to and following both interventions for all eight participants. The background level of activation during the imposed plantarflexion perturbations did not differ significantly pre and post for either of the two intervention (BCI$_{passive}$: t_7 = -1.348, p = 0.22; pre: 150±43 μV, post: 156±43 μV; BCI$_{FES}$: t_7 = 0.075, p = 0.943; pre: 203±39 μV, post: 202±38 μV).

3. Discussion

We exposed healthy participants to an established associative intervention where the peripheral input consisted of either FES or a passive dorsiflexion movement timed such that the afferent inflow reached M1 during the peak negative phase of the MRCP. Both types of interventions resulted in a significant increase in the excitability of the corticospinal tract to the TA as assesses by TMS that outlasted the stimulation period by at least 30 minutes. No such changes were observed for the short latency component of the TA stretch reflex.

3.1. Reliability of the MRCP

Prior to the exposure to the interventions, the participants performed 30 dorsiflexion movements timed to a cue. This served to quantify the repeatability of the MRCP and, the timing of the peak negative phase in relation to the cue. Since participants will differ in their reaction to the visual cue (Mrachacz-Kersting et al. 2012), in the current protocol the afferent inflow was timed to arrive at the PN of the MRCP rather than at the onset of the visual cue shown to the participants. The results demonstrated that participants performed the movement in a similar manner across days since the PN did not vary significantly between the two sessions. This confirms previous reports where the MRCP was repeatable across sessions in both healthy participants (Mrachacz-Kersting et al. 2012) and chronic stroke patients (Mrachacz-Kersting et al. 2016).

3.2. Changes in the output properties of the motor cortex for the BCI$_{FES}$ and BCI$_{passive}$ interventions

An associative BCI induces significant plasticity of M1 when applied to healthy participants in a single session (Mrachacz-Kersting et al. 2012; Xu et al. 2014). In our previous study we have demonstrated that neither electrical stimulation alone, passive dorsiflexion alone or imagery alone when repeated over such few trials have a significant effect on MEP sizes. Rather it is the combination of the afferent induced signal that is timed to arrive at the PN of the MRCP that is imperative for plasticity induction (Mrachacz-Kersting et al. 2012). The necessity of timing the peripheral stimulus so that the generated afferent volley arrives at precisely the PN phase of the MRCP is based on a theory proposed by (Hebb 1949). This implies that when an action potential arrives at a presynaptic neuron just prior to or concomitantly to the postsynaptic cell firing, the

synapse is strengthened. In intact humans this may be tested by a protocol termed paired associative stimulation (PAS – (Stefan et al. 2000; Mrachacz-Kersting et al. 2007)). Here, a single stimulus is applied to the nerve that innervates the target muscle and once the generated afferent signal arrives at M1, a TMS stimulus is applied over that area of M1 that has projections to the target muscle. Since the initial reports, the peripheral stimulus as part of the PAS protocol has been modified to include either ipsilateral voluntary contraction of the target muscle (Kujirai et al. 2006), contralateral voluntary muscle activation (Kennedy and Carson 2008) or FES (Mrachacz-Kersting 2013). As for PAS, results from the current study confirm that plasticity induction occurs irrespective of the type of afferent volley induced artificially. Since neither a passive movement alone (Mrachacz-Kersting et al. 2012) nor FES applied alone (Knash et al. 2003; Khaslavskaia et al. 2002) can induce significant changes in MEP size when applied over such a short time window (less than 10 minutes), the effects reported here are likely due to the continuous pairing of the afferent signal with the PN of the MRCP.

Afferent input arising from FES and passive movements presumably arrive at M1 via the somatosensory cortex, although it is known that M1 also receives afferent input from thalamocortical circuits (Kaneko et al. 1994a; Kaneko et al. 1994b). The afferent input between the BCI_{FES} and $BCI_{passive}$ interventions is likely to differ significantly since a passive movement will not only unload the target muscle but also stretch the antagonist and the activated muscle and cutaneous afferents will fire in an asynchronous manner. This is in contrast to FES where the intensity and frequency of the input would lead to recruitment of all afferents within the target nerve in a synchronous manner. However, somatosensory evoked potentials induced by a passive movement as performed here have an average onset latency of 47 ms (Petersen et al. 1998) which is similar to that following electrical stimulation (44 ± 2 ms, (Mrachacz-Kersting et al. 2007). Thus, for both the BCI_{FES} and $BCI_{passive}$ intervention, the initial afferent volley arrived at the same time in relation to the PN of the MRCP. If we assume that it is the arrival of the first afferent volley timed precisely with the PN of the MRCP that lead to the plasticity induction, it may not be surprising that both interventions showed a similar effect.

In our previous studies we applied a single peripheral nerve stimulus at MT (Mrachacz-Kersting et al. 2012; Mrachacz-Kersting et al. 2016) or a dorsiflexion movement (Xu et al. 2014) timed so that the generated afferent signal arrived at M1 during the PN phase of the MRCP. As for the BCI_{FES} and

BCI$_{passive}$ intervention presented here, significant alterations in the excitability of the cortical projections to the TA are evident after a single session that outlast the stimulation period. It appears that MEP changes may be induced regardless of the type of proprioceptive input supplied. From a clinical perspective this is desirable as some clinics may not have robotic actuators available but most use FES as part of the weekly rehabilitation of stroke patients. However, the type of proprioceptive input may have different effects on the motor cortical network (Rosenkranz and Rothwell 2006) when applied as part of an intervention. For example, PAS increases the MEP size of the target muscle, yet has no effect on short interval intracortical inhibition (SICI) or sensorimotor reorganization, while actual movement performance also increases MEP size, decreases SICI, and significantly changes sensorimotor reorganization (Rosenkranz and Rothwell 2006). SICI and sensorimotor reorganization were not investigated in the current study, thus it remains speculative whether the FES or the passive movement targeted different components of the motor cortical network.

3.3.   Changes in spinal excitability for the BCI$_{FES}$ and BCI$_{passive}$ intervention

Alterations in MEP size can result due to changes in either cortical or spinal networks. However, since there were no changes in the spinally mediated component of the TA stretch reflex, it is unlikely that subcortical sites contributed to the effect. Recent reports suggest that at least some of the effects following PAS targeting upper limb muscles may occur at the spinal level (Meunier et al. 2007). However, not all participants that showed an increase in MEP size also had a concomitant increase in the H-reflex. The H-reflex only probes that pathway arising from muscle spindle Ia afferents while the TA stretch reflex has various components believed to arise from different muscle afferents (Kearney and Hunter 1984; Petersen et al. 1998) and may therefore be better suited to quantify spinal pathway changes. However, even with the stretch reflex it is not possible to probe the entire network of spinal pathways and thus it cannot be excluded that some of the changes may have occurred at subcortical or spinal sites.

4. Conclusion

Here we present an associative intervention with two types of peripherally applied proprioceptive inputs typically implemented in the clinical setting, FES and robot controlled passive movements. The two types of proprioceptive feedback induced similar changes in the excitability of the cortical

projections to the TA, with no changes in the spinal stretch reflex. While these results provide strong support for the associative nature of the interventions, further studies are required to assess whether BCI$_{FES}$ and BCI$_{passive}$ have similar effects on the motor cortical network.

5. Methods and Materials

5.1. Participants

Twelve participants (six females; aged 25.3 ± 3.0 years, mean ± SD) provided written informed consent to take part in this study. At the time of the study, all participants were free of any known physical or neurological disorders. Approval for the study was provided by the scientific ethics committee for Nordjylland (Reference Number: N-20130039). The study was performed in accordance with the declaration of Helsinki.

During all experiments described below, participants were seated in a chair (Hip 90°, Knee 130°) with their right and left foot resting on separate footplates (see Figure 5A-C for the experimental set-up).

5.2. Movement related cortical potential (MRCP)

Ten channels of monopolar EEG were recorded using an active EEG electrode system (g. GAMMAcap [2], Austria) and g.USBamp amplifier (gTec, GmbH, Austria) from FP1, Fz, FC1, FC2, C3, Cz, C4, CP1, CP2, and Pz according to the standard international 10-20 system. The channel selection was based on the large Laplacian with Cz as the central channel (McFarland et al. 1997). The reference electrode was placed on Fz and the ground on the left earlobe. A single channel surface electromyography (EMG) was recorded from the tibialis anterior (TA) muscle to control for the participant's movement. All signals were sampled at a frequency of 256 Hz (16 bits accuracy) and hardware filtered from 0 to 100 Hz.

Next, participants were asked to perform 30 dorsiflexions of their dominant foot in relation to a visual cue. A custom made Matlab script (R2014b, Mathworks®) provided this cue via a screen positioned 2 m in front of the participant on when to mentally prepare, execute, and release the movement (Figure 5D). Participants were instructed to perform a single dorsiflexion movement as fast as possible when the cursor had reached the upwards turn and to maintain the new position for 2 s, following which they relaxed again for 4-5 s prior to the next cue being provided. Data from

recorded EEG trials was used to quantify the time of peak negativity of the MRCP's before proceeding to one of two interventions described under the section 'Interventions'.

5.3.   Feature extraction from the MRCP

Matlab software (R2014b, Mathworks®) was used to filter continuous EEG signals using a 2nd order band-pass Butterworth filter from 0.05-10 Hz. EEG data were then divided into epochs of 4 s (from 2 s before to 2 s following the visual cue) for each movement and subsequently a Laplacian channel (McFarland et al. 1997) was used to enhance the MRCP in each epoch. Next, a window of 500 ms on either side of task onset was chosen. If any epoch's peak negativity was outside the selected window it was discarded. Epochs with EOG activity exceeding 140 µV were also discarded. The remaining epochs were averaged and the mean peak negativity (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.

5.4.   Recording and Stimulation

The EMG activity was recorded by surface Ag/AgCl electrodes (Ambu Neuroline 720, Ambu A/S, Denmark) placed over the belly of the right tibialis anterior (TA). The electrodes were placed in accordance with the recommendations of Cram et al. (Cram et al. 1998). Surface EMGs were pre-amplified and sampled at 2 kHz using scientific software Mr. Kick II 2.3 (Knud Larsen, Center for Sensory-Motor Interaction, Aalborg University, Denmark) for recordings of the TA stretch reflex. The EMG amplifier pod supplied by Rogue Research Inc.as part of the Brainsight™ system (Rogue Research inc.), was used to collect MEP data. During the BCI intervention, EMG data were collected using the g.USBamps (g.tec GmbH, Austria) at a sampling frequency of 256 Hz.

A Magstim 200 (Magstim Company, Dyfed, UK) with a focal figure of eight double cone coil (110 mm diameter) was used to apply single transcranial magnetic stimulation (TMS) pulses to elicit a motor evoked potential (MEP) in the TA. The direction of the current was directed from posterior to anterior.

Peripheral nerve stimulation was performed during one of the interventions. Stimulation of the common peroneal nerve (CPN) was applied using a NoxiTest isolated peripheral stimulator (IES 230). Stimulating electrodes (32 mm, PALS® Platinum, Patented Conductive Neurostimulation Electrodes, Axelgaard Manufacturing Co., Ltd. USA) were placed on the skin overlying the deep branch of the right common peroneal nerve (CPN – L4 and L5) with the cathode proximal. A suitable position for stimulation, defined as the site where a maximal M-wave was produced in the TA with no activity from the synergistic peroneal muscles and no activity from the antagonist soleus (SOL), was identified. Palpation of SOL and peroneal muscles was performed during stimulation trials to ensure that this was occurring. The stimulation site corresponded to a point just anterior to the level of the caput fibulae. The pulse width was 1 ms. Initially, the motor threshold (MT) was determined as that intensity where an M-wave became visible in the EMG signal. During the BCI$_{FES}$ intervention, the frequency was set to 20 Hz, the intensity was adjusted to produce a dorsiflexion of the ankle joint corresponding to approximately 30 degrees and the duration of the stimulus train was one second. For the BCI$_{passive}$ intervention, a custom-made robotic actuator performed a passive ankle movement with parameters set to induce the ankle trajectory during a normal gait cycle (Figure 7C).

5.5.   Experimental procedures

Initially, the intensity for the magnetic stimulation was set at approximately 50% of the stimulator output (SO) to find the optimal site for evoking a MEP in the TA. The best spot for stimulation (also termed the hot-spot) was defined as the coordinate where the peak-to-peak amplitudes of the MEPs were greater in the target muscle than the amplitudes of adjacent coordinates for a given stimulus intensity. For all participants, this site was approximately 2-3 cm anterior to the vertex and a stimulation applied to this area also evoked a response in the SOL. Once the hot-spot was identified, it was marked using Brainsight™ (Rogue Research inc.) to ensure that the coil position was maintained so that the stimulation was always applied over the same area of M1.

Subsequently, the resting motor threshold (RMT), defined as the highest stimulus intensity that produced no more than five of ten consecutive TA MEPs with a peak to peak amplitude of ~50 μV while the muscle was at rest, was identified. Next, 12 MEPs were elicited in the resting TA at each of six TMS intensities; 90, 100, 110, 120, 130, and 140% of RMT (72 MEPs in total). The TMS stimuli

were delivered every 5-7 s in a randomized order. The mean peak-to-peak TA MEP amplitudes were extracted pre, post, and 30 minutes following the cessation of the intervention.

5.6.   The interventions: BCI$_{FES}$ vs BCI$_{passive}$

All participants attended two intervention sessions spaced at least 48 hours apart. These consisted of either FES (BCI$_{FES}$ intervention) or a passive dorsiflexion movement (BCI$_{passive}$ intervention) being imposed and timed so that the artificially generated afferent flow arrived at the PN of the MRCP as outlined in our previous publication (Mrachacz-Kersting et al. 2012). 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). All participants were asked to imagine a dorsiflexion movement 30 times according to the cue in Figure 5D. An example from a single participant during the BCI$_{FES}$ intervention is shown in Figure 6. Figure 6A shows an example of the MRCP generated during a voluntary dorsiflexion (obtained during the MRCP acquisition as described under section 5.2), the associated EMG activity of the TA is shown in Figure 6B, and the EMG during the BCI$_{FES}$ activated dorsiflexion movement (i.e. during the intervention) in Figure 6C. The vertical line indicates the PN of the MRCP. An example of the same participant during the BCI$_{passive}$ intervention is shown in Figure 7. Figure 7A shows an example of the MRCP generated during a voluntary dorsiflexion (obtained during the MRCP acquisition as described under section 5.2), Figure 7B the EMG activity during the BCI$_{passive}$ intervention, and Figure 7C the ankle angle during the BCI$_{passive}$ intervention. The vertical line indicates the PN of the MRCP.

It has to be noted that the experimental procedure in this study did not include the online detection of MRCP. Therefore, the interventions were not BCI sessions but rather cue-based imagination or execution and triggering of peripheral stimuli. However, the trigger was timed based on a preliminary assessment of the MRCP timing with respect to the cue. This choice was preferred over the online detection of MRCPs since the focus of the study was to study the effect of the type of afferent feedback elicited and variability in detection accuracy of MRCPs among subjects and among conditions would have not made it possible to compare the interventions. Despite the interventions are not based on BCI decoding, we have still denoted them as BCI$_{FES}$ and

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BCI$_{passive}$ since their comparison according to the experimental procedure proposed in this study has a direct implication for the design of an associative BCI based on the same principles but with online detection.

5.7.　Control experiment: Stretch reflex recording

It is not possible to differentiate if alterations in the MEP are due to changes in spinal or cortical circuitry based on the MEP alone. For this purpose, TA stretch reflexes were elicited prior to and following the BCI$_{FES}$ and BCI$_{passive}$ interventions in eight participants (five males, three females; age: 26.3 ± 3.1 years). The right leg was affixed to an electrically controlled custom made actuator such that the anatomical ankle axis of rotation was aligned with the fulcrum of the actuator. The foot segment of the right leg of the participant was firmly strapped to a custom-made plate that extended from the actuator, thus producing a tight interface between the arm of the motor and the foot of the participant, ensuring that the movement of the actuator was transmitted solely to the ankle joint. The angular position of the actuator was monitored by an angular displacement transducer (Transtek DC ADT series 600). The participants were asked to produce three maximum voluntary contractions (MVC) of the TA, separated by three minutes of rest. The greatest of the three MVC forces was used as the reference MVC. The root mean square value of the rectified TA EMG for the MVC over a 1s period was calculated. Subsequently, the participants were provided with visual feedback via a computer screen displaying horizontal markings set at 5% MVC and a vertical bar displaying the participant's current level of TA activation. Participants were asked to maintain the bar between the horizontal markings while the perturbations were applied without interfering with the imposed plantarflexion perturbations.

Thirty stretches were randomly applied at intervals ranging from 5 to 7 s (velocity: 200°s^{-1} to 300°s^{-1}; amplitude: 4° to 6°; hold-time: 200 ms). The angular velocity and the amplitude of the imposed perturbations were adjusted for each participant prior to the intervention so that the amplitude of the three response peaks observed in the TA EMG trace were approximately the same and also similar to the amplitude of the TA MEP prior to the intervention. The latency of the first response peak (termed M1 or alternatively SLR in the literature) was extracted from the data both prior to and immediately following the intervention. The root mean square (RMS) value of a

window extending 10 ms on either side of the SLR was calculated and used as an indication of the size of this component of the TA stretch reflex.

5.8.   Statistical analysis

A Student's paired *t*-test was used to establish the reliability of the PN of the MRCP and changes in the size of the SLR component of the TA stretch reflex for both intervention days. A two-way repeated analysis of variance (rmANOVA) was conducted on the pre-intervention measures with the factors intervention (BCI_{FES} and $BCI_{passive}$) and TMS stimulation intensity (90, 100, 110, 120, 130 and 140% RMT). The effectiveness of the two interventions in inducing alterations of the corticospinal tract excitability was tested using a three-way rmANOVA with the factors time (pre, post and 30 minutes post intervention), intervention (BCI_{FES} and $BCI_{passive}$) and TMS stimulation intensity (90, 100, 110, 120, 130 and 140% RMT). Greenhouse-Geisser corrections were used in the case of sphericity being violated. The significance level was set to $p < 0.05$.

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Figure Legend

Figure 1: The mean PN time (and SE) for each participant for the two training part of the BCI$_{FES}$ and BCI$_{passive}$ interventions.

Figure 2: Single trial MRCPs and the average MRCP for one participant for the BCI$_{FES}$ **(A)** and BCI$_{passive}$ **(B)** interventions days respectively.

Figure 3: Changes in motor output following the BCI$_{FES}$ and BCI$_{passive}$ interventions. (A) and **(B)** The average of 12 TA MEP traces for 90-140% RMT prior to, following, and 30 minutes after the BCI$_{FES}$ and BCI$_{passive}$ interventions for one participant. **(C)** and **(D)** Mean TA MEP amplitudes for 110-140% RMT across all participants immediately following and 30 minutes after both interventions. Data are expressed as a percentage of pre-intervention values (black dashed line). Black bars represent the BCI$_{FES}$ intervention and the white bars represent the BCI$_{passive}$ intervention. Error bars represent SEM.

Figure 4: Stretch reflex data. **(A)** and **(B)** Right ankle angle (°) for the BCI$_{FES}$ and BCI$_{passive}$ interventions, respectively. The vertical dashed line indicates the onset of the imposed plantarflexion perturbation. **(C)** and **(D)** TA rectified EMG trace prior to (thin line) and following (thick line) the BCI$_{FES}$ and BCI$_{passive}$ interventions. Each trace is the mean of 30 trials. Data are for n = 1. **(E)** The mean SLR amplitude across all participants prior to and following the BCI$_{FES}$ and BCI$_{passive}$ interventions. Error bars represent standard deviations.

Figure 5: Schematic of the experimental set-up. (A) Pre-intervention quantification of the excitability of the cortical projections to the target muscle tibialis anterior (TA) using non-invasive transcranial magnetic stimulation (TMS). Participants were seated with the TA relaxed while 72 stimuli at six different intensities were applied. **(B)** Schematic of the BCI$_{FES}$ and BCI$_{passive}$ interventions. Participants watched a screen placed 2 m in front of them on which a cue provided information on when to imagine the dorsiflexion movement. Relevant brain activity was measured, detected and converted into an output command for an electrical stimulator or a robotic actuator. 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 electroencephalographic (EEG) signal. Thirty such pairs were performed. **(C)** Immediately post-intervention and 30 minutes later, measures as for A. **(D)** The visual display shown to the
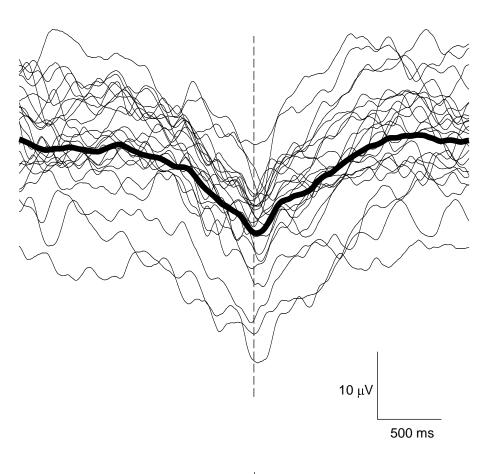
participants during the intervention. FOCUS appeared on the screen initially followed by the schematic of a step-function. Participants were required to start the imagined movement once the moving cursor (triangle) reached the upward slope. The word REST appeared last on the screen.

Figure 6: The MRCP and associated TA EMG activity recorded during the dorsiflexion movement and for the BCI$_{FES}$ intervention. (A) The mean MRCP trace for one participant following 30 dorsiflexion movement. **(B)** The associated TA EMG activity and **(C)** the TA EMG trace during the application of FES during the BCI$_{FES}$ intervention. The vertical dashed line indicates the time of PN of the MRCP.

Figure 7: The MRCP and associated TA EMG activity recorded during the dorsiflexion movement and for the BCI$_{passive}$ intervention. (A) The mean MRCP trace for one participant (the same participant as for Figure 2) following 30 dorsiflexion movement. **(B)** The associated TA EMG activity and **(C)** the TA EMG trace during the application of the passive movement during the BCI$_{passive}$ intervention. The vertical dashed line indicates the time of PN of the MRCP.

Figure 1

Figure 2

Figure 2

10 μV

500 ms

Figure 3

Figure 4

Figure 5

A Pre-measures

TMS

Pre – TA MEP

B Intervention

EEG → MRCP

Visual Display

1. Functional Electrical Stimulation

2. Passive Ankle Movement

C Post-measures

TMS

Post – TA MEP

D Visual display during intervention

FOCUS

2 s   2 s

REST

Figure 6

A

4 µV

500 ms

20 µV

500 ms

B TA EMG activity
during active dorsiflexion

200 µV

500 ms

C TA EMG activity during
the BCI$_{FES}$ intervention

Figure 7

Figure 7

A

4 μV

500 ms

B

TA EMG activity during the
BCI$_{passive}$ intervention

10 μV

500 ms

C

Ankle Angle during the
BCI$_{passive}$ intervention

5°

500 ms