Towards a neurorehabilitation system combining neural interfaces, peripheral stimulation, and sleep

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Table of Contents

Abstract

Stroke is the leading cause of motor impairment worldwide. In the last decade, brain-machine interfaces (BMIs) have emerged as a promising tool for stroke rehabilitation, but the rehabilitative outcome is still moderate. This thesis capitalizes on the neuroscience behind sensorimotor integration and memory formation that occurs during stroke rehabilitation to design a novel BMI-based rehabilitation intervention that includes peripheral electrical stimulation and a sleep interface. The first study investigates the challenges and biases interpreting EEG during a visuomotor task, revealing confounding factors that might bias EEG analyses of a reaching task, a critical part of a BMI-based stroke rehabilitation. The second study builds upon the neuroscientific knowledge on memory consolidation and uses state-of-the-art techniques, like targeted memory reactivation (TMR), to manipulate memory consolidation after neuroprosthetic learning, the type of learning that takes place during a BMI training. In line with previous animal studies, increased spindle and slow wave activity was found over the brain area linked to the BMI control in the intervention night, showing that, after TMR, the occurrence of such neural correlates was more lateralized towards that brain area. The third and final study integrates peripheral electrical stimulation (PES) in a BMI system and explores how this stimulation, when properly synchronized with supraspinal activity, can excite the sensorimotor system, potentially leading to a better rehabilitative outcome. The findings of this study clearly show a higher excitatory state of the sensorimotor system, but no behavioural changes (in a motor learning task) could be found. Finally, both studies ended with a test on stroke population. Study 2 studied how sleep changes after a BMI-based rehabilitation intervention and looked for the same patterns of memory consolidation that could be found in healthy participants. Study 3, on the other hand, implemented and tested in a two-month intervention a portable home-based BMI system for rehabilitation that delivered closed-loop PES to increase excitability of the sensorimotor system. Altogether, this thesis sets the basis towards a neurorehabilitation system combining neural interfaces, peripheral stimulation, and sleep.

1 Introduction

1.1 Stroke

Stroke is a medical emergency that occurs when the blood supply to a part of the brain is interrupted or reduced. This can be caused by the rupture of a blood vessel (hemorrhagic stroke) or by a blocked artery (ischemic stroke). Its consequences vary widely depending on factors like lesion location, extent of the brain damage or promptness of the medical intervention. Thus, some stroke survivors may experience mild and temporary impairments, while others may encounter more severe and long-lasting effects. Stroke is, indeed, one of the main causes of long-term motor disability, leading to functional deficits in motor control in more than 85% of the cases (Langhorne et al., 2011).

The consequences of a stroke can be devastating, affecting not only the individuals who experience it but also their loved ones and caregivers. Following a stroke, survivors may encounter physical and cognitive difficulties, including speech and language comprehension issues, memory problems and paralysis. These changes result in important changes in the lives of both patients and their families since patients often require daily life support. Additionally, post-stroke treatments represent a financial burden both for the families, as well as the healthcare system (Kolominsky-Rabas et al., 2006; Lee et al., 2010). Thus, patient autonomy is a crucial factor that needs to be addressed to improve the quality of life of the patients and their families.

Strategies like compensating or substituting the lost motor function, by adjusting the remaining motor abilities or using technical aids like canes or walkers, can assist stroke patients to become more independent. Alternatively, rehabilitation therapies focus on restoring the motor function. In this context, physical and behavioral therapy is the overall accepted method for motor rehabilitation after stroke. However, the efficacy of motor rehabilitation is limited when it comes to severely paralyzed and patients in a chronic stage (Byblow et al., 2015; Winters et al., 2015). In the last decade, a novel rehabilitation approach based on the use of brain-machine interfaces (BMIs) has emerged as an effective tool for motor stroke rehabilitation (Ramos-Murguialday et al., 2013).

1.2 Brain machine interfaces

A brain-machine interface is a system that translates neural signals into commands that are used to control an external device (e.g., cursor movement, exoskeleton or wheelchair). Brain activity can be recorded using a plethora of techniques that measure electrical or magnetic fields (i.e., functional MRI, PET or functional near-infrared imaging), but the most commonly used method is electroencephalography (EEG). EEG records the electrical fields at the scalp that result from brain activity. This technique is commonly used due to its relatively low cost, short setup time and non-invasiveness. However, this technique presents two main limitations. First, it is more susceptible to external electrical contamination, leading to lower signal to noise ratio (Buzsáki et al., 2012). Thus, electrooculographic and electromyographic activity are prone to pollute the EEG recordings, as well as impedance shifts resulting from sensor movements (Kline et al., 2015; Lebedev & Nicolelis, 2017; Snyder et al., 2015). Additionally, EEG recordings must deal with low spatial resolution a limited frequential range.

The detection of motor intention from EEG signals has been successfully achieved using two brain movement-related brain signatures: event related desynchronizations (ERD) and motor related cortical potentials (MRCP). ERDs, also referred as sensorimotor rhythms, reflects the decrease in power resulting from the desynchronization of neural activity between 8-30 Hz that occur over the sensorimotor cortices when a movement is performed, planned, or imagined (Pfurtscheller & Lopes da Silva, 1999). MRCPs, on the other hand, represent slow (0.1-1 Hz) changes in amplitude occurring up to 1.5 seconds before execution or imagination voluntary of movements (Shibasaki & Hallett, 2006). Both, and their combination, have been extensively used to discriminate between rest and movement states, as well as motor imagery both for healthy (Ibáñez et al., 2015; Niazi et al., 2011) and paralyzed individuals (Antelis et al., 2013; E. Lew et al., 2012;

López-larraz et al., 2014). Interestingly, some invasive studies have shown that information related to arm movement directions can be identified from low frequency components (<4 Hz) (Ball et al., 2009; Waldert et al., 2008). Indeed, in the last years, several studies have shown promising results on the decoding of different movements from the EEG (Iturrate et al., 2016; Ofner et al., 2017; Pereira et al., 2017; Shiman et al., 2015). These findings could help design the next generation of BMIs for rehabilitation since a precise and accurate decoding of both MRCP and/or ERDs plays a pivotal role on such technologies.

BMIs for rehab

The key idea behind BMI-based rehabilitation approaches is that consistently linking brain activity with the afferent feedback might induce, and guide, activity-dependent brain plasticity that ultimately leads to a restoration of lost motor functions. Thus, Ramos and colleagues showed, in a study involving 32 severely paralyzed stroke patients, that BMI training led to significantly larger improvements in upper limb motor function, when compared to a control group where the feedback, hand orthosis movement, was not linked to the brain activity (Ramos-Murguialday et al., 2013). These results elegantly demonstrate that it is the contingency between brain activity and feedback (the central point of the BMIs) and not the feedback itself, what induces, and guides, activity-dependent plasticity to restore motor function. Since then, several studies have replicated those results but also explored the use of other feedback modalities.

Other studies that rely on proprioception by the means of moving the paretic limb either using an orthoses, hand knobs or exoskeletons (Ang et al., 2014, 2015; Frolov et al., 2017; Hu et al., 2020; Ono et al., 2014). Alternatively, several groups have focused on visual, achieving remarkable results (Mottaz et al., 2018a; Pichiorri et al., 2015; Rayegani et al., 2014). Lastly, the use of peripheral electrical stimulation (PES), electrical impulses stimulating specific muscles or nerves, to close the loop with the brain activation of the patients has also achieved promising results (Biasiucci et al., 2018; T. Kim et al., 2016; Mrachacz-Kersting et al., 2016; Ono et al., 2014). Remarkably, as revealed by the meta-analysis of Nojima and colleagues, the studies using visual feedback, and especially the ones relying on PES, tended to show higher clinical improvements when compared with the group of proprioception studies (Nojima et al., 2022).

However, the effect of these studies on motor recovery is still moderate. Several reasons might be hampering the effectiveness of BMI-based rehabilitation. Firstly, the attention span of this type of patients, together with a relatively long set up time of these BMIs result in a short training time. Indeed, in the study of Frolov and colleagues, most of the patients reported fatigue after 20-30 minutes of training. Another limitation comes from the characteristics of the EEG signals (low SNR, low spatial resolution), as they only enable to reliably extract whether the subject is attempting to initiate movement or not during the training. This is a critical step since it limits the contingency between the brain activity and the feedback, a crucial element in BMIs and BMI-based rehabilitation. Thus, a more precise decoding of the EEG signals that could, for example, extract information about the type of movement being performed, would allow for more specific feedback/afference, potentially improving the rehabilitative outcome. Thus, the first study of this thesis will focus on the extraction of motor-related neural patterns to discriminate among reaching directions.

Peripheral electric stimulation

Focusing on the optimization of the afferent feedback during a BMI-based rehabilitation, PES holds a great potential since it can be tailored to deliver contingent feedback that both closes the loop and excites sensorimotor pathways, thus enhancing the activity-dependent plasticity that is behind the efficacy of rehabilitative BMIs (Sur & Rubenstein, 2005). During PES, the electrical currents trigger a sensory response by depolarizing sensory axons, directly influencing the sensorimotor cortex, and enhancing cortical excitability (Bergquist et al., 2011; Burke et al., 1983; Insausti-Delgado et al., 2020). This approach is already being explored by several groups with promising results.

Biasiucci and colleagues showed that a BMI coupled with functional electrical stimulation (FES), a type of PES that is focused on elicit muscle contraction to provide function, leading to significant and lasting motor recovery in stroke patients (Biasiucci et al., 2018). Interestingly, this recovery remained 6-12 months after the therapy, adding evidence to the stability of such recovery that Ramos-Murguialday and colleagues confirmed in a follow-up measurement 6 months after the intervention (Ramos-Murguialday et al., 2019). Authors also found a correlation between connectivity of motor areas of the affected hemisphere and recovery, suggesting that functional recovery is driven by the plasticity produced by the contingency between efference and afference. However, this approach can be further improved by understanding the interaction between the afference generated by the electrical stimulation and the sensorimotor integration produced during the rehabilitation therapy. Although these studies showed a closed-loop PES where stimulation is delivered only during the motor attempt of the patients, some studies have recently explored the relationship between the phase of certain ongoing sensorimotor rhythms and higher excitatory states (Van Elswijk et al., 2010; Zrenner et al., 2018a). These closed-loop approach holds a great potential for BMIs for stroke rehabilitation since a stimulation delivered in higher excitatory states might facilitate that the impaired sensorimotor system to create new connections, a crucial factor in the rehabilitative BMIs.

1.3 Sensorimotor integration and sensory gating

During a movement action, the central nervous system (CNS) integrates different sources of stimuli to, simultaneously, generate motor commands (Machado et al., 2010). Thus, a correct voluntary movement execution depends on the integration of the relevant sensory inputs. In fact, every body movement stimulates peripheral receptors, activating neurons in the spinal cord and brain via afferent feedback. This flow of afferent feedback during movement is not constant but modulated by top-down mechanisms. This effect, known as sensory gating, where afferent information is intermittently reduced has been extensively studied in voluntary movements (Angel & Malenka', 1982; Bays et al., 2006; Blakemore et al., 1998). This afference modulation has also been also found in the modulation of cortical Somatosensory-evoked potentials (SEPs): attenuation of the evoked responses has been found during several phases of self-initiated, but not passive, movements (Andrew et al., 2015; Dancey et al., 2016; Haavik & Murphy, 2013; Macerollo et al., 2018). This top-down modulation is of special interest for a rehabilitative BMI since an afference timed with a higher excitatory state of the sensorimotor system is more likely to drive activity-dependent plasticity. The modulation of the excitability of the sensorimotor cortex and how to exploit it to improve BMIs for rehabilitation will be investigated in Study 3 of this thesis.

1.4 Neuroprosthetic learning and sleep

In the context of motor rehabilitation after stroke, where the impaired sensorimotor system might not be capable of fully encoding and consolidating the newly learned information, understanding how memories are being formed (and consolidated) is of paramount importance to further improve the outcome of any rehabilitative intervention. It is known that memories are formed in a labile state, susceptible to interferences and forgetting, and need to go through a consolidation process. Sleep plays a major role in memory consolidation (Diekelmann & Born, 2010). There is an extensive body of research showing the behavioral benefits of sleep on declarative and procedural memories. More specifically, the consolidation effect of sleep has been shown in several motor learning, a type of procedural learning similar to the one that occurs during a BMI task (Fischer et al., 2005; Korman et al., 2007; Walker et al., 2003).

Neuroprosthetic learning

The process of learning to control a neuroprosthetic device with a BMI can be considered as a specific type of instrumental learning (Carmena, 2013; Green & Kalaska, 2011) in which a subject learns to control the underlying brain activity by feedback and reward, reinforcement and error-based learning, based on the achievement of task-relevant goals (Wander et al., 2013). During training of a neuroprosthetic skill, the central nervous system modifies the connections of the neuronal populations participating in the control of the neural interface (e.g., the neuronal ensembles that produce an EEG signal that is translated into robotic movement) and minimizes error of the output (e.g., by visuo-motor and proprioceptive feedback) through plasticity in the cortex and in the striatum, which is associated with reward behavior (Ganguly et al., 2011; Koralek et al., 2012). The direct control of feedback based on activity of neural populations makes neural interfaces an exquisite neuroscientific tool to study learning at the level of brain activity, as represents a causal link between brain activity and behavior. Considering BMI control as a form of sensorimotor learning helps us to study how to optimize BMI systems using the extensive body of research on sensorimotor learning as a framework (Krakauer & Mazzoni, 2011). Conversely, BMI learning also allows us to investigate processes happening during motor learning, such as changes in directional tuning of neurons with learning (Ganguly et al., 2011). Improving control of neural interfaces is critical, since it has been shown that only a subset of the population is able to learn to control a brain-machine interface and that neural activity is easily contaminated by other physiological and non-physiological signals (Bibián et al., 2021; Vidaurre & Blankertz, 2010). Hence, a better understanding on how these neuroprosthetic memories are created and consolidated would lead to very promising applications, especially in the population of patients that could potentially benefit from BMIs as assistive or rehabilitative platforms.

Sleep and memory consolidation

Sleep plays a paramount role in the retention of memories, or memory consolidation, through the reactivation of prior experiences (Diekelmann & Born, 2010; Mander et al., 2011; Schönauer et al., 2015). More specifically, Slow-wave sleep has been associated with a replay of behaviors learned during the day (Rasch and Born, 2007). This process is understood to be responsible for the reinforcement of new neuronal connections made during the day, thereby leading to improvements in task performance. Several brain correlates of sleep have been identified to play a role in this memory consolidation: slow-wave and spindle activity in sleep increases after motor learning, specifically over motor areas (Huber et al., 2004; Latchoumane et al., 2017; Vahdat et al., 2017), and the increasing slow-wave activity strengthens memory consolidation (Marshall et al., 2006; Ngo et al., 2013). These processes have been largely studied for both declarative and procedural memories (Korman et al., 2007; Vahdat et al., 2017), but the mechanisms behind the consolidation of neuroprosthetic memories are now starting to be unveiled.

Recent work has demonstrated in rodents that successful neuroprosthetic learning (direct neural control of an external actuator) is linked to enhanced phase-locking and emergence of coherent task-related activity during post-learning slow-wave sleep (SWS) (Gulati et al., 2014; J. Kim et al., 2019). In contrast, task-unrelated activity either remained unchanged or experienced a reduction in phase-locking and coherent activation. Other studies have also evaluated activity during SWS after learning to control an invasive BMI in humans (Johnson et al., 2012). They showed a local increase in sleep spindle occurrence and during SWS after BMI training, with higher spindle rate located close to the electrodes used for training. In contrast, control subjects did not present such local modulation of spindle activity. These results are in line with the findings observed in other motor learning paradigms and give additional support for viewing sensorimotor rhythm-based BMI control (and BMI-based motor rehabilitation) and its effects on sleep as a surrogate of motor learning and consolidation (Landsness et al., 2009; Nishida & Walker, 2007).

Targeted memory reactivation

Manipulating memory consolidation has been proven to be possible through the reactivation of the previously learned memories during sleep (Schouten et al., 2017). This technique, called targeted memory reactivation (TMR), can be achieved through stimulation during sleep with odor cues (Rasch & Born, 2007) or auditory stimuli that have been associated with the learning material before sleep (Antony et al., 2012; Antony & Paller, 2017; Schönauer et al., 2013). However, most of these studies have been done on healthy young individuals, and little is known about the older population or stroke patients. Thus, a better understanding of these new memories being formed could allow us to exploit the potential of targeted memory reactivation to help the impaired sensorimotor system consolidate the newly acquired rehabilitative memories.

1.5 Objectives and hypothesis

A decade after the first BMI for stroke rehabilitation was presented, the clinical outcome of such intervention remains modest. This thesis aims at redefining some aspects of a BMI-based rehabilitation that could have a major impact on the effectiveness of such interventions.

The first study, published in Bibian et al. 21, was conducted to investigate the challenges that arise in the analysis and interpretation of EEG data from a visuomotor task, similar to what is used in BMI-based rehabilitative interventions. The main hypothesis of this study is that, when eye and head movements concurrently occur with the motor task of study (i.e., reaching movements), the EEG recordings are contaminated with external activity that might bias the interpretation of the data.

The second study explores how learning occurs during a BMI task, how this learning is consolidated throughout the night and explores state-of-the-art techniques, such as TMR, to manipulate memory consolidation. The hypothesis is that after sensorimotor-rhythm BMI training, sleep can be manipulated to enhance the consolidation of this learning. Additionally, we hypothesize that PES is an effective stimuli modality to apply targeted memory reactivation.

The third study of this thesis investigates the top-down modulation of the excitability of the sensorimotor system. Additionally, it develops and tests, both in healthy and stroke participants, a closed-loop system to further excite the sensorimotor system during a BMI task, potentially improving the behavioral outcome. We hypothesize that if PES is synchronized with supraspinal activity from sensorimotor cortex, targeting higher states of excitability, the plasticity effect of BMI intervention can be fully exploited (Zanos et al., 2018).

2 Studies

2.1 Study 1: Extraction of neural correlates of upper limb visuomotor task

2.1.1 Introduction

In this study, we explore how "natural" head and eye movements that naturally occur during a visuomotor task affect the recorded EEG signals and if and how they influence the analysis and interpretation of EEG data. The primary objective is to evaluate the impact of concurrent eye and head movements on EEG during reaching movements conducted under constrained versus unconstrained conditions without specific instructions. We comprehensively analyzed EEG, electro-oculographic (EOG) signals, and accelerometer data to discern the contributions of head and eye movements to EEG recordings. Additionally, we employ a multivariate pattern classification (MVPC) approach to gain insights into the nature of the recorded EEG signals.

2.1.2 Materials & Methods

2.1.2.1 Experimental design

Ten healthy participants (four females, mean age: 29.3 ± 6.3 years) took part in a cross-over study where they engaged in reaching tasks under two conditions: 1) deliberately avoiding head and eye movements during the task, and 2) performing the task naturally, allowing their gaze and head to follow their arm movements in a natural manner. All the procedures were approved by the Ethics Committee of the Faculty of Medicine of the University of Tübingen, Germany.

The setup aimed to assess the impact of head and eye movements on EEG-based movement decoding for the same limb in a realistic BMI-based rehabilitative scenario. Subjects were comfortably seated with their right arm wearing a rehabilitative exoskeleton (ISMORE exoskeleton, Tecnalia) (Figure 1a). In the present study, however, we used a passive version of the exoskeleton to reduce its weight as the subjects are meant to actively move their arms. Thus, in addition to recreating a rehabilitative scenario, this version of the exoskeleton was used as a tracking device for the reaching movements. More details on the exoskeleton can be found in (Bibián et al., 2021).

The subjects performed reaching movements under two different conditions: a constrained approach in which the subjects were explicitly instructed to avoid head and eye movements; and an unconstrained approach in which the subjects' head and gaze could follow their arm movements freely, as a patient would do during a rehabilitative intervention. This way, we aimed at testing if and how head and eye movements influence the EEG-based decoding of movements from the same limb. All the subjects performed the two conditions in two separate sessions following a cross-over design (half of the subjects performed the constrained approach first, and the other half performed the unconstrained approach first).

During these sessions, participants were asked to perform reaching movements to four different targets and returning to the starting position. Each experimental session comprised five runs, each consisting of 40 trials arranged in a randomized sequence, with 10 trials for each target. The average trial duration was approximately 7.5 seconds and included a rest period (2–3 seconds), a readiness period (2 seconds), and a movement period (3 seconds), all signaled by auditory cues (see Figure 1b).

2.1.2.2 Data Acquisition and Preprocessing

We recorded brain activity using a 64-electrode EEG cap and collected EOG data with four passive electrodes (see montage in Figure 1b). Impedance for both EEG and EOG signals was maintained below 1kohm. Additionally, an accelerometer sensor on the head monitored head movements. After synchronization, all data was downsampled to 100 Hz (after antialiasing filtering below 45 Hz) and a common average reference (CAR) spatial filter was applied to the sensorimotor cortex electrodes (highlighted in yellow in Figure 1b). Movement onset used in later analyses was determined based on the kinematic data from the exoskeleton. Thus, a movement onset was defined as the time point in which the exoskeleton position crossed the threshold =5% of its maximum distance moved during each trial (Niazi et al., 2011). After that, the signal from all the sensors was segmented into 8-second trials from -5 to 3 seconds, with t=0 indicating movement onset.

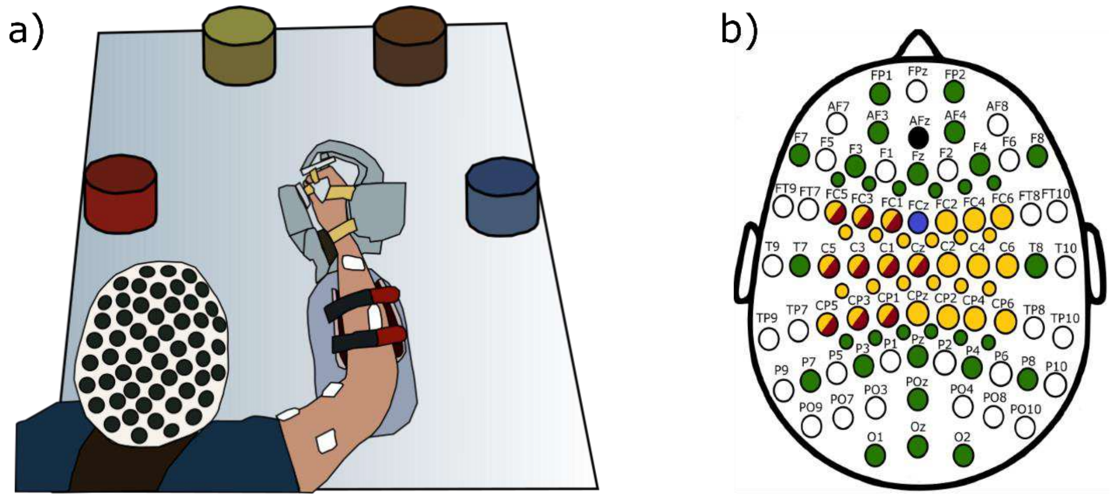

Figure 1. (a) Experimental setup. Subject performing a reaching movement toward a target while wearing the exoskeleton. (b) Trial structure. (c) EEG setup. Electrodes included in the recording displayed in green. Yellow color indicates the electrodes used for the CAR filter. The electrodes colored as red, further referred to as contralateral motor cortex electrodes, were used for the subsequent feature extraction and classification processes. (d) Positioning of the EOG electrodes. Figure adapted Bibian et al. 21.

2.1.2.3 Topography of the EEG Activation

First, we wanted to examine how reaching directions were represented in the topography of the EEG recordings. To do that, we generated topographical plots or "topoplots" of EEG activation for each electrode. The raw EEG signals were filtered within the movement-related cortical potential (MRCP) frequency band (0.05–2 Hz). After that, we created individual topoplots for each 0.5 seconds calculating the amplitude of each electrode in 0.5-seconds intervals, after baseline correction (subtracting the mean amplitude value between -5 and -4 seconds). We did that separately for each reaching direction (see Figure 2).

2.1.2.4 Disentangling Brain' External and Internal Activities from EEG

For this analysis, we selected four categories of features that are characteristic from movement-related neural activity, eye movements and head motion. The first two features are the most used to describe the neural activity during a motor task: MRCP (Shibasaki & Hallett, 2006) and ERD (Pfurtscheller & Lopes da Silva, 1999). Secondly, to assess the influence of eye movement artifacts on EEG recordings, we extracted low-frequency features from FP1 and FP2 electrodes, which are typically most affected by ocular activity. Lastly, for characterizing head movements during the task, we extracted features from the accelerometer signal.

1) MRCP Features: We processed epochs from the 10 electrodes over the contralateral motor cortex (highlighted in red in Figure 1b) by applying a causal first-order Butterworth band-pass filter in the [0.05 to 2] Hz, following (Bibian et al., 2017). After that, the filtered signal was down sampled to 10 Hz to extract temporal, resulting in 10 features per electrode and thus 100 features per epoch.
2) ERD Features: Power spectral density was computed for epochs extracted from the 10 electrodes over the contralateral motor cortex (also marked in red in Figure 1b). These epochs were windowed using a Hamming function, followed by power spectrum estimation using a 16th-order autoregressive model solved with the Burg's algorithm. Subsequently, we calculated the mean logarithmic power within the alpha ([7–13] Hz) and beta ([14–25] Hz) bands, resulting in 2 features per electrode and 20 features per epoch.
3) FP1 and FP2 Features: Temporal features were extracted from FP1 and FP2 electrodes using the same procedure as for the MRCP features, yielding 20 features per epoch (10 features per electrode, covering two electrodes).
4) Accelerometer Features: After applying a first-order Butterworth filter to the accelerometer signal within the [0.1 to 1] Hz range, we extracted the orientation of the accelerometer on the horizontal plane and computed the signal magnitude vector from its three components (Zhang et al., 2012).

To better distinguish the variations in EEG activity between the two conditions, we applied a MVPC technique utilizing a linear Support Vector Machine (SVM) (Mika et al., 1999). Our approach involved two distinct phases of EEG analysis for both conditions: 1) The discriminability between EEG from movement and rest periods, in a binary classification task. 2) The discriminability among the EEG activity of four reaching directions, in a multiclass classification approach. To assess the performance, we employed a block-based 5-fold cross-validation, training the SVM in four blocks and testing in the remaining block. In the training data, we used two 1-second epochs for training the binary SVM classifier: [−5, −4] for rest and [0, 1] for movement. For training the multiclass SVM classifier, only the movement epochs, [0, 1] seconds, were considered. To evaluate the SVM in the test block, we used sliding windows with a sliding step of 50 miliseconds (López-Larraz, Figueiredo, et al., 2018).Thus, each 1-second epoch was classified by both the binary and multiclass classifiers, yielding two classification outputs every 50 milliseconds from -4 to 3 seconds (indicating rest/movement for the binary classifier and one of the four movement directions for the multiclass classifier). These SVM classifiers were trained and tested independently for each of the features groups (explained before) explained before. After extracting the features, and right before training/testing the SVM, we applied z-score normalization resulting in distributions with a mean of zero and unit variance.

Finally, to assess the performance obtained using each group of features, for the binary classification we calculated the percentage of outputs correctly identified as movement during the movement task period, we refer to this metric as "performance" (López-Larraz et al., 2017).In the case of the multiclass classifier, we evaluated its accuracy using decoding accuracy (DA), indicating the number of correctly identified target directions (E. Y. L. Lew et al., 2014) .

2.1.2.5 Impact of the Ocular and Movement Artifacts on the DA

Finally, we wanted to assess the impact of eye and head movements on the performance by using artifact reduction techniques and applying the MVPC methods described before on the cleaned data. It is important to note that this analysis does not aim at completely removing the effect of eye and head movements on the performance but to rather evaluate how the discriminability changes when the most contaminated trials are removed from the analysis. To do so, we incorporated an artifact removal pipeline into the training dataset processing (López-Larraz, Figueiredo, et al., 2018). This pipeline consists of two main steps:

1) Ocular Artifacts Removal: To reduce the influence of eye movements on EEG signals, we employed a linear regression-based method that decorrelates EEG and EOG recordings (Schlögl et al., 2007). This algorithm was applied to all EEG channels before feature extraction in the training data and later in the test data using coefficients obtained from the training data.

2) Motion and Muscular Artifacts Rejection: To quantify to what extent the trials were contaminated with motion or muscular artifacts, we computed power in the delta ([0.1–4] Hz) and gamma ([30–45] Hz) frequency bands, known to be susceptible to motion and muscular artifacts (Castermans et al., 2014; Muthukumaraswamy, 2013). A trial was rejected if delta or gamma power exceeded three standard deviations above the mean in any of the classes contained in the trial (e.g., rest and one of the reaching directions). Additional details on the application of this method can be found in (López-Larraz, Figueiredo, et al., 2018).

2.1.2.6 Statistical Analysis

The primary outcome measures for the previously described analysis were the performance and the DA, for the binary and multiclass analysis respectively. For the statistical analysis, we focused on the early movement epochs so we calculated the average performance and DA between [0.5–1.5] seconds obtaining one value per metric (2), condition (2) and feature (4). Thus, to evaluate whether any of the conditions and features had a significant effect on the metrics, we calculated a two-way repeated measures ANOVA with the factors “feature” (four levels: MRCP, ERD, FP1 and FP2, and accelerometer) and “condition” (two levels: constrained and unconstrained) separately for the binary and multiclass metrics. We calculated the Mauchly’s test of Sphericity was computed and applied the Greenhouse–Geisser correction when assumption of sphericity was violated. After that, one-way repeated measures ANOVAs were calculated for each factor if significant interactions were detected. If a significant effect was found for any factor, we compared the different levels of that factor by calculating a paired t-test with Bonferroni correction was calculated. Finally, to evaluate the effect of the artifact removal methods we computed a three-way repeated measures ANOVA with the factors “feature” (four levels: MRCP, ERD, FP1 and FP2, and accelerometer), “condition” (two levels: constrained and unconstrained) and “artifact removal” (two levels: with and without applying the artifact removal).

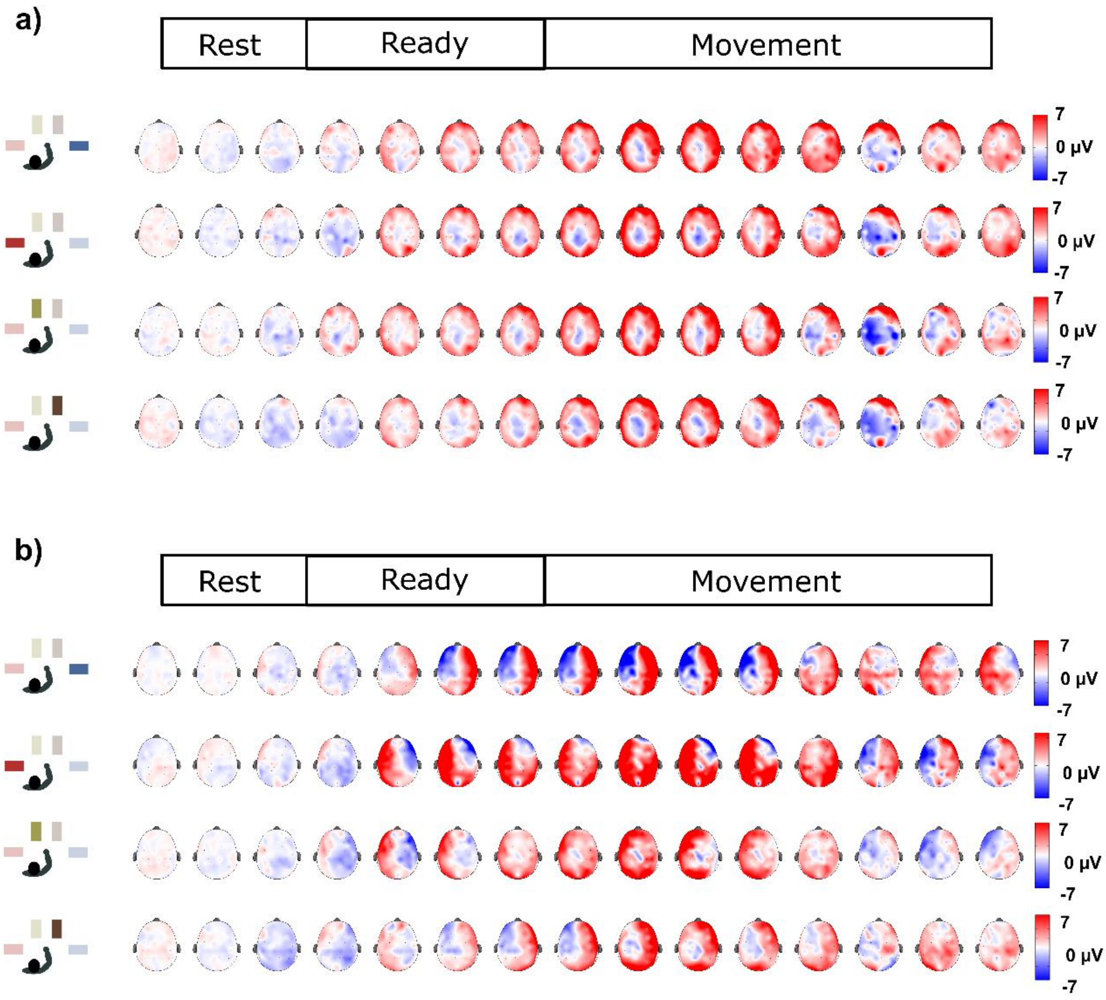

Figure 2. Topography EEG activation: amplitude of the EEG, in µV (color bar), every 0.5 s, for each reaching for each target in the constrained (a) and unconstrained (b) conditions in the low-frequency band (MRCP, FP1, and FP2). The upper part of each panel shows the EOG, accelerometer, and arm kinematic activations through the trial. The lower part of each panel shows the topographic distribution of the EEG for each of the reaching directions. The activations are divided by its SD so that scales are similar and fit into the same plot (thus, the only information this plot provides is when the activation happens for each signal). Figure adapted Bibian et al. 21.

2.1.3 Results

2.1.3.1 Topography of the EEG Activation

From the topographical analysis of the EEG low-frequency activation in the non-constrained condition, where subjects were moving freely, the activation shows a clear laterality among the reaching directions that starts around 1.5 seconds before the movement onsets and that co-occurs with the eye and head movements. This activity seems to overlap the MRCP characteristic negativity over the central electrodes and that can be clearly seen in the data from the constrained condition when this eye and head movements were minimized (see Figure 2).

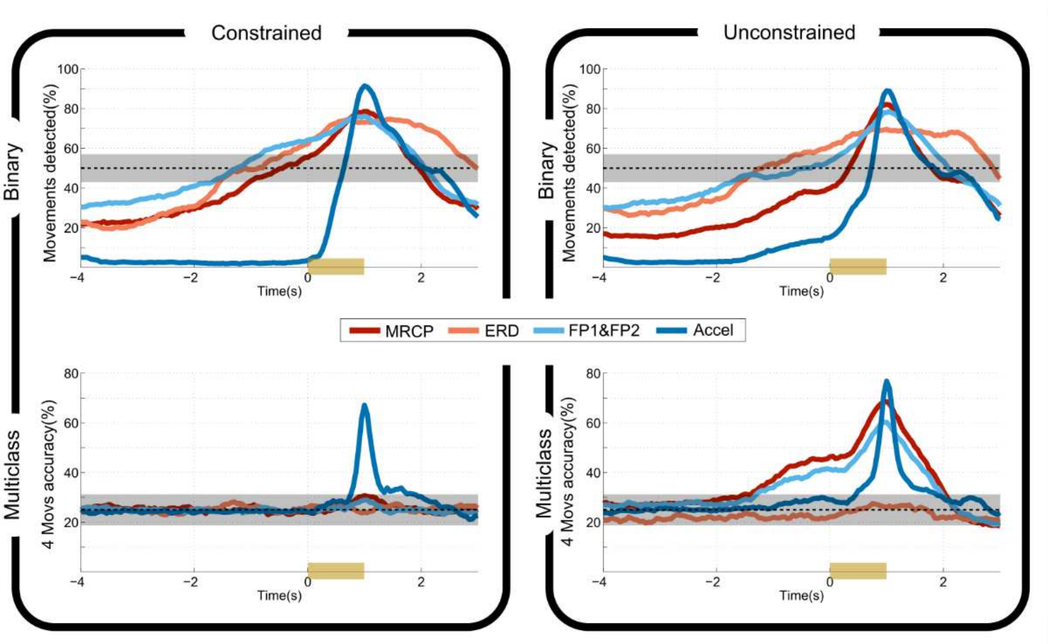

Figure 3. Binary and the multiclass classifier performances for both conditions. The Y-axes represent performance in %, and the X-axes the trial time, with time = 0 being the time in which the "GO" cue was presented to the participants. The performance of the binary and multiclass analyses are shown in the first and second row respectively. Constrained and unconstrained conditions are shown in the first and second column respectively. The 95% confidence interval (gray shaded area) of the chance level (black dotted line), calculated according to Müller-Putz et al. (2008). Brown shaded area on the time axis shows the window used to train the classifier. Figure adapted Bibian et al. 21.

2.1.3.2 Disentangling Brain's External and Internal Activities from EEG

In the binary classification analysis, that focuses on the discrimination between rest and movement conditions, we observed that the four features (MRCP, ERD, FP1 and FP2, and accelerometer) showed similar performances, all above 70% in both conditions and that no important differences were found between constrained and unconstrained conditions (see Figure 3). In the multiclass classification analysis, we found that no EEG features could identify reaching directions in the constrained conditions. Interestingly, in the unconstrained condition, both MRCP, eyes (FP1 and FP2 features) and accelerometer led to accuracies above 50 % (see Figure 3).

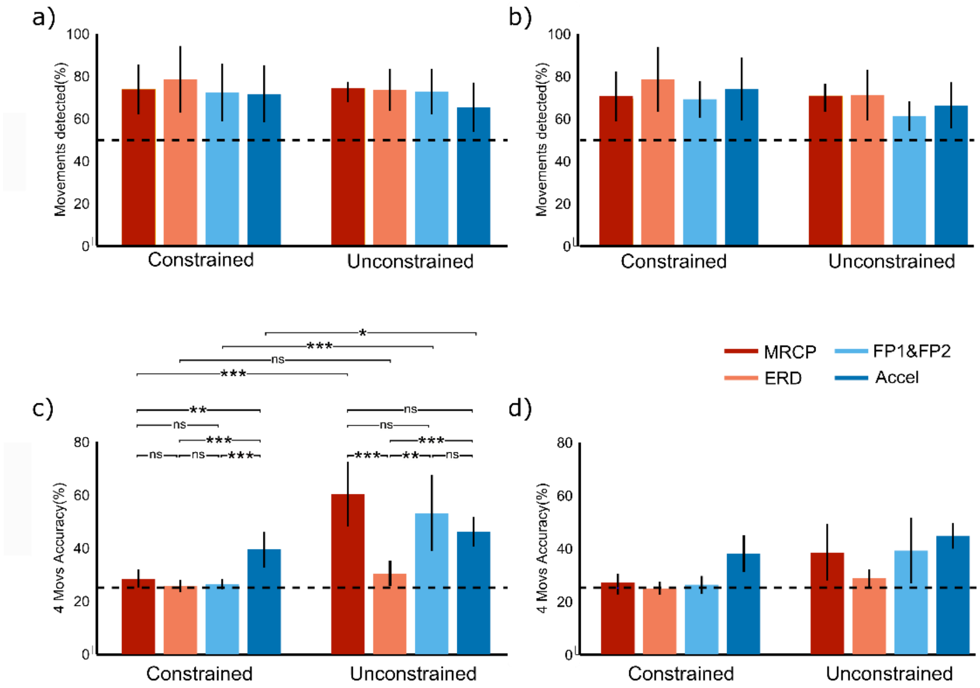

Figure 4. Average performances, between 0.5 and 1.5 s, for binary and multiclass classifies with and without artifact removal. Binary and multiclass classifiers are showed in the first and second rows, respectively. First column shows the results without applying artifact removal, and second one, corresponds to the results after applying it. Dotted lines show the theoretical chance level for each classification problem. Figure adapted Bibian et al. 21.

2.1.3.3 Influence of the Constrained/Unconstrained Conditions

In line with the results of previous sections, the binary classification analysis showed no significant interaction between the factor conditions and feature ($F_{3,27} = 0.402$, $P = 0.753$) nor a significant effect of condition ($F_{1,9} = 1.338$, $P = 0.277$) or feature ($F_{3,27} = 1.094$, $P = 0.369$) (two-way repeated measures ANOVA) (Figure 4a).

For the multiclass classification, both condition and type of feature influenced the classification accuracy—significant interaction between condition and feature ($F_{3,27}$ = 20.66, $P < 0.001$) as well as a significant effect of condition ($F_{1,9}$ = 80.301, $P < 0.001$) and feature ($F_{3,27}$ = 19.123, $P < 0.001$). In the constrained condition, accelerometer features showed significantly better performances than all the other features ($P < 0.01$, post hoc paired samples t-test calculated after finding a significant effect of feature under both conditions in the subsequent one-way ANOVA). In the unconstrained condition, the low-frequency features (MRCP, FP1 and FP2, and accelerometer) performances were significantly higher than the ERD features ($P < 0.01$, post hoc paired samples t-test) (Fig. 4b). Lastly, the comparison between conditions for each feature showed that the results were significantly lower in the constrained condition for all the low-frequency features: MRCP ($P < 0.001$), FP1 and FP2 ($P < 0.001$) and accelerometer ($P < 0.044$) features.

2.1.3.4 Influence of the Artifact Removal

Applying the artifact removal techniques had no effect on the binary classification --no significant interaction between artifact removal, experimental condition, and feature ($F_{1.67,14.55}$ = 1.179, $P = 0.325$) (Figure 4a,c). For the multiclass classification we found a significant interaction among the three factors (condition, feature, and artifact removal). However, the subsequent two-way ANOVA found no interaction between feature and condition after applying artifact removal ($F_{1.6,27}$ = 2.452, $P = 0.129$) (Fig. 4d).

2.1.4 Discussion

In this study, published in Bibian et al. 21, we have addressed the potential challenges and biases that can affect the interpretation of EEG data when studying brain oscillatory activity during motor tasks. Here, we have found that visuomotor tasks (task that involve a visuo-motor response) can introduce confounding factors in the EEG data collections. We designed a study to evaluate the impact of concurrent external activations that occur during reaching movements (i.e., eye and head movements) and how that affects EEG data. More specifically, we found that reaching directions could only be discriminated from EEG signals when subjects followed their arm movements with their gaze and head movements. This concerning finding suggests that, in this context, EEG signals mostly reflect the influence of head and eye movements and not the neural correlates of upper-limb motor-related activity. Although some technical improvements could help isolating the two sources of information (e.g., wireless sensors that reduce cable artifacts, source localization algorithms…), this study highlights the importance of taking head an eye movement into account when designing an EEG study of reaching movements, or any other visuo-motor task. Especially, when designing rehabilitative EEG based BMIs, in which the contingency from the brain and the feedback are key to induce instrumental and Hebbian learning.

2.2 Study 2: Neuroprosthetic learning and sleep

2.2.1 Introduction

Ever since the first study BMI for motor rehabilitation was presented, several research groups have investigated ways to maximize the rehabilitative outcome of such intervention. However, although significant and promising, the functional motor recovery achieved by stroke patients with novel BMI rehabilitative interventions (as well as with long-dose intensive therapies) remains modest (López-Larraz, Sarasola-Sanz, et al., 2018). Most of the attempts to improve these therapies have focused on improving the technology, for instance proposing algorithms to more precisely detect the brain commands, improve the link between brain and muscles to enhance Hebbian learning and plasticity or developing more sophisticated devices to mobilize the paralyzed limb of the patients. However, little attention has been paid to the process of learning and consolidation of the new skill being trained with the BMI, which could play a key role in how this new skill is integrated to the cerebro-spinal network (Dimyan & Cohen, 2011; Krakauer, 2006; Krakauer & Mazzoni, 2011).

In this study, we hypothesize that sleep after sensorimotor-rhythm-based BMI training can be manipulated to reinforce and consolidate this learning both in healthy young and stroke individuals. Furthermore, we hypothesize that peripheral electrical stimulation (PES) during sleep can be used to trigger memory reactivation and consolidation, thus contributing to further improving the neuroprosthetic learning and the rehabilitation outcome. We used the EEG-based BMI as a neuroscientific tool to study the oscillatory neurophysiology of sensorimotor recall during sleep and its effect on learning and sensorimotor integration. Furthermore, we developed the hardware, investigated the effect of post-learning sleep, and tested the efficacy of PES to boost neuroprosthetic learning in healthy participants. Lastly, we investigated the effect BMI-based rehabilitation has on the sleep of stroke patients.

2.2.2 Methods

To test the hypotheses mentioned before, two studies were conducted on healthy and stroke participants respectively. First, we designed a study on healthy participants to investigate the effects of neuroprosthetic learning on sleep and to evaluate the effect of TMR using PES to boost memory consolidation. Secondly, we studied the changes of sleep in a stroke population that underwent a BMI-based rehabilitative experiment.

2.2.2.1 TMR to boost neuroprosthetic learning

Experimental design

Twelve healthy participants without cognitive and neurologic impairment or sleep disorders (10 females, 23 and 2.4 years) took part in a within subject design that consisted of 2 conditions separated in different sessions (plus a calibration session). Subjects who perform any kind of shift work are excluded from taking part in this study. Participants were randomly divided in 2 groups: one group receiving condition 1 (experimental session B: closed-loop PES during NREM sleep targeting memory reactivation) in the first session and condition 2 (experimental session A: control sleep without any stimulation) in the second, while the second group will receive condition 2 the first session and condition 1 the second session. Before and after both nights, the subjects trained in a neuroprosthetic task for around 30 minutes (see Figure 5a). From those, two subjects had to be removed from the study due to technical difficulties.

Data acquisition

During the neuroprosthetic task, we recorded brain activity using a 32-electrode EEG cap and collected EOG data with four passive electrodes. The Impedance for EEG and EOG signals was kept below 1 kOhm. EEG signals were re-referenced (during the post processing) using a common average reference (CAR) over the sensorimotor cortex electrodes similarly to the study 1 (Bibián et al., 2021).

During the night, a standard polysomnographic recording, that included electroencephalographic (EEG), electromyographic (EMG), and electrooculographic (EOG) was recorded with a sampling rate of 200 Hz. EEG was recorded from six scalp electrodes (F3, F4, C3, C4, P3 and P4 following the International 10–20 System) with the reference on combined signal from the left and right mastoids. Additionally, a PES electrode was placed on the extensor digitorum of the dominant arm during the task and during the night. This electrode was used to deliver electrical stimulation both during the neuroprosthetic task as well as for the TMR during the night.

BMI Task

Participants underwent a neuroprosthetic task (hereafter referred to as BMI), designed as surrogate of sensorimotor learning. Here, the participants had to control the movement of a robotic exoskeleton along a predefined trajectory based on their sensorimotor rhythm activity. Subjects were instructed to perform a motor imagery task with their upper limb to move the exoskeleton. Thus, the alpha-band ERD (neural activity associated with movement execution/imagery) was computed for contralateral electrode over the motor cortex (C3 or C4, hereafter referred as “feedback electrode”) and a threshold was applied. If the value of power was below the threshold, the exoskeleton would move towards the target. This threshold was customized for every subject on the first training day (during adaptation session). Both experimental sessions started with the threshold from the adaptation session. After that, within each training period, the threshold was continuously updated using the signal of the last every 2 minutes to account for the within session variability of the EEG signals. Subjects had 15 seconds to reach the target position before trial was finished and the exoskeleton moved back to the initial position. Each block training (i.e., evening and training) consisted of 6 runs of around 5 minutes (trial duration depends on the performance of the subject) leading to a total training length of around 30 minutes. During the training, feedback was provided to the subjects in two ways: movement of the exoskeleton and PES was delivered on the extensor digitorum at the sensory threshold (interstimulus interval of 1 second). Both things occurred simultaneously.

Targeted memory reactivation

Directly after the BMI task, subjects slept 8 h with targeted memory reactivation during the first sleep cycle. The TMR consisted in PES pulses (on the same muscle and intensity as during the BMI task) delivered in a closed-loop manner following Antony et all. For that, we implemented a real-time algorithm that detected spindles and generated a trigger so that the PES was delivered 2.5 seconds after the spindle was detected. For the spindle detection algorithm (Antony et al., 2018), the signal from the feedback electrode was filtered between 11 and 16 Hz using a 2^{nd} order Butterworth filter. After that we calculated the root-mean-square (RMS) values with sliding 200-ms intervals and compared the signal with two different thresholds. The lower and upper thresholds were set to, respectively, 2 and 4.5 times the RMS of the data from the previous 600 s of signal without artifacts. When the RMS values stayed between the lower and upper thresholds for a period between 0.5-3 seconds, a spindle was detected.

Behavioral data analysis

First, we wanted to check whether neuroprosthetic learning had occurred. This is a critical in our protocol step since EEG data can easily be contaminated by electrical artifacts due to the closed-loop PES. Since the neuroprosthetic task was designed so that subjects learn to desynchronize their sensorimotor rhythm (lowering the power of alpha oscillations during movement), we first evaluated whether we could observe a decrease in alpha power during the training, used to control the neuroprosthesis, by calculating the difference in power between early and late trials (first and last 20% of the trials respectively) of the evening training for each experimental day. The EEG from the subjects showing an increased in alpha power was further analyzed to confirm that the feedback was, indeed, corrupted by electrical artifacts. Thus, subjects that did not show a decrease in alpha power in both experimental days were excluded from further analysis regardless of whether they showed behavioral changes (improvement in exoskeleton control) or not. To quantify the task performance from the behavioral data, we used the distance to target. This metric represents the distance from the final position to the target at the end of each trial. Thus, a distance to target of value zero would mean that the subject has reached the target within this trial whereas a value of one implies that the subject has not moved during the trial. All the intermediate values represent how far in the trajectory the subject has got in each trial. To assess whether both experimental conditions had different learning levels before the experimental nights we compared the learning at the end of the evening sessions in the control and intervention days. We did that by comparing both the distance to target and alpha power in the late trials (last 20% of the training) of the evening trainings. Finally, to evaluate the effect of TMR, we compared the drop in performance (distance to target) from the late evening trials to the early morning trials between control and intervention experimental days. We used a paired t-test to assess statistical significance.

Additionally, we assessed the learning by calculating the alpha power evolution. For that, we calculated the alpha power during the movement period of each trial. After that, the power of the first and last 20% of the trials within the training block were calculated (hereafter referred as alpha power early/late values). Then, the evolution of the ERD value was assessed subtracting the alpha power late – alpha power early values.

Sleep data analysis

The neurophysiological data recorded during the night was used to study learning-related sleep activity (occurrence of spindles, SWS and lateralization indexes) and to investigate whether reactivation of sensorimotor neural networks can be influenced during sleep using TMR.

In the offline analysis, we detected discrete slow oscillations (SO) and sleep spindles within the NREM periods. The detection algorithm both for the SO and spindles was based on the work by Mölle and colleagues (Mölle et al., 2011). The signal of the feedback electrode was filtered between 0.05 and 3.5 Hz. A peak-to-peak amplitude larger than 75 microV, a SO was marked as detected. For the spindle detection, the same procedure as for the online detection was implemented (see Targeted memory reactivation section). Finally, we calculated the density of SO-spindle complexes by detecting the amount of SO with a nested spindle in the upstate of the SO (Staresina et al., 2015).

We also wanted to test the influence of neuroprosthetic learning on any of those brain signatures. To do that, we calculated the SO and spindle occurrence also for the ipsilateral electrode (opposite to the feedback electrode). Thus, we can compare the values between the brain area where the learning is expected to occur (feedback electrode) and those of the opposite hemisphere. Lastly, to investigate whether these differences between hemispheres were affected by the TMR, we calculated the laterality index by dividing the SO and spindle density values of the feedback electrode and the ipsilateral electrode. Separately for the learning hemisphere and the lateralization, we performed a two-way repeated-measures ANOVA with multiple comparisons with the factors experimental night (intervention or control) and sleep signature (SO, spindle or SO-spindle complex), to evaluate the effect of TMR on any of the sleep signatures extracted.

2.2.2.2 Effect of BMI-based stroke rehabilitation on sleep

We next wanted to investigate the effects of a BMI-based rehabilitation on sleep. For that, we performed polysomnographic recordings on stroke patients right before and after they participated in a BMI-base rehabilitation intervention.

Experimental design

All the chronic stroke subjects underwent a 10-sessions BMI-based rehabilitative intervention. In this intervention, subjects learned to control a robotic exoskeleton (described in Study 1) using their brain and muscle activity. In this approach, the EEG signal recorded in the perilesional motor cortex was use activate/de-activate the movement of the exoskeleton whereas the EMG activity recorded over the paretic arm was used to define the trajectory and velocity of the exoskeleton's movement. More details on the BMI intervention can be found in (Sarasola-Sanz et al., 2017). Right before and after the subjects participated in the BMI rehabilitation, we recorded the polysomnographic activity. In those recordings, referred as to Pre and Post respectively, we performed polysomnography recordings that included EEG, EMG and EOG activity. For the EEG recordings, we recorded six as in the study 2.1: F3, F4, C3, C4, P3 and P4, according to the International 10-20 system.

Sleep data analysis

In this study, we used the same methods as in Study 2.1 on healthy participants. Thus, we calculated SO and spindle occurrences as well as the SO amplitude over the ipsilesional motor cortex, used for the control of the BMI. Additionally, we calculated the lateralization indexes for those brain signatures. For these analyses we also evaluated which subjects showed a successful BMI learning, reduction of the distance to target after the 10 sessions. Thus, the sleep signatures and their lateralization were reported indicating whether that subject learned or not. In these analyses SO-spindle complexes were not calculated due to the lower occurrence of SO. Additionally, as it greatly differs from sleep on healthy participants, we analyzed the sleep architecture by calculating the percentage of time spent in each of the sleep stages both for the Pre and Post sessions.

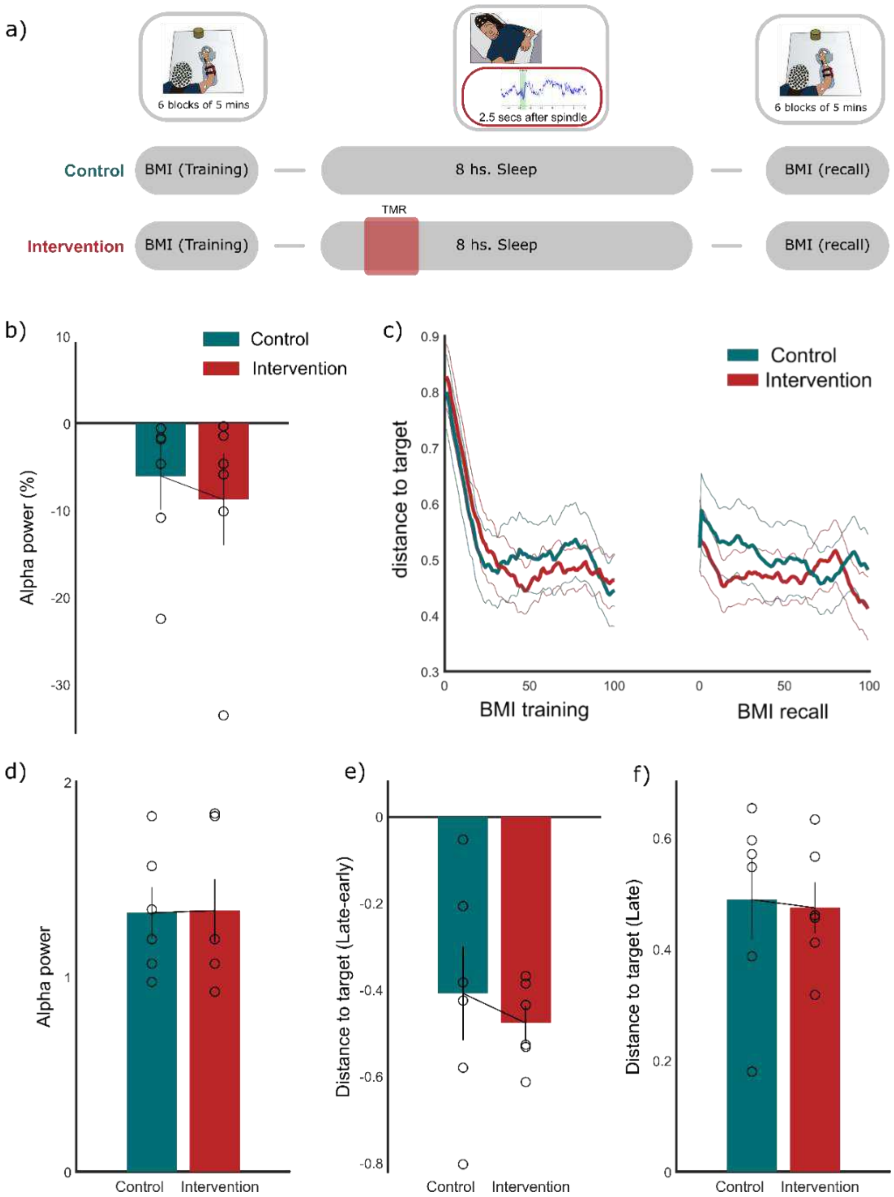

Figure 5. Neuroprosthetic task. (a) Experimental design. BMI training was performed before and after a 8hrs sleep period. TMR was only performed in the night of the intervention experimental day, and not in the control. (b) Change of alpha power (%) during the control of the BMI during the evening training session (c) Distance to target as a function of the percentage of session completed. For each group (Control and Intervention) average and s.e.m are showed. The gap between BMI training and BMI recall correspond to the night sleep. (d) Comparison of alpha power during the neuroprosthetic task at the end of the evening training sessions for the intervention and control day. (e) Changes in distance to target within the evening training sessions for intervention and control day. (f) Comparison of distance to target during the neuroprosthetic task at the end of the evening training sessions for the intervention and control days.

2.2.3 Results

2.2.3.1 TMR to boost neuroprosthetic learning

Behavioral data

The offline analysis showed that four subjects learned to control the BMI without a modulation of the neural activity of the motor cortex for at least one of the training sessions (Intervention and control). These subjects were discarded for further analyses since neuroprosthetic skill learning cannot be assured. The remaining subjects (six) showed a consistent reduction in alpha power that led to improvement in the behavioral results (Figure 5b, 5c and 6a). Over the course of the average 30 minutes of training sessions, all the remaining participants showed improvement in performance (i.e., neuroprosthetic skill learning) as shown by a significant reduction in distance to target (paired t-test, $P < 0.001$; Figure 5e). Additionally, the pre-sleep learning showed no differences between control and intervention conditions both in alpha power reduction (paired t-test, $P = 0.94$; Figure 5d) and distance to target (paired t-test, $P = 0.85$; Figure 5f). Furthermore, we did not find statistical difference between control and intervention (were TMR was applied) in the overnight drop in performance (paired t-test, $P = 0.31$; Figure 6b).

Sleep data

The repeated measures two-way ANOVA on the density of SO, spindle and SO-spindle complex over the learning hemisphere revealed no significant interaction between condition (control and intervention) and sleep signature (SO, spindle and SO-spindle complex), ($F_{(2,12)}$= 0.025, $P = 0.975$; Figure 6c) as well as non-significant main effect of condition ($F_{(1,6)}$= 0.091, $P = 0.774$; Figure 6c). Similarly, we found no significant interaction on the lateralization between condition and sleep signature, ($F_{(2,12)}$= 3.002, $P = 0.09$; Figure 6c), and no significant main effects ($F_{(1,6)}$= 0.013, $P = 0.914$; Figure 6c). Lastly, we calculated the evoked spindle response after the TMR was delivered (Figure 6d). We found that around 15% of the cues evoked a spindle with a peak of occurrence between 2 and 3 seconds. No correlations could be found between this value and any of the behavioral results.

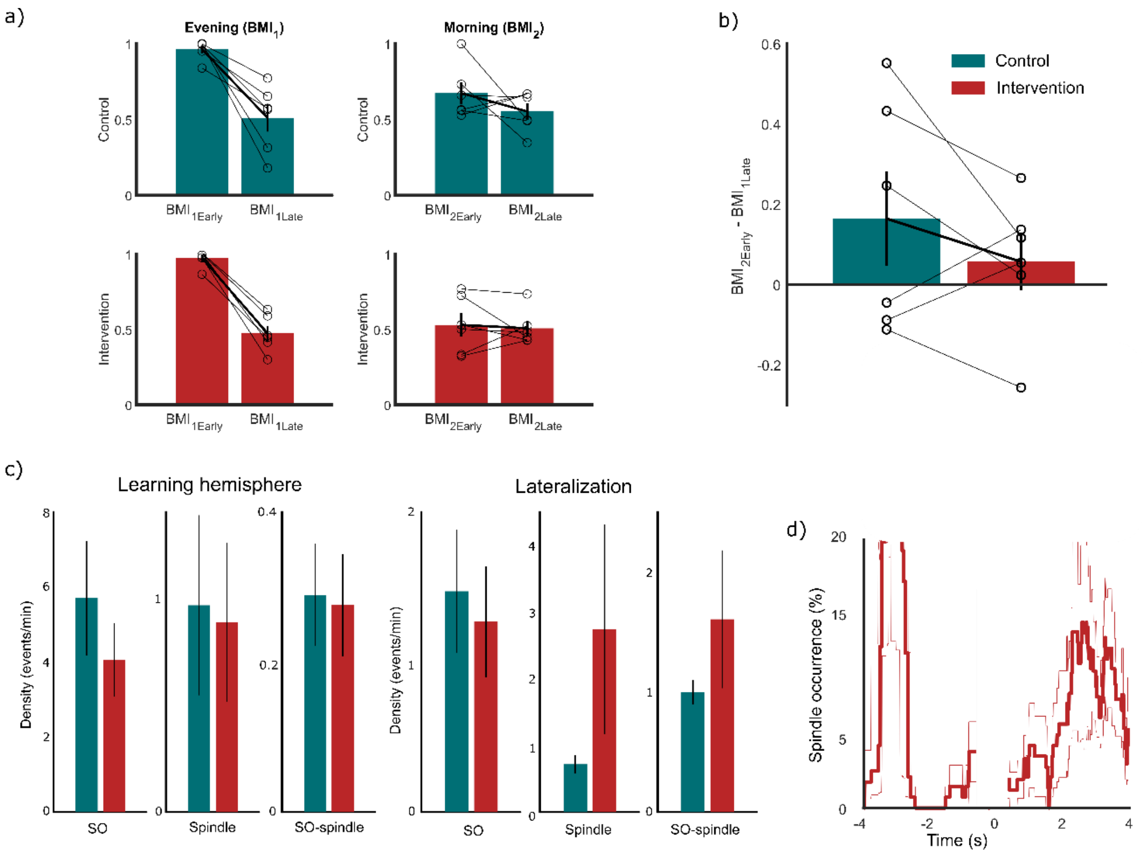

Figure 6. Effect of TMR on neuroprosthetic learning. a) Evolution of distance to target for each training session (evening and morning) and experimental day (control and intervention). The performance of the first and last 20% of the training, referred as BMI early and BMI late respectively, was calculated for each training session. (b) Drop in performance after the 8-hours sleep period as the change in distance to target from the last 20% of the evening training to the first 20% of the morning training. (c) Density over the learning hemisphere (left) and Lateralization (right) of the occurrence of each neural correlate of sleep (SO, Spindle and SO-spindle complex) after neuroprosthetic learning. Lateralization (calculated as contra/ipsilateral motor cortex) of the density of SO, spindle and SO-spindle complexes respectively, for control (blue) and intervention (red) nights. A lateralization value higher than one indicates that the density is higher over the contralateral motor cortex, where the learning is expected to occur. (d) Spindle occurrence around the TMR cues. Y-axis represents the percentage of cues that evoked a spindle for a given time point relative to the time where the cue was presented, x-axis, being t=0s the time of the stimulus.

2.2.3.2 Effect of BMI-based stroke rehabilitation on sleep

In line with previous research, stroke subjects spent 70-80% of the night in NREM sleep. For these patients, NREM sleep was mostly composed of S2 (Figure 7a), with very short time spent in SWS (<10%). After the rehabilitation intervention, we found a small increase in percentage of the sleep spent in REM and S2, together with a slightly shorter time spent in SWS and NREM. We first assessed the learning of each subject as distance to target reduction from the first to the last 2 experimental sessions (Figure 7b). Interestingly, we found an increase in the spindle density in the ipsilesional (contralateral) hemisphere after the rehabilitation only for the subjects who showed a consistent learning (dotted lines in the Figure 7c). However, spindle density lateralization did show lower values after the intervention, and with values lower than one, showing an increase in spindle activity towards the contralesional hemisphere, opposite to the learning hemisphere, and to what we found in healthy individuals (Figure 7f). Both the ipsilesional SO density and its lateralization did not show any change after the rehabilitation (Figure 7d and g). However, SO amplitudes over the ipsilesional motor cortex were lower after the rehabilitation for the subjects who did show a decrease in distance to target (Figure 7e). In line with these findings, the laterality also showed a decrease in the laterality of the SO amplitude for the subjects who did not manage to reduce the distance to target, indicating a higher SO amplitude change over the contralesional motor cortex after the BMI learning/rehabilitation (Figure 7h).

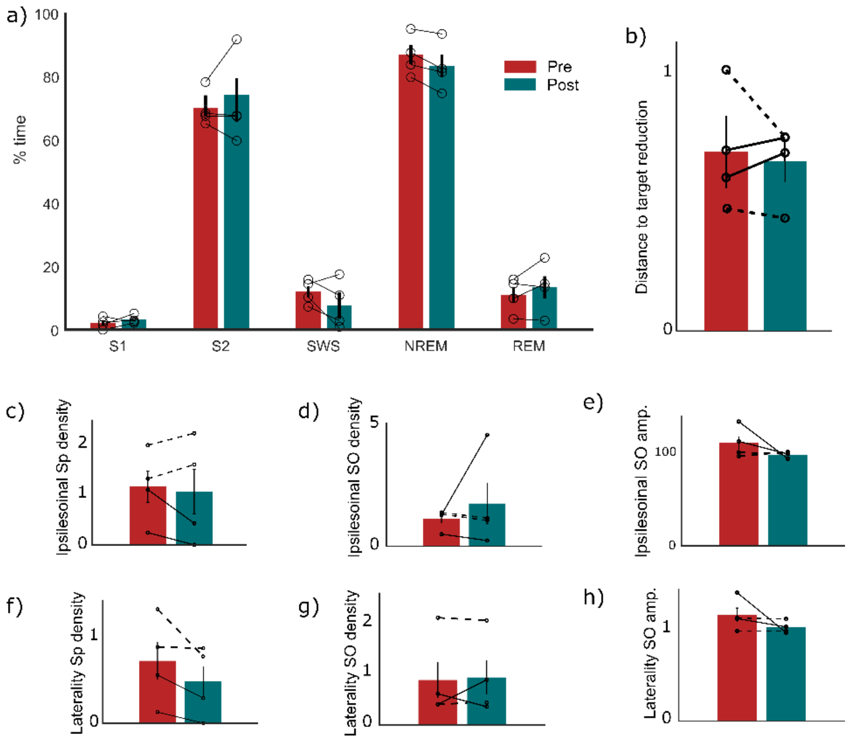

Figure 7. Sleep after a BMI-based stroke rehabilitation. (a) Sleep architecture before (red) and after (blue) the block of ten sessions of BMI-based stroke rehabilitation. (c) Spindle density over the ipsilesional motor cortex. Dotted lines show the patients that showed positive behavioral results in the control of the exoskeleton (d) SO density over the ipsilesional motor cortex (e) SO amplitude over the ipsilesional motor cortex (f) Laterality of the spindle density calculated as the ipsi/contralesional motor cortex density, being the ipsilesional the one used for the BMI-control. (g) Laterality of the SO density calculated as the ipsi/contralesional motor cortex density (h) Laterality of the SO amplitude calculated as the SO amplitude of the ipsi/contralesional motor cortex.

2.2.4 Discussion

In this study, we explored the potential of Targeted memory reactivation to enhance sensorimotor-rhythm-based neuroprosthetic learning on healthy and stroke individuals. Firstly, we explored whether post-training sleep could be used to reinforce the consolidation of the newly acquired sensorimotor skills on healthy participants. To do so, we introduced peripheral electrical stimulation (PES) during sleep as a task-associated stimulus for the targeted memory reactivation (TMR). A sleep interface was implemented to deliver closed-loop PES following the findings by Antony et al 2018. Here we could study how neuroprosthetic learning affects post-learning sleep. Lastly, we investigated the changes in sleep of stroke patients that underwent a BMI-based rehabilitative therapy.

Learning to control a neuroprosthetic device can be considered a type of instrumental learning where the subject learns to modulate the brain activity by feedback and reward (Carmena, 2013; Green & Kalaska, 2011). During the training, subjects adapt their neural connections to minimize output errors, through plasticity in the cortex and striatum (Ganguly et al., 2011; Koralek et al., 2012). The direct link between the neural activity and the feedback makes neuroprosthetic learning an invaluable tool to study the relationship between brain activity and behavior. Thus, although our behavioral results showed that subjects could learn the task, a subsequent analysis on the evolution of sensorimotor rhythm alpha activity during the learning showed that an important portion of our participants (around 40%) could not learn the neuroprosthetic task in any of the sessions. This could be due a lower EEG signal quality that led to the electrical artifact corrupting the alpha band, or to their inability to modulate their brain activity to successfully control the neuroprosthetic. The latter concept, defined as "BMI illiteracy" has been extensively investigated and refers to the idea that a subset of the population might find challenging to learn and proficiently use a BMI (Vidaurre & Blankertz, 2010). However, another alternative is that some of the subjects could have found alternative ways to complete the task: artifact-driven control of the neuroprosthetic. As we discussed in the previous study, it is crucial to improve the control of neural interfaces and to ensure that the control is not biased by external activity, from both physiological and non-physiological origins (Bibián et al., 2021; Vidaurre & Blankertz, 2010).

Successful learning could be achieved by all the remaining subjects, as shown by the significant reduction in distance to target (Figure 5e). Contrarily to our hypothesis, we did not find statistical difference between control and intervention (where TMR was applied) in the overnight drop in performance (Figure 6b). However, the mean differences we obtained in a relatively low sample size are promising and further experiments with a larger sample size could potentially result in significant differences between conditions. A growing body of evidence supports the effectiveness of TMR for procedural memories (Cellini & Capuozzo, 2018). Laventure and colleagues presented task-related odor cues to successfully improve the post sleep performance of a motor sequence learning (MSL) task (Laventure et al., 2016). On the other hand, several studies have used auditory cues in a finger tapping task, showing the same positive effects (Antony et al., 2012; Schönauer et al., 2013). Interestingly there is very little evidence on the use of somatosensory feedback for TMR. In fact, Pereira and colleagues found no effect of tactile stimulation on post sleep MSL performance (R. Pereira et al., 2017). The variance in outcomes across these studies may be attributed to the differences in brain areas related to the afferent stimuli of each TMR modality. TMR can stimulate the memory reactivation network indirectly (a stimulus non-related directly to the neural network recruited during the task, e.g., an auditory stimulus during a motor task) or directly (e.g., electrical stimulation of the motor neurons involved in the motor task). In our protocol we have implemented a PES-based TMR because the afference during PES directly targets sensorimotor areas, where neuroprosthetic learning is produced, potentially enhancing the slow-waves and spindles activity in sensorimotor areas pushing sensorimotor learning and consolidation.

When investigating the effect of PES-based TMR on sleep, we did not find any statistical differences between control and intervention (when TMR was used) in the occurrence of slow oscillations, spindles or SO-spindle complexes over the learning hemisphere or in its lateralization (density over the learning hemisphere divided by the density over the opposite hemisphere). However, a larger sample size is necessary to better understand the efficacy of PES for TMR. In fact, although no differences in average are observed over the learning hemisphere (Figure 6c, left), we can observe a promising effect of TMR on the spindle density lateralization with an increased lateralization in the intervention night, where TMR was applied. Spindle activity, and more specifically spindle activity over the learning areas, has been linked to the memory reactivation processes occurring during sleep (Bergmann et al., 2012; Schönauer, 2018). Cairney and colleagues found that when previously learned words were presented during NREM, on top of the expected improvement on memory compared to non-presented words, they evoked a higher spindle response than non-studied words (Cairney et al., 2018). Additionally, this study showed that it is possible to decode the category of the word being presented from the evoked spindle response (2-3 seconds after the word was presented). In our study, we found a high occurrence of spindles 2-3 seconds after the presentation of the stimulus, around 15%. This, together with the TMR-driven lateralization of the spindle density towards the learning hemisphere suggests a biasing effect of the TMR on the reactivation processes, enhancing the neural reprocessing of the neuroprosthetic skill trained. Similarly, the lateralization of SO-spindle complexes seems to increase towards the learning hemisphere when TMR is presented, adding further evidence to the reactivation processes occurring over the learning hemisphere due to the TMR. The validation of PES as an effective method for TMR is especially relevant since, to the best of our knowledge, no single study has evaluated the efficacy of electrical stimulation as a cueing mechanism for memory reactivation during sleep. PES-based TMR holds a great potential for the reactivation of rehabilitative memories after stroke, where the impaired sensorimotor is not capable of fully encoding and consolidating newly acquired memories.

Additionally sleep quality indicators decrease with age, while the incidence of stroke shows the opposite trend. Aging is typically associated by a decrease of the time spent in slow-wave sleep and a decrease in the amplitude and occurrence of slow waves. Importantly, stroke patients exhibit significant disruptions of their sleep patterns (Bakken et al., 2012, Terzoudi et al., 2007). Indeed, chronic stroke patients spent similar time in REM but more time in S2 than age-matched individuals. Here we investigated the sleep patterns of 5 stroke patients that participated in a 10-day BMI-based rehabilitation intervention. The baseline sleep, measured before they started the rehabilitation, showed sleep architecture very similar to what has been previously reported: 15% REM and 85% NREM (with a very low, <10%, presence of SWS). When comparing the sleep architecture before and after the BMI intervention, we found an increase in time spent in REM and S2 with a small reduction in the time spent in SWS. These results are in line with previous research on motor memory consolidation that showed increases in REM during post learning sleep. Additionally, the amount of REM sleep has also been linked to the improvement of performance in a finger tapping sequence (Siengsukon & Boyd, 2009). In line with our results, a study on stroke patients showed that time spent in REM sleep was positively correlated with offline motor learning while NREM3 showed a negative correlation (Siengsukon et al., 2015). REM sleep has also been associated with poor rehabilitative outcome in acute stroke patients (Pace et al., 2018).

On the other hand, procedural motor learning has been associated with sleep S2 (Walker et al., 2002). It has been proposed that this association between S2 and motor learning derives from the presence of sleep spindles during S2 (Rauchs et al., 2005). Interestingly, in our study we observed that spindle occurrence was higher after the rehabilitative intervention for the subjects that showed a successful learning (i.e., reduction in distance to target). Interestingly, Gottselig and colleagues showed a positive correlation between ipsilesional spindles in an acute phase after stroke and functional outcome after one year, thus highlighting the relevance of sleep spindles for stroke rehabilitation (Gottselig et al., 2002). This effect could also be found for the SO amplitude: subjects who learned to control the exoskeleton (reduction of the distance to target), showed a higher amplitude SOs over the ipsilesional cortex. Interestingly, the change of lateralization of SO amplitude showed a higher amplitude SO over the contralesional motor cortex (opposite to the control) after the rehabilitation for those subjects who did not learn to control the neuroprosthesis. Altogether, these results are in line with previous studies that have associated both spindle occurrence and SO amplitude with the reactivation of previously learned memories.

In summary, in this study we have investigated the relationship between neuroprosthetic learning and sleep. We have found an effect of our BMI learning reflected on the sleep data, with an increase in spindle and slow-wave activity over the contralateral hemisphere, used to control the BMI, in healthy subjects and stroke patients. Furthermore, we showed a consolidation effect of sleep in a neuroprostehtic skill learning and the potential benefit of TMR to improve the speed of learning. Additionally, our findings present PES-based TMR as a highly promising tool, especially suitable for memory consolidation of sensorimotor tasks, like BMI-based rehabilitation, and patients with an impaired sensorimotor system, like stroke patients. However, our findings also raise questions regarding BMI literacy and highlight the importance of a highly controlled BMI task, to ensure that subjects rely on the target neural population to control the neuroprosthetic and not on compensatory strategies (e.g., head or eye movements or neck and cranial muscles contractions). Future studies might further investigate the effects of neuroprosthetic learning on a larger number of healthy subjects and will need to be expanded to stroke patients to test the possibility to manipulate memory consolidation during sleep.

2.3 Study 3: Subliminal rehabilitation for stroke patients

2.3.1 Introduction

Neuromuscular electrical stimulation (NMES) is a type of PES technique that consists of applying electrical currents on the skin to depolarize motor and sensory nerves beneath the stimulating electrodes (Bergquist et al., 2011). NMES has been used as a neuroscientific tool to study sensorimotor neural mechanisms and structures (Carson & Buick, 2019), and as a clinical application, e.g., in neurorehabilitation to reduce muscle atrophy, and to improve muscle tone and motor function in patients with paralysis after stroke (Knutson et al., 2016). The working principle of rehabilitative PES is based on: 1) the direct effect on muscle tone; and 2) the activation of receptors that generate afferent volleys that induce cortical excitation. The expected neuromodulation is based on the pioneering work from Fetz and Baker (1973), who used operant conditioning to generate patterns on precentral activity units and correlated responses in adjacent cells and contralateral muscles (Fetz & Baker, 1973). One promising area of application for PES is the oscillation dependent (closed-loop) electrical stimulation to induce plasticity measured by evoked responses. Recent studies have investigated its application in animal models (Capogrosso et al., 2016; Nishimura et al., 2013) and humans (Van Elswijk et al., 2010; Zrenner et al., 2018a). Most of those works assume a gain modulation of the incoming stimulus depending on the phase of the ongoing oscillation, thus a neuro facilitatory/inhibitory effect. Together, brain machine interfaces and PES, BMI-PES therapies have been proven to drive significant functional recovery and purposeful plasticity in stroke patients (Biasiucci et al., 2018).

However, one of the problems to be addressed when translating these findings into a rehabilitative scenario is the short intervention time due to, among other factors, the dose effect of the stimulation (Insausti-Delgado et al., 2020). Previous work described that brain activation due to PES, studied by functional magnetic resonance imaging (fMRI) and near infrared spectroscopy (NIRS), is proportional to the applied intensity (Blickenstorfer et al., 2009; Smith et al., 2003). These findings rely on the fact that as the stimulation intensity increases, there is a progressive recruitment of more afferent receptors that modulate brain activity (Golaszewski et al., 2012; Maffiuletti, 2010). However, there is evidence suggesting that below-motor-threshold stimulation activates cutaneous mechanoreceptors that provide feedback to areas 3b and 1 in the somatosensory cortex (S1), while above-motor-threshold stimulation generates muscle contractions that activate muscle spindles and Golgi tendon organs that send afference to areas 3a and 2 in S1 (Carson & Buick, 2019). It is believed that muscle spindles can directly project to the motor cortex (M1) via area 3a, whereas neural transmission due to cutaneous activation from area 3b to M1 is scarce (Carson & Buick, 2019). Thus, the presence or absence of muscle contraction elicited by PES has a direct impact on somatosensory cortex, and in turn, on motor cortex excitability (Sasaki et al., 2017). Recent findings further support this approach, reporting that a low-intensity sensory threshold PES can also induce significant activation over sensorimotor areas and enhance brain connectivity patterns (Mottaz et al., 2018b). This low intensity PES could offer a solution for longer interventions in which subjects are subliminally stimulated while performing daily tasks. In fact, low-intensity PES showed treatment effects like high-intensity PES in stroke patients (Hsu et al., 2010; Tu-Chan et al., 2017). Additionally, understanding how the afferent feedback is modulated in the sensorimotor system increases the excitatory effect of PES. Indeed, it is known that afferent information is reduced during voluntary movements (Angel & Malenka', 1982; Bays et al., 2006; Blakemore et al., 1998). This afference modulation is a critical factor in a BMI-PES since a stimulation timed with higher excitatory states of the sensorimotor system is more likely to drive activity-depended plasticity (Mrachacz-Kersting et al., 2012). Here we hypothesize that the plasticity effect of ultra-low-sensory ("subliminal") peripheral electrical stimulation on corticomuscular connections can be fully exploited if this is synchronized with supraspinal activity from the sensorimotor cortex.

2.3.2 Methods

2.3.2.1 Modulation of the excitability of the sensorimotor system

This part of Study 3 constitutes the first step towards a rehabilitative closed-loop PES that optimally excites the sensorimotor system to improve the rehabilitative outcome. Here, we studied the if, and how, the excitability of the sensorimotor system is modulated by endogenous sensorimotor cortical oscillations.

Experimental paradigm

Fourteen right-handed subjects with no history of nervous or cognitive disorders were recruited for a one-session experiment (age: 22 ± 3 years, 12 females). All the procedures were approved by the Ethics Committee of the Faculty of Medicine of the University of Tübingen, Germany. During the experiment, the subjects were comfortably seated in front of a computer screen with the hand positioned on a pressure-sensitive device and the palm facing upwards. The screen subsequently cued blocks of 60 seconds of either rest or isometric contraction conditions (20% of a maximal voluntary contraction). During the blocks, peripheral electrical stimulation was delivered on the median nerve with the parameters described below.

Electrophysiological recordings and Peripheral electrical stimulation

We recorded Electroentephalographic activity (EEG) data from 64 Ag/AgCl electrodes (Easycap GmbH, Germany) and 24bit EEG amplifiers (NeurOne Tesla, Bittium, Finland) in DC mode at a sampling rate of 5 kHz. The electrodes for the PES were placed on the surface of the skin of the non-dominant hand in the center of the wrist above the median nerve with the cathode being more distal. Single pulses of 1 ms with a biphasic square wave at an intensity that produced H-reflexes with half of the H-maximal amplitude.

Data preprocessing

Firstly, to improve the quality of the data applied, we implemented an automated trial-rejection algorithm. This algorithm, used also in the first study (section 2.1.2.5) and similar to (López-Larraz, Figueiredo, et al., 2018), computes the mean and standard deviation power for the frequency bands that contain movement and muscle artifacts: [0.1–4] Hz and [30–45] Hz, respectively (Castermans et al., 2014; Muthukumaraswamy, 2013). If the power of, at least, one of the bands exceeds three standards deviations from the mean, the trial is marked as noisy and removed from further analysis. Additionally, after noisy trials were removed, we calculated the mean and standard deviation of each channel for each condition and, after visual inspection, removed the channels that showed abnormal oscillations.

Modulation of somatosensory excitability during motor activation

To assess the somatosensory excitability during rest and movement conditions, we selected the parietal N20 and the frontal N30. We selected those potentials because they have been described to provide insights on the integration of sensory information during motor task and the integration between sensory and motor processing (Macerollo et al., 2018). Thus, we first extracted the somatosensory evoked potentials both for the rest and motor condition from the contralateral F and CP channels (for the frontal N30 and parietal N20 respectively). Once the data was cleaned, following the procedures described before, trials were extracted from -1000 to 120 ms, being t = 0 the delivery of the PES. A baseline correction method was applied. To do so, the mean value of the 1 second before the stimulation [-1000 0] ms was calculated and subtracted to all the values of the trial. After that, we calculated the grand averages for each condition and subject. From the grand averages of each subject and condition, we extracted the amplitude of the N20 and N30 potentials (from the parietal and frontal electrodes respectively) by visually finding the latency of each potential for each subject.

Cortical modulation of somatosensory excitability during rest and during movement

We subsequently assessed whether the somatosensory excitability during rest was modulated by sensorimotor cortical oscillations. We calculated the pre stimulus phase of the EEG for each frequency between 3 and 35 Hz. To do so, the EEG signal was individually bandpass filtered for each frequency (f) using a 1st order Butterworth filter between (f-1.5, f+1.5). Then, from each of those signals and only for the stimulations delivered during the rest condition, we extracted pre-stimulation trials of 2 wavelength durations [-2/f,0] seconds, being t = 0 seconds the moment of the stimulation. Then, the pre-stimulus phase for each trial and frequency was calculated using the Fast Fourier transform (FFT) on the windowed (Hanning) trials of each frequency, resulting in a value of pre-stimulus phase for each trial and each frequency.

To evaluate if, and how, the phase of the cortical oscillations was related to the sensorimotor excitability, we divided the 400 trials based on the pre-stimulus phase into eight clusters (with centroids: -180º : 45 : 145º). To do so, the 100 trials that were closest to the centroid value, were assigned to that cluster, averaged and the N20 and N30 potentials were calculated following the methods explained before. To quantify the phase modulation of each potential a least-squares cosine function was fitted to each the values for of each cluster. Thus, the amplitude of the fitted cosine was defined as the modulation depth (no relation between cluster phase and amplitude of the potential would result in a low amplitude whereas if the phase modulates the amplitude of the potential the cosine amplitude will reflect that modulation). This process was repeated for each frequency and each potential independently, resulting in a value of modulation depth for each frequency and potential. Lastly, a cluster-based permutation test was performed to look for significant differences between the modulation depth of each frequency against a bias estimate. The bias estimate was calculated following the same steps as the modulation depth but on trials clustered based on a randomized pre-stimulus phase. Finally, to evaluate the pre-stimulus phase modulation effect during the movement condition, we repeated the same procedure on the movement condition trials.

Changes of somatosensory excitability after a motor task

We next determined whether somatosensory excitability changes after a motor task has been performed. Given that both conditions are intercalated throughout the experimental session, we analyzed the evolution of N20 and N30 potentials when the time of the experiment (and the time of execution of the motor task) increases. Thus, for the rest and movement condition separately, we divided the data into eight groups of 100 trials. After that, we extracted the N20 and N30 potentials with the methods explained before. Then we used a linear regression to evaluate whether there is any trend in the change of N20 and N30 potentials through the session. We then wanted to evaluate whether this evolution of the somatosensory excitability throughout the experimental session, investigated in the previous analysis, had any effect on the modulation of somatosensory excitability. To do so, we divided the dataset into an early and late blocks (first and second half of the dataset). Thus, we repeated the aforementioned analysis separately for each 20-min block (see Cortical modulation of somatosensory excitability during rest and during movement subsection) but using separately the first and the last 20 minutes of data.

2.3.2.2 PES to boost motor learning

Building upon the findings of the previous study, where we confirmed the modulation of sensorimotor excitability by the motor cortex oscillations, and following previous work on somatosensory excitability modulation, this study evaluates the use of a closed-loop BMI to boost motor learning (Van Elswijk et al., 2010; Zrenner et al., 2018a). This BMI has been designed to deliver PES precisely during the optimal phase of the SMC activity (alpha oscillations in particular). Additionally, since the work of our research group goes towards the use of this technology at home, we have integrated the BMI into a portable setup that relies on a dry EEG cap. Thus, we designed an experiment to test our primary scientific hypothesis, while evaluating the functionality of the setup. Our primary hypothesis is that PES optimally locked to the negative phase of the SMC alpha oscillations will increase the excitability of the sensorimotor system and facilitate motor learning.

Experimental design

Subjects. 14 subjects (10 females, 25 ± 3.81 years) without any neurologic disease history participated to the study. All the procedures were approved by the Ethics Committee of the Faculty of Medicine of the University of Tübingen, Germany. During the experiment, subjects were comfortably seated on a chair in front of a computer screen and a keyboard. To test our scientific hypothesis, we designed a between subject experiment where participants were randomly assigned to either the intervention or the control group. In both groups, subjects had to learn a finger tapping sequence (more details below). While no PES was delivered in the control group, the intervention group received closed-loop PES during the finger tapping training. To evaluate whether the closed-loop PES could be used as feedback in a BMI system and further improve motor learning by exciting the sensorimotor system, the stimulation was only delivered during the finger tapping time, and not within the rest periods. Right before and after the training, to evaluate the changes in the excitability of the sensorimotor system due to the intervention SEPs were recorded by delivering PES on the medial nerve as we did in the study 3.1. Finally, a pre and post finger tapping assessment block was performed so that learning withing the session could be assessed.

Finger tapping task

To evaluate whether motor learning could be boosted by closed-loop PES, we implemented a finger tapping task based on (Schönauer et al., 2013). The task consists in a 12 digits sequence (423121432413) that is performed with digits 2 to 5 (index to little finger). This sequence has been designed so that each transition between fingers is present only once. In our experiment, subjects had to perform this sequence as fast and as accurately as possible with their non-dominant hand. During the learning, subjects had 3 blocks of 12 trials of finger tapping. Each of those trials consisted on 30 seconds of rest followed by 30 seconds finger tapping. Additionally, before and after the three blocks, a pre and a post assessment block was performed. Those blocks consisted of 3 trials each, following the same structure as the learning blocks.

Closed-loop PES

Given that the previous study has showed that PES could excite the sensorimotor system if delivered in the right phase of sensorimotor oscillations, we implemented a closed-loop algorithm that extracted the phase of alpha oscillations over the motor cortex and delivered PES accordingly. Thus, when the alpha oscillations were detected to be in the depolarising phase, a triplet of PES stimulations was delivered (Zanos et al., 2018**)**. This triplet was delivered with an interstimulus interval of 100 ms, so that stimulations hit the consecutive depolarising phases (assuming no phase drift occurs within the period). The stimulation was delivered on the extensor digitorum and at the sensory threshold intensity.

Behavioural analysis

To assess the learning that occurred during the training session, we calculated the number of successful full sequences within each block. Additionally, we also calculated the number of successful tapping transitions. To do that, we calculated, within each attempt, how far in the sequence the subjects got before making a mistake. Once a mistake is detected, the sequence is considered as restarted and the algorithm looks for correct transitions, starting again from the first digit of the sequence. To evaluate the learning change within the session, both metrics were calculated for Pre and Post blocks and the relative change (calculated as (Post-Pre)/Pre) was computed for each experimental group.

Lastly, to investigate how the learning occurred during the training, these metrics were also computed for each of the three training blocks independently. Here, to assess the learning that occurred in each training block we computed the behavioral metrics for the first and last 20% of the trials withing each block. Then we calculated the relative change for each block, following the methods used before.

Physiological data analysis (SEP)

We subsequently wanted to investigate whether the closed-loop PES resulted in any changes on the excitability of the sensorimotor cortex. We characterized the excitability of the sensorimotor cortex as the amplitude of the N20 potential, calculated from the pre and post SEP recordings. Thus, we calculated the relative changes of N20 amplitude for each experimental group and compared those values with an independent samples t-test.

2.3.2.3 Subliminal PES for home rehabilitation

The aim of this final part of Study 3 is to evaluate the viability of a closed-loop ultra-low-sensory stimulation to improve motor rehabilitation in stroke patients. To investigate that, ultra-low-sensory stimulation was delivered on the median and ulnar nerves, while the patients were performing rehabilitative exercises with their paretic limb. This study was part of a European project in collaboration with two partners (Bitbrain, Spain and Promotion Software, Germay).

Experimental design

In this pilot study, a repeated measures experimental design was used to assess whether closed loop ultra-low sensory stimulation can be used to enhance motor rehabilitation in stroke patients. Thus, all the subjects were assigned to the same experimental group, in which the PES was delivered based on the ongoing brain activity of the subjects. Although the number of subjects (2) is insufficient to statistically validate our main scientific hypothesis, this pilot study allowed us to test the viability of the system as well as to get feedback from the subjects on the usage of the rehabilitative system, ergonomics and its overall acceptance.

Before and after the two months, the patient was invited to our lab to conduct the evaluation, where clinical scales, as well as usability, ergonomics and satisfaction questionnaires were measured. After that, the patients were able to use the system autonomously at their homes. During the first days, daily phone calls were made to interview the patient, solve questions about the system and make sure that everything worked as intended. During the whole process, the participants and their caregivers were able to contact (via phone call or message) a member of the lab at any time to solve any question or report any issues with the system.

Experimental setup

In the project, together with our partners, we designed a fully portable system that included EEG, inertial recordings (IMU) and PES. To analyze the signals and store the data, a computational unit and a battery were included in a small side bag that the subject carries. To record the EEG signals, we used a portable dry-electrodes EEG HeroTM system (Bitbrain, Spain). Additionally, the system included a programmable neuromuscular stimulator Bonestim (Tecnalia, Serbia) and a bracelet that included electrodes to deliver the PES as well as an IMU sensor. The EEG and IMU data was processed in a Raspberry Pi 4 Model B, that stored the data and generated the outputs for the PES to be delivered. The system was extensively tested (technically and ergonomically) on healthy participants before the field evaluation at home started.

Lastly, a serious game was developed to engage the patients in the rehabilitation process. For that, a tablet was provided to the subject for the duration of the experiment. The game generated cues for the rehabilitative exercises and provided feedback on the quality of the movements. These movements were designed in collaboration with a physiotherapist and customized for every subject after the first session. We also designed a therapist interface so that the therapist could access information on quality of the movements and number of repetitions performed. This information was used by the therapist to track the progress of the patient and to update the exercise list after some weeks of training.

Rehabilitative intervention

The game provided a battery of upper limb movements to perform. Those movements, and the feedback about them, were presented via visual feedback in the tablet/laptop. As we described before, this list of movements was designed in collaboration with physiotherapists with experience in rehabilitation of stroke patients. Subjects were able to start and stop the intervention anytime during the day. However, they are encouraged to use the system for at least 20-30 minutes per day. Once the training was finished, the participants could move to a "subliminal" use of the system, in which they continue wearing the system during their daily life activities. In this mode of use, the system continued delivering ultra-low PES based on their detected arm movements and EEG activity. Additionally, some extra points in the game were provided. This subliminal use was limited to 1 hour maximum each day.

Outcome measures

Both before and after the rehabilitative intervention, we collected information about the ergonomics of the system, the time that it took the subjects to set the system working and the overall feel. Clinical scales like Ashworth, Fugl-Meyer and Broetz scales were also performed. Additionally, to evaluate the acceptance of the system, we analyzed the number of sessions that patients used the system at home, the length of the rehabilitative sessions, the quality of the IMUs and EEG data, and the duration of the setup time.

2.3.3 Results

2.3.2.1 Modulation of the excitability of the sensorimotor system

Modulation of somatosensory excitability during motor activation

We first evaluated the differences between the rest and active conditions for the N20 and N30 potentials. The results show significantly smaller N30 potentials, but not N20, during movement, when compared to the rest condition (paired samples t-test, $P < 0.001$ and $P = 0.44$ respectively).

Changes of somatosensory excitability after a motor task

We next investigated the relationship between the motor task length and the changes in excitability. Here, we found a significant positive relation between the amount of movement trials performed and the N30 amplitude during the rest condition ($p = 0.042$). Interestingly, during the movement condition, this correlation was also significant, but negative ($p=0.026$; Figure 9d). We did not find any correlation for N20 potential (see Figure 9c).

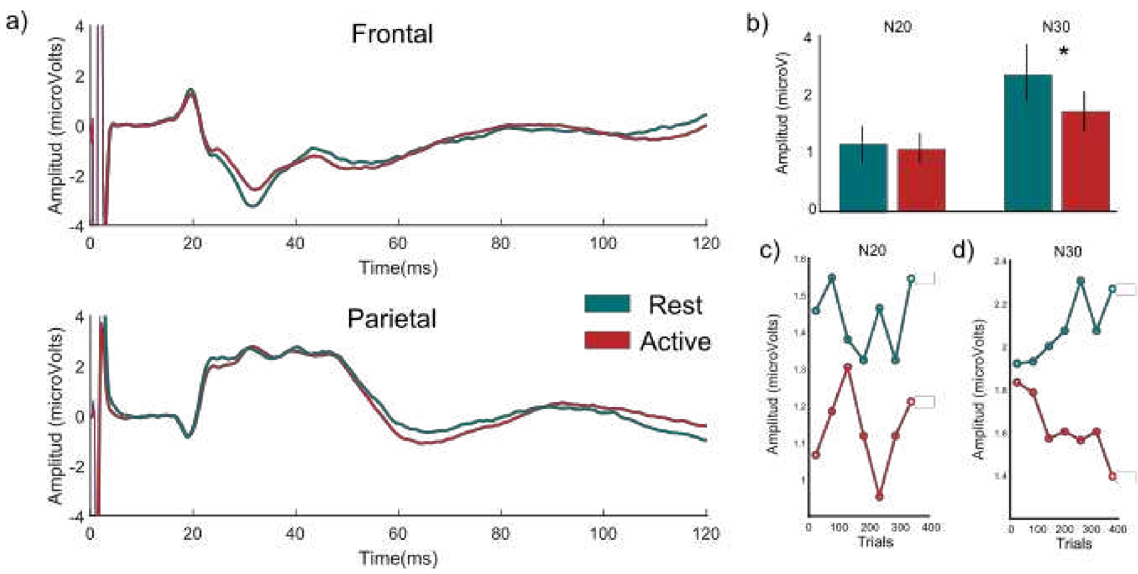

Figure 8 Sensory gating. (a) Somatosensory evoked potentials for frontal and parietal contralateral cortex during rest (blue) and active condition (red). (b) Amplitude of N20 and N30 potentials for rest and active conditions. (c) N20 potential for every 100 trials of experiment for rest (blue) and active (red) condition. (d) Same as (c) but for N30 potentials.

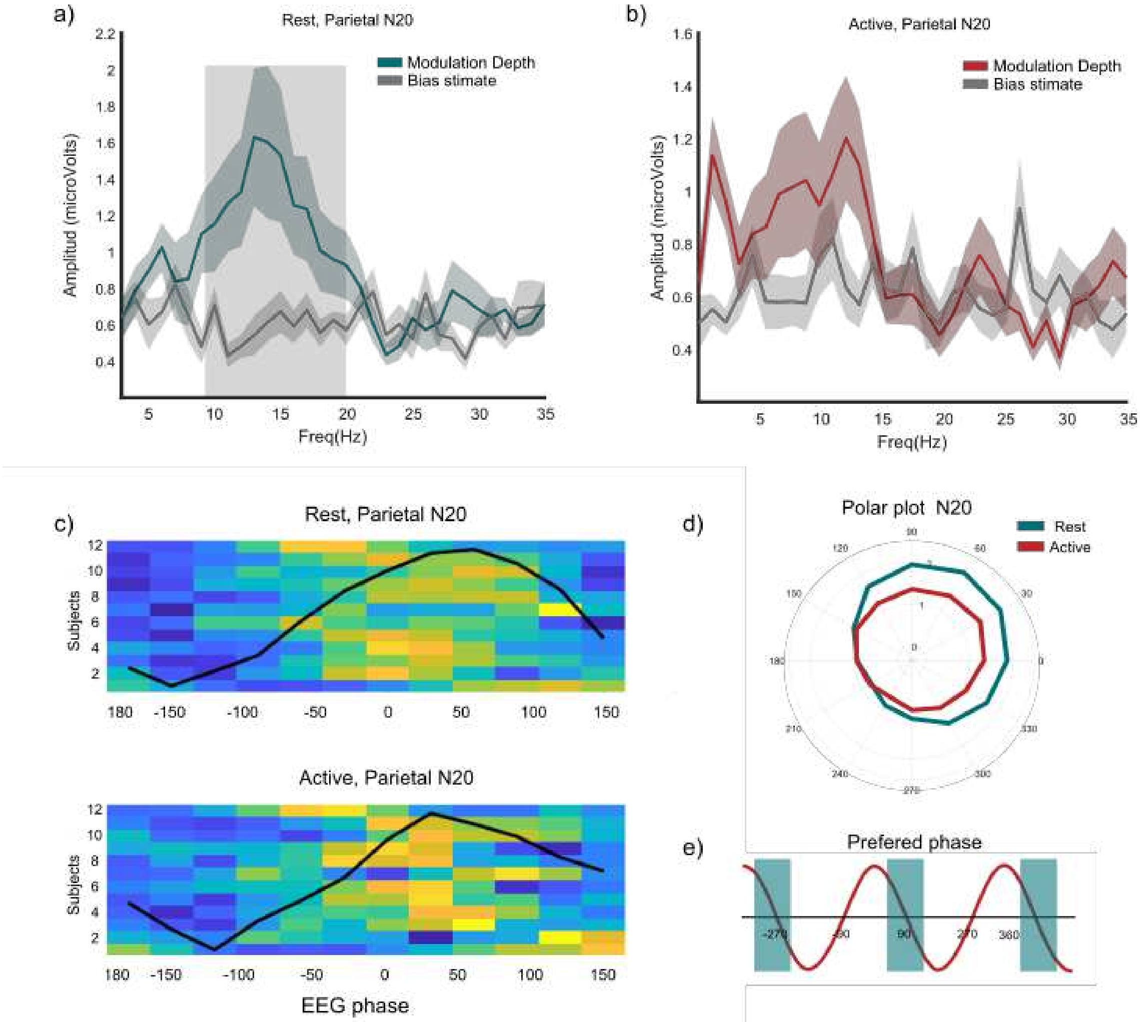

Figure 9. Modulation of excitability of the sensorimotor system. (a) Modulation of the N20 potentials by cortical oscillation within the frequency range 3-35 Hz (x-axis) during the rest condition. Blue line represents the mean and s.e.m. of the modulation depth, and dark grey line the bias estimate. Grey area indicates significant differences between Modulation depth and bias estimate for a specific cluster of frequencies. (b) Same as a) but during the active condition. (c) Modulation of N20 by cortical oscillations (12 Hz) during rest and active condition for every subject. Color plot shows the N20 modulation for each subject (y-axis) as the amplitude of N20 (yellow representing the higher values and blue the lower) based on the pre-stimulation phase (x-axis). The black line shows the grand average of the N20 modulation 12 Hz for all the subjects. (d) Polar plot of the N20 modulation by cortical oscillations (12Hz) for rest and active conditions. (e) Preferred phase of stimulation. It shows, blue area, the pre-stimulation phase that evoked a higher amplitude N20.

Cortical modulation of somatosensory excitability during rest and during movement

Finally, we studied whether pre-stimulus cortical oscillation is related to the modulation of the excitability of the sensorimotor system. During rest, we found a significant modulation of the N20 potentials by cortical oscillations between 8 and 20 Hz (see Figure 10a). During the movement condition, we did not find any significant modulation of N20 for any of the frequencies studied (see Figure 9b). The N30 potentials did not show any significant modulation for any of the conditions (rest and active). However, given that we observed a change in N30 amplitude during the experiment, we wanted to assess whether the modulation was also changed throughout the experiment (Figure 10). As expected, no differences in modulation could be found for N20 modulation between the early and late stimulations, first and last 20 minutes (Figure 10a). Interestingly, in the early stimulations, the N30 potential showed a significant modulation during the active condition for the frequencies between 8 and 16 Hz. This modulation could not be found for the late stimulations, where subjects had already performed at least 20 minutes of isometric contractions.

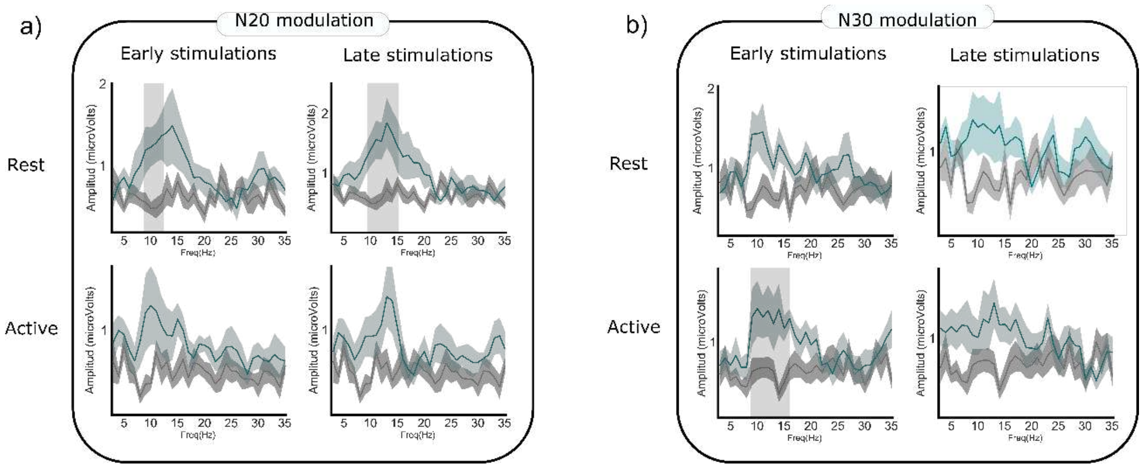

Figure 10. Changes in the modulation of somatosensory excitability. (a) Modulation of N20 potential in the first and last half of the experiment, Early and Late stimulations respectively, (columns) during the rest and active conditions (rows). (b) Same as (a) but for the modulation of the N30 potential.

2.3.2.2 PES to boost motor learning

We found no significant differences in performance between the Control and Intervention for the successful transitions or the number of successful sequences (Figure 11c). When comparing between conditions withing the 3 blocks of training data, no differences could be found in the learning of each block.

The physiological data revealed no significant differences in the N20 changes (pre to post) between the intervention and the control group (p = 0.09, independent samples t-test; Figure 11b).

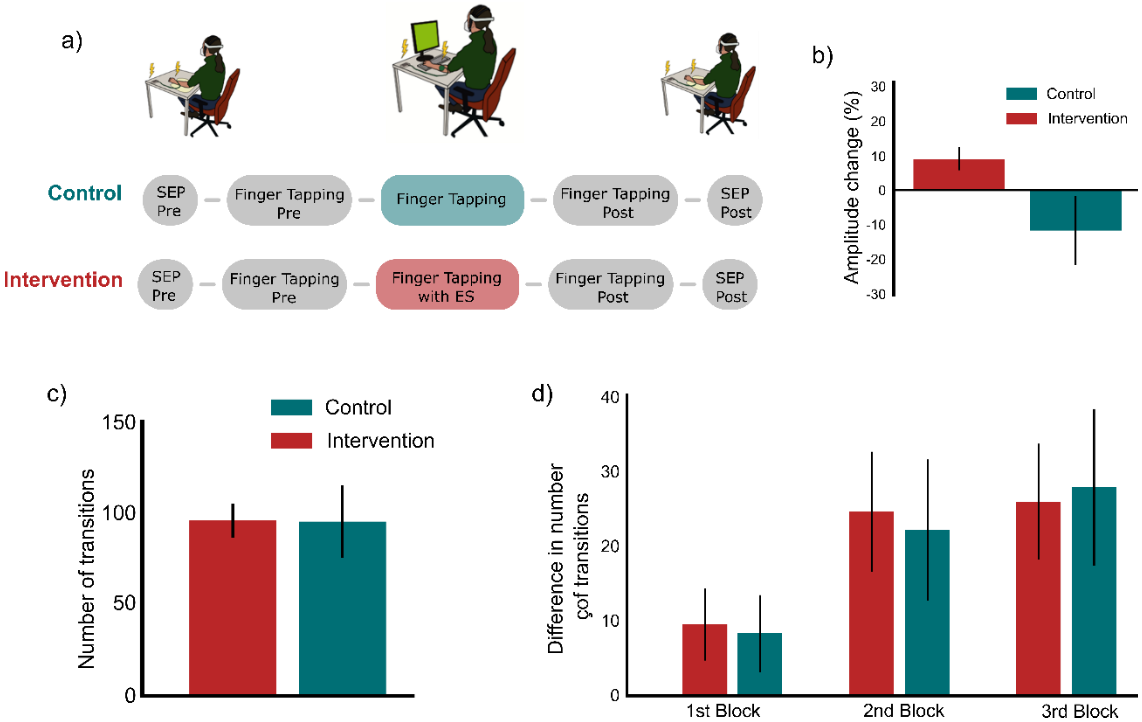

Figure 11. Peripheral electrical stimulation to boost motor learning. (a) Experimental protocol. (b) SEP change. It shows the change, as 100(Post-Pre)/Pre, of the N20 potentials for the control (blue) and intervention (red) groups. (c) Performance of the Finger tapping post training assessment for the control (blue) and intervention (red) condition as the number of successful typing transitions. (d) Difference in number of successful transitions withing for each of the three training blocks, calculated as the change between the first and last 20% of the block.*

2.3.2.3 Subliminal PES for home rehabilitation

The usability evaluation revealed positive ergonomic scores for our system. Both patients found the system easy to set up and comfortable to use. While overall acceptance was high, a common concern centered around the cable connecting the bracelet to the backpack, which was seen as somewhat inconvenient. This feedback provides valuable insights as we continue to refine our system for improved user experience. Another important factor was the setup of the EEG headset because patients had to be able to set it up themselves without any external help. As shown by the questionnaires, in both cases the participants felt comfortable about using the headset and found the instructions clear and easy to learn. In fact, the setup time for each patient was measured at 3.1 ± 2. 5 and 7.46 ± 14 minutes, respectively. We consider these times as adequate and realistic for a home environment use of the system.

The questionnaire about usability of the system showed that subjects felt “highly confident” about setting the system up without any external help. Indeed, after the two months, subjects reported that they were able to finish the setup process in less than 10 minutes, something we confirmed in the post session, when they repeated the whole process with us in the lab. When analyzing the data generated by their patients during the use of the system at home, we could confirm that setup time was 3.1 ± 2. 5 and 7.46 ± 14 minutes for each subject.

The acceptance of the system and the rehabilitation approach was also high as shown by the feedback received by the patients as well as the usage data. Thus, patients actively used the system for a total of 51 sessions (16 and 35 sessions each). Withing these sessions, the participants were training for 15. 7 ± 5.3 and 23.6 ± 11.3 minutes respectively. Lastly, the subliminal mode was used for an average of 10 ± 4.6 minutes after the training sessions.

No changes were found in Fugl-Meyer or Ashworth scales. However, one subject showed improvement in the Broetz score, a more functional and sensitive scale presenting an improvement from 19 to 29 pre to post intervention (Broetz et al., 2014).

2.3.4 Discussion

The integration of peripheral electrical stimulation (PES) into BMI-based rehabilitation interventions represents a promising alternative to enhance the outcome of rehabilitative interventions. Thus, a system that promotes the use of the paralyzed limb into daily life activities, while incorporating closed-loop PES would both increase the amount of training while exciting the sensorimotor system, thus creating optimal conditions for plasticity-driven rehabilitation. In this study, we investigated the interaction between PES and sensorimotor cortical oscillations to take advantage of the endogenous modulation of the excitability of the sensorimotor system. After that, we evaluated the if the increase in excitability could be translated into behavioural outcomes (i.e., motor learning in healthy participants and clinical improvement in stroke patients). Finally, we tested a home-based closed-loop PES rehabilitative system that allowed patients to train at home while optimizing the excitability of the sensorimotor system to help rehabilitation. This line of research holds a great potential for rehabilitative BMIs for stroke, where the sensorimotor integration is compromised.

Modulation of the excitability of the sensorimotor system

Sensory gating is a well-studied neurophysiological process where the central nervous system actively attenuates the processing of sensory inputs and plays an important role of sensorimotor integration. In our study, we compared the excitability of the sensorimotor pathways during a motor task and a resting state. In line with previous findings, we found a sensory gating effect during the motor task with significantly lower N30 potentials (Figure 8b). Interestingly, we did not find a significant attenuation of the N20 potentials, which is also in line with previous sensory gating studies that showed no gating effects on N20 (Akaiwa et al., 2023; Kirimoto et al., 2014; Lei & Perez, 2017). This idea is supported by the fact that N20 is generated in areas of the primary somatosensory cortex, without connexions to the primary motor cortex (Macerollo et al., 2018). On the other hand, N30 represents early somatosensory input into non-primary motor areas with potential oscillatory contributions from primary motor cortex and prefrontal cortex and has been previously reported to show modulation during movement preparation and execution, suggesting the interaction between somatosensory processing and motor control in non-primary motor areas (Macerollo et al., 2018).

This modulation of the sensorimotor excitability could be further investigated in this study by analysing if, and how, pre-stimulus cortical oscillations are related to the excitability of the sensorimotor pathways. We did that both for rest and motor condition. Interestingly, we could only find a significant modulation effect for the rest condition and only on N20, but not on N30 potentials. Here, we found that N20 potentials were significantly modulated according to the phase of the cortical oscillations of the motor cortex that include alpha and low beta activity (8 – 20 Hz). The fact that N20 was modulated during rest but not during motor execution might suggest that during rest intervals (intercalated between movement periods), subjects are in an "alert" preparatory state that might involve integrating sensory information with motor planning. Thus, N20 is primarily originated in S1, and has been linked to this integration between sensory processing and motor planning (Lei & Perez, 2017).

On the other hand, N30 showed no modulation during rest or movement conditions when analysing the 40 minutes of experimental data as a whole. An important factor that could result in lower modulation during the movement task that should be considered, especially for future experiments with patients, is the lower signal-to-noise ratio during the motor task (with lower amplitude of alpha and beta oscillations due to ERD and higher occurrence of movement and muscle artifacts), that could have resulted in a less accurate phase estimation. However, a more interesting explanation for this lack of N30 modulation is the dynamic nature of the N30 gating. Previous studies have linked N30 gating with the degree of movement skill, suggesting this dynamic nature of the degree of gating, linked to the learning stage (Akaiwa et al., 2022). In our protocol, subjects are required to perform an isometric contraction of the flexors to keep the angle of a pressure-sensitive between a certain range. This task, although it is not a typical motor learning task, did require for the subjects to learn how to keep the angle withing this range for the 30 seconds of each movement interval. Thus, we decided to analyze the N30 gating and its modulation after dividing the data in two blocks, thus being able to explore the differences in the early and late learning blocks (first and second half of the dataset). In line with previous studies like Akaiwa et al 2022, we found a greater modulation of N30 during the late block of movement, suggesting a more refined motor execution (Akaiwa et al., 2022, 2023).

Earlier stages of motor learning heavily rely on premotor area (PMA) activation when somatosensory information feedback is more important, whereas in later stages of motor learning, when switching to a more automatic performance, the supplementary motor area (SMA) shows greater activation (Jenkins, et al., 1994). In this line, it has been shown that during motor learning, changes in S1 excitability precede those in M1 (Bernardi et al., 2015; Ohashi et al., 2019). This transition from a preference of S1 during early stages of learning would support the heavier modulation of N30 potentials by cortical oscillations between 8-16 Hz that we found only in the first half of the experiment (Figure 10b), suggesting an increased integration of sensory information in these early stages of motor learning.

For that frequency range (8-16 Hz), we found that when the PES was delivered in the depolarizing phase, the stimulation resulted in a larger potential, indicating a state of higher excitability. On the other hand, when the stimulation was delivered in the hyperpolarizing phase, we found a smaller evoked response. These results go in line with a growing body of research that showed that TMS stimulation, when delivered on the depolarizing phase of the ongoing sensorimotor alpha rhythms, lead to larger motor evoked potentials (Zrenner et al., 2018b). Altogether, these results suggest a cumulative effect on N30 only, which indeed is the potential related to M1 neural activity. Some studies have previously reported the change of both N20 and N30 after movement execution suggesting that this inhibitory/excitatory effect has cumulative effects resulting in short-term plasticity-like changes (Andrew et al., 2015; Dancey et al., 2016; Haavik & Murphy, 2013).

PES to boost motor learning

Based on these findings, we implemented a closed-loop PES that increases the excitability of the sensorimotor system and evaluated whether this could be translated into behavioral results. Although, we ultimately wanted to use the system for motor rehabilitation of stroke patients, we first designed an experiment in healthy participants where motor learning was used as a “healthy” surrogate of motor recovery in stroke patients. Here, we compared the motor learning between two groups of healthy subjects, one that received closed-loop PES during training and one that received no PES. Interestingly, although we could find a significant trend that suggests higher excitability of the sensorimotor system for the subjects that received PES, we did not find any difference in motor learning performance.

As revealed in our offline analysis, PES was consistently delivered on the depolarizing phase of the sensorimotor rhythms during the training. However, the stimulation of the phase heavily depends on the quality of the data. In the context of our experiment, subjects were performing a finger tapping task and freely moving during the task. This, as we studied in Study 1, has a great impact in the SNR of the EEG signal. Additionally, as we discussed above, during the movement ERD is produced and the amplitude of both alpha and beta oscillations is reduced, making the phase estimation even harder in a noisy environment. Altogether, these factors result in a PES that, although delivered predominantly on the depolarizing phase, is quite spread along the phase of the SMR. Thus, one potential hypothesis for the lack of behavioral results could be the cancelling effect produced by stimulations being accidentally delivered on the hyperpolarizing phase.

Further investigation is needed to confirm whether these technical challenges are behind our results. Some factors, like the quality of the signal, can be improved in a more controlled experimental setup. However, we wanted to evaluate our scientific hypothesis in a more realistic scenario, since the next step in our project was to test it on stroke subjects while they perform rehabilitation at home. In any case, a more controlled experimental setup that ensures a more precise PES might be necessary. Additionally, an additional experimental group where subjects receive the stimulation on the hyperpolarizing phase would help to better evaluate the effect of PES on excitability and motor learning. Lastly, in our experiments PES was delivered during the training as we ultimately want to apply this approach to a BMI-based rehabilitation for stroke patients, where the link between brain activation during movement and feedback is crucial. However, our previous study showed that the excitability of the sensorimotor system is modulated mostly during rest. Thus, a potential alternative would be to apply the closed-loop PES during rest, before the training occurs, exciting the sensorimotor system potentially enhancing the learning capabilities of the system, in an even more practical and ergonomic fashion.

Subliminal PES for home rehabilitation

The closed-loop PES system used in the previous study was then evaluated in a rehabilitative scenario. This evaluation included the participation of two severely paralyzed stroke patients, who were actively engaged with the system at home for a period of two months. It is important to note that, although the sample size in the study is small, the results represent an important step towards a home-based rehabilitation system that optimizes PES to boost rehabilitation.

The evaluation of the usability showed very positive feedback about the ergonomics and usability of the system. Both patients found the system easy to set up and comfortable to use. While overall acceptance was high, some minor issues were reported (like the inconvenience of a cable connecting the bracelet to the backpack). This feedback provides valuable insights as we continue to refine our system for improved user experience in the future. Another important factor was the setup of the system since patients had to be able to set it up themselves without any external help. Both participants felt comfortable about using the headset and found the instructions we provided clear and easy to learn. In fact, we could see that the setup time for each patient was measured at 3.1 ± 2. 5 and 7.46 ± 14 minutes, respectively. We consider these times as adequate and realistic for a home environment use of the system.

The favorable response to the system could also be seen in the usage of the system. Indeed, both participants actively engaged in numerous sessions, with a combined total of 51 sessions (16 and 35 sessions each). Each session extended to 15.7 ± 5.3 and 23.6 ± 11.3 minutes respectively, showing a clear commitment to integrate the system into their daily routines. Another innovative feature of the system was its subliminal mode, that allowed participants to engage in rehabilitation subtly throughout the day. This feature was used for an average of 10 ± 4.6 minutes daily. Altogether, this data highlights the system's potential to be integrated into patients' daily lives, potentially promoting consistent and valuable engagement in their rehabilitation routine.

Even though no changes could be found in Fugl-Meyer or Ashworth scales, one of the subjects showed a notable improvement in the Broetz score, indicating a positive response to the intervention (Broetz et al., 2014). However, it's important to be very cautious in interpreting this result considering the limited sample size. In conclusion, while the current findings are preliminary and limited by the small sample size (but very intensive, longitudinal-like design), the early results obtained from the participation of two patients are very promising. Additionally, the feedback obtained represents a vital first step towards a more mature home-based rehabilitation system.

3 Conclusions and general discussion

The studies conducted in this thesis constitute the first steps towards a rehabilitative system that includes PES and sleep. First, they show the challenges and biases that EEG analyses face in a visuomotor task, what is of upmost importance for a rehabilitative BMI system that relies on the contingency of the link between brain activity and afference. In this line, optimizing the afference produced by a BMI system has the potential to further improve the outcome of a rehabilitative intervention.

In this thesis, we have confirmed the top-down regulation of the excitability of the sensorimotor system and build a system that delivers PES so that the afference y synchronized with states of higher excitability, potentially enhancing the plasticity effect of the BMI. Behavioural results could not confirm this hypothesis in healthy participants, so further investigation is needed. Additionally, we designed, implemented, and tested on stroke participants a home-based rehabilitative BMI for stroke patients that relies on PES and includes a serious game to further engage the patients. This pilot study provided unvaluable feedback and constituted a great step towards a more mature system that can be used in a clinical by a larger population of patients.

Before that, some additional studies might be needed to elucidate why the closed-loop stimulation did not show any effect on the behavioural results. For that, an experiment that includes two control groups: one with PES on the depolarizing phase (higher excitability state), one with PES on the hyperpolarizing phase (lower excitability state) and one with no stimulation would give us a better understanding of the neuroscience behind the behavioural results. Another alternative that could be explored is to deliver

PES during rest, before the training occurs, since our study showed a significant modulation of the excitability of the sensorimotor system only during rest. This way, we might use the periods in right before the training to excite the sensorimotor system for the upcoming training period. Interestingly, this approach could be tested for any type of training, and it is not necessarily restricted to BMI rehabilitation, since it does not rely on the link between afference and brain activation during the training.

Lastly, the memory consolidation processes that occur after BMI have been explored in this thesis both in healthy and stroke participants. We also implemented a closed-loop system to trigger TMR during sleep that aims at enhancing memory consolidation. The results of this study, although preliminary, are promising and encourage us to continue developing a sleep interface that improves the memory consolidation processes that occur after a BMI-based rehabilitation system for stroke. A larger study on healthy participants, ensuring that neuroprosthetic learning occurs, is necessary to investigate the effects of TMR on this type of memories before it is applied in a clinical study with stroke participants.

In summary, the findings in this thesis suggest the potential for an ideal rehabilitation system that integrates brain-machine interfaces (BMIs) with closed-loop PES, to enhance the activity-dependent plasticity effects that occur during a BMI training, and targeted memory reactivation during sleep, to help the impaired sensorimotor system consolidate those memories.

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5 Statement of contributions

The dissertation work was carried out under the supervision of Dr. Ander Ramos-Murguialday. The people involved in each of the studies contained in this dissertation are detailed below. I confirm that I wrote the dissertation myself under the supervision of Dr. Ander Ramos-Murguialday.

Study 1. The following people participated in the study: Carlos Bibián, Nerea Irastorza-Landa, Monika Schönauer, Niels Birbaumer, Eduardo Lopez-Larraz and Ander Ramos-Murguialday. I designed the experiment together with ARM and ELL. I collected the data. I analyzed the data with the supervision of ELL, MS and ARM. ELL, ARM, NIL, MS, and NB helped with the interpretation of the results.

Study 2. The study was performed with the help of Elaina Bolinger, Julia Carbone, Fridos Bouraima, Yannina Siré, Andreas Ray and Ander Ramos-Murguialday. I designed the experiment together with ARM, EB, and JC. I collected the data together with JC, FB, YS, AR, and EB. I analyzed the data with the supervision of JC and ARM.

Study 3. The following people were involved in the study: Karolina Poczopko, Carlo Caruso, Wala Jaser and Ander Ramos-Murguialday. I designed the experiment together with WJ and ARM. I collected the data together with KP, WJ and CC. I analyzed the data with the help of CC and KP and the supervision of ARM.

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