A different state of mind: neural activity related to volitionally up- versus downregulating cortical excitability

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Abstract

To date there exists no reliable method to non-invasively upregulate or downregulate the state of the resting motor system over a large dynamic range. Here we show that an operant conditioning paradigm which provides neurofeedback of the size of motor evoked potentials (MEPs) in response to transcranial magnetic stimulation (TMS), enables participants to self-modulate their own brain state. Following training, participants were able to robustly increase (by 83.8%) and decrease (by 30.6%) their MEP amplitudes. This volitional up- versus downregulation of corticomotor excitability caused an increase of late-cortical disinhibition (LCD), a read-out of presynaptic $GABA_B$ disinhibition which was accompanied by an increase of gamma and a decrease of alpha oscillations in the trained hemisphere. This approach paves the way for future investigations into how altered brain state influences motor neurophysiology and recovery of function in a neurorehabilitation context.

Introduction

Rhythmic oscillatory brain activity at rest is associated with high versus low neuronal responsiveness, or 'excitability' of a region [1,2]. Measuring these momentary fluctuations of neural activity via electro- or magnetoencephalography (EEG/MEG) over human primary motor cortex (M1), it has been demonstrated that frequency, amplitude and phase of the ongoing oscillation cycle systematically modulate responses evoked by transcranial magnetic stimulation (TMS) [3-5] [6-8]. In particular, it has been shown that corticomotor excitability is significantly higher when the power (amplitude) of sensorimotor rhythms in the alpha band (8-14 Hz, also called the 'mu'-rhythm), or beta band (15-30 Hz) are low, or when M1 is stimulated during the trough of the oscillatory cycle of these rhythms [9]. This concept has inspired neurofeedback interventions whereby, for example, stroke patients learn to volitionally desynchronize sensorimotor rhythms with the goal of bringing the

sensorimotor system into a more excitable state as a precursor for enhanced neural plasticity and accelerated recovery [10-12].

Previous research has focussed on interactions between corticomotor excitability and cortical dynamics at rest, but much less is known about whether it is possible to *voluntarily* control the excitability of sensorimotor circuits while keeping motor output and sensory feedback constant. In the case of stroke rehabilitation, this mechanism may become particularly relevant as patients are unable to move or receive sensory feedback from the paretic limb. Therefore, interventions that optimally harness the residual ability to voluntarily and endogenously activate relevant brain circuits in the days and weeks early after the incident, may provide the crucial innervation necessary to promote re-wiring for functional recovery [13].

It is well known that primates [14,15], and humans [10,eg. 16-19] can gain volitional control of neural activity by receiving neurofeedback via a brain-computer interface (BCI). Here, we used a BCI-neurofeedback approach as an effective method for training participants to both volitionally upregulate and downregulate corticomotor excitability as reflected by the size of TMS-evoked motor potentials (MEPs), with the aim to modulate their amplitudes over a much larger dynamic range than observed during rest. Using this approach enabled us to investigate the neural mechanisms that underlie volitional up- versus down-regulation of corticospinal excitability in the motor system and the associated oscillatory signatures. By modulating one neural marker, i.e. motor evoked potential amplitude, while measuring independent modalities using EEG, or paired-pulse TMS, this approach allows us to causally relate voluntary rather than incidental changes of corticomotor excitability to cortical dynamics.

To achieve this goal we developed a BCI by stimulating the cortex with TMS and providing neurofeedback of MEP amplitudes (Fig. 1). The feedback was designed such that participants were rewarded for larger than average MEPs in one condition, and smaller than average in another condition.

In a within-subject cross-over design, participants performed four training sessions with TMS-neurofeedback of MEP amplitudes in order to learn how to up- and downregulate their corticomotor excitability (two ‘UP’ sessions, two ‘DOWN’

sessions, order counterbalanced across participants). After the training we characterised the neural underpinnings of these two distinct activity states in detail by conducting a series of multimodal experiments using EEG and paired pulse TMS to profile the associated oscillatory and neurophysiological processes. As it has been proposed that dynamic modulation of neuronal activity is realized via synchronization of high frequency rhythms [20] which are tightly coupled to desynchronizing sensorimotor rhythms [21], we hypothesised that Gamma synchronisation (31-80Hz) and alpha (8-13Hz) /beta (14-30Hz) desynchronization play a critical role in actively determining the state of the motor cortex.

RESULTS

Bidirectional changes in corticospinal excitability were observed in the MEP neurofeedback group but not in a control group.

We first tested whether participants could learn to volitionally increase or decrease corticomotor excitability when using a motor imagery strategy shaped by neurofeedback of MEP amplitudes. Across two training sessions, we found that MEP amplitudes increased during UP training (Fig. 2A, orange symbols) and decreased during DOWN training (Fig. 2A, blue symbols) relative to the baseline measurement (BS), revealing a significant dissociation over time (*neurofeedback type* x *block number* interaction during training session 1 [$F(4,115.9)=3.87$, $p=0.006$], session 2 [$F(4,125.0)=3.7$, $p=0.007$] and EEG session [$F(2,70)=6.9$, $p=0.002$], *F tests following mixed effects models*, $n=15$; see supplementary Fig. 1 for additional analyses). Since MEP amplitudes are a compound measure of excitability influenced by multiple neural elements [22], including background muscle activity [23,24], we repeated this analysis using the root mean squared (rms) background muscle activation (EMG) recorded in the 100ms prior to each TMS pulse. Importantly, this control analysis revealed no such interactions on any of the sessions, suggesting that the observed modulation was not driven by changes in activity of the target muscle, nor any of the additional 3 control muscles (OP, ADM, left FDI) (all $p>0.18$, see Supplementary Table 2).

In order to isolate the effect of the neurofeedback, we included a control group who undertook the same protocol, using the same mental imagery strategies, but with

feedback that was not contingent on the MEP amplitudes. This group exhibited no systematic changes of corticomotor excitability across training (Fig. 2B) and mixed effects models revealed no significant *neurofeedback type* x *block number* interactions on any of the separate testing sessions in the control group (all $p>0.06$, note that statistics approached significance for the second session because MEPs were randomly higher in the DOWN than in the UP condition ; see Supplementary Fig. 1B for further details). Additionally, there were no significant differences in background EMG (All p>0.09, Supplementary Table 2). Next we compared the performance of the experimental and the control group, by normalizing MEP amplitudes to baseline (% change) and calculating the difference between UP and DOWN (Fig. 2C). The differences were substantial in the experimental group, who exhibited on average MEP amplitudes twice as large during UP than during DOWN, and differed significantly from the control group where systematic differences were virtually absent (effect of 'Group' [$F(1,25.6)=13.32$, $p=0.001$], *F tests following mixed effects*, $n=28$). The effect sizes (Cohen's d) of the between-group differences were small for the first two blocks (<0.5), but consistently increased during training (d= 1.27 for block 8), and remained high in the two blocks of the EEG session ($d>0.97$). As the control group were executing the same mental imagery strategies as the experimental group, this comparison demonstrates that veridical TMS neurofeedback was essential for gaining volitional control over corticomotor excitability.

Neurofeedback training effects are retained for at least 6 months

In a follow-up investigation approximately 6 months following initial neurofeedback training, we showed for a subset of the participants (n=11) that they had retained the ability to upregulate and downregulate their MEP amplitude with neurofeedback (Fig.3; significant effect of neurofeedback type (UP vs DOWN) in a retention block carried out with no top-up training ($F(1,10)=6.64$, $p=0.028$). Measurements of resting MEP amplitude taken 5 and 10 minutes following the retention block indicated no after-effects (all p>0.2) indicating that subjects could acutely control corticomotor excitability without long-lasting after-effects. Having verified that the ability to modulate brain states had been robustly retained, we next tested whether participants could sustain this performance even when feedback was removed. Performing a feedback-free block, we found that MEP amplitude was significantly larger in the UP versus DOWN condition ($F(1,10)=12.32$, $p=0.006$), indicating that when participants

have reached a sufficient level of training they have optimised their mental strategies and no longer require continuous feedback.

In order to measure whether the feedback-induced changes in corticospinal excitability were specific to the hemisphere targeted by the neurofeedback, we used two TMS coils simultaneously and performed one block of 40 trials, where half of the TMS pules were applied to the left hemisphere (i.e. the usual feedback hemisphere), and the other half to the right (i.e. the opposite hemisphere). We found that the same pattern of upregulation and downregulation of MEP amplitudes was observed in the opposite hemisphere, an effect that approached significance ($F(1,20)=4.032$, $p=0.07$) but was much smaller than in the neurofeedback hemisphere particularly for the UP condition (UP $d = 1.01$ for neurofeedback hemi, $d=0.27$ for opp. hemi, DOWN $d=0.40$ for neurofeedback hemi, $d=0.35$ for opp. hemi, Fig. 3).

Paired pulse TMS investigation of mechanisms

Finally, we investigated which excitatory/inhibitory circuits may have contributed to the changes in corticomotor excitability, using paired pulse TMS measures of three different neurophysiological processes: (i) Short-interval intracortical inhibition (SICI), believed to reflect postsynaptic $GABA_A$ inhibition [25]; (ii) Long interval intra-cortical inhibition (LICI), considered as a marker for postsynaptic $GABA_B$ inhibition; and (iii) late-cortical disinhibition (LCD), which is thought to measure presynaptic $GABA_B$ disinhibition, and manifests as a period in which MEP amplitude returns to and typically overshoots baseline levels, in a time window following LICI (~220ms after a suprathreshold conditioning TMS pulse) [26-28]. In the following analyses we determined the time point (baseline vs during NF) x neurofeedback type (UP, DOWN) interaction and applied FDR correction for multiple testing. Single pulse MEPs collected during these measurement blocks (25% of all trials) revealed significantly larger MEP amplitudes for the UP than the DOWN condition, replicating the findings of the main experiment (Fig. 4A; significant time point x neurofeedback type interaction: $F(1,27.67)=14.36$, $p_{FDR}=0.001$). Surprisingly, there were no significant differences in the magnitude of SICI (% of single pulse MEPs) between the resting baseline data and the SICI MEPs collected during neurofeedback, nor

between the UP versus DOWN states (time point x neurofeedback type interaction [$F(1,28.31)=0.08$, $p_{FDR}=0.77$]). The same was true for LICI (time point x neurofeedback type interaction [$F(1,28.90)=0.02$, $p_{FDR}=0.88$]). Thus, circuits controlling postsynaptic inhibition did not seem to be differentially modulated by the UP versus DOWN state. However, for LCD there was a significant time point x neurofeedback type interaction ($F(1,28.35)=12.09$, $p_{FDR}=0.002$, Fig.4B).

Pairwise comparisons revealed that LCD was significantly elevated in the UP condition, when compared to the baseline measurement taken immediately before neurofeedback (Fig. 4B, right panel, MeanDiff=50.9%, df=28.35, $p<0.001$) and when compared to the equivalent data recorded in the DOWN condition (MeanDiff=56.1%, df=28.76, $p<0.001$). For the DOWN condition LCD did not differ significantly from baseline ($p=0.45$).

Distinct oscillatory signatures for high versus low corticospinal excitability

As part of the initial training study (see Figure 2A, 'Ses 3' for the behavioural results), we investigated whether the two different activity states evoked differential cortical dynamics extracted from EEG recordings which were acquired simultaneously while TMS was being performed to provide neurofeedback of MEP amplitude. As distinct functions have been ascribed to 8 different sub-frequency bands across the known range of brain signals (0.1 - 80Hz), we now probed whether volitional changes in corticospinal excitability of M1, drives neural activity measured in the delta (0.1-4Hz), theta (5-7Hz), low alpha (8-10Hz), high alpha (11-13Hz), low beta (14-21Hz), high beta (22-30Hz), low gamma (31-50Hz) and high gamma (51-80Hz) bands. Using the portion of EEG data collected in the 1.5 seconds prior to each TMS pulse, we calculated relative power in the UP and DOWN states for the eight frequency bands of interest. Figure 5a-f (n=15) shows that UP- versus DOWN regulating corticomotor excitability caused reduced band-limited power in the theta and alpha band (blue areas in Fig.5b-d) while gamma power was clearly increased. (red areas in Fig.5e,f). For each participant, we extracted the information for the electrode closest to their individual motor hotspot (Figure 5a shows the different locations across participants) and calculated whether the UP-DOWN difference (Δ relative power %) deviated significantly from 0 (Fig. 5g). Wilcoxon signed rank tests revealed significantly higher power for the UP than DOWN condition in the Delta

(p=0.024, d=0.754), Low Gamma (p_{FDR}=0.024, d=0.753) and High Gamma (p_{FDR}=0.016, d=0.712) band and significantly lower power for UP than DOWN in the theta (p_{FDR}=0.003, d=0.947), low alpha (p_{FDR}=0.004, d=0.805) and high alpha band (p_{FDR}=0.007, d=0.714). Although the feedback was lateralised to MEPs from the right limb (left hemisphere motor hotspot), we also quantified the same neural oscillations at the corresponding location in the opposite hemisphere. Here, only the theta rhythm showed significantly lower power for the UP than the DOWN state (p_{FDR}<0.001, d=1.071, see Supplementary Fig. 2).

Next, we tested whether the amplitude of neural oscillations recorded at the hotspot at the time of each TMS pulse could predict the amplitude of the resulting MEPs. For each participant, MEP amplitudes of the 120 trials (60 UP, 60 DOWN) were entered as the outcome variable in a robust regression model with trial-by-trial relative power values for each frequency band as predictor variables. Regression slopes (beta values) for each participant were carried forward into a group level analysis (Fig. 5m), and Wilcoxon signed rank tests were used to establish whether the slopes were significantly different from 0 (a 0 slope would indicate no statistical relationship between predictor and outcome variable). Lower amplitude oscillations in theta (p_{FDR}=0.024, Fig.5h), low alpha (p_{FDR}<0.001) and high alpha (p_{FDR}=0.002) were predictive of larger MEP amplitudes, and higher amplitude oscillations in low gamma (p_{FDR}=0.020) and high gamma (p_{FDR}=0.020) were significant predictors of larger MEP amplitudes. In a previous study, it was reported that a strong predictor of cortical excitability was the low gamma : high alpha ratio [3]. We replicated this finding, demonstrating that this ratio was a significant predictor of MEP amplitude (p_{FDR}=0.016) with larger ratios predicting larger MEP amplitudes.

EEG data classification

We next tested whether the distinction between the two trained states was large enough that the individual data trials could be successfully predicted as 'UP' state or 'DOWN' state, using machine learning based solely on the EEG power values (relative power data, scaled by 1/f transformation) of the 8 frequency bands of interest. A linear support vector machine (SVM) was applied to the data of each participant (60 UP 60 DOWN epochs). The SVM has been shown to be particularly

powerful on EEG data, which is noisy and contains many features that are correlated. This approach additionally allowed us to perform feature selection, to quantify which EEG features most heavily contributed to the distinction between the two states. Using only data from the electrode closest to the hotspot (8 rhythms plus LowGamma:HighAlpha ratio) the SVM was capable of classifying the brain states with an average accuracy of 81.5% (±5.1%) based on 10-fold cross validation which differed significantly ($p=0.001$, $n=14$) from a null model revealed by permutation testing (accuracy null model: 49.0% ±13). Additionally, incorporating data from the same rhythms recorded at the corresponding electrode in the opposite hemisphere increased this accuracy to 85.1% (±4.6%) across participants (see Supplementary Table 1). Using feature ranking based on Recursive Feature Elimination (RFE), taking the mode of the top ranked features across participants revealed that the strongest contribution to the high classification accuracy of the latter SVM was the High Gamma rhythm in the hotspot electrode, followed by High Alpha at the hotspot, then the LowGamma:HighAlpha ratio (for full ranking order see Supplementary Table 1).

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Discussion

Here we aimed to uncover neural activity evoked by voluntarily facilitating or suppressing excitability within sensorimotor circuits, while keeping motor output and sensory feedback constant. We show that using a bidirectional TMS-neurofeedback approach is critical to gain volitional control over MEP amplitudes, a skill that is retained for at least 6 months without further training. This voluntary state-setting with a large dynamic range is causally related to modulating pre-synaptic $GABA_B$ mediated disinhibition and to a prominent increase of gamma power in sensorimotor cortex for the UP state which was accompanied by a clear reduction of power in the theta, low and high alpha bands.

Volitional control of corticomotor excitability

Previous studies have shown that it is possible to gain voluntary control over activity in the central nervous system if appropriate neurofeedback is embedded in a

reinforcement learning task, with food rewards for animals [14,15] and visually rewarding stimuli for humans [19,29]. Here we confirm that this approach is also suitable for learning how corticomotor excitability can be bidirectionally up- or down-regulated. Our participants were initially familiarized with two motor imagery strategies which are known to modulate corticospinal excitability in the required manner [29-32]. Learning, however, indicated by progressively stronger dissociation between the UP and the DOWN state, only took place when direct low-latency feedback regarding the MEP amplitude was provided. After training, participants were able to modulate corticomotor excitability across a large range so that MEP amplitudes were approximately twice as large during the UP than the DOWN condition. UP training, in particular, resulted in an 83.8% increase of MEP amplitudes from baseline, while downregulation of MEP amplitude was possible [eg. see 29] but more difficult (30.6% decrease from baseline). Once acquired, volitional control of corticomotor excitability was retained for at least 6 months and could be performed even without online feedback indicating true, long-term learning [33].

Once participants could control their corticomotor excitability, we uncovered the electrophysiological underpinnings by applying measurements that were independent of the feedback modality (single pulse TMS) and investigated whether there were differences between the UP versus DOWN state. This approach cancelled out the effects that were common to both mental strategies, isolating the mechanisms underlying the MEP modulation. This revealed two key novel electrophysiological findings, involving presynaptic $GABA_B$ disinhibition, and gamma oscillations. Additionally, the pronounced changes of cortical physiology despite the absence of EMG activity or changes in sensory input suggests that the increase vs decrease of corticomotor excitability was -a least partly- of cortical origin rather than mediated by spinal cord mechanisms.

Different excitability states cause modulation of $GABA_B$ mediated inhibitory circuits

The UP state was associated with a significant increase of LCD while other measurements probing inhibitory M1 circuits failed to reveal differential effects for

the UP versus DOWN state. LCD is thought to represent a read-out of the presynaptic self-inhibition of GABAergic neurons which is thought to be mediated by extrasynaptic $GABA_B$ auto-receptors [3435]. This mechanism is hypothesized to result in a net facilitatory effect as observed during the UP condition in our study. Previously, LCD was found to be elevated during motor imagery (MI), but this increase relative to rest was observed irrespective of whether participants imagined voluntarily activating or relaxing hand muscles [36]. However, this investigation was conducted in a single session, and did not employ neurofeedback, so MEP modulation by these two imagination conditions could be expected to be substantially smaller than observed in our study, particularly for the voluntary relaxation condition which had a similar excitability state as the rest condition. Thus, it is possible that the clear modulation of LCD observed here only manifested after neurofeedback training, i.e. when the two excitability states became clearly distinct. It is important to note here that group level results indicated no LCD at rest, and in fact it was only evident during the UP state. While LCD is elicited more readily during contraction [28], some studies have reported LCD at rest [27,36], whereas others have only reported occasional or non-significant facilitation occurring beyond 200ms after the suprathreshold conditioning stimulus, ie. in the period immediately following LICI (Valls-Sole et al, 1992). In our search procedure (to decide upon the optimum conditioning stimulus (CS) intensities), we prioritized SICI and LICI, finding a CS intensity that elicited as close to 50% inhibition of the test MEP as possible. We tested intensities between 106-114% RMT for LICI (and above or below this if no appropriate inhibition was found), and applied these parameters also to LCD (such that the only difference between the LCD and LICI protocols was the ISI). This may simply have been too low to elicit strong LCD at rest. Other studies have reported no LCD at 110% RMT, neither at rest [27] nor with contraction [37]. It is nonetheless interesting that the lack of LCD at rest in the current study was overshadowed by the strong facilitation observed while in the TMS-feedback induced UP state, indicating that future indepth investigation into this effect with a larger range of conditioning stimulus intensities may be warranted.

399 ***Different excitability states cause distinct neural dynamics in motor cortex***
400
401 We observed significant modulation of the alpha and gamma rhythms close to
402 M1 of the trained hemisphere. Focusing on data from the recording electrode closest
403 to each individual's hotspot revealed a significant association between low alpha and
404 high gamma power for the UP versus DOWN state. Trial-by-trial modulation of these
405 rhythms correlated significantly with MEP amplitude, and a support vector machine
406 (SVM) classifying the two states based on EEG data ranked the high gamma and high
407 alpha band as the two top features characterizing the distinction. Our observation of
408 reciprocal changes in the alpha and gamma band are in line with previous studies
409 using transcranial as well as intracranial recording methods [1]. The 'pulsed inhibition'
410 theory suggests that repeated bursts of inhibitory alpha activity serve to temporarily
411 silence gamma oscillations [1]. Thus, these two rhythms are seen to exhibit a reciprocal
412 relationship, whereby when alpha is high, gamma is low. In periods of high alpha,
413 gamma may still burst periodically, but only at the troughs of the oscillation cycle,
414 meaning that the gamma 'duty cycle' (window for neural processing) is short, and
415 only brief messages can be sent. By contrast, in periods of low alpha power, the
416 gamma duty cycle is longer, and more extensive neuronal processing and inter-
417 regional communication may occur. Our finding of increased gamma activity is also
418 consistent with previous animal literature, showing that the pharmacological removal
419 of $GABA_B$-mediated inhibition (by receptor blockage) in rats results in increased
420 gamma oscillations [38] which have been shown to be largest in M1's layer V [39].
421
422 Gamma has often been considered difficult to detect using scalp electrodes
423 because it is highly localised [40] and may also reflect non-cortical sources when
424 recorded with EEG [41,42]. However, it is tempting to speculate that, in our experiment,
425 gamma activity was strongly synchronized as a consequence of the neurofeedback
426 training, where participants learned to substantially facilitate corticomotor excitability
427 while keeping EMG activity constant, such that EMG amplitude differed only
428 minimally between the UP and DOWN conditions. This suggestion is in line with
429 previous neurofeedback studies that provided direct feedback of gamma activity,
430 showing that gamma power could be upregulated to a substantial amount which even
431 exceeded power values observed during movement execution [15,43]. By keeping the

visual feedback for the two conditions identical, we ensured that differences in eye movements between the UP and DOWN states were minor. As we were particularly interested in gamma oscillations, we additionally performed all EEG recordings in an electromagnetically shielded room, using a gel-based electrode system to maximize signal to noise ratio.

Previous studies have taken a correlational approach to investigating the relationships between brain rhythms and corticomotor excitability. These have shown that low alpha [4,44] or beta power [45] as well as high gamma power [3] during natural fluctuations at rest are associated with larger MEP amplitudes. We confirm and extend these results by introducing causality to this relationship for the first time, showing that experimentally driving excitability into two distinct states causes specific patterns of neural dynamics in the volitionally controlled cortical area.

While changes in alpha and gamma were specific to the hemisphere from which feedback was provided, theta showed a bilateral pattern of modulation, being higher in the DOWN than the UP state in motor areas in both hemispheres. While mid-frontal theta activity has been linked to error monitoring [46] the role of lateralized theta activity close to the sensorimotor hotspot electrode and its symmetric counterpart is less clear. Slower rhythms exert effects over larger distances, and are thought to be involved in long-range communication [40]. A similar pattern of upregulation and downregulation was observed in the homologous muscle in the opposite limb, albeit weaker and not statistically significant. This is likely a reflection of the extensive transcallosal structural connectivity and functional coupling of homologous regions of the cortical motor network [47-49]. It is tempting to speculate that the bilateral theta activity observed in the current study served to regulate the inhibition/facilitation of functional coupling or 'spillover' of activation from motor areas in the target hemisphere to their homologous counterparts.

Surprisingly we did not observe differential modulation of the Beta band, which is the predominant oscillatory frequency in sensorimotor cortical regions [50,51]. It typically desynchronizes (together with alpha) during motor execution and motor

imagery [52-55] and has been associated with corticomotor excitability at rest [3]. As our results represent the direct contrast between the UP and DOWN states, the lack of Beta involvement may firstly be due to the fact that both conditions involved a mental strategy targeted at the sensorimotor system and, secondly, that no temporal structure was imposed so that we could not perform analyses which are, for example, time-locked to the potential onset of these mental strategies. However, our data further confirm that the two 'inhibitory' rhythms alpha and beta might serve different functions in selecting and activating the appropriate sensorimotor representations [56].

Conclusion and future applications

Here we present an innovative approach to voluntarily and bidirectionally change the state of the motor cortex, by directly targeting MEP amplitudes in a neurofeedback paradigm. This method provided a unique opportunity to reveal the oscillatory and neurochemical underpinnings of the two distinct trained brain states, using concurrent TMS EEG measurements, and mechanistic follow-up investigations using paired-pulse TMS. The results comparing UP and DOWN states indicate that voluntary upregulation of corticomotor excitability causes increased presynaptic $GABA_B$-mediated disinhibition, elevated neural oscillations in the gamma frequency range, and reduced alpha and theta rhythms.

This paves the way for new technologies that allow the user to regulate aspects of their own brain function in order to reach desired states that are, for example, associated with enhanced motor performance. In the context of stroke rehabilitation, training volitional modulation of corticomotor excitability may hold promise as a rehabilitative therapy early after stroke, i.e. when patients are deprived of rehabilitation training because they are unable to execute overt movements with the impaired upper limb. As it is known that LCD is recruited during actual movement [28,57,58], the elevated LCD we observed in the UP condition may reflect that the neurofeedback had engaged similar mechanisms to those involved in movement execution, using only voluntary endogenous processes. Furthermore, as pathological hyperexcitability of the non-damaged hemisphere has been hypothesized to limit recovery in some patients [59], the TMS-neurofeedback protocol can be individually

tailored either to upregulate the damaged hemisphere, down-regulate the intact hemisphere, or a combination of both, depending on the patient's specific needs.

FIGURE LEGENDS

Figure 1. Outline of experimental setup. Each trial of neurofeedback training commenced with a display of four circles (A), each representing the background EMG in one of the recorded hand muscles (right FDI, ADM and OP, and left FDI). The circles were red if the root mean squared (rms) EMG at rest was greater than 7 microvolts. It was essential that all four circles were green for at least 500ms before the trial could proceed. When this condition was met a fixation cross appeared for a random period (in order to prevent anticipation of the TMS pulse). During the fixation cross, it was still essential to keep the background EMG below 7 microvolts in order for a TMS pulse to be delivered. (B) The peak-peak amplitude of the motor evoked potential (MEP) evoked by the TMS was calculated in real-time and displayed immediately to the participant on screen in the form of a rectangular bar.
(C) Different feedback for UP training and DOWN training. In the UP training If the MEP was greater than the baseline mean, the rectangle was green, with a green tick, a dollar sign to indicate a small financial reward, a display of the current score, and a positive encouraging sound bite was heard. If the MEP did not meet the criterion amplitude, the bar was red, there was no dollar sign, and a negative sound bite was heard. (D) A custom 3D printed 'coil spacer' device was used to prevent direct contact of the TMS coil on the EEG electrodes and allow the pre-TMS EEG period to be recorded artefact free.

Figure 2. MEP amplitudes during neurofeedback. Panel A depicts MEP amplitude in millivolts during the two types of MEP neurofeedback. UP training is shown in orange and DOWN training in blue, across all 10 training blocks. Filled triangles labelled 'BS' indicate the baseline measurement block that occurred at the beginning of that particular session, prior to any neurofeedback. Dotted vertical lines indicate the separation of the blocks into different 'sessions', which occurred on separate days. Panel B shows the same data for the control group who received no veridical

neurofeedback. Panel C shows the UP-DOWN difference (in the normalised % change from baseline data) for each block in the experimental group and the control group. Higher values represent greater deviations between the UP and DOWN data points and therefore more modulation of MEP amplitude. Thus, these values are significantly higher in the experimental group than in the control group. # symbols indicate blocks in which the Cohen's *d* effect size for the difference between the experimental and control group was large-very large (>0.8). All data are shown as mean ±SEM.

Figure 3. Retention, aftereffects and feedback-free measurements. Filled bars represent blocks of neurofeedback, and unfilled bars represent MEPs collected at rest. Panel A shows MEP amplitudes with their preceding resting baseline values subtracted. Values above 0 represent increases relative to baseline, and below 0 represent decreases. State-dependent neurofeedback training feedback effects were still evident in a retention block carried out approximately 6 months following the initial experiment. No aftereffects were observed on resting MEP amplitude 5 and 10 minutes later. In a separate block participants were capable of upregulating and downregulating MEP amplitudes with feedback removed (FB free). MEPs measured from the opposite hemisphere during neurofeedback exhibited a similar pattern of modulation.

Figure 4. Investigation into mechanisms of MEP neurofeedback. The data show paired pulse TMS measurements taken during neurofeedback blocks to probe distinct neurophysiological processes. In all subsequent panels, unfilled bars represent baseline MEP amplitudes collected at rest prior to the block. Panel A Shows that MEP amplitudes from the single pulses (from which neurofeedback was provided) exhibited the same state-dependent modulation as observed previously. In Panel B MEP amplitudes are expressed as a percentage of the single pulse MEPs. While expected levels of inhibition were observed for both SICI and LICI paired pulses, there was no state-dependent modulation. LCD was, however, significantly increased in the UP condition relative to baseline, and relative to the DOWN condition.

Figure 5. Neural oscillations associated with the trained brain states. Panels b-f show topographical representations of the relative power (in % of whole spectrum) in the UP condition minus the DOWN condition, for 5 distinct frequency bands (Averaged group data, n=14, 3 other frequency bands shown in Supplementary Fig 3). Red colours indicate regions that demonstrated greater synchronisation in the UP condition. Blue colours indicate greater synchronization in the DOWN condition. The location of the electrode nearest to the TMS hotspot varied between participants but was always within the region indicated in a). Colours are scaled from blue-red by minimum-maximum (range shown to right of each plot). Panel g shows the same data (UP-DOWN) extracted for each participant's hotspot electrode. Values greater than 0 indicate larger amplitude oscillations in the UP condition, and lower than 0 indicate larger oscillations in the DOWN condition. Stars indicate significant deviations from 0 (Wilcoxon Signed Rank tests). Panel h shows group level data for regression analyses performed on MEP amplitudes with relative power in each frequency band. This included all 120 trials (60 UP, 60 DOWN) collected during the combined TMS-EEG recording session. The Y axis depicts the slope of the regression model. Stars indicate significant deviations from 0 (0 would indicate no slope, Wilcoxon Signed Rank test). Individual regression plots are shown for one representative participant in Supplementary Fig. 4.

Materials and Methods

Participants

Fifteen healthy volunteers (age 23± 3.14 s.d, 7 female) participated in the experimental group. An additional thirteen participants (age 25± 3.06 s.d, 3 female) formed a control group. All participants were right handed according to the Edinburgh Handedness Inventory [60], and gave informed consent to procedures. The experiments were approved by the Kantonale Ethikkommission Zürich, and were conducted in accordance with the Declaration of Helsinki (1964).

TMS-based neurofeedback

Participants undertook five sessions of TMS-based neurofeedback, on separate days. The first four days comprised of neurofeedback training, and on the fifth day neurofeedback was performed with simultaneous electroencephalography (EEG) recording to investigate state specific neural dynamics. On two of the training days neurofeedback was adjusted so that a rewarding visual stimulus was displayed when MEPs were larger than baseline (the 'UP' condition) and on the other two days, the rewarding stimulus was displayed when MEPs were smaller than baseline (the 'DOWN' condition). On each of the training days, 4 separate blocks of neurofeedback were preformed, each comprising of 30 individual MEP feedback trials (total 120 trials per day). The format of individual trials and feedback is described in more detail below. Baseline corticospinal excitability was measured on each day prior to training (20 MEPs) and post-measurements were taken during the rest periods between each of the 4 blocks (12 MEPs per measurement).

Subjects sat in a comfortable chair with both arms and legs resting in a neutral position supported by foam pillows. Surface electromyography (EMG, Trigno Wireless; Delsys) was recorded from right First Dorsal Interosseous (FDI), Abductor Digiti Minimi (ADM), Opponens Pollicis (OP), and left FDI. EMG data were sampled at 2000Hz (National Instruments, Austin, Texas), amplified and stored on a PC for off-line analysis.

TMS was performed with a figure-of-eight coil (internal coil diameter 50mm) connected to a Magstim 200 stimulator (Magstim, Whitland, UK). The coil was held on the left hemisphere over the 'hotspot' of the right FDI at the location with the largest and most consistent MEPs, and with the optimal orientation for evoking a descending volley in the corticospinal tract (approximately 45 degrees from the sagittal plane in order to induce posterior-anterior current flow). Once the hotspot was established, the lowest stimulation intensity at which MEPs with peak-to-peak amplitude of approximately 50μV were evoked in at least 5 of 10 consecutive trials was taken as Resting Motor Threshold (RMT).

The stimulation intensity used to evoke MEPs during the experiment was chosen using the following procedure in order to obtain a baseline MEP amplitude that was 50% of the participant's maximum. A recruitment curve [eg. 61] was performed at the beginning of the first experimental session, whereby 6 TMS pulses were applied at 10 different intensities relative to RMT (90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 180%, 190%) in a randomized order. MEP amplitude at each intensity was plotted to determine the point on the curve at which plateau occurs and the MEPs do not continue to increase. Maximal MEP amplitude was recorded, and the intensity required to evoke 50% of this amplitude was used for all subsequent testing. With this approach, there was scope for MEP amplitude to both increase and decrease to similar extents from this 'intermediate' value. Post-hoc analyses revealed that this procedure resulted in an average stimulation intensity corresponding to 130% RMT. Immediately following this procedure and prior to the first block of neurofeedback, 20 MEPs were collected at rest at the chosen intensity to determine 'baseline' corticospinal excitability. The mean MEP amplitude at baseline was recorded and used during neurofeedback to establish the criterion amplitude that determined whether participants received either positive or negative feedback.

Format of neurofeedback

Neurofeedback was performed using custom written MATLAB software. Participants kept eyes open with attention directed to a monitor in front of them. They were instructed to relax their limbs and avoid tensing any muscles throughout the experiment. In order to ensure that MEP amplitude could not be influenced by background muscle activation, the root mean square (rms) of the EMG signal for each muscle for the previous 100ms of data was calculated and displayed in real-time on screen at the beginning of each trial in the form of four coloured 'traffic lights', representing each muscle (Fig. 1A). If the background EMG in a muscle exceeded 7μV, the corresponding light turned red. Participants were instructed that a trial could not begin unless all four lights were green (all muscles relaxed below 7μV) for at least a continuous 500ms period. When a trial commenced the traffic lights disappeared, but background EMG continued to be monitored and the trial was automatically paused if any muscle exceeded the threshold. At the beginning of each trial a fixation cross appeared in the center of the screen. After a variable period of time (between 5.5

664 - 8.5 seconds, or longer if muscle activation delayed the trial) a TMS pulse was fired.
665 The MEP amplitude for the target muscle (right FDI) was immediately measured and
666 displayed to the participant on screen within 500ms. The display consisted of a
667 vertical bar indicating MEP amplitude relative to a horizontal line in the middle of the
668 screen representing the mean recorded at baseline (Fig. 1B). In 'UP' sessions if the
669 MEP was larger than the criterion amplitude, the bar was shown as green with a tick
670 beside it, a positive soundbyte was heard, and a number adjacent to a dollar sign
671 incremented to indicate that a small financial reward had been gained. If the MEP was
672 smaller than the criterion amplitude, the bar was red with a cross beside it, a negative
673 soundbyte was heard, and no financial reward was shown. The reverse was true in the
674 'DOWN' sessions (Fig. 1C). The feedback remained on screen for 4 seconds, before
675 being replaced by the traffic lights display preceding the next trial. Participants were
676 instructed to attend to the feedback and that the goal was to increase (or decrease) the
677 size of the MEP represented by the bar. Prior to the experiment participants read an
678 instruction sheet explaining the procedures above and providing recommended mental
679 strategies that were reported in previous literature in which corticospinal excitability
680 was downregulated [29] and upregulated [31] by motor imagery (Specific task instructions
681 are provided in Supplementary Material). Initially the criterion amplitude
682 corresponded to the baseline MEP measure. After each block of 30 MEPs,
683 performance was quantified and the task difficulty was adjusted if necessary. If the
684 success rate was >70% difficulty was increased by raising (or lowering in the DOWN
685 condition) the criterion MEP amplitude that needed to be reached by 10% in order for
686 the positive reward to be presented. If performance was > 90%, this was adjusted by
687 20%.

688 *EEG session*

689

690 On the fifth day neurofeedback was provided during simultaneous EEG
691 recording. The participant's TMS hotspot was determined and marked on the scalp
692 prior to EEG capping. EEG signals were recorded using a 64 channel gel-based TMS-
693 compatible cap (Electrical Geodesics Inc., Oregon, USA), and the channel closest to
694 the TMS hotspot was noted. EEG data were amplified and sampled at 1000hz. In
695 order to minimize artefacts associated with the direct contact of the TMS coil resting
696 on the electrodes of the EEG cap, we designed and 3D-printed a custom plastic 'coil

spacer' device [62], which has four wide legs positioned to provide a platform distributing the weight of the TMS coil, so that it hovers over the electrodes without contact (Fig. 1D). This allowed quality recordings to be obtained even from the channel of interest closest to the participant's 'hotspot'. The participants RMT was established while wearing the EEG cap with TMS coil spacer, and the same % above threshold that was used for all previous sessions was applied for neurofeedback. Impedances were monitored throughout and maintained below 50kΩ.

Baseline corticospinal excitability was measured in the same fashion as for the first four sessions, followed by two blocks of neurofeedback (UP or DOWN, counterbalanced) with brief (12 MEP) post measurements following each. After the final post measurement, a 15 minute rest break was scheduled for the participant. Following this, the procedure was repeated and baseline excitability was measured again, followed by two blocks of either UP or DOWN neurofeedback (whichever was not performed in the first half of the session). At the end of this session participants were debriefed.

Control group

Participants were blinded as to whether they were allocated to the experimental or control group. The control group experienced identical conditions to the experimental group, with the exception that direct neurofeedback was not provided. The visual feedback bar demonstrating MEP amplitude was always the same height (reaching the 'mean' horizontal line). 'Positive' feedback/rewards were presented in the same proportion as in the experimental group (66% of all trials - calculated upon completion of experimental group), but at a fixed and predicable rate in order to prevent the development of illusory correlations. Participants were instructed to attend to the visual feedback on screen, and that rewards would occur at a fixed rate. Aside from this, they were otherwise given identical instructions as the participants in the experimental group- i.e. the same recommended mental strategies were provided on control 'UP' and 'DOWN' blocks.

Data processing and analysis

MEP data

EMG data from all four hand muscles were band-pass filtered (30–800 Hz), separately for the portion of data containing the 100ms of 'pre-TMS' background EMG, and for the portion of EMG containing the MEP, in order to prevent smearing of the MEP into the background EMG data chunk. The root mean squared (rms) of the background EMG was calculated, and peak-peak MEP amplitude was measured. Trimming (removal) of the maximum and minimum MEP in each block was performed in order to screen out extreme values. MEP amplitude is known to be modulated by EMG background activation [23,24]. Therefore, the rms pre-stimulus EMG recordings were used to assess the presence of unwanted background EMG activity in the period 110 to 10ms preceding the magnetic pulse. MEPs preceded by background EMG higher than 0.01mV were excluded. For each subject and over all trials we calculated the mean and standard deviation of the background EMG. MEPs that occurred when the background EMG value exceeded 2.5 standard deviations above the mean, and MEPs with a peak-to-peak amplitude which exceeded Q3 + 1.5 x (Q3 - Q1) were removed from further analysis, with Q1 denoting the first quartile and Q3 the third quartile computed over the whole set of trials for each subject.

Inferential statistics were computed using Mixed Effects Models in SPSS (Version 16.0, SPSS Inc. Chicago, US), as they account for covariances between related data samples in repeated measures designs, and have greater flexibility for modeling effects over time than traditional ANOVA approaches [63]. Fitting of the mixed effects models employed restricted maximum likelihood estimation (REML) and a compound symmetry covariance matrix. Model fit indices (Akaike Information Criterion and Schwarz Bayesian Criterion) were considered prior to choosing the covariance matrix and model type. Fixed effects were *neurofeedback type* (UP or DOWN) and *block number* (1-10). The influence of each of the fixed effects on the model was estimated using *F* tests. In all models *subject* was designated as a random effect with random intercepts.

The criterion alpha value was set to 0.05 for all inferential tests. In cases where multiple comparisons were performed, *p* values were false discovery rate (FDR) corrected.

EEG data

Signals from all 64 channels were first epoched to extract only the data on each trial from the 4 seconds before the TMS pulse. This was to remove the substantial artefacts that arise during the magnetic pulses, prior to conducting any filtering or further processing. These separate chunks of unpolluted data were then concatenated into one continuous epoch, and highpass filtered at 1Hz, prior to conducting an independent components analysis (ICA). Independent components were visualized and those containing artefacts arising from eye movements, facial EMG, cardiac signals, bad channels or other non-brain activity related signals were removed.

The cleaned data were average-referenced, and re-epoched into chunks of data containing only the 1.5s on each trial prior to the TMS pulse (ie. to capture the ongoing oscillatory activity at the instance in which the TMS occurred, while the fixation cross was on screen and the 'traffic lights' had disappeared).

A power spectrum was computed for each single epoch and the mean power (and relative power) in each of the relevant bandwidths were extracted (delta (0.1-4Hz), theta (5-7Hz), low alpha (8-10Hz), high alpha (11-13Hz), low beta (14-21Hz), high beta (22-30Hz), low gamma (31-50Hz) and high gamma (51-80Hz). Power values were computed separately for UP and DOWN trials, and non-parametric Wilcoxon signed rank tests (with FDR correction) were used to compare neural oscillations in these two states.

We also analysed whether trial-by-trial variation of EEG data was associated with trial-by-trial variation of MEP amplitudes. Therefore, relative power in each bandwidth for each epoch was entered into a multiple regression model with MEP amplitudes measured in the muscle from which neurofeedback was provided (right FDI). The beta (slope) values resulting from each regression model for each participant were forwarded into a group-level analysis.

Classification of distinct brain states

Individual epochs of EEG data (60 UP 60 DOWN) were classified by a linear support vector machine (SVM, 10-fold cross validation), to test separately for each participant whether the epochs could be successfully predicted as 'UP' state or 'DOWN' state based solely on the power values (scaled by using 1/f transformed relative power) of

the 4 frequency bands of interest. The SVM was chosen as it is known to perform particularly well in BCI settings using EEG data which is noisy and has features that are correlated. In order to validate the results the same procedure was repeated with randomly permuted labels, and this null model was statistically compared to the model with true labels (C=1). Feature selection was conducted using feature ranking based on Recursive Feature Elimination [64].

Follow-up experiment 6 months later

A sub-set of 11 participants from the experimental group returned approximately 6 months later to participate in a follow-up experiment probing retention and mechanisms underlying the two distinct states. This was conducted over a further 4 days of testing. On one day, retention, aftereffects, and excitability in the opposite 'untrained' hemisphere were tested for the 'UP' condition. On another, neurophysiolocial mechanisms were probed using paired pulse TMS. These two days were repeated for the 'DOWN' condition, and the order of these sessions was counterbalanced. We additionally tested whether trained participants were able to upregulate and downregulate when feedback was temporarily removed.

Retention testing & aftereffects measurement

After a 6-month break and no top-up training, participants were tested with one block of TMS-neurofeedback (20 MEPs) in order to assess retention of learning. All other procedures were identical to those carried out in the main experiment.

Following this block, 12 MEPs were collected at rest after 5 and 10 minutes.

Excitability in the opposite hemisphere

During one block, two TMS coils were used, placed over the right and left motor hotspots (as described previously). This block contained 40 trials, 20 of which were normal TMS neurofeedback trials. The other 20 were trials where TMS was applied to the opposite hemisphere, rather than to the hemisphere that was the target for neurofeedback. No feedback was given in these trials. The presentation of left and right hemisphere TMS pulses was randomized.

Feedback- free measurements

We additionally tested whether trained participants were able to upregulate and downregulate when feedback was temporarily removed. In this feedback-free block, the timing of trials and participant instructions were identical to normal neurofeedback blocks, but in place of the usual feedback bar showing MEP amplitude, the white fixation cross simply turned red during this period. The onset of trials was still contingent on muscles being completely relaxed, and the traffic lights display still preceded every trial.

Paired pulse TMS measurements

On separate days (one 'UP' one 'DOWN) from the measurements described above, we performed three additional blocks of TMS neurofeedback (24 trials per block x 3 = 72 total trials), in which just 25% of trials were standard single pulse TMS-neurofeedback trials, with the usual feedback. The remaining trials contained paired pulses in place of the usual single pulse TMS. For all paired pulse measurements, the test stimulus intensity was identical to that which had been chosen for the TMS neurofeedback (ie. that produced MEPs that were 50% of the maximum on the recruitment curve). On 25% of trials Short Interval Intracortical Inhibition (SICI) was measured. This was with a conditioning stimulus intensity that was chosen using a personalized search procedure testing intensities ranging from 50%-90% RMT, to achieve as close to 50% inhibition as possible, and an inter-stimulus interval of 1.97ms [65]. The reduction in the size of the test MEP is believed to represent postsynaptic $GABA_A$ inhibition [25]. On 25% of trials Long Interval Intracortical Inhibition (LICI) was measured. This was with two suprathreshold pulses, with the conditioning stimulus intensity chosen using a search procedure between 106-114% RMT, and an inter-stimulus interval of 100ms [27]. This is believed to reflect postsynaptic $GABA_B$ inhibition [66]. On the remaining 25% of trials, Late Cortical Disinhibition (LCD) was tested. This was with the exact same pulse intensities as used for LICI, but with a 220ms inter-stimulus interval [27], and is thought to measure presynaptic $GABA_B$ disinhibition [26-28]. The order of presentation of paired pulses and single pulses was randomized.

Baseline measurements were taken at rest with each of these three paired-pulse TMS protocols, prior to the beginning of neurofeedback blocks (20 of each type of paired pulse measurement, and 20 single pulse MEPs).

1

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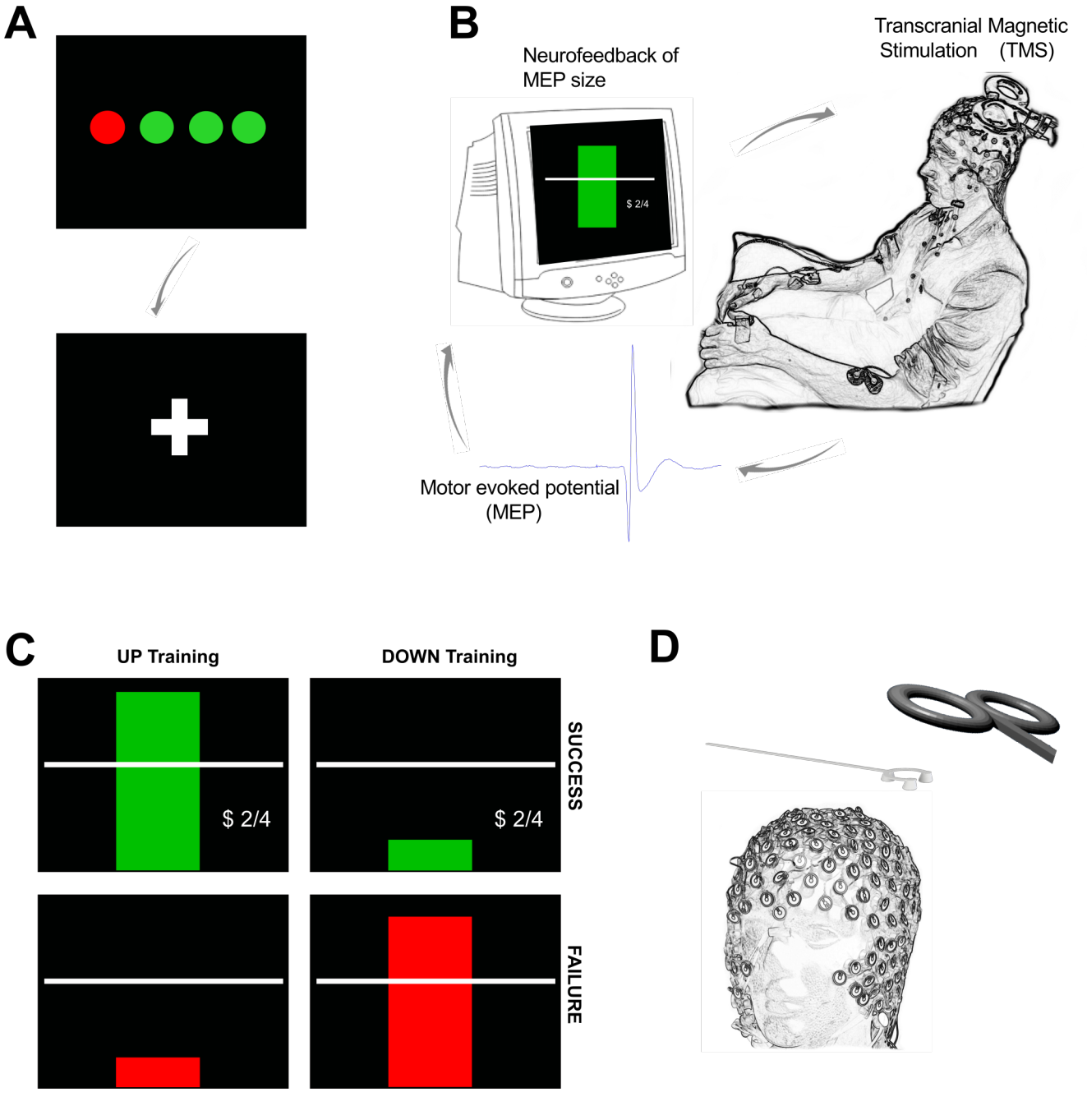
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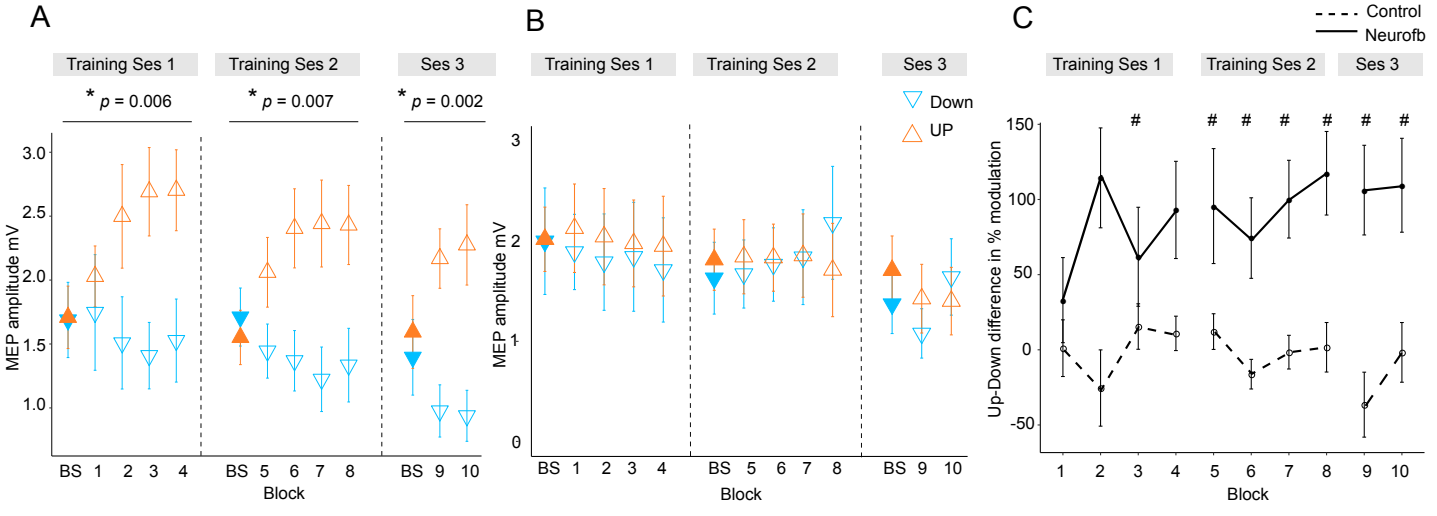
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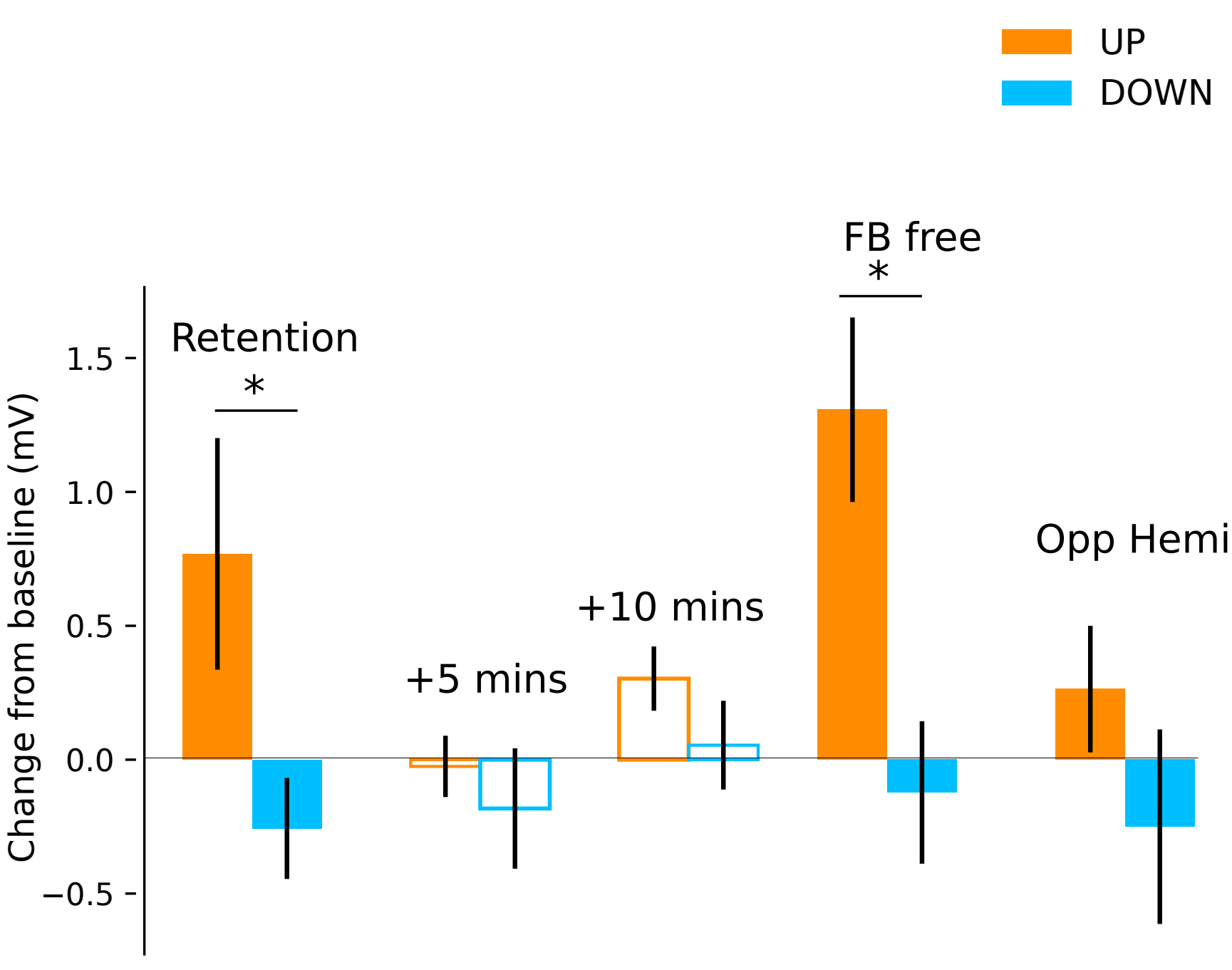
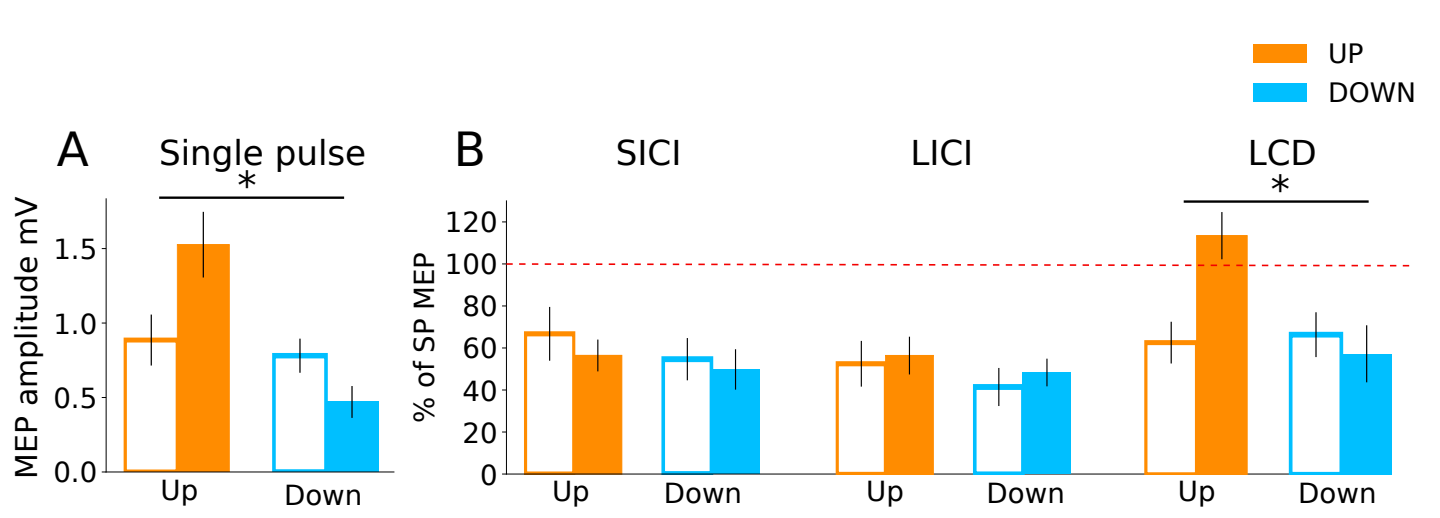
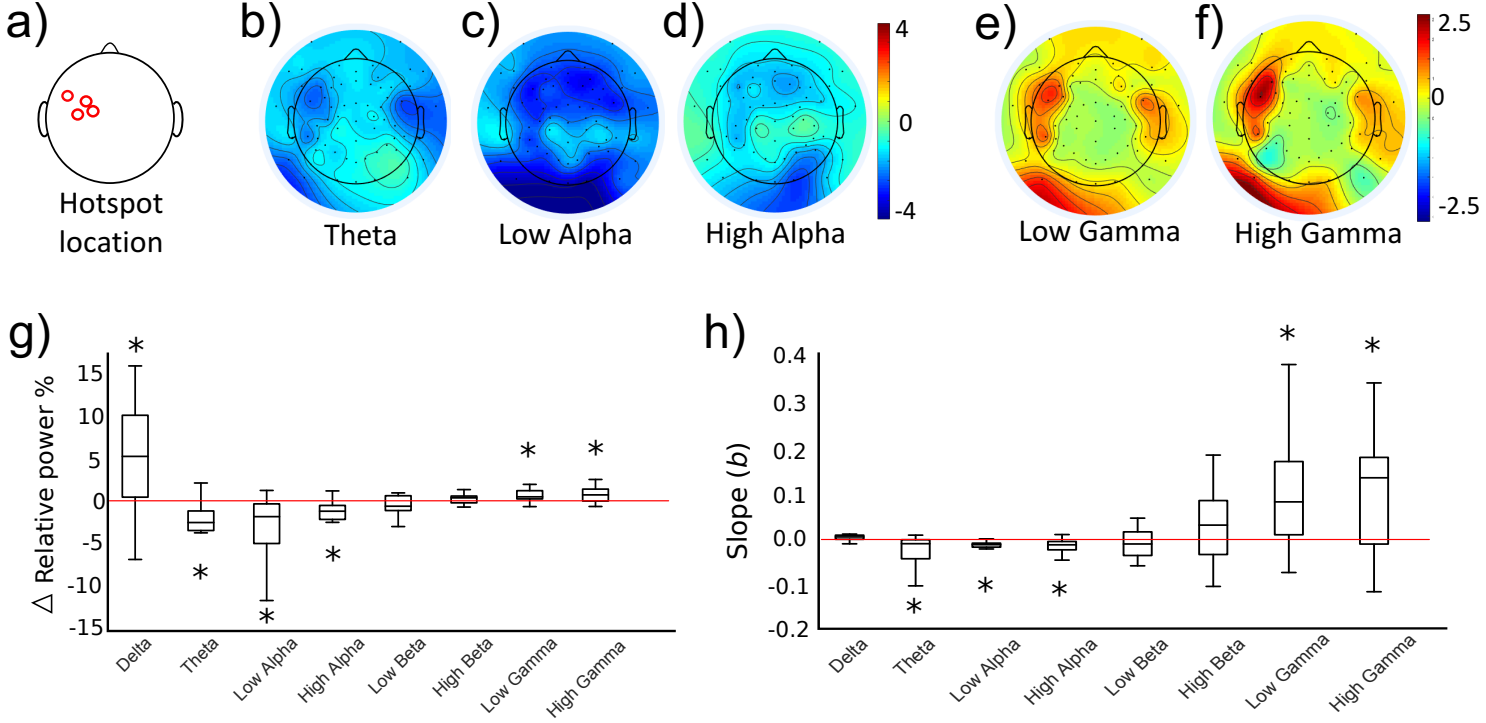
BS 9 10
Block
B
Training Ses 1
Training Ses 2
Ses 3
Down
UP
MEP amplitude mV
3
2
1
0
BS 1 2 3 4
BS 5 6 7 8
BS 9 10
Block
C
Control
Neurofb
Training Ses 1
Training Ses 2
Ses 3
Up-Down difference in % modulation
150
100
50
0
-50
#
1 2 3 4 5 6 7 8 9 10
Block


UP
DOWN
Retention
*
FB free
*
+5 mins
+10 mins
Opp Hemi
Change from baseline (mV)
1.5
1.0
0.5
0.0
−0.5


UP
DOWN
A
Single pulse
*
MEP amplitude mV
1.5
1.0
0.5
0.0
Up
Down
B
SICI
LICI
LCD
*
% of SP MEP
120
100
80
60
40
20
0
Up
Down
Up
Down
Up
Down


a)
Hotspot location
b)
Theta
c)
Low Alpha
d)
High Alpha
4
0
-4
e)
Low Gamma
f)
High Gamma
2.5
0
-2.5
g)
△ Relative power %
15
10
5
0
-5
-10
-15
Delta
Theta
Low Alpha
High Alpha
Low Beta
High Beta
Low Gamma
High Gamma
h)
Slope (b)
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
Delta
Theta
Low Alpha
High Alpha
Low Beta
High Beta
Low Gamma
High Gamma