Transcranial Magnetic Stimulation Following A Paired Associative Stimulation Protocol Based On A Videogame Potentiates Cortical Plasticity And Motor Behavior

Arantzazu San Agustín (✉ arantzazusanagustinperez@gmail.com)

Consejo Superior de Investigaciones Científicas <https://orcid.org/0000-0003-1417-5461>

Guillermo Asín-Prieto

Gogoa Mobility Robots

Juan C Moreno

Consejo Superior de Investigaciones Científicas

Antonio Oliviero

Hospital Nacional de Paraplégicos

José L Pons

Shirley Ryan AbilityLab

Research Article

Keywords: Transcranial Magnetic Stimulation, Paired Associative Stimulation, Videogame, Reaction Time, Corticospinal plasticity, Movement-related Cortical Stimulation, Fatigue

Posted Date: September 7th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-775978/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background

Transcranial Magnetic Stimulation (TMS) can induce synaptic plasticity potentiation following a paired associative stimulation (PAS) protocol, synchronizing a TMS single pulse with a movement task, named movement-related cortical stimulation (MRCS). However, MRCS plasticity induction and performance potentiation has been related exclusively to single movement tasks.

Method

In order to unveil the changes in motor learning produced by the MRCS protocol in complex movements, associated to Activities of Daily Living (ADL), we induced PAS changes in synchronization with a movement-related dynamic task by performing a customized videogame. We measured the task performance as well as nervous system excitability neuromodulation in 22 healthy subjects, analyzing Reaction Time (RT) and the peak-to-peak amplitude of the Motor Evoked Potentials (MEPs) respectively. The MEPs were recorded in the main task executor muscle, Abductor Pollicis Brevis (APB), and a secondary muscle, Abductor Digiti Minimi (ADM), before, right after, and 30 minutes after the intervention, in a real against sham group experimental parallel design.

Results

PAS application in synchronization with a complex task resulted in a motor performance potentiation effect, inducing shorter RTs when compared to the sham group. Moreover, it triggered long-term corticospinal plasticity mechanisms reflected in a MEP amplitude depression for the APB muscle at the higher intensity of recruitment curve and an enhancement of the corticospinal excitability of ADM muscle at around threshold intensity. RTs and ADM MEP amplitudes correlated positively in around threshold and high intensity assessments.

Conclusions

We conclude that the proposed PAS protocol facilitated the learning of time-accuracy movement in complex movement tasks, even if fatigue could be affecting the executor muscle excitability, and enhanced potentiation towards a passive muscle. This phenomenon can be very useful to develop neurorehabilitation strategies with complex movements (more similar to ADLs) and to avoid maladaptive plasticity related likely to fatigue.

Introduction

Synaptic plasticity is the cellular basis of learning and memory processes [1]. Spike-timing-dependent plasticity (STDP) induction has been experimentally observed by the synchronized activation of two neurons synaptically connected, leading to a modulation in the synaptic strength [2]. This plastic

mechanism depends on the relative timing and temporal order of pre- and post-synaptic depolarization to trigger Long Term Potentiation (LTP) or Long Term Depression (LTD) processes [3; 4; 5; 6; 7; 8; 9].

The emergence of non-invasive brain stimulation techniques such as Transcranial Magnetic Stimulation (TMS) [10, 11] enabled the study of STDP induction in the human brain. LTP associative model studied *in vitro* slice preparation was proven *in vivo* human brain using a Paired Associative Stimulation (PAS) protocol, i.e. the synchronization between low-frequency peripheral stimulation of somatosensory afferent pathway 25ms before TMS single pulse over contralateral motor cortex [12]. This paradigm implies a synchronization between sensory afferences and the TMS pulse over the motor cortex, resulting in an enhancement of the cortico-spinal pathway excitability and plastic changes [13]. PAS protocols enhancing motor cortex excitability have been shown to correlate with improved motor performance in healthy [14], schizophrenia [15], chronic traumatic tetraplegia [16] and stroke patients [17; 18].

Moreover, PAS paradigm has been applied associating TMS single pulse with the physiological activation of motor cortex, induced by a thumb abduction during a simple Reaction Time (RT) task [19]. This movement-related cortical stimulation (MRCS) protocol resulted on an enhancement in the corticospinal excitability and a facilitation of the RT [20]. Pairing TMS with an endogenous cortex activation, induced by a motor task, provides a method that might trigger plastic mechanisms without peripheral electrical stimulation and the possibility to pair the TMS pulse with any activity that the user is performing, in order to enhance the function of the task itself [21].

Nevertheless, the main findings in MRCS PAS motor applications has been in regard to a shorter RT in a simple movement task. Our goal is to investigate if this TMS intervention also promotes motor learning in a complex motor task, which is closer to the Activities of Daily Living (ADL). Therefore, we have designed a movement related dynamic task, framed in a videogame and aimed at performing a functional motion goal. This allowed us to assess precision and motor learning, in addition to being able to measure RT. For the task to be applied as a future tool in therapy, our intention has been to stablish a level of motivation and comfort in the subjects towards the therapeutic intervention.

Thus, we have studied the consequences on movement precision, motor learning and RT performance applying a PAS intervention paired with a movement dynamic task and analyzed the performance relationship with corticospinal excitability. We evaluated the motor task performance and the Motor Evoked Potentials (MEPs) before, immediately after and 30 minutes after the PAS intervention in order to unveil change rates due to the MRCS PAS dependent plasticity in the complex task performance.

Materials And Methods

Subjects. 22 healthy volunteers (Age 21-63; 9 females, 13 males) participated in this study. Eleven subjects (Age 22-39; 2 females) participated in the real condition when the stimulation of the motor cortex was applied using a real TMS pulse (110% RMT) over the APB hot spot. Other eleven subjects (Age 21-63; 7 females) participated in the sham condition. The recruited subjects were not the same as the ones that participated in the real condition experiment (to avoid learning and carry-over effects). None of

the subjects had past or present neurological disorders and they were not having any pharmacological treatment on the experiment day. All participants gave informed consent before the experimental intervention. The Spanish National Research Council (CSIC) Ethics Committee approved all procedures.

Transcranial Magnetic Stimulation. The stimulation was applied using a figure-of-eight double 70mm remote control coil and a single pulse, monophasic stimulator (Magstim 200² stimulator). TMS stimulation was applied always to induce a posterior to anterior current direction.

In the assessment phase, TMS was applied in order to evaluate motor pathway excitability, eliciting MEPs in Abductor Pollicis Brevis (APB) and Abductor Digiti Minimi (ADM) muscles. The subjects were seated in a chair and their dominant hand was placed comfortably in the armrest. Firstly, we found in the scalp the location where the thumb stimulation (APB muscle) was optimal (hot-spot). We started with a TMS pulse of 50% of the maximum stimulator output intensity and the scalp localization of 10mm anterior and 20mm right or left to *bregma*, depending on the subject's dominant hand. This point was marked on the subject's scalp to ensure the same coil position through the experiment. Subsequently, the Resting Motor Threshold (RMT) was estimated by the variation of the stimulation intensity. The RMT is defined as the stimulation intensity that evokes 5 out of 10 times a MEP with a peak-to-peak amplitude of at least 50 μ V while the muscle is at a resting state [22].

Then, 50 MEPs were elicited at different intensities (10 pulses of 90%, 100%, 110%, 120% and 130% of the RMT), in a randomized order (recruitment curve). We obtained a recruitment curve also for ADM muscle (adjacent muscle not primarily involved in the motor task) by using the same hot-spot and intensity than for APB. Thus, the ADM MEPs were obtained in a sub-optimal condition. We measured the peak-to-peak amplitude of the MEP in each intensity.

In the intervention phase, motor cortex stimulation was induced by the synchronization of TMS and the movement task in order to generate an excitability enhancement and induce plastic changes. Monophasic pulses at 110% of RMT intensity over the APB hot-spot were given synchronized to the dynamic motor task. TMS pulses were delivered every 10 seconds. The relationship between the motor task onset and the TMS is described below, in subsection *Movement related dynamic task*.

In order to achieve sham stimulation, a TMS coil was placed on the subjects' APB hot-spot location, although the pulse was not administered. The sound of the stimulation was simulated with another coil connected to the stimulator. The subjects were blind to the kind of TMS they received (real or sham).

Electromyography (EMG) recordings. We used bipolar surface EMG electrodes. The EMG signals were amplified by g.USBamp biosignal amplifier (g.tec). 50 Hz noise was removed with a notch filter, digitized at a rate of 2400 Hz and stored for offline analysis.

Movement related dynamic task. The aim of the programmed videogame was the player to follow the trajectories depicted via visual collectible items ("bottles") avoiding other visual objects ("tubes")

presented on the screen. The subject had to follow the trajectories (not directly showed) by controlling the movement of the character (gyrocopter).

The motor task is based on five different trajectories, distributed randomly across 30 trials in two intervention phases. Each trial lasts 10 seconds and demands six repetitive movements. Thus, the subject had to fulfill 360 short APB contractions in 0.6 Hz during 10 minutes. The user was able to command the up and down movement of the “gyrocopter” on the screen by moving the instrumented hand thumb to the left or right, respectively.

The thumb of the subject was attached to a custom designed mechanism in a platform with an absolute position sensor. The angle of the movement was read by a custom designed electronic board and sent to a BeagleBone Black (BBB) board via CAN that acts as CAN to UDP converter for the main computer to run the visual paradigm. The main computer read the angle and represented it as the movement of the “gyrocopter” on the screen through MATLAB program [23 (**Figure 1.A.**)].

Each trial consisted of four phases (**Figure 1.B**): 1. Resting phase: the subject had to wait for the character (“gyrocopter”) to appear (1500ms); 2. Pre-movement phase: the character appeared on the screen, and the subject had to remain between the dotted lines (1500ms); 3. Movement phase: the subject moved in order to collect the items (“bottles”) on the screen avoiding the “tubes” (5000ms); 4. Feedback phase: the subject was given feedback, i.e., the performance in the trial (2000ms). The movement phase is visually cued when the “gyrocopter” enters in the “sky”, i.e., beginning of the movement phase (the screen background changes from white to blue).

Subjects were asked not to move until the movement phase in order to collect bottles and avoid tubes. The aim of the exercise was to train the RT and the maximum recollection of items following the ideal trajectory, i.e., the best linear path between bottles.

The variables we used to characterize the motor learning were: **1) RT** for the assessment of time-accuracy movement learning: we analyzed the elapsed time from the first EMG activation of APB to the movement cue established in the visual interface in each trial. **2) Trajectory error (TE)** for trajectory-accuracy movement learning: we analyzed the error between the angular position of the subject and the ideal target angle. **3) Collected and avoided items** for the object-directed motor learning assessment index: we calculated the collected and avoided items amount.

During the two intervention phases, 30 trials per phase, the BBB received a command via UDP from the computer at the onset of the movement phase of the task to trigger the TMS (real or sham, see below, in *Experimental design* subsection). One single TMS pulse was applied at the onset of each movement phase.

Experimental design. This is a real/sham parallel experimental design (**Figure 2**). The experimental session began with an assessment of basal skills in task performance during 30 trials (PrePre-Assessment). Separately, we assessed the cortico-spinal excitability of APB and ADM of the dominant

hand eliciting MEPs by means of TMS. We repeated the PrePre-assessment MEPs phase in order to ensure measurement stability and replicability (PrePre-Assessment and Pre-Assessment). Next, the intervention phase was carried out with the subject performing the task in two experimental phases of 30 trials each (the first intervention and the second intervention phase; Int1 and Int2). TMS was applied at the onset of task movement phase. A new excitability assessment was made with the same parameters as the Pre-intervention assessments immediately and 30 minutes after the intervention (Post-assessment and Post-30'-assessment).

Statistical Analysis. RT, TE, collected items and avoided items data, rated the motor dynamic task performance learning success. We transformed the data in absolute values for the time and error calculation. As the task was performed during each assessment and each intervention phase, we compared the RT between every experimental phase time (Pre-assessment, Intervention 1, Intervention 2, Post-assessment and Post-30'-assessment). A repeated-measures ANOVA with the factors Time (Pre-assessment, Int1, Int2, Post-assessment and Post-30'-assessment) x Group (Real Group and Sham Group) and a Post Hoc analysis with Bonferroni correction was applied.

The corticospinal pathway excitability was assessed by MEPs amplitude data from each applied intensity of each assessment phase. We normalized the raw μV data (individual value-mean/standard deviation) and applied a repeated-measures ANOVA with Time (PrePre-assessment, Pre-assessment, Post-assessment and Post-30'-assessment), Intensity (90, 100, 110, 120, 130) and Muscle (APB and ADM) as intra-subject factors and Group (Experimental Group and Control Group) as inter-subject factor. Post Hoc analysis using Bonferroni correction was applied.

The correlation between task performance and corticospinal excitability was measured for data that were significantly different between groups and in the intensities significantly different between Times.

Finally, we analyzed the following correlations: RT data of the first intervention time phase and MEP amplitude in the Post-assessment and Post-30'-assessment phases. For MEP amplitude we used only the intensities that were significant between Times (see MEP amplitude analysis at the *Results* section). All data were transformed in proportional data based on Pre-assessment phase for correlation analysis. The Grubbs' test was applied to detect and delete outlier scores. Finally, Pearson correlation coefficient was calculated.

The statistical procedures were calculated with the aid of statistical software IBM SPSS for every statistical analysis [24].

Results

There are no significant differences between the ages of the subjects in the real group (Mean = 28.54 SD = 6.28) and the sham group (Mean = 29.72 SD = 11.92).

Behavioral results. Comparing performance indices between phases and groups, we calculated the intervention effects on the task performance ability.

Mean RT at baseline was similar between the real and sham group (real group: Mean = 0.262s Standard Error (SE) = 0.072s; sham group: Mean = 0.282s SE = 0.069s; $p > 0.5$). In RT data, a significant main effect of Time $F(4, 80) = 9.134$ ($p = .000$), Group $F(1,20) = 5.984$ ($p = .024$) and interaction effect of Time x Group $F(4,80) = 2.637$ ($p = .040$) was found. At the post-hoc analysis (Bonferroni), the real group, compared to the Pre-assessment baseline state, significantly shortened RT in every time phase: first intervention (Mean = 0.128s SE = 0.019s, $p = .000$), second intervention (Mean = 0.159s SE = 0.026s, $p = .001$), post assessment (Mean = 0.194s SE = 0.022s, $p = .008$) and the post-30-minutes assessment (Mean = 0.169s SE = 0.025s, $p = .001$). Compared to first intervention time, the RT in post assessment time significantly augmented ($p = .006$).

Regarding the sham group, it significantly shortened the RT score compared to the baseline only in the post-30-assessment time (Mean = 0.172s SE = 0.022s, $p = .009$).

The real TMS group reduced significantly the RT compared with the sham group (sham group: Mean = 0.245s SE = 0.019s, $p = .000$) in the first intervention phase ($D = 0.117$ s, SE 0.026s) (Fig. 3).

Regarding the TE, real and sham group significantly improved their adjustments to the trajectory through time. A significant main effect in Time $F(4,80) = 34.59$ ($p = .000$) was found while no significant differences were found between groups (**REAL**: Pre, 1.52; Int1, 1.20; Int2, 1.19; Post, 1.15; Post30, 1.08; **SHAM**: Pre, 1.78; Int1, 1.45; Int2, 1.32; Post, 1.28; Post30, 1.19). Concerning collected items variable in task performance, both groups significantly changed overtime. A significant main effect in Time $F(4, 80) = 54.48$ ($p = .000$) was found. However, no changes between groups were observed (**REAL**: Pre, 10.05; Int1, 11.50; Int2, 12.01; Post, 12.36; Post30, 12.75; **SHAM**: Pre, 9.23; Int1, 10.50; Int2, 11.25; Post, 11.70; Post30, 11.93). Moreover, in avoided item index a significant main effect in Time $F(4, 80) = 18.36$, $p = .000$ was found as well, although no significant differences were seen between groups (**REAL**: Pre, 1.23; Int1, 0.79; Int2, 0.72; Post, 0.74; Post30, 0.66; **SHAM**: Pre, 1.29; Int1, 0.93; Int2, 0.76; Post, 0.82; Post30, 0.68). All with $p > 0.05$.

To summarize, the obtained results demonstrate that, since the first intervention, the real group has been able to perform shorter RTs. Compared to the sham group, the first TMS application time generated a significant difference in task performance. The time-accuracy movement learning was immediate when applying TMS.

Cortico-spinal excitability. The Mean $\pm$ SE of data for both muscles in separated condition groups are represented in **Fig. 4**.

Firstly, we normalized the data and analyzed it by a three intra-subject factors (Muscle, Time, and Intensity) and one inter-subject factor (Group condition) ANOVA. A significant main effect of Muscle ($F(1, 20) = 7.855$, $p = .011$); Time ($F(3, 60) = 3.565$, $p = .019$); and Intensity ($F(4, 80) = 298.606$, $p = .000$) was

found, as well as Muscle x Intensity ($F(4, 80) = 6.456, p = .000$); Time x Intensity ($F(12, 240) = 2.483, p = .004$); Muscle x Time x Intensity x Group ($F(12, 240) = 3.030, p = 0.001$).

The post-hoc pair comparison with Bonferroni correction for multiple comparison revealed that in the real group at PrePre-assessment time, the APB muscle MEP amplitude induced at 130% of RMT (Mean = 2.456 SD = 0.388) was significantly higher than in Post-assessment time (Mean = 0.977 SD = 0.263, $p = .021$). In contrast, at Post-assessment time of the ADM muscle MEP amplitude induced at 110% of RMT (Mean = -0.451 SD = 0.102) was significantly lower than in Post-30'-assessment (Mean = 0.056 SD = 0.197, $p = .047$). There are not significant differences between time phases in the sham group.

These results indicate that the TMS intervention synchronized with the dynamic task movement was depressing the motor excitability in the target APB muscle right after the task at 130 % RMT intensity and inducing an opposite mechanism enhancing motor excitability of the control ADM muscle in a long lasting manner, at 110% RMT intensity.

Correlation between behavior performance and cortico-spinal excitability changes induced by TMS-PAS application. The application of MRCS PAS induced changes in motor performance as well as in the excitability levels of motor cortico-spinal pathway. The correlation between these two different changes will explain the same induction from TMS intervention or the possible causality relation between the factors.

As far as the real group concerns, we found positive lineal correlations (Fig. 5) between the RT of the first intervention time (the significantly different RT condition between groups) and the MEP amplitude induced in ADM muscle in Post-assessment time at 110% of RMT intensity ($r = 0.775, p = .008$) and 130% of RMT intensity ($r = 0.668, p = .035$), as well as in Post30-assessment time at 130% of RMT intensity ($r = 0.634, p = .049$). This indicated that the lower is the MEP amplitude in ADM muscle, the shorter the RT.

Regarding the same comparison in sham group (Fig. 6), a tendency for negative lineal correlation was observed between the RT of the first intervention time and the amplitude of MEP induced in ADM muscle in Post-assessment time at 110% of RMT intensity ($r = -0.495, p = .122$), at 130% of RMT intensity ($r = -0.575, p = .064$) and in Post-30'-assessment time at 130% of RMT ($r = -0.559, p = 0.074$).

No correlation was found comparing the RT of first intervention time with APB MEPs. No other correlations between RT and MEP amplitude of the same time were found.

Discussion

The main goal of this study was to evaluate the effects of TMS application over the motor cortex in associative synchronization with the activation of the main muscle performing a dynamic movement related exercise. The main result was the improvement of the task performance (i.e. RT reduction) by MRCS PAS. This effect is not present when sham TMS is used (with the same associative synchronization). As far as the other studied variables concern, the trajectory-accuracy and object-

directed performance improved over time, suggesting a learning effect. However, the PAS intervention we used did not further enhance these indices.

PAS effects on RT

PAS intervention decreased the RT respect to the baseline in both intervention phases of the real group, meaning that PAS is effective immediately. Furthermore, the facilitation of RT performance is also observed immediately after and 30 min after the application of PAS protocol. This suggests both short and long lasting effects of PAS protocol on the motor performance.

The shortening of the RT in both intervention phases does not necessarily imply plasticity. The RT was around 120 ms shorter in the real group compared to sham in the first intervention (faster than the response in second intervention that was 70 ms shorter in the real than in sham group). A possible explanation is that the TMS pulse directly or indirectly facilitates the motor execution of the task. The shortening of the RT 30 min after the intervention more likely implies plasticity and it built up over time.

PAS effects on MEPs

APB muscle

The changes in excitability of the motor cortico-spinal pathway, measured by the amplitude of the MEPs, showed that PAS stimulation induced a depression at the Post-stimulation phase when applying an intensity of 130% of the RMT in APB, the target of the stimulation and main muscle developing the motor task. At least two different factors may contribute to the excitability changes, namely, the plastic changes induced through the PAS protocol and fatigue mechanisms. As far as the plastic changes induced through the PAS protocol concern, we could expect a facilitation of the APB and less or null facilitation of the ADM, however, the opposite results were found.

We propose two alternative explanations: 1) MEP facilitation is occluded by fatigue; Our protocol reduced cortical excitability and the RT shortening depends on improved time-accuracy learning more than to motor cortex cortical excitability changes. Fatigue in the motor corticospinal pathway correlates with decreased MEP amplitude [25, 26, 27, 28, 29, 30]. Our task was highly demanding and required a series of muscle activation repetitions that could be responsible of generating central fatigue. In our experiment, the APB muscle participated more actively to the task and so it is conceivable that APB and its corticospinal connections were more fatigued than the ADM. When a PAS protocol and a fatigue task, or vice versa, are consecutively applied, processes of potentiation due to the intervention and depression of fatigue are occluded by the opposing processes, remaining without differences from previous to posterior assessments [31]. This is in agreement with our results, which suggest that plasticity induction was not producing the MEP enhancement effect, at the first four TMS intensities, likely due to the **occlusion of plasticity processes by fatigue**. At the highest intensity, we could observe a reduction of the MEP amplitude suggesting that, at this stimulation level, fatigue was overcoming the effects of PAS induced plasticity.

We suggest that PAS plasticity was important to induce the improvement of the performance, but the MEP evaluation of a fatigued muscle was not representing completely these effects due to fatigue factor contribution to the amplitude. Fatigue is not occluding RT improvement as the MRCP PAS is inducing at the same time both phenomena, making both indivisible in the measure of the MEP amplitude.

2) It is well known that plasticity is state dependent. PAS protocols at rest usually increase cortical excitability [12]. The PAS during a fatigued state may induce a different plasticity that produces cortical excitability reductions while the time-accuracy learning is improved.

ADM muscle

We demonstrated that there was a potentiation in ADM excitability although the PAS intervention had APB as the target muscle (TMS pulses over the APB hot spot). Interestingly, the potentiation was only evident at around threshold intensity (110% RMT). As this MEP potentiation correlated with the task performance in a positive direction specifically in real group, we can suggest that the PAS was the main mechanism to produce both phenomena: RT reduction and ADM long-term potentiation. However, the correlation showed a smaller MEP linked with a shorter RT. This can be showing that **PAS was contributing to performance potentiation as well as fatigue process or decreased MEP amplitude**. Although PAS was likely inducing a more pronounced fatigue, it also promoted a better time-accuracy movement learning.

The differential effects on ADM and APB cortical excitability were probably due to two main mechanisms: 1) PAS effects are not muscle specific; 2) ADM was not that much fatigued (as it was not primarily involved in the task execution) so we could measure the amplitude increase due to PAS. However, the more the task performance was improved, the more the MEP was reduced in ADM and therefore more fatigue induced.

Why the MEP effects are different depending on the TMS pulse intensity?

Patton and Amassian (1954) characterized the electrical stimulation descending volley through the direct recording from pyramidal tract of the motor cortex in cats and primates. They suggested two type of waves in the induced electrical activity: the initial volley produced by direct stimulation of the pyramidal neuron axon, called D wave, and the later volleys produced by indirect activation through the synapsis of this corticospinal neurons, called I waves [32]. Posterior studies found that, while TMS recruits I waves at threshold intensity, activating the presynaptic neuron that triggers the pyramidal activation indirectly, at supra-threshold intensity activates both neurons, the cortico-cortical and the pyramidal nervous cells [33; 34; 35; 36; 37, 38, 39].

We found MEP potentiation of the ADM muscle at 110% of the RMT and MEP depression in APB muscle at 130% of RMT. As far as ADM MEP facilitation concerns, we suggest that at 110% of RMT PAS was indirectly activating corticospinal neurons. This effect was not evident when TMS pulse intensity was

higher, probably because we could not see the facilitation when a larger number of corticospinal neurons were stimulated directly. Thus, PAS induction could affect cortico-cortical plasticity to a greater extent.

Regarding the depression of APB at 130% of RMT, as discussed above, the PAS effects may not be observed due to the fatigue. D wave contributes to the MEP amplitude and be sensitive to fatigue [40], so it is conceivable that fatigue could affect more the MEP obtained at higher TMS intensity, when the pyramidal neuron axons are recruited. Thus, fatigue may affect more the corticospinal cells.

In conclusion we suggest a mechanism in which plastic enhanced changes are likely induced by PAS (behavioral effects demonstrated it), and that the fatigue affecting pyramidal neurons may explain why these effects are not always paralleled by MEP amplitude changes.

Methodological considerations and limitations of the study

The PAS study we presented is an intermediate step between the research of the neurological and behavioral effects of the MRCS protocols and its practical application to the neurorehabilitation of complex movements involved in ADLs. The results reflected the capacity of improving functional and dynamic movements and thus, the direction for future studies towards the application of this protocols in motor processes triggered for a specific activity.

In addition, the results of our study facilitated the design of these therapies by relating the importance of fatigue with nervous system excitability changes. The functional movements synchronized in PAS protocols in order to improve RTs, should be integrated in the least fatigue-inducing way to enhance the excitability of the target motor pathway.

The empirical results reported herein should be considered in the light of some limitations that could be addressed in future research.

The fatigue assessment was not present in our research; therefore, we did not precisely define the specific extend of this factor, which would be of great interest since it is likely a fundamental factor interacting with the results of the task execution. In future studies, an evaluation of this would be necessary in addition to research the effects of rest and recovery within the plasticity induction protocol.

Overall, we conclude that the applications of MRCS PAS can be beneficial for the motor rehabilitation of movements involved in ADLs and the necessity of a design that considers fatigue for future validation of dynamic PAS protocols.

Conclusion

We can conclude that MRCS protocol had immediate and long-term motor performance effects on more complex movement tasks. The plastic changes induced by this protocol are maintained in a long-lasting manner. This could lead to future research in the promotion of an enhancement PAS application regarding ADL tasks.

Moreover, we demonstrated that the MRCS PAS protocol has not muscle specific effects on corticospinal excitability when paired with complex task. PAS effects induced changes in other adjacent muscles. We suggested that fatigue might interfere with the PAS protocol occluding the possible optimal long-lasting effects in corticospinal excitability of the main muscle and that PAS is likely contributing to increase it although enhances the task at the same time.

Besides, we detected that MRCP PAS plasticity effects are induced over cortico-cortical network while induced fatigue is likely triggered in the pyramidal tract of central nervous system.

These conclusions can be of great help when applying PAS protocols, in order to rehabilitate movement in ADL tasks considering fatigue process and preventing a maladaptive potentiation on the nervous system.

Abbreviations

Transcranial Magnetic Stimulation (TMS);

Paired Associative Stimulation (PAS);

Movement-Related Cortical Stimulation (MRCS);

Reaction Time (RT);

Activities of Daily Living (ADL);

Motor Evoked Potentials (MEPs);

Abductor Pollicis Brevis (APB);

Abductor Digiti Minimi (ADM);

Spike-Timing-Dependent Plasticity (STDP);

Long Term Potentiation (LTP);

Long Term Depression (LTD);

Resting Motor Threshold (RMT);

Beagle Bone Black (BBB);

Trajectory error (TE);

Standard Error (SE).

Declarations

Ethics approval and consent to participate

All participants gave informed consent before the experimental intervention. The Spanish National Research Council (CSIC) Ethics Committee approved all procedures.

Consent for publication

Every individual person gave consent in order to publish their data and, in concrete, the individual detail image in Figure 1A (a hand) is aware and gave consent for publication.

Availability of data and material

Large part of data generated or analysed during this study are included in this published article. The complete datasets during and/or analyzed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by European social fund through the Youth Employment Operational Program and the Youth Employment Initiative (YEI) of the Community of Madrid and Cajal Institute of the Spanish National Research Council (CSIC), Madrid, Spain.

Authors' contributions

ASA designed and performed the experiments applying TMS methodology; recorded, analyzed and interpreted the data; and was a major contributor in writing the manuscript. GA developed the new software of videogame task, assisted the experiments and contributed to the manuscript and figures drafting and correction. AO analyzed and interpreted the data regarding cortical plasticity, motor cortex excitability and behavioral potentiation results. JCM and JLP provided materials needed, have drafted the work and substantively revised it. All authors read and approved the final manuscript.

Acknowledgements

We would like to thank all the subjects that have participated in this study.

References

1. Mayford, M., Siegelbaum, S. A., & Kandel, E. R. (2012). Synapses and memory storage. Cold Spring Harbor perspectives in biology, 4(6), a005751. doi: 10.1101/cshperspect.a005751.

2. Dan, Y., & Poo, M. M. (2006). Spike timing-dependent plasticity: from synapse to perception. *Physiological reviews*, 86(3), 1033-1048. <https://doi.org/10.1152/physrev.00030.2005>.
3. Bliss, T. V. (1973). P. Lomo T. Long-lasting potentiation of synaptic transmission in the anaesthetized rabbit following stimulation of the perforant path. *J physiol*, 32-331. <https://doi.org/10.1113/jphysiol.1973.sp010273>.
4. Egger, V., Feldmeyer, D., & Sakmann, B. (1999). Coincidence detection and changes of synaptic efficacy in spiny stellate neurons in rat barrel cortex. *Nature neuroscience*, 2(12), 1098-1105. <https://doi.org/10.1038/16026>.
5. Song, S., Miller, K. D., & Abbott, L. F. (2000). Competitive Hebbian learning through spike-timing-dependent synaptic plasticity. *Nature neuroscience*, 3(9), 919-926. <https://doi.org/10.1038/78829>.
6. Sjöström, P. J., Turrigiano, G. G., & Nelson, S. B. (2001). Rate, timing, and cooperativity jointly determine cortical synaptic plasticity. *Neuron*, 32(6), 1149-1164. [https://doi.org/10.1016/S0896-6273\(01\)00542-6](https://doi.org/10.1016/S0896-6273(01)00542-6).
7. Tzounopoulos, T., Kim, Y., Oertel, D., & Trussell, L. O. (2004). Cell-specific, spike timing-dependent plasticities in the dorsal cochlear nucleus. *Nature neuroscience*, 7(7), 719-725. <https://doi.org/10.1038/nn1272>.
8. D'amour, J. A., & Froemke, R. C. (2015). Inhibitory and excitatory spike-timing-dependent plasticity in the auditory cortex. *Neuron*, 86(2), 514-528. <https://doi.org/10.1016/j.neuron.2015.03.014>.
9. Froemke, R. C. (2015). Plasticity of cortical excitatory-inhibitory balance. *Annual review of neuroscience*, 38, 195-219. <https://doi.org/10.1146/annurev-neuro-071714-034002>.
10. Barker, A. T., Jalinous, R., & Freeston, I. L. (1985). Non-invasive magnetic stimulation of human motor cortex. *The Lancet*, 325(8437), 1106-1107. [https://doi.org/10.1016/S0140-6736\(85\)92413-4](https://doi.org/10.1016/S0140-6736(85)92413-4).
11. San Agustín, A., & Moreno, J. C. (2021). Ethical Aspects of Transcranial Magnetic Stimulation for Neuroenhancement. *Dilemata*, (34), 121-132.
12. Stefan, K., Kunesch, E., Cohen, L. G., Benecke, R., & Classen, J. (2000). Induction of plasticity in the human motor cortex by paired associative stimulation. *Brain*, 123(3), 572-584. <https://doi.org/10.1093/brain/123.3.572>.
13. Classen, J., Wolters, A., Stefan, K., Wycislo, M., Sandbrink, F., Schmidt, A., & Kunesch, E. (2004). Paired associative stimulation. *Suppl Clin Neurophysiol*, 57(563), 9. [https://doi.org/10.1016/S1567-424X\(09\)70395-2](https://doi.org/10.1016/S1567-424X(09)70395-2).
14. Ziemann, U., Ilić, T. V., Pauli, C., Meintzschel, F., & Ruge, D. (2004). Learning modifies subsequent induction of long-term potentiation-like and long-term depression-like plasticity in human motor cortex. *Journal of Neuroscience*, 24(7), 1666-1672. <https://doi.org/10.1523/JNEUROSCI.5016-03.2004>.
15. Frantseva, M. V., Fitzgerald, P. B., Chen, R., Möller, B., Daigle, M., & Daskalakis, Z. J. (2008). Evidence for impaired long-term potentiation in schizophrenia and its relationship to motor skill learning. *Cerebral Cortex*, 18(5), 990-996. <https://doi.org/10.1093/cercor/bhm151>.

16. Tolmacheva, A., Savolainen, S., Kirveskari, E., Lioumis, P., Kuusela, L., Brandstack, N., ... & Shulga, A. (2017). Long-term paired associative stimulation enhances motor output of the tetraplegic hand. *Journal of neurotrauma*, 34(18), 2668-2674. <https://doi.org/10.1089/neu.2017.4996>.
17. Wessel, M. J., Zimerman, M., & Hummel, F. C. (2015). Non-invasive brain stimulation: an interventional tool for enhancing behavioral training after stroke. *Frontiers in human neuroscience*, 9, 265. <https://doi.org/10.3389/fnhum.2015.00265>.
18. Brihmat, N., Tarri, M., De Boissezon, X., Gasq, D., Loubinoux, I., Marque, P., & Castel-Lacanal, E. (2018). Effect of the association of motor imagery exercises and paired associative stimulation in stroke patients (MIPAS). *Annals of Physical and Rehabilitation Medicine*, 61, e28. <https://doi.org/10.1016/j.rehab.2018.05.062>.
19. San Agustín, A., & Pons, J. L. Paired associative stimulation protocols with transcranial magnetic stimulation for motor cortex potentiation. In *School And Symposium On Advanced Neurorehabilitation (SSNR2018)* (p. 44).
20. Thabit, M. N., Ueki, Y., Koganemaru, S., Fawi, G., Fukuyama, H., & Mima, T. (2010). Movement-related cortical stimulation can induce human motor plasticity. *Journal of Neuroscience*, 30(34), 11529-11536. <https://doi.org/10.1523/JNEUROSCI.1829-10.2010>.
21. San Agustín, A., & Pons Rovira, J. L. (2019). Paired Associative Stimulation For Memory Facilitation. In *School And Symposium On Advanced Neurorehabilitation (SSNR2019)* (p. 37-39).
22. Groppa, S., Oliviero, A., Eisen, A., Quartarone, A., Cohen, L. G., Mall, V., ... & Rossini, P. M. (2012). A practical guide to diagnostic transcranial magnetic stimulation: report of an IFCN committee. *Clinical Neurophysiology*, 123(5), 858-882. <https://doi.org/10.1016/j.clinph.2012.01.010>.
23. The Math Works, Inc. (2013). MATLAB (Version 2013b) [Computer software]. <https://www.mathworks.com/>
24. IBM Corp. (2019). IBM SPSS Statistics for Windows. (Version 2019) [Computer software]. <https://www.ibm.com/analytics/spss-statistics-software>.
25. Brasil-Neto, J. P., Pascual-Leone, A., Valls-Solé, J., Cammarota, A., Cohen, L. G., & Hallett, M. (1993). Postexercise depression of motor evoked potentials: a measure of central nervous system fatigue. *Experimental Brain Research*, 93(1), 181-184. DOI: 10.1007/BF00227794.
26. Samii, A., Wassermann, E. M., & Hallett, M. (1997). Post-exercise depression of motor evoked potentials as a function of exercise duration. *Electroencephalography and Clinical Neurophysiology/Electromyography and Motor Control*, 105(5), 352-356. [https://doi.org/10.1016/S0924-980X\(97\)00033-7](https://doi.org/10.1016/S0924-980X(97)00033-7).
27. Sacco, P., Newberry, R., McFadden, L., Brown, T., & McComas, A. J. (1997). Depression of human electromyographic activity by fatigue of a synergistic muscle. *Muscle & Nerve: Official Journal of the American Association of Electrodiagnostic Medicine*, 20(6), 710-717. DOI: 10.1002/(sici)1097-4598(199706)20:6<710::aid-mus8>3.0.co;2-b.
28. Zijdwind, I., Zwarts, M. J., & Kernell, D. (2000). Potentiating and fatiguing cortical reactions in a voluntary fatigue test of a human hand muscle. *Experimental brain research*, 130(4), 529-532. DOI:

29. Di Lazzaro, V., Oliviero, A., Tonali, P. A., Mazzone, P., Insola, A., Pilato, F., ... & Rothwell, J. C. (2003). Direct demonstration of reduction of the output of the human motor cortex induced by a fatiguing muscle contraction. *Experimental brain research*, 149(4), 535-538. DOI: 10.1007/s00221-003-1408-6.
30. Perretti, A., Balbi, P., Orefice, G., Trojano, L., Marcantonio, L., Brescia-Morra, V., ... & Santoro, L. (2004). Post-exercise facilitation and depression of motor evoked potentials to transcranial magnetic stimulation: a study in multiple sclerosis. *Clinical neurophysiology*, 115(9), 2128-2133. <https://doi.org/10.1016/j.clinph.2004.03.028>.
31. Milanović, S., Filipović, S. R., Blesić, S., Ilić, T. V., Dhanasekaran, S., & Ljubisavljević, M. (2011). Paired-associative stimulation can modulate muscle fatigue induced motor cortex excitability changes. *Behavioural brain research*, 223(1), 30-35. <https://doi.org/10.1016/j.bbr.2011.04.013>.
32. Patton, Harry D., and Vahé E. Amassian. "Single-and multiple-unit analysis of cortical stage of pyramidal tract activation." *Journal of neurophysiology* 17.4 (1954): 345-363. <https://doi.org/10.1152/jn.1954.17.4.345>.
33. Day, B. L., Thompson, P. D., Dick, J. P., Nakashima, K., & Marsden, C. D. (1987). Different sites of action of electrical and magnetic stimulation of the human brain. *Neuroscience letters*, 75(1), 101-106. [https://doi.org/10.1016/0304-3940\(87\)90083-8](https://doi.org/10.1016/0304-3940(87)90083-8).
34. Di Lazzaro, V., Restuccia, D., Oliviero, A., Profice, P., Ferrara, L., Insola, A., ... & Rothwell, J. C. (1998). Magnetic transcranial stimulation at intensities below active motor threshold activates intracortical inhibitory circuits. *Experimental Brain Research*, 119(2), 265-268. DOI: 10.1007/s002210050341.
35. Di Lazzaro, V., Oliviero, A., Pilato, F., Mazzone, P., Insola, A., Ranieri, F., & Tonali, P. A. (2003). Corticospinal volleys evoked by transcranial stimulation of the brain in conscious humans. *Neurological research*, 25(2), 143-150. <https://doi.org/10.1179/016164103101201292>.
36. Di Lazzaro, V., Oliviero, A., Pilato, F., Saturno, E., Dileone, M., Mazzone, P., ... & Rothwell, J. C. (2004). The physiological basis of transcranial motor cortex stimulation in conscious humans. *Clinical neurophysiology*, 115(2), 255-266. <https://doi.org/10.1016/j.clinph.2003.10.009>.
37. Berardelli, A., Inghilleri, M., Cruccu, G., & Manfredi, M. (1990). Descending volley after electrical and magnetic transcranial stimulation in man. *Neuroscience letters*, 112(1), 54-58. [https://doi.org/10.1016/0304-3940\(90\)90321-Y](https://doi.org/10.1016/0304-3940(90)90321-Y).
38. Thompson, P. D., Day, B. L., Crockard, H. A., Calder, I., Murray, N. M., Rothwell, J. C., & Marsden, C. D. (1991). Intra-operative recording of motor tract potentials at the cervico-medullary junction following scalp electrical and magnetic stimulation of the motor cortex. *Journal of Neurology, Neurosurgery & Psychiatry*, 54(7), 618-623. DOI: 10.1136/jnnp.54.7.618 .
39. Burke, D. J., Hicks, R., Stephen, J., Woodforth, I., & Crawford, M. (1993). The site of activation of the corticospinal system by transcranial magnetic and electrical stimulation of the human motor cortex. In *Spasticity* (pp. 57-66). Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-78367-8_5.
40. Di Lazzaro, V., Oliviero, A., Profice, P., Saturno, E., Pilato, F., Insola, A., ... & Rothwell, J. C. (1998). Comparison of descending volleys evoked by transcranial magnetic and electric stimulation in

Figures

Figure 1

A. Task phases of movement related dynamic task. 1. Resting phase; 2. Pre-movement phase; 3. Movement phase; 4. Feedback phase, and TMS application time indicated by the lightning signal. B. Customized videogame device based on position sensor, electronic board and BeagleBone Black board.

Figure 2

Experimental phases time line. 1. Pre-intervention phase; we assessed the task basic skills and pathway excitability. 2. Intervention phase; we applied the PAS protocol synchronizing thumb movement with TMS pulse. 3. Post-intervention phase: we assessed task learned skills and pathway excitability changes. 4. 30

Figure 3

Behavioral measurement. Mean and Standard Error of the elapsed time from the cue until the first movement, defined as RT, for real and control groups between every phase: Pre (Pre-intervention assessment phase), Int1 (First 30 trials of intervention phase), Int2 (Second 30 trials of intervention phase), Post (Post-assessment intervention phase), Post30 (Post-30 minutes intervention assessment phase). One asterisk corresponds to significance between $p=0.05$ and $p=0.01$; two asterisks correspond to significance below $p=0.01$.

Figure 4

Recruitment curves. Raw data of Main, SE and significant differences of the peak-to-peak MEP amplitude for APB and ADM muscles represented in real and sham groups separately, and the different experimental times in each intensity levels. One asterisk corresponds to significance between $p=0.05$ and $p=0.01$; two asterisk correspond to significance below $p=0.01$.

Figure 5

Real group's correlation between % of MEP amplitude and % of RT based on Pre-assessment time. Simple linear regression and the coefficient of determination of the correlation of behavior RT performance in the first intervention with MEPs amplitudes of ADM in post- and post-30'-assessment time at 110 % and 130 % of RMT. R and p value are reported in the results. One asterisk corresponds to significance between $p=0.05$ and $p=0.01$; two asterisk correspond to significance below $p=0.01$.

Figure 6

Sham group's correlation between % of MEP amplitude and % of RT based on Pre-assessment time. Simple linear regression and the coefficient of determination of the correlation of behavior RT performance in the first intervention with MEPs amplitudes of ADM in post- and post-30'-assessment time at 110 % and 130 % of RMT.