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Exploration of cortical effect of PAS

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Abstract

Context: Transcranial Magnetic stimulation (TMS) is a non-invasive stimulation technique of the brain, which allows the investigation of the neuronal plasticity and cortical circuits in humans. This method is based on the generation of an impulse by using an electromagnetic induction (1). Paired associative stimulation (PAS) is a non-invasive technique used to induce and to study the plasticity in the human motor cortex. Essentially, it is the repeated pairing of a peripheral stimulation with a cortical stimulation (2). PAS induces an LTP-like effect with an inter-stimulus interval of 25ms. The latencies of afferent signals from the median nerve stimulation at the wrist to reach the motor cortex (M1) after 20-25ms in order to produce synchronous pre- and postsynaptic activation (3). This change of excitability in the motor cortex induced by PAS is quantified by recording motor evoked potentials (MEP) before and after the intervention. Another way to study alterations in the motor cortex with TMS is to record the long interval intra-cortical inhibition (LICI) before and after PAS. It is very important to know that the PAS response is characterized by a high inter/intra-subject variability (4). This variability could be partially explained by the fact that every subject has a different level of excitability before the PAS. So, a reason to investigate the effect of PAS on MEP and LICI is to understand if PAS could be a method to set every subject at the same level of excitability before studying his plasticity in order to have more comparable results.

Objectives: This study is part of a larger project that investigates potential interactions between different techniques exploring focal dystonia in patients with the Complex Regional Pain Syndrome (CRPS). This study explores and compares two protocols of PAS, the conventional low frequency PAS (0.2 Hz) with a high frequency (5Hz) PAS.

Method: In our study, we will investigate the plasticity of the motor cortex and inhibition in 15 healthy subjects. The PAS protocol is a 2 stimuli sequence separated by a specific interval (25ms). We will deliver an electric stimulus on the median nerve followed by a TMS pulse at the level of the motor cortex with an 8-shaped magnetic coil. we record the MEP from the abductor digiti minimi muscle (ADM), Dorsal interossei of the hand (FDI). The paired-pulse TMS paradigm (ppTMS) resides in the combination of a subthreshold conditioning stimulus with a suprathreshold test stimulus at different intervals. LICI will be recorded by ppTMS using 100ms as interval with two suprathreshold stimulus.

Conclusions: Both 5Hz and 0.2Hz PAS protocols have comparable effects on motor cortex, about ¾ of the subjects show lpong-term potentiation. There were no side effects, but some subjects found 5Hz unpleasant **Keywords:** LTP, TMS, PAS, hand dystonia, LICI

Introduction

1. Basic principles of transcranial magnetic stimulation

A non-invasive investigation of the neuronal plasticity, cortical and spinal circuits in humans is possible thanks to Transcranial Magnetic Stimulation (TMS). This method is based on the principles of electromagnetic induction and allows a non-invasive and painless stimulation of the brain. An electric Current in the coil creates a perpendicular magnetic field, which induces then an electric current that can be directed to activate a specific brain cortex area, in this case the motor cortex. (1) A muscle response termed Motor Evoked Potential (MEP) can be measured. MEPs are characterized by an important variability due to variable number of recruited α-motor neurons depending on the cortical excitability.(5) On the other hand there is the desynchronization of the descending discharges which leads to a phase cancellation phenomenon whereby the positive and negative phases of the desynchronized action potentials cancel each other out which determine the MEPs. (6)

2. Paired associative stimulation and cortical plasticity

The paired associative stimulation (PAS) is a non-invasive technique to study the plasticity in the human motor cortex. The PAS consists of the repeated pairing of peripheral nerve stimulation with cortical stimulation with a defined inter-stimulus interval (ISI). The peripheral pulse is an electric stimulation, while the cortical one is single pulse TMS. Depending on the inter-stimulus interval between those two stimulations, we can observe an excitatory or an inhibitory effect on the motor cortex (2) corresponding to a long-term potentiation (LTP) and long-term depression (LTD) which underlie neuronal plasticity (7). The molecular mechanism in LTP relies on *N*-methyl-D-aspartate (NMDA)sub-class of glutamate receptor (NMDAR) (8). Premedication with a NMDAR blocker (dextromethorphan) suppresses the potentiation of the motor evoked potentials (MEP) (9). Furthermore, there are evidence that PAS25 does not decrease the intra-cortical inhibition mediated by $GABA_A$ receptors (9,10). These findings indicate that under the mechanism for the induction of plasticity there is an LTP-like effect. The PAS induces an LTP-like effect with the inter-stimulus interval (ISI) of 25ms, as the afferent signal from the median nerve stimulation reaches the motor cortex (M1) within 20-25ms producing presumably a synchronous pre- and postsynaptic activation (11). This LTP-like change of excitability in the motor cortex induced by PAS is quantified by recording MEP before and after the intervention. The results from Stefan et al. (2000) showed that on average 55% of the subjects have a facilitator effect after PAS25, passing from

1.1 ± 0.3 mV to a 1.7 ± 0.8 mV MEP. (2) PAS excitatory effect last up to 90 min, but the strongest and more consistent effect duration is 60 min. (12)

The PAS is characterized by a high inter-/intra-subject variability, which limits its use in research and clinical practice. As detailed by Suppa et al. many technical and subject-related factors influence the intensity of the facilitation effect of this technique. (13)

3. Paired-pulse TMS and intracortical inhibition

Another way to study changes of excitability in the motor cortex with TMS is to record the paired-pulse stimulation paradigm of long interval intra-cortical inhibition (LICI) before and after the PAS protocol. The paradigm consists in the combination of two supra-threshold stimulus of 120% of rMT (rest Motor-Threshold). The first one is the conditioning stimulus (CS), while the second one is the test stimulus (TS). The rest motor threshold is defined as the intensity required to produce a minimal MEP response in 5 of 10 trials (> 50µV).

When the CS precede the test stimulus (TS) from 50ms to 200ms, the amplitude of the motor evoked potential (MEP) provoked by the TS is decreased by the activation of long-interval intracortical inhibitory circuits (LICI) (14). This mechanism is due to the recruitment of the inhibitory interneurons which are mediated by $GABA_B$ receptor activity. (15)

4. Interests of our study

The objectives of this study are to compare the effect of PAS25 in human cortex of healthy subjects by measuring LICI and MEP before and after the PAS intervention. Furthermore, we compare two different PAS paradigms: the conventional 0.2Hz PAS protocol with a faster PAS protocol at a rate of 5Hz, which has been principally applied by Quartarone et al. who demonstrated the efficacy of the 5Hz protocol to induce an LTP-like effect in healthy subjects. (16)

This study explores and compares two protocols of PAS, the conventional low-frequency PAS (0.2 Hz) with a high-frequency (5Hz) PAS. Particularly, we were interested whether the 5Hz and the 0.2Hz protocol are comparable in inducing cortex plasticity, which could reduce the time of stimulation from 20 to 2 minutes.

Method

Subjects

17 healthy subjects participated in the study, 14 did both protocols (0.2 and 5Hz), 17 did the 0.2Hz and 14 did 5Hz. MEP Recording was done in the dominant hand. All subjects gave written informed consent and the local ethics committee approved the experiment. The international safety standards for TMS (17) were followed and used as exclusion criteria.

TMS

A figure-of-eight 70mm hand-held coil connected to a Magstim BiStim2 apparatus (Magstim Company Ltd., Spring-Gardens, Whitland, UK) was used to obtain MEPs (TMS). First, we looked for the optimal cortical stimulation spot («motor hot-spot») holding the coil tangentially to the skull. Minimal displacements following a grid were necessary to find the best spot with the largest MEP. The coil was then manually kept in the same position throughout the experiment. Resting motor threshold (RMT) was determined using the maximum likelihood threshold hunting procedure described by Awiszus (2003) (Motor Threshold Assessment Tool, version 2.0: http://www.clinicalresearcher.org/software.htm). Motor evoked potentials of peak-to-peak amplitude of >50 µV were considered valid responses and fed back accordingly to the software

EMG recording

We recorded the MEP from the abductor digiti minimi muscle (ADM) and the Dorsal interossei of the hand (FDI) with surface electromyography (EMG) electrodes. The EMG was recorded with the Viking Select (Nicolet Biomedical, Madison, WI, USA). Signals were amplified, band-pass filtered (1–5 kHz), and then sampled at a rate of 25 kHz and stored for off-line analysis. We applied a Fourier transformation analysis of the digitalized signal to exclude 50-Hz (AC) artefacts. In order to explore the central effect of the paradigm, we recorded LICI by using a paired-pulse transcranial magnetic stimulation paradigm combining two suprathreshold (120% of the rest motor threshold) stimuli given with an inter-stimulus interval (ISI) of 50,100 and 200ms.

Procedure

The TMS can be set as described before and the rest motor threshold (rMT) is determined. For LICI the 120% of this value is used for the recording of the baseline and for the stimulation. The baseline values of MEP and LICI can now be recorded: 12 MEPs and 2x12 LICI .

For the peripheral nerve stimulation, we use a Grass Stimulator (Grass S88 – Astro-Med Inc. Grass Instrument Division, West Warwick, RI, USA) with the electrodes placed on the ulnar nerve at wrist

level. The stimulation intensity is progressively increased until the subject feels the stimulus in his little finger. The stimulation intensity for the PAS is set at the 2.5 sensitivity threshold level.
We performed both the PAS stimulation protocols in a random order, (for 20 minutes for the 0.2Hz protocol and for 2 min for the 5Hz PAS protocol). We recorded the baselines measures (12 MEPS and 2x12 LICI) immediately (T1) , and 20 minutes after the PAS protocol (T2).

Analysis

Each signal of the EMG recordings was visually inspected to reject "noisy" recordings, the peak-to-peak amplitudes of the MEPs were automatically extracted with a MATLAB script.

Results

1. Effect of PAS on single pulse

Comparison of the effect of both protocols

The main objective of this study was to compare the 0.2Hz PAS protocol with the 5Hz PAS protocol and analyse if they are equivalent. We found that the ratio of responders is comparable: 71% for both protocols. The mean response across subject before and after PAS25 protocol as a percentage of the baseline response was processed (Figure 1). The 0.2 Hz showed a strong effect at T1, that weakens at T2 (+ 57% and +28% respectively), while the 5 Hz had a smaller effect at T1 that reached at T2 a effect size similar to 0.2Hz at T1 (+15% and +53% respectively). A two-way ANOVA showed no effect of PAS_TYPE (p=0,59) but an effect of TIME (p=0,02) and no effect of interaction PAS_TYPE * TIME (p=0,62). Both protocols showed thus a comparable effect across time. Concerning inter-subject variability, both paradigms had a similar variability, with a standard deviation of the percentage of change at T1 and T2 of 101,7 % for 0.2 Hz and 107,7% for 5 Hz.

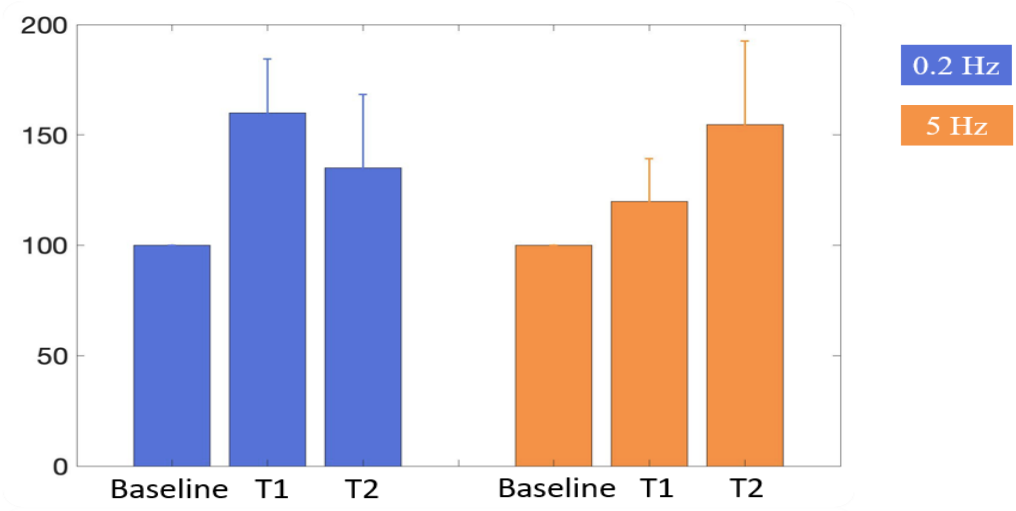

Figure 1. The mean response of the 14 subjects that participate to both protocols is shown here as a percentage value. The baseline corresponds to the 100%. Is also notable the variation coefficient, which is represented over the columns.

Effect of the paradigms at the subject level

Figure 2 illustrates the variability of effect of each protocol across subject. A majority of subjects demonstrated a facilitation after both PAS protocols, either immediatly after, 20 minutes after or both (10 out of 14 in both paradigms). For each subject, the effect of both paradigms was not necessarily the same. At T1 only 5 out of 14 subjects demonstrated the same effect in both PAS, and at T2, 7 out of 14 subjects (Figure 3).

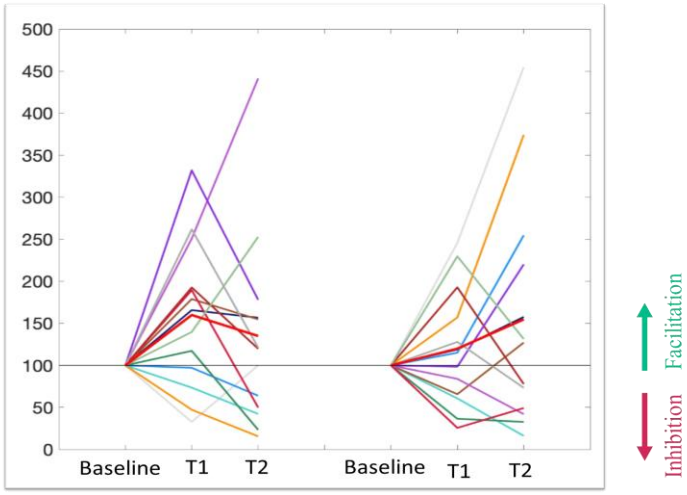

Figure 2. MEP amplitude in response to single pulse expressed as the percentage of baseline response (at right we can found 0.2Hz protocol and at left the 5Hz protocol).Each line represents a subject's response immediately after PAS (T1) and 20 minutes after (T2).

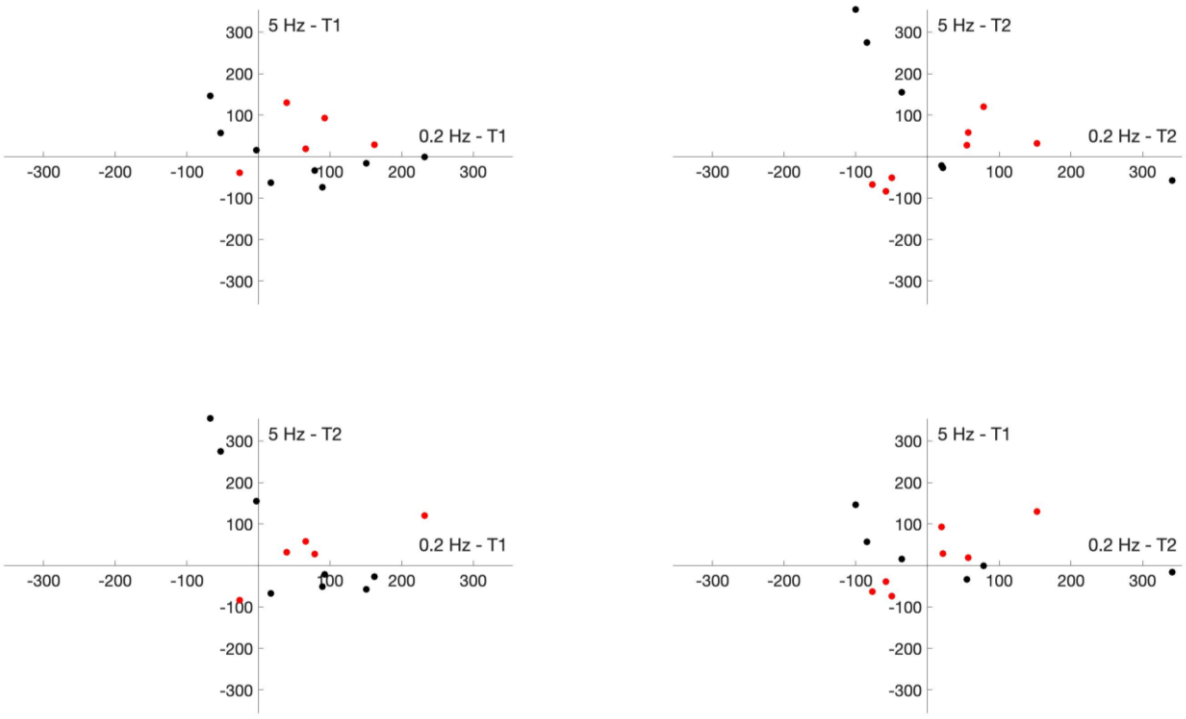

Figure 3. Correlation of theeffects of PAS 0.2 and 5 Hz at the individual level. Each point represents a subject and the change in MEP amplitude size at T1 and T2 compared to the baseline in regard to the two PAS protocols, the PAS 5Hz is shown on y axis and PAS 0.2 Hz on x axis. (A) effect of each protocol at T1, (B) effect of each protocol T2, (C) effect of 5 Hz at T2 and 0.2 Hz at T1 (D) effect of 5 Hz at T1 and 0.2 Hz at T2. Red dots represent subjects who presented a comparable change of MEP amplitude in the same direction (facilitation or inhibition) in both PAS paradigms.

2. Interaction between PAS and intra-cortical inhibition

Intra-cortical inhibition

Three different paradigms of LICI were tested, with three ISI: 50ms, 100ms, 200ms. Only the first 12 subjects were tested with the three intervals. In figure 4, mean group data at baseline show a SP response for the 0.2Hz protocol of 0.545mV, 0.698mV to the TS in PP50, 0.094mV in PP100 and 0.638mV in PP200. For the 5Hz protocol: 0.821mV for SP, 0.929mV PP50, 0.119mV PP100, 0.610mV PP200.

Furthermore, is possible to notice that standard deviation (SD) in PP100 is smaller than any other. SD PP100 in 0.2Hz protocol 0.05mV, 0.15mV SP, 0.20mV for PP50 and 0.16mV for PP200. SD PP100 in 5Hz protocol 0.1mV, 0.18mV SP, 0.25mV for PP50 and 0.19mV for PP200. A one-way ANOVA demonstrated that there was a significant effect of LICI (p=0,029). Post-hoc test demonstrated that only

LICI100 was significantly different from baseline (p<0,0001). All subjects had a reduction of MEP after the test stimulus lower than the baseline SP with LICI100. The two other paradigms variability across subjects precluded such a clear effect (figure 4).

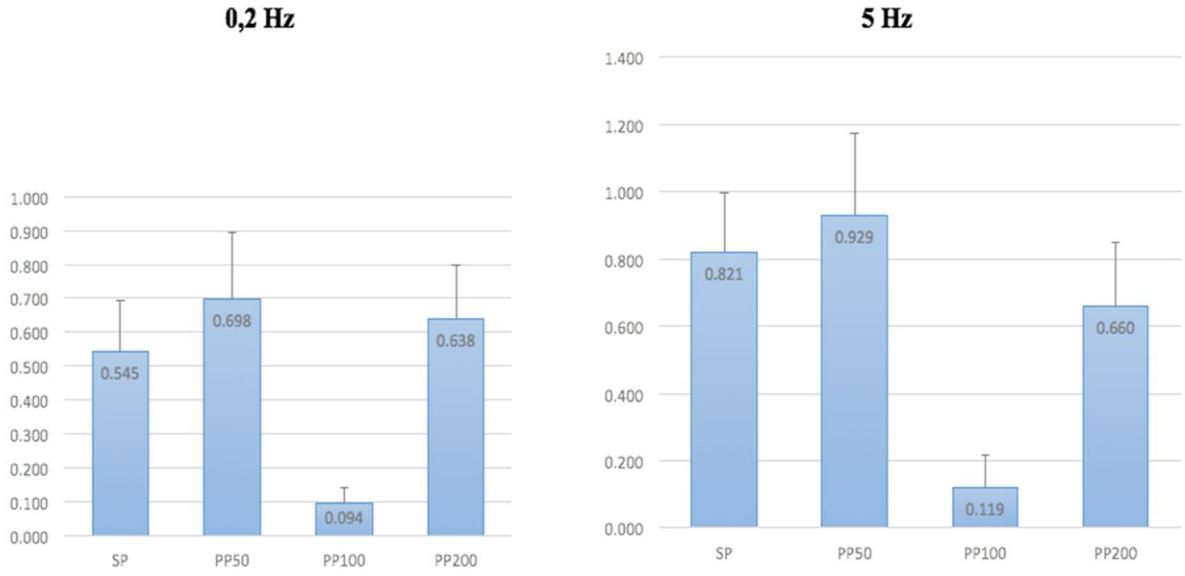

Figure 4: Baseline average values with standard deviation

Interaction between PAS protocols and intracortical inhibition

At the figure 5, the columns represent the average percentage inhibition of MEP amplitude in PP compared to SP at each point time. The 0.2 Hz protocol shows a slightly increment of the inhibition, while the 5Hz shows a decrease. Furthermore, the coefficient of variation is represented. Comparing this result with the figure 4 it seems that in PAS 5 Hz the decrease in inhibition is quite like the increase in MEP amplitude while in PAS 0.2 inhibition and MEP amplitude both increase in a similar way. A repeated measure ANOVA demonstrated that there is a significant effect of LICI (p=0,019) but no effect of TIME, PAS or interaction. Even when considering only LICI100 which was the most consistent, only the factor LICI was significant. There is thus no interaction between the effect of any of the two PAS paradigm and LICI.

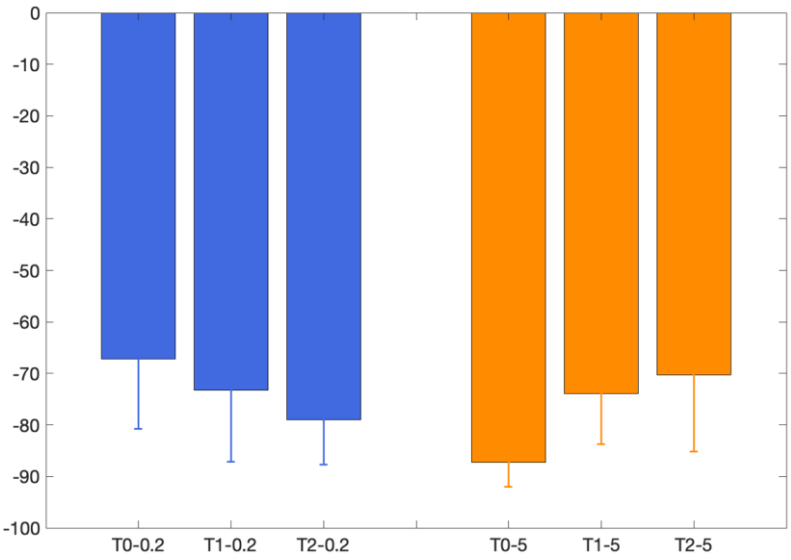

Figure 5: LICI represented as average percentage inhibition of MEP amplitude in PP compared to SP at each point time

Discussion

PAS25 as an effective facilitator protocol

As we expected the 0.2 Hz PAS25 protocol is an efficient facilitator protocol, in fact we found that 71,4% of the subjects have a facilitation. Some subjects show inhibition. Looking at the literature we can find that around 55 % of the subjects respond to the PAS25. (2,4)

5 and 0.2Hz protocol are comparable

This is the first study to compare two PAS protocols with a different frequency. For the first time we demonstrate that the percentage of responders in both protocols is equivalent, it is possible to see that 3/4 of the subjects show a faciliatory effect after both PAS25ms protocols. This is the most important result. In fact, it means that is possible to reduce by 10 the time of stimulation (20min for 0.2Hz protocol and 2min for 5Hz). A shorter time means a more precise and less operator-dependent stimulation. Moreover, the attention of the subject is more consistent and as a result leads to a lower variability intra-subject. The 5 Hz protocol demonstrate an increase still at T2 while there is already a decrease in 0.2 Hz at T2. However, the amplitude change at T2 with the 5 Hz protocol is comparable to the change seen at T1 in the 0.2 Hz protocol (Figure 1). An explanation could be that the mechanism for PAS 0.2 might already be starting after a few minutes after the start of the stimulation, this means that T1 corresponds

to the T2 of the 5Hz protocol. This could explain the slow progression into facilitation seen in 5 Hz in comparison to 0.2 Hz. It might be that if we paused in the PAS 0.2 we would have seen an equivalent of the T1 of PAS 5Hz, and if we recorded 40 minutes after the end of the 5Hz, we could have seen an equivalent of T2 of PAS 0.2 Hz (Figure 6).

It is also important to notice that some subjects described the 5Hz protocol as more disagreeable to support due to the high frequency of stimulation.

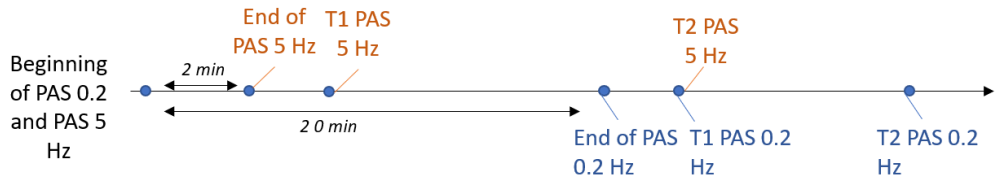

Figure 6. Hypothesis of the difference of peak effect seen in both paradigms.

It is important to consider that the PAS protocol has an important intra and inter-subject variability that depends on various factors such as the position of the magnetic coil which depends essentially by the operator and some subject factors (age, attention, alcohol use, …) as described in the review by Suppa et al. (13) We also found that the variability intra and inter-subject for this technique is very important. As explained in the introduction the reasons are multiples. First, we must consider the variability on MEP recording as described before, and second the variability due to the PAS itself.

Differences between the subjects in the same recording session were found in both protocols, in fact we can see that not all the subjects respond to PAS25 by having a facilitator effect, but some show inhibition. When comparing the effect of the two protocols we can see there is an important intra-subject variability. Indeed, between ¼ to ½ of the subjects only presented the same effect (facilitation or inhibition) with both paradigms. One might be tempted to explain this difference by a different mechanism of induction of both techniques; however, it was previously demonstrated that even the same paradigm repeated several times in the same subject is susceptible to variability (18).

LICI 100 as inhibition protocol

To analyze the effect of PAS25 on LICI only the 100ms ISI is considered. The paradigm with 50ms and 200ms ISI were too variable and show even facilitatory effect. Therefore, the 100ms ISI LICI is more consistent. In literature we can find multiple studies over different intervals ranges to study and produce a LICI, and most are with an ISI of 50 and 200ms. (19,20) For most of the studies the 100ms ISI is used because produce the strongest inhibition of the MEP. (21)

Effect of PAS 25 on LICI

No significant modulation of LICI was observed with either PAS protocol. Thus, the facilitation observed with both PAS 0.2Hz and PAS 5Hz cannot be due to the modulation of the long-intracortical inhibitory pathways (LICI) that we tested in this protocol.

Limit of the study

A limit of this study is the fact that T1 and T2 of the two protocols are do not correspond to the same laps of time as mentioned before and visible in figure 8. A solution to have more comparable results between the 5 and 0.2Hz protocol could be a further record at 40 or 60 minutes after the end of PAS25. As described in the introduction this could be possible thanks to the fact that the facilitation last up to 60 minutes. (12)

Clinical applications of PAS:

PAS is not only important as a method of research and study of Hebbian plasticity of the pathophysiology of some neurodegenerative diseases such as Parkinson's disease (22,23,24), Huntington's disease (25) or focal hand dystonia(26), but it could also be considered as a therapeutic method.
Various studies have applied PAS as a method for treating post-stroke brain injured patients. For example, Carson et al. (2018) demonstrated that PAS can increase the excitability of corticospinal projections to wrist flexor and extensor muscles. (27) Castel-Lacanal et al. (2008) showed that patients with subcortical infarcts respond to PAS in earlier than later delay after a stroke, furthermore a facilitation effect was initially found in 73% of the subjects immediately after stroke and 30% 12 months after. (28) More recent studies like Palmer et al. (2018) suggest that PAS can increase motor cortex excitability that could positively impact on motor skills performance in chronic post-stroke patients. (29)
All those findings are promising and this technique may allow more applications. Moreover, the possibility to reduce the time of stimulation with the same results could encourage more searchers to investigate more therapeutic applications in different fields.

Conclusions

The first conclusion that emerge from this work is that the classical PAS25 protocol effect is confirmed with 71,4% of the subjects responding with a facilitation. Furthermore, we found that the 5Hz has a

comparable effect as 0.2 Hz PAS protocol. The main difference is also the time, PAS 5Hz has a stimulation duration 10 times shorter than the conventional PAS to induce this facilitation. But, we also found that for some subjects it was more difficult to support.

There is an important intra-/inter-subject variability in the 17 subjects who underwent 2 sessions.

PAS allows induce and evaluate cortical plasticity non-invasively. This is of interest in clinical research to better understand the pathophysiology of certain diseases and the effects of various therapies. Previous studies have shown an altered PAS which is normalized by medication in Parkinson's Disease (22), an enhanced plasticity in focal hand dystonia (25) and a reduced plasticity in Huntington's Disease (26).

Additionally, the shorter time of stimulation would allow its use as an "excitability booster" before physical rehabilitation.

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