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Exploring Brain Inhibition and Facilitation by Transcranial Magnetic Stimulation:

Is there a role of repetitive spinal motor neuron discharges (repMND) in the conventional paired-pulsed paradigm of short intra-cortical inhibition (SICI) and intra-cortical facilitation (ICF)?

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Abstract

***Objective*:** To study the role of spinal repMND in the paired-pulsed (PP) paradigms of SICI and ICF by combining PP with TMS, TST, QuadS and QuintS techniques and to explore their variability.

***Background*:** Transcranial magnetic stimulation (TMS) leads to repetitive spinal motor neuron discharges (repMNDs). The paired-pulse TMS (PP-TMS) paradigm allows the exploration of the motor cortex physiology. The triple stimulation technique (TST) and an extended TST-technique including a 4^{th} and 5^{th} stimulation, Quadruple (QuadS) and Quintuple (QuintS) stimulation, respectively, allow a more precise exploration of the central motor conduction and of repMND.

Design/Methods: We explored the PP TMS paradigms of short intracortical inhibition (SICI) with an inter-stimulus interval (ISI) of 2ms and intracortical facilitation (ICF) with an ISI of 10ms in the conventional way (TMS), combined with the TST, and with the QuadS and QuintS in a randomized design in 20 healthy volunteers

Results: About half of the subjects have repMND following a single pulse TMS in the QuadS and QuintS condition (60% and 40%, respectively) and generally more in the QuadS than in the QuintS condition. In both the QuadS and the QuintS, there appear more repMND in the PP-TMS paradigm of ICF than with a single pulse TMS and less than latter in SICI. The variability differs considerably between subjects, but combining the PP paradigms with the TST reduced variability of SICI by -27% but not for ICF or SP. There were subjects showing inhibition and facilitation when the opposite was expected.

***Conclusions*:** These results suggest that there is a contribution of repMND in the conditioned responses of PP-TMS. The large variability precludes their utility in clinical practice. This needs to be further explored.

Keywords: TMS, TST, PP-paradigms, MEP, repMND, cortico-spinal excitability

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Introduction

Transcranial magnetic stimulation (TMS) allows explore brain's physiology. Various TMS measures can be used to evaluate different aspects of cortical excitability. The paired-pulse (PP) paradigms contribute to a better understanding of the presumed inhibitory and facilitatory circuits in the brain [1]. It works by combining two stimulations in a conditioning-test paradigm. The intensity of the conditioning stimulus (CS) and test stimulus (TS), and the inter-stimulus interval (ISI) determine the effect and can lead to inhibition or facilitation of the motor-evoked potential (MEP). At ISI of 1 to 6ms, we obtain short intra-cortical inhibition (SICI) and at ISI of 10 to 15 m we obtain intra-cortical facilitation (ICF) [2]. Their mechanism remain yet undetermined.

Abnormal SICI and/or ICF can be observed in various disorders, such as amyotrophic lateral sclerosis, Parkinson disease or focal hand dystonia, and could be considered of potential diagnostic utility [3]. However, the variability precludes their use in clinic. A number of factors may contribute to this variability [4] such as change in the number of recruited alpha motor neuron (MN), the desynchronization of their discharges or the occurrence of repetitive motor-neuron discharges (repMND). Desynchronization can be corrected by the triple stimulation technic (TST) collision technic [5] and repMND can be quantified with the quadruple (QuadS) and quintuple (QuintS) stimulation collision technique [6].

This research comes in line with precedent Master theses. [7, 8]. The general aims of these were, on one side, to explore PP paradigms mechanism, and, on the other, to assess whether applying TST enhance diagnostic accuracy and consistency of responses.

The objective of our study is to combine PP paradigms of SICI and ICF with QuadS and QuintS to explore whether repMNDs could contribute to the mechanism in the PP paradigms of SICI and ICF and to explore their variability.

This research was done in a team formed with Eleni Batzianouli and Nathalie Nguepnjo Nguissi. I participated in the execution of the experiment, analysis of results and focused my work and research on variability of PP-TMS measures.

Methods

Subjects

Twenty healthy subjects participated in the study (men, n=17). Their mean age was 27.25 years (range 22-56 years). All subjects, except one, were right-handed according to the Edinburgh Inventory of Handedness [9]. All of them were screened for TMS contraindications and declared no co-morbidities or regular medication intake. All subjects gave written informed consent and the local ethics committee approved the study.

EMG recordings

Recordings were obtained from the abductor digiti minimi (ADM) muscle using the belly-tendon montage with surface electrodes. A ground electrode was placed at the wrist. Subjects were comfortably seated in an armchair, fingers II to V were taped together and their hand placed over a cushion. A Viking Select apparatus was used for the measurement (Nicolet, Madison; WI, USA). Bandpass filter were set at 1 Hz – 5 kHz [10]. Signal acquisition and pre-processing was done with a software called "EMG triggering and acquisition" coded on LabVIEW (National Instrument Corporation, LabVIEW, Austin) by Sci-Consulting. Post-processing was done with another software coded on LabVIEW by Nguyet Dang (National Institutes of Health, Bethesda, MD, USA).

Peripheral nerve stimulations

We obtained compound muscle action potentials (CMAP) by stimulating supramaximally the ulnar nerve at the wrist with a bipolar electrode and the brachial plexus at Erb's point using a monopolar hand-held electrode and a copperplate electrode attached on the back.

Transcranial Magnetic Stimulation

Transcranial magnetic stimulation was performed using Magstim bistim2 stimulator (Magstim Compagny Limited, Spring Gardens, Withland, UK) with a figure-of-eight 70 mm hand-held coil. The intensity was expressed as a percentage of the maximal

stimulation output (MSO). The localization of the optimal cortical stimulation spot (motor hotspot) was determined in accordance with the IFCN guidelines [10] with the help of a 16-point grid as proposed by Kimiskidis [11], as follows: we first determined the Cz spot and then attached to the head a 16-point grid, separated by 1cm, allowing to move precisely the coil. Starting 5cm lateral and 1 cm frontal to Cz, we then moved the coil in order to elicit a MEP of 0.5 to 1 mV. We then moved the coil 1cm frontal, posterior, lateral and medial and elicited 3 MEPs at each site. The site with the largest MEP was chosen as the motor hotspot and marked on the cap. The coil was then kept in the same position, hand-held, throughout the experiment. We determined the resting and active motor threshold using a procedure described by Awiszus [12] (Motor Threshold Assessment Tool (MTAT, version 2.0: http://www.clinicalresearcher.org/software). We visually assessed and determined a valid MEP response as > 50 uV peak-to-peak amplitude and fed it back to the software.

Triple Stimulation Technique

TST is a collision method developed by Magistris et al. [5] It corrects for the de-synchronization of the descending discharges [19], which leads to a phase cancellation phenomenon whereby the positive and negative phase of de-synchronized action potentials cancel each other out. It has been previously detailed by Magistris et al. [5]. In short, it consists in a sequence of three stimuli. The first stimulus is applied to the brain, the second to the ulnar nerve at the wrist and the third to the brachial plexus at Erb's point. First, there is a collision of orthodromic (TMS) and antidromic (wrist) impulses and then a resynchronization of the dispersed descending volleys from the brachial plexus stimulation leading to synchronous action potentials. This technique allows a precise quantification of the number of motor neurons units discharging after TMS by comparing test response (sequence: brain – wrist – Erb's point) with control response (sequence: Erb's point – wrist – Erb's point). It can therefore quantify the integrity and eventual loss of corticospinal conduction.

Quadruple and quintuple stimulation (QuadS, QuintS)

The quadruple and quintuple stimulation can be considered an extension of TST. These technics allow quantification of repetitive motor neuron discharges (repMNDs), as a number of MNs discharging more than once in response to single TMS. In the quadruple stimulation, an additional stimulus is given at the wrist after the first stimulus at the wrist and before the stimulus at Erb's point (sequence: head – wrist 1 – wrist 2 – Erb's point) with an inter-stimulus of 3ms between stimuli at the wrist. This interval was used, as described by Z'Graggen et al [6], because it is longer than the refractory period of peripheral nerves and shorter than the earliest repMNDs. The quintuple stimulation consists in another additional stimulation at wrist (sequence: head – wrist 1 – wrist 2 – wrist 3 – Erb's point).

Paired-pulse paradigm

Kujirai originally described paired-pulse paradigms in 1993 as inhibition or facilitation of MEPs after a paired magnetic stimulation of the motor cortex [2]. The effect depends on the interstimulus interval (ISI) and the intensity of the two stimuli. The first stimulus, called conditioning stimulus (CS), is set at a sub-threshold intensity of 80% of rMT. The latter is followed by a second stimulus, called test stimulus (TS), which is set at a supra-threshold intensity of 120% of resting motor threshold (rMT). When ISI is short (1-5 ms), we obtain the so-called Short Intra-Cortical Inhibition (SICI) resulting in smaller (inhibited) MEPs compared to those evoked by single pulse TMS. In the contrary, with longer ISI (10-15 ms), we obtain Intra-Cortical Facilitation (ICF) with larger (facilitated) MEPs compared to those evoked by single pulse TMS. These phenomena are thought to result from interneurons circuits in the brain. In our study, the CS was set at 80% of rMT, the TS at 120%, the inhibitory ISI at 2 ms (SICI) and the facilitatory ISI at 10 ms (ICF).

Procedure

Firstly, we connected all the electrodes to the Viking Select ENMG apparatus. Then we determined the supra-maximal responses at wrist and Erb's, a TMS and a control and then a TST. The Viking Select ENMG apparatus has a specific TST program that triggers the stimulations at appropriate delays previously calibrated as follow: Delay I = minimal MEP latency – CMAPwrist latency. Delay II = CMAPerb latency – CMAPwrist latency. However, the Viking Select apparatus doesn't include QuadS and QuintS, therefore we needed a new setup. We added two stimulators synchronized by a specific software on Labview: "EMG triggering and acquisition". The first stimulator allows multiple discharges at the wrist (Grass S88 – Astro-Med Inc. Grass Instrument Division, West Warwick, RI, USA) and the second to trigger the electrode at Erb's point (Digitimer DS7AH – Digitimer Ltd, Welwyn Garden City, Hertfordshire, UK). We then entered the values calculated by Viking in the software "EMG triggering and acquisition" which then randomly triggered the stimulators at a specific time and order depending on the condition. We assessed 12 conditions using four technics (TMS, TST, QuadroS and QuintoS) with three methods (single pulse (SP), inhibitory (PP2) and facilitatory (PP10) paired-pulse). We recorded 12 MEPs for each stimulus condition and added 4 TST control for a total of 148 stimulations.

Analysis

For each signal, using the Nguyet application of LabVIEW, we visually inspected the correct response, adapted the time window for the analysis and finally measured the peak-to-peak amplitude of the MEP, the difference between the two (MaxMin), the area under the curve (Area) and the root mean square (RMS). We calculated the mean, median and the standard deviation (SD) for each of the 12 conditions. To compare the variability of the different measures, we applied the coefficient of variation (CV), defined as the standard deviation divided by the mean, as previously described by Kiers et al. [13].

Literature

A literature review was performed using PubMed, Ovid Medline and Embase 1990 trough 2016. A description of the exact search terms used: *variability, reproducibility, repeatability, reliability, paired-pulse, trial-to-trial, inter-session, inter-individual, intra-individual, between-session, within-session.* Associated with: *transcranial magnetic stimulation, TMS, TST, ppTMS, ppTST, SICI, ICF, inhibition, facilitation, repetitive motor neuron discharge.*

Results

Stimulation parameters

Table 1: Stimulation parameters				
	aMT (%MSO)	rMT (%MSO)	Wrist stimulus (mV)	Erb's point stimulus (mV)
Mean (±SD)	34.15 (±8.12)	52.3 (±9.64)	132.5 (±37.67)	226 (±13.79)
Min	20	36	75	115
Max	55	73	200	351
rMT = resting motor threshold, %MSO = percentage of the maximal stimulation output of Magstim bistim				

MEP amplitudes

The mean values of peak-to-peak MEP amplitudes, for each subject and each condition are shown in table 2 and figure 1. Responses with TST are approximately one third higher than with TMS (32% for SP, 45% for SICI and 22% for ICF). *Prevalence of response* stands for the percentage of subjects showing a response out of 20 subjects and *prevalence of trials* stands for the percentage of trials showing responses out of 148 trials.

Table 2: Mean values (mV)						
	TMS			TST		
	SP	SICI	ICF	SP	SICI	ICF
Mean (±SD)	2.013 (±1.55)	0.881 (±0.85)	2.563 (±1.87)	3.342 (±2.27)	1.897 (±1.61)	3.508 (±2.51)
Prevalence of responses	100%			100%	90%	100%
Prevalence of trials	82.5%	66.5%	81.2%	82.5%	61.7%	82%
Mean values (mV)						
	QuadS			QuintS		
	SP	SICI	ICF	SP	SICI	ICF
Mean (±SD)	0.791 (±1.53)	0.669 (±1.26)	0.936 (±1.64)	0.619 (±1.32)	0.450 (±0.98)	0.551 (±1.04)
Prevalence of responses	60%	55%	70%	40%	30%	45%
Prevalence of trials	38.75%	30%	37.3%	22.5%	13.75%	22.5%

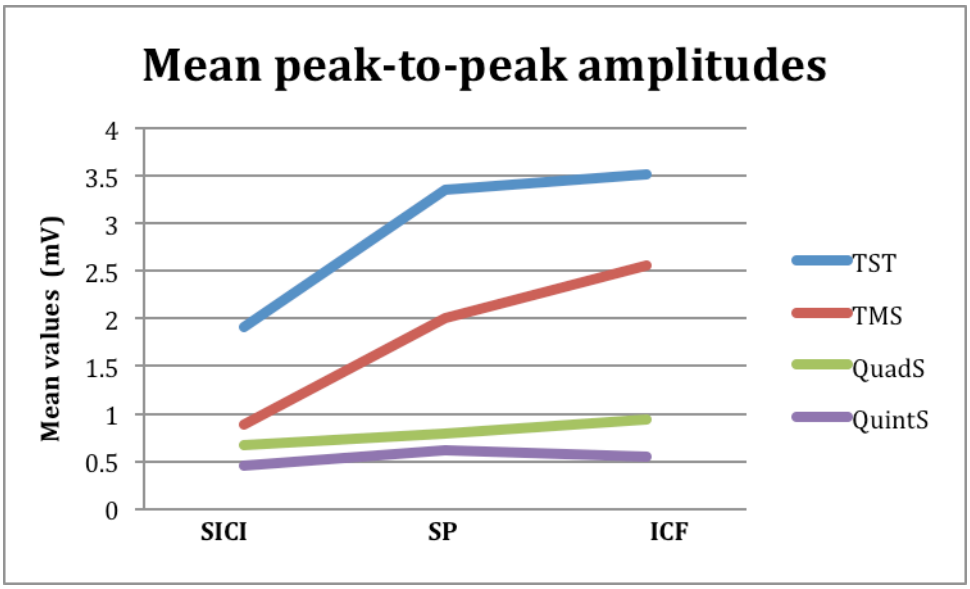

Figure 1. *This graph shows the mean amplitudes in the different stimulation conditions of SICI, ICF and SP with the four different techniques of TMS, TST, QuadS and QuintS*

Intracortical inhibition and facilitation

SICI and ICF are expressed in percentage of the ratio conditioned / test pulse (peak-to-peak amplitude of PP2 and PP10). SICI is defined when the ratio is <100% .and ICF when >100. In PP2, SICI was found, with TMS, in 19/20 subjects with a mean ratio of 48.5%. With TST, it was found in 17/20 subjects with a mean ratio of 73%. In PP 10ms, ICF was found, with TMS, in 13/20 subjects with a mean ratio of 166.7%. With TST, it was found in 13/20 subjects with a mean ration of 121.8%. The other subjects were showing inhibition and facilitation when the opposite was expected, with the four methods and with all ISI: 2 subjects with TMS and 4 with TST showed facilitation with ISI of 2ms and 5 subjects in with TMS and 8 with TST showed inhibition with ISI of 10ms. Results are presented in table 3, 4 and in figure 2, 3

Table 3: SICI and ICF				
	TMS		TST	
	SICI	ICF	SICI	ICF
Mean (±SD)	48.5 (±26)	166.7 (±140)	64.3 (±41)	121.8 (±71)
Median	45.7	130.7	73	109.2
Max	109.6	622.4	159.4	387.6
Min	7.7	48.4	<1	37.8
This table displays the mean values of the inhibition and facilitation obtained with both TMS and TST methods				

Table 4: SICI and ICF : individual data				
Subject	TMS		TST	
	SICI	ICF	SICI	ICF
1	45.8	131.8	41.2	143.6
2	34.0	144.9	112.9	116.0
3	109.7	175.6	<1	119.7
4	60.9	622.4	85.3	92.3
5	30.1	90.3	159.4	99.2
6	10.6	480.0	<1	387.6
7	33.4	98.4	75.1	182.5
8	83.7	224.1	93.3	155.2
9	85.5	48.3	93.4	99.4
10	68.8	129.7	41.1	104.4
11	26.9	98.4	13.0	114.1
12	7.8	171.0	21.2	95.5
13	68.6	154.5	109.5	104.8
14	45.6	81.7	87.4	111.7
15	19.8	150.2	40.9	52.8
16	35.0	129.0	80.5	62.4
17	56.2	134.2	56.0	112.7
18	60.6	95.7	78.5	137.8
19	56.3	123.6	71.0	106.8
20	30.2	52.2	25.9	37.9
This table displays the mean values of SICI and ICF obtained for each subject with each condition.				

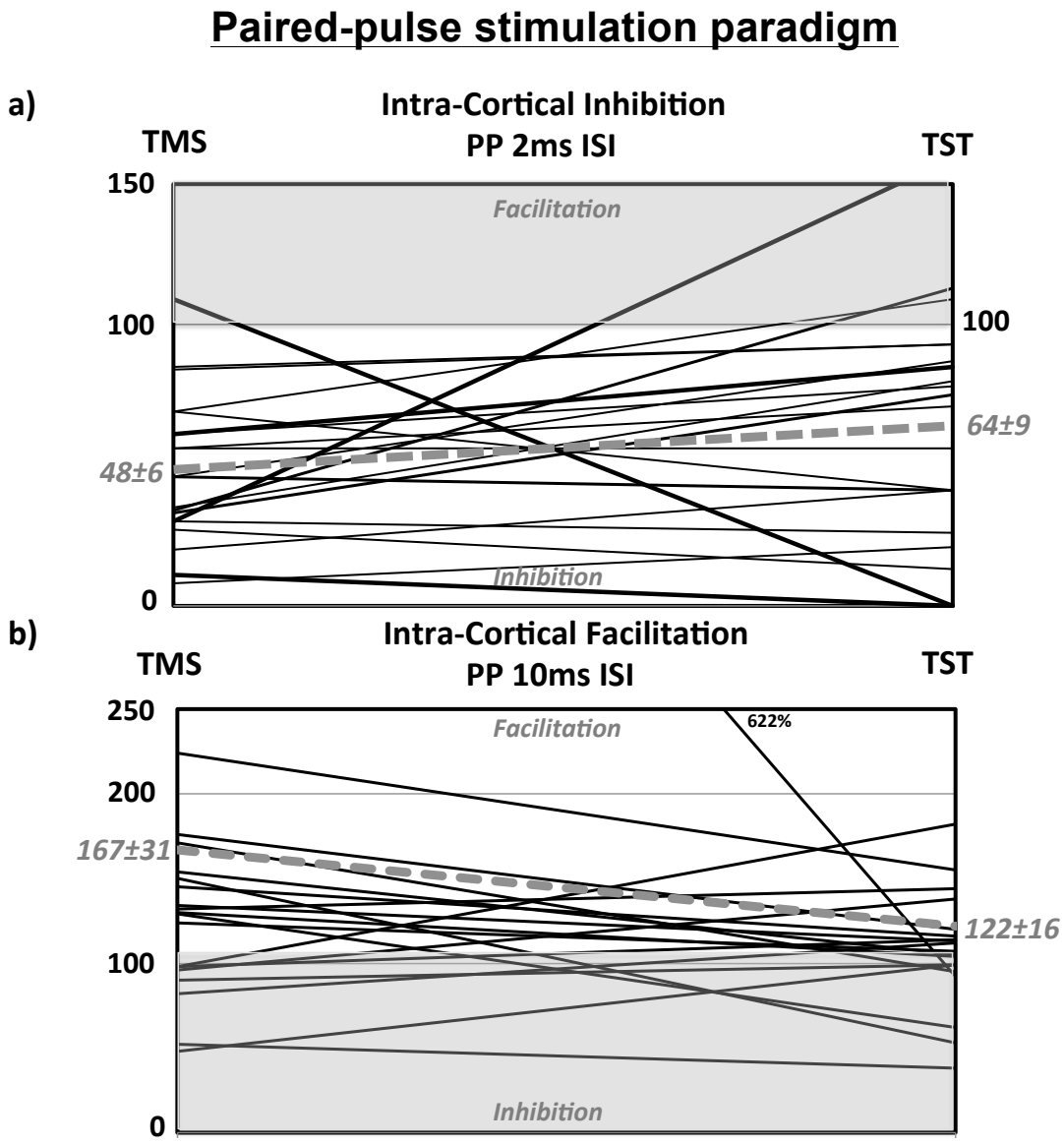

Figure 2. *Results of the paired pulse stimulation paradigms. a) SICI b) ICF. Each line represents a participant's response and the dotted line the group mean. For better clarity, in blank: areas of expected; and in grey: areas of unexpected responses.*

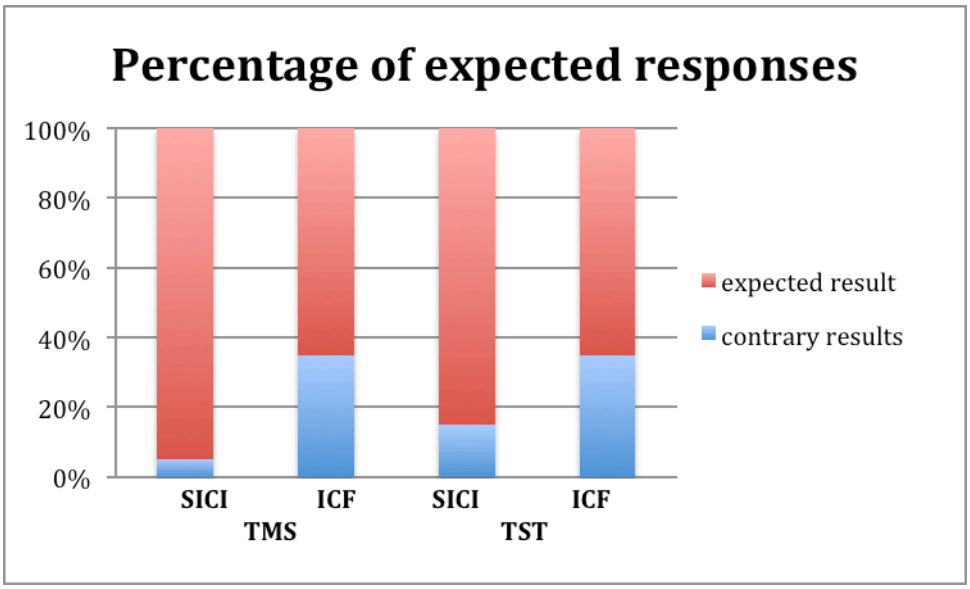

Figure 3. *This chart shows the percentage of subject showing or not the expected result according to the method and the PP paradigm.*

QuadS and QuintS

The mean percentages of positive responses out of the 12 trials and the percentage of subject showing response to QuadS or QuintS for each condition are presented in table 6 and figure 4 and 5.

Concerning the prevalence of the occurrence of repMNDs, we found 60% of the subjects to have one repMND after a single pulse TMS (QuadS) and 40% a double repMND (QuintS).

In QuadS, there appear more repMND in the PP-paradigm of ICF (37.24%) rather than in the SICI (30%).

There seems to be a correlation between the conditioned response of PP-TMS (as described by Kurijai [2]) and occurrence of repMNDs, suggesting a contribution of the latter to PP-TMS.

Mean percentage of trial responses						
	QuadS			QuintS		
	SP	SICI	ICF	SP	SICI	ICF
Mean	38.75	30	37.24	22.5	13.75	22.27
Percentage of subject showing response						
	QuadS			QuintS		
	SP	SICI	ICF	SP	SICI	ICF
	60	55	70	40	30	45

***Table 6**: This table shows the mean percentage of trials responses, including subjects showing no response to QuadS or QuintS.*

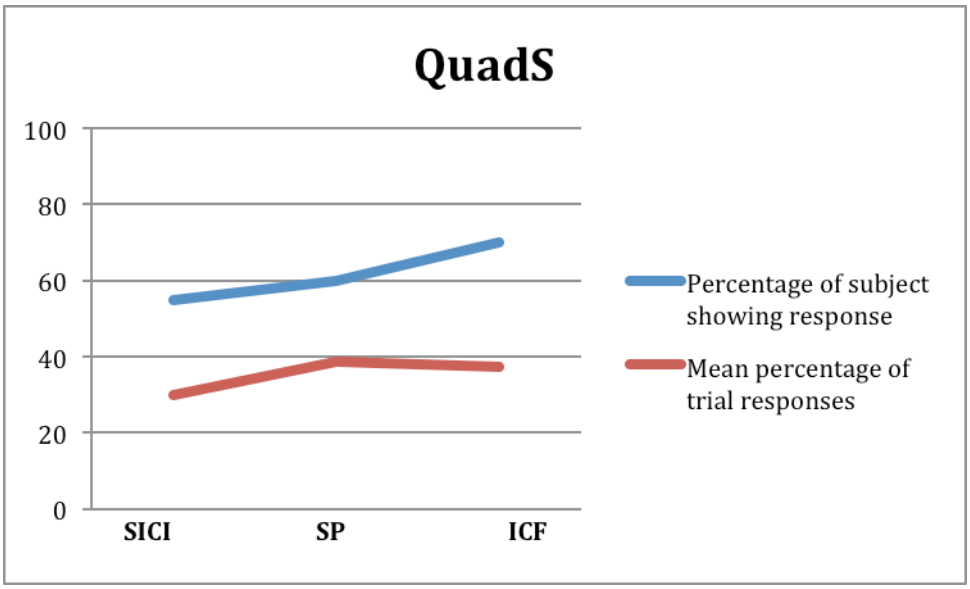

Figure 4. *This graph shows the prevalence of QuadS responses according to the PP paradigm, comparing the percentage of subject showing responses with the mean percentage of trials showing responses.*

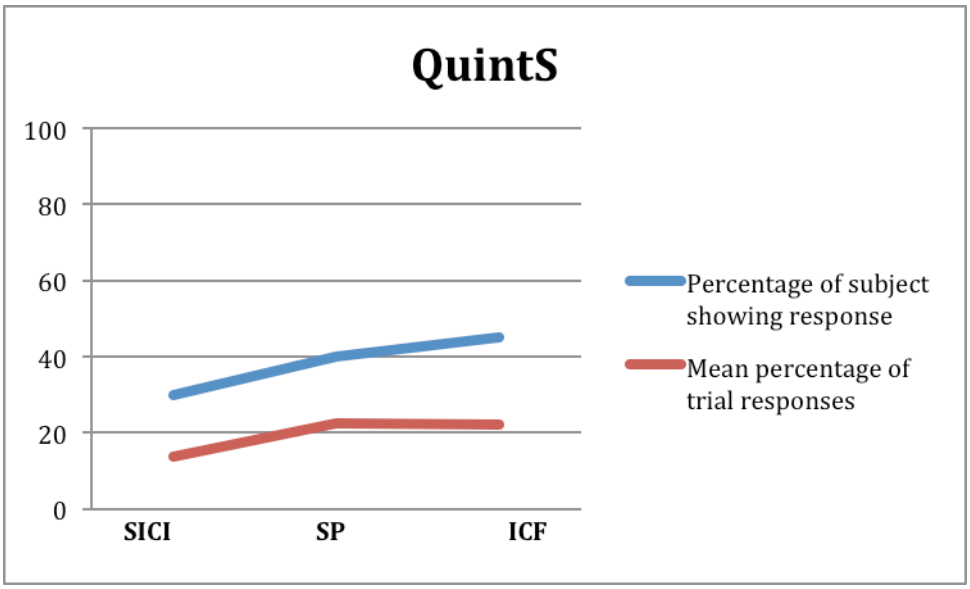

Figure 5. *This graph shows the prevalence of QuintS responses according to the PP paradigm, comparing the percentage of subject showing responses with the mean percentage of trials showing responses.*

Coefficient of variation

For single stimulations (SP), the mean CV is 37.2 for TMS, 35.7 for TST. For PP2 paradigm, the mean CV is 54.1 for TMS, 39.2 for TST. For PP10 paradigm, the mean CV is 35.3 for TMS, 35.9 for TST. There is reduction of the CV with TST compared with TMS for SICI (from 54.1 with TMS to 39.2 with TST (-27%)) but not for SP (-4%) and ICF (+1%).

Variability differed considerably between subjects.

The results are presented in table 7 and in the figure 6.

Table 7. Coefficient of Variation						
	TMS			TST		
	SP	SICI	ICF	SP	SICI	ICF
Mean (±SD)	37 (±30)	54 (±36)	35 (±35)	36 (±24)	39 (±35)	36 (±33)
Median	54	52	41	36	38	40
Max	129	144	140	100	112	135
Min	18	20	13	5	0	8
Variation of stimulation using TMS, TST, QuadS and QuintS. Coefficient of variation = standard deviation divided by the mean						

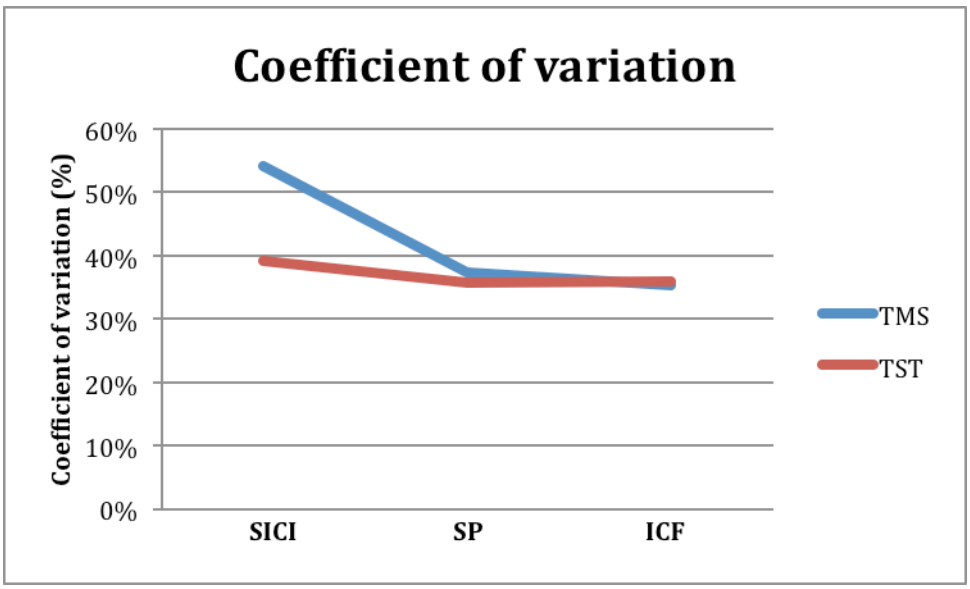

Figure 6. *This graph shows the coefficient of variability of SICI and ICF for TMS and TST.*

Discussion

The objectives of our study were to explore the cortical excitability by studying the role of spinal repMND in the paired-pulsed paradigms of SICI and ICF using conventional TMS, TST, QuadS and QuintS techniques, the two latters allowing exploration of repMND contribution to SICI and ICF. Our results are in line with what was already demonstrated by Kujirai in 1993 [2], namely inhibition with ISI of 2ms and facilitation with ISI of 10ms. Our principal finding is that repMND appears to result fromthe conditioned response of PP-TMS since, in parallel with the size of MEPs, their occurrence decreases with ISIs of 2ms and increases with ISIs of 10ms. The Utility of these techniques in clinical practice is limited by high inter- and intra-individual variability. We evaluated the variability by measuring the CV and found that TST, compared with TMS, reduces variability by -27% for SICI, globally increasing the consistency of responses. However, variability was very high and there were effects opposite to the expected, with subjects showing inhibition instead of facilitation at ISI of 10ms and facilitation instead of inhibition with ISI of 2ms, which need to be further explored. We managed to detect repMND in both QuadS and QuintS, in contrast with Z'Graggen et al. [6] who didn't record any QuintS responses using three levels of facilitatory ADM contractions (0%, 5% and 20% of maximal voluntary force) and two different stimulus intensities (120% and 150% of RMT). We found them greater and occurring more frequently in QuadS compared to QuintS responses. Their prevalence as well as amplitude responses are smaller compared to TMS and TST.

The concept of PP paradigms of ICF and SICI allows exploration of intra- and inter-regional physiological interaction of various motor areas [1] and the pathophysiology of various disorders [14]. These measures are considered to be of potential diagnostic utility by a consensus of the IFCN [3], however their use in clinical practice remain limited by their intra- and inter-individual variability.

These two paradigms have different mechanisms, which remain unknown. There is some consensus among experts about an intra-cortical origin of SICI being mediated by a population of inhibitory interneurons acting on the motor neurons [3]. Support for these mechanisms come from comparison with TMS versus TES [2], which activates directly the axons, and epidural recordings [15, 16]. Origin of ICF also appears to take place in the motor cortex, mediated by a distinct interneuronal population, but there are observations that suggest spinal mechanisms [17].
This experiment comes in line with precedent master theses. Bedulli et al. [7] found that CS of 80% rMT excites spinal MN. Therefore, they concluded that CS modifies the excitability of the cortico-spinal tract or the spinal MN, possibly priming the spinal motor neuron and facilitating repMNDs. Caranzano et al. [8] found that PP TST protocol confirms the inhibition and facilitation of MEPs as with the conventional PP TMS paradigms. Therefore, they assumed that there was no contribution of desynchronization of MN discharge in the conditioning effect of PP-paradigms of SICI and ICF. They also found reduction of variability by using TST compared to TMS. It was significant for SP and SICI but not for ICF. Our results suggest that repMNDs contribute to the occurrence of facilitation and inhibition in the TMS PP paradigm.

There is an intra- and inter-individual variability of MEPs compared with peripherally-evoked CMAP. This also applies to PP paradigms and limits their application in clinical practice [4]. Our results confirm this variability.
Many different factors may contribute to variability of MEPs. One possible explanation is the presence of spontaneous fluctuations of the cortical or anterior horn motor neuron excitability levels resulting in the activation of a variable number of MN unit [13, 18, 19]. We believe this could be defined by stable biological differences between individuals, such as gender [4], age [25], genetics [4], behavioral traits [20] or oscillatory activity of the brain [21, 22, 23]. It could be modulated by varying biological differences between individuals, such as arousal, mental activity [18, 19, 24], attention [26], behavioral states, such as anxiety [27], but also by the time of the day (circadian hormones levels like cortisol [28]), menstrual cycle phase (varying levels of estradiol and progesterone [29, 30]) and pharmacological influences [31, 32]. Another source of response variability is inherent to biological properties of the

corticospinal tract. This includes desynchronization of MN discharges, causing a variable phase cancellation [5], and variable occurrence of repetitive MN discharges [6]. Finally, variability depends also on methodological and experimental conditions, such as varying degrees of target relaxation, background contraction [13, 16, 33] or position of the coil [18, 31].

Variability can be reduced by using the TST. It corrects the effect of desynchronization of the repMNDs [5, 6]. TST reduces variability of MEP by one third [19] and variability of SICI by -59% [8]. Our results are in line with the third of variability but we find a lesser decrease (-27%) of variability for SICI by using TST. As suggested by Kiers et al. [13], the variability related with variable excitability level of MN could be modulated with manoeuvers raising this level of excitability or increasing the probability of MN firing, such as an increase of stimulus intensity or prestimulus voluntary muscle contraction. This was successfully done by Magistris et al. [5] who used the TST SP with high stimulation intensities (supra-maximal) and facilitation maneuvers resulting in a lowering of variability. However, these conclusions run on SP stimulation but can only partially be implemented in a PP protocol, since the conditioning pulse of PP applies a sub-maximal response.
Some authors [34, 35] found less variability when applying the aMT rather than the rMT in the determination of CS and TS. This effect could be due to the fact that physical and mental resting condition is more complex to define than an active condition. For the AMT, they suggest contraction of 5 to 20% of maximal ipsilateral muscle contraction. In addition, they determined the individual's threshold for SICI and ICF rather than a percentage of aMT or rMT and found lower variability. In order to determine the individual threshold for SICI, the authors explored the inhibition with different CS intensities (CSI) (for example, Orth et al. used 60, 70, 80, 90, 100, 110% of aMT). Percentage inhibition is then plotted against the intensity of the CSI (in % stimulator output). Finally, Individual threshold is defined as the CSI that produced at least 10% inhibition, above which further increases in CSI produced progressively greater levels of SICI, increasing consistency of responses, therefore lowering variability.

There are other precautions to take such as the use of randomized trials in order to minimize the effects of the oscillatory activity, to give the subjects a specific task or a defined activity to concentrate on in order to control for mental activity. Effects of age and circadian rhythms can possibly influence the variability of response, therefore we could turn to subjects of similar age or carry out the experiment in the similar time of the day.

In order to assess and to control variability, it would be interesting to create a questionnaire including various possible factors of variability and screen the subjects. In such a questionnaire, we could include the smoking, alcohol, caffeine and drug consumption, medication use, menstrual cycle, habits and last session of exercise or behavioral traits using, as proposed by Wassermann [4], the NEO-PI-R inventory, a personality inventory. It would thus be interesting to explore the behavioral state during the ongoing experiment, especially the effect of anxiety, stress or other negative emotions.

In this study, we intended to combine the PP paradigms with QuadS and QuintS protocols to investigate the physiology of SICI and ICF. Our findings confirm the presence of repMND after TMS. The fact that more repMND are seen in ICF than in SICI suggests a possible contribution of repMND in the conditioned response of PP-TMS. Both SICI and ICF are of a potential diagnostic utility in various disorders [3, 36], but their inter-individual variability precludes their use in clinical practice. TST increases the consistency which could be helpful in clinical research. Further studies are needed in order to conclude to the benefits of TST, particularly in patients as well as combined with other protocols. Inter- and intra-individual variability needs to be further explored.

Abbreviations

ADM	Abductor Digiti Minimi
aMT	active Motor Threshold
CMAP	Compound Muscle Action Potentials
CS	Conditionning Stimulus
CV	Coefficient of Variation
ICF	Intra-Cortical Facilitation
ISI	Inter-Stimulus Intensity
MEP	Motor-Evoked Potential
MN	Motor-Neuron
MSO	Maximal Stimulation Output
PP	Paired-Pulse
PP-TMS	Paired-Pulse TMS
PP-TST	Paired-Pulse TST
QuadS	Quadruple Stimulation
QuintS	Quintuple Stimulation
repMND	Repetitive Motor Neuron Discharges
RMS	Root Mean Square
rMT	resting Motor Threshold
SD	Standard Deviation
SICI	Short Intra-Cortical Inhibition
TES	Transcranial Electric Stimulation
TMS	Transcranial Magnetic Stimulation
TS	Testing Stimulus
TST	Triple Stimulation Technic

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