Optimization of temporal parameters of repetitive transcranial magnetic stimulation to improve its efficacy

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Statement of Originality

I hereby declare that this thesis has been written independently with no other sources and aids that quoted in the text, references and acknowledgments.

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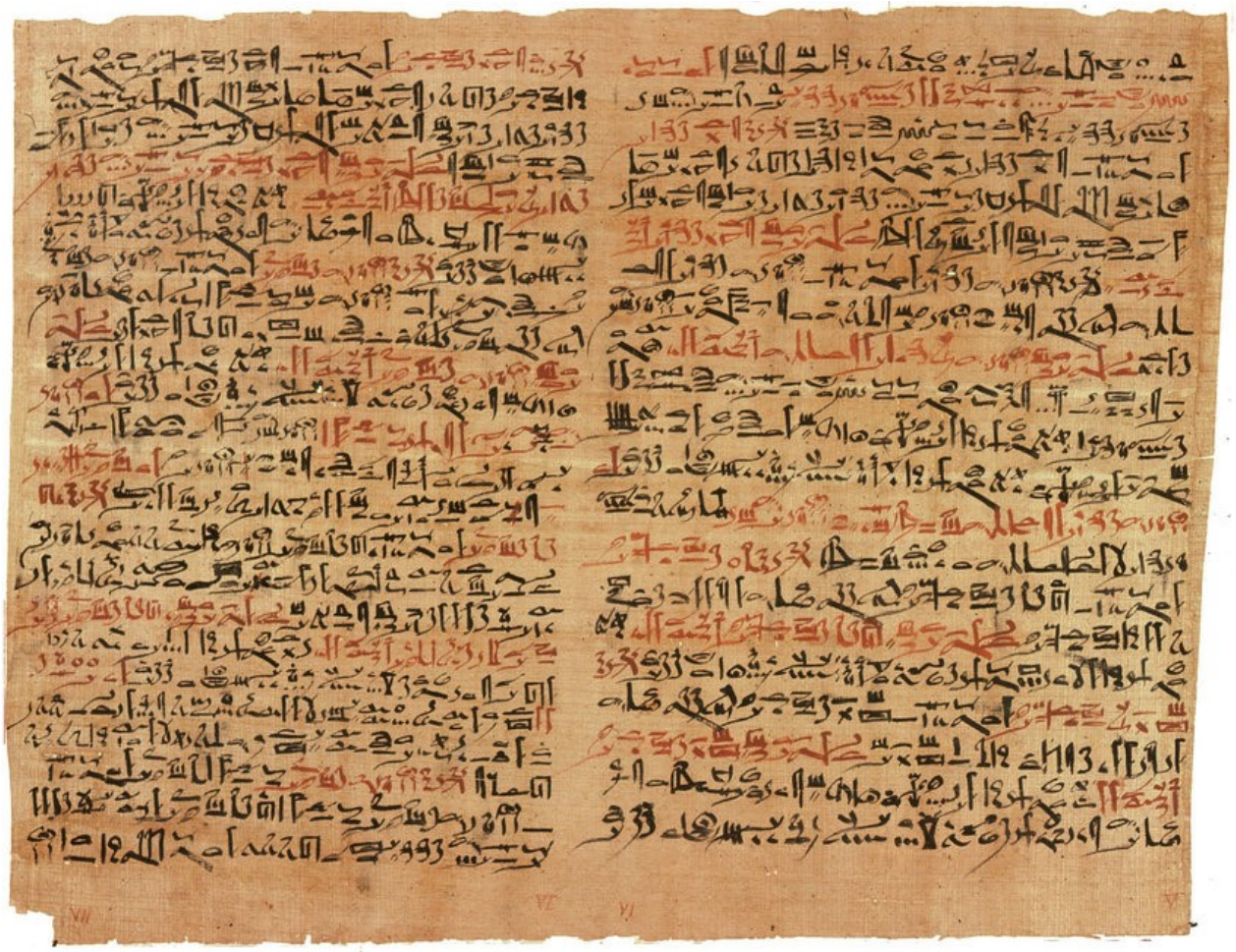
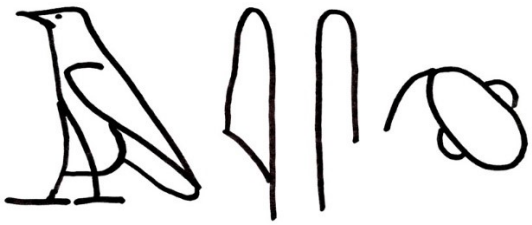


First mention of the brain in written History by ancient Egyptians.



From James Henry Breasted, 1930. The Edwin Smith Surgical Papyrus, 2 volumes, Chicago: The University of Chicago Press. The papyrus is describing the symptoms, diagnosis and prognosis of compound skull fractures 700 years B.C. Prof. Eric Kandel used this papyrus in the epilogue to his book “Principles of neural science”.

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1.Introduction

Noninvasive excitation of brain regions is possible by delivering a rapidly varying magnetic field in the brain (Barker et al. 1985). Barker and colleagues made use of Faraday's principles of electromagnetic induction to stimulate a subject's head by a wire coil over the head, through which brief pulses of current flow were delivered generating a magnetic field through the subject's scalp and skull with insignificant resistance. The magnetic field in turn induces a secondary electric field which is perpendicular to the direction of the magnetic field inducing an electric current in the brain (Hallett 2000). This electric current could then modify membrane potentials and excitability of the stimulated neurons. The propagation of such potentials through the pyramidal fibers in response to suprathreshold stimuli elicits Motor Evoked Potentials (MEPs) which we could use to trace motor cortex excitability and conduction times (Hallett 2007).

Accordingly, trans magnetic stimulation (TMS) of the motor cortex is particularly convenient for estimating excitability of descending corticospinal tracts as motor thresholds and MEP amplitudes are known to reflect membrane excitability of the pyramidal neurons (Klomjai et al. 2015). This was proven through multiple studies testing the effects of different pharmacological agents acting through known neuronal channels and receptors pathways on motor threshold, MEP amplitudes and MEP recruitment curves (Ziemann et al. 2015). Most excitability studies use a multitude of neurophysiological measures as resting motor threshold (RMT), MEP amplitudes and latencies, cortical silent period and paired TMS pulses paradigms as short interval intracortical inhibition (SICI) and short intracortical facilitation (SICF).

All these measures are significantly altered in cases with changes in motor function whether experimentally in healthy subjects or pathologically in patients and all are made possible through MEP recordings of the motor cortex (Rossini et al. 2015).

For regions outside the motor cortex where MEP recording is not possible as the visual cortex for example, other measures as phosphene threshold and effect of TMS on visual evoked potential are used (Reichenbach et al. 2011). For other cortical areas as the dorsolateral prefrontal and the temporal cortices, other tools as TMS-EEG and TMS-fMRI are used to non-invasively assess the cortical excitability and connectivity changes in response to magnetic stimulation (Siebner et al. 2009; Ziemann 2011).

1.1 Applications of rTMS:

When the induced electric field within brain tissue is used in a repetitive manner, it induces excitability changes (Pascual-Leone et al. 1993) and long lasting changes through neuroplasticity (Rossini et al. 2015) by restructuring the neural connections both structurally and functionally.

That opened the field for clinical trials to examine the long lasting modulatory effects of repetitive trans-cranial magnetic stimulation (rTMS) which proved to be promising for a variety of neuropsychiatric conditions (Wassermann and Zimmermann 2012; Lefaucheur et al. 2014); the number of applications continues to increase as indicated by the increase in numbers of ongoing clinical trials in a variety of diseases (773 studies registered at https://clinicaltrials.gov/). Therapeutic utility of

rTMS is graded with class A evidence (definite efficacy) for treatment of depression and chronic pain. Other disorders such panic disorders, hallucinations, obsessions/ compulsions, schizophrenia, Parkinson's disease, dystonia and stroke have been less convincing for being treated with rTMS so far (Lefaucheur et al. 2014).

This less convincing results in treatment of the above mentioned diseases originate from the reproducibility problem, with studies sometimes using almost similar protocols but failing to reproduce the afore reported beneficial effects of rTMS (Ridding and Rothwell 2007; Héroux et al. 2015). This variability is the reason behind efforts to evaluate the quality of the data by massive reviews as (Lefaucheur et al. 2014) and to improve this quality through more systematic handling of the data (Wilson and St George 2016).

While this variability is noticed across rTMS protocols in cortical excitability response in healthy subjects (Maeda et al. 2000; López-Alonso et al. 2014; Nettekoven et al. 2014), the factor of a pathological affection of cortical function expectedly adds another source of variability as noticed in clinical trials for depression (McClintock et al. 2018), for which is rTMS therapy is FDA approved (Lefaucheur et al. 2014). That emphasizes that this variability is brought by the interaction between a multitude of factors affecting the outcome, those factors could be attributed to two sources:

1) Biological variability of rTMS effects: through physiological and anatomical differences whether within same individual based on metaplasticity functions as the basic activation state (Huang et al. 2008; Goldsworthy et al. 2014; Karabanov et al.

2015) or across subjects based on variable biological factors such as age, gender, genetics and brain anatomy also affect the rTMS effects (Ridding and Ziemann 2010; Pellegrini et al. 2018). The discrepancy between AP-PA latency which reflects different brain anatomy in the form of axonal orientations also showed a significant correlation with rTMS outcomes (Hamada et al. 2013). Brain-derived neurotrophic factor gene (BDNF) variations has been associated with different outcome of theta burst stimulation (Cheeran et al. 2008). Recently two gene variations were identified as a partial source of variation for rTMS outcome (Raginis-Zborowska et al. 2019).

2) Stimulation parameters variability: rTMS aftereffects exhibit sensitivity to the physical characters of stimulation as intensity (Modugno et al. 2001; Fitzgerald et al. 2002), frequency (Ziemann et al. 2008), pattern (Huang et al. 2005; Hamada et al. 2008), orientation (Rothkegel et al. 2010; Sommer et al. 2013) and total duration of stimulation. Also, some less investigated parameters pulse width and phasicity (Goetz et al. 2016) and inter train intervals (Rothkegel et al. 2010; Cash et al. 2017) influence the outcome the stimulation.

While the biological factors involved in inter-individual variability could be controlled to an extent in healthy subjects, this becomes more difficult in clinical trials where some pathological activation states may be present or simply by absence of possible pathways of action. This depicts rTMS parameter testing as more optimal and reliable in efforts to understand rTMS underlying mechanisms (Klomjai et al. 2015). As the variability is dependent on rTMS parameters, a better standardization of those parameters should be unified across rTMS devices (Peterchev et al. 2012).

1.2 Mechanism of rTMS:

The main mechanism of action of rTMS is induction of synaptic plasticity whether long term potentiation (LTP) or long term depression (LTD) (Huerta and Volpe 2009; Vlachos et al. 2017). This is supported by the fact that this interaction exhibits the Hebbian properties of synaptic plasticity (Hebb 1949), through the closely related characteristics in response to different stimulation parameters (Bliss and Cooke 2011; Pell et al. 2011). More detailed discussion about those characteristics follows in the next section emphasizing individual rTMS parameters.

This is supported by the experimental rTMS effects on learning (Muellbacher et al. 2000; Baraduc et al. 2004) that were closely related to the effects of LTP and LTD established in animal experiments (Rioult-Pedotti et al. 2000). Another proof of this correlation is the recognized role of different alleles of brain derived neurotrophic factor gene (BDNF) which lead to different modulation of LTP (Lu et al. 2008) and LTD differently (Woo et al. 2005) in animal studies. This role is reflected into human studies, where BDNF allele variations had similar effects on iTBS and cTBS after effects (Cheeran et al. 2008; Mastroeni et al. 2013).

The large parameter space and the interaction between their underlying mechanism probably play a major role in the variability of the outcome reported from different studies (Rubens and Zanto 2012). A deeper comprehension of those mechanisms would enable us to understand the underlying mechanism by which they affect synaptic plasticity and thus optimize the outcome of rTMS protocols.

1.3 Parameters of rTMS:

Frequency:

When it was first discovered in earlier experiments using electrical stimulations in rabbits, LTP was originally referred to as “frequency potentiation” (Andersen et al. 1966). Its role in determining LTP or LTD in response to rTMS was demonstrated in rodents (Wang et al. 1996). Similarly, rTMS stimulation frequency is the main deciding factor on the direction of the cortical excitability modulation induced by the rTMS (Cooke and Bliss 2006; Pell et al. 2011), where frequencies lower than 3 Hz are inhibitory and frequencies of 5 Hz and above are excitatory (Ziemann et al. 2008). Note that frequencies needed for producing excitation in humans (usually 10 Hz) are significantly lower than stimulation frequencies that induce LTP in neuronal culture studies (100Hz) (Vlachos et al. 2012). This is probably because of the wider activation effect of the magnetic field (Funke and Benali 2011) and the resulting cortical amplification (Hay and Segev 2015).

This frequency dependence was attributed to the tetanic response where high frequency stimulation allows for summation of excitatory post synaptic potentials, causing influx of larger amounts of calcium thus triggering LTP. While low frequency stimulation might allow for a lower calcium influx, leading to LTD. This is mediated through activation of different receptors (Vlachos et al. 2012; Lenz et al. 2015). An additional explanation might be the spectral responsivity properties of dendrites leading to their preferential stimulation by high frequency rTMS (Ledergerber and Larkum 2010; Das et al. 2017).

Intensity:

The Hebbian plasticity concepts of cooperativity and associativity or in more modern words synchronization; have been illustrated by increasing pulse widths (as an analogue for intensity) of external stimulation of rat cortices by Mcnaughton and colleagues as early as 1978 (McNaughton et al. 1978). That was proven true for high frequency rTMS where increasing the intensity lead to more excitation (Modugno et al. 2001). For low frequency rTMS, increasing the stimulation intensity leads to more efficient inhibition (Fitzgerald et al. 2002; Lang et al. 2006; Nojima and Iramina 2017).

Patterned protocols:

Driven by the afore mentioned variability of outcome of regular low and high frequency rTMS, the search for more consistently efficacious protocols continued. Huang and colleagues presented theta burst stimulation (TBS) in the motor cortex (Huang et al. 2005), inspired by its merit in producing LTP in neuronal culture experiments (Larson et al. 1986; Capocchi et al. 1992; Hernandez et al. 2005; Larson and Munkácsy 2015). TBS was found to have longer lasting effects on cortical excitability with less variability than classic low or high frequency rTMS protocols (Di Lazzaro et al. 2011; Iezzi et al. 2011), but its effects are now found to be variable in relation MEP latencies and direction of stimulation (Hamada et al. 2013; Huang and Mouraux 2015)

Another patterned form of rTMS is the repetitive paired pulse stimulation delivered at I wave intervals to produce either inhibition using inter stimulus intervals (3 milliseconds) of short interval intracortical inhibition (SICI) (Sommer et al. 2002; Khedr et al. 2004), or excitation using inter stimulus intervals (1.5 milliseconds) of short interval intracortical facilitation (SICF) (Thickbroom et al. 2006). Using the same interval of 1.5 milliseconds., Hamada and colleagues added two more pulses to the train to create the quadripulse stimulation (QPS) which produced longer lasting facilitation in comparison to paired pulse stimulation (Hamada et al. 2008). Jung and colleagues combined theta burst and QPS to produce quadripulse theta burst stimulation (qTBS) (Jung et al. 2016).

Duration of stimulation:

The duration of stimulation must be differentiated into two categories: one including daily stimulation sessions and number of pulses per day, and the other category including duration and repetition of individual stimulation trains with closely related underlying mechanisms.

Daily stimulation: experimentally, daily sessions of stimulation in animal experiments lead to increased excitation in a form of LTP known as kindling (Goddard et al. 1969; Racine 1978), this is reflected in humans as two sessions proved to be more efficacious than one session per day in treatment of depression (Modirrousta et al. 2018; Schulze et al. 2018).

Train duration: As LTP is associated with learning, it made sense that repeating stimulation trains would increase LTP as demonstrated by (Huang and Kandel 1994), however that was disputed both in animal experiments where even single stimulation trains provoked long lasting LTP (Villers et al. 2012) and in humans where TBS aftereffects were reversed when the duration of stimulation was doubled (Gamboa et al. 2010).

Inter train intervals:

The significance of intertrain intervals (ITIs) where brought to our attention because of the different outcome of two large multicenter studies studying the efficacy of 10 Hz rTMS in treatment of depression using almost the same protocols but with different ITIs (Herwig et al. 2007; O'Reardon et al. 2007). I then correlated ITIs and average frequency with the outcome of all the reportedly efficacious high frequency protocols in treatment of depression and chronic pain from the Lefaucheur and colleagues review (Lefaucheur et al. 2014), we found some patterns demonstrating that protocols with longer ITIs and lower average frequencies had more significant therapeutic effects (Halawa et al. 2018).

That supported previous findings for 5 Hz rTMS where breaks during stimulation succeeded in producing the expected excitatory aftereffects while continuous 5Hz rTMS failed to do so (Rothkegel et al. 2010). However it contradicted findings from a 20 Hz rTMS study focusing on the effect of ITIs of high frequency rTMS on cortical excitability, where they examined ITIs of 4, 8 16 and 32 seconds on the outcome of 20 Hz rTMS, and even though the 8 second ITI was less efficient

than the 16 and 32 second ITI protocols, the most efficient was the protocol with the shortest ITI of 4 seconds (Cash et al. 2017). This highlights the additional mechanisms underlying the therapeutic effects of rTMS (Chervyakov et al. 2015).

Pulse widths:

Using a novel device that could readily change the widths of individual pulses as well as their directionality (Peterchev et al. 2011), Goetz and colleagues examined the effect of combining increasing both the pulse width and directionality on 1 Hz rTMS with both parameters leading to more inhibition (Goetz et al. 2016). My second publication however, was the first study to separately test the effect of pulse widths on low frequency rTMS, where wider unidirectional pulses with 120μs wide main component changed the expected inhibitory outcome of 1Hz rTMS into excitation (Halawa et al. 2019b).

We also examined the effect of pulse widths on high frequency rTMS, where pulses wider than 100μs produced more excitatory aftereffects than shorter pulses (Halawa et al. 2019a). I propose that pulses wider than 100μs were more efficient in producing excitation in HF rTMS and caused excitation in LF rTMS because they stimulated dendrites. 100μs seems like the cut off value above which excitation occurs as demonstrated in electrical stimulation experiments in rabbit cortices (McNaughton et al. 1978). Rattay and colleagues demonstrated in a neuronal model that dendrites are not at all excitable by any pulses shorter than 100μs (Rattay et al. 2012).

Pulse phasicity and directionality:

This indicates the directionality of different phases within the pulse, which understandably makes it dependent on the coil orientation. For example, biphasic pulses proved more efficient in producing excitation than monophasic pulses only when used in antero-posterior (AP) 5 Hz frequency (Sommer et al. 2013). As for 1Hz frequency only the AP directed pulse shapes produced any effect outlasting the stimulation, with biphasic pulses producing excitation and monophasic pulses resulting in inhibition (Sommer et al. 2013), an effect I was able to reproduce in the second experiment of my second paper (Halawa et al. 2019b).

Stimulation direction:

As for the direction of stimulation, the two mainly used coil orientations for motor area rTMS are posterioanterior (PA) with a 45-degree angle to the middle line and anteroposterior (AP) with reversed current flow. Across a wide range of neuromodulatory protocols, AP directed current had more evident outcome. This was noted across numerous modalities of rTMS, for example continuous theta-burst stimulation (cTBS) (Hamada et al. 2013; Huang and Mouraux 2015), Anodal transcranial direct current stimulation (Wiethoff et al. 2014; Davidson et al. 2016), short latency afferent inhibition protocol (Ni et al. 2011), qTBS (Jung et al. 2016), 5 Hz rTMS (Rothkegel et al. 2010; Sommer et al. 2013), and 1Hz rTMS (Sommer et al. 2013). This may be due to the direction or the intensity effect. In other words, AP stimulation is less efficient in producing MEPs but with subthreshold stimulation, we can get longer lasting and more prominent aftereffects.

This effect highlights the orientation effect on threshold as axons have a certain orientation and subsequently an optimum direction of stimulation while smaller branches and dendrites do not have a specific orientation and could be stimulated in any direction by magnetic stimulation as described in neuronal cultures (Stern et al. 2015; Lee and Fried 2017) and models (Aberra et al. 2018). This was supported by the fact that more activation could be achieved by rotating magnetic fields or wider pulses (Rotem et al. 2014).

This thesis will focus on the effects of changing temporal organization of rTMS whether within the protocol by examining the effects of inter train intervals on the efficacy of rTMS protocols in the first paper (Halawa et al. 2018), or within pulse shapes by testing the effects of changing the pulse widths and directionality in low frequency (Halawa et al. 2019b) and high frequency rTMS (Halawa et al. 2019a). The aim is to better understand the underlying physiological mechanisms of outcome variability by investigating less commonly investigated stimulation parameters so we could eventually make more efficient use of rTMS modulatory effects in treatment of diseases with cortical origin, or prospectively enhance learning and increase mental abilities.

2. Role of inter train intervals in rTMS protocols:

Intertrain intervals (ITIs) are used in order to avoid overheating of the coil, so understandably they are only used with high frequency rTMS. We were particularly interested in studying them because of the different outcome of two large multicenter studies studying the efficiency of 10 Hz rTMS in treatment of depression, both used almost similar protocols with the positive study using 4 second trains with ITIs of 26 seconds (O'Reardon et al. 2007) and the negative study administering 2 second trains with 8 second ITIs (Herwig et al. 2007).

That lead us to start a collaboration with the physics department from Bar Ilan University to examine those two protocols in neuronal cultures, which showed that shorter ITIs lead to neuronal response failures, especially when the 'average frequency' (obtained by dividing the total number of pulses over the total stimulation duration) exceeded the neuronal critical frequency (Halawa et al. 2018). We then correlated ITIs and average frequency with the outcome of all the reportedly significantly efficient high frequency protocols in treatment of depression and chronic pain (with level A efficiency) from then the latest evidence based review (Lefaucheur et al. 2014), for that I had to extract detailed stimulation protocol parameters and percentage of improvement of prognostic scores (Hamilton depression rating score in case of depression and visual analogue scale for chronic pain) from each paper. I then calculated the average frequency from the extracted parameters and correlated it, the ITIs length the total number of pulses and the total duration of stimulation to the percentage improvement of prognostic scores. I found some patterns

demonstrating that protocols with longer ITIs and lower average frequencies had more efficient therapeutic effects (Halawa et al. 2018).

I must state though that they used a cocktail of synaptic blockers in order to obtain this effect in single neurons, methodology is described in (Vardi et al. 2015) and modeled the effect of the neuronal response failures into multiple layers, making the saturation or as we called the critical frequency much lower. That would explain the different outcome from the MEP study with 20 Hz rTMS where shortest ITI of 4 seconds were the most efficient in producing MEP amplitude facilitation (Cash et al. 2017). That apparently contradicts our conclusion that longer ITIs in rTMS are needed for better clinical outcome for depression and chronic pain, but that is not a simple relationship as I highlighted before because of the different mechanisms underlying long lasting effects of rTMS especially in pathological state cortices. Also the different rTMS frequency plays a role, as for 5 Hz rTMS for example, continuous stimulation with no ITIs produced no significant facilitation while trained stimulation produced the expected facilitation (Rothkegel et al. 2010).

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Less Might Be More: Conduction Failure as a Factor Possibly Limiting the Efficacy of Higher Frequencies in rTMS Protocols

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Introduction: rTMS has been proven effective in the treatment of neuropsychiatric conditions, with class A (definite efficacy) evidence for treatment of depression and pain (Lefaucheur et al., 2014). The efficacy in stimulation protocols is, however, quite heterogeneous. Saturation of neuronal firing by HFrTMS without allowing time for recovery may lead to neuronal response failures (NRFs) that compromise the efficacy of stimulation with higher frequencies.

Objectives: To examine the efficacy of different rTMS temporal stimulation patterns focusing on a possible upper stimulation limit related to response failures. Protocol patterns were derived from published clinical studies on therapeutic rTMS for depression and pain. They were compared with conduction failures in cell cultures.

Methodology: From 57 papers using protocols rated class A for depression and pain (Lefaucheur et al., 2014) we extracted Inter-train interval (ITI), average frequency, total duration and total number of pulses and plotted them against the percent improvement on the outcome scale. Specifically, we compared 10 Hz trains with ITIs of 8 s (protocol A) and 26 s (protocol B) *in vitro* on cultured cortical neurons.

Results: In the *in vitro* experiments, protocol A with 8-s ITIs resulted in more frequent response failures, while practically no response failures occurred with protocol B (26-s intervals). The HFrTMS protocol analysis exhibited no significant effect of ITIs on protocol efficiency.

Discussion: In the neuronal culture, longer ITIs appeared to allow the neuronal response to recover. In the available human dataset on both depression and chronic pain, data concerning shorter ITIs is does not allow a significant conclusion.

Significance: NRF may interfere with the efficacy of rTMS stimulation protocols when the average stimulation frequency is too high, proposing ITIs as a variable in rTMS protocol efficacy. Clinical trials are necessary to examine effect of shorter ITIs on the clinical outcome in a controlled setting.

Keywords: HfrTMS, rTMS, ITIs, NRFs, neuronal cultures

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INTRODUCTION

Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive therapeutic tool for a variety of neuropsychiatric conditions (Lefaucheur et al., 2014). There are presently 617 ongoing clinical trials registered at https://clinicaltrials.gov/ (accessed 05.03.2018). There is class A evidence of the therapeutic utility of rTMS in the treatment of depression and chronic pain (Lefaucheur et al., 2014) which led the FDA to approve the treatment in the USA and Canada for depression (Lefaucheur et al., 2014).

On the other hand, rTMS used in the treatment of other disorders, such as panic disorders, hallucinations, obsessive-compulsive disorder, schizophrenia, Parkinson's disease, dystonia and stroke has been less promising so far (Lefaucheur et al., 2014). To increase the use of rTMS and its acceptance in the medical community across different medical specialties requires a better understanding of the potential pitfalls of the employed protocols.

As far as stimulation frequency is concerned, there is some consensus on the excitatory effects at higher frequencies and inhibitory effects at lower frequencies (Fitzgerald et al., 2006), although further temporal variants also play a role.

However, the temporal organization of rTMS pulses, including the inter-train interval (ITI) or intra-burst interval, has attracted less attention, despite its seemingly cardinal role in determining rTMS efficacy. The relevance of the ITI has been more intensively studied in so-called "patterned stimulation protocols" such as theta burst (Huang et al., 2005) or quadripulse (Hamada et al., 2008) stimulation. In these paradigms, a smaller number of stimulations was more efficacious than rTMS with a constant inter-stimulation interval. However, the influence of the ITI needs to be better addressed in the conventional stimulation protocols used in the treatment of depression or chronic pain.

Different time ranges play different roles in this context. In TBS it seems that the introduction of an 8-s ITI between ten bursts of three high-frequency pulses at 5 Hz (i.e., in a theta range) was facilitatory, while cTBS without this 8-s ITI was inhibitory (Huang et al., 2005). With QPS the effect on plasticity induction is quite the opposite when intra-burst intervals within the burst of four are crossed over from facilitation when using 5 ms to inhibition when using 50 ms (Hamada et al., 2008). Even for conventional 5 Hz rTMS protocols the introduction of a longer ITI switched the aftereffects from inhibition to excitation (Rothkegel et al., 2010).

Another way to view the issue of ITI is to apply information gained from paired-pulse TMS protocols. For example, repetitive paired-pulse stimulation using 10–15 ms intervals, which can cause facilitation in the intracortical facilitation (ICF) protocol, was more excitatory than 2–3 ms intervals, which would cause inhibition in the short-interval intracortical inhibition (SICI) (Sommer et al., 2001; Shirota et al., 2016). In this way, even though ITIs were initially introduced for practical reasons, such as to avoid coil overheating or for probably mistaken safety considerations to reduce the risk of seizures, it can be a critical parameter that determines the clinical efficacy of rTMS.

The problem is that infinite possibilities exist for manipulating intervals. For example, using 10 ms intervals in the short intra-cortical facilitation (ICF) range, repetitive paired-pulse stimulation was more excitatory than with 2 ms intervals both at 5 and 2 Hz repetition frequency (Sommer et al., 2001).

Other strategies for increasing efficacy are increasing the duration of the stimulation and concomitantly the total number of pulses, e.g., 54,000 stimuli over 3 days (George et al., 2014). But this strategy may fail: when the motor evoked potential is used as the biomarker with theta burst, prolonged, intermittent theta burst stimulation (1,200 pulses instead of 600) is inhibitory, i.e., efficacy not only declined, but the direction of the changes was even reversed when the number of stimulation pulses was doubled (Gamboa et al., 2010). tDCS also showed similar behavior with a reversal of aftereffects when two sessions were applied back-to-back compared to a single session (Monte-Silva et al., 2013).

Also the very simple parameter of rTMS frequency still remains insufficiently investigated. Frequencies higher than 10 Hz have rarely been used in conventional rTMS protocols. One reason for this being the increasing technical difficulties, in particular coil heating with higher frequencies or a possibly increased risk of seizures. The safety guidelines (Rossi et al., 2009) at 110% motor threshold assume a safe train duration of more than 5 s at 10 Hz that decreases to 1.6 s at 15 Hz and 0.84 s at 25 Hz.

Neuronal Response Failures: From Cell Cultures to Human Brains

Given the uncertainty of the significance of the temporal organizations in rTMS, we hypothesized that delayed neuronal response latency (NRL) or a resulting neuronal response failure (NRF) should play an important role in the biological effects of rTMS. When a neuron is subjected to supra-threshold stimulation, it typically produces an action potential, which can be measured extracellularly several milliseconds after the stimulation. The time-gap between the stimulation and the corresponding recorded evoked spike is known as neuronal response latency (Vardi et al., 2012; Goldental, 2014; Sardi et al., 2017, 2018). If the stimulus frequency is low enough, NRL is stable and there are no response failures. With repeated stimulations above a so-called critical frequency (f_c), the NRL was found to stretch gradually (**Figure 1A**) (Goldental et al., 2016) until it fluctuated around an average value, and stochastic neuronal response failures (NRFs) appeared, i.e., the average firing rate is saturated and is equal to f_c, even if the external stimulation frequency is higher. The neuron functions like a low-pass filter. At a stimulation frequency higher than f_c NRFs appear randomly and independently with a probability of f_c/f (Goldental et al., 2015). After several minutes without stimulation, the NRL approaches its initial value (Vardi et al., 2012). On the other hand, when a neuron is stimulated below its f_c, the NRL is stable (Vardi et al., 2015), and the probability of neuronal response failure is negligible (Vardi et al., 2015).

Repetitive Transcranial Magnetic Stimulation and Neuronal Plasticity

Thus, at the single neuron level, the response probability decreases when the stimulation frequency exceeds f_c (**Figure 1B**;

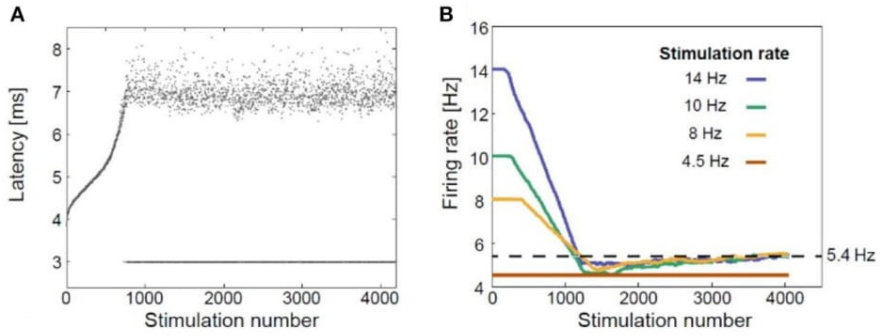

FIGURE 1 | Neuronal plasticity. **(A)** The neuronal response latency (NRL) of a cultured cortical neuron functionally separated from its network using a pharmacologic block, stimulated at a frequency of 10 Hz. Response failures are represented by dots at NRL = 3 ms. **(B)** Firing rates for different stimulation rates using a sliding window of 1,000 stimulations, indicating a saturated firing rate (~5.4 Hz, dashed line) independent of the applied stimulation rate. Adapted from Goldental et al. (2016) with the author's permission.

Goldental et al., 2016). At the population level, e.g., in recurrent or feed-forward networks, this effect is even enhanced because a sufficient number of neurons must fire in synchrony for the signal to propagate among populations of neurons representing perceptual entities (Vardi et al., 2012).

As a result, on the one hand, we expect rTMS with a lower frequency to be more reliable and thus more efficient than higher frequency stimulation, in which neuronal response lead to less synchronized responses. On the other hand, we must differentiate between intended inhibitory effects that occur with rTMS frequencies around 1 Hz, and rTMS stimulation with frequencies higher than 1 Hz, which are excitatory. The frequency/response curve should therefore resemble an inverted U. The situation is however more complicated when considering intervals, which allow a recovery of conduction reliability as outlined in more detail in the comparison of two trials on rTMS in depression.

The influence of the inter-train interval in rTMS has been investigated less than that of stimulation frequency (e.g., 1 or 5 Hz) even in the context of basic research, so we examined the premise using the neuronal culture developed by Vardi and co-workers (Vardi et al., 2015). The greatest incremental success in the treatment of depression was seen in a key trial (O'Reardon et al., 2007) using a special patterned paradigm based on 10 Hz rTMS, while the results of a different multicenter trial conducted at the same time using 10 Hz rTMS in the treatment of refractory depression were negative (Herwig et al., 2007). Among the many reasons for the contrary outcomes, e.g., total stimulation duration, number of stimuli per session, total duration in days and intensity, the two studies differed in the pattern of their rTMS stimulation sequence. The successful data set (Protocol B) applied 3,000 10 Hz stimuli per day with 4 s of stimulation and an interval of 26 s. The unsuccessful study (Protocol A) applied 2,000 10 Hz stimuli per day with 2 s of stimulation and an 8-s interval without stimulations. It should be noted that the Herwig Protocol (Herwig et al., 2007) used an accelerated treatment protocol of 15 days, while the O'Reardon Protocol (O'Reardon et al., 2007) used a full course of 4–6 weeks of treatment. Treatment duration is also known to be a factor that influences treatment efficacy (Lefaucheur et al., 2014). Nonetheless, ITI duration is also an important parameter consideration, which may influence the efficacy of clinical protocols.

We compared both protocols using a single neuron *in vitro* approach in a cell culture model (Goldental et al., 2016).

In summary, we investigated in a literature review whether conduction failure of cortical neurons limited reliable spike conduction by comparing the published results of clinical studies with *in vitro* experiments.

METHODS

Protocols A and B were compared in a cell culture (Vardi et al., 2012; Sardi et al., 2017). All cell culture experimental procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and Bar-Ilan University Guidelines for the Use and Care of Laboratory Animals in Research and are approved and supervised by the Institutional Animal Care and Use Committee. Cortical neurons are obtained from newborn rats within 48 h after birth using mechanical and enzymatic procedures (Vardi et al., 2012; Sardi et al., 2017). The neurons are plated directly onto substrate-integrated multi-electrode arrays (MEAs) and are allowed to develop functionally and structurally mature networks over a period of 2–3 weeks *in-vitro*, prior to the experiments. The number of plated neurons in a typical network is in the order of 1,300,000, covering an area of about 380 mm^2. In order to conduct experiments in which cultured cortical neurons are functionally isolated from their network, a pharmacological block of glutamatergic and GABAergic synapses is performed. This cocktail does not block the spontaneous network activity completely, but rather makes it sparse. At least 1 h is allowed for stabilization of the effect. For stimulation and recording an array of 60 extracellular electrodes, 30 μm in diameter, and spaced 200–500 μm from each other (Multi-Channel Systems, Reutlingen, Germany) is used. Mono-phasic square voltage pulses were used, in the range of [−800, −500] mV and [60, 400] μs and each

channel was sampled at a frequency of 50 k samples/s (Vardi et al., 2015). Post-experiment analyses are performed in a Matlab environment (MathWorks, Natwick, MA, USA).

For a more realistic setting, considering that neurons are not at rest before rTMS, they were primed with 300 stimulations at 10 Hz followed by 30 repetitions of the stimulation patterns according to the protocols A or B (**Figure 2**).

For the simulation, a critical frequency (f_c) was randomly chosen from range of 1 to 10 Hz frequency for each neuron. The neuronal response latency was simulated as a constant increase per firing, 0.03 ms, for stimulation frequencies above the neuronal f_c and an exponential decay with a time constant of 40 s for lower frequencies. Above the critical neuronal response latency increase of 3 ms neuronal response failures occur and the NRL is set as a random value between 3 and 4 ms for each firing of the neuron. The connections between layers in the feed-forward networks were randomly chosen with a probability of 15/N and a strength equal to 0.1 of the neuronal threshold, where N is the number of neurons in a layer. All other details are the same as in Vardi et al. (2015).

We simulated protocol A neurons in layer 1 with supra-threshold stimulations at 10 Hz for 2 s, every 10 s, and for protocol B we stimulated neurons in layer 1 with supra-threshold stimulations at 10 Hz for 4 s, every 30 s.

To verify the ITI-influence hypothesis from available data on depression studies, we obtained 59 detailed protocols from 57 papers with class A level of evidence. We also focused on the number of pulses per train, number and duration of trains, interval duration, and number of sessions (**Tables 1**, **2**). Forty of the protocols were for depression, with two studies (George et al., 2000; Su et al., 2005) directly comparing two protocols, and 19 were for the treatment of pain as summarized in Lefaucheur et al. (2014). High frequency rTMS stimulation was applied to the left DLPFC for depression, and to the contralateral motor area for pain (Lefaucheur et al., 2014).

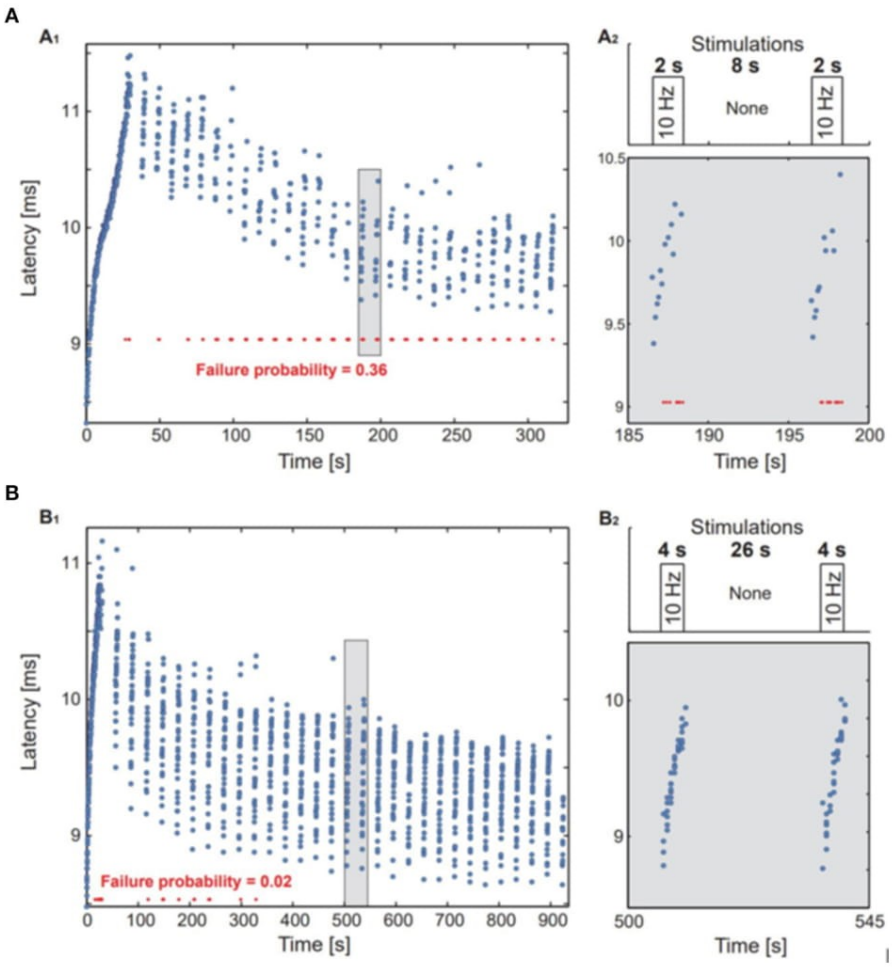

FIGURE 2 | *In vitro* experiments in single neurons. A neuron with an fc = 1.3 Hz was stimulated above the threshold using the stimulation timings of protocols A and B. **(A1,B1)** The neuronal response latency was measured for each response, response failures are represented by red dots. **(A2,B2)** upper panel: an illustration of the stimulation protocol. Lower panel: a zoom-in of the marked area at **(A1,B1)**.

TABLE 1 | Detailed stimulation patterns and percentage improvement in Hamilton depression scores from all papers using the efficacy A classified HFrTMS protocol in management of depression.

Study	Stimulation frequency /Hz	Pulse/burst	Burst duration /s	Inter train interval/s	Repetitions	Average frequency /Hz	Total duration/s	Total number of pulses	Intensity RMT	Session number	Hamilton % improv	Hamilton % improv sham	Difference
Pascual-Leone et al., 1996	10	80	8	52	15	1.33	848	1,200	0.9	5	45	7	38
George et al., 1997	20	40	2	60	20	0.65	1,180	800	0.8	10	23	−15	38
Loo et al., 1999	10	50	5	30	30	1.43	1,020	1,500	1.1	10	23	16	7
Padberg et al., 1999	10	50	5	30	5	1.43	145	250	0.9	5	19	1	18
Berman et al., 2000	20	40	2	58	20	0.67	1,142	800	0.8	10	32	3	29
Eschweiler et al., 2000	10	100	10	50	20	1.67	1,150	2,000	1.1	10	20	−6	26
George et al., 2000	5	40	8	22	40	1.33	1,178	1,600	1	10	48	21	27
	20	40	2	28	40	1.33	1,172	1,600	0.9	10	27	21	6
Garcia-Toro et al., 2001a	20	40	2	30	30	1.25	930	1,200	0.9	10	40	9	31
García-Toro et al., 2001b	20	40	2	30	30	1.25	930	1,200	0.8	10	21	15	6
Manes et al., 2001	20	40	2	60	20	0.65	1,180	800	0.9	5	40	28	12
Boutros et al., 2002	20	40	2	58	20	0.67	1,142	800	0.9	10	32	18	14
Padberg et al., 2002	10	100	10	30	15	2.50	570	1,500	1	10	33	13	20
Nahas et al., 2003	5	40	8	22	40	1.33	1,178	1,600	1.1	10	32	25	7
Fregni, 2004	15	75	5	55	40	1.25	2,345	3,000	1.1	10	32	0	32
Hausmann et al., 2004	20	200	10	90	10	2.00	910	2,000	1.1	10	51	40	11
Jorge et al., 2004	10	50	5	60	20	0.77	1,240	1,000	1	10	40	5	35
Koerselman et al., 2004	20	40	2	30	20	1.25	610	800	0.8	10	40	18	22
Mosimann et al., 2004	20	40	2	28	40	1.33	1,172	1,600	1	10	18	16	2
Rossini et al., 2005a	15	30	2	28	20	1.00	572	600	1	10	72	14	58
Rossini et al., 2005b	15	30	2	28	30	1.00	872	900	1	10	52	25	27
Rumi et al., 2005	5	50	10	20	25	1.67	730	1,250	1.2	20	63	30	33
Su et al., 2005	5	40	8	22	40	1.33	1,178	1,600	1	10	54	16	38
	20	40	2	28	40	1.33	1,172	1,600	1	10	58	16	42
Avery et al., 2006	10	50	5	30	32	1.43	1,090	1,600	1.1	15	30	19	11
Herbsman et al., 2009	10	50	5	30	32	1.43	1,090	1,600	1.1	15	32	13	19
Anderson et al., 2007	10	50	5	30	20	1.43	670	1,000	1.1	20	44	15	29
Bortolomasi et al., 2007	20	40	2	28	20	1.33	572	800	0.9	5	52	18	34
Herwig et al., 2007	10	20	2	8	100	2.00	992	2,000	1.1	10	43	38	5
Loo et al., 2007	10	50	5	25	30	1.67	875	1,500	1.1	20	39	26	13
O'Reardon et al., 2007	10	40	4	26	75	1.33	2,224	3,000	1.2	15	25	14	11
Bretlau et al., 2008	8	64	8	52	20	1.07	1,148	1,280	0.9	10	35	23	12
Jorge et al., 2008	10	60	6	60	20	0.91	1,260	1,200	1.1	13	35	15	20
Mogg et al., 2008	10	50	5	55	20	0.83	1,145	1,000	1.1	10	25	10	15
Lisanby et al., 2009	10	40	4	26	75	1.33	2,224	3,000	1.2	20	22	6	16
George et al., 2010	10	40	4	26	75	1.33	2,224	3,000	1.2	20	18	11	7
Paillère Martinot et al., 2010	10	80	8	60	20	1.18	1,300	1,600	0.9	10	42	27	15
Triggs et al., 2010	5	40	8	22	50	1.33	1,478	2,000	1	10	32	22	10
Ray et al., 2011	10	60	6	24	20	2	576	1,200	0.9	10	85	33	52
Baeken et al., 2013, 2014	20	40	2	12	39	2.86	534	1,560	1.1	20	23	18	5

TABLE 2 | Detailed stimulation patterns and percentage improvement in visual analog scales from all papers using the efficacy A classified HFrTMS protocol in management of chronic pain.

Study	Stimulation frequency/Hz	Pulse/burst	Burst duration/s	Inter train intervals/s	Repetitions	Average frequency/Hz	Total duration/s	Total number	Intensity RMT	Number of sessions	VAS % improv	VAS % improv sham	Difference
Lefaucheur et al., 2001a	10	50	5	55	20	0.83	1,145	1,000	0.8	1	20	7	13
Lefaucheur et al., 2001b	10	50	5	55	20	0.83	1,145	1,000	0.8	1	29	−10	39
Lefaucheur, 2004	10	50	5	55	20	0.83	1,145	1,000	0.8	1	35	0	35
Khedr, 2005	20	200	10	50	10	3.33	550	2,000	0.8	5	45	5	40
André-Obadia et al., 2006	20	80	4	84	20	0.91	1,676	1,600	0.9	1	11	8	3
Hirayama et al., 2006	5	50	10	50	10	0.83	550	500	0.9	1	29	5	24
Krishnan and Nestler, 2008	5	500	100	0	1	5.00	100	500	0.95	5	5	10	−5
Lefaucheur et al., 2006	10	60	6	54	20	1.00	1,146	1,200	0.9	1	36	1	35
Saitoh et al., 2007	5	50	10	50	10	0.83	550	500	0.9	1	33	3	30
Saitoh et al., 2007	10	100	10	50	5	1.67	250	500	0.9	1	38	3	35
André-Obadia et al., 2008	20	80	4	84	20	0.91	1,676	1,600	0.9	1	17	2	15
Lefaucheur et al., 2008	10	60	6	54	20	1.00	1,146	1,200	0.9	1	24	9	15
Kang et al., 2009	10	50	5	55	20	0.83	1,145	1,000	0.8	5	23	−3	26
Ahmed et al., 2011	20	200	10	50	10	3.33	550	2,000	0.8	5	54	−2	56
André-Obadia et al., 2011	20	80	4	84	20	0.91	1,676	1,600	0.9	1	12	−2	14
Lefaucheur et al., 2011	10	100	10	30	20	2.50	770	2,000	0.9	1	33	13	20
Hosomi et al., 2013	5	50	10	50	10	0.83	550	500	0.9	1	22	7	15
Jetté et al., 2013	10	50	5	25	40	1.67	1,175	2,000	0.9	1	17	8	9
André-Obadia et al., 2014	20	80	4	84	20	0.91	1,676	1,600	0.9	1	15	3	12

The parameters studied were average stimulation frequency and total duration of stimulation. In the published depression studies, we assessed efficacy as the percentage improvement in the Hamilton depression rating scale (HDRS) compared to sham conditions. In the studies on chronic pain, we assessed changes in pain severity quantified with a visual analog scale (VAS). We plotted the results against average stimulation frequency, interval duration, total duration and total number of pulses. We excluded the study by Fregni (2004) from the analysis because it did not compare rTMS to sham stimulation but only to drug therapy with both showing an equal degree of improvement.

RESULTS

In the cell culture study, protocol A with the shorter ITIs was associated with a substantial fraction of response failures, while these were much less frequent in protocol B (**Figure 3**). The average stimulation frequency in protocol A was 2 Hz (20 stimulations per 10 s) as compared to the average stimulation frequency of 1.33 Hz in protocol B (40 stimulations per 30 s).

Figure 3 illustrates, through simulations, the superiority of protocol B over protocol A for both single neurons and networks, i.e., higher response probability when applying protocol B. Simulations of feedforward, layered networks increasingly demonstrated this effect, which was also visible in simulations including more than two layers (**Figure 4**).

HFrTMS Protocol Evaluation

Our evaluation of the available clinical data in a literature review showed that ITI protocols using ITIs of 20 s and longer were superior to shorter ITI protocols in the treatment of depression and chronic pain. For depression, the relationship between ITIs and improvement in HDRS was not linear and followed an inverted U-curve. We must, however, emphasize that only two studies used ITIs shorter than 20 s in the treatment of depression, and that there was only one such protocol for chronic pain.

We plotted the efficacy against isolated parameters, clustering the data into four groups and connecting the centers of the clusters. The graphs, with the exception of **Figure 6A**, showed low coefficients of determination (R^2), which suggests poor fitting of the graph to the data points. Only the plot of ITIs against VAS improvement in **Figure 6A** showed an R^2 of 0.53 and a P of 0.019.

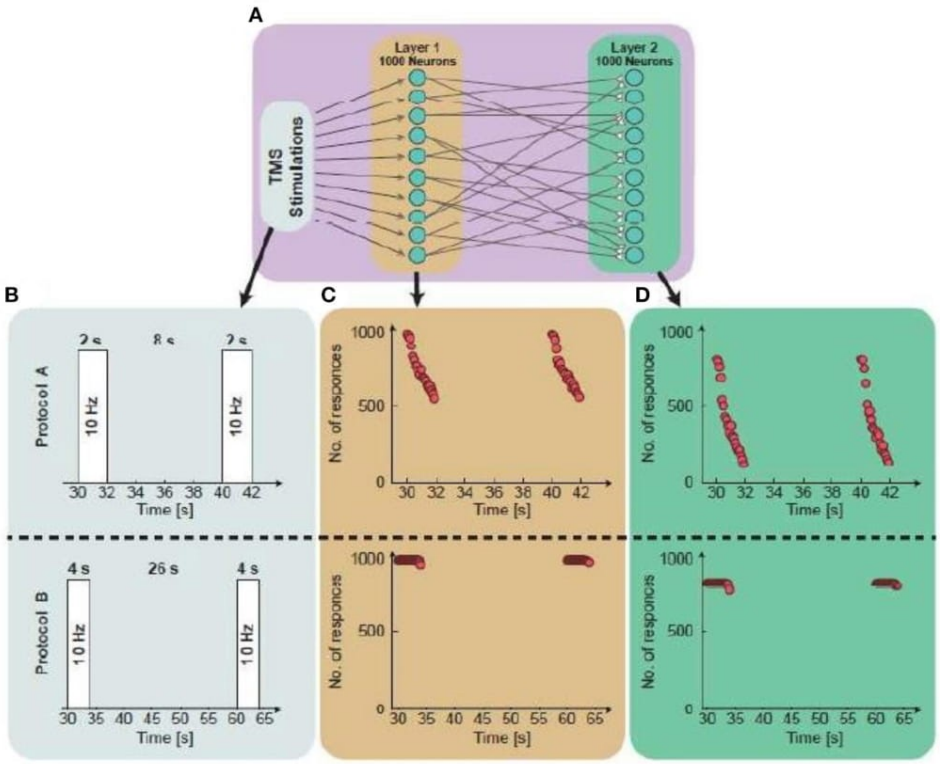

FIGURE 3 | Simulations from a single neuron to population dynamics. **(A)** A scheme of the simulated two-layered feed-forward network: Two populations, 1st layer is randomly connected presynaptically to the 2nd layer. Each layer consists of 1,000 neurons and the 1st layer is stimulated repeatedly. **(B)** An illustration of the simulated protocol. Upper panel: Neurons in the 1st layer are stimulated at 10 Hz for 2 s, every 10 s. Lower panel: Neurons in the 1st layer are stimulated at 10 Hz for 4 s, every 30 s. **(C)** The number of firing neurons in the 1st layer as a function of the stimulation time. The average number of responses in the 1st layer for protocol A and protocol B is 0.75 and 0.999, respectively. **(D)** The number of firing neurons in the 2nd layer as a function of the stimulation time. The response probability in the 2nd layer for protocols A and B is 0.44 and 0.85, respectively.

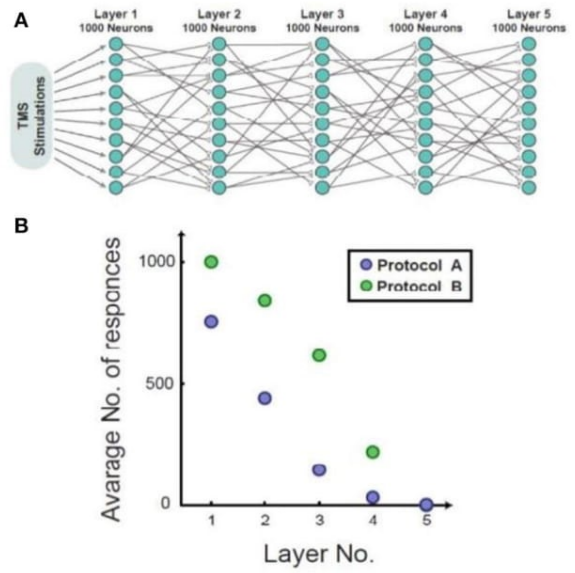

FIGURE 4 | Simulation of a feed-forward network consisting of five layers. **(A)** A diagram of the simulated feed-forward network. **(B)** The average number of responses as a function of the layer number. In the presented case, protocol B demonstrated fewer response failures in comparison to protocol A up to the 5th layer.

Plotting the inter-train intervals against effect showed that the most effective duration between 10 Hz trains was approximately 50 s ($R^2 = 0.147$; **Figure 5A**).

Moreover, the average frequency in the sessions (calculated by dividing the total number of pulses by the total session time in seconds) showed a negative correlation with the efficacy measure, i.e., the higher the average frequency, the less effective the treatment (**Figure 5B**). As mentioned above, protocol B employed a lower average frequency than protocol A ($R^2 = 0.143$).

The evaluated studies on chronic pain revealed similar tendencies (**Figures 6A,B**) with an optimum inter-train interval also around 50 s ($R^2 = 0.53$) and an average frequency of about 2.5 Hz ($R^2 = 0.36$).

Both total duration of stimulation and the number of pulses showed a negative correlation with efficiency in depression studies. Protocols lasting more than 20 min were less effective than shorter ones (**Figure 5C**) (P-value = 0.041). Protocols using more than 2,000 pulses had a lower efficacy than protocols with a smaller number of pulses (**Figure 5D**) (P-value = 0.113 using independent samples t-test). Thus, it seems that the average frequency is a more important measure than simply the number of pulses. For example, in our comparison protocol B employed 3,000 pulses lasting approximately 37 min while protocol A, used 2,000 pulses over 16.5 min.

This was also evident in the pain studies (**Figure 6C**), which showed a decrease in efficiency with longer stimulation ($R^2 = 0.33$). No correlation was found with the total number of pulses (**Figure 6D**).

DISCUSSION

We evaluated the hypothesis that an increase in the effect magnitude of rTMS cannot be achieved by deliberately increasing the number of TMS pulses per time unit because of the provocation of conduction failures. We compared the results of published evidence level A studies in pain and depression with data obtained in cell cultures. In chronic pain protocols we found an inverse relationship between excitatory rTMS frequency and efficacy as predicted, but we were unable to verify this for the depression protocols. We believe that this is because of the differences in the neuronal circuitries involved in the two disorders, with different optimum firing and stimulation frequencies (Krishnan and Nestler, 2008; Simons et al., 2014).

The analysis of the depression studies revealed that most research groups used more or less similar protocols leading to the data being clustered around certain points. Other significant points, e.g., ITIs of ca. 50 s and an average frequency of ca. 1.5 Hz are lacking and should be tested. Despite the fact that relevant points are missing and that the data is noisy, we were still able to detect a pattern in the graphical representations, in which the missing testable variables are easily visible.

In the studies on chronic pain, that of Irlbacher et al. (2006) seems to stand out and illustrates the importance of the ITIs. They used a 100-s continuous train of 5 Hz rTMS (hence the short duration and small number of pulses) without any intervals. Of all the pain studies reviewed here this is the only one that actually showed a worsening of visual analog scales scores compared to sham stimulation. This supports the results of an earlier study that described inhibition using 5 Hz rTMS without intervals (Rothkegel et al., 2010). In all other pain studies the number of pulses per burst ranged from 50 to 200 (for 20 Hz).

We are aware that conduction failures play only one, and probably a minor role compared to the multitude of other plasticity mechanisms. The pleomorphism in response can be mediated by the effect of such patterns and parameters on the behavior of intracellular calcium (Huang et al., 2011; Wilson et al., 2016). Glutamatergic transmission, in particular NMDA receptor mechanisms also play key roles.

A low-pass filter effect of single neurons and, consequently, of neuronal networks is demonstrated by the observation that when a single neuron is stimulated at frequencies of between 20 and 100 Hz, its actual maximum firing frequency is capped at, for example, 17 Hz (Sardi et al., 2016). Even at a continuous 10 Hz stimulation frequency, the neuronal response latencies increase with significant response failures occurring after 700 stimulations.

Neuronal cell culture results showed protocol B to be more efficient than protocol A, with the difference attributed to the longer silence period of 26 s used in protocol B that allowed recovery and relaxation of the NRL toward its initial value. i.e., the higher average stimulation frequency in protocol A is interpreted as a main source of the difference between their efficiencies.

A second finding derived from the cell culture data was that firing was initially stable but that conductions failures occurred after a few hundred stimuli. It is unclear whether this can be

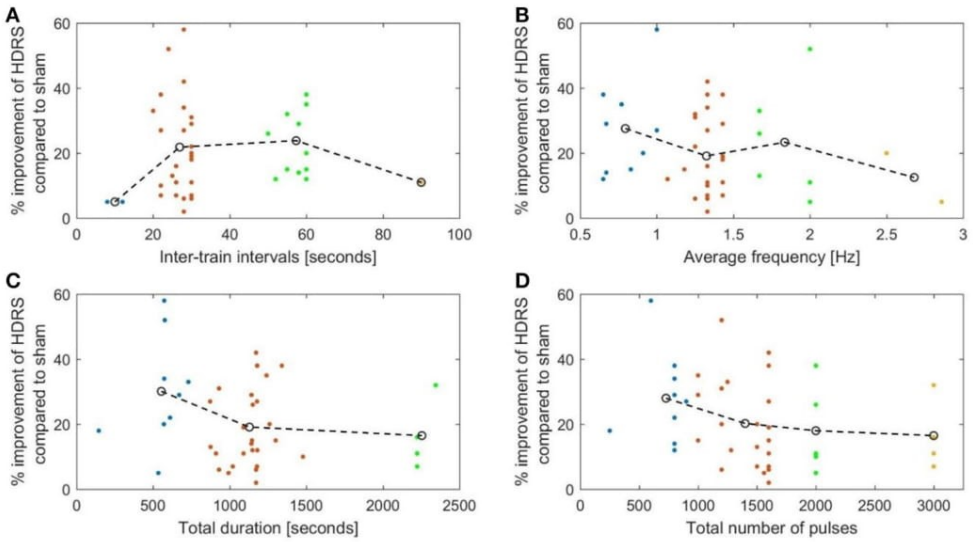

FIGURE 5 | The percentage reduction of the Hamilton depression rating score compared to sham plotted against. **(A)** The duration of the inter-stimulation intervals ($R^2 = 0.147$). **(B)** The average stimulation frequency ($R^2 = 0.143$). **(C)** The total duration of stimulation ($R^2 = 0.037$). **(D)** The total number OF pulses ($R^2 = 0.113$).

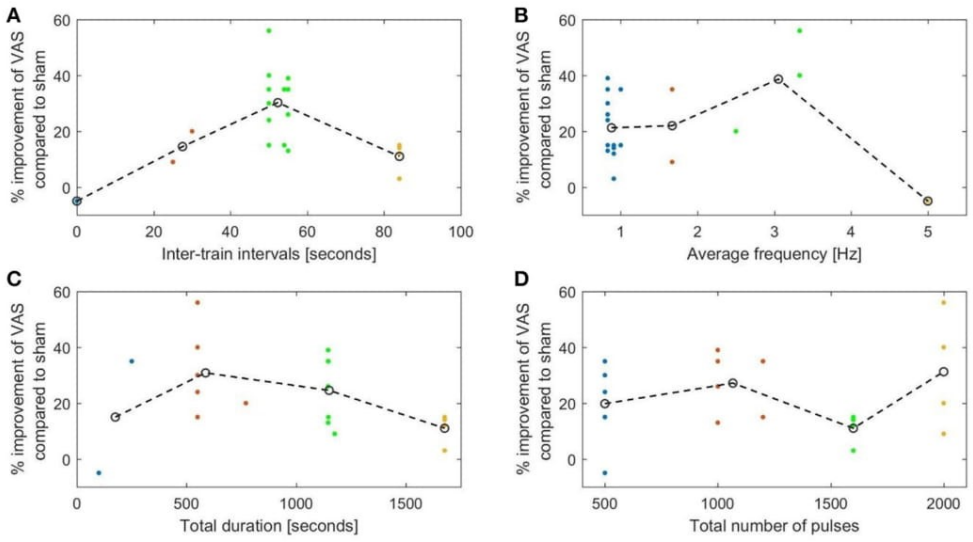

FIGURE 6 | The percentage decrease in VAS rated pain compared to sham plotted against. **(A)** The duration of the inter-stimulation intervals ($R^2 = 0.53$). **(B)** The length of the average stimulation frequency ($R^2 = 0.36$). **(C)** The total duration of stimulation ($R^2 = 0.33$). **(D)** The total number of pulses ($R^2 = 0.24$).

transferred to clinical studies, since no similar protocols have been used in patients.

Theoretically, the effect of response failures on the activity of a feed-forward network is enhanced in case of subthreshold synapses. While in the case of a single neuron, the neuron can fire or not-fire, in this case several neurons have to fire synchronously in order to transmit the signal to the next population.

A recent experiment investigated in normal subjects inter-train intervals ranging from 4 to 32 s for a 20 Hz protocol (Cash et al., 2017). At first glance the results seem to argue against our hypothesis; the shortest ITI using 4-s generated the highest MEPs, the 8 s intervals stimulation resulted in the smallest MEPs, which increased again with 16 and 32 s ITI. The 8 to 32 s ITI findings are however in line with our hypothesis. For the 4 s ITI finding a different mechanism may apply: Since SICI was disinhibited most by the shortest ITI of 4 s with a smaller disinhibition at 8 s and almost none at 16 and 32 s. The large 4 s ITI disinhibition of SICI may overrun conduction failures and dominate the 4 s results in the MEP study (Cash et al., 2017). Almost certainly more than one mechanism is involved in the production of the net outcome.

Controlled studies are also here necessary to split up the involved mechanisms.

Conduction failures are also determined by the original firing rate of the network so that the upper frequency limit might also apply to inhibitory protocols using 1 Hz stimulation frequencies. Repetition suppression (i.e., the decrease in the amplitude of subsequent MEPs compared to the first stimulus) is the measure to test for inhibitory protocols with lower frequencies (Pitkänen et al., 2017). With higher frequency facilitatory protocols using 5 Hz, the MEP amplitudes increased during the train but inter-train suppression was also detected as a latent period stretching with each TMS pulse (Berardelli et al., 1999).

More data is required in order to optimize the protocol further. In future studies, one might consider starting stimulation with a higher rTMS pulse frequency incorporating a decay over time in order to remain below the critical frequency at which conduction blocks arise. rTMS protocols employing a higher frequency may be more efficient initially but become less reliable over time.

We conclude that depending on the disorder and the desired outcome it should be possible to optimize both the intervals between stimulation trains and the average stimulation frequencies.

In the examined clinical data set for class A rTMS protocols, a lot of heterogeneity were observed in other stimulation variables such as stimulation frequencies, intensities, number of pulses and treatment duration, each one of which might solely explain the discrepancy between the clinical outcome of the O'Reardon et al. (2007) and Herwig et al. (2007) protocols. The focus of our work here was on ITIs, which were so far not considered as an isolated rTMS variable in all studies. Based on the neuronal culture results we propose ITIs as a noteworthy variable in therapeutic rTMS protocols. As such, it would be helpful to initiate specific studies in which only the ITI parameter is modified with a clinical outread.

AUTHOR CONTRIBUTIONS

IH gathered and analyzed data and wrote Manuscript. AG carried out the neuronal culture experiments, analyzed data and idea. YS gathered and analyzed data and revision. IK oversight and review. WP idea, oversight, and review.

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3. Role of pulse width and directionality in low frequency rTMS:

Using the new cTMS device that could readily change the widths of individual pulses as well as their directionality (Peterchev et al. 2011), I was able to isolate and test the effect of two properties of individual pulses namely pulse widths and directionality on low frequency rTMS (Halawa et al. 2019b). To test pulse width, I fixed the directionality index known as M ratio at 0.2 and changed the pulse widths from 40 to 80 and 120μs, in other words I tested unidirectional pulses with the above-mentioned widths. The I fixed the pulse width at 80μs and changed the M ratio from 0.2 to 0.6 and 1.0 to test effect of directionality. M ratio is a directionality index which refers to the relation between pulses components.

For the directionality, unidirectional pulses were inhibitory and bidirectional pulses were excitatory similar to reported data (Sommer et al. 2013). The new important finding however, was for the varying pulse widths where the unidirectional pulse shape with 120μs wide main component changed the expected inhibitory outcome of 1Hz rTMS into excitation (Halawa et al. 2019b). Which lead me to believe that wider pulses are stimulating an additional component in the target area namely dendrites as they have been found not to respond to pulse shapes shorter than 100μs (Rattay et al. 2012). I then plotted a strength duration curve for the motor cortex for my subjects and found a significant correlation between the PwTh (shortest pulse width able to produce 50μV MEP) to the aftereffects of the 40μs pulse shape 1 Hz rTMS (Halawa et al. 2019b).

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Neuronal tuning: Selective targeting of neuronal populations via manipulation of pulse width and directionality

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Dendrites

ABSTRACT

Introduction: Motor evoked potentials (MEP) in response to anteroposterior transcranial (AP) magnetic stimulation (TMS) are sensitive to the TMS pulse shape. We are now able to isolate distinct pulse properties, such as pulse width and directionality and evaluate them individually. Different pulse shapes induce different effects, likely by stimulating different populations of neurons. This implies that not all neurons respond in the same manner to stimulation, possibly, because individual segments of neurons differ in their membrane properties.
Objectives: To investigate the effect of different pulse widths and directionalities of TMS on MEP latencies, motor thresholds and plastic aftereffects of rTMS.
Methods: Using a controllable pulse stimulator TMS (cTMS), we stimulated fifteen subjects with quasi-unidirectional TMS pulses of different pulse durations (40 μs, 80 μs and 120 μs) and determined thresholds and MEP AP latencies. We then compared the effects of 80 μs quasi-unidirectional pulses to those of 80 μs pulses with different pulse directionality characteristics (0.6 and 1.0 M ratios). We applied 900 pulses of the selected pulse shapes at 1 Hz.
Results: The aftereffects of 1 Hz rTMS depended on pulse shape and duration. 40 and 80 μs wide unidirectional pulses induced inhibition, 120 μs wide pulses caused excitation. Bidirectional pulses induced inhibition during the stimulation but had facilitatory aftereffects. Narrower pulse shapes caused longer latencies and higher resting motor thresholds (RMT) as compared to wider pulse shapes.
Conclusions: We can tune the aftereffects of rTMS by manipulating pulse width and directionality; this may be due to the different membrane properties of the various neuronal segments such as dendrites.
Significance: To date, rTMS frequency has been the main determinant of the plastic aftereffects. However, we showed that pulse width also plays a major role, probably by recruiting novel neuronal targets.
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Introduction

Repetitive transcranial magnetic stimulation (rTMS) is considered a promising therapeutic tool in neuropsychiatric diseases and has demonstrated a definite efficacy in the treatment of depression and chronic pain [1]. However, there are patients who are resistant to rTMS therapy. This may be due to a differing sensitivity to rTMS [2]. To this date, the rTMS parameters contributing to such variability are not clear. A better understanding of the underlying mechanisms affecting the outcome of rTMS is expected to enhance its reliability as a therapeutic tool [3,4].

Variable rTMS aftereffects are thought to depend on different physiological mechanisms of the neuronal responses; intracellular due to alterations of Ca^{2+} spiking [5], extracellular by shifts in levels of factors and kinases [6], or on the network level by eliciting near and more distant related cortical potentials [7]. Various models have been proposed to combine the physical parameters of the stimulation and their various physiological interactions [8–10]. Those models address distinct parts of the pyramidal cells together with their connecting inhibitory neurons, primarily in order to

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model TMS effects on the temporal evolution of cortico-spinal volleys (direct and indirect waves), and to a lesser extent the expected plastic aftereffect in response to such stimulation. There is a multitude of proposed mechanisms for the therapeutic effects and this leads to ambiguity with regard to the neuronal mechanism of rTMS-induced plasticity, particularly when considering their interaction [11,12].

Only a few rTMS parameters involved in modulating its effects have been closely investigated, among which are e.g. stimulation frequency, intensity and number of pulses and sessions [1]. However, the impact of individual pulse shapes on rTMS outcome is unknown because technical limitations prevented systemic variation of pulse shape. Aside from rare exceptions we have only been able to change pulse frequency and patterns [13,14].

A controllable pulse parameter TMS (cTMS) device opens up a new parameter-space for TMS, i.e. pulse shape. It is capable of producing near rectangular pulse shapes by the use of two capacitors, and two bipolar semiconductor transistors that alternate the current between the capacitors [15]. The cTMS rectangular pulse platform provides a sufficient flexibility of pulse shapes by altering the phase widths, heights (intensity) and also the relation between positive and negative components of the pulse (directionality, defined as the M ratio). Such customized pulses may be more efficient [16] and could be applied in repetitive trains. In particular, symmetrical, bidirectional pulses, which require little or no capacitor discharging, can be applied at repetition rates of up to 1 kHz [13]. In this study, we made use of the cTMS to test the effect of pulse width and directionality on the sign and temporal evolution of plastic aftereffects.

Across a wide range of neuromodulatory protocols, AP current gives a more evident outcome in correlation with latencies. This was observed with numerous modalities of rTMS, e.g. continuous theta-burst stimulation (cTBS) [17,18], anodal transcranial direct current stimulation [19,20], short latency afferent inhibition protocol [21], and 5 Hz [22] and 1 Hz rTMS [23]. While this latency to after effects correlation was very strong in some cases to the extent of claiming the possibility of identifying responders from non-responders to cTBS based on it [17,18], this correlation however was not significant with 1 Hz rTMS [23]. In this study, 900 pulses of 1 Hz rTMS from a MAGpro stimulator produced plastic MEP changes only if the current was induced in the anteroposterior (AP) but not the posterioanterior (PA) direction. Biphasic, symmetrical pulses with the central, decisive AP-directed component induced facilitatory aftereffects, while monophasic AP-directed pulses produced inhibitory aftereffects [23].

This differing behavior in response to AP stimulation is probably because different populations of neurons are responding. This can be seen in recordings of the descending corticospinal volleys which showed that AP stimulation preferentially recruited late I waves while PA stimulation more readily recruited earlier I waves [24].

Using cTMS enabled D'Ostillio and colleagues to increasing the pulse width from 30 to 60 and then to 120 μs which led to a significant shortening of the latency but only with AP stimulation [25]. Additionally, 30 μs pulses applied in the AP direction in the short latency afferent inhibition protocol gave longer latencies and produced more inhibition than 120 μs pulses [26].

We were particularly interested in AP stimulation because only this orientation had produced plastic aftereffects in response to 1 Hz rTMS [23], and had shown significant variation in the latencies to different pulse widths [25].

In this study, we systematically explored the influence of pulse shape on the aftereffects of a low frequency (1 Hz) rTMS train, which is thought to induce inhibitory aftereffects [27,28]. We focused on pulse duration and pulse directionality, both of which presumably play an important role in the selective stimulation of neurons in the primary motor cortex (M1).

We used quasi-unidirectional pulses with an M ratio of 0.2 while varying the width of the main pulse component. The M ratio is a measure of directionality or asymmetry that expresses the ratio between the intensities of the first and second phase as shown in Fig. 1. We then kept the width of the main pulse component constant and compared the most asymmetrical, i.e. the quasi-unidirectional pulses with two other levels of pulse directionality (ratios of 0.6 and 1.0 M).

We also plotted the strength duration curve for the motor cortex by defining the pulse width threshold (PwTh) as the narrowest pulse width required to elicit a 50 μV MEP in five out of ten trials at 100% maximum stimulator output (MSO) of the cTMS intensity. We used 60 μs as a reference for the RTM defined it as 100% and then went in 10% steps into the widest possible pulse in one direction and the 100% MSO in the other.

Methods

Participants

Fifteen healthy volunteers were recruited for the main experiments (five males and ten females, mean age ± standard deviation was 25.2 ± 3.7 years). All participants were right-handed and free from any neurological or psychiatric disorders, took no centrally acting medications, and had no contraindications to TMS. Because the power cap of the device and the standard coil for the wider pulse shapes prevented intensities above 50% MSO, a higher resting motor threshold, i.e. above 70% MSO for the Magstim device was an exclusion criterion.

We obtained written informed consent from each subject before participation. The local ethics committee of the University Medical Center Göttingen approved the study protocol, which conformed to the Declaration of Helsinki.

Recordings

Motor evoked potentials (MEPs) were recorded from the first dorsal interosseous (FDI) muscle of the right hand with surface Ag−AgCl electrodes in a belly-tendon montage. The electromyography signals were amplified, band-pass filtered (2 Hz−2 kHz), and digitized at a sampling rate of 5 kHz with a micro-1401 AD converter (Cambridge Electronic Design Ltd., Cambridge, UK). All signals were stored in a computer for offline analysis. The peak-to-peak MEP amplitude served as an index for M1 excitability. The participants were asked to relax the right FDI during the measurements. If recordings were contaminated by voluntary muscle contraction before the TMS pulse(s), they were excluded from analysis.

A Magstim 200 (Magstim Co. Ltd., Whitland, UK) for measurement and a cTMS prototype 3 (cTMS3; Rogue Research Inc., Montreal, Canada) for intervention were used to deliver TMS over the M1.

- Initial session: motor thresholds and MEP latencies were measured using 25 pulses of AP 110% active motor threshold (AMT) for each pulse shape (Fig. 1).
- Five repeated, randomized sessions were separated by at least one week to avoid carry-over effects:
  Step 1: measuring the thresholds and the baseline:

For each session, we determined the Magstim RMT and the MSO intensity that gives approximately 1 mV amplitude for the pre-measurement intensity in the PA direction. In addition, we

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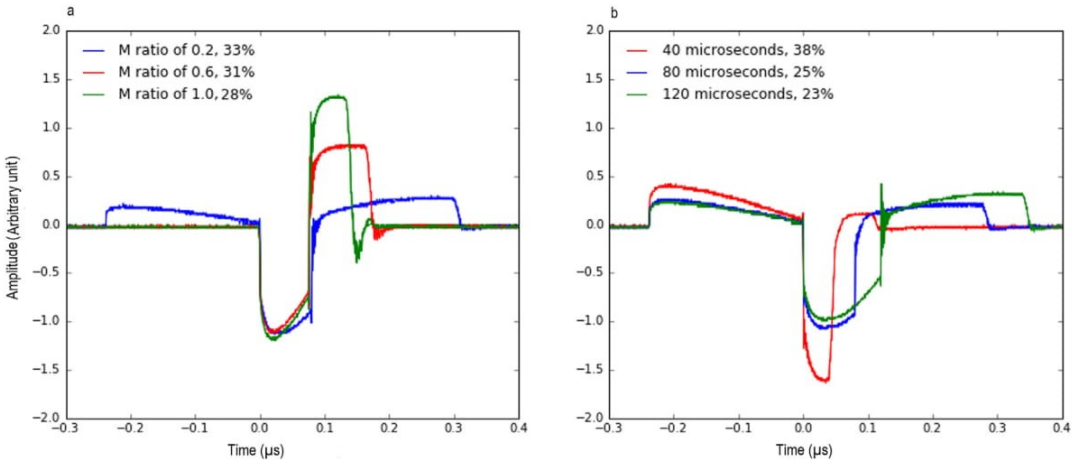

Fig. 1. Pulse shapes used in the 1 Hz stimulation as measured by an external pickup coil and oscilloscope, calibrated to the threshold level of each pulse shape expressed in percentage of the maximum stimulator output.

measured RMT for the cTMS pulse shape being used for the intervention in AP direction by rotating the coil 180°, and then we used 90% of that RMT for the rTMS in the same direction. The baseline measurement consisted of 50 pulses at 0.25 Hz with the previously determined 1 mV intensity.

Step 2: The interventional cTMS stimulation and in-between measurements as shown in Fig. 2: We applied 900 rTMS pulses at 1 Hz and 90% RMT intensity in five 180-pulse blocks separated by ten 1 mV MEP pulses using Magstim for the in-between measurement. The pulse shapes used were unidirectional with the main component in the AP direction with pulse widths of 40 μs, 80 μs, and 120 μs and a fixed 0.2 M ratio, as well as with different pulse directions, i.e. 0.2, 0.6 and 1.0 M ratios and a fixed width of 80 μs.

Step 3: After the final 180 rTMS pulse block, we recorded 25 pulses targeting a 1 mV amplitude every 5 min for 30 min using Magstim.

Statistical analysis: We averaged the RMT values and MEP latencies for each subject for each pulse shape. RMT was analyzed using repeated measures ANOVA with the pulse shape of TMS as the independent variable. To analyze MEP latencies, we averaged the 25 trials, and then we visually evaluated and marked the values. We analyzed the results using repeated measures ANOVA with the pulse shapes. For the MEP changes, we performed multiple paired, two-tailed t-tests on the MEP amplitudes from the normalized 1 mV baseline for each condition with the baseline, then we used Benjamini and Hochberg false discovery rate analysis for correction of multiple comparisons p values into Q values. For correlation analysis, we correlated MEP latencies and amplitudes across all data points using linear regression. The level of significance was set at $p < 0.05$.

After data analysis, the pulse width threshold was determined in a separate session in eleven of the fifteen participants. This was done by defining the RMT at 60 μs.as 100% and then increasing or decreasing in 10% steps until the limits of the stimulator were reached; pulse width of 120 μs in one direction and 100% MSO in the other. We defined the pulse width threshold as the shortest pulse width at 100% MSO that produced MEPs with a 50-μV amplitude in five out of ten trials. We correlated the PwTh to the average MEP change for each subject using linear regression.

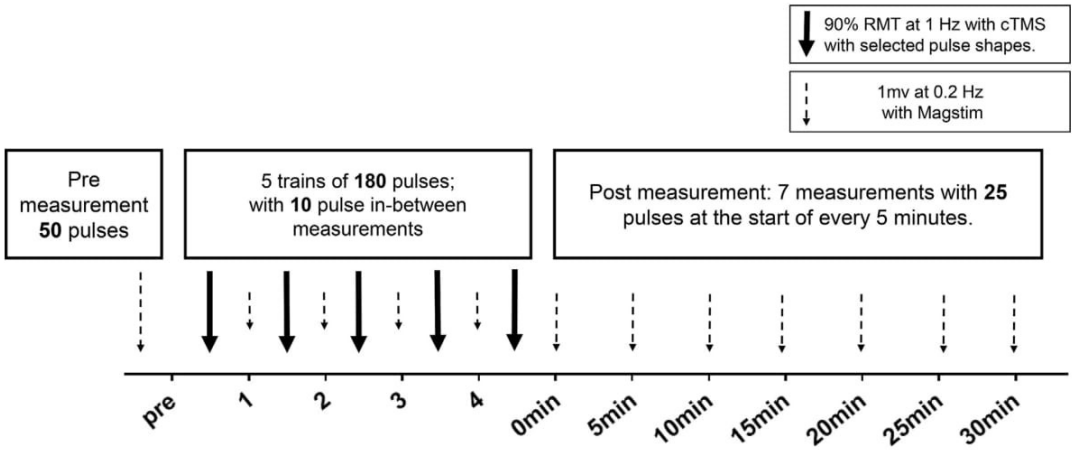

Fig. 2. Diagram of the experiment timeline for each session. Each subject had five such sessions, one with each pulse shape.

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Results

RMT: Longer pulse shapes had lower RMTs than shorter pulse shapes. Pulse width F value was 232.5 with $p < 0.0001$ (Fig. 3a). Pulses with a higher M ratio had a lower threshold than quasi-monophasic pulses. M ratio F value was 75.97 with $p < 0.0001$. (Fig. 3b).

Latencies: AP latencies were significantly longer for the narrower pulse shapes, particularly for the 40 μs pulse shape, which had the longest latency of all examined pulse shapes with a mean of 22 ± 1.2 ms. The 80 μs pulse shape had a mean latency of 21.3 ± 1.1 ms., and the 120 μs pulses expressed the lowest latency of the monophasic pulses with a mean latency of 20.5 ± 0.9 ms. Pulse width F was 27.23 with $p < 0.0001$. (Fig. 4a).

For directionality, pulses with an M ratio 0.2 had the longest latency with a mean of 21.3 ± 1.1 ms. M ratio of 0.6 had a mean latency of 19.8 ± 1.1 ms, and M ratio of 1 had the shortest latency of all tested pulse shapes with a mean of 19.4 ± 1.0 ms. M ratio F was 23.29 with $p < 0.0001$ (Fig. 4b).

Plastic aftereffects: We plotted the averaged normalized MEP data in the pre-stimulus, baseline phase, during stimulation [1,2,3and4] and after stimulation (0 min−30 min). We compared all subsequent data points to the baseline using a multiple comparison, paired *t*-test.

Shorter, monophasic pulses with a main component duration of 40 and 80 μs induced predominantly significant inhibition ($q < 0.001$, corrected paired two tailed *t*-test) at all of the time points during and after stimulation when compared to the baseline. The 120 μs pulse shape showed a different pattern that tended towards excitation. But this was only significant from the 15 to the 30 min time points ($q < 0.001$, corrected paired two tailed *t*-test) (Fig. 5).

As for the M ratio plastic aftereffects, pulses with an M ratio of 1.0 produced inhibition during stimulation ($q < 0.001$, corrected paired two tailed *t*-test). The effect shifted to excitation at the 25 min time point ($q < 0.001$, corrected paired two tailed *t*-test) (Fig. 6). An M ratio of 0.6 induced minor inhibition, but only during stimulation at the in-between measurements ($q < 0.02$, corrected paired two tailed *t*-test) with no further significant shift from the baseline. The 0.2 M ratio induced significant inhibition ($q < 0.001$, corrected paired two tailed *t*-test) at all the data points up (Fig. 6). Note that the 0.2 M ratio data is the same as in the pulse width comparison with the 80 μs pulse.

We correlated the average increase in MEPs across all time points to the latency differences using linear regression analysis and found no significant correlation for the different pulse widths (Fig. 7a, b).

Using the pulse width threshold data, we plotted a pulse width/strength (MSO %) curve by defining the RMT at 60 μs.as 100% and then shifting it in 10% steps in both directions. The limits of the stimulator were reached, with a pulse width of 120 μs of the main pulse component in the longer pulse direction and 100% MSO in the shorter one. In the latter, PwTh ranged from 17 to 26 μs (Fig. 8a). We then plotted its correlation with the average MEP percentage change for 40, 80 and 120 μs. The 40 μs showed significant negative correlation with an r value of −0.71 and a P value of 0.0145 demonstrating that subjects with higher PwTh values had more inhibition in response to shorter pulse stimulation. (Fig. 8b).

Discussion

Many approaches are currently in use to improve the efficacy of rTMS. In this study one of our aims was to elucidate the effects of prolonging the pulse duration of a unidirectional pulse from 40 μs to 120 μs, which is now technically possible with the recently developed cTMS device [13]. A secondary goal was to study the underlying mechanisms of current flow direction by changing the pulse shape from unidirectional to bidirectional while keeping the pulse duration constant. We used a repetition rate of 1 Hz, a protocol that robustly produces inhibitory aftereffects [28]. We applied rTMS in the anteroposterior direction as defined by the higher single pulse threshold as compared with PA direction. At the 1 Hz repetition frequency we reproduced the inhibitory effects of conventional TMS with short unidirectional AP pulses and the facilitatory effects of bidirectional AP pulses [23]. The novel key finding is that 1 Hz rTMS with 120 μs wide unidirectional pulses increased cortical excitability although significance was reached only 15 min after the end of the stimulation.

To our knowledge this is the first study to systematically and independently alter one pulse parameter (duration or directionality ratio) while keeping the other constant. Our results are in agreement with those in the available literature. Our 80 μs unidirectional pulse, which is identical to the "RU-N" pulse applied at 1 Hz by Goetz and colleagues [29], caused robust inhibition very similar to the effect they reported. Also the inhibitory aftereffects found in numerous studies using conventional TMS pulses at 1 Hz [28,30] compare very well with the effects we observed with 40 and 80 μs

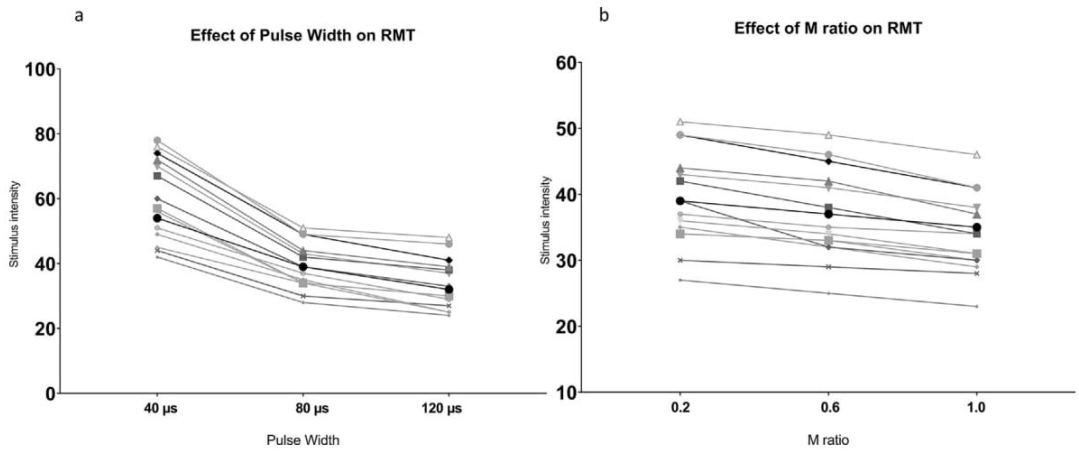

Fig. 3. Effect of pulse width (a) and directionality (b) on resting motor threshold.

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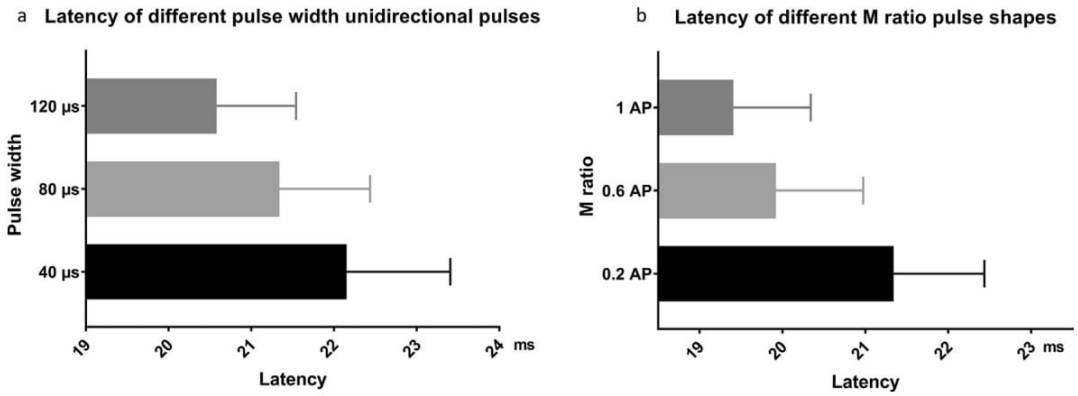

Fig. 4. Anteroposterior latencies for pulse shapes with different pulse widths (a) and M ratio (b).

unidirectional pulses. Thus, our finding of excitatory rather than inhibitory aftereffects with the 120 µs pulses was not anticipated and requires further investigation of the underlying physiological mechanisms of rTMS aftereffects.

In all subjects, RMT (Fig. 3) and MEP latency (Fig. 4) decreased with increasing pulse width and directionality, i.e. increasing M ratio. The completely bidirectional pulse shape had the shortest latency, which was similar to PA latencies. This supports the notion that not the first 'negative' phase of the bidirectional pulse but the second 'positive' PA-oriented phase of the pulse may have had determined latency and threshold [31].

The lower threshold of longer pulses as described by Ref. [25] is associated with a higher total pulse energy [32,33]. However, the higher pulse energy alone could not account for the shift from inhibitory to excitatory plastic aftereffects. Earlier studies that tested 1 Hz rTMS applied with constant pulse duration, reported that an increase in the pulse amplitude above the 90% RMT used here, resulted in even stronger inhibition [34–36].

The aftereffects seem to differ to some extent from intra-stimulation effects. All three directionality conditions induced inhibition during stimulation. This persisted after the unidirectional pulses but there was a shift to post stimulation facilitation with the fully bidirectional pulses. Most likely, the unexpected shift from intra-stimulation inhibition to post-stimulation facilitation with bidirectional pulses is caused by the second pulse phase. We propose that both the first and the second phase induce a paired pulse stimulation effect. This may either occur by the simultaneous induction of spike-time dependent plasticity in various nearby neurons at the network [37] or the neuronal level [38]. This could occur if one phase stimulated the axon hill or the axon at the bend in the white matter and the second one preferentially targeted dendrites [39]. Dendritic morphology and synapse locations has been found

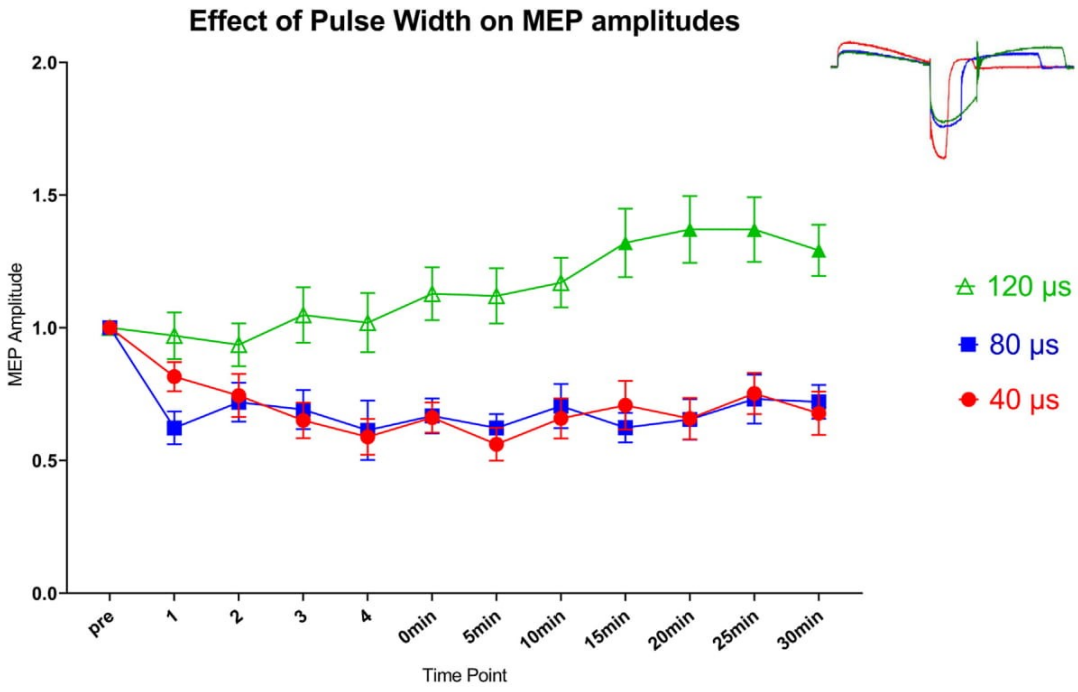

Fig. 5. Aftereffects of 1 Hz AP stimulation using monophasic pulses with 40, 80 and 120 µs main component durations, pulse shapes are illustrated in corresponding colors in the top right panel. Solid shapes indicate significance. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

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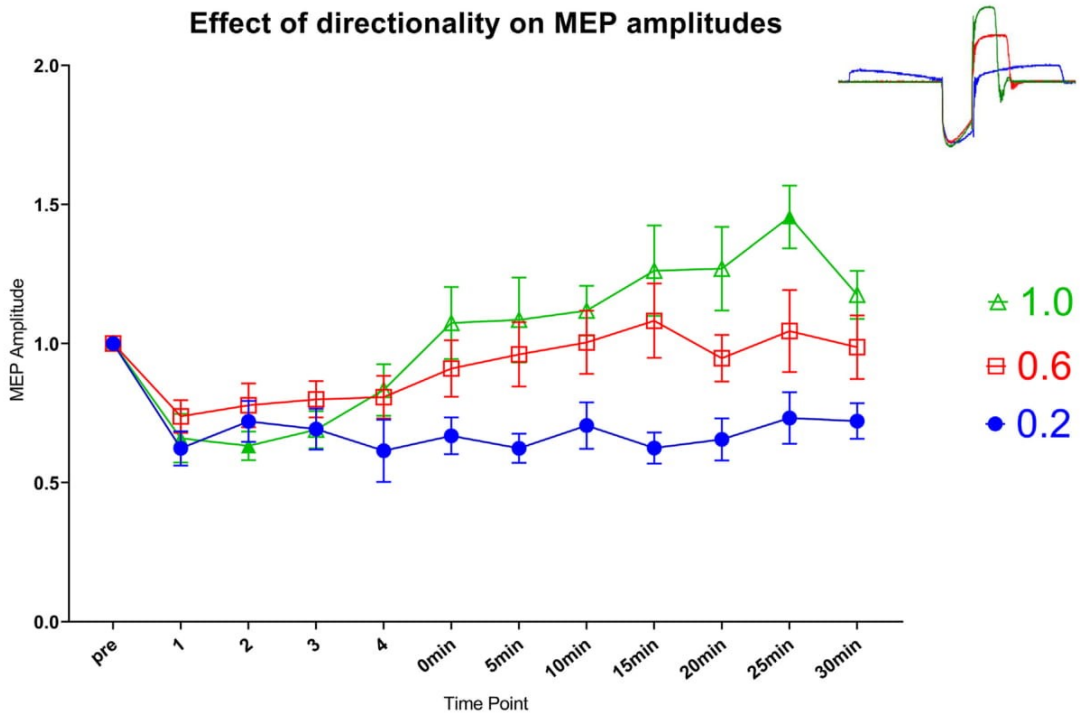

Fig. 6. Aftereffects of 1 Hz AP stimulation using 80 μs pulses with m ratios of 0.2, 0.6 and 1. Pulse shapes are illustrated in corresponding colors in the top right panel. Solid shapes indicate significance. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

to affect the spike-time dependent plasticity [40]. Another explanation would be that the inhibition induced by the monophasic 0.2 M ratio pulses could be attributed to repeated hyperpolarization of pyramidal cells, while the biphasic 0.6 and 1.0 M ratio pulses induced a similar initial hyperpolarization followed by a steeper polarization [14] thus producing some facilitation.

As described by Ref. [23] we were also unable to detect a correlation between the modulatory effect strength of 1 Hz rTMS and MEP latencies. This differs from the results of studies on cTBS [17,18] or anodal tDCS [20,26] which showed a significant correlation between AP MEP latencies and the neuromodulatory outcome of those protocols. We understand that this correlation is not intuitive, but the previous significant correlation with cTBS aftereffects attempt to identify a possible biomarker for plastic aftereffects (17,18) and the different latencies of different pulse widths [25], lead us to examine if this variability in latency in response to

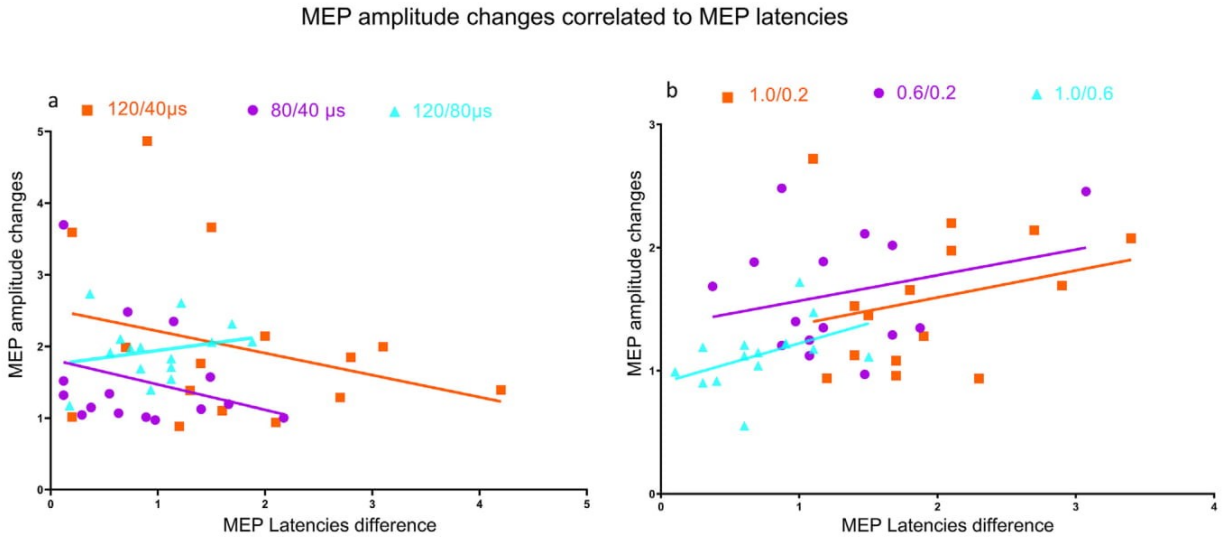

Fig. 7. Correlation between the average change in MEPs across all time points and the latency differences among: a) different pulse widths and b) different M ratios. There was no significant correlation.

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writing of the report; and in the decision to submit the article for publication.

Declaration of interest

None declared.

Acknowledgments

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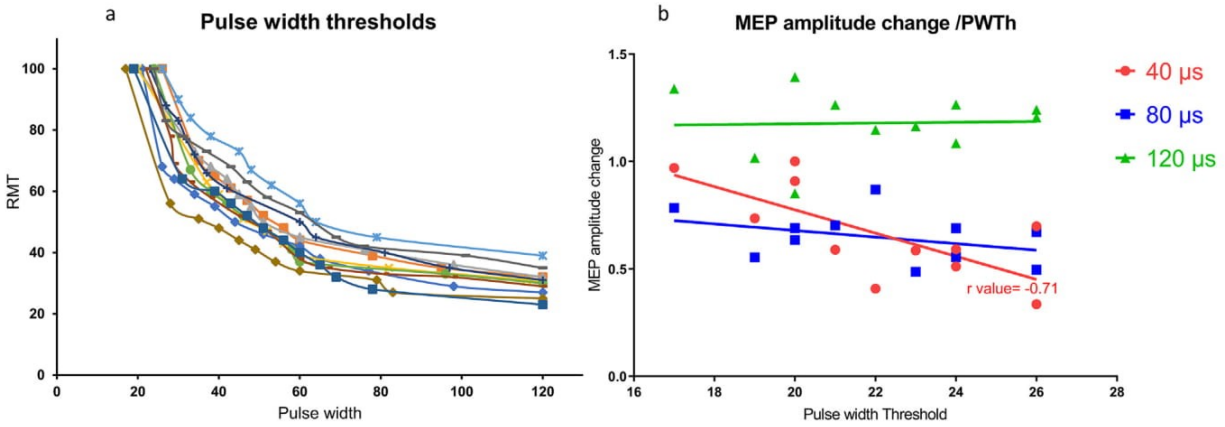

Fig. 8. a) Pulse width/strength RTM curves for eleven subjects. b) Correlation between the average change in MEPs across all time points and the PwTh, only the 40 µs condition showed significant negative correlation.

different pulse shapes would also act as a determinant for plastic aftereffects. A neurocomputational model by Rusu and colleagues showed that increase of dendrite length and concentration lead to a higher spiking rate [10], explaining how wider pulses have shorter latencies through summation of dendritic spikes.

It was demonstrated experimentally [41,42] and by modelling [43] that axons have orientation specific threshold as neuronal fibers respond more readily to perpendicular currents [44]. If we transfer this effect to the in vivo setting, this could attest that dendrites and smaller branching axons have a higher likelihood of responding to stimulation in any direction and thus also to AP stimulation with a smaller percentage of larger axons being stimulated as those have a higher threshold in this orientation [45]. So even though dendrites generally have a higher threshold and require more energy to be stimulated, the threshold of axons can exceed the dendritic threshold in certain orientations.

Lee and Fried used an implanted micro magnetic coil to selectively stimulate cultured layer 5 neurons to demonstrate that tuft dendrites were harder to stimulate, as in they required a longer stimulation duration of the same intensity 10 Hz stimulation [41]. However, when finally activated, the firing was higher in amplitude and outlasted the magnetic stimulation. While axonal stimulation only resulted in firing during the stimulation, indicating that dendrites have more plasticity than axons [41]. This emphasizes the significance of dendritic stimulation in producing lasting aftereffects even at the single neuron level. It is already known that higher frequency stimulation increases excitability, and if prolonged can lead to epileptic fits [12]. And conversely, patients with epilepsy have been found to carry genes for abnormal dendritic spines, branching and arborization [46].

Changes in thresholds, latencies and the inversion of aftereffects all suggest that wider and bidirectional TMS pulses are recruiting additional neuronal targets. In experiments and biophysical models of neurons and neural compartments a strength-duration analysis of excitability has proven useful to study recruitment of different neuronal targets [42,47]. In such analysis, rheobase is defined as the minimum intensity required to trigger a response to arbitrarily long stimuli. The chronaxie is defined as the minimal pulse width that can still trigger a response, if the stimulus intensity is set to twice rheobase.

In this study we tried to fully plot the strength duration curve for the human primary motor cortex (Fig. 8a). Since the width of the determinant phase of the cTMS pulse shapes has an upper limit of 120 µs, we were unable to obtain data on the rheobase of the motor cortex. The graph in (Fig. 8a) shows different slopes across the range of pulse widths from 20 to 120 µs, which might suggest that at least two different neuronal populations with different chronaxies are being stimulated. This is however uncertain as it impossible to isolate those effects on the strength duration curve as in neuronal culture results simply because the brain has a much more complicated neuronal architecture.

The properties of our two shorter unipolar pulse shapes with 40 and 80 µs resemble most closely the conventional monophasic pulse shape produced by the Magstim device with a main component of 82 µs [48] which failed to produce any firing in layer 5 dendrites in the mouse cortex [49] also being in line with the existing literature on conventional pulses [27,28].

The shorter pulse duration end of the strength duration curve signifying our PwTh (the pulse width below which it is not possible to excite the neuron) varied with our equipment between 17 µs and 26 µs (Fig. 8a). In a neuronal model, pulse width shorter than 100 µs was not able to stimulate dendrites any more, while the axon and soma were still responsive for pulses as short as 10 µs [47]. Accordingly, our 120 µs pulse shape should have the highest likelihood of stimulating dendrites.

We found a significant negative correlation between the PwTh (at 100% MSO) and the plastic aftereffects of the 40 µs conditions as subjects with higher PwTh had more inhibition (Fig. 8b). Since we assume that 40 µs only stimulate axons or the cell body this might reflect individual variation of percentages of larger axons and smaller branches and dendrites at the stimulated area.

Here we propose that AP stimulation more readily target dendrites, as dendrites do not have a particular orientation as axons. The selective membrane properties of the dendrites render anteroposterior stimulation more sensitive to temporal TMS parameters, producing those differences in latencies, thresholds and plastic aftereffects in response to different pulse widths and directionalities. This might be the underlying reason for increased excitability in response to wider pulse rTMS. If that is true, we could lead to more fine tuning of the aftereffects of different stimulation protocols theoretically through activation of different neuronal components.

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4. Role of pulse width and direction in high frequency rTMS:

For the third publication, we examined the effect of coil direction, stimulation intensity and pulse widths on high frequency 5 Hz rTMS, we demonstrated that AP is more effective than PA as expected (Rothkegel et al. 2010; Sommer et al. 2013). We also demonstrated that wider pulses with higher intensity are more efficient in producing the projected excitatory aftereffects. Where 90% RMT AP 5Hz rTMS was more effective than 80%. The 120μs pulse width stimulation produced more excitation than 80μs pulse width AP 5Hz rTMS at 90% RMT (Halawa et al. 2019a).

This supported my hypothesis of the 100μs cut off value for dendritic activation specially as I also found evidence from animal studies examining the effect of increasing pulse widths of high frequency electrical stimulation on rabbit cortices, where stimulation needed pulses longer than 100μs to significantly potentiate neurons (McNaughton et al. 1978). While the effect of increasing the pulse widths seems comparable to the effects on increasing stimulation intensity, it involves another mechanism as I demonstrated in my second publication, where wider pulses changed the expected inhibitory outcome of 1 Hz rTMS into excitation (Halawa et al. 2019b). While it is known that increasing the intensity of 1 Hz rTMS increases inhibition (Fitzgerald et al. 2002; Lang et al. 2006; Nojima and Iramina 2017).

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Increasing pulse widths and intensity increase the efficacy of high frequency rTMS in inducing excitatory aftereffects.

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Abstract:

Introduction: High frequency repetitive transcranial magnetic stimulation induces excitation (i.e. post-stimulation increase of motor evoked potential (MEP) amplitudes) when applied to the motor cortex. The effects differ according to pulse width, probably by selectively stimulating distinct segments of neurons due to their different membrane properties. Here we focus on the aftereffects generated with high frequency controllable pulse TMS (cTMS).

Objectives: To investigate the influence of different stimulation intensities, pulse widths and direction on the excitatory plastic aftereffects of high frequency (HF) rTMS reflected by MEP amplitudes.

Methods: Using a controllable pulse stimulator TMS (cTMS), we stimulated the hand motor cortex of 20 healthy subjects with 5 Hz rTMS applying 1200 bidirectional pulses with the main component widths of 80 and 120 microseconds. 80% resting motor threshold (RMT) intensity was investigated for both anterior-posterior (AP) and posterior-anterior (PA), and in a second experiment at 90% RMT in the AP direction.

Results: 80% HF rTMS did not produce any significant excitation in either AP or PA direction. 90% RMT AP stimulation with 120 microsecond-wide pulses induced more significant excitatory aftereffect, when compared to the 80 microsecond-wide pulses.

Conclusions: HF rTMS with wider pulses is more effective in producing excitatory aftereffects, which may be due to the different membrane properties of the various neuronal segments such as dendrites whose activation plays a major role in long term potentiation.

Significance: The findings here may contribute to better results in future clinical studies performed with longer cTMS pulses.

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Introduction:

Therapeutic use of repetitive transcranial magnetic stimulation (rTMS) has been shown to have level A efficacy in the treatment of depression and chronic pain (Lefaucheur et al., 2014). More efficacious protocols are needed and are the subject of two ongoing NIH research initiatives. The key rTMS parameters involved in its efficacy, stimulation frequency and intensity, and number of pulses and sessions have been closely investigated (Rossini et al., 2015). However, there are few studies on the impact of pulse width on rTMS outcome because of the scarcity of devices with adjustable pulse widths. However, we now have the technology that allows us to change pulse widths as easily as changing frequency and stimulation patterns (Peterchev et al., 2011).

The controllable pulse parameter TMS (cTMS) device can change the width of its near rectangular pulses using two capacitors, and two bipolar semiconductor transistors that alternate the current between the capacitors (Peterchev et al., 2014). Such customized pulses may be more efficient in delivering energy to the cortex by making use of the leaky properties of neuronal membranes (Goetz et al., 2013), and they can be applied in repetitive trains. Here, we used cTMS to test the effect of different pulse widths on the excitatory aftereffects of HF rTMS. With a rTMS frequency of 1 Hz we have already shown that only the widest pulse duration of 120μs leads to a significant increase in excitability, while the two shorter pulses shapes (40 and 80 Hz) produced significant inhibition (Halawa et al., 2019).

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In this study, we explored the interplay of coil orientation, pulse width, and pulse intensity on the aftereffects of a high frequency (5 Hz) rTMS train, a protocol well known to induce excitatory aftereffects (Hallett, 2007; Ziemann et al., 2008).

Figure 1: Diagram of the experiment timeline for each session of the experiment. Sessions were randomized and with at least one week gaps to avoid possible carry over effects.

***Methods*:**

Participants

For the first experiment, we recruited fourteen subjects, four males and ten females, mean age 23.5 ± 2.6 SD years. For the second experiment, a different set of fifteen healthy volunteers was recruited, four males and eleven females, mean age 24.1 ± 3.1 SD years. All participants were right-handed and free from any neurological or psychiatric disorders, took no centrally acting medications, and had no contraindications to TMS. With this device and using the standard coil for the wider pulse shapes intensities above 50% of maximal stimulator output (MSO) were not possible, a resting motor threshold (RMT) above 70% MSO for a Magstim 200^2 device was an exclusion criterion.

We obtained written informed consent from each subject before participation. The local ethics committee of the University Medical Center Göttingen approved the study protocol, which conformed to the Declaration of Helsinki.

Recordings

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Motor evoked potentials (MEPs) were recorded from the first dorsal interosseous (FDI) muscle of the right hand with surface Ag–AgCl electrodes in a belly-tendon montage. The electromyography signals were amplified, band-pass filtered (2 Hz–2 kHz), and digitized at a sampling rate of 5 kHz with a micro-1401 AD converter (Cambridge Electronic Design Ltd., Cambridge, UK). All signals were stored digitally for offline analysis. The peak-to-peak MEP amplitude served as an index for M1 excitability. The participants were requested to relax the right FDI during the measurements. Individual traces contaminated by voluntary muscle contraction before the TMS pulse(s) were excluded from the analysis.

A Magstim 200^2 (Magstim Co. Ltd., Whitland, UK) for measurement and a cTMS prototype 3 (cTMS3; Rogue Research Inc., Montreal, Canada) for intervention were used to deliver TMS over the M1 representation.

Repeated, randomized sessions were separated by at least one week to avoid carry-over effects. There were six sessions in the first experiment and two in the second (fig 1):

Step 1: Determining thresholds and baseline:

For each session, we use the Magstim 200^2 with the D70 coil to determine the RMT and the MSO intensity that gave a resonse of approximately 1 mV for the baseline measurement intensity in the PA direction. In addition, we determined RMT for the cTMS pulse shape being used for the intervention, both for the PA and the AP direction (coil rotated by 180 degrees), as a reference for the 5 Hz rTMS stimulation. The baseline measurements consisted of two 25-pulse measurements at 0.25 Hz with the previously determined MSO intensity giving a 1 mV response.

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Step 2: The interventional cTMS stimulation:

In order to isolate the effect of altering the pulse width without interference from the directionality of the individual pulses, we used custom, balanced bidirectional pulse shapes which are easier to fire at higher frequencies (Peterchev et al., 2011). We used three widths of the main component (80µs, 100µs, and 120µs) in the PA and the AP direction as shown in Fig 2. We used 80% of the appropriate RMT for the rTMS in the corresponding direction. For the second experiment, we used 90% RMT with 80 and 120µs pulse shapes for the 5 Hz rTMS only in the AP direction.

We triggered the cTMS device at 5Hz using an external trigger to fire the customized pulse shapes. For the first experiment we applied 1200 pulse of rTMS in both the AP and the PA direction at 5 Hz with 80% RMT intensity in six 200-pulse blocks with 15 MEP measurements in between as breaks necessary to produce significant excitatory aftereffects (Rothkegel et al., 2010). For the second experiment, we used the same protocol but with 90% RMT in the antero-posterior (AP) direction, which is more effective with 5Hz rTMS (Sommer et al., 2013).

Step 3: After the final rTMS pulse block, we recorded 25 pulses with the baseline MSO intensity for 1mA response every five minutes for 30 minutes using Magstim 200^2.

Figure 2: Pulse shapes detected by an external pickup coil and oscilloscope, used in the: a) 5 Hz PA Stimulation. b)5 Hz AP Stimulation

Statistical analysis: We averaged the RMT values for each subject for each pulse shape. RMT was analyzed using repeated measures ANOVA to test the significance of

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changing the pulse widths. For the MEP changes we used multiple-way ANOVA for the pulse width, stimulation direction and evolution across time for baseline and after measurement time points. Then for each time point, we performed post-hoc multiple paired, two-tailed t-tests on the MEP amplitudes from the normalized 1 mV baseline for each condition with the baseline, using the Bonferroni-Dunn method to correct for multiple comparisons.

***Results*:**

RMT: For the first experiment longer pulse shapes had significantly lower RMTs than shorter pulse shapes in the PA direction and the AP direction (Fig 3.a and 3.b, respectively; $p < 0.0001$ for both). The same was true for the second group of subjects with the 80 and 120µs pulse shapes used for the 90% RMT AP 5 Hz rTMS ($p<0.0001$) (Fig 3.c).

Figure 3: Effect of pulse width on the thresholds for individual pulse shapes: a) PA-directed for the three pulse widths used in the first experiment, b) AP-directed for the three pulse widths used in the first experiment, c) AP directed for the two pulse widths used in the second experiment.

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Plastic aftereffects: We plotted the averaged normalized MEP data in the baseline phase, during stimulation and after stimulation (0 min to 30 min). We used ANOVA to detect significant effects of changing the pulse width. We also compared all subsequent data points to the baseline using corrected multiple comparison, paired t-tests.

For the first experiment, ANOVA revealed that 80% RMT, PA-directed 5 Hz rTMS did not produce any significant effects outlasting the stimulation, and there was no significant effect of changing the pulse widths ($p>0.1$; Fig.4). For AP-directed stimulation, there was no significant change over time ($p =0.4769$, $F=0.9530$) and no significant shift from the baseline for any time point ($p >0.2$; Fig.5), but there was a significant effect of pulse width change with ($p<0.001$; $F=12.76$). Additionally, AP-directed stimulation produced significantly greater excitation than PA-directed stimulation ($p<0.05$; $F= 0.9826$). Post-hoc, multiple paired t-tests showed no significant difference across all time points. No significant difference was found in the baseline measurements.

Figure 4: Aftereffects of 80% RMT 5 Hz stimulation using 80,100 and 120 µs main component in the PA direction. Pulse shapes used for stimulation are illustrated in corresponding colors in the top right panel.

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Figure 5: Aftereffects of 80% RMT 5 Hz stimulation using 80,100 and 120 µs main component in the AP direction. Pulse shapes used for stimulation are illustrated in corresponding colors in the top right panel.

For the second experiment, two-way ANOVA revealed significant effects for pulse widths ($p=0.0278$; $F= 4.876$) and change over time ($p=0.0135$; $F=2.098$). Post-hoc t-tests showed that the 120 µs pulse shape produced significant excitation at six time points (corrected $p <0.009$, paired two-tailed t-test) compared to the 80µs pulses, which only exhibited significant excitation at one time point (corrected $p= 0.02$, paired two-tailed t-test; Fig.5). No significant difference was found in the baseline measurements.

Figure 6: Aftereffects of 90% RMT 5 Hz AP stimulation using 80 and 120 µs main component durations, pulse shapes are illustrated in corresponding colors in the top right panel. Solid shapes indicate significance.

***Discussion*:**

In this study we showed that prolonging the pulse duration of HF rTMS led to significant excitation for 90% RMT 5 Hz AP rTMS. Although there were no significant aftereffects after 80% RMT 5 Hz rTMS with pulse widths of 80, 100 and 120µs in either direction, we found that AP stimulation is more sensitive to pulse width change ($p <0.001$) and more effective in producing plastic aftereffects than PA stimulation ($p<0.05$). This has been demonstrated in many rTMS protocols especially for 5 Hz rTMS (Rothkegel et al., 2010; Sommer et al., 2013).

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Only the AP-directed stimulation was used with the 90% RMT employing the longest (120µs) and shortest (80µs) pulse shapes. Again, we reproduced the excitatory effects of bidirectional AP pulses when using an interval design (Rothkegel et al., 2010). However, the key finding is that the 120 µs pulses with 5 Hz rTMS increased cortical excitability more than the 80µs pulses.

In all subjects, RMT (Fig 3) decreased with increasing pulse width in agreement with previous reports (D'Ostilio et al., 2016). While the thresholds as percentage of the device's MSO decreased, this apparent decrease in the threshold is not actually correct. The read-out amplitude does not reflect the total energy of the pulse, which is a function of the area of the pulse shape and not just the stimulation intensity. Wider pulses require more energy to elicit responses in the cortex (Barker et al., 1991; Peterchev et al., 2013; Grill, 2015). The higher total pulse energy could explain the seemingly greater efficacy of wider pulses when only pulse amplitude is considered (Modugno et al., 2001). Using pulse width as an analogue for intensity, high frequency stimulation of rabbit cortex required pulses longer than 100µs to significantly potentiate neurons (McNaughton et al., 1978). However, pulse width and intensity work through different mechanism, since increasing the pulse width of 1 Hz rTMS above 100µs changed the inhibitory outcome into an excitatory one (Halawa et al., 2019), which is contrary to the conventional observation that increasing the stimulation intensity in 1 Hz rTMS increases inhibition (Fitzgerald et al., 2002; Lang et al., 2006; Nojima and Iramina, 2017).

Neuronal models examining membrane properties for different parts of the neuron showed that pulses shorter than 100µs are usually unable to stimulate smaller

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dendrites, even using exceedingly high amplitudes, while the axon and soma are still responsive for pulses as short as 10μs (Rattay et al., 2012). Because dendrites lack myelin (Aberra et al., 2018) and have a small diameter (Pashut et al., 2011), they respond preferentially to wider pulses. That could imply that pulse shapes wider than 100μs should have a higher likelihood of stimulating dendrites.

While dendrites are more difficult to activate, when they are eventually activated, they fire with higher amplitudes and for a longer period after stimulation ceases (Lee and Fried, 2017), which shows that dendrites have a greater plasticity than axons. A similar effect was shown in single neurons where current injection into dendrites furthest from the soma produced longer and larger action potentials compared to somatic current injection (Larkum et al., 2007), especially in response to higher frequency and longer pulse width stimulation (Ledergerber and Larkum, 2010).

Those special plastic properties could be mediated by their high resistance spines which allow them to passively amplify local synaptic depolarization up to 50-fold, where the higher spine neck resistance leads to increased cooperativity (Harnett et al., 2012). The number of those dendritic spines also increased in response to high frequency rTMS (Vlachos et al., 2012).

Dendritic stimulation also produces back propagating potentials that would potentiate the anterograde potentials arising from somatic stimulation, thus producing LTP through associativity (Larkum, 2004) or cooperativity and spike timing dependent plasticity (Lenz et al., 2015). This was supported by the fact that distal dendritic stimulation was more effective in producing cooperativity mediated LTP compared to proximal dendrite stimulation (Weber et al., 2016).

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The significance of dendritic stimulation in producing lasting plastic aftereffects through LTP in response to rTMS is emphasized (Sjöström et al., 2008; Müller-Dahlhaus and Vlachos, 2013). In this study we showed that increasing the pulse width increased the excitatory efficacy of HF rTMS. This is probably due to a stronger dendritic activation, resulting from their unique membrane properties and their important role in inducing synaptic plasticity.

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Declaration of interest:

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None declared.

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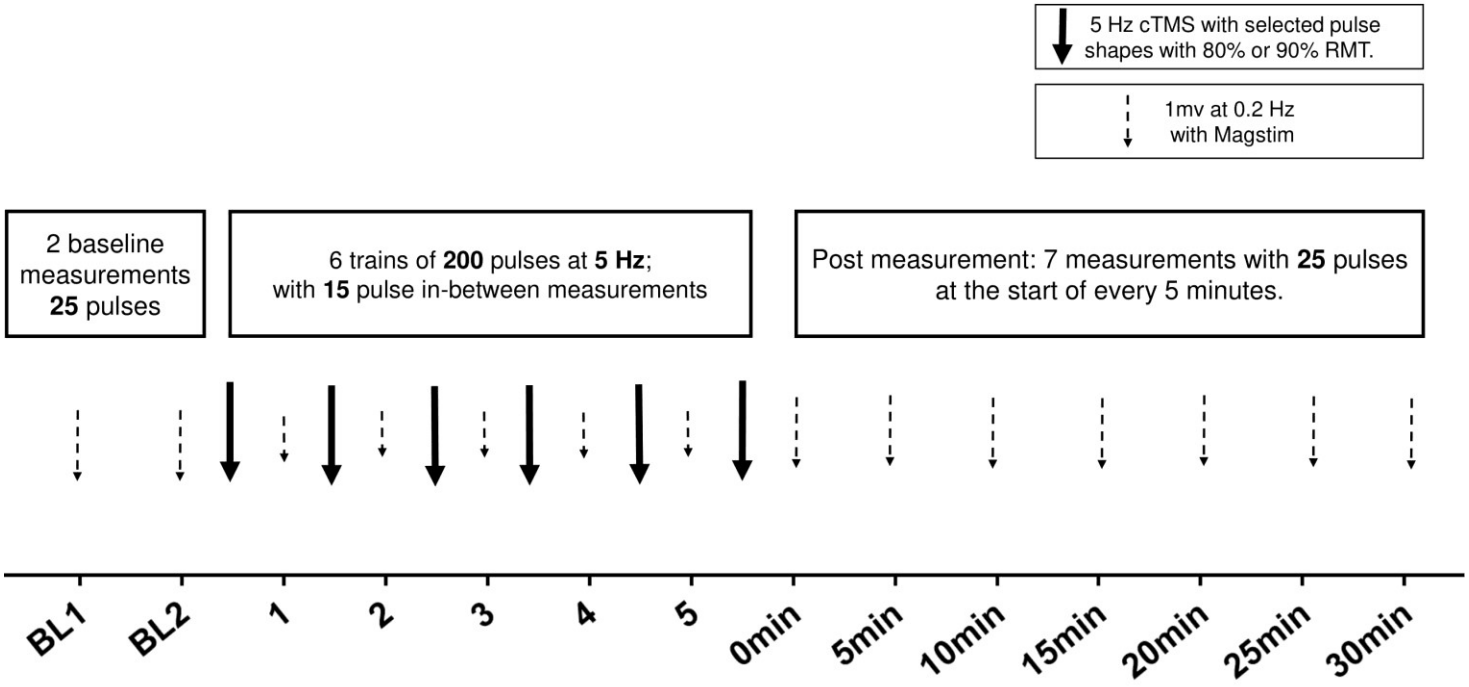
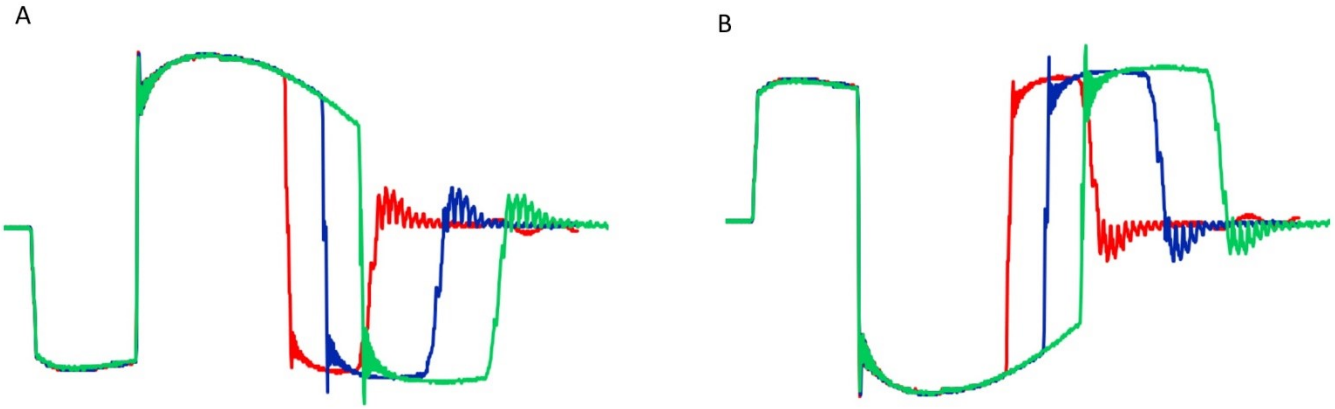
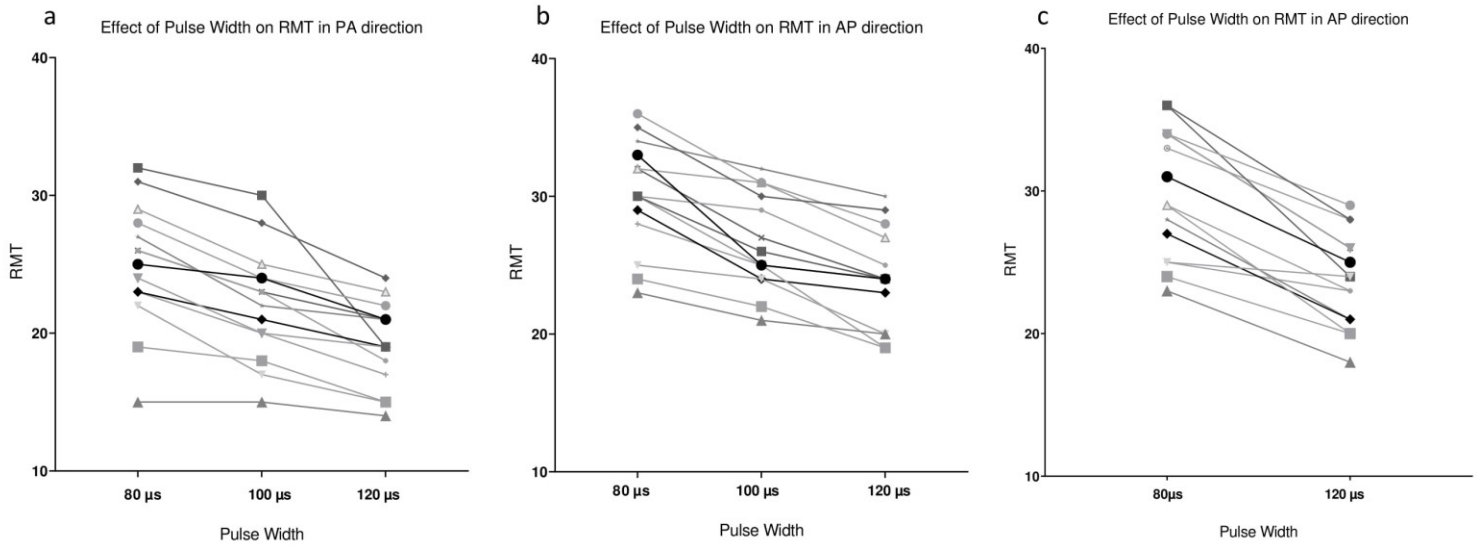

5 Hz cTMS with selected pulse shapes with 80% or 90% RMT.
1mv at 0.2 Hz with Magstim
2 baseline measurements 25 pulses
6 trains of 200 pulses at 5 Hz; with 15 pulse in-between measurements
Post measurement: 7 measurements with 25 pulses at the start of every 5 minutes.
BL1
BL2
1
2
3
4
5
0min
5min
10min
15min
20min
25min
30min


A
B


a
Effect of Pulse Width on RMT in PA direction
RMT
40
30
20
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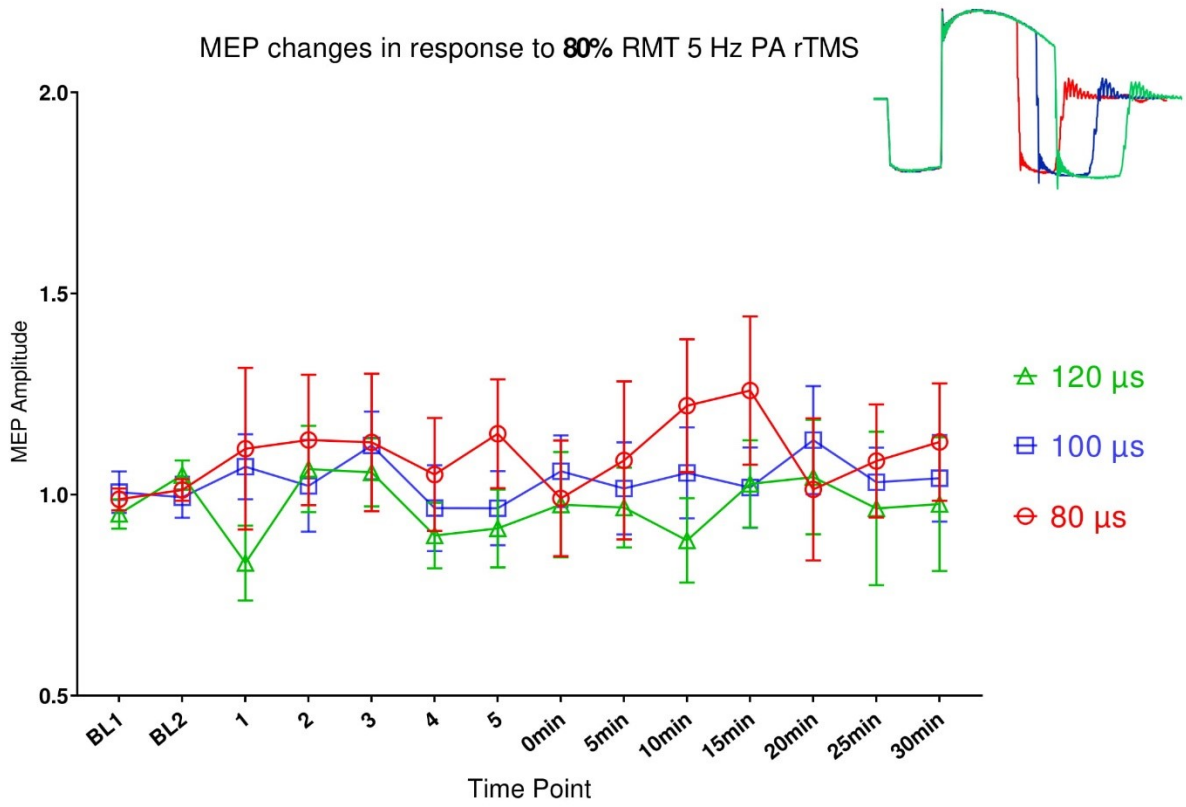
80 µs
100 µs
120 µs
Pulse Width
b
Effect of Pulse Width on RMT in AP direction
RMT
40
30
20
10
80 µs
100 µs
120 µs
Pulse Width
c
Effect of Pulse Width on RMT in AP direction
RMT
40
30
20
10
80µs
120 µs
Pulse Width


MEP changes in response to 80% RMT 5 Hz PA rTMS
MEP Amplitude
2.0
1.5
1.0
0.5
BL1
BL2
1
2
3
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5
0min
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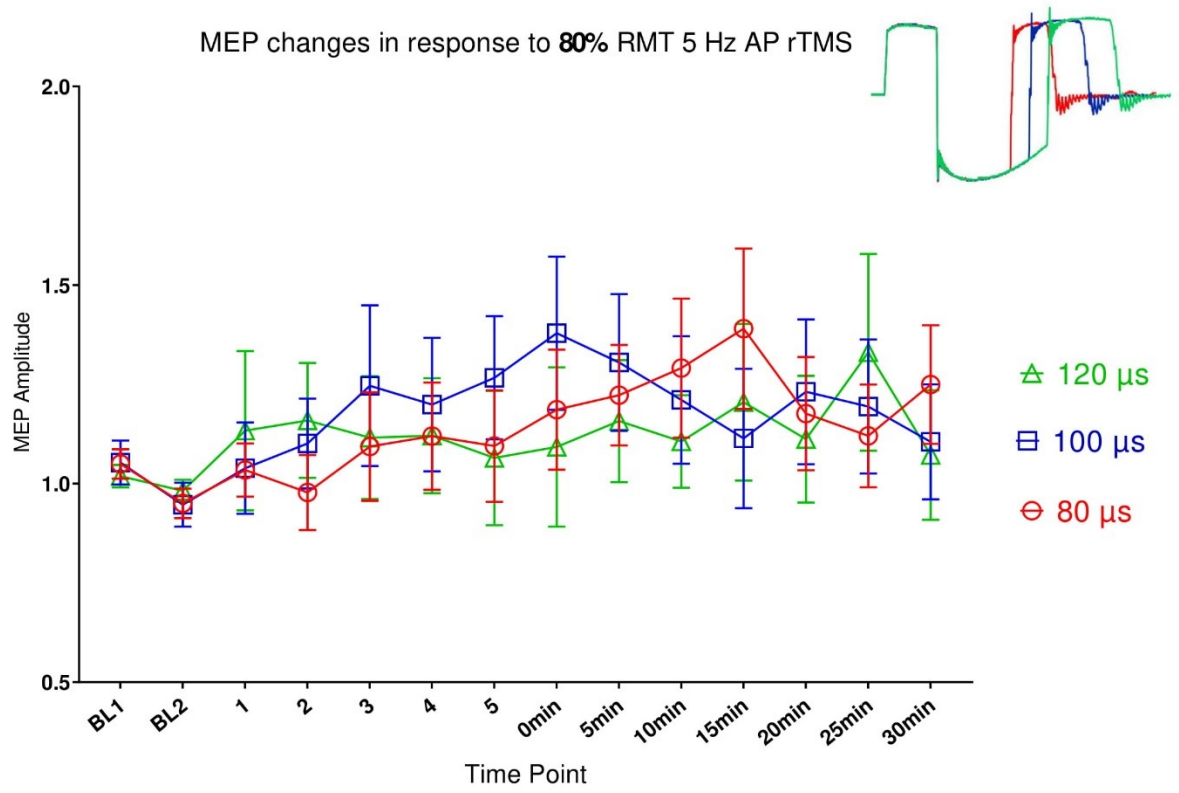
25min
30min
Time Point
120 µs
100 µs
80 µs


MEP changes in response to **80%** RMT 5 Hz AP rTMS
MEP Amplitude
2.0
1.5
1.0
0.5
BL1
BL2
1
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0min
5min
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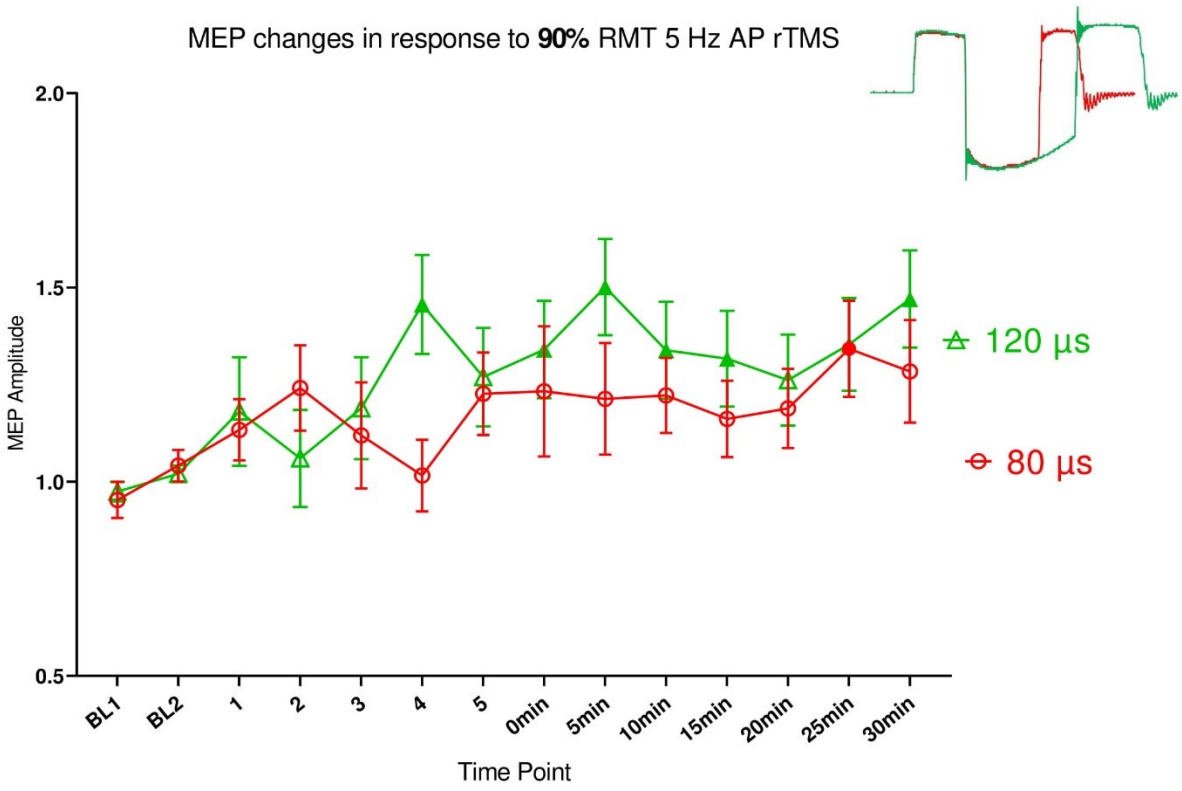
25min
30min
Time Point
120 µs
100 µs
80 µs


MEP changes in response to **90%** RMT 5 Hz AP rTMS
MEP Amplitude
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1.0
0.5
BL1
BL2
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2
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4
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0min
5min
10min
15min
20min
25min
30min
Time Point
120 µs
80 µs

5. Summary and concluding remarks:

Repetitive transcranial magnetic stimulation (rTMS) is now being increasingly used as a therapeutic modality against drug resistant disorders involving the central nervous system because it demonstrated promising outcomes in the treatment of some neurological disorders specially depression and chronic pain (Lefaucheur et al. 2014). Extensive efforts to understand mechanisms underlying the effects and variability of rTMS are necessary to fully understand its therapeutic potential and to use it efficiently in therapy of different disorders (Chervyakov et al. 2015).

In this project I wanted to understand the role of less commonly tested rTMS parameters, I focused on the temporal properties either for trains or pulse shapes as a step further from frequency which is the cardinal parameter deciding the direction of the outcome (Bliss and Cooke 2011). In the first publication, I examined the effect of ITIs on 10 Hz rTMS protocols rated with level of efficiency “A” by Lefaucheur and colleagues (Lefaucheur et al. 2014). We discovered that if neurons are stimulated above their critical frequency, they exhibit increased percentage of response failures, we hypothesized that could be the reason behind the reduced efficiency of rTMS protocols with shorter ITIs. Correlation between ITIs and efficacy outcome showed apparent correlation however not significant, probably because of the small number and dispersion of the available data points (Halawa et al. 2018).

For my second publication I focused on the effects of pulse width and directionality on the aftereffect of 1 Hz rTMS. For pulse directionality, I reproduced previous data showing monodirectional pulses 1 Hz rTMS in the AP direction

produced inhibition while bidirectional pulses produced excitation(Sommer et al. 2013). For the second part I examine the effect of changing the pulse widths of monodirectional AP directed 1 Hz rTMS. The novel finding was the reversal of the 120μs wide 1 Hz rTMS in comparison to the shorter pulse shapes 0f 40 and 80μs which exhibited the expected inhibition. I attributed that finding to the interplay between two factors relating to dendritic properties:

1- The orientation effect on threshold as axons have a certain orientation and subsequently an optimum direction of stimulation while smaller branches and dendrites do not. So, when we use a higher intensity in AP stimulation, we stimulate a larger percentage of narrower neuronal fibres than axons. This orientation threshold dependency was described in neuronal cultures (Stern et al. 2015; Lee and Fried 2017) and models (Aberra et al. 2018).

2- The special membrane properties of dendrites: as dendrites lack myelin (Aberra et al. 2018) and have a small diameter (Pashut et al. 2011), they respond preferentially to wider pulses. Meaning that dendrites are not at all stimulated by shorter pulses as demonstrated by the neuronal models examining the different membrane properties for different parts of the neuron showing that pulses shorter than 100μs were not able to stimulate dendrites, while the axon and soma were still responsive for pulses as short as 10μs (Rattay et al. 2012). My two shorter pulse shaped were close in properties to conventional monophasic pulse shapes produced by the Magstim device with a main component of 82 μs, which were unsuccessful to produce any firing in layer 5 dendrites in the mouse cortex (Murphy et al. 2016).

For my third publication, we wanted to verify this finding so we examined the effect of pulse widths on high frequency rTMS, so we examined the effect of changing the pulse widths of 5 Hz rTMS from 80μs and 120μs and accordingly, only the wider pulse stimulation produced significant excitation (Halawa et al. 2019a). I found out that this effect that was originally demonstrated as early as 1978 by Mcnaughton and colleagues on rabbit cortices where they demonstrated that only stimulation wider than 100μs produced response potentiation (McNaughton et al. 1978).

So, this implies that pulse shapes wider than 100μs are stimulating dendrites while shorter pulses could not do so. Dendrites are more difficult to activate, but when they eventually activated, they fire in higher amplitudes and for a longer period after cessation of stimulation (Lee and Fried 2017), highlighting the known role of dendritic activation in LTP (Frick et al. 2004). Those special plastic properties could be mediated through the dendritic high resistance spines which allow them to passively amplify local synaptic depolarization up to 50 folds, where higher spine neck resistance lead to increased cooperativity (Harnett et al. 2012).

Concluding, this research demonstrated that the less famous temporal parameters of rTMS protocols play a role in its outcome, whether within a stimulation train as inter train intervals or on the level of individual pulses expressed by pulse width and morphology. In my first paper, about clinical therapeutic protocols I proposed that we do not always need longer stimulation for better clinical outcome. I

emphasized the importance of the length of the inter train intervals and its role in varying study results.

Yet, I think the most meaningful finding in my project is the cut off value of 100μs above which pulses had a different outcome than pulses shorter than 100μs which was also proven in animal experiments (McNaughton et al. 1978). This lead me to believe that is mainly because of added stimulation of dendrites which were proven to be not responsive to pulses shorter than 100μs (Rattay et al. 2012). This opens up the opportunity to selectively target and test the dendritic role in plasticity and different brain functions by integrating this knowledge to explore more of the parameter space. For example, therapeutic protocols utilizing high frequency rTMS could benefit from wider pulses to increase their efficiency.

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7.Contributions:

For all the articles and manuscript contained in this thesis, I designed, recruited participants and collected data. I also analyzed the data, plotted all the results, created diagrams and wrote the first draft of manuscripts.

In the first article, Amir Goldental carried out the neuronal culture experiments, collected data and revised the manuscript. Yuichiro Shirota, Ido Kanter and Walter Paulus co-designed, supervised and reviewed all manuscript drafts.

In the second article, Yuichiro Shirota co-designed the study, contributed to the manuscript writing and reviewed all the drafts. Martin Sommer supervised the study and reviewed the manuscript. Walter Paulus supervised the study and co-wrote the manuscript.

In the third article, I co-designed the study, implemented the experimental configuration and setup, supervised Katharina Reichert and taught her how to collect the data and analyze it. I did the statistical analysis, prepared the figures and wrote the manuscript. Martin Sommer supervised the study and reviewed the manuscript. Walter Paulus supervised the study and co-wrote the manuscript.

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Education

PhD, Universität Göttingen, Brain stimulation 2015-2019

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Focus: novel rTMS protocols with different intervals, pulse shapes and widths.

Dissertation: Optimization of temporal parameters of repetitive transcranial magnetic stimulation to improve its efficacy.

MSc, Clinical Neurophysiology 2008-2014

Kasr Elainy Cairo University of Medicine, Egypt

Concentrations: Psychophysiology, Children with special needs

Dissertation: Quantitative EEG changes in the frontal areas during concentration in children with attention deficit hyperactive disorder.

MBBCh, Bachelor of internal Medicine and Surgery with Honors
2000-2008

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Employment

Researcher in Brain stimulation, Universität Medizin Göttingen
2015-2019

Clinical Neurophysiology

Responsibilities: Conduction of MEP experiments for probing of excitability in response to different neuromodulatory techniques. Data analysis and manuscript writing. Supervising and teaching masters and medical doctoral students

Assistant clinical researcher, National Research Centre, Egypt
2012-2015

Special Needs Children Department

Responsibilities: Recording and reviewing EEG record for the, Recording and reviewing Evoked potentials (VEP, BAEP and SSEP) and evoked related potentials (P300). NCS and EMG examination and reporting. Both for clinical and research purposes.

Research assistant, National Research Centre, Egypt 2011-2012

Special Needs Children Department

Responsibilities: History taking and clinical examination of special needs children, carrying out parents' questionnaires for ADHD and Autism spectrum disorders. Recording and reviewing EEG record.

Neurophysiology Resident. Kasr El-Ainy Teaching Hospital Cairo University 2008-2011

Responsibilities: EEG, EMG, Evoked potentials and Polysomnography training.

Publications

Neuronal tuning: optimizing rTMS aftereffects by selectively targeting neuronal populations via manipulation of pulse width and phase 2019.

Less Might Be More: Conduction Failure as a Factor Possibly Limiting the Efficacy of Higher Frequencies in rTMS Protocols 2018.

Frontal theta/beta ratio changes during TOVA in Egyptian ADHD children 2017. Continuous performance task in ADHD children 2016.

Quantitative electroencephalographic changes in attention deficit hyperactivity disorder children 2015.

Presentations

Controllable parameters TMS Manual: Practical aspects of pulse selection for cTMS experiments. Brainbox Initiative Conference London 2018.

Importance of intervals in high frequency rTMS protocols. Göttingen 2017.

Transcranial direct current stimulation and transcranial magnetic stimulation for rehabilitation of Stroke patients. The 15th annual conference of the Egyptian society of Neurology, Psychiatry and Neurosurgery, Alexandria 2015.

Brain computer interface as a rehabilitation tool for the disabled. The 14th annual conference of the Egyptian society of Neurology, Psychiatry and Neurosurgery, Alexandria 2014.

EEG recording during sleep in young children with aphasia, for early detection of electrical status epilepticus during sleep causing aphasia. Medical Conference at the National research center Cairo 2013.

Spinal muscle atrophy type I in a 4-month-old, a case report. Cairo University clinical Neurophysiology workshop, 2012.

The differentiation of learning-disabled adolescents and delinquent children using Quantitative EEG. Medical Conference at the National research center 2011.

Posters

Effect of pulse width and phase on MEP latency and its significance in inducing cortical plastic changes. International congress of Clinical Neurophysiology, Washington 2018.

Where less is more: Conduction failures may limit the upper frequency in rTMS induced plasticity and in cell culture data. ECCN Budapest 2017.

Funding and academic awards

DAAD German Egyptian research long-term PhD scholarship 2015-2019.
Young Investigator Award European Congress of Clinical Neurophysiology, Budapest 2017.
Honorary Degree, awarded by Cairo University at graduation 2006.
High achievement award by Dar Eltarbiah school for scoring straights A*s and As in the IGCSE sponsored by the British council in Egypt.

Professional memberships

Member of the Egyptian society of Neurology, Psychiatry and Neurosurgery.
Member of the Egyptian Clinical Neurophysiology society.
Member of the Neurowissenschaftlichen Gesellschaft (German neuroscience society).

Skills

Clinical Neurophysiology Modalities:
Recording, reviewing and reporting EEG.
EMG examination and Nerve conduction studies.
Scoring of Polysomnograms.
Recording and assessment of ABR, VEP, ERG, SSEP and MEPs.
Non-invasive Brain stimulation: Transcranial magnetic stimulation, recording motor evoked potentials and rTMS as a therapeutic tool through neuromodulatory protocols as Paired pulse paradigms, theta burst and Quadripulse. Extensive experience with the controllable parameters TMS machine. Transcranial direct and alternation currents stimulation.

Language skills: Arabic Native English C2 German C1.