Influence of inter-train interval on the plastic effects of rTMS

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ABSTRACT

Background: High frequency repetitive transcranial magnetic stimulation (rTMS) elicits plastic effects in excitatory and inhibitory circuits. Inter-train intervals (ITI) were initially incorporated into rTMS paradigms to avoid overheating and for safety considerations. Recent studies have shown that inclusion of ITI, as opposed to continuous stimulation, is essential for eliciting excitatory effects, but the optimal ITI remains unknown. Moreover, if ITI duration has no effect, it may be possible to substantially reduce treatment time for rTMS.

Hypothesis: ITI duration modulates the excitatory and disinhibitory effects of rTMS.

Methods: rTMS (20 Hz, 2 s trains, 1200 pulses, 100% RMT) was applied in 14 healthy individuals with ITI of 4s (duration: ~3 min), 8s (~5 min), 16s (~9 min) or 32s (16.5 min) in sessions separated by ≥5 days. Effects on cortical excitability and GABAa receptor mediated short interval intracortical inhibition (SICI) were measured for 75 min following rTMS.

Results: The time-course of increased cortical excitability following rTMS was independent of ITI duration. There was a striking influence of ITI on SICI, whereby disinhibition increased with shorter ITI duration. Changes in cortical excitability and SICI were independent of each other.

Conclusion: These findings provide the first evidence to suggest that ITI may be substantially shortened without loss of rTMS effects, and warrant further investigation where rTMS is applied therapeutically. Furthermore, shorter ITIs result in greater disinhibitory effects which may be desirable in some clinical disorders and accelerated treatment paradigms. The tuning of the plasticity of cortical excitatory and inhibitory circuits to rTMS parameters in human cortex are independent.

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1. Introduction

Over the past 20 years, various forms of repetitive transcranial magnetic stimulation (rTMS) have been developed aiming to induce plastic changes in human cortex. These largely mimic cellular plasticity protocols. For example, rTMS delivered at high frequencies induces long-term potentiation (LTP) -like increases in cortical excitability while low frequency rTMS elicits long-term depression (LTD) -like decreases in cortical excitability [11]. High frequency rTMS also elicits LTD-like disinhibitory effects in GABAergic circuits [3]. Recent studies have aimed to understand [4–6] and reduce inter-individual variability and increase the efficacy of non-invasive brain stimulation (NIBS) protocols [7]. This is especially pertinent for rTMS, which is an approved treatment for depression and is also used in other neurological disorders. Several parameters that influence efficacy and variability have been characterised. Higher rTMS frequencies, in particular 20 Hz, have been shown to induce more robust excitatory effects [8], and higher stimulus intensities are more likely to elicit LTP-like effects across a range of rTMS protocols [4,9,10]. One factor that remains largely overlooked is the influence of inter-train interval (ITI). In early studies, ITIs were introduced to avoid overheating of stimulation coils and as an important safety consideration [11]. More recent studies have shown that incorporation of an ITI (as opposed to continuous stimulation) is essential for eliciting excitatory effects [12,13], but the optimal ITI for excitatory and disinhibitory effects remains unknown. One modelling study indicated that short ITIs, such as that used in intermittent theta-burst stimulation (iTBS, 8 s),
may favour the build-up of excitatory effects with reduced
strengthening of "inhibitory" effects, relative to continuous stim-
ulation [14]. This 8 s ITI was consequently incorporated into
another recently developed plasticity protocol [7]. Cellular studies
have demonstrated that different processes favouring LTP and LTD
of excitatory synapses may occur simultaneously [15], and it may be
that certain parameters such as ITI could tip the balance. Alterna-
tively, it has been suggested that more robust effects are induced
when the ITI and total stimulation period are longer. Nakamura and
colleagues [16] have argued that the effects of repetitive stimulation
protocols may be more robust if delivered over a relatively long
stimulation time period (~30 min). This argument appears con-
tradicted by adaptations of plasticity protocols such as paired
associative stimulation (PAS) and I-wave TMS (iTMS) which were
found to be equally effective when delivered over shorter time
periods [7,17]. Nonetheless, no study has systematically investi-
gated the influence of ITI on rTMS effects, and it remains unknown
whether the tuning curves and sensitivity of inhibitory and excit-
atory effects to ITI might differ. There is a paucity of basic science
literature on this point. If ITI duration has no effect, then this would
provide the first indication that it may be possible to substantially
reduce treatment length of clinical rTMS protocols, and conse-
quently reduce the personal and societal economic cost of clinical
rTMS treatment.

In the present study we investigated the influence of ITI (4, 8, 16
and 32 s) on the modulation of cortical excitability and GABA-A
mediated cortical inhibition by rTMS, and furthermore investigated
whether these plastic changes are related or entirely distinct.

2. Methods

2.1. Participants

14 healthy volunteers participated (5 women, mean age 23 ± 3.3
years, range 18–29 years). All participants provided written
informed consent in accordance with the Declaration of Helsinki.
The protocol was approved by the University Health Network
(Toronto) Research Ethics Board. Handedness was confirmed using
the Edinburgh Handedness Inventory [18]. Participants had normal
neurological examination by a trained clinician. Exclusion criteria
included self-reported history of neurological or psychiatric disor-
ders, metallic implants, cardiac pacemakers, pregnancy or post-
partum state, family or personal history of seizures, any medica-
tion affecting the nervous system and history of substance
dependence in the last six months.

2.2. Surface electromyography (EMG) recording

Surface EMG was recorded from the first dorsal interosseous
(FDI) muscle of the dominant hand with disposable surface
Ag–AgCl electrodes in a tendon-belly arrangement. The signal was
amplified 1000× (Intronix Technologies Corp., Model 2024F, Bol-
ton, Ontario, Canada), filtered (bandpass 20 Hz - 2.5 kHz), digitized
at 5 kHz (Micro 1401, Cambridge Electronic Design, Cambridge, UK)
and stored in a laboratory computer for off-line analysis.

2.3. Transcranial magnetic stimulation (TMS)

TMS was performed with a figure-of-eight shaped coil (central
diameter of each loop was 7 cm) and four Magstim 200² stimula-
tors (The Magstim Company, Whitland, UK) connected via a custom
connector box (Magstim, Whitland, Dyfed, UK), generating mono-
phasic current.

TMS was delivered with the handle of the coil pointing back-
ward at 45° from the midsagittal line, approximately perpendicular
to the central sulcus. The optimal position for activating the first
dorsal interosseous (FDI) muscle of the dominant hand was iden-
tified and marked with a pen. The hot spot coordinates were retained
and rechecked in subsequent sessions. Resting motor threshold
(RMT) was determined by the relative frequency method [19] and
was defined as the lowest intensity eliciting MEPs >50 μV peak-to-
peak amplitude in at least 5 of 10 trials. Test stimulus (TS) intensity
of 1 mV was defined as the lowest intensity that generated an
average MEP of 1 mV in the FDI muscle.

2.4. Motor cortical excitability

At baseline, 3 blocks of 10 TMS pulses (30 pulses, intensity set to
give MEPs of ~1 mV, 5s intervals) were delivered to establish
baseline motor cortical excitability. After intervention, blocks of 10
TMS pulses were delivered at 1, 10, 20, 30, 45, 60 and 75 min at the
same intensity as at baseline. One block per time-point was
recorded following rTMS.

2.5. Short interval intracortical inhibition (SICI)

SICI was recorded with two blocks at baseline and the mean of
these was used. Following rTMS, SICI was recorded at two time-
points comprising 14–16 min (T1) and 40–42 min (T2). A single
block comprised 10 TS and 10 conditioned-test stimuli (CS:TS), with
both intermixed in randomised order. TS intensity was set to induce
1 mV MEP and adjusted following rTMS if necessary. SICI was eli-
cited with a conditioning stimulus (CS) delivered at approximately
65% RMT, adjusted to produce ~50% MEP inhibition with TS deliv-
ered 2.1 ms later. This reliably evokes SICI without contamination by
excitatory circuitry [20–25]. We aimed for ~50% inhibition of TS
MEP to avoid floor or ceiling effects.

2.6. Repetitive TMS (rTMS)

This phase of the study required the use of a Magstim Super
Rapid stimulator (The Magstim Company, Whitland, UK). A 70 mm
air cooled figure-of-eight coil (biphasic pulse, Posterior-Anterior
followed by Anterior-Posterior induced current direction in the
brain) was held over the optimal scalp position for activating the
right FDI, and orientated 45° from the midsagittal line with the coil
pointing backwards. RMT was determined according to the criteria
described above.

A frequency of 20 Hz was chosen as this was shown to be more
effective at eliciting plastic changes with less inter-individual
variability than lower frequencies [8]. To determine 20 Hz rTMS
stimulation intensity and burst duration, we followed the rTMS
safety guidelines [11,26–28]. 20 Hz rTMS was therefore applied at
100% RMT, with trains of 2 s duration (40 pulses per train). 1200
pulses were delivered in 30 trains. Note that due to safety limita-
tions, 20 Hz rTMS in motor cortex should not be applied with trains
longer than 2 s, or with higher intensity (unless train duration is
shortened) (readers are encouraged to refer to 27, 28). To monitor
potential spread of muscle activity, a computer screen displayed
EMG activity from four muscles during rTMS (first dorsal inter-
seous, abductor pollicis brevis, extensor carpi radialis longus, flexor
carpi radialis).

Participants attended 4 sessions (Fig. 1), separated by 5 days
apart, in which rTMS was administered with one of the following
ITIs, the order of which was pseudorandomised and counter-
balanced: 4s (duration: ~3 min); 8s (duration: ~5 min); 16s (dura-
tion: ~9 min); 32s (duration: 16.5 min). These ITIs were also within
the safety guidelines [11,27]. Participants were instructed to focus
their attention on the index finger [29] and to avoid contraction of
hand muscles during or following rTMS, as this may interact with
plastic effects [30,31]. Female participants were asked to avoid bookings study sessions in the first days of the menstrual cycle to minimize potential hormonal confounding effects on neuroplasticity between sessions [32].

2.7. Data analysis

MEP amplitudes were measured peak-to-peak. Data analysis was performed blinded to experimental condition on values normalised to baseline. Averages are expressed as mean ± SEM. Statistical analysis was performed using repeated measure analysis of variance (ANOVA) and two-tailed paired t-tests (statistical significance defined as P < 0.05) with SPSS (version 19.0). Data analysis was performed on raw MEP amplitudes to incorporate comparisons to baseline (T0) values. SICI was calculated from the MEP amplitude ratio [CS.TS ÷ (CS + TS)]. As CS was subthreshold the equation simplifies to CS.TS ÷ TS. For Fig. 3, SICI T1 and T2 were normalised to baseline (T0) which was defined as 100%, then all values were divided by 2 to give values relative to 50%. This procedure facilitated visualisation of results and was only employed after ensuring that there were no statistically significant differences in baseline SICI across ITIs. Responders and non-responders were classified according to the grand average of rTMS response over 75 min, below and above 100% of baseline [33–35]. Our dataset with 14 participants and 4 conditions is underpowered for cluster analyses used elsewhere [36]. To assess the correlation between changes in single pulse MEP amplitude and changes in SICI at T1, single pulse MEP amplitude values were averaged for surrounding intervals (i.e. 10 and 20 min). Pearson’s correlation coefficient was determined using linear regression analysis.

3. Results

One way ANOVA confirmed no difference in baseline (T0) MEP values (0.92 ± 0.09, 1.02 ± 0.09, 1.13 ± 0.18, 0.98 ± 0.09 mV) or SICI (49 ± 6, 56 ± 6, 52 ± 6, 49 ± 4%) between 4, 8, 16 and 32 s ITI conditions respectively. RMT was 46 ± 2, 45 ± 1.9, 46 ± 1.7 and 46 ± 1.6% for 4, 8, 16 and 32 s ITI conditions respectively, and was not altered by rTMS in agreement with previous studies.

3.1. rTMS cortical excitability changes were independent of ITI

A two-way repeated measures ANOVA with within-subject-factors “ITI” (4, 8, 16, 32 sec) and “Time” (0, 1, 10, 20, 30, 45, 60 and 75 min) on MEP amplitude indicated a significant effect of Time (F(7,91) = 3.1, P = 0.006) but not of ITI (F(3, 39) = 0.517, P = 0.67) or the Time*ITI interaction (F(21, 7) = 0.85, P = 0.65). Fig. 2 displays the change in MEP amplitude over time, individual changes in average MEP amplitude by ITI and proportion of responders. The number of responders for 4, 8, 16 and 32 sec ITIs were 79, 71, 64 and 71% respectively (Fig. 2c).

3.2. Influence of rTMS ITI on inhibitory circuitry

A two-way repeated measures ANOVA with within-subject-factors “ITI” (4, 8, 16, 32 sec) and “Time” (T0, T1, T2) on SICI indicated a significant effect of ITI (F(3,36) = 5.3, P = 0.004), Time (F(2,24) = 3.62, P = 0.044) and their interaction (F(6,72) = 2.57, P = 0.027). This relationship is displayed in Fig. 3. One way repeated measures ANOVA showed a significant effect of time on SICI for rTMS at ITI 4 s (F(2,24) = 4.1, P = 0.03), and ITI 8 s (F(2,24) = 4.32, P = 0.03), but not other ITIs. Disinhibition of SICI was significant at T1 (t = 4.6, P = 0.001) and ITI 32 s (P = 0.01) with a return to baseline by T2 (Fig 3). Note that one outlier (noneetheless showing disinhibition following rTMS) was rejected due to a value that exceeded 4 SD from the mean. The number of participants showing disinhibition for 4, 8, 16 and 32 sec ITIs were 71, 64, 43 and 43% respectively (Fig. 3B).

3.3. No relationship between plasticity of motor cortical excitability and SICI

A linear regression analysis showed no relationship between changes in cortical excitability and inhibition at T1 (r² = 0.026, P = 0.25) or T2 (r² = 0.032, P = 0.19). We performed additional analysis, based on the notion that SICI at T1 might influence MEP amplitude at T2 [37], but did not find such a relationship in our dataset (r² = 0.007, P = 0.56). These analyses were also performed for ITI 4 s alone for which the greatest changes in MEP amplitude and SICI were observed, but no significant relationships were found: T1 (r² = 0.029, P = 0.56); T2: (r² = 0.000, P = 0.92); T1 → T2: (r² = 0.008, P = 0.75).

4. Discussion

Our results present several interesting findings that have relevance to the experimental and clinical application of rTMS.

4.1. rTMS cortical excitability changes were independent of ITI

While rTMS increased cortical excitability at all ITIs, there was no influence of ITI on the magnitude of this increase despite stimulation duration that varied from ~3 to 16 min and ITI from 4 to 32 s. Therefore, at least within the ITI range of 4–32s, the increase in cortical excitability from rTMS is not influenced by ITI and total duration of stimulation. Interestingly, previous studies showed that continuous stimulation without incorporation of an ITI, using either rTMS or TBS, led to LTD-like effects [12,13]. In the case of rTMS and TBS, short bursts of stimuli (1.5 or 2s, respectively) led to LTP-like effects [12,35]. These previous findings were partially confounded by different ITI durations and it remained unclear to what extent these results could be attributed to train length or ITI duration. In the context of the present data, the
distinct influence of train and inter-train duration are now more clearly defined: there is no influence of ITI duration on excitatory effects, and the LTD-like effects previously observed [12,38] were likely due to longer train duration, rather than specific choice of ITI. Interestingly, cellular studies suggest that depletion of glutamate during continuous trains could reduce NMDAR activation [39], and the resultant sustained reduction in calcium levels could result in LTD-like effects, or at least reduce the propensity for LTP [40].

The present data suggest that when rTMS is used therapeutically, with the aim of increasing cortical excitability, it may be possible to vastly shorten treatment duration without reducing rTMS efficacy. Commonly used protocols require 20–40 min per session even for unilateral stimulation [41,42]. Shorter protocols of 3–5 min, if equivalent in efficacy, would enable a several-fold improvement in both treatment capacity and per-session cost.

4.2. Influence of rTMS ITI on inhibitory circuitry

Our second interesting finding is the influence of ITI on cortical inhibition. One of the most robust effects of rTMS is the reduction in GABA_A receptor mediated cortical inhibition, SICI [43]. In the review by Fitzgerald et al. (2006) [43], seven of eight studies using high frequency rTMS and investigating SICI found disinhibition following rTMS. Interestingly, the data indicate a significant relationship between rTMS ITI and the level of disinhibition, with greater disinhibition at shorter ITIs (Fig. 3). It is conceivable that disinhibition would increase and consequently also be observed at longer intervals if a higher number of rTMS pulses (i.e., >1200) were used [43], however the present data indicate that disinhibition of GABA_A circuitry is most robustly elicited at shorter ITIs. The disinhibition of SICI circuitry observed here is in agreement with recent evidence from murine entorhinohippocampal slice cultures that high frequency rTMS reduces the strength of functional GABA_A mediated postsynaptic inhibition via mechanisms congruent with short and long term depression of GABA_A receptor mediated synapses [3]. Previous studies also suggested that disinhibition is greater with increasing rTMS frequency [44]. The present findings expand upon this data by demonstrating the influence of ITI when rTMS is applied in human in vivo. This finding may be relevant in the selection of parameters where rTMS is used for major depressive disorder (MDD) which is associated with modulated expression of GABA_A (but not GABA_B, [45,46]) receptor-subunit genes in the dorsolateral prefrontal cortex (DLPFC; [47]) and other cortical regions [48]. The exact mechanism(s) by which rTMS exerts its effects in MDD remains unclear, but it may be beneficial to optimise parameters for the reduction of cortical inhibition in DLPFC, and further explore these findings in DLPFC using combined TMS and electroencephalography [49,50].

As a separate consideration, disinhibition increases the efficacy of plasticity induction both in animal [51,52] and in human studies [7,53,54], when applied concurrently. Furthermore, the level of inhibition during plasticity induction influences inter-individual variation in plastic effects [4,55] and modulates the magnitude and direction of plastic changes in cell slice [15,56] and human studies [4]. The duration of inhibitory effects observed here (>15 min, < 40 min) suggests that these effects are mediated by a plasticity phenomenon such as short term plasticity. As plastic effects take some time to develop, they would not be expected to influence the effects of a single session of rTMS. However, recent treatment approaches have incorporated repeated successive rTMS sessions for accelerated treatment [57,58]. Cell slice studies have shown that single applications of high frequency stimulation are sufficient to induce early-phase LTP, but repeated trains applied in a spaced manner (10–60 min apart) may be required to elicit
consolidated late-phase plasticity [59–61], lasting up to several months [62]. If the effect of rTMS ITI on cortical inhibition is utilised in protocol design, such that the second session is delivered during disinhibitory time window, such approaches could be especially effective. The present data indicate that disinhibitory effects, following a single session of rTMS at short ITIs, were present by 15 min and resolved by 40 min, providing a time-window of disinhibition that appears well suited for exploring further facilitation of subsequent rTMS sessions in accelerated treatment protocols.

4.3. Relationship between the plasticity of cortical excitatory and inhibitory circuits

Our data provide new evidence that the plasticity of cortical excitatory and inhibitory circuits as probed by rTMS in human cortex are independent. Cortical excitability was increased in response to all rTMS conditions whereas cortical inhibition was decreased only at short ITIs. Moreover, there was no relationship between changes in cortical excitability and inhibition across individuals. The plasticity of excitatory and inhibitory circuits also differed in terms of their time-course of effects with disinhibitory effects subsiding earlier than changes in cortical excitability. The specificity of disinhibition to the shortest ITIs here adds support to previous suggestions that changes in cortical excitability following rTMS are not secondary to changes in GABAergic inhibition [20]. Furthermore, recent cellular studies showed that rTMS elicited LTP of excitatory synapses [63] and LTD of inhibitory circuits [3]. The present data indicate that the plasticity of excitatory and inhibitory circuits have different sensitivity or tuning curves to rTMS ITI duration and that ITI may be chosen to facilitate the intended effects.

4.4. Relation to previous studies and technical considerations

Some previous studies found no influence of rTMS on SICI [44,64]. Due to parameter choice these may have been prone to floor and ceiling effects, regression to the mean (for discussion see 44) or selection of intensities or ISIs that expose SICI to contamination by excitatory changes [22]. We minimized these risks by taking two SICI baselines ten minutes apart for T0 to give a more stable baseline, adjusted SICI to ~50% inhibition to avoid floor or ceiling effects, and chose parameters that avoid contamination by excitatory circuitry. Several studies that found no change in cortical excitability following rTMS included the measure of cortical silent period (CSP) [44,64]. Muscle contraction prior to, during or following plasticity induction paradigms, as required for CSP measures, can abolish the effects of plasticity paradigms on cortical excitability ([30,65–67], although see Ref. [68]) and SICI [67] in motor cortex.

4.5. Limitations

The present study demonstrates that rTMS ITI could be shortened to 4 s when applied to motor cortex with no loss of effects on cortical excitability and inhibition. It remains an important question whether this finding is specific to motor cortex, or whether it applies more broadly to other brain regions that are widely used as...
targets for therapeutic rTMS, such as the DLPFC [42] or the dorso-medial prefrontal cortex (DMPFC) [69]. Further work will be required to assess whether shorter ITIs can be used while maintaining or improving therapeutic effects. Another limitation is that systematic investigations of safe parameters have not been conducted for non-motor regions, and it should be noted that with the current paradigm (20 Hz, 2 s burst, 100% RMT) the ITI of 4 s was at the lowest end of safety margins in motor cortex [11,28].

In the present study, the 20 Hz frequency was selected based on previous work indicating that 20 Hz stimulation had a more consistent direction of effect and less inter-individual heterogeneity, compared to 10 Hz or lower frequencies. This greater consistency for 20 Hz stimulation has been demonstrated for cortical excitability measures [8,44,70] and for functional MRI resting-state connectivity [71]. While the most widely used protocols for rTMS in clinical disorders apply stimulation at 10 Hz [42], other frequencies such as 18 Hz or theta-burst stimulation are increasingly entering routine use [41]. There is no known physiological basis that would limit the present ITI results to 20 Hz. However, future studies will be required to ensure that the present results extend to other stimulation frequencies or paradigms such as theta burst stimulation [12] or disinhibition stimulation [7], although the duration of such protocols is already much shorter than conventional rTMS.

4.6. Conclusion

The present data suggest that the ITI used in rTMS may be substantially shortened without loss of excitatory effects, potentially giving vastly reduced treatment times and therefore lower cost. Furthermore, shorter ITIs resulted in greater disinhibitory effects, which may be desirable in some clinical disorders and may further facilitate summed effects of rTMS in accelerated treatments. Lastly, the tuning of the plasticity of cortical excitatory and inhibitory circuits to rTMS parameters in human cortex are entirely independent.

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