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Ongoing cumulative effects of single TMS pulses on corticospinal excitability: An intra- and inter-block investigation

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HIGHLIGHTS

- MEPs amplitude evoked by TMS is an indirect measure of corticospinal excitability.
- Single TMS pulses, delivered at random or fixed inter-trial interval (ITI), induce cumulative changes in neural activity.
- Temporal summation of neuronal depolarisation induced by several single TMS pulses increases MEPs amplitude.

ABSTRACT

Objective: To evaluate the effects of several single TMS pulses, delivered at two different inter-trial intervals (ITIs), on corticospinal excitability.
Methods: Twelve healthy volunteers participated in two experimental sessions, during which TMS pulses were delivered at random or at fixed ITIs. The TMS single pulse-induced modulation of corticospinal output (motor evoked potential amplitude – MEP) was evaluated on-line. Each session began with a baseline block, followed by 10 blocks, with 20 TMS pulses each. Intra- and inter-block effects were valuated using an ANOVA model, through nested random effect on subjects considering the subject-specific variability.
Results: The delivery of successive TMS pulses significantly changed both intra-block and inter-block cortical excitability, as demonstrated by an increase in the amplitude of MEPs ($p < 0.001$) and supported through trend analyses, showing a perfect linear trend for inter-block levels ($R^2 = 1$) and nearly linear trend for intra-block levels ($R^2 = 0.97$). The MEPs significantly increased when the TMS pulses were delivered at both random and fixed ITIs.
Conclusions: Single TMS pulses induce cumulative changes in neural activity during the same stimulation, resulting in a motor cortical excitability increase.
Significance: Particular attention should be taken when several single TMS pulses are delivered in research and clinical settings for diagnostic and therapeutic purposes.

1. Introduction

Transcranial magnetic stimulation (TMS) is both a tool to measure the state of the human motor cortex, using a single TMS pulse approach, and a tool to induce cortical excitability changes, using a repetitive TMS (rTMS) approach (Wagner et al., 2009). However, it has recently been suggested that single TMS pulses also induce significant changes in cortical excitability. Specifically, studies have shown that a single TMS pulse modulates brain activity, inducing cortical oscillations (Paus et al., 2001; Van Der Werf and Paus, 2006; Rosanova et al., 2009; Kawasaki et al., 2014) and neuronal activity changes (Moliadze et al., 2003; Funke and Benali, 2010; Stamoulis et al., 2011).

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In this context, we generally consider that "single TMS pulses", delivered at random or in fixed inter-trial intervals (ITIs), are commonly used to evaluate the corticospinal state and do not produce any changes in cortical excitability per se (Kiers et al., 1993; Pell et al., 2011; Julkunen et al., 2012). Nevertheless, the ITIs used in several experimental designs (generally between 0.15 and 0.3 Hz) do not guarantee the independence of a subsequent neurophysiological response from the previous response (Nielsen 1996; Schmidt et al., 2009). Therefore, the effects of several single TMS pulses, delivered in sequence, on corticospinal excitability are currently undervalued. Specifically, we might underestimate the dependence of a second single TMS pulse on the previous pulse. Support for this hypothesis comes indirectly from neuroimaging studies, reporting not only specific neuronal activation induced through a TMS pulse (Denslow et al., 2004; Hanakawa et al., 2009; Chen et al., 2013) but also a specific haemodynamic time course of the response (Bohning et al., 1999; 2000). However, direct evidence for changes in corticospinal excitability is lacking.

Although the after effects (off-line) induced through a short train of single pulse TMS have been well established, in terms of both haemodynamic (Allen et al., 2007) and blood oxygenation changes (Thomson et al., 2012), the effects of several single TMS pulses and more specifically the role of the ITIs on the ongoing enrollment of corticospinal excitability is still under-investigated. Only a recent study, investigating whether the single-trial MEP amplitude distribution was time invariant, has highlighted that the individual MEP amplitudes are strongly dependent on ITI (Julkunen et al., 2012). In this scenario, understanding the effect of the ITI on the functional state of cortical neurons during stimulation is key, considering that the single-pulse TMS approach is typically used as a tool to measure the corticospinal excitability state. The repeated application of TMS pulses over many trials at random or fixed intervals is commonly used to measure the corticospinal excitability state, although the effects of non-specific factors, such as habituation or anticipation to TMS pulses, on motor cortical excitability and on the subsequent corticospinal responses are yet under-investigated. In this study, we examined whether repeated single TMS pulses, delivered over the primary motor cortex at random and fixed ITIs, could induce ongoing changes of corticospinal excitability, measured as MEPs amplitude. Corticospinal excitability modulation was also evaluated within and between blocks. We hypothesised that TMS pulses, singularly delivered, but spaced by specific ITIs, could modulate the excitability of the resting human motor cortex, inducing cumulative changes in neural activity.

2. Materials and methods

2.1. Subjects

Twelve healthy volunteers (6 females; ages 23.5 ± 4.3 years) participated in this study. None of the participants had a history of neurological, psychological or other relevant medical diseases, and these individuals were not taking CNS-active medication at the time of the experiments. None of the participants had any contraindication for TMS (Rossi et al., 2009), and all participants were right-handed according to the Edinburgh Handedness Inventory test (Oldfield, 1971). The study was approved through the Ethics Committee of IRCCS Centro San Giovanni di Dio, Fatebenefratelli, Brescia, Italy, and written informed consent was obtained from all participants before the experiment.

2.2. Experimental design

Each participant took part in two experimental sessions, during which the subjects received either single TMS pulses at a random ITI of 0.18–0.4 Hz, with one pulse between 5.5 and 2.5 s (one pulse every 4 s on average – Experiment 1); or single TMS pulses at a fixed ITI of 0.25 Hz (one pulse every 4 s – Experiment 2). The two experimental sessions were conducted on different days. The schedule was maintained across participants to control for potential circadian effects (Sale et al., 2007). Corticospinal excitability was investigated by recording MEPs from the abductor pollicis brevis (APB) of the left hand. The choice to evaluate the motor cortex of the non-dominant hemisphere was determined using the initial experimental protocol, and these data represented the control condition. Fig. 1 shows the experimental protocol.

To obtain baseline measurements, each experimental session was initiated with a baseline MEP block, followed by 10 experimental blocks. In each block, 20 single TMS pulses were applied with an intensity of 120% of the resting motor threshold (RMT). A 100-s break separated each TMS block.

During the experiment, the participants were seated on a comfortable armchair in a shielded sound-proofed room. During MEP recordings, the participants were instructed to keep their hands completely relaxed, while passively sitting and fixing their eyes on the visual target located directly in front of them. Each experimental session lasted approximately 60 min.

2.3. Motor cortical excitability

TMS-elicited MEPs were recorded to measure the motor cortical excitability of the left APB representation area. Single-pulse TMS was performed using a Magstim Super Rapid magnetic stimulator (Magstim Company, Whitland, UK) and a standard figure-of-eight shaped coil with an outer winding diameter of 70 mm that generates 2.2 T as a maximum output. The current waveform was biphasic and posterior-anterior directed. The coil was placed tangentially on the scalp with the handle pointing backwards and laterally, approximately 45° from the midline. The stimulation started at supra-threshold intensity. The optimal stimulus site to elicit MEPs in the left APB was selected after positioning the coil approximately over the central sulcus and moving it along the scalp in 0.5 cm steps in the right primary motor area. On the motor hot spot, RMT was assessed as the lowest stimulus intensity required to produce a response of at least 50 μV in amplitude in the relaxed muscle for at least five out of ten consecutive stimulations, at a resolution of 1% of the maximal stimulator output (Rossini et al., 1994, 2015). A TMS neuronavigation system (SofTaxic, EMS, Bologna, Italy) was used to ensure a high degree of reproducibility across separate experimental sessions (Cincotta et al., 2010; Carducci and Brusco, 2012).

Surface electromyographic (EMG) activity was recorded from the left APB muscle (Brain Products GmbH, Munich, Germany), with the active electrode mounted on the belly of the muscle and the reference electrode placed over the base of the metacarpal-phalangeal joint. EMG activity was monitored throughout the experiment to ensure complete muscle relaxation. The EMG signal was acquired using a band-pass filter at 0.1–1000 Hz and digitised at a sampling rate of 1 kHz using a 16 bit A/D-converter. The skin/electrode impedance was maintained below 10 kΩ. The data were analysed off-line using BrainVision Analyzer software (Brain Products GmbH, Munich, Germany).

2.4. Corticospinal excitability analysis

Changes in corticospinal excitability induced through TMS were evaluated using MEP amplitudes as the dependent variable. Firstly, the continuous EMG signal was divided into epochs (from 400 ms before, to 400 ms after) for each TMS pulse and subsequently baseline corrected (50 ms before the TMS pulse). Epochs containing muscle artefacts were rejected (overall 5.5% of epochs). The

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