Immediate Effects of Intermittent Theta Burst Stimulation on Primary Motor Cortex in Stroke Patients: A Concurrent TMS-EEG Study

Zhongfei Bai, Jack Jiaqi Zhang, and Kenneth N. K. Fong

***Abstract*— The neurophysiological effect of intermittent theta burst stimulation (iTBS) has been examined with TMS-electromyography (EMG)-based outcomes in healthy people; however, its effects in intracortical excitability and inhibition are largely unknown in patients with stroke. Concurrent transcranial magnetic stimulation and electroencephalogram (TMS-EEG) recording can be used to investigate both intracortical excitatory and inhibitory circuits of the primary motor cortex (M1) instantly and the property of brain networks at once. This study was to investigate the immediate effects of iTBS on intracortical excitatory and inhibitory circuits, neural connectivity, and network properties in patients with chronic stroke, using TMS-EEG and TMS-EMG approaches. In this randomized, sham-controlled, crossover study, 20 patients with chronic stroke received two separate stimulation conditions: a single-session iTBS or sham stimulation applied to the ipsilesional M1, in two separate visits, with a washout period of five to seven days between the two visits. A battery of TMS-EMG and TMS-EEG measurements were taken before and immediately after stimulation during the visit. Compared with sham stimulation, iTBS was effective in enhancing the amplitude of ipsilesional MEPs ($p = 0.015$) and P30 of TMS-evoked potentials located at the ipsilesional M1 ($p = 0.037$). However, iTBS did not show superior effects on ipsilesional intracortical facilitation, cortical silent period, or short-interval intracortical inhibition. Regarding the effects on TMS-related oscillations, and neural connectivity, comparisons of iTBS and sham did not yield any significant differences. iTBS facilitates intracortical excitability in patients with chronic stroke, but it does not show modulatory effects in intracortical inhibition.**

***Index Terms*— Cortical excitability, intracortical inhibition, theta burst stimulation, transcranial magnetic stimulation-evoked potentials.**

List of Abbreviations

cSP	Cortical silent period.
dwPLI	Debiased weighted phase lag index.
ERSP	Event-related spectral perturbation.
GMFP	Global mean field power.
ICF	Intracortical facilitation.
iTBS	Intermittent theta burst stimulation.
LMFP	Local mean field power.
M1	Primary motor cortex.
MEPs	Motor-evoked potentials.
rmANOVA	Repeated measures analysis of variance.
RMT	Resting motor threshold.
SICI	Short-interval intracortical inhibition.
TEPs	TMS-evoked potentials.
TMS	Transcranial magnetic stimulation.

I. Introduction

STROKE is a neurological condition caused by cerebral ischemia or intracerebral hemorrhage and results in neurological dysfunction persisting for more than 24 hours or until death. Previous studies have shown that corticospinal excitability of the ipsilesional primary motor cortex (M1) decreases immediately following stroke, and poststroke upper limb motor recovery is parallel with the enhancement of the ipsilesional corticospinal excitability [1]. Therefore, driving the brain into a state of enhanced corticospinal excitability would facilitate optimal motor recovery for poststroke patients [2]. Conventionally, excitability of the ipsilesional M1 is often assessed by motor-evoked potentials (MEPs) elicited by single-pulse transcranial magnetic stimulation (TMS) [3], and the presence of ipsilesional MEPs at the acute stage have been proved to be a prognostic biomarker in predicting poststroke motor recovery [4]. GABAergic intracortical inhibition is also engaged in poststroke motor learning and recovery,

Manuscript received 5 December 2022; revised 6 April 2023; accepted 26 May 2023. Date of publication 5 June 2023; date of current version 23 June 2023. The work of Zhongfei Bai was supported in part by the Shanghai Sailing Program, Shanghai, China, under Grant 20YF1445100; and in part by the Shanghai Municipal Health Commission Clinical Research Program under Grant 20224Y0220. The work of Kenneth N. K. Fong was supported in part by the General Research Fund (GRF), Research Grants Council, University Grants Committee, Hong Kong SAR, under Grant 15105919M. *(Corresponding author: Kenneth N. K. Fong.)*

This work involved human subjects or animals in its research. Approval of all ethical and experimental procedures and protocols was granted by the Human Research Ethics Committee of the Hong Kong Polytechnic University under Application No. HSEARS20200621001, and performed in line with the Declaration of Helsinki.

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Digital Object Identifier 10.1109/TNSRE.2023.3282659

and can be assessed using TMS-electromyography (EMG) outcomes, including short-interval intracortical inhibition (SICI) and cortical silent period (cSP) – mediated by GABAa [5] and GABAb receptors [6], respectively. Notably, previous studies have documented disinhibition over the ipsilesional M1, which might play a compensatory role in facilitating poststroke functional recovery from the acute to chronic stage [7], and a better motor outcome is associated with gradually normalized ipsilesional intracortical inhibition at the chronic stage [8]. A local stroke lesion also disrupts the functions of brain networks. Patients with chronic stroke show reduced efficiency of brain networks over the ipsilesional hemisphere compared with the contralesional hemisphere [9]. Reorganization of large-scale neural networks among cortical and subcortical regions and the cerebellum facilitates motor recovery and motor re-learning after stroke [10]. These findings indicate the roles of intracortical circuits (excitatory and inhibitory) and large-scale brain networks in poststroke recovery.

Noninvasive brain stimulation has been widely used to bidirectionally modulate brain activity. Theta burst stimulation, an artificial neurophysiological rhythmic stimulation, has been shown to be optimal in inducing synaptic plasticity in hippocampal neurons, as its stimulation pattern mimics the natural firing pattern of a rat's hippocampus during spatial learning [11]. Theta burst stimulation delivered via a magnetic stimulator has been later employed as a neuromodulation method for human brain, such as the primary motor cortex (M1) [12]. Compared with conventional repetitive TMS, theta burst stimulation demonstrates a more robust effect but with a much shorter conditioning period and lower stimulation intensity [12]. Intermittent theta burst stimulation (iTBS) has demonstrated a reliable effect in enhancing the MEP amplitude of stimulated M1 in healthy adults [13]. The clinical effect of iTBS on poststroke motor outcomes shows promising preliminary results [14], believed to be related to the modulation in cortical excitability and intracortical inhibition. Previously, iTBS applied to the ipsilesional M1 has been shown to enhance ipsilesional cortical excitability in patients with stroke, indexed by significantly enlarged ipsilesional MEPs [15]. However, how iTBS modulates ipsilesional intracortical inhibitory circuits has been rarely investigated. A limited number of studies show a null effect of iTBS on SICI and cSP over the ipsilesional M1 in patients with stroke [16], [17], indicating that the modulatory effect of iTBS on cortical inhibitory circuits has not yet been well studied.

Concurrent TMS-electroencephalography (TMS-EEG) is a novel neurophysiological technique that records brain activity in response to TMS pulses, termed TMS-evoked potentials (TEPs). The TEPs consist of a sequence of positive and negative peaks, including P30, N45, P60, N100, and P180 [18]. Pharmacological-TMS experiments have confirmed that these peaks are mediated by glutamatergic and GABAergic neurotransmitters, thus reflecting the functions of intracortical excitatory and inhibitory circuits [19]. The TMS-EEG approach also offers a solution to characterize connectivity from the perturbed site to other remote brain regions [20]. By computing phase synchronization of neural oscillations, it is possible to analyze the network property of the interaction among brain regions [21]. Unlike TMS-EMG measurements, a prominent advantage of TEPs is that they reflect a direct readout of cortical neuronal responsiveness and are not affected by the neural activity of the spinal cord [22].

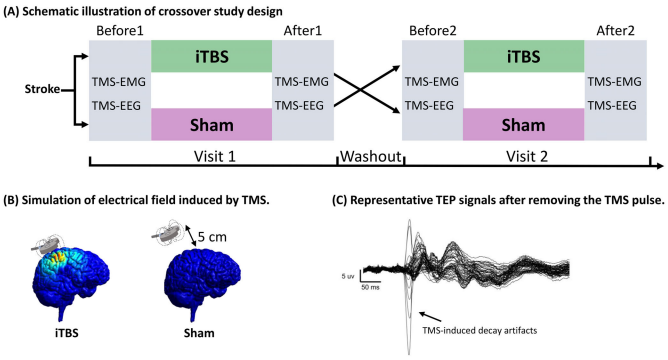

Fig. 1. Study design, TMS and TMS-EEG. (A) Patients with stroke received iTBS or sham stimulation randomly in two separated visits, and measurements were conducted accordingly. (B) The simulation of electrical field induced TMS-EEG recording, iTBS, and Sham. In the sham stimulation, the coil was placed 5 cm away from the scalp. (C) After removing TMS pulses (−2 – 15 ms) and applying data interpolation, several time-locked deflections can be observed.

Therefore, combining TMS-EEG and TMS-EMG measurements to examine the neurophysiological effects of iTBS would advance our understanding of how iTBS modulates intracortical circuits (excitatory and inhibitory), large-scale neural connectivity, and related brain networks in patients with stroke instantly. To do this, patients with stroke were randomized to receive a single session of iTBS and sham stimulation respectively in two separate visits before crossover, and both TMS-EEG and TMS-EMG were measured before and after the stimulation at each visit.

II. Materials and Methods

A. Study Design

Fig. 1 illustrates the experimental procedure of this randomized, sham-controlled, crossover study. Each participant visited our laboratory for two experimental conditions with a washout interval of five to seven days. Each session consisted of both TMS-EMG and TMS-EEG measurements before and after each stimulation.

B. Participants

Twenty patients with stroke were included (right-handed; age = 62.3 ± 5.8 years; 5 females; time since stroke = 78.4 ± 67.4 months). Patients were eligible to join the experiment if they met all the following inclusion criteria: (1) suffered from first-ever, ischemic or hemorrhagic, unilateral stroke; (2) time since stroke more than 6 months, i.e., chronic stroke. The reason for enrolling patients with chronic stroke was that they have limited potential of spontaneous neurological recovery, but their brains are still responsive to iTBS and that it would be valuable if plasticity takes place after treatment [23];

TABLE I
CHARACTERASTICS OF PATIENTS WITH STROKE

Participant	Age (years)	Gender	Time since stroke (months)	Unaffected RMT	Affected RMT	Lesion type	Lesion location	FMA
Pt1	60	M	60	70	70	Ischemic	R lentiform region	60
Pt2	60	M	60	60	60	Hemorrhagic	R corona radiata and lentiform	63
Pt3	58	M	21	61	72	Hemorrhagic	L parietal and occipital region	66
Pt4	72	M	49	58	58	Hemorrhagic	L putamen	66
Pt5	59	M	62	60	80	Hemorrhagic	R frontoparietal lobe (M1, S1 involvement)	56
Pt6	63	M	72	56	58	Hemorrhagic	L lentiform	58
Pt7	67	M	49	54	62	Ischemic	L pons and parietal-occipital lobe	59
Pt8	53	F	82	50	60	Ischemic	R anterior temporal, insular, inferior frontal lobes, and putamen	65
Pt9	63	F	117	68	81	Hemorrhagic	L thalamus and posterior limb of the internal capsule	59
Pt10	63	M	53	65	78	Ischemic	L corona radiata and putamen	63
Pt11	72	M	176	66	68	Ischemic	R posterior limb of the internal capsule	61
Pt12	69	M	312	50	55	Ischemic	R anterior limb of the internal capsule	66
Pt13	54	M	10	70	80	Ischemic	L corona radiata	60
Pt14	62	F	69	64	76	Ischemic	L basal ganglia	60
Pt15	61	F	57	68	76	Hemorrhagic	L corona radiata, posterior limb of the internal capsule, thalamic regions	60
Pt16	63	M	120	55	55	Hemorrhagic	R lentiform and external capsule	66
Pt17	68	M	44	64	56	Ischemic	R basal ganglia	63
Pt18	67	M	11	80	80	Ischemic	L inferior frontal gyrus	66
Pt19	61	F	47	62	62	Ischemic	L midbrain	66
Pt20	51	M	96	66	68	Ischemic	R frontal lobe and pons	66

Abbreviations: F: female; M: male; RMT: resting motor threshold; L: left; R: right; M1: primary motor cortex; S1: primary somatosensory cortex; FMA: Fugl-Meyer Assessment for upper extremity.

(3) detectable MEPs from the affected first dorsal interosseous muscle; and (4) provided written informed consent. Patients were excluded if they met any of the following exclusion criteria: (1) had any contraindication to TMS [24]; (2) had a known neurological disease excluding stroke, or a psychiatric disease; or (3) were using a psychostimulant, sedative, antidepressant, or antiepileptic medication. The detailed demographics of included patients with stroke are provided in Table I. This clinical study was registered (Ref. no.: NCT05509686) in (URL: http://www.clinicaltrials.gov, and approved by the Human Research Ethics Committee of the Hong Kong Polytechnic University (Ref. no.: HSEARS20200621001), and conducted following the Declaration of Helsinki [25].

C. Transcranial Magnetic Stimulation

Participants were seated in a TMS-specific adjustable chair with head and back supports and kept awake with their eyes open. To maintain consistency throughout the experiment, all TMS procedures were performed over an EEG cap. Biphasic TMS pulses were always delivered to the motor hotspot of the first dorsal interosseous muscle, using a figure-of-eight cooling coil (Cooling B-65, external diameter of each wing: 75 mm) connected to a magnetic stimulator (MagPro X100, MagVenture, Denmark). The motor hotspot was defined as the position where the largest and most reliable MEPs could be obtained from the first dorsal interosseous muscle. Ipsilesional MEPs were recorded from the contralateral muscle using disposable Ag-AgCl surface electrodes positioned in a belly-tendon montage, and a ground electrode was placed on the ulnar styloid process. The coil positioning and orientation on the scalp were continuously monitored by a frameless stereotactic neuronavigation system (Localite, Bonn, Germany). Resting motor threshold (RMT) was defined as the minimum intensity (% of maximal stimulator output) that could elicit peak-to-peak MEP amplitudes higher than 50 μV in at least five out of ten trials [26].

D. Intermittent Theta Burst Stimulation

The iTBS protocol was delivered to the motor hotspot over the ipsilesional hemisphere [12], [27]. In view of the dramatically enhanced RMT in the ipsilesional M1, the intensity of iTBS was set at 70% of the contralesional RMT. The sham stimulation was the same as that of iTBS, but the coil was held in the air 5cm above the scalp. Electrical field simulation showed that the setups for sham stimulation would not induce any valid cortical activation (Fig. 1). For either real or sham conditions, the participants could not see the coil placement.

E. TMS-EMG Recording

Four TMS-EMG measures for the ipsilesional M1, namely, cortical silent period (cSP), MEPs, intracortical facilitation (ICF), and SICI, were collected before and immediately after a session of iTBS or sham stimulation. Eight trials were recorded for each protocol, with inter-trial intervals of four to five seconds. The intensity of test pulses was fixed at 120% of the RMT. Single-pulse MEPs were used to measure corticospinal excitability at rest. The cSP was the disruption of background EMG activity by a suprathreshold test pulse while sustaining 30% of the maximal voluntary strength of thumb-index finger contraction [28]. The SICI was obtained by delivering a suprathreshold test pulse after a subthreshold conditioning pulse at 80% of RMT, with an inter-pulse interval of two milliseconds, and same intensities were set for ICF with an inter-pulse interval of ten milliseconds. The raw signals of MEP-based measures were recorded by a bipolar channel of the EEG system (SynAmps, NeuroScan, USA), digitized at 5 kHz and stored on a laptop for offline analysis.

F. TMS-EEG Recording

Concurrent TMS-EEG was recorded using a TMS-compatible DC EEG system (SynAmps, NeuroScan, USA) with 64 Ag/AgCl electrodes mounted according to the international 10-10 system. The raw data were online referenced to FCz, grounded to AFz, digitized at a sampling rate of 5 kHz, and online filtered below 2 kHz. The impedance between the scalp and the electrodes was maintained below 5 kΩ to optimize the signal-to-noise ratio. During TMS-EEG recording, 90 TMS pulses at 110% of the contralesional RMT were applied to the ipsilesional M1 with intertrial intervals of 4.5s with a jitter of ± 0.5s. To suppress auditory evoked potentials produced upon coil discharges, all participants wore an inserted earphone, and white noise was played throughout all TMS-EEG recoding [22]. The volume of the noise was as loud as it could be for all participants so that they could not hear the TMS coil 'click' sound. To minimize TMS-decay artifacts, a thin piece of foam was placed underneath the coil to prevent direct contact with the electrodes [22], and the direction of lead wires near the coil was rearranged so that they were perpendicular to the coil [29]. To avoid eye movements, participants were required to gaze at a black cross with a white background almost two meters away.

G. Data Processing and Analysis

The TMS-EMG and TMS-EEG signals were offline-preprocessed using EEGLAB 14.1.2 [30], TESA extension [31], FieldTrip [32], and custom-made MATLAB scripts (The MathWorks, Inc., Natick, MA). First, a Butterworth bandpass filter (fourth-order, 10 Hz–2 kHz) was applied to continuous EMG signals. Second, the signals were segmented into individual trials (−1000 – 999 ms) and baseline-corrected (−500 – −20 ms). Third, cSP and MEP amplitudes were identified from the individual trial. The MEP amplitudes at rest were log-transformed to decrease their variability. The cSP was defined as the time from TMS pulse onset to the first point of a five-millisecond window at which 50% of EMG signal samples returned to a level at least three-fold standard deviations away from the silent period. ICF and SICI were calculated as the ratio of MEPs produced by paired-pulse protocols to MEPs by single pulses at rest. Finally, valid trials were averaged to obtain grand mean values.

Following the steps proposed by Rogasch et al. [31], the continuous EEG signals were segmented into individual trials (−2000 – 1999 ms) and baseline-corrected (−500 – −10 ms). The trials and/or channels were excluded due to large artifacts or consistent noise. The data around TMS pulses (−2 – 15 ms) were removed and interpolated using a cubic method, followed by a down-sampling procedure to 1 kHz. Thereafter, two rounds of independent component analysis based on FastICA (systematic approach and tanh contrast function) were carried out. The first round was to remove the largest TMS-decay artifact detected by a semi-automated component classification algorithm implemented in TESA. The EEG signals were bandpass filtered (1–80 Hz) and bandstop filtered (48–52 Hz) using a fourth-order Butterworth filter, followed by another segmentation from −1000 to 999 milliseconds. FastICA was conducted again to remove remaining physiological artifacts. Excluded channels were interpolated back, and the reference channel was also recovered. Lastly, the EEG data were referenced to a common average, and TEPs were obtained by averaging across trials.

In the temporal domain, we defined five peaks with reference to previous literature [22]: P30 (28–35 ms), N45 (40–50 ms), P65 (55–75 ms), N100 (90–130 ms), and P180 (160–220 ms). Global mean field power (GMFP) of TEPs was computed using the following formula to explore the global brain reactivity following TMS pulses [33]:

$$\mathrm{GMFP(t)} = \sqrt{\left[\sum_{\mathrm{i}}^{\mathrm{K}} \left(\mathrm{V_i(t)} - \mathrm{V_{mean}\,(t)}\right)^2\right]/\mathrm{K}} \qquad (1)$$

where t is time, V is the voltage at channel i, and K is the number of channels.

In the time-frequency domain, event-related spectral perturbation (ERSP) was computed by decomposing individual trials based on the Morlet wavelet transform (three cycles, a frequency step of 1 Hz between 4 and 48 Hz, baseline-corrected [-625–100 ms], time resolution of ∼3 ms) and then averaging across trials. In accordance with a recent study [34], we also defined early (15–150 ms) and late (150–350 ms) stages for analysis of ERSP in the theta (4–8 Hz), alpha (8–13 Hz), beta-1 (13–20 Hz) and beta-2 (20–30 Hz) bands. Neural connectivity was also investigated in the two stages (15–150 ms, 150–350 ms) based on debiased weighted phase lag index (dwPLI) which is weighted by the magnitude of the imaginary component of cross-spectrum and insensitive to noise and volume conduction [35]. Four regions of interest were predefined, including stimulated M1 (C4), nonstimulated M1 (C3), prefrontal region (F2, F4, AF4), and parietal region (P2, P4).

H. Statistical Analysis

Statistical analysis was performed using SPSS22 (IBM, NY, USA) and FieldTrip in MATLAB 2016a. The alpha

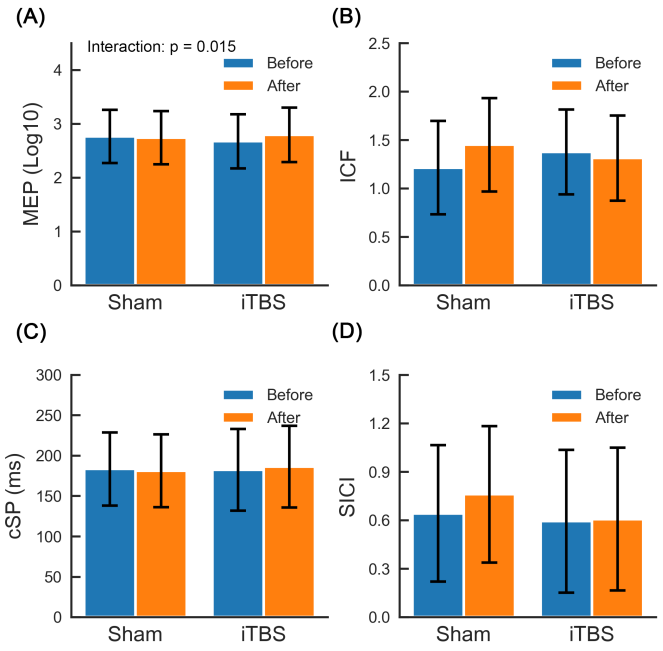

Fig. 2. The effects of iTBS on TMS-EMG measures. The MEP amplitudes at rest were log-transformed to decrease their variability. The error bar indicates one standard deviation. Abbreviations: MEP: motor-evoked potentials; ICF: intracortical facilitation; cSP: cortical silent period; SICI: short interval intracortical inhibition.

threshold was set was at 0.05 (two-tailed). The normality of variables prior to parametric tests was checked using both one-sample Kolmogorov-Smirnov tests and histogram plots. The effects on TEPs and TMS-related oscillations were separately explored by cluster-based permutation tests for iTBS and sham conditions, and then verified by two-way repeated measures analysis of variance (rmANOVA). Outcome measures including the MEP amplitude, cSP, ICF, SICI, GMFP of P30, and neural connectivity were subjected to two-way rmANOVA with two main effects (condition and time) and one interaction effect (condition-by-time). The correlation between the MEP amplitude and the GMFP of P30 was evaluated using Pearson's correlation.

III. Results

Fig. 2 presents the effects of iTBS in patients with stroke. Two-way rmANOVA revealed a significant condition-by-time interaction effect on the MEP amplitude ($F = 7.09$, $p = 0.015$), suggesting that iTBS enhanced corticospinal excitability. However, there was no significant condition-by-time interaction effect on ICF ($F = 2.10$, $p = 0.164$), cSP ($F = 1.26$, $p = 0.276$), or SICI ($F = 1.33$, $p = 0.264$).

Regarding the comparison of TEPs before and after iTBS, a cluster-based permutation test found a significant positive cluster in the time window of P30 ($p = 0.003$) located around the stimulated site (Fig. 3). However, sham stimulation did not induce any significant changes on TEPs. Furthermore, two-way rmANOVA showed a significant condition-by-time interaction effect ($F = 5.06$, $p = 0.037$) on the GMFP of P30, confirming the effect of a single-session iTBS in enhancing local excitability of the stimulated M1. As the relationship between MEPs and P30 is still unclear, we explored this further using the whole dataset, including the measures before and after the sham and iTBS conditions. A significant but modest correlation between MEP amplitudes and the GMFP of P30 was found ($r = 0.35$, $p = 0.002$), whereas the differences (after – before) of MEPs and the GMFP of P30 were not significantly correlated ($r < 0.01$, $p = 0.991$).

Cluster-based permutation tests failed to identify any significant clusters on ERSP either in the early or the late stage, suggesting that iTBS did not significantly modulate TMS-related oscillations (Fig. 4, data shown in the early stage only). Regarding the connectivity between the ipsilesional M1 and remote regions, two-way rmANOVA showed that iTBS produced no significant effects on any connectivity pairs in the early stage, reflected by a nonsignificant condition-by-time interaction effect in the analyses of M1–prefrontal (theta: $F = 1.84$, $p = 0.191$; alpha: $F = 0.19$, $p = 0.667$; beta-1: $F = 0.04$, $p = 0.844$; beta-2: $F = 1.16$, $p = 0.294$), M1–M1 (theta: $F = 0.03$, $p = 0.872$; alpha: $F = 4.28$, $p = 0.052$; beta-1: $F = 2.29$, $p = 0.146$; beta-2: $F = 1.70$, $p = 0.208$), and M1–parietal (theta: $F = 0.38$, $p = 0.544$; alpha: $F = 0.97$, $p = 0.338$; beta-1: $F < 0.01$, $p = 0.925$; beta-2: $F = 2.32$, $p = 0.144$). Similarly, iTBS did not significantly modulate any connectivity pairs in the late stage (data not shown).

IV. Discussion

Clinically, iTBS has been used as a motor priming method to enhance the treatment benefits from rehabilitation intervention in chronic stroke patients; however, the neurophysiological mechanism underlying its clinical effects is still under exploration [36]. Our study demonstrated that a single session of iTBS, but not sham stimulation, was significantly effective in enhancing the excitability of the ipsilesional M1 in patients with chronic stroke, reflected by increased amplitudes of P30 and MEPs. Furthermore, the amplitude of MEPs and the GMFP of P30 were cross-sectionally correlated, but their longitudinal changes before and after stimulation were not. Compared with sham stimulation, iTBS did not significantly change other intracortical measures, including ICF, cSP, or SICI. Similarly, there were no significant iTBS-induced effects on TEP peaks (such as N45 or N100) which reflect GABAergic intracortical inhibition. Regarding neural connectivity and networks, it was disappointing to find nonsignificant effects of iTBS on ERSP or dwPLI-based connectivity. Together, iTBS was effective in facilitating intracortical excitability, but not for intracortical inhibitory circuits, neural connectivity, or brain networks.

In line with previous studies in healthy people [13], the excitability of the stimulated M1 was significantly enhanced following iTBS. Gedankien et al. [37] found that the amplitude of MEPs was enhanced after iTBS, but no significant modulatory effect was found on the amplitude of the N15-P30 complex in older adults. Conversely, our study in patients with stroke not only confirmed the effects of iTBS in enhancing MEPs, but also supported its effects on P30 around the stimulated M1. This is in line with previous studies in which high frequency repetitive TMS and anodal transcranial direct current stimulation significantly enhanced the early peak of

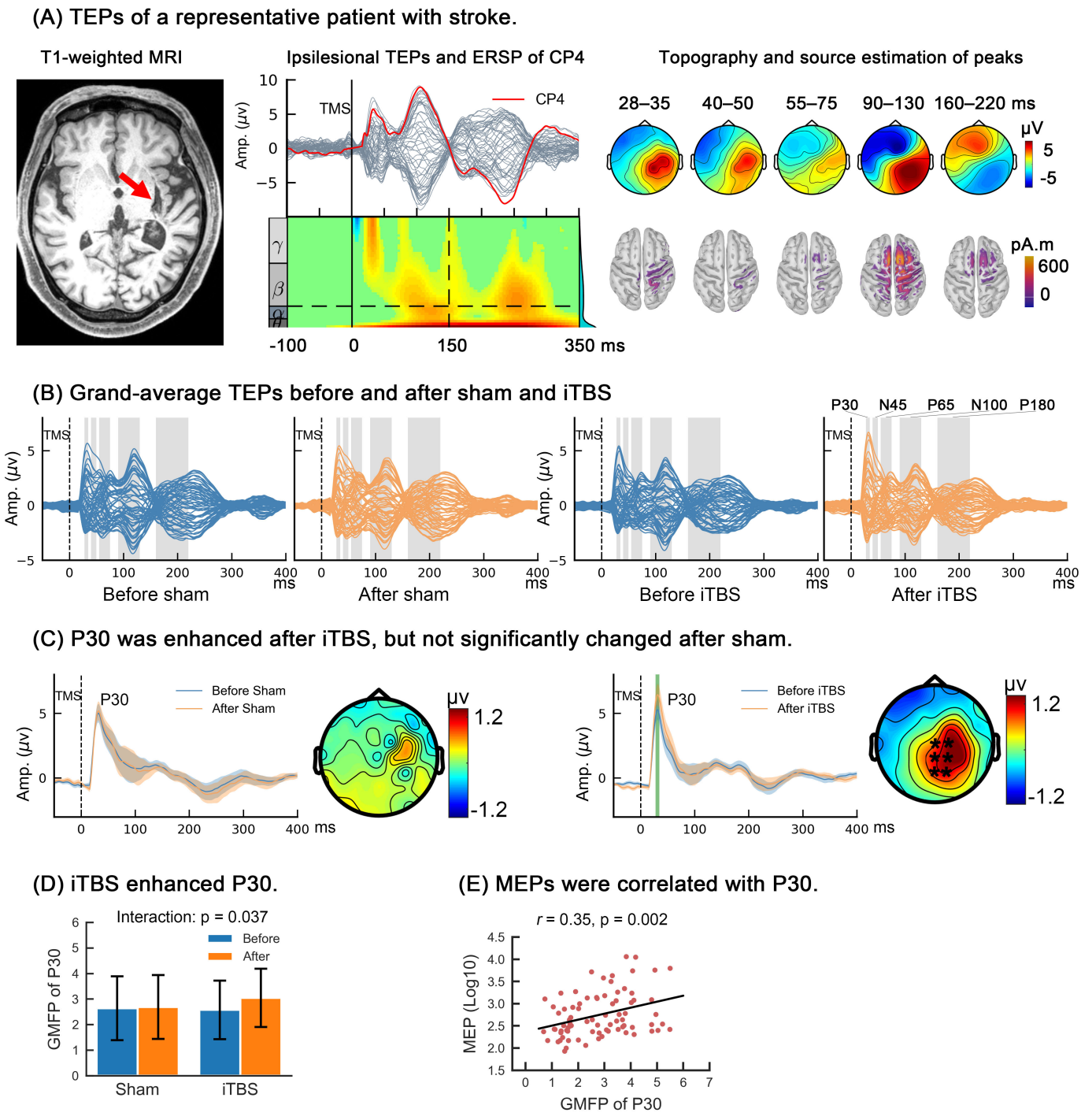

Fig. 3. The effects of iTBS on TEPs. (A) TEPs produced from the ipsilesional M1 of a representative patient with stroke. The patient suffered from a hemorrhagic stroke at the right corona radiata and lentiform. The butterfly plot of TEPs is presented. In a time-frequency plot for CP4, an early (15–150 ms) and a late (150–350 ms) stage following TMS pulses were predefined. Last, the typography and source estimation of five peaks are shown. The source estimation was completed in the Brainstorm toolbox using the linear minimum-norm inverse method. (B) Grand-average TEPs before and after sham and iTBS. (C) P30 was significantly enhanced after iTBS, but not significantly changed after sham stimulation. Topographies represent the difference (after − before) of P30, and line plots show the time courses of a representative channel (CP2). A green rectangle in the right line plot indicates significantly enhanced P30 after iTBS. The curve shadings are mean ± standard error. (D) iTBS enhanced the global mean field power of P30. (E) MEPs correlated with P30, and a total of 80 data samples (20 ∗ 4) were included in the correlation analysis. The error bar indicates one standard deviation. Abbreviations: iTBS: intermittent theta burst stimulation; MEP: motor-evoked potential; TEP: TMS-evoked potential; global mean field power (GMFP).

TEPs in healthy adults [38], [39]. For the origin of P30, recent studies found that its amplitude was not mediated by the blockers of NMDA [40] or AMPA [41] receptors permeable to calcium and/or sodium. Voltage-gated sodium channels also mediate neuronal depolarization [42], and become maximally open in the late stage of the rising phase. Darmani et al. [43]

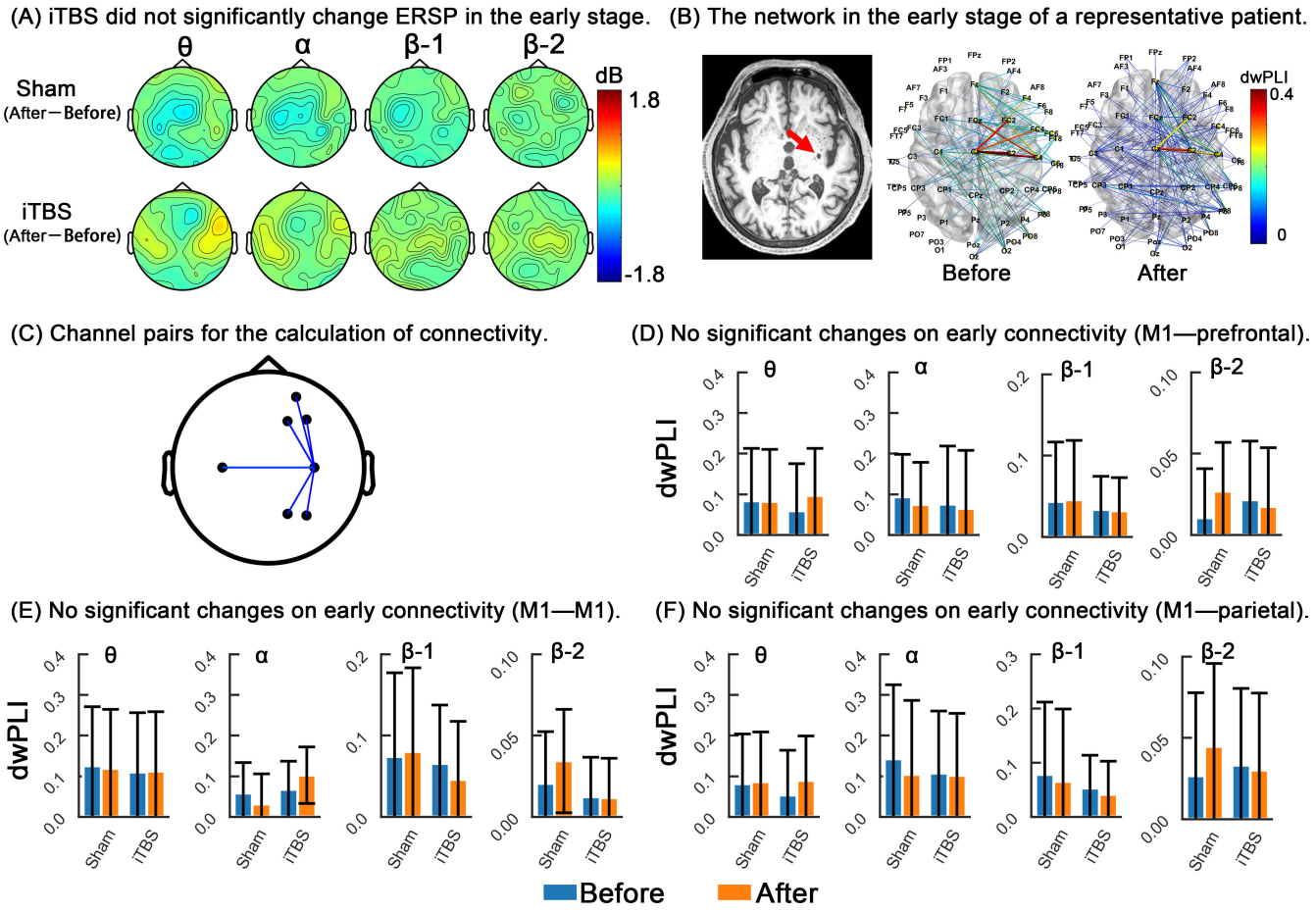

Fig. 4. The effects of iTBS on TMS-related oscillations, connectivity, and network measures. Only data in the early stage following TMS pulses were shown. (A) iTBS did not significantly change ERSP in the early stage. (B) The network in the early stage of a representative patient with stroke. (C) Channel pairs for the calculation of connectivity are shown. Taking right stimulation for example, dwPLI of three channel pairs (C4-F2, C4-F4, C4-AF4) were averaged to denote the connectivity between the M1 and the prefrontal region, and two channel pairs (C4-P2, C4-P4) were averaged for the connectivity between the M1 and the parietal region. The C4-C3 channel pair served as the connectivity between the bilateral M1. (D – F) iTBS did not significantly change neural connectivity. The error bar indicates one standard deviation. Abbreviations: ERSP: event-related spectral perturbation, dwPLI: debiased weighted phase lag index; M1: primary motor cortex.

found that the amplitude of P30 was decreased at the site of stimulation by voltage-gated sodium channel blockers, indicating that P30 is likely to be relevant to the late process of action potential initiation rather than the process of postsynaptic excitatory potentials [42]. Previous studies have indicated that the effect of iTBS relies on NMDA receptor-dependent calcium influx [44]. Thus, the increase of P30 found in our study further suggests that iTBS may have a specific effect in upregulating the efficiency of voltage-gated sodium channels, thereby facilitating intracortical excitability within the stimulated M1 of patients with stroke.

Because both MEPs and P30 are believed to represent excitability of the cortex, many previous studies have attempted to verify their cross-sectional and longitudinal relationship. However, no significant linear correlation was found between the amplitude of P30 and the amplitude of MEPs in previous studies with small samples [37], [45], [46]. Our study with a larger sample size found a significant but modest cross-sectional relationship between them, in line with a previous study (r: around 0.3) [47]. This modest correlation indicates that both outcomes may reflect similar underlying mechanisms. For the longitudinal correlation, a significant correlation between the difference of MEPs and the differences of P30 after iTBS was found in a study with older adults [37]; however, we failed to replicate this in patients with stroke. The above inconsistency could be attributed to various reasons. According to previous literature [22], P30 usually occurs around the stimulated site, but its topographical distribution is not perfectly overlaid across people. Therefore, the P30 amplitude of arbitrarily selected channels or regions of interest may not be suitable for everyone. By contrast, the GMFP of P30 may be an optimal solution that avoids the above arbitrary selection. On the other hand, TMS-evoked decay artifacts can last up to 50 milliseconds after TMS pulses [48], and rigorous analysis pipelines are necessary to clean them up without distortion to the early TEP peaks (e.g., N15, P30).

Previous studies with young healthy adults showed that a single session of iTBS decreased the N100 amplitude of TEPs [49] and GABA concentration measured by magnetic resonance spectroscopy [50], indicating that iTBS resulted in a reduction of GABAergic intracortical inhibition (i.e., low level of activity in the intracortical inhibitory circuits). However, the

effect in intracortical inhibition was not observed in the present study with patients with stroke. This might be caused by the different baseline neuronal activities in healthy people and patients with stroke. As shown previously, baseline neuronal activity accounts for the variability of the effects of brain stimulation [51], [52]. In patients with stroke, the ipsilesional M1 undergoes a complex process of neuroplastic changes, including cortical disinhibition after stroke [7]. Compared with healthy controls, early TMS-EEG studies found that patients with stroke showed a reduction of the N100 amplitude [53], [54], further suggesting intracortical disinhibition in the ipsilesional M1. As the baseline state of the ipsilesional M1 has been disinhibited [53], [54], so it becomes difficult to further suppress the activity of the inhibitory circuits in the ipsilesional M1 by iTBS. From a neurophysiological perspective, the reduction of GABAergic activity may have improved the brain response to excitatory noninvasive brain stimulation, supported by the finding that iTBS-induced neuroplastic changes are mediated by GABA concentration [50].

TMS-EEG-based connectivity is not widely investigated in patients with stroke, and its underlying mechanism has not been clear. In our study, the effects of iTBS in remote brain regions or network measures were not observed in patients with stroke. The results appear inconsistent with previous observations that iTBS not only enhances the excitability of stimulated M1, but also has an effect in facilitating brain connectivity using resting-state EEG [55] and resting-state fMRI [56]. We speculate that this inconsistency may be due to the different approaches used: resting-state functional connectivity and TMS-EEG-based connectivity reflect different aspects of brain networks. Most recently, Momi et al. [57] demonstrated that the signals of TMS-EEG recording were better explained by individuals' brain structural connectivity than by functional connectivity. Therefore, the nonsignificant changes on TMS-EEG-based connectivity may imply limited effects of a single session of iTBS on the structural networks in patients with stroke, but multiple sessions of stimulation plus conventional rehabilitation seem to be effective in increasing the microstructure of white matter in patients with stroke [58].

Several limitations of the present study should be acknowledged. First, due to the limited number of patients, we could not subgroup them based on the lesion locations. Majority of our included patients had a chronic stroke involving subcortical areas or white matter, thus limiting the generatability of our results to cortical stroke. Further studies could employ more patients to explore the relationship between TMS-EEG outcomes and residual brain structures after stroke, using structural MRI. Second, a behavioral measurement was not included in the current study, which could decrease the interpretability of our findings. Third, white noise was used to eliminate contamination of auditory-evoked potentials from TEPs, but somatosensory-evoked potentials following TMS pulses were not controlled. The TEP components, particularly the N100, might be confounded by the multi-sensory inputs during TMS-EEG experiments [59], [60], [61] To minimize the potential confounds to the modulatory effect (i.e., before and after iTBS), a within-subject design had already been used in this study. Further study may employ a realistic sham TMS system as the sham control to validate the results.

V. Conclusion

In conclusion, iTBS applied to the ipsilesional M1 enhances corticospinal excitability through facilitating local excitatory circuits, but does not modulate intracortical inhibitory circuits. Additionally, a single session of iTBS does not significantly modulate TMS-EEG-based neural connectivity or network measures, probably suggesting its limited effects in structural networks.

Acknowledgment

The authors thank K. H. Ting, Kevin Yu, Celia Dong, and the University Research Facility in Behavioral and Systems Neuroscience (UBSN) at The Hong Kong Polytechnic University for facility support.

Declaration of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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