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A high-density theta burst paradigm enhances the aftereffects of transcranial magnetic stimulation: Evidence from focal stimulation of rat motor cortex

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ABSTRACT

Background: Theta burst stimulation (TBS) is an efficient noninvasive neuromodulation paradigm that has been widely adopted, clinically. However, the efficacy of TBS treatment remains similarly modest as conventional 10 Hz repetitive transcranial magnetic stimulation (rTMS).
Objective/hypothesis: To develop a new TBS paradigm that enhances the effects of TMS administration while maintaining high time-efficiency.
Methods: We describe here a new TMS paradigm, named High-Density Theta Burst Stimulation (hdTBS). This paradigm delivers up to 6 pulses per burst, as opposed to only 3 in conventional TBS, while maintaining the inter-burst interval of 200 ms (or 5 Hz) – a critical parameter in inducing long-term potentiation. This paradigm was implemented on a TMS stimulator developed in-house; its physiological effects were assessed in the motor cortex of awake rats using a rodent specific focal TMS coil. Microwire electrodes were implanted into each rat's limb muscles to longitudinally record motor-evoked potential (MEP). Four different TBS paradigms (3, 4, 5 or 6 pulses per burst, 200 s per session) were tested; MEP signals were recorded immediately before (baseline) and up to 35 min post each TBS session.
Results: We developed a stimulator based on a printed-circuit board strategy. The stimulator was able to deliver stable outputs of up to 6 pulses per burst. Animal experiments (n = 15) revealed significantly different aftereffects induced by the four TBS paradigms (Friedman test, $p = 0.018$). Post hoc analysis further revealed that, in comparison to conventional 3-pulse TBS, 5- and 6-pulse TBS enhanced the aftereffects of MEP signals by 56% and 92%, respectively, while maintaining identical time efficiency.
Conclusion(s): A new stimulation paradigm is proposed, implemented and tested in the motor cortex of awake rats using a focal TMS coil developed in the lab. We observed enhanced aftereffects as assessed by MEP, with no obvious adverse effects, suggesting the translational potentials of this paradigm.

1. Introduction

Transcranial magnetic stimulation (TMS) has evolved into an important neuromodulation tool for basic and clinical neuroscience. Early clinical trials documented the antidepressant effect of 10 Hz repetitive TMS (rTMS) to the dorsolateral prefrontal cortex (dlPFC) [1–3], resulting in US Food and Drug Administration clearance and broad clinic adoption. Notably, compared to the sham control groups, the efficacy of TMS treatment in these early clinical trials were modest, and have remained disappointingly moderate in the years that followed [4,5]. Many approaches have been reported to enhance the effectiveness of TMS treatment, including patient stratification [6,7], stimulation targets beyond dlPFC [8,9], more condensed TMS treatment sessions [10], multisite stimulation [11,12], and new TMS coil designs that can access deeper brain structures [13,14], etc.

From a technical perspective, TMS employs a brief but strong magnetic field pulse, inducing electrical stimulation in the brain (a few thousand amperes and a few thousand volts) [15]. Perhaps due

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to the technical demands as well as patient safety considerations [16,17], new TMS paradigm designs, which often necessitate hardware modifications, have been a less explored domain in the quest for superior TMS outcomes [18–21]. One exception was the successful development of Theta Burst Stimulation (TBS) [22], a variation of the classical theta burst paradigm in slice physiology that is known to optimally induce long term potentiation (LTP) [23], consistent with a long-standing notion that excitatory rTMS works by increasing net excitability through an LTP-like phenomenon. In animal experiments, LTP induction is a complex function of the intensity and temporal pattern of tetanic stimulation [24–26]. Classical theta burst electrical stimulation that is known to efficiently induce LTP applies multiple pulses at 100 Hz per burst [23]. In contrast, TBS applies 3 pulses per burst, presumably due to the technical limitations of the TMS system. Unlike the conventional 10 Hz rTMS paradigm, which requires 37.5 min for each treatment session, intermittent TBS requires only 200 s per session, drastically improving the time efficiency [22]. Unfortunately, the efficacy of the TBS paradigm remains similarly modest [27]. Furthermore, simply increasing the duration of the TBS sessions does not necessarily lead to stronger neuromodulation effects. Indeed, Gamboa and colleagues [28] documented a reversal of the theta burst aftereffects with prolonged stimulation: by doubling the duration of an intermittent or continuous TBS session (iTBS or cTBS), the conventional facilitatory aftereffect of iTBS became inhibitory; and the normally inhibitory aftereffect of cTBS converted to facilitatory. These data, seemingly counterintuitive, highlight the complexity of TMS-induced plasticity.

Physiologically, there is a limited understanding of how TMS exerts its effects in the stimulated loci and in the interconnected network, which also hinders the development of more efficacious TMS paradigms [5,29]. The electric field produced by a TMS coil is invariably stronger in superficial than in deep cortical layers [30]. Locally, upper layer interneurons and layer 2/3 pyramidal neurons likely experience the strongest electric field modulation; layer 5 pyramidal dendrites that extend to upper layers and synapse with interneurons will likely experience strong electric field modulation as well. At a network level, the activation of layer 2–3 long-range fibers and deep layer projecting neurons likely modulate cellular activity of interconnected brain areas. While human research can shed light on the physiological process underlying TMS [31], animal models permit invasive manipulations and could be valuable in understanding the cellular and neurochemical processes induced by acute and longitudinal TMS [32–38].

However, for preclinical studies to be translational, the spatial focality and temporal patterns of animal TMS should mimic human TMS conditions. So far, the best-achieved focality with commercially available rodent TMS coils was half-hemispherical stimulation [39]. Given the complex effects of TMS on local neural circuits and interconnected networks as described above, and considering that human TMS modulates anatomically specific brain regions (such as the thumb representation of the motor cortex), the lack of focality in an animal TMS coil, in comparison, raises the question of face validity [37]. This limits our ability to draw spatially relevant neurobiological conclusions from preclinical models and, ultimately, our ability to inform clinical intervention. Perhaps, due to the lack of consensus on a valid animal model for TMS, most studies aiming at enhancing therapeutic efficacy of TMS treatment have been conducted in human subjects, largely on a trial-and-error basis.

The goal of this study is to develop a new TMS paradigm that enhances the efficacy while maintaining high time efficiency, as is in conventional TBS. We hypothesize that stronger aftereffects can be induced by increasing the number of pulses per burst, while maintaining the inter-burst interval at 200 ms (5 Hz). To this end, we have developed a new stimulator that is capable of generating bursts of TMS pulses, with the number of pulses per burst ranging from 3 to 6, herein coined High-Density Theta Burst Stimulation (hdTBS); in the meantime, the inter-burst interval remained at 200 ms – a critical parameter that has been experimentally demonstrated to optimally induce LTP [23,26]. We have assessed the aftereffects of the hdTBS paradigm in rat motor cortex. The animal experiments leverage recent technological developments within the lab, which includes a focal TMS coil specific for rodent animals [40] and the platform of TMS administration in awake rats [41], allowing for consistent and precise TMS administration across sessions and across animals, avoiding confounds from anesthesia [38]. Motor-evoked potential (MEP) was longitudinally measured in the activated muscles and was used as the metric to assess the effects of TMS, in line with human literature [19,21,22,29,42–44]. Results demonstrate that, in comparison to conventional TBS, the new TMS paradigm produces stronger modulation in MEP signal. To the best of our knowledge, this is the first report of a TMS device that delivers condensed TBS up to 6 pulses per burst. This technology, along with the focal TMS coil and the awake rat model, opens novel avenues for developing safe and more efficacious TMS paradigms and for investigating the neurobiological mechanism of TMS.

2. Material and methods

2.1. Longitudinal MEP recording in rats

MEP, a measure of electromyographic (EMG) signal in the activated muscle induced by stimulation of the corresponding motor cortex, has been conventionally employed as the metric to quantitatively and conveniently assess TMS effects [42,45]. While an EMG signal can be readily acquired in humans using surface electrodes, consistent EMG recording in an awake rat is challenging since rats do not readily comply with the motionless requirement. Thus, we have adopted a rodent EMG recording approach previously reported by Tysselling and colleagues [46], as detailed below.

EMG electrodes were constructed in-house: soft 7-strand annealed stainless steel microwires, 0.025 mm in diameter (A-M systems, Washington, USA, cat. No. 793200), were cut to 13 cm in length; the insulation coat from one end of the wire was stripped for 3 mm, and then press-connected to a female socket (Model E363/0, P1 Technology, USA). Then, two or more sockets were inserted into a 6-channel electrode pedestal (Model: MS363, P1 Technology, USA). The pedestal and the microwires were attached to a circular Marlex mesh and secured with dental cement. Following, a small portion (about 2 mm) of the insulation coat, 5 cm away from the other end, was carefully stripped. This de-insulated portion was the active contact to sense the EMG signal. Fig. 1B shows 4 electrode wires connected to a pedestal.

The rats were anesthetized using isoflurane and electrodes were implanted as a “backmount,” using a method described previously [46]. After one week of surgical recovery, the microelectrodes were interfaced to a BIOPAC system (BIOPAC Systems Inc, CA, USA) via a 6-pin male connector (Model: 363–441/6, P1 Technology, USA). A standard EEG pad was also connected to the tail to serve as the ground electrode.

2.2. Headpost implantation for consistent TMS positioning

We previously reported the design of a focal TMS coil specific for a rodent brain [40]. The key to this novel design was the introduction of a small magnetic core that enhanced and focused the magnetic field (see Fig. 2). The high coil focality raises a challenge for TMS administration, namely, how to consistently position the

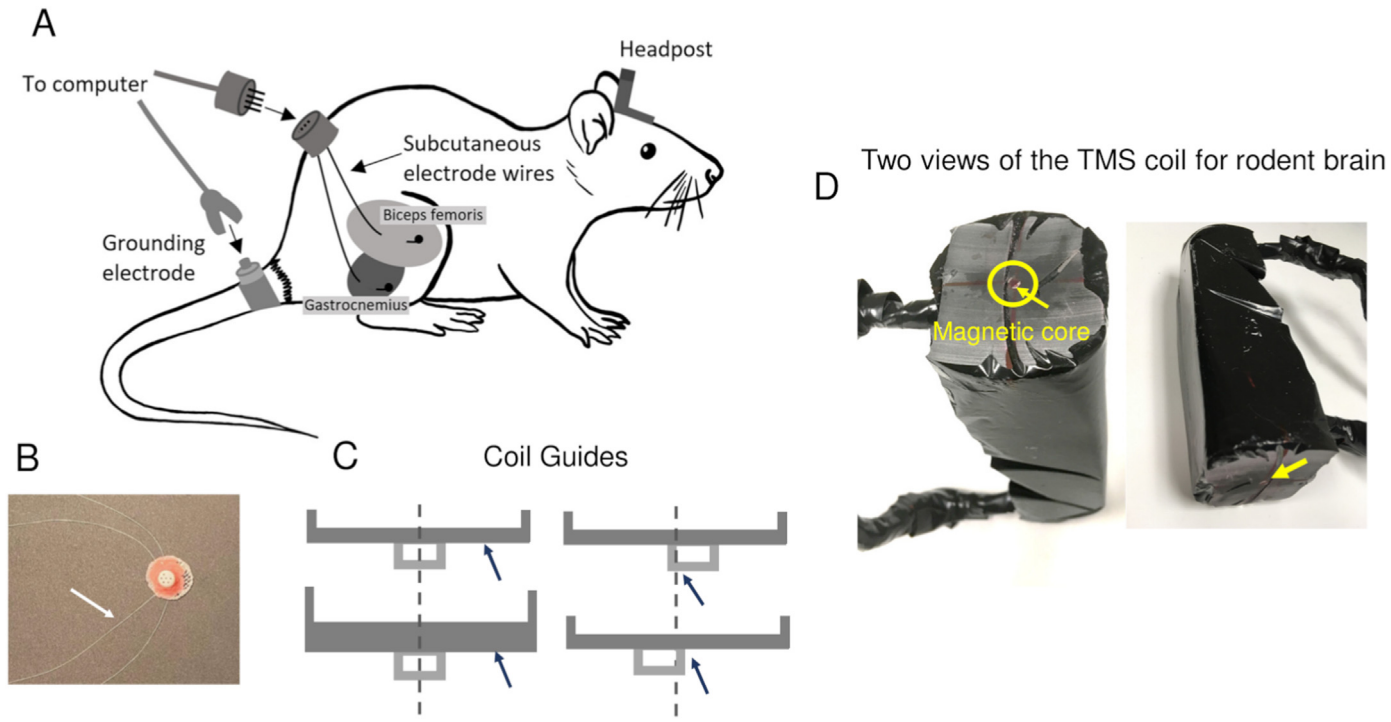

Fig. 1. Customized microwire electrodes (A) were surgically implanted into the rat biceps femoris and gastrocnemius muscles (B) for longitudinal EMG recording. The white arrow in (B) indicates the microwire electrode. A headpost was implanted on the rat skull, serving as the reference to consistently position the customized focal TMS coil [40] (shown in C) across sessions and across animals. The circle and arrow in (E) indicate a small magnetic core that enhances and focuses the magnetic field. The headpost was carefully designed such that the hotspot of the TMS coil targeted the region of interest (the hindlimb motor cortex).

coil to the region of interest. We have developed a strategy to address this question [41]: implanting a headpost onto the rat skull to serve as a reference and a detachable coil guide to efficiently position the TMS coil to the region of interest (hindlimb motor cortex, M1HL). Fig. 1A and B illustrate the surgical preparation.

2.3. Animal habituation for TMS administration

To mitigate animal stress during TMS administration, naive rats were handled and habituated to the TMS environment for one week using the procedures reported previously [41]. Sham TMS

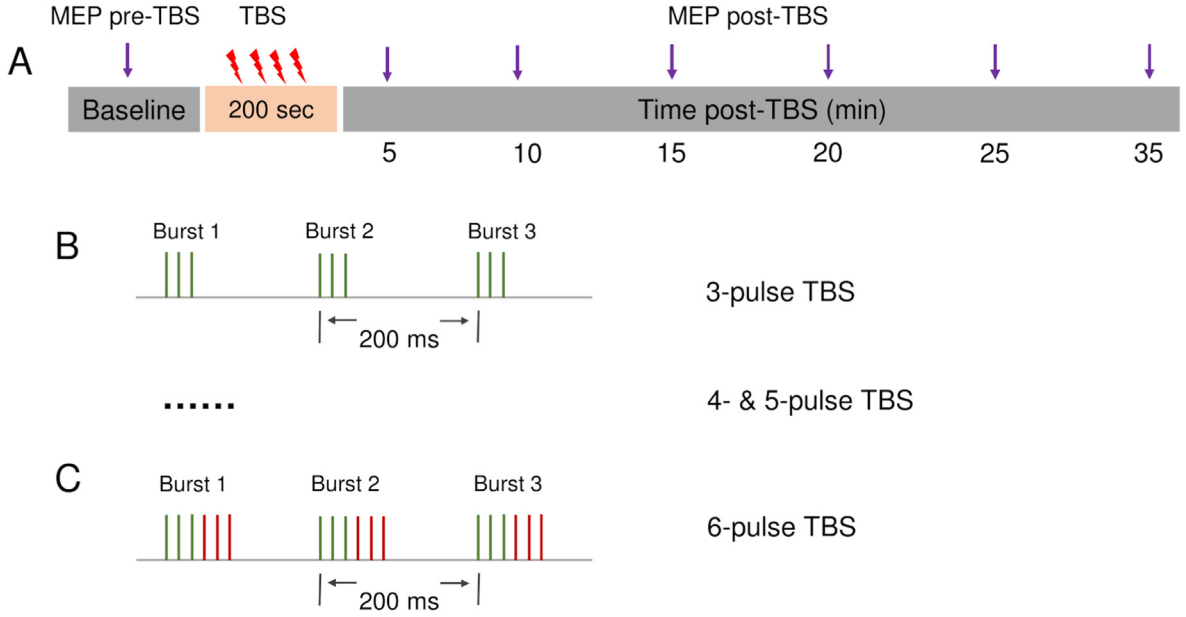

Fig. 2. (A) Experimental design demonstrating the effects of TMS on MEP signal. Rats received one type of TBS administration on a given day; the order of TBS paradigm was randomized. MEP was recorded at pre-TBS baseline and up to 35 min post-TBS. (B) shows conventional TBS: each burst consists of 3 pulses, with an inter-burst interval of 200 ms. (C) Shows hdTBS with 6 pulses per burst. Inter-burst interval remained at 200 ms; inter-pulse-interval within each burst was 22 ms. hdTBS with 4 and 5 pulses per burst are not shown for visual clarity.

was administered at 5% of the motor threshold for 5 min per day with the rat being held underneath the coil. Fruit treats were given as a reward following the habituation session to reduce stress associated with habituation training.

2.4. Acute aftereffects of intermittent TBS

TBS pulses were delivered at 2 s ON and 8 s OFF with a total of 20 repetitions [22]. The number of pulses per burst was either 3, 4, 5 or 6. We employed a within-subject design: for each rat, one TBS paradigm was randomly assigned on a given day; the inter-burst-interval remained constant at 200 ms. Each TBS session lasted for 200 s (Fig. 2).

The stimulation was delivered using a stimulator developed in-house (see below). During TMS administration, 3D printed coil guides attached to the implanted headposts on awake rats were used to direct the focal point of the coil to the target region on the head surface (Fig. 1C). Rats were held under the TMS coil for the full treatment session with the same holding method used in habituation. Motor threshold for each rat was measured on day 0, which was defined as the TMS power that caused contralateral hindlimb movement in 50% of the stimulation. TMS power was at 100% motor threshold across the 4 testing days.

We measured MEP at the following time points: pre-TBS baseline, 5, 10, 15, 20, 25 and 35 min post-TBS. This was done by delivering single-pulse TMS every 5 s, with a total of 10 pulses. The EMG signal was band-pass filtered (100–5000 Hz), amplified by × 2000, and sampled at 10,000 Hz (BIOPAC system).

Sixteen adult Sprague-Dawley rats (12 male, 4 females) were used in this study. Two rats received bilateral electrode implantation (for mapping the focality of the TMS coil), while the rest received unilateral implantation (for studying the aftereffects of TBS). One rat with bilateral electrodes also completed the TBS study (n = 15). All experimental procedures were approved by the Animal Care and Use Committee at NIDA.

2.5. Development of the hdTBS stimulator

A TMS stimulator typically employs high-voltage capacitors to store energy. Semiconductor switches such as an insulated gate bipolar transistor (IGBT) or a silicon-controlled rectifier (SCR) are used to control energy discharge from the capacitor to a TMS coil, generating desired pulses [45–50]. Pulsed electrical current could cause voltage spikes within the system. Two recent studies [51,52] evaluated the insulation properties and stray inductance of bus designs based on the printed-circuit board (PCB) in high-voltage (up to 16 kV), pulsed current applications. Inspired by these two studies, we implemented a PCB bus design for the high-voltage components of the TMS system as shown in Fig. 3. The PCB bus contains 16 sub-layers. Each of the two high-voltage layers and the ground layers contain 7 sub-layers (thickness 0.1 × 7 mm). These connections were insulated from each other, which were designed to sustain at least 4.5 kV. The coordinates of the contact pads on the PCB bus were carefully designed to match the layouts of the high-power semiconductor modules. The PCB bus board was 6.5 mm in thickness and weighed 1.56 kg. All the high-power components were integrated on the PCB bus, resulting in a compact high-power unit for TMS (Fig. 3B). The three-dimensional layout of the stimulator was also optimized to reduce stray inductance to as small as 20 nH at 5 kHz (the connection between the capacitor and the IGBT collector, not including the capacitors). Pulse timing was programmed on a microprocessor. Energy storage capacitors (C1, C2) were charged with direct-current power supplies (PSU1, and PSU2, model: 152 A-3 KV, TDK Lambda Americas, New Jersey, USA), and the stimulator delivered up to 6 pulses per burst at an inter-burst interval of 200 ms. The inter-pulse-interval within each burst was 22 ms. A further increase in frequency and the number of pulses per burst is possible with more powerful direct-current power supplies. Fig. 3C illustrates the stimulator circuit. Biphasic TMS pulses were generated by sequentially turning ON and OFF the two IGBT units, allowing for energy transfer among C1, L, and C2. The high-voltage circuit topology is similar to Ref. [53] except that the active snubber circuits across L were eliminated because we observed only minor oscillation following each pulse. Notably, a stray-inductance minimizing the PCB-based snubber circuit and improved laminated bus bars were recently described in a multi-level TMS device that delivered wide output ranges and ultra-brief pulses [54].

2.5.1. Data analysis

The amplitudes of MEP signals (peak-to-peak) were identified using BIOPAC software. We first examined the normality of the data distribution. This was done by pooling the data across time windows and across animals, which were subject to Shapiro-Wilk and Chi-Square statistics. We found that the MEP data were non-normally distributed ($p < 0.0001$). We thus performed two types of statistical analyses: 1) a non-parametric test on the raw data; (2) we pre-processed the data using the Box-Cox transformation so that the data were approximately normally distributed [55], followed by parametric statistical analysis as detailed below.

2.5.1.1. Non-parametric statistical analysis. Given that the primary goal of our animal experiment was to examine whether hdTBS induced stronger aftereffects than conventional TBS, we calculated the area-under-curve (AUC) of the MEP signal across the 35 min time window post-TBS under each pulse-type condition (3-, 4-, 5- or 6-pulse TBS) for each individual animal, which was normalized to each animal's pre-TBS baseline. We performed the Friedman test with Pulse Type as the factor to examine the main effect, followed by post-hoc analyses using the Wilcoxon signed rank tests, which were subjected to multiple-comparison correction using the Benjamini-Hochberg method.

2.5.1.2. Parametric statistical analysis. To further investigate whether there is any TIME effect and Time × Pulse Type interaction, we performed Box-Cox transformation [55] of the raw data to reduce skewness. A range of β values were tested to examine the normality of the data using Shapiro-Wilk and Chi-Square statistics. We found that data were approximately normally distributed after Box-Cox transformation with β=0.1 ($p > 0.2$).

We subsequently performed two-way repeated measures ANOVA with the Time and the Pulse Type as the two factors. This was followed by post-hoc pairwise two-sided t-tests, corrected for multiple comparisons (Benjamini-Hochberg). Statistical computing was carried out in R package. A corrected $p < 0.05$ was considered significant.

3. Results

3.1. Stable current output from the hdTBS stimulator

Since energy loss is inevitable in pulse generation, a critical question is: how stable can the output current be as the number of pulses per burst increases? We measured coil current by programming the system to output up to 6 pulses per burst. Fig. 4 shows raw plots on a Tektronix Oscilloscope (Model DP02024B) using a Rogowski current waveform transducer (Power Measurements, Ltd., Nottingham, UK. Model #: CWT30, peak current 6000 A, frequency range: 2 Hz to 30 MHz, sensitivity: 1 mV/A). The first pulse had a peak-to-peak amplitude of ± 3.0 kA; the last one

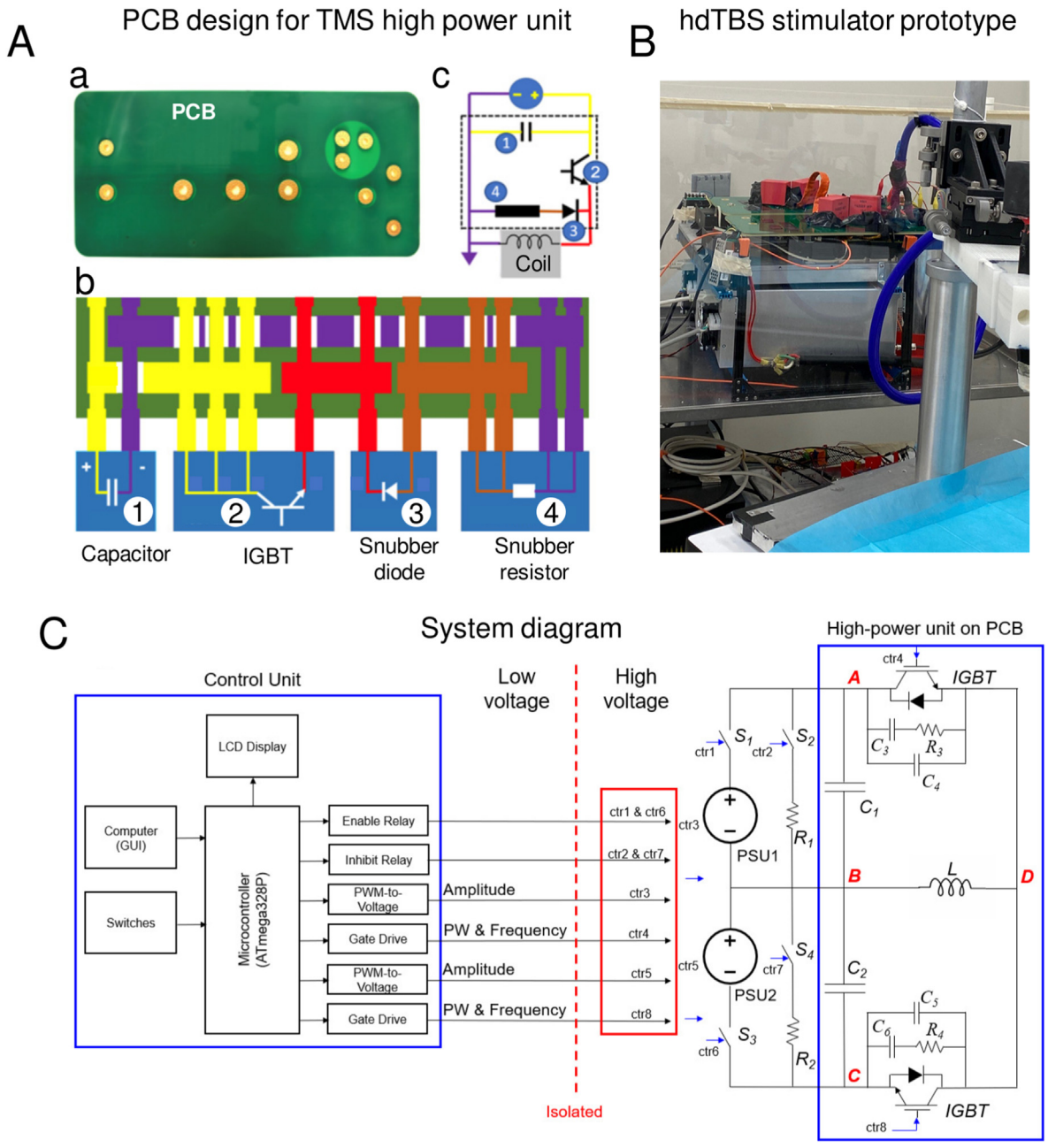

Fig. 3. Illustration of the hdTBS stimulator. The stimulator was based on the printed circuit board (PCB) design to reduce circuit inductance and resistance. (**A**) PCB bus board is shown in (a), with the copper layouts of the high-voltage layers and the ground layers illustrated in (b). High-voltage connections are in the middle layers except the contact pads are exposed on the surface layer. As an example, (c) illustrates circuit connections with the PCB board. (**B**) A prototype of the hdTBS stimulator. (**C**) Schematic diagram of the control unit for the stimulator. The high-power unit was mounted on the PCB board specifically designed to sustain high current and high voltage and was isolated from low voltage units.

had +2.92/-2.88 kA. The maximum difference in pulse amplitude across the 6 pulses was 4%. As we further increased the number of pulses per burst to 7, an unstable current output to the TMS coil was detected and was thus not explored. We concluded that the unstable output was due to the constraints of the power supply units (PSU1 and PSU2), whose maximum capacitor-charging power was limited to 1500 W.

3.2. MEP induced by TMS of the motor cortex in the hindlimb region

We next conducted animal experiments using this stimulator. As a first step, we measured the MEP signal in the rat motor cortex. With the headpost serving as the reference and the coil guide, we directed the TMS coil to M1HL. We mapped coil focality by applying different coil guides to offset the positioning of the TMS coil by 1 mm along 4 directions (rostral, caudal, left, and right), and by measuring the MEP signal at each location.

As shown in Fig. 5, MEP signal of up to 1.6 mV peak-to-peak was detected when the TMS coil was aimed at the center of the hindlimb motor cortex (coordinates relative to bregma: anterior-posterior 1.8 mm; medial-lateral 2.5 mm) [56]. The amplitude diminished substantially as we offset the coil by 1 mm. These data are consistent with our previous estimation: the rodent coil had a focality of 2 mm [40,41].

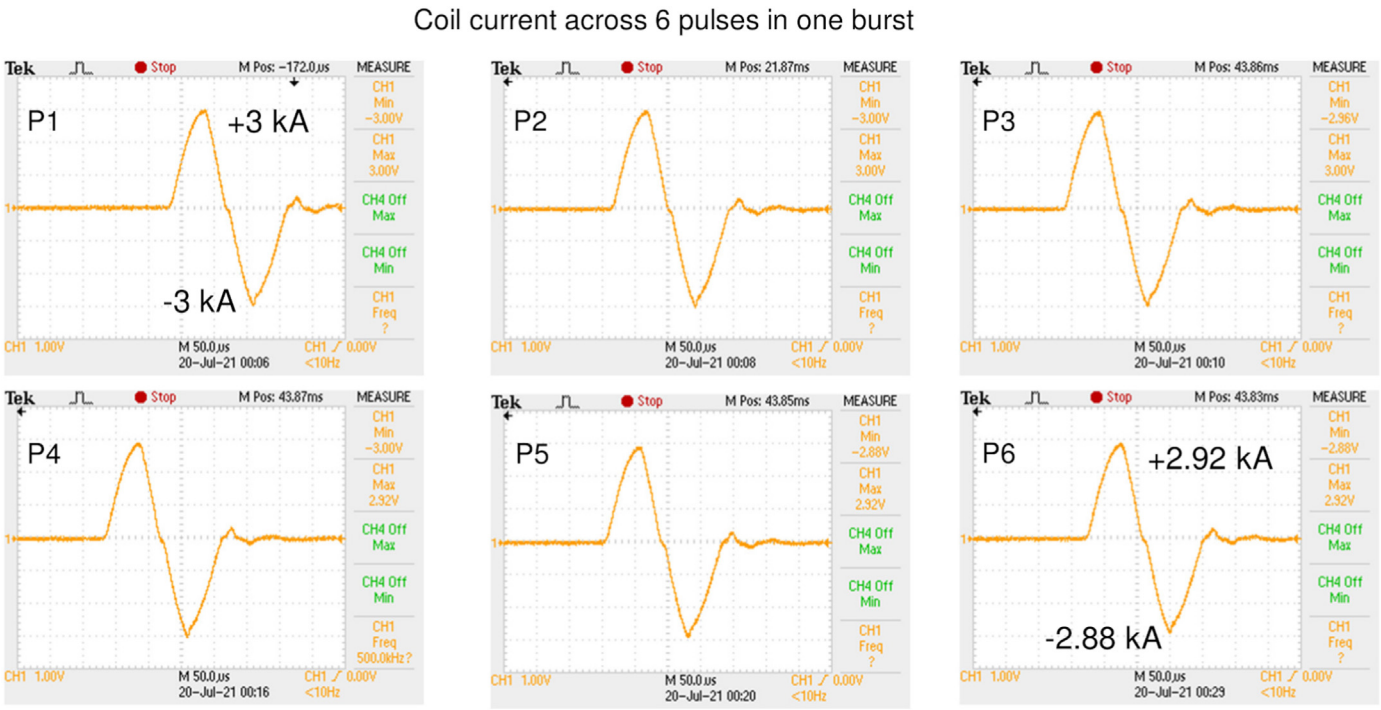

Fig. 4. Consistency in electric current output of the hdTBS stimulator. The stimulator was programmed to output 3 kA pulsed electric current to the TMS coil shown in Fig. 1. The amplitude of the last pulse reduced by 4% in comparison to the first one.

3.3. Comparisons of the aftereffects following a single session of intermittent TBS

Previous human TMS studies measured the MEP signal pre- and post-TMS administration as the metric to assess the effects of TMS [22,57]. We have adopted a similar approach. The duration of the stimulation was kept constant (200 s), while the number of pulses per burst varied. As an example, Fig. 6 shows the MEP signal pre- and post-TBS under two conditions. With 6 pulses per burst, apparent enhancement in MEP amplitudes was seen at 10 and 25 min post-TMS; modest enhancement was seen with 3 pulses per burst, as indicated by the arrows.

We observed variability in the baseline MEP signal across animals and across days within the same animal. This is not unexpected, given that the specific locations of electrode implantation cannot be guaranteed to be identical across animals, and that the electrode contact could experience minor displacement within leg muscles across days due to the animals' movement. We normalized the post-TMS MEP signal to the pre-TMS baseline value and performed non-parametric statistical analysis. Results are summarized in Fig. 7.

Fig. 7A shows the MEP signal across the 35 min post-TBS windows, averaged across animals (mean ± standard deviation, n = 15). Since the waveforms under the 4 pulse types did not follow a specific temporal pattern (see Fig. 7A), we calculated the AUC under each TBS condition, which was subject to the Friedman test with Pulse Type as the factor. There was a significant main effect of Pulse Type ($p = 0.018$). Post-hoc Wilcoxon signed rank tests revealed significant enhancement in the AUC values in both the 5-pulse and 6-pulse TBS conditions compared to those in the 3-pulse TBS: before multiple-comparison correction: $p = 0.012$ for 3-P *vs.* 5-P;

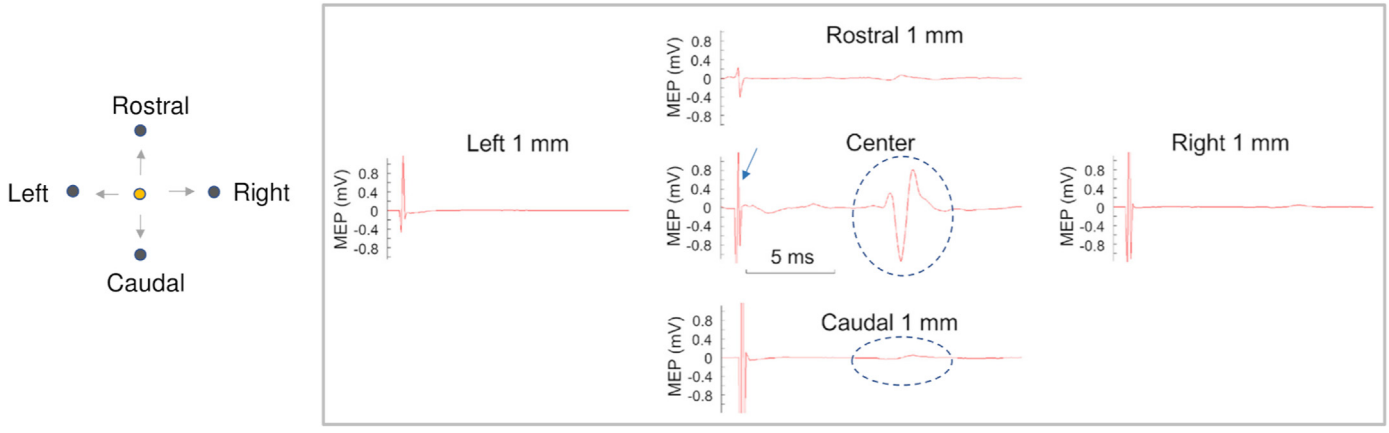

Fig. 5. Mapping the focality of the rodent specific TMS coil. The headpost (see Fig. 2) served as the reference to direct the TMS coil to the desired location. As the coil aimed at the center of the hindlimb representation of the motor cortex, up to 1.6 mV (peak-peak) MEP signal was detected, which diminished substantially when the coil was offset by 1 mm. The blue arrow indicates artifacts resulting from the TMS pulse. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

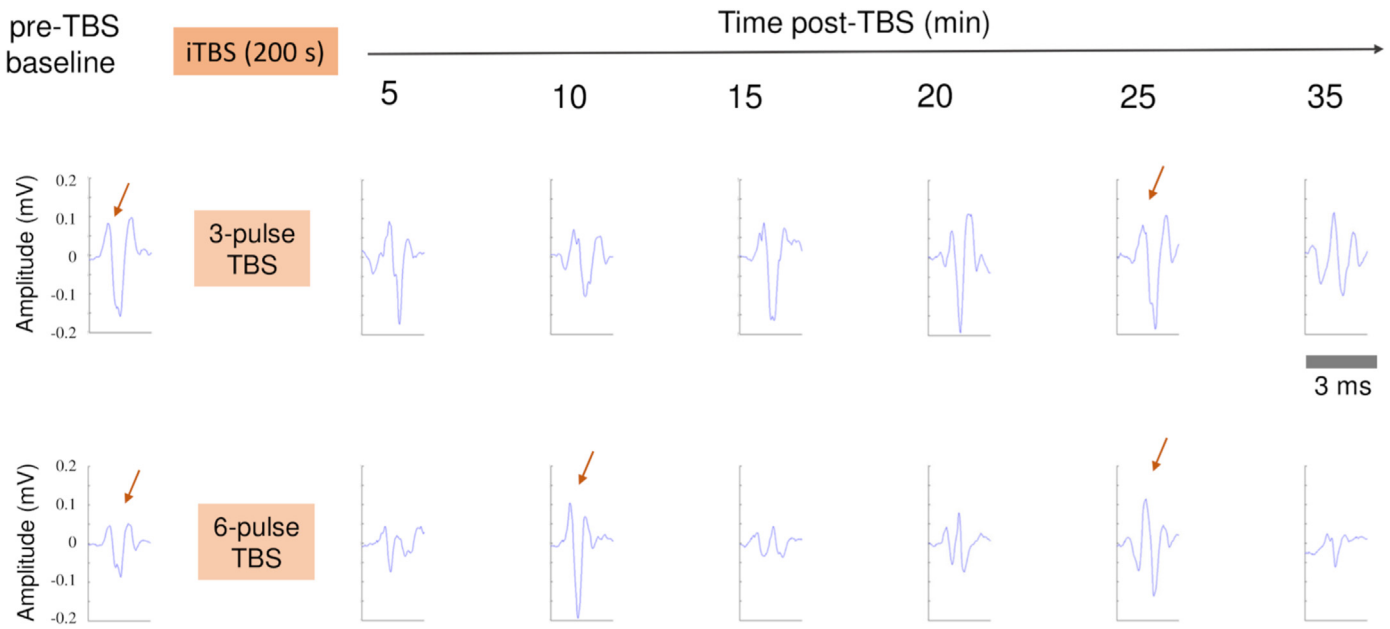

Fig. 6. Raw MEP signal under two TBS conditions (3 and 6 pulses per burst) are shown. Red arrows indicate enhancement in MEP amplitude post-TBS administration relative to pre-TBS baseline. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

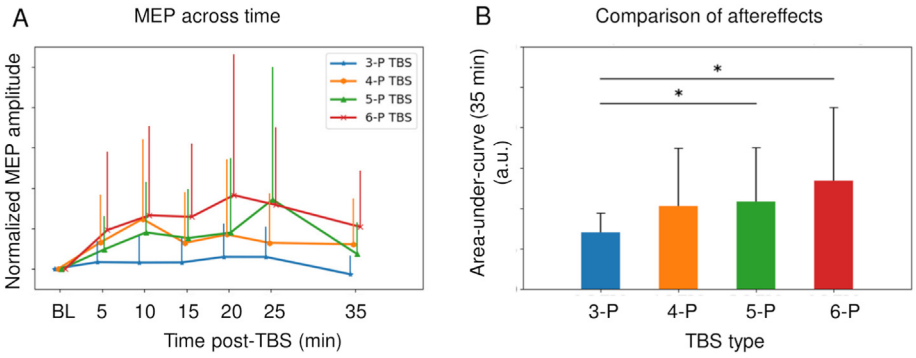

Fig. 7. (A) Averaged MEP signal across animals under the conditions of 3, 4, 5, and 6 pulses per burst. MEP amplitude was normalized to pre-TBS baseline. Area under the 35 min post-TBS window was calculated (area-under-curve, AUC) for each TBS condition, which was subject to non-parametric statistical comparisons. Friedman test revealed significant difference in AUC among the 4 TBS conditions ($p = 0.018$). Post-hoc Wilcoxon signed rank tests further revealed significantly higher AUC values under 5- and 6-pulse TBS than under the 3-pulse TBS condition (before multiple-comparison correction: $p = 0.012$ for 3-P *vs.* 5-P and $p = 0.010$ for 3-P *vs.* 6-P. $p = 0.037$ for both conditions after correction for multiple-comparisons). Data are presented as mean ± standard deviation across animals (n = 15). Abbreviation: a. u., arbitrary unit.

$p = 0.010$ for 3-P *vs.* 6-P. Furthermore, $p = 0.037$ for both conditions after a correction for multiple comparisons (Fig. 7B). The difference in AUC values under 3-P *vs.* 4-P was non-significant ($p = 0.082$ after multiple-comparison correction, and $p = 0.018$ before the correction).

We also performed a Box-Cox transformation so that the data reached normal distribution, followed by parametric statistical analyses. The results of this transformation are summarized in the **Supplemental Materials**. Briefly, two-way repeated measured ANOVA revealed significant main effects in both the Time ($p = 0.002$) and Pulse Type ($p = 0.046$), but no significant interaction of Time × Pulse Type was expressed.

4. Discussion

Enhancing the therapeutic efficacy of TMS treatment has been an active research theme in the neuromodulation field. Most studies were conducted in human subjects using existing rTMS technologies, and largely on a trial-and-error basis. While clinical trials ultimately decide the fate of any interventions, perhaps a more systematic approach is to develop and test a TMS technology in animal models first, and then, hopefully, translate the results from animals to humans.

In the present study, we have developed a stimulator that is able to deliver the theta burst paradigm with up to 6 pulses per burst. The utility of the hdTBS paradigm was demonstrated in the rat motor cortex: a significant enhancement in aftereffects was detected in 5- and 6-pulse TBS, more than in conventional 3-pulse TBS. Two logical questions remain. First, since 6-pulse Theta Burst effectively doubles the dose of a conventional 3-pulse TBS. Will this new TBS paradigm be tolerated by patients? Of the 16 rats tested, we observed no indication of seizure events, nor any behavioral abnormality after TBS, such as eating, drinking, or grooming, etc. Second, the aftereffects were assessed with a MEP signal in the activated muscles. Will the enhanced aftereffects be translated to improved treatment outcomes when applied to conventional TMS targets, such as the dlPFC? Future studies in human subjects can address these important questions.

Our hdTBS is based on adding more pulses to the burst period, while keeping the interval between two neighboring pulses constant, which increases the burst duration. Alternatively, the burst period could have been kept constant by shortening the inter-pulse interval, potentially enabling 100 Hz per burst, as is in classical electrical TBS. In this initial implementation, the peak capacitor-charging power (PSU1, PSU2 in Fig. 3) was limited to 1500 W. In principle, employing more powerful capacitor-charging power supplies or interleaving two 50 Hz hdTBS units could realize 100 Hz hdTBS. Experiments on animals would be ideal to study the safety profile and potential therapeutic effects of 100 Hz hdTBS.

The standard deviation of the MEP signal induced by 5- and 6-pulse TBS are visibly higher than that induced by 3-pulse TBS (Fig. 7). Indeed, our data normality analysis revealed that the degree of skewness in the pooled MEP data was primarily attributed to 5- and 6-pulse TBS; MEP data from 3- and 4-pulse TBS were normally distributed. The reasons for this observation are unknown but might be related to the possibility that 5- and 6-pulse TBS perturbs the balance of excitatory and inhibitory local circuits more aggressively than 3-pulse TBS does, inducing a strong non-linear neural output from the motor cortex. The implications of such variability in TMS treatment are of interest to explore.

5. Face-validity of the rat TMS model

Rodent animals, such as rats and mice, have been the dominant species in preclinical research, with many cellular and molecular tools specifically developed over the past decades. There have been efforts to develop a rodent model of TMS, and to back-translate findings from human studies to rodent animals with variable successes, for example, Refs. [39,58–62]. Given the dramatic differences in brain sizes between rodent animals and humans, a critical technical question is the spatial focality of the TMS coil [63], as pointed out by Rotenberg and colleagues [37].

Due to poor coil efficiency, electromechanical stress and Joule heating [64], it would appear impractical to design a rodent-specific TMS coil by simply reducing the coil size. We previously proposed a new coil design strategy to deal with this technical challenge [40]: instead of planarly distributing energy as seen in most human TMS coils, we distributed energy vertically and used a magnetic core to guide, enhance and focus the magnetic flux to the end of the coil, and achieved suprathreshold, unilateral motor response in anesthetized mice [40] and in awake rats [41]. Based on the neuroanatomy of the rat motor cortex, we estimated the coil focality to be about 2 mm. In the present study, we mapped the focality of the TMS coil based on MEP response, and further confirmed this (see Fig. 5). We routinely observe unilateral motor response to TMS stimulation, which we believe, to some degree, mimics TMS in humans.

6. Limitations

Due to technical complexity, we employed a within-subject design to evaluate the aftereffects of TBS administration. Since TMS has an accumulative effect in the stimulus loci and in the interconnected network, our data acquired at the last (fourth) day might be confounded by TMS effects from the previous 3 days. However, since the specific TMS paradigm (3-, 4-, 5- or 6-pulse TBS) at a specific date was randomized across animals and across days, such accumulative TMS effects should not affect the conclusion. But this design likely contributed to the high data variability seen in Fig. 7.

In summary, this study reports a novel TMS paradigm. It maintains a time efficiency identical to conventional TBS, but delivers up to 6 pulses per burst, doubling the TMS dose. This new paradigm significantly enhanced the aftereffects of TBS. This technology and the new animal model open novel avenues for developing a safe and more efficacious TMS paradigm.

CRediT authorship contribution statement

Qinglei Meng: Formal analysis, Writing – original draft, designed experiment, performed experiment, analyzed data, wrote the paper. **Hieu Nguyen:** Formal analysis, Writing – original draft, designed experiment, performed experiment, analyzed data, wrote the paper. **Antonia Vrana:** Writing – original draft, performed experiment, wrote the paper. **Simone Baldwin:** Writing – original draft, performed experiment, wrote the paper. **Charlotte Qiong Li:** Writing – original draft, performed experiment, wrote the paper. **Antonia Giles:** Writing – original draft, performed experiment, wrote the paper. **Jun Wang:** Writing – original draft, designed experiment, performed experiment, wrote the paper. **Yihong Yang:** Conceptualization, Writing – original draft, conceptualized the study, wrote the paper. **Hanbing Lu:** Conceptualization, Formal analysis, Writing – original draft, Conceptualization, designed experiment, performed experiment, analyzed data, wrote the paper.

Declaration of interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

US patent about the high-density theta burst stimulation was filed in December of 2021 (application number: 63/286,229; NIDA EIR: 07627).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brs.2022.05.017.

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