EFFECTS OF THE METAPLASTICITY-BASED THETA-BURST TRANSCRANIAL STIMULATION PROTOCOLS ON WORKING MEMORY PERFORMANCE

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The study of the metaplasticity-based transcranial magnetic stimulation (TMS) protocols is an extensively studied approach to increase the effectiveness of stimulation. However, the effects of protocols with different intervals between the TMS blocks on cognitive functions are poorly understood. The study was aimed to assess the effects of two theta-burst transcranial stimulation (iTBS) protocols with short and long intervals between blocks on the working memory (WM) performance in healthy volunteers. A total of 16 participants were undervent a single TMS session of each protocol, wich were applied in random order (iTBS 0–15 — two iTBS blocks over the left dorsolateral prefrontal cortex (DLPFC) iTBS with an interval of 15 min between blocks followed by stimulation of the vertex area in 60 min after the first block; iTBS 0–60 — iTBS block over the left DLPFC iTBS, block of the vertex stimulation after 15 min, and the second block of iTBS over the left DLPFC iTBS 60 min after the first one; iTBS 0 — one block of iTBS over the left DLPFC iTBS and two blocks of the vertex stimulation; control protocol — three blocks of the vertex stimulation with similar intervals). WM was assessed using the n-back test before the first block and after the second and the third stimulation blocks. No significant effects of protocols on WM or differences between protocols in alterations of test results and the responder rates to TMS between protocols were observed. The trend toward statistical signficance was reported for the protocol with short interval (iTBS 0–15). Furthermore, low reproducibility of individual iTBS effect was reported. The study of protocols with short intervals between blocks involving larger cohort of volunteers and taking into account the other factors potentially influencing the effect of the protocol (number of blocks and duration of a single block) seems to be promising.

Keywords: transcranial magnetic stimulation, theta-burst stimulation, non-invasive brain stimulation, metaplasticity, working memory, left dorsolateral prefrontal cortex

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ЭФФЕКТ ОСНОВАННЫХ НА МЕТАПЛАСТИЧНОСТИ ПРОТОКОЛОВ ТРАНСКРАНИАЛЬНОЙ СТИМУЛЯЦИИ ТЕТА-ВСПЫШКАМИ НА ПОКАЗАТЕЛИ РАБОЧЕЙ ПАМЯТИ

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Исследование протоколов транскраниальной магнитной стимуляции (ТМС), основанных на метапластичности, является интенсивно изучаемым подходом к улучшению эффективности стимуляции. Однако эффекты протоколов с разным интервалом между блоками ТМС в отношении когнитивных функций изучены недостаточно. Целью работы было оценить эффект двух протоколов стимуляции тета-вспышками (iTBS) с коротким и длинным интервалами между блоками на показатели рабочей памяти (РП) у здоровых добровольцев. В случайном порядке 16 участникам проводили по одной сессии ТМС каждым протоколом (iTBS 0–15 — два блока iTBS левой дорсолатеральной префронтальной коры (лДЛПФК) с интервалом 15 мин между ними и последующей стимуляцией области вертекса через 60 мин после первого блока; iTBS 0–60 — блок iTBS лДЛПФК, блок стимуляции вертекса через 15 мин и второй блок iTBS лДЛПФК через 60 мин после первого, iTBS 0 — один блок iTBS лДЛПФК с двумя блоками стимуляции вертекса и контрольный протокол — три блока стимуляции вертекса с аналогичными интервалами). РП оценивали с помощью теста n-back перед первым, после второго и третьего блоков стимуляции. Статистически значимых эффектов протоколов на РП, а также различий между протоколами по изменению показателей теста или количеству участников, ответивших на ТМС, обнаружено не было. Тенденция к статистической значимости показана для протокола с коротким интервалом (iTBS 0–15). Кроме того, подтверждена низкая индивидуальная воспроизводимость эффекта iTBS. Перспективными представляются исследование протоколов с коротким интервалом между блоками на более крупных выборках добровольцев, а также учет других факторов, потенциально влияющих на эффект протокола (количество блоков и длительность одного блока).

Ключевые слова: транскраниальная магнитная стимуляция, стимуляция тета-вспышками, неинвазивная нейромодуляция, метапластичность, рабочая память, левая дорсолатеральная префронтальная кора

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Transcranial magnetic stimulation (TMS) is widely used in clinical practice and research [1, 2]. However, high variability of effects is still an important limitation of the TMS use [3]. Protocols that are based on metaplasticity mechanisms are being actively developed in order to improve the effectiveness of TMS. According to this concept, the magnitude, direction, and duration of the synaptic plasticity processes depend on the previous synaptic activity. There can be additive or homeostatic metaplasticity [4, 5]. It has been shown that metaplasticity has a significant impact on the effects of the combinations of TMS protocols [6].

The effects of combined TMS protocols depend on both the type of individual blocks of stimulation and the intervals between blocks. The effects of intervals between blocks can be seen from the protocols that include several blocks of the same type [6–9]. These data provided the basis for the hypothesis of "critical window", according to which homeostatic metaplasticity can be induced when applying the second stimulation block within an interval representing a middle third of the expected duration of the effect of a single block, and additive metaplasticity is induced when using a shorter or longer interval [6].

The protocols with short intervals between blocks (up to 20 min) were primarily studied in healthy volunteers, and these studies yielded conflicting data [7, 10, 11]. Our study of two combined intermittent theta-burst stimulation (iTBS) protocols with short (15 min) and long (60 min) intervals between the primary motor cortex stimulation blocks revealed no significant effects of individual protocols or differences between protocols when assessing the effects on the amplitude of motor evoked potentials (MEPs) and the responder rates [12].

The authors of the majority of papers studied effects on the motor cortex excitability. Despite the fact that stimulation of motor cortex provides a convenient model, the results should be extrapolated to other cortical areas with caution. Variability of the MEP amplitude is an important limitation of the neurophysiological assessment of the motor cortex stimulation effect [13]. It is therefore reasonable to study stimulation of non-motor areas and use behavioral and other measurements for assessment of the effect.

Considering these limitations, the study was aimed to assess the effects of iTBS protocols with short and long intervals between the blocks of stimulation over the left dorsolateral prefrontal cortex (DLPFC) on the scores the n-back test for assessment of verbal working memory (WM) in healthy volunteers and to perform comparison with the standard iTBS protocol and stimulation of the control site (vertex). The combined protocols were selected based on the "critical window" hypothesis [6].

METHODS

Subjects

The study was performed in the Research Center of Neurology in 2021–2022. Participants completed a questionnaire on contraindications to TMS before inclusion in the study. Medical history of each participant was obtained and demographic data were acquired, the subjects underwent routine electroencephalography (EEG) with standard functional tests in order to exclude epileptiform activity.

Inclusion criteria: informed consent; age 18–40 years.

Non-inclusion criteria: refusal to participate; contraindications to MRI and TMS [14]; epileptiform activity on EEG; the use of medications that exert effects on the central nervous system; neurological or mental disorders; chronic somatic disease.

Exclusion criteria: severe side effects revealed during the TMS procedure (epileptic seizure, syncope, etc.); the onset of somatic, mental or neurological disorder after inclusion; pacemaker implantation, intracardiac catheter insertion or brain surgery involving placing metal objects in the cranial cavity; getting pregnant; refusal to continue participating in the study.

A total of 22 volunteers were screened, among them two people had the non-inclusion criteria, the other two were unable to continue participating in the study for logistical reasons. Two people dropped out due to poor tolerability of TMS. Thus, a total of 16 subjects completed the study (6 males; average age 28.1 years).

Stimulation protocols

To construct an individual 3D model of the brain for navigated TMS, MRI was performed in the 3D-T1-MPR mode using the MAGNETOM Verio and MAGNETOM Prisma scanners (Siemens Healthcare GmbH; Germany) (voxel size 1.0 – 0.977 – 0.977 mm^3, 176 sagittal slices).

The volunteers underwent four TMS sessions with an interval of at least 72 h (Fig. 1A). Such an interval seemed to be sufficient to minimize the impact of the previous session considering the duration of the single iTBS block effect [15]. The protocol sequences were randomized according to a Latin square approach to minimize the sequence effects. All attempts were made to perform sessions at the same time interval of the day (9–13 or 14–18 h). The participants were not informed about the sequence of protocols applied.

The following protocols were studied (Fig. 1B):

– the combined protocol with a short interval between blocks (iTBS 0–15): two consecutive blocks of active stimulation with a 15 min interval between blocks and a control stimulation block 60 min after the first block;

– the combined protocol with a long interval between blocks (iTBS 0–60): a block of active stimulation followed by a control stimulation block with an interval of 15 min and a block of active stimulation 60 min after the first block;

– the standard protocol (iTBS 0): a block of active stimulation followed by the control stimulation blocks in 15 and 60 min;

– the control protocol (Control): three control stimulation blocks with intervals of 15 and 60 min.

The iTBS procedure was performed using the MagPro X100 + MagOption stimulation device (Tonica Elektronik A/S; Denmark) with a liquid-cooled figure-eight coil in combination with the Localite TMS Navigator System (Localite GmbH; Germany) and the Axillum Robotics TMS-Cobot robotic positioning system (Axillum Robotics; France). Each stimulation block consisted of 20 cycles that included 10 bursts of three stimuli with a frequency of 50 Hz, applied with a frequency of 5 Hz and divided into 2-second trains with an intertrain interval of 8 s. The number of stimuli per block was 600. The left DLPFC, defined on MRI scans as a region of superior or middle frontal gyrus located about 5 cm from the "hot spot" of the first dorsal interosseous muscle cortical representation, was used as a target for active stimulation. The vertex area defined as a zone located halfway between the glabella and the occipital protuberance in the midsagittal plane was used as a target for control stimulation. The iTBS intensity constituted 75% of the resting motor threshold (rMT) defined using the Rossini-Rothwell algorithm, an intensity, for which the most prominent effect was previously shown [16]. rMT was determined before each session of stimulation. The questionnaires on adverse events (AEs) were completed during the TMS procedure and within 24 h after TMS in order to assess tolerability.

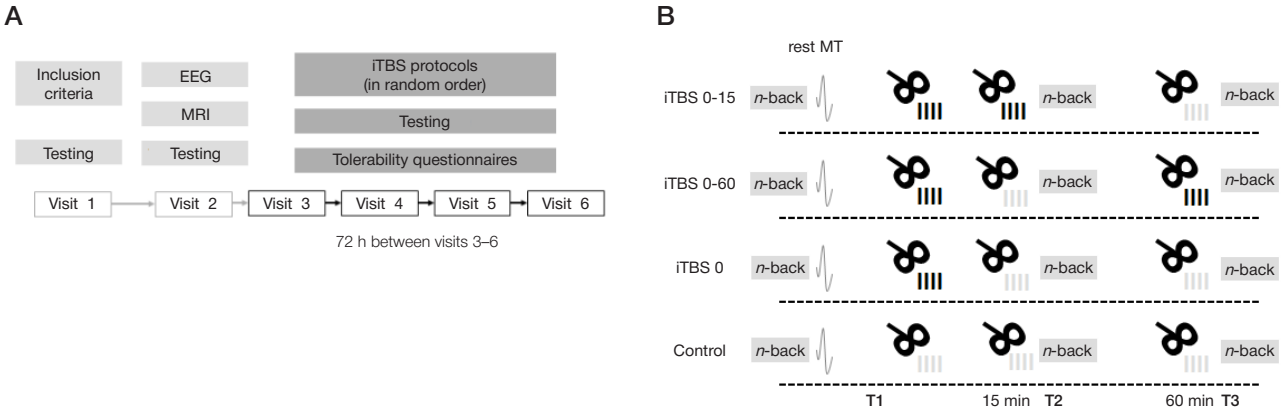

Fig. 1. A. Study design. **B**. Theta-burst stimulation protocols

Cognitive tests

Tests were performed using the Psychology Experiment Building Language (PEBL) open source software [17]. The *n*-back test involving presentation of verbal stimuli (Latin consonants) was performed with *n* = 2, 3, 4 (22, 23 and 24 stimuli per task, 6 matching letters per *n*). The training test was conducted twice in order to minimize the learning effect; furthermore, preliminary training test with *n* = 1 and 2 was performed prior to each session at the first testing. Performance was assessed three times: before the start of the first stimulation block (T1) and immediately after the second (T2) and the third (T3) stimulation blocks.

The *n*-back task accuracy was assessed by calculating *d*'-value [18].

$$d' = Z(\text{hit rate}) - Z(\text{false alarm rate}).$$

The calculation took into account the number of correct keystrokes in response to the concordant stimulus normalized to the total number of concordant stimuli (hit rate) and the number of false keystrokes in response to the discordant stimulus normalized to the total number of discordant stimuli for each n (false alarm rate). Z transformation was applied to each normalized measurement.

Statistical analysis

The IBM SPSS Statistics (v.23) software package (IBM, SPSS Inc.; USA) was used for statistical analysis. Individual effects of each protocol at T2 and T3 (comparison of d' scores with T1) were assessed using the Wilcoxon's signed-rank test. The effects of the protocol at T2 and T3 were estimated as the difference between d' at this time point and the value at T1. The Friedman test was used to compare the effects of different protocols at T2 and T3.

Depending on the changes of d' at T2 and T3 the subjects were divided into responders (facilitators, when the difference was above 0, or inhibitors, when the difference was below 0) and non-responders (the difference between the values was 0). The proportions of responders were compared between the protocols using the binomial test (exact McNemar's test).

In addition, reproducibility of the effect of the combination of active stimulation block with the vertex stimulation (T2 in the iTBS 0–60 and iTBS 0 protocols) was assessed twice using Spearman's rank correlation coefficient and the

Table 1. The effects of iTBS protocols on the *n*-back test accuracy

Protocol	T2–T1	*p*	T3–T1	*p*
n = 2				
iTBS0-15	0	0.058	0	1
iTBS0-60	0	0.874	0.0435	0.2
iTBS0	0	0.502	0	0.866
Control	0	0.331	0	0.362
n = 3				
iTBS0-15	–0.003	0.363	0.555	0.054
iTBS0-60	–0.397	0.094	0.208	0.865
iTBS0	0.129	0.507	–0.106	0.851
Control	–0.268	0.495	0	0.944
n = 4				
iTBS0-15	0.186	0.28	0.292	0.624
iTBS0-60	0.058	0.875	–0.360	0.293
iTBS0	0.405	0.094	–0.484	0.14
Control	0	0.826	–0.405	0.078

Note: T2–T1 — median difference (d') between T2 and T1; T3–T1 — between T3 and T1; uncorrected *p*-values are provided.

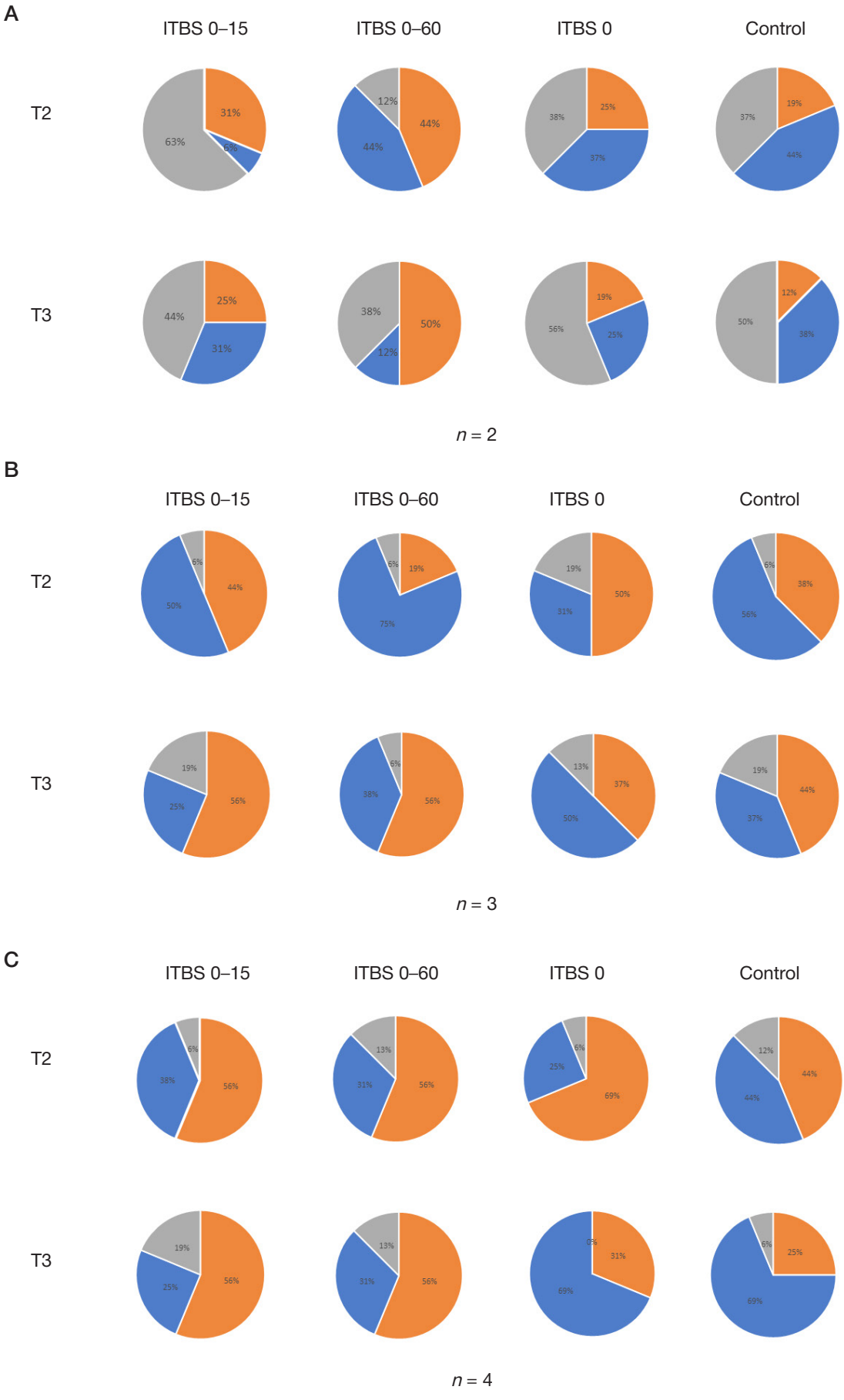

Fig. 2. Percentage of participants with various types of response to TMS protocols (A for *n* = 2, B for *n* = 3, C for *n* = 4). Facilitation is highlighted in orange, inhibition in blue, and no response in grey

Table 2. *P*-values for comparison of the number of subjects with various response types between protocols (uncorrected)

n	T	iTBS 0–15 vs. iTBS 0–60	iTBS 0–15 vs. iTBS 0	iTBS 0–15 vs. Control	iTBS 0–60 vs. iTBS 0	iTBS 0–60 vs. Control	iTBS 0 vs. Control
Facilitation							
n = 2	T2	0.726	1	0.688	0.549	0.289	1
	T3	0.289	1	0.688	0.18	0.07	1
n = 3	T2	0.289	1	1	0.227	0.375	0.754
	T3	1	0.508	0.754	0.508	0.688	1
n = 4	T2	1	0.727	0.688	0.727	0.754	0.219
	T3	0.289	0.289	0.125	1	1	1
Inhibition							
n = 2	T2	0.031	0.125	0.07	1	1	1
	T3	1	1	0.453	0.688	0.289	0.688
n = 3	T2	0.219	0.549	1	0.039	0.375	0.289
	T3	0.688	0.289	0.727	0.754	1	0.727
n = 4	T2	1	0.727	1	1	0.727	0.375
	T3	0.07	0.065	0.039	1	1	1

association analysis of the response type at T2 using the Fisher's exact test.

RESULTS

Assessing the effects of individual protocols

Assessment of the effects of protocols on the *n*-back accuracy at T2 and T3 revealed no significant differences (Table 1). The lowest *p*-values were obtained for the accuracy of *n*-back test with $n = 2$ after the second stimulation block of the iTBS 0–15 protocol ($p = 0.058$), and for $n = 3$ after the third stimulation block of the same protocol ($p = 0.054$); the Bonferroni adjusted *p*-value were 1.

The percentage of subjects showing different response to TMS at T2 and T3 was calculated for each protocol (Fig. 2)

Comparing the effects of protocols

No significant differences in the effects between protocols for any of *n*-values were revealed when performing comparison at T2 (Friedman test; uncorrected $p = 0.6$, 0.62 and 0.428 for $n = 2$, 3, 4, respectively) and T3 ($p = 0.283$, 0.294 and 0.13). No differences were found when comparing the effects immediately after two blocks of active stimulation, i.e. between iTBS 0–15 at T2 and iTBS 0–60 at T3 (Wilcoxon's signed-rank test; uncorrected $p = 0.372$; $p = 0.535$; $p = 0.211$ for $n = 2$, 3 and 4).

Asssessing the differences in the direction of the TMS protocol effects

As for the rate of participants showing facilitation, no significant differences were revealed (Table 2). Uncorrected *p*-values lower than 0.05 were obtained when comparing the percentage of subjects showing inhibition in the iTBS 0–15 and iTBS 0–60 protocols at T2 for $n = 2$ and the iTBS 0–15 and Control protocols at T3 for $n = 4$. Furthermore, comparison of inhibition in the iTBS 0–15 and iTBS 0–60 protocols at T2 yielded a p-value lower than 0.05. The Bonferroni adjusted *p*-values for these tests were 1.

Assessing reproducibility of the effect

A *p*-value of 0.02 was obtained for $n = 2$ (negative Spearman's sample correlation coefficient), the Bonferroni adjusted *p*-value was 0.06 (Table 3).

Association analysis of both facilitation and inhibition revealed no significant correlation between the iTBS 0–60 and iTBS 0 protocols (Table 4). Furthermore, only 6 subjects out of 16 (37.5%) showed facilitation at T2 for $n = 4$ in both protocols, iTBS 0–60 and iTBS 0, while the lower complexity tests revealed no subjects showing similar facilitatory response to both protocols.

Tolerability of protocols

The studied TMS protocols were characterized by favorable safety profile. No serious AEs were reported. Two volunteers discontinued participation in the study due to poor tolerance (one case of severe pain during stimulation of the left DLPFC and one case of headache during stimulation of the vertex persisting for a few hours after stimulation and resolving after taking ibuprofen). The AEs reported during the 67.2% of session and within 24 h after 8% of the assessed sessions were mild and had no impact on the desire to continue participation in the study. Pain and sleepiness were most often reported during stimulation (28.3% each), along with the contraction of

Table 3. Correlation of the effects of two iterations of the combination of active stimulation block with the vertex stimulation (T2 in the iTBS 0–60 and iTBS 0 protocols) with the *n*-back test accuracy

n	*n* = 2		*n* = 3		*n* = 4	
Parameter	ρ	*p*	ρ	*p*	ρ	*p*
iTBS 0–60 vs. iTBS 0 (T2)	–0.573	0. 02	–0.157	0.563	0.274	0.304

Note: ρ — Spearman's rank correlation coefficient, *p* — uncorrected *p*-value.

Table 4. Association analysis of the direction of responses to two iterations of the combination of active stimulation block with the vertex stimulation (T2 in the iTBS 0–60 and iTBS 0 protocols)

Facilitation				
n = 2		Facilitation in iTBS 0	No facilitation in iTBS 0	
	Facilitation in iTBS 0–60	0/16	7/16	*p* = 0.088
	No facilitation in iTBS 0–60	4/16	5/16	
n = 3		Facilitation in iTBS 0	No facilitation in iTBS 0	
	Facilitation in iTBS 0–60	0/16	3/16	*p* = 0.200
	No facilitation in iTBS 0–60	8/16	5/16	
n = 4		Facilitation in iTBS 0	No facilitation in iTBS 0	
	Facilitation in iTBS 0–60	6/16	3/16	*p* = 1
	No facilitation in iTBS 0–60	5/16	2/16	
Inhibition				
n = 2		Inhibition in iTBS 0	No inhibition in iTBS 0	
	Inhibition in iTBS 0–60	2/16	5/16	*p* = 0.633
	No inhibition in iTBS 0–60	4/16	5/16	
n = 3		Inhibition in iTBS 0	No inhibition in iTBS 0	
	Inhibition in iTBS 0–60	4/16	8/16	*p* = 1
	No inhibition in iTBS 0–60	1/16	3/16	
n = 4		Inhibition in iTBS 0	No inhibition in iTBS 0	
	Inhibition in iTBS 0–60	2/16	3/16	*p* = 0.546
	No inhibition in iTBS 0–60	2/16	9/16	

Note: uncorrected *p*-values are provided (Fisher's exact test).

facial muscles in the vicinity of the left DLPFC (9%); within 24 h headache was the only AE reported (8%).

DISCUSSION

The aim of the study was to assess the effects of two metaplasticity- based theta-burst stimulation protocols of the left DLPFC with short and long intervals between blocks on the WM performance in healthy individuals. The effects were also compared with that of the standard and control protocols. We estimated the differences in the number of participants with the same direction of stimulation effects in various protocols as well. The protocols applied were safe and well tolerated. No convincing data to confirm the effectiveness of individual protocols on the WM or variability of the response to stimulation were obtained. Low reproducibillity of individual iTBS effects was reported.

The effect of a single iTBS block on the WM performance in healthy individuals was explored in a number of projects, however, these studies yielded inconsistent results [16, 19–21]. Variability of response to stimulation confirmed for the effect on the motor cortex excitability can be one of the sources of differences [22, 23]. At the same time, variability of the iTBS effects in terms of WM is still poorly understood.

The use of metaplasticity-based protocols is a method to potentially increase the effectiveness of TMS, however, the problem of optimal interval between blocks of stimulation is not resolved. We compared the effects of protocols with short and long intervals between active stimulation blocks on the WM performance. No significant differences between the test results for individual protocols were reported. Furthermore, comparison of metaplasticity-based protocols with the standard and control protocols revealed no significant differences in alterations of the n-back test accuracy at both time points. There were also no significant differences in the number of subjects who showed better (facilitation) or worse (inhibition) performance during testing between protocols. Such results are consistent with our previously reported data obtained for the effects on the motor cortex excitability [12].

At the same time, a possible trend toward statistical significance of the effects of the protocol with short interval between blocks (iTBS 0–15) on the *n*-back test with $n = 2$, when performing measurement after the second block, and $n = 3$, when performing measurement after the third block, is noteworthy. In the studied sample, a lower number of participants, who showed inhibitory response after the second stimulation block in this protocol, compared to the iTBS 0–60 protocol for $n = 2$, and after the control protocol for $n = 4$, was also observed. It is interesting to note that the stimulation protocol consisting of three blocks with an interval of 15 min between blocks significantly improved the visuospatial WM, executive functions [24], and decision-making in healthy individuals [25]. Furthermore, it was shown that 14 sessions of stimulation using this protocol improved cognitive functions in patients with Alzheimer's disease [26]. In our opinion, it seems appropriate to continue studying the effects of protocols with short intervals between blocks (15 min) on cognitive functions.

In addition, we assessed reproducibility of the iTBS 0–60 and iTBS 0 protocol effects after the second stimulation block. No significant correlation of the effect or association of the response direction between two protocols was reported. Assessment of the tests with $n = 2$ and 3 yielded 0% of participants showing facilitation, while the percentage for $n = 4$ was 37.5%. The findings are consistent with the results of earlier studies focused an assessing variability of the response to a single block of the motor cortex theta-burst stimulation [7, 22, 23]. We can conclude that the response to iTBS has low intra-individual variability in terms of both motor cortex excitability and cognitive performance. In our study, the sources of variability associated with anatomical features and the changes in the coil position were minimized by MRI navigation and the use of robotic coil positioning system, therefore, it can be assumed that intra-individual variability of the response to iTBS is the cause of insufficient stimulation effect reproducibility.

The lack of stimulation effect reported in our study may result from insufficient number of active stimulation blocks. The earlier reported study also showed no significant effect of two blocks of the left DLPFC iTBS with an interval of 15 min between blocks on the n-back test results [21]. The assumption of the higher effectiveness of protocols consisting of three blocks is in line with the results of the earlier study showing a significant effect of three, not two, blocks of motor cortex stimulation with an interval of 15 min between blocks [27], and with the earlier reported data on the effectiveness of the DLPFC stimulation protocol consisting of three blocks with an interval of 15 min [24]. It should be noted that the metaplasticity-based stimulation protocols than have shown some clinical efficacy, for example in drug-resistant depression [28, 29] or spasticity associated with multiple sclerosis [30], consist of 10 and three stimulation blocks, respectively.

Furthermore, the duration of a single block may have an impact on its effect. The protocols that have shown clinical efficacy comprise prolonged stimulation blocks (1800 stimuli compared to 600 in the standard one) [28–30]. However, to date, the effects of prolonged iTBS blocks on cognitive functions are poorly understood.

Small cohort size can be considered one of the limitations of the study, however, in the current pilot study the sample size may be enough for detection of large effects and selection of the most effective protocols to be studied in the larger cohorts. Furthermore, the crossover study design can affect the test results due to learning effect. On the other hand, the impact of this effect seems to be minimal: first, when performing re-tests within the protocol the effect is controlled by comparison with the protocol comprising the same number of the vertex stimulation blocks. Second, the average effect values reported during sessions do not depend on possible effects of the session sequence number due to Latin square randomization, i.e. possible learning effect between sessions does not cause bias in estimates of the differences between protocols.

The use of only one *n*-back test with verbal stimuli can be considered one more limitation of the study. However, it is widely used in neuropsychological research for assessment of WM. It is also necessary to bear in mind the ceiling effect observed when performing the lowest complexity test ($n = 2$). Such an effect can explain a high non-responder rate observed at this n. A small number of stimuli per task can be considered one more limitation that should be taken into account when performing further research. Furthermore, we assessed the effects of stimulation immediately after the second and the third block. This does not exclude possible delayed effects [19].

It should be noted that the lack of effects of metaplasticity-based protocols on both cognitive test results and neurophysiological parameters in healthy volunteers does not mean a lack of clinical efficacy. It is important to consider that metaplasticity patterns observed in patients and healthy volunteers may be different, that is why the findings should be translated into clinical practice with caution.

CONCLUSIONS

The study yielded no convincing data to support the effectiveness of the metaplasticity-based protocols on the WM performance and direction of the response to stimulation in healthy individuals. Considering the findings and limitations, further study of the effects of protocols with short intervals between blocks consisting of the larger number of stimulation blocks and comprising prolonged iTBS blocks seems to be promising.

References

1. Beynel L, Appelbaum LG, Luber B, Crowell CA, Hilbig SA, Lim W, et al. Effects of online repetitive transcranial magnetic stimulation (rTMS) on cognitive processing: A meta-analysis and recommendations for future studies. Neurosci Biobehav Rev. 2019; 107: 47–58. DOI: 10.1016/j.neubiorev.2019.08.018. PMID: 31473301; PMCID: PMC7654714.
2. Lefaucheur JP, Aleman A, Baeken C, Benninger DH, Brunelin J, Di Lazzaro V, et al. Evidence-based guidelines on the therapeutic use of repetitive transcranial magnetic stimulation (rTMS): An update (2014–2018). Clin Neurophysiol. 2020; 131 (2): 474–528. DOI: 10.1016/j.clinph.2019.11.002. PMID: 31901449.
3. Goldsworthy MR, Hordacre B, Rothwell JC, Ridding MC. Effects of rTMS on the brain: is there value in variability? Cortex. 2021; 139: 43–59. DOI: 10.1016/j.cortex.2021.02.024. PMID: 33827037.
4. Abraham WC, Bear MF. Metaplasticity: the plasticity of synaptic plasticity. Trends Neurosci. 1996; 19 (4): 126–30. DOI: 10.1016/s0166-2236(96)80018-x. PMID: 8658594.
5. Bakulin IS, Poydasheva AG, Zabirova AH, Suponeva NA, Piradov MA. Metaplasticity and non-invasive brain stimulation: the search for new biomarkers and directions for therapeutic neuromodulation. Annals of clinical and experimental neurology. 2022; 16(3): 74–82. Russian. DOI: 10.54101/ACEN.2022.3.9.
6. Hassanzahraee M, Zoghi M, Jaberzadeh S. How different priming stimulations affect the corticospinal excitability induced by noninvasive brain stimulation techniques: a systematic review and meta-analysis. Rev Neurosci. 2018; 29 (8): 883–99. DOI: 10.1515/revneuro-2017-0111. PMID: 29604209.
7. Tse NY, Goldsworthy MR, Ridding MC, Coxon JP, Fitzgerald PB, Fornito A, et al. The effect of stimulation interval on plasticity following repeated blocks of intermittent theta burst stimulation. Sci Rep. 2018; 8 (1): 8526. DOI: 10.1038/s41598-018-26791-w. PMID: 29867191.
8. Thomson AC, Sack AT. How to Design Optimal Accelerated rTMS Protocols Capable of Promoting Therapeutically Beneficial Metaplasticity. Front Neurol. 2020; 11: 599918. DOI: 10.3389/fneur.2020.599918. PMID: 33224103.
9. Yu F, Tang X, Hu R, Liang S, Wang W, Tian S, et al. The After-Effect of Accelerated Intermittent Theta Burst Stimulation at Different Session Intervals. Front Neurosci. 2020; 14: 576. DOI: 10.3389/fnins.2020.00576. Erratum in: Front Neurosci. 2021; 15: 687972. PMID: 32670006.
10. Gamboa OL, Antal A, Laczo B, Moliadze V, Nitsche MA, Paulus W. Impact of repetitive theta burst stimulation on motor cortex excitability. Brain Stimul. 2011; 4 (3): 145–51. DOI: 10.1016/j.brs.2010.09.008. PMID: 21777874.
11. Murakami T, Müller-Dahlhaus F, Lu MK, Ziemann U. Homeostatic metaplasticity of corticospinal excitatory and intracortical inhibitory neural circuits in human motor cortex. J Physiol. 2012; 590 (22): 5765–81. DOI: 10.1113/jphysiol.2012.238519. PMID: 22930265.
12. Bakulin I, Zabirova A, Sinitsyn D, Poydasheva A, Lagoda D, Suponeva N, et al. Adding a Second iTBS Block in 15 or 60 Min Time Interval Does Not Increase iTBS Effects on Motor Cortex Excitability and the Responder Rates. Brain Sci. 2022; 12 (8): 1064. DOI: 10.3390/brainsci12081064. PMID: 36009127.
13. Vallence AM, Goldsworthy MR, Hodyl NA, Semmler JG, Pitcher JB, Ridding MC. Inter- and intra-subject variability of motor cortex plasticity following continuous theta-burst stimulation. Neuroscience. 2015; 304: 266–78. DOI: 10.1016/j.neuroscience.2015.07.043. PMID: 26208843.
14. Rossi S, Hallett M, Rossini PM, Pascual-Leone A, Safety of TMS Consensus Group. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in

clinical practice and research. Clin Neurophysiol. 2009; 120 (12): 2008–39. DOI: 10.1016/j.clinph.2009.08.016. PMID: 19833552.
15. Rounis E, Huang YZ. Theta burst stimulation in humans: a need for better understanding effects of brain stimulation in health and disease. Exp Brain Res. 2020; 238 (7–8): 1707–14. DOI: 10.1007/s00221-020-05880-1. PMID: 32671422.
16. Chung SW, Rogasch NC, Hoy KE, Sullivan CM, Cash RFH, Fitzgerald PB. Impact of different intensities of intermittent theta burst stimulation on the cortical properties during TMS-EEG and working memory performance. Hum Brain Mapp. 2018; 39 (2): 783–802. DOI: 10.1002/hbm.23882. PMID: 29124791.
17. Mueller ST, Piper BJ. The Psychology Experiment Building Language (PEBL) and PEBL Test Battery. J Neurosci Methods. 2014; 222: 250–9. DOI: 10.1016/j.jneumeth.2013.10.024. PMID: 24269254.
18. Haatveit BC, Sundet K, Hugdahl K, Ueland T, Melle I, Andreassen OA. The validity of d prime as a working memory index: results from the "Bergen n-back task". J Clin Exp Neuropsychol. 2010; 32 (8): 871–80. DOI: 10.1080/13803391003596421. PMID: 20383801.
19. Hoy KE, Bailey N, Michael M, Fitzgibbon B, Rogasch NC, Saeki T, et al. Enhancement of Working Memory and Task-Related Oscillatory Activity Following Intermittent Theta Burst Stimulation in Healthy Controls. Cereb Cortex. 2016; 26 (12): 4563–73. DOI: 10.1093/cercor/bhv193. PMID: 26400923.
20. Viejo-Sobera R, Redolar-Ripoll D, Boixadós M, Palaus M, Valero-Cabré A, Marron EM. Impact of Prefrontal Theta Burst Stimulation on Clinical Neuropsychological Tasks. Front Neurosci. 2017; 11: 462. DOI: 10.3389/fnins.2017.00462. PMID: 28867993.
21. Chung SW, Rogasch NC, Hoy KE, Fitzgerald PB. The effect of single and repeated prefrontal intermittent theta burst stimulation on cortical reactivity and working memory. Brain Stimul. 2018; 11 (3): 566–74. DOI: 10.1016/j.brs.2018.01.002. PMID: 29352668.
22. Perellón-Alfonso R, Kralik M, Pileckyte I, Princic M, Bon J, Matzhold C, et al. Similar effect of intermittent theta burst and sham stimulation on corticospinal excitability: A 5-day repeated sessions study. Eur J Neurosci. 2018; 48 (4): 1990–2000. DOI: 10.1111/ejn.14077. PMID: 30022548.
23. Boucher PO, Ozdemir RA, Momi D, Burke MJ, Jannati A, Fried PJ, et al. Sham-derived effects and the minimal reliability of theta burst stimulation. Sci Rep. 2021; 11 (1): 21170. DOI: 10.1038/s41598-021-98751-w. PMID: 34707206.
24. Wu X, Wang L, Geng Z, Wei L, Yan Y, Xie C, et al. Improved Cognitive Promotion through Accelerated Magnetic Stimulation. eNeuro. 2021; 8 (1): ENEURO.0392-20.2020. DOI: 10.1523/ENEURO.0392-20.2020. PMID: 33452108.
25. Wang L, Wu X, Ji GJ, Xiao G, Xu F, Yan Y, et al. Better modulation for risk decision-making after optimized magnetic stimulation. J Neurosci Res. 2021; 99 (3): 858–71. DOI: 10.1002/jnr.24772. PMID: 33617027.
26. Wu X, Ji GJ, Geng Z, Wang L, Yan Y, Wu Y, et al. Accelerated intermittent theta-burst stimulation broadly ameliorates symptoms and cognition in Alzheimer's disease: A randomized controlled trial. Brain Stimul. 2022; 15 (1): 35–45. DOI: 10.1016/j.brs.2021.11.007. PMID: 34752934.
27. Nettekoven C, Volz LJ, Kutscha M, Pool EM, Rehme AK, Eickhoff SB, et al. Dose-dependent effects of theta burst rTMS on cortical excitability and resting-state connectivity of the human motor system. J Neurosci. 2014; 34 (20): 6849–59. DOI: 10.1523/JNEUROSCI.4993-13.2014. PMID: 24828639.
28. Poydasheva AG, Bakulin IS, Sinitsyn DO, Zabirova AH, Suponeva NA, Maslenikov NV, et al. Experience of Stanford neuromodulation therapy in patients with treatment-resistant depression. Bulletin of RSMU. 2022; (4): 31–7. Russian. DOI: 10.24075/brsmu.2022.044.
29. Cole EJ, Phillips AL, Bentzley BS, Stimpson KH, Nejad R, Barmak F, et al. Stanford Neuromodulation Therapy (SNT): A Double-Blind Randomized Controlled Trial. Am J Psychiatry. 2022; 179 (2): 132–41. DOI: 10.1176/appi.ajp.2021.20101429. PMID: 34711062.
30. Bakulin IS, Poydasheva AG, Zabirova AH, Lagoda DY, Rimkevichus AA, Zakharova MN, et al. Use of a metaplasticity-based protocol of therapeutic transcranial magnetic stimulation in patients with progressive multiple sclerosis and spasticity: first experience. Neuromuscular Diseases. 2022; 12 (3): 26–35. DOI: 10.17650/2222-8721-2022-12-3-26-35. Russian.

Литература

1. Beynel L, Appelbaum LG, Luber B, Crowell CA, Hilbig SA, Lim W, et al. Effects of online repetitive transcranial magnetic stimulation (rTMS) on cognitive processing: A meta-analysis and recommendations for future studies. Neurosci Biobehav Rev. 2019; 107: 47–58. DOI: 10.1016/j.neubiorev.2019.08.018. PMID: 31473301; PMCID: PMC7654714.
2. Lefaucheur JP, Aleman A, Baeken C, Benninger DH, Brunelin J, Di Lazzaro V, et al. Evidence-based guidelines on the therapeutic use of repetitive transcranial magnetic stimulation (rTMS): An update (2014–2018). Clin Neurophysiol. 2020; 131 (2): 474–528. DOI: 10.1016/j.clinph.2019.11.002. PMID: 31901449.
3. Goldsworthy MR, Hordacre B, Rothwell JC, Ridding MC. Effects of rTMS on the brain: is there value in variability? Cortex. 2021; 139: 43–59. DOI: 10.1016/j.cortex.2021.02.024. PMID: 33827037.
4. Abraham WC, Bear MF. Metaplasticity: the plasticity of synaptic plasticity. Trends Neurosci. 1996; 19 (4): 126–30. DOI: 10.1016/s0166-2236(96)80018-x. PMID: 8658594.
5. Бакулин И. С., Пойдашева А. Г., Забирова А. Х., Супонева Н. А., Пирадов М. А. Метапластичность и неинвазивная стимуляция мозга: поиск новых биомаркеров и направлений терапевтической нейромодуляции. Анналы клинической и экспериментальной неврологии. 2022; 16 (3): 74–82. DOI: 10.54101/ACEN.2022.3.9.
6. Hassanzahraee M, Zoghi M, Jaberzadeh S. How different priming stimulations affect the corticospinal excitability induced by noninvasive brain stimulation techniques: a systematic review and meta-analysis. Rev Neurosci. 2018; 29 (8): 883–99. DOI: 10.1515/revneuro-2017-0111. PMID: 29604209.
7. Tse NY, Goldsworthy MR, Ridding MC, Coxon JP, Fitzgerald PB, Fornito A, et al. The effect of stimulation interval on plasticity following repeated blocks of intermittent theta burst stimulation. Sci Rep. 2018; 8 (1): 8526. DOI: 10.1038/s41598-018-26791-w. PMID: 29867191.
8. Thomson AC, Sack AT. How to Design Optimal Accelerated rTMS Protocols Capable of Promoting Therapeutically Beneficial Metaplasticity. Front Neurol. 2020; 11: 599918. DOI: 10.3389/fneur.2020.599918. PMID: 33224103.
9. Yu F, Tang X, Hu R, Liang S, Wang W, Tian S, et al. The After-Effect of Accelerated Intermittent Theta Burst Stimulation at Different Session Intervals. Front Neurosci. 2020; 14: 576. DOI: 10.3389/fnins.2020.00576. Erratum in: Front Neurosci. 2021; 15: 687972. PMID: 32670006.
10. Gamboa OL, Antal A, Laczo B, Moliadze V, Nitsche MA, Paulus W. Impact of repetitive theta burst stimulation on motor cortex excitability. Brain Stimul. 2011; 4 (3): 145–51. DOI: 10.1016/j.brs.2010.09.008. PMID: 21777874.
11. Murakami T, Müller-Dahlhaus F, Lu MK, Ziemann U. Homeostatic metaplasticity of corticospinal excitatory and intracortical inhibitory neural circuits in human motor cortex. J Physiol. 2012; 590 (22): 5765–81. DOI: 10.1113/jphysiol.2012.238519. PMID: 22930265.
12. Bakulin I, Zabirova A, Sinitsyn D, Poydasheva A, Lagoda D, Suponeva N, et al. Adding a Second iTBS Block in 15 or 60 Min Time Interval Does Not Increase iTBS Effects on Motor Cortex Excitability and the Responder Rates. Brain Sci. 2022; 12 (8): 1064. DOI: 10.3390/brainsci12081064. PMID: 36009127.
13. Vallence AM, Goldsworthy MR, Hodyl NA, Semmler JG, Pitcher JB, Ridding MC. Inter- and intra-subject variability of motor cortex plasticity following continuous theta-burst stimulation. Neuroscience. 2015; 304: 266–78. DOI: 10.1016/j.neuroscience.2015.07.043. PMID: 26208843.
14. Rossi S, Hallett M, Rossini PM, Pascual-Leone A, Safety of TMS Consensus Group. Safety, ethical considerations, and application

guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clin Neurophysiol. 2009; 120 (12): 2008–39. DOI: 10.1016/j.clinph.2009.08.016. PMID: 19833552.
15. Rounis E, Huang YZ. Theta burst stimulation in humans: a need for better understanding effects of brain stimulation in health and disease. Exp Brain Res. 2020; 238 (7–8): 1707–14. DOI: 10.1007/s00221-020-05880-1. PMID: 32671422.
16. Chung SW, Rogasch NC, Hoy KE, Sullivan CM, Cash RFH, Fitzgerald PB. Impact of different intensities of intermittent theta burst stimulation on the cortical properties during TMS-EEG and working memory performance. Hum Brain Mapp. 2018; 39 (2): 783–802. DOI: 10.1002/hbm.23882. PMID: 29124791.
17. Mueller ST, Piper BJ. The Psychology Experiment Building Language (PEBL) and PEBL Test Battery. J Neurosci Methods. 2014; 222: 250–9. DOI: 10.1016/j.jneumeth.2013.10.024. PMID: 24269254.
18. Haatveit BC, Sundet K, Hugdahl K, Ueland T, Melle I, Andreassen OA. The validity of d prime as a working memory index: results from the "Bergen n-back task". J Clin Exp Neuropsychol. 2010; 32 (8): 871–80. DOI: 10.1080/13803391003596421. PMID: 20383801.
19. Hoy KE, Bailey N, Michael M, Fitzgibbon B, Rogasch NC, Saeki T, et al. Enhancement of Working Memory and Task-Related Oscillatory Activity Following Intermittent Theta Burst Stimulation in Healthy Controls. Cereb Cortex. 2016; 26 (12): 4563–73. DOI: 10.1093/cercor/bhv193. PMID: 26400923.
20. Viejo-Sobera R, Redolar-Ripoll D, Boixadós M, Palaus M, Valero-Cabré A, Marron EM. Impact of Prefrontal Theta Burst Stimulation on Clinical Neuropsychological Tasks. Front Neurosci. 2017; 11: 462. DOI: 10.3389/fnins.2017.00462. PMID: 28867993.
21. Chung SW, Rogasch NC, Hoy KE, Fitzgerald PB. The effect of single and repeated prefrontal intermittent theta burst stimulation on cortical reactivity and working memory. Brain Stimul. 2018; 11 (3): 566–74. DOI: 10.1016/j.brs.2018.01.002. PMID: 29352668.
22. Perellón-Alfonso R, Kralik M, Pileckyte I, Princic M, Bon J, Matzhold C, et al. Similar effect of intermittent theta burst and sham stimulation on corticospinal excitability: A 5-day repeated sessions study. Eur J Neurosci. 2018; 48 (4): 1990–2000. DOI: 10.1111/ejn.14077. PMID: 30022548.
23. Boucher PO, Ozdemir RA, Momi D, Burke MJ, Jannati A, Fried PJ, et al. Sham-derived effects and the minimal reliability of theta burst stimulation. Sci Rep. 2021; 11 (1): 21170. DOI: 10.1038/s41598-021-98751-w. PMID: 34707206.
24. Wu X, Wang L, Geng Z, Wei L, Yan Y, Xie C, et al. Improved Cognitive Promotion through Accelerated Magnetic Stimulation. eNeuro. 2021; 8 (1): ENEURO.0392-20.2020. DOI: 10.1523/ENEURO.0392-20.2020. PMID: 33452108.
25. Wang L, Wu X, Ji GJ, Xiao G, Xu F, Yan Y, et al. Better modulation for risk decision-making after optimized magnetic stimulation. J Neurosci Res. 2021; 99 (3): 858–71. DOI: 10.1002/jnr.24772. PMID: 33617027.
26. Wu X, Ji GJ, Geng Z, Wang L, Yan Y, Wu Y, et al. Accelerated intermittent theta-burst stimulation broadly ameliorates symptoms and cognition in Alzheimer's disease: A randomized controlled trial. Brain Stimul. 2022; 15 (1): 35–45. DOI: 10.1016/j.brs.2021.11.007. PMID: 34752934.
27. Nettekoven C, Volz LJ, Kutscha M, Pool EM, Rehme AK, Eickhoff SB, et al. Dose-dependent effects of theta burst rTMS on cortical excitability and resting-state connectivity of the human motor system. J Neurosci. 2014; 34 (20): 6849–59. DOI: 10.1523/JNEUROSCI.4993-13.2014. PMID: 24828639.
28. Пойдашева А. Г., Бакулин И. С., Синицын Д. О., Забирова А. Х., Супонева Н. А., Масленников Н. В. и др. Опыт применения Стэнфордской нейромодулирующей терапии у пациентов с терапевтически резистентной депрессией. Вестник РГМУ. 2022; (4): 35–42. DOI: 10.24075/vrgmu.2022.044
29. Cole EJ, Phillips AL, Bentzley BS, Stimpson KH, Nejad R, Barmak F, et al. Stanford Neuromodulation Therapy (SNT): A Double-Blind Randomized Controlled Trial. Am J Psychiatry. 2022; 179 (2): 132–41. DOI: 10.1176/appi.ajp.2021.20101429. PMID: 34711062.
30. Бакулин И. С., Пойдашева А. Г., Забирова А. Х., Лагода Д. Ю., Римкевичус А. А., Захарова М. Н. и др. Первый опыт терапевтической транскраниальной магнитной стимуляции при прогрессирующем рассеянном склерозе и спастичности по протоколу, основанному на метапластичности. Нервно-мышечные болезни. 2022; 12 (3): 26–35. DOI: 10.17650/2222-8721-2022-12-3-26-35.