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Double the dose, double the impact? Effects of iTBS on salivary cortisol in stressed healthy volunteers

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ABSTRACT

There is a growing interest in applying double-dose repetitive transcranial Magnetic Stimulation (rTMS) as a therapeutic tool for stress-related psychiatric disorders. Such stimulation protocols may shorten the treatment duration and may result in faster symptom improvement. Currently, theta-burst stimulation (TBS) protocols have gained attention because of their significantly reduced treatment duration, compared to conventional rTMS. However, the effect of one or twice daily rTMS sessions remains unclear in relation to stress. Using a two-period cross-over design, we examined the impact of double-dosed intermittent (TBS) over the left dorsolateral prefrontal cortex on stress responses (salivary cortisol) in thirty-eight healthy participants after being stressed by a validated psychosocial stress task: the Trier Social Stress Test. After the first active iTBS session, as contrasted to sham, no differential effects on salivary output were observed. However, after the second active session, there was a significantly smaller decrease of salivary cortisol concentrations in the active iTBS condition compared to sham. Our results suggest that double-dosed iTBS after being stressed might differently affect stress recovery compared to a single session of iTBS.

1. Introduction

The clinical parameters of repetitive transcranial magnetic stimulation (rTMS) in stress-related psychiatric disorders are evolving rapidly [1,2]. Intermittent theta-burst stimulation (iTBS), a more recent form of rTMS, has increasingly gained attention [3] as iTBS can significantly shorten treatment duration and in depressed patients it produces comparable clinical outcomes compared to conventional high frequency rTMS protocols [4]. Considering that the FDA-approved once-daily iTBS treatments quickly extends to six weeks, this imposes a considerable logistic burden on patients and caregivers and could delay therapeutic effects. Hence twice-daily or double-dose stimulation has gained popularity [5]. However, the neuro-endocrinological effects of a single and repeated/second session remains to be determined.

Of interest, given that the dorsolateral prefrontal cortex (DLPFC), the most frequently stimulated target of rTMS, plays an essential role in regulating the hypothalamic-pituitary-adrenal (HPA) system [6], here we focused on the neuro-endocrinological effects of double-dose iTBS on the stress response after a stressful event. We hypothesized that a second iTBS-session would reinforce the effects on cortisol secretion compared to a single session, and sham iTBS (single or double) would not show this pattern. We did not expect that mood would be influenced by active or sham iTBS.

2. Methods & materials

Thirty-eight healthy right-handed females (mean age = 23.53 years, *SD* = 3.00, age-range = 18–28 years), all taking hormonal contraceptives, were invited to participate in the study on two separate experimental days. At baseline, all participants were randomly assigned to an active-first or sham-first condition following simple randomization procedures using computerized random numbers to 1 of 2 stimulation groups. Except for the stimulation part (active vs. sham), the protocol on both days was identical (see Fig. 1).

There was at least a one-week interval between the two experimental days. Since circadian rhythm influences cortisol levels, the sessions were

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carried out in the afternoon (between 14:00 and 20:00), and every individual participant started both sessions at the same time.

To provoke an acute response of the HPA axis to stress, before being stimulated, participants performed a modified version of the Trier Social Stress Test (TSST; see Ref. [7] for a detailed description). In the first part (preparation phase), participants have 3 min to prepare a 5-min presentation. During the second part, the presentation itself, the participants were asked to convince a jury to hire them for the job of their choosing. During the third part, participants performed a challenging arithmetic test for another 5 min. Cortisol and mood were measured before and after a single iTBS session (i.e., T_1 and T_2 respectively) and before and after a second iTBS session (i.e., T_3 and T_4 respectively) and before and after both sessions (i.e., T_1 and T_4 respectively).

To evaluate the endocrinological stress responses, we collected saliva samples using an insert containing a sterile polyester swab for collecting saliva, yielding a clear and particle-free sample. The salivettes were used according to the instructions provided by the manufacturer (Sarstedt, Germany). Saliva cortisol levels (µg/L) were determined by Cortisol Saliva luminescence immunoassay (IBL International GmbH, Germany). The limit of Quantification was 0.12 µg/L, and the within-run and between-run variation coefficients were less than 5%.

To evaluate potential influences on mood, participants rated six feelings ('fatigued', 'vigorous', 'angry', 'tensed', and 'cheerful') on a 100-mm line. Positive mood scales were reversed, therefore high scores on all the subscales indicate more negative affect.

The stimulation was applied using a Magstim Rapid2 Plus1 magnetic stimulator (Magstim Company Limited, Minneapolis, USA) connected to a 70 mm "figure-of-eight" shaped coil. We used a special designed coil for the sham condition that looks identical to the coil for active stimulation and imitates feeling and sound delivering a very shallow magnetic field to mimic the sensation of the active magnetic stimulation. The region of interest, the left DLPFC, was identified with the Brainsight neuronavigation system (BrainsightTM, Rogue Research, Inc) using visual identification of the area in the center of the mid-prefrontal gyrus [Brodmann 9/46] based on individual neuroanatomical data. For each session, the following parameters were used: frequency 50Hz, burst frequency 5Hz, 1620 pulses in total a spread over 54 cycles in which each cycle includes 10 bursts of 3 pulses each, with a train duration of 2 s and an inter-train interval of 6 s (7 min and 12 s for one session), with a power output of 110% of the resting motor threshold. On both experimental days, the participants received a block of either two active stimulation or two sham stimulations. Given that the stress-induced cortisol increases after TSST can be observed usually 5–20 min after stress-induction, with a peak after 10–30 min [8], we left a time interval between both iTBS sessions of 5 min.

Statistical analyses were performed using SPSS 24.0 (IBM SPSS Statistics 24.0). The significance level was set at $p < 0.05$, two-tailed, for all analyses. The iTBS effects on HPA-functioning and mood were analyzed using the balanced two-period cross-over design described by Ref. [9] used in relatively small samples (to test for stimulation-by-period (order) and group interactions). The two treatment, two-period cross--over design overcomes the difficulty of potential carry-over effects by having half of the subjects receive treatment A followed by treatment B while the other half receive B followed by A. Carryover (or residual) effects may occur when the effect of a stimulation condition given in the first time period persists into the second period and distorts the effect of the second [10]. Using the balanced two-period cross-over design, the order of stimulation is incorporated, so any temporal change that might favour B over A in one group will favour A over B in the other group and cancel out the stimulation comparison. Stimulation (active and sham) are compared by combining the difference between A and B from within each group. Periods (order) are compared by looking at the difference between the measurements in period one and those made in period two. Importantly, if period effects are present, they do not influence the comparison of stimulation. Moreover, given that we directly compare the effects on changes in cortisol levels between the first and second stimulation sessions and between both days, the effects of stimulation around the cortisol peak are observed during the first sham/active session, and the effects of stimulation after the cortisol peak are observed during the second sham/active session.

The [9] approach was also used for the mood analysis. Of note, for mood analyses, a total mood score was calculated, making a weighted average of all subscales. Positive mood scales were reversed, with higher scores indicating more negative affect.

3. Results

3.1. The effect of iTBS on mood

The balanced two-period cross-over design showed no significant stimulation-by-visit (order) and group interactions (p's > 0.05). This indicates that there was no influence of iTBS on mood after a single or a double dose of iTBS. An overview of mood scores can be found in Table 1.

3.2. The effect of iTBS on HPA-functioning

We refer to Table 1 for an overview of all cortisol concentrations. After the single stimulation session, using the Hills and Armitage approach, we found a significant period effect, indicating that the first visit significantly influenced the second visit ($p < 0.01$), with as a result less cortisol output at the second visit. However, the interaction effect between stimulation and visit was not significant ($p = 0.12$). Furthermore, stimulation (active vs sham) was not significant ($p = 0.94$).

After the second stimulation session, using this balanced two-period cross-over analysis, we observed once more a significant period effect, again implying that the first visit influenced the second ($p < 0.01$), resulting in less cortisol output at the second visit. Crucially, the

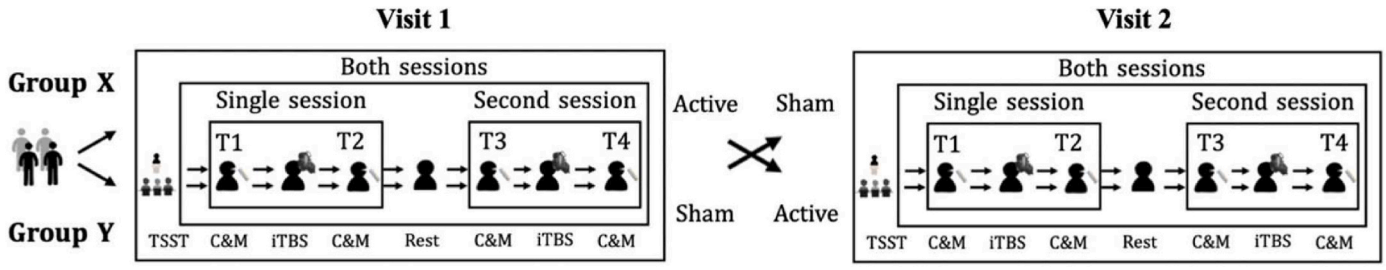

Fig. 1. Overview of the protocol. Abbreviations: iTBS, intermittent theta burst stimulation; TSST, Trier Social Stress Test, T1-T2-T3-T4: timepoint 1-2-3-4, C&M: cortisol and mood. All participants were randomly divided into two groups, X and Y. Those in group X received a block of two active iTBS stimulation sessions (condition A) on the first day (i.e., visit 1) and a block of two sham stimulation sessions (condition B) on the second day (i.e., visit 2). In contrast, in group Y, the order was reversed. Cortisol and mood were measured before and after a single iTBS session (i.e., T_1 and T_2), before and after a second iTBS session (i.e., T_3 and T_4) and before and after both sessions (i.e., T_1 and T_4).

Table 1
Mean ratings and standard error for the change in salivary cortisol (expressed in mg/L) and mood after a single session (T1-T2), a second session (T3-T4), and double sessions (T1-T4) for the active and sham group during the first and second visit.

		Group X		Group Y			P- value	
		Visit 1 Active	Visit 2 Sham	Visit 1 - 2 Active - Sham	Visit 1 Sham	Visit 2 Active	Visit 2 - 1 Active - Sham	Effect Stimulation
Mood	Single session	1.13 (6.15)	-.32 (2.71)	.73 (6.22)	.45 (4.05)	-.09 (3.66)	.00 (4.61)	P = 0.83
	Second session	.45 (4.51)	.14 (2.44)	.76 (4.39)	-.31 (2.62)	-.85 (2.76)	.99 (3.88)	P = 0.33
	Both sessions	2.52 (5.98)	.07 (3.19)	1.99 (6.95)	1.04 (5.49)	-.34 (4.92)	.42 (6.07)	P = 0.60
Cortisol	Single session	-.30 (1.47)	-.21 (.14)	-.08 (.11)	-.12 (.06)	−0.6 (.06)	-.49 (.06)	P = 0.11
	Second session	.09 (.10)	-.22 (.08)	-.12 (.10)	.06 (.03)	.12 (.06)	.06 (.05)	P < 0.01
	Both sessions	-.21 (.17)	.15 (.14)	-.37 (.16)	.54 (.08)	.12 (.09)	.07 (.07)	P < 0.01

interaction effect between stimulation and visit was not significant ($p > 0.05$). However, stimulation was significant ($p < 0.01$), indicating that the second active iTBS as compared to sham led to a significantly lesser decrease in salivary cortisol output.

When examining both stimulation sessions together, similar to both iTBS sessions separately, we found a significant period effect, showing again that the first visit influenced the second ($p < 0.01$), yielding less cortisol concentrations at the second visit. However, the interaction effect between stimulation and visit was not significant ($p > 0.05$). Importantly, stimulation was significant ($p < 0.01$), showing that active iTBS led to a significantly lesser decrease in salivary cortisol responses immediately after both simulations, as compared to sham.

4. Discussion

This study examined the effect of a single and a following second iTBS session on salivary cortisol in healthy female volunteers after being stressed. As expected, mood was not differentially altered after active or sham stimulation throughout the protocol. This agrees with previous research examining the effect of single and multiple rTMS sessions on mood in non-stressed healthy participants [11] indicating that the effects of iTBS after being stressed was also not influenced by changes in self-reported mood.

Concerning the effects of iTBS on cortisol secretion, we found a significant period effect, indicating that independently of stimulation type (active or sham) participants display less cortisol output during the second experimental visit. This is in line with previous research looking into the repeated use of TSST on psychophysiological responses without the use of non-invasive brain stimulation [12,13]. Boesh and colleges (2014) observed a smaller increase in cortisol levels when performing the TSST for the second time. Importantly, since period effects in the [9] approach do not influence the comparison of stimulation, this does not influence our results.

One session of active or sham iTBS applied to the left DLPFC, preceded by the TSST, showed no differential effects on the HPA-system. This is in line with previous healthy volunteers studies examining the impact of one session of high frequency rTMS on cortisol secretion, however, this was without stress induction [14].

When adding a second (double dose) iTBS session 5 min later, this resulted in a significantly less attenuation in salivary cortisol output in the active condition only. This suggests that instead of a faster stress recovery by double dose iTBS, applying this second active iTBS session after only 5 min may hamper the stress recovery after being stressed. Although speculative, these results could be explained by mechanisms of homeostatic metaplasticity, where the alteration of synaptic plasticity of the targeted neurons during a second iTBS session is dependent on the prior activity, (which has been altered due to the first iTBS session) [15]. Alternatively, short intervals between two sessions might lead to null-effects or to opposite results [16]. For instance, previous motor evoked potential (MEP) research showed that when using two consecutive iTBS sessions applied to the motor cortex, with either a 5 min interval or no interval (i.e. iTBS session two starts directly after session one, creating one session with the double number of pulses); this may lead to a decrease in excitability [16]. In contrast, two iTBS sessions with an interval of 15 min or longer led to similar or enhanced effects on MEP when compared to only a single session [17]. Although we did not introduce MEP measurements into our protocol, it may be possible that the relatively short time between our two active iTBS sessions might have led to inhibition - instead of excitation - of the DLPFC, resulting in a significantly slower stress recovery compared to sham stimulation. At this point, we can only conclude that stimulation protocols applying iTBS twice daily with only a short time of intersession interval may produce rather inhibitory effects on stress regulation instead of a facilitation to stress recovery. However, research giving 10 sessions of iTBS using 1800 pulses/session (i.e., giving three sessions of 600 pulses without a break) to depressed patients, show a significant drop in depressive symptoms [4].

Even though this study has several strengths, some limitations should be discussed. First, only healthy young women using hormonal contraceptives were included, so these results cannot be generalized to a broader population. Secondly, since the participants received stimulation after being stressed, the stimulation effects could only be assessed during the stress recovery. Thirdly, because we only used two active and two sham stimulations to evaluate the difference between a single or double dose of (active/sham) iTBS on salivary cortisol, we did not evaluate possible placebo effects in the HPA-system in relation to active iTBS or vice versa.

In conclusion, the application of double dose iTBS with only a 5 min intersession interval significantly delayed stress recovery in healthy female volunteers after being stressed, irrespective of mood influences. Our results suggest that double dose iTBS with short session intervals might result in an inhibitory effect on the HPA-system, delaying stress recovery.

Declaration of interests

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 paper.

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