Baseline 'state anxiety' influences HPA-axis sensitivity to one sham-controlled HF-rTMS session applied to the right dorsolateral prefrontal cortex

Running title: 'state anxiety' influences HPA-axis sensitivity

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Summary

Although negative results have been reported, an important aspect of the physiology of repetitive Transcranial Magnetic Stimulation (rTMS) could be related to the endocrinological response of the hypothalamic-pituitary-adrenal (HPA) axis, such as cortisol secretion. Because endocrinological responses are influenced by anxiety states, this could influence the effect of rTMS in healthy individuals. In this sham-controlled, "single blind" crossover study, we examined whether one session of HF-rTMS could affect the HPA-system, when taking into account individual state anxiety scores based on the State-Trait Anxiety Inventory (STAI). Twenty-four healthy rTMS naïve females received one sham-controlled high frequency (HF)-rTMS session delivered on the right dorsolateral prefrontal cortex (DLPFC). The Profile of Mood States (POMS) questionnaire, together with salivary cortisol samples, was collected before, just after and 30 minutes post HF-rTMS. To examine whether state anxiety could influence endocrinological outcome measurements, we administered the STAI-state just before each HF-rTMS experiment started. Based on the POMS questionnaire, no mood changes were observed. Without taking individual state anxiety scores into account, one sham-controlled right-sided HF-rTMS session did not influence the HPA–system. When taking into account individual STAI-state scores, we found that healthy women scoring higher on the STAI-state displayed a significantly more sensitive HPA-system, resulting in salivary cortisol concentration increases after real HF-rTMS, compared to those scoring lower on this anxiety scale. Our results indicate that healthy women scoring high on state anxiety display a more sensitive HPA-system when receiving one right-sided HF-rTMS session. Our findings suggest that the incorporation of individual anxiety states in experimental rTMS research could add further information about its neurobiological influences on the HPA-system.

Keywords: HF-rTMS; right dorsolateral prefrontal cortex; salivary cortisol; mood; healthy volunteers; state anxiety

Introduction

In the last two decades, a considerable amount of research has been conducted to investigate the effects of repetitive transcranial magnetic stimulation (rTMS) on mood in both healthy and depressed individuals. However, the underlying neurobiological working mechanisms as to how this application might affect mood remains largely unclear (George et al., 2003). Asking participants to state subjectively how they feel might to some extent be indicative of emotional processes, but provides little insight into the underlying neurobiological mechanisms of mood regulation (Buck, 1999). Endocrinological responses (e.g. cortisol) operate rather independently of consciously experienced mood and could provide more insight into the neurocircuitry of emotion processing and emotional reactivity (Buck, 1999). Animal and human models suggest that an important aspect of the physiology of rTMS could be related to the endocrinological response of the hypothalamic-pituitary-adrenal (HPA) axis, such as cortisol secretion (Ji et al., 1998; Keck et al., 2001; Evers et al., 2001; Hedges et al., 2003). Further, the neurobiological modulation of stress-regulatory systems such as the HPA-axis are predominantly regulated by the right prefrontal cortex (Sullivan and Gratton, 2002; Cerqueira et al., 2008).

Few studies have examined the endocrinological rTMS response on the HPA-system in healthy volunteers: whereas George et al (1996) found a slight increase of serum cortisol levels post left-sided prefrontal suprathreshold stimulation, Evers et al (2001) observed a decrease of serum cortisol concentrations by using left-sided infrathreshold high frequency (HF)-rTMS. However, in both studies carry-over effects could not be excluded. We recently reported negative results on salivary cortisol changes after one sham-controlled session of left and right-sided HF-rTMS in healthy female subjects (Baeken et al., 2009a).

However, individual differences such as in state anxiety have been found to correlate with neuroendocrine responses (Chida and Hamer, 2008). Because endocrinological responses are by definition influenced by emotional stress, this could imply that individual anxiety states may influence rTMS outcome results. To our knowledge, there are no published healthy volunteer placebo-controlled studies examining a possible influence of individual differences in mood or tension states, such as state anxiety, to endocrinological responses in experimental HF-rTMS designs. Consequently, in a new study we wanted to evaluate whether one sham-controlled HF-rTMS session could influence the HPA-system, measured with easy to obtain salivary cortisol samples, when taking individual anxiety scores into account before the start of the experiment. The State-Trait Anxiety Inventory (STAI) is an appropriate and adequate measure for studying anxious tension in research and clinical settings (Oei et al., 1990; Bieling et al., 1998).

Because the effects of rTMS on the right (and not the left) dorsolateral prefrontal cortical (DLPFC) are specifically related to negative emotional processing and difficulties to inhibit negative affect in healthy subjects (e.g. Leyman et al., 2009), we targeted this prefrontal area under MRI guidance. To ensure that our endocrinological measurements would not be influenced by HF-rTMS induced mood changes, we assessed mood on each salivary cortisol collection. As gender and age could be a possible confounder in HPA-axis reactivity protocols (Seeman et al., 2001; Kudielka et al., 2004) and as the intra-individual stability of baseline salivary cortisol levels is reported to be more stable in women (Kirschbaum et al., 1992), we chose to use a 'uniform' group of non-depressed young female subjects.

In line with our former research (Baeken et al, 2009), we hypothesized that without taking into account individual differences on state anxiety one HF-rTMS session would not affect HPA-system measurements. In contrast, when integrating individual state anxiety scores, we hypothesized that women scoring higher on state anxiety would display a more sensitive HPA-system and less HPA-system inhibition, resulting in a significantly higher cortisol output. In line with our former research on HF-rTMS, (Baeken et al., 2008), we did not expect any mood changes after one session.

Materials and Subjects

The ethics committee of the University Hospital (UZBrussel) approved the study and all subjects gave written informed consent. Subjects were financially compensated. All 24 volunteers (mean age= 22.29, SD= 2.58 years) were right-handed (van Strien & Van Beeck, 2000) and all were naïve to the rTMS procedure. No drugs were allowed, except birth-control pills. All participants used oral contraceptives at the time of the study. Psychiatric disorders were assessed by the Dutch version of the Mini-International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998). A clinical psychiatric interview was performed before a subject's inclusion in the study. Subjects with a psychiatric disorder and/or a score higher than eight on the Beck Depression Inventory (BDI; Beck et al., 1984) were excluded. On the morning before the start of the HF-rTMS experiments, all female volunteers were assessed using the Dutch version of State-Trait Anxiety Inventory for adults, state version (20 items) (STAI; Van der Ploeg et al., 1980).

A sham-controlled, 'single' blind, crossover design was used. The order of the HF-rTMS sessions (real versus sham) was counterbalanced. Twelve participants first received real HF-rTMS before sham and the twelve other volunteers received sham HF-rTMS followed by the real condition. To avoid carry-over effects from the previous stimulation, the second session was carried out after an interval of one week. All volunteers were

stimulated within the same time schedule, between 10 am and 14 noon. Subjects were kept unaware of the type of stimulation they received; they wore earplugs and were blindfolded. On the days of the two stimulation sessions, subjects were asked to deliver a salivette just before the start of HF-rTMS (T_1). All subjects then received sham or active HF-rTMS targeted on the right DLPFC. Immediately after stimulation, subjects delivered another salivette (T_2) and again after 30 minutes (T_3). Saliva samples were collected using a salivette (Sarstedt, Germany), with an insert containing a sterile polyester swab for collecting saliva, yielding a clear and particle-free sample. Additionally, participants completed a Dutch version of The Profile of Mood States (POMS; Wald and Mellenbergh, 1990), a 32-item inventory that assesses five mood dimensions, just before (T_1) and just after (T_2), as well as just after 30 minutes (T_3). Feelings of 'depression' (8 items: minimum score 0; maximum score 32), 'fatigue' (6 items: minimum score 0; maximum score 24), 'tension' (6items: minimum score 0; maximum score 24), 'anger' (7 items: minimum score 0; maximum score 28) and 'vigor' (5 items: minimum score 0; maximum score 20) were rated. Ratings were made on four-point scales: a rating of 0 indicates a low level and a rating of 4 indicating a high level of a given mood.

We used a Magstim high-speed magnetic stimulator (Magstim Company Limited, Wales, UK), connected to a figure-of-eight-formed double 70mm coil. Before each application, the motor threshold (MT) of the right abductor pollicis brevis muscle of each individual was determined. In order to accurately target the right DLPFC (Brodmann area 9/46), taking into account individual anatomical brain differences, the precise stimulation site and position of the coil was determined using MRI non-stereotactic guidance (Peleman et al., 2010). Perpendicular to this point the precise stimulation site on the skull was marked and stimulated. In each high-frequency (10 Hz) stimulation session, at stimulation intensity of 110 % of the subject's MT, subjects received 40 trains of 3.9 s duration, separated by an intertrain interval of 26.1 s (1560 pulses per session). For the sham condition, the coil was held at an angle of 90°, only resting on the scalp with one edge. Subjects were kept unaware of the type of stimulation they received; they wore earplugs and were blindfolded. The study was conducted conform the current safety guidelines (Rossi et al., 2009).

This study was part of a large project investigating the influence of HF-rTMS on different neurocognitive markers.

Statistical analysis

All collected data were analyzed with SPSS 15 (Statistical Package for the Social Sciences). The significance level was set at $p \leq 0.05$ for all analyses. Where necessary, we applied the Greenhouse-Geisser correction to ensure the assumption of sphericity.

Mood ratings were analysed separately for each POMS subscale, using a two-way repeated measures ANOVA. Within-subject factors were stimulation (real or sham HF-rTMS) and time (T_1, T_2 and T_3).

We analyzed salivary cortisol samples at three different time points (at T_1, T_2 and T_3) and as proposed by Pruessner et al (2003), we calculated the area under the curve (*AUC*) with respect to ground (*AUCg*) and the *AUC* with respect to increase (*AUCi*). The *AUCg* measures, in endocrinological terms, the total 'hormonal output', whereas the *AUCi* measures the hormonal changes over time. Therefore, the latter index is especially suitable to evaluate HPA-axis sensitivity (Fekedulegn et al., 2007). In a first step, the effects of HF-rTMS on the HPA-axis were analyzed using the real and sham *AUC* values as dependent variables in a repeated measures ANOVA analysis with stimulation (real HF-rTMS-sham HF-rTMS) as the within subjects factor. Analyses were performed for the *AUCg* and the *AUCi* separately.

In a second step, to examine the effects of state anxiety to this experimental procedure, we performed a repeated measures ANCOVA using the real and sham *AUC* values as dependent variables and stimulation (real HF-rTMS vs. sham HF-rTMS) as the within subjects factor. Because a paired *t*-test did not show baseline (T_1) STAI-state score differences between real (mean= 30.95, *SD*=6.28) and sham (mean= 29.43, *SD*=5.75) STAI-state scores ($t(20)=1.59$, $p=0.13$), the mean individual scores of the STAI-state version over the two stimulation sessions (mean= 30.40, *SD*=5.86) were used as covariate. Again, analyses were performed for the *AUCg* and the *AUCi* separately. Pearson correlation analysis completed the analyses.

Results

During the determination of the individual MT, one volunteer experienced a dermatological reaction to the physiological patches and did not further participate in the second part of the study. Furthermore, at some time points for four participants some incomplete POMS data sets were delivered and for two subjects insufficient saliva needed for analysis was produced. POMS mood ratings and salivary cortisol data are summarized in Table 1.

Mood effects

The ANOVA for the POMS-depression scale showed no significant overall effect for stimulation ($F(1,18)=0.32$, $p=0.58$). No main effect was found for time ($F(2, 17)=0.99$, $p=0.35$) and also the crucial interaction between time and stimulation was not found ($F(2, 17)=0.04$, $p=0.86$). The ANOVA for the POMS-anger subscale did not reveal significant main effects for stimulation ($F(1,18)=0.55$, $p=0.47$), time ($F(2, 17)=1.33$, $p=0.29$), nor for the interaction between both variables ($F(2, 17)=0.65$, $p=0.54$). The ANOVA for the POMS-tension scale showed no significant overall effect for stimulation ($F(1,18)=1.34$, $p=0.26$). On the other hand, we found a significant main effect of time ($F(2, 17)=4.33$, $p=0.03$). However, the important interaction between time and stimulation was not found ($F(2, 17)=0.41$, $p=0.67$). The ANOVA for the POMS-fatigue scale showed no significant overall effect for stimulation ($F(1,18)=0.91$, $p=0.35$), no significant main effect for time ($F(2, 17)=2.42$, $p=0.12$), and no interaction between time and stimulation ($F(2, 17)=0.05$, $p=0.95$). The ANOVA for the POMS-vigor scale showed a significant main effect of stimulation ($F(1, 18)=5.11$, $p=0.04$). No main effect was found for time ($F(2, 17)=1.76$, $p=0.20$). Also the crucial significant interaction effect between time and stimulation was not significant ($F(2, 17)=1.90$, $p=0.13$). We also applied a last observation carry-forward (LOCF) analysis of variance (ANOVA) to control for missing POMS values. This approach did not affect the outcome results.

Salivary cortisol

Concerning the salivary cortisol analysis, a paired *t*-test did not show baseline (T_1) cortisol differences between real and sham HF-rTMS ($t(22)=1.34$, $p=0.19$). The results of the *AUCg* ANOVA with stimulation (real HF-rTMS-sham HF-rTMS) as the within subjects factor showed no significant main effect ($F(1,21)=1.23$, $p=0.28$). Also the results of the *AUCi* ANOVA showed no significant main effect ($F(1,21)=0.02$, $p=0.88$).

For the *AUCg* ANCOVA with stimulation (real vs.sham HF-rTMS) as the within subjects factor and the individual scores of the mean STAI-state as covariate, we observed no statistically significant main effects for stimulation ($F(1,20)=1.06$, $p=0.32$), mean STAI-state ($F(1,20)=0.85$, $p=0.37$) or interaction effect ($F(1,20)=0.72$, $p=0.41$). The results of the *AUCi* ANCOVA showed a significant main effect of stimulation ($F(1,20)=4.65$, $p=0.04$), but no main effect of state anxiety ($F(1,20)=0.61$, $p=0.45$). However, the interaction effect between stimulation and mean STAI-state was significant ($F(1,20)=4.7$, $p=0.04$). To further clarify the meaning of this interaction effect, we calculated delta *AUCi* (real *AUCi*-sham *AUCi*) and performed a Pearson correlation analysis. We found a significant positive correlation between delta *AUCi* and mean STAI-state ($r= .44$, n=22, $p=0.04$), which means that the larger the difference between real and sham anxiety scores the larger the increase in salivary cortisol concentrations. See Fig 1. In addition, Pearson correlation analysis revealed that the mean STAI-state correlated significantly with the real *AUCi* ($r= .45$, n=23, $p=0.03$) but not with the sham *AUCi* ($r= -.11$, n=22, $p=0.62$). See Fig 2. As we collected a STAI-state version before each sham and real session, consequently, we performed Pearson correlation analysis with sham STAI-state before sham HF-rTMS and the real STAI-state before real HF-rTMS. Again, we found a significant correlation between real STAI-state and the real AUCi (r= .50, n=21, p=0.02), but not between the sham STAI-state and sham AUCi (r= -.13, n=22, p=0.57). These results indicate that more anxious women display a more sensitive or reactive HPA-axis response to the HF-rTMS application without a global difference in cortisol output.

Discussion

First of all, one sham-controlled HF-rTMS session applied to the right DLPFC did not result in a subjectively aware mood change in our healthy female subjects, even without correction for multiple comparisons, which is indicative for the true absence of subjectively experienced mood effects. This corresponds with our previous report of a single sham-controlled session of right-sided dorsolateral prefrontal HF-rTMS, causing no immediate or delayed mood changes in a comparable group of healthy women (Baeken et al., 2008).

Concerning the salivary cortisol results, without taking individual state anxiety scores into account, the current results confirm our former data that in healthy women the HPA–system was not influenced by one session of HF-rTMS applied to the right DLPFC (Baeken et al., 2009), a conclusion which is in line with former sham-controlled suprathreshold HF-rTMS volunteer studies (Evers et al., 2001). When taking into account individual state anxiety scores, one real as well as one sham HF-rTMS session did not affect cortisol secretion patterns (*AUCg* analysis), suggesting that in these kinds of rTMS experiments the global endocrinological output is not immediately affected. Hedges et al (2002) also demonstrated that in animals one real HF-rTMS was not related with changes in neuroendocrine stress responses, such as corticosterone and prolactine increases. In support of this assumption, our volunteers did not feel more 'tense' or 'depressed' after one such a HF-rTMS session, not after real nor sham stimulation. In contrast to the lack of global changes in cortisol secretion patterns, we found that healthy women scoring higher on the STAI-state displayed a more sensitive HPA-system, resulting in higher salivary cortisol concentrations, compared to those females scoring lower on this anxiety scale (*AUCi* analysis). Importantly, this endocrinological sensitivity was only observed during the real HF-rTMS session and not during sham. Because we used a sham-controlled counterbalanced design, the differences in HPA-system sensitivity can not be attributed to the circadian variation of cortisol (Hanson et al., 2000). In the current study, our volunteers were two times stimulated within the same time schedule and during our HF-rTMS protocol all subjects performed standard cognitive tasks before T_1 and also between T_2 and T_3. Our *AUCi* results are in line with healthy volunteer studies incorporating individual information which observed a stronger HPA-system reactivity in individuals scoring higher on neuroticism or harm avoidance, both personality features closely linked to anxiety proneness (Zobel et al., 2004; Tyrka et al., 2008).

Because cortisol effects are frequently observed in stress induction paradigms (i.e. Dickerson and Kemeny, 2004), one could argue that the 'rTMS procedure' by itself could have been a stressful event especially for individuals experiencing some form of anxiety. However, we have two major arguments that withstand this assumption. First of all, as the relationship between predominantly negative mood changes and cortisol

responses has been well documented (Smyth et al., 1998; Buchanan et al., 1999), it could be argued that possible HF-rTMS induced mood effects could have interfered with the endocrinological measurements. But as mentioned before one such a HF-rTMS session did not influence mood states. If some kind 'discomfort' due to pain sensations would have interfered with our endocrinological measurements, we would have detected an increase in tension or other mood changes on the POMS, which we did not observe. Moreover, after both real and sham HF-rTMS the main effects suggested decreases rather than increases on tension scores in both conditions. Secondly, if stress-related responses would have interfered with our endocrinological measurements, we would also have expected cortisol influences in the 'more anxious' females during sham stimulation. In contrast, sham stimulation did not affect HPA-axis reactivity (*AUCi* analysis). As the *AUCg* analysis showed, the general cortisol output was not affected by the HF-rTMS experiment, indicating that in line with animal studies stress-responses did not interfere with our data (Hedges et al., 2002). Furthermore, to reduce possible stress-related responses, we used of salivettes as this has certain advantages over blood samples: sampling is non-invasive, it can frequently be repeated, and it avoids stress induction (painless) (Castro et al., 2000).

In short, when compared to less anxious individuals one real HF-rTMS session in more anxious women resulted in a more sensitive or reactive HPA-axis response, resulting in higher cortisol concentrations, without a global difference in cortisol output. So what is the catch here? The current findings should be interpreted within a cortico-limbic network that plays a central role in the top-down prefrontal cortical regulation of the HPA-system (Herman and Cullinan, 1997; Herman et al., 2003). Current hypotheses on possible rTMS working mechanisms suggest that the neurobiological influence occurs at the subcortical level, such as the paraventricular nucleus (PVN) of the hypothalamus (Post and Keck, 2001; Keck, 2003). Prefrontal cortical stimulation not only results in enhanced neuronal activity under the stimulation coil locally, but spreads through transsynaptic transmission further to other prefrontal cortical areas, such as the anterior cingulate cortex (ACC), in subcortical structures implicated in the neurocircuitry of emotional processing (Paus et al., 2001; Nahas et al., 2001; Kimbrell et al., 2002; Barrett et al., 2004) and in the neurobiological mechanisms of controlling HPA-system responsiveness (Herman et al., 2005; Radley et al., 2006; Jankord and Herman, 2008). These cortico-subcortical innervations which project to the hypothalamus are branches of the basic loop that is connected to autonomic expression of emotion (Gray, 1999; Zahm, 2006).

As mentioned before, the neurobiological modulation of stress responses has been reported to be lateralized to the right prefrontal cortex (Sullivan and Gratton, 2002; Cerqueira et al., 2008). Our findings of a higher HPA-system sensitivity in more anxious individuals after HF-rTMS applied over the right DLPFC might

be especially germane in light of the therapeutic applications of rTMS as a treatment for anxiety disorders and/or posttraumatic stress disorder (PTSD) (Cohen et al., 2004; Pallanti and Bernardi, 2009; Zwanzger et al., 2009; Handwerger, 2009; Yue et al., in press). Because of the overwhelming evidence that GABAergic mechanisms regulate neuroendocrine stress responses (Herman et al., 2002; Herman et al., 2004; Kovács et al., 2004; Cullinan et al 2008; Radley et al., 2009) and the importance of GABA receptor involvement in the pathophysiology and treatment of anxiety disorders (Lydiard, 2003; Nemeroff, 2003; Kalueff and Nutt., 2007), it is tempting to assume that in our study the observed HPA-axis sensitivity to one HF-rTMS session in more anxious females was mediated by the GABA system.

Because heterogeneous subject sampling such as age and gender could be an important methodological issue in TMS research, the choice of more 'homogeneous' sample of young female subjects should be considered as a major advantage (Martin et al., 2003; Kajantie and Phillips, 2006). Another major advantage in this study is the use of 3D-MRI guidance to target the right DLPFC, correcting for individual cortical anatomical differences (Peleman et al., 2010). Nevertheless, there are important limitations to our study that have to be considered. Firstly, it is possible that oral contraceptives (OC) and the menstrual cycle phase could have had an impact on salivary measurements (Kirschbaum et al., 1999). Secondly, the interpretation of our results is limited to relatively young healthy females and cannot be generalized to a broader population. Thirdly, although all participants were blindfolded and ear-plugged, and in spite that the sham stimulation was performed with the coil at a 90° angle, ensuring minimal stimulation of the DLPFC, it is possible that a partially active placebo effect was present (Loo et al., 2000). Although we didn't find any indications for elevated tension or other mood changes during real rTMS, as measured with the POMS, we cannot completely rule out that this might be related to the higher HPA-system reactivity pattern in more anxious female subjects. More anxious females might display lower pain thresholds, resulting in enhanced HPA-system reactivity (Stones et al., 1999). However, it has been shown that HF-rTMS applied to the left DLPFC results in reduced pain perceptions in healthy subjects (Borckardt et al., 2007; Fierro et al., 2010). In addition, the possibility that 'more anxious' healthy subjects are more susceptible to pain sensations, resulting in a more sensitive HPA system reactivity due to the real HF-rTMS application, remains an open question as contrasting findings on the relationship between anxiety, stress and pain perception have been reported (Bement et al., in press). It is important to mention that sham coils completely mimicking a real HF-rTMS session are not yet available, a problem that affects almost all sham-controlled rTMS studies. Sham devices could be improved by reproducing not only the identical external appearance of the coil and wires, but an ideal sham control should also produce similar sensations (discharging

noise, scalp muscle contractions and electrical paresthesias) on the scalp (Epstein, 2008). Moreover, the interpretation of this study should be limited to state anxiety levels present before the start of the experiment and not to individual anxiety traits. It has to be noticed that in our sample no participants were diagnosed with any anxiety or mood disorders and of course it remains questionable whether rTMS effects observed in healthy volunteers can be transferred to neurobiological rTMS effects found in psychiatric patients (Zwanzger et al., 2009). Other individual differences, such as state anxiety and personality features (Boudarene et al., 2002; Zobel et al., 2004; Tyrka et al., 2007, 2008; Hauner et al., 2008) might be involved in the HF-rTMS related HPA-system reactivity.

Altogether, our results suggest that women scoring higher on state anxiety display a more sensitive HPA-system and one real HF-rTMS session applied to the right DLPFC consequently resulted in enhanced cortisol responses. However, whether this HPA-system sensitivity in more anxious females is the result of general or local disinhibition processes involving the GABAergic system remains unanswered. As no left-sided HF-rTMS studies incorporating individual anxiety states and/or traits have been carried out, it is not clear whether these anxiety influences only occur during right sided-HF-rTMS. In conclusion, when investigating the HPA-system by rTMS methods, individual state anxiety scores could add further information about its neurobiological impact on affective processing.

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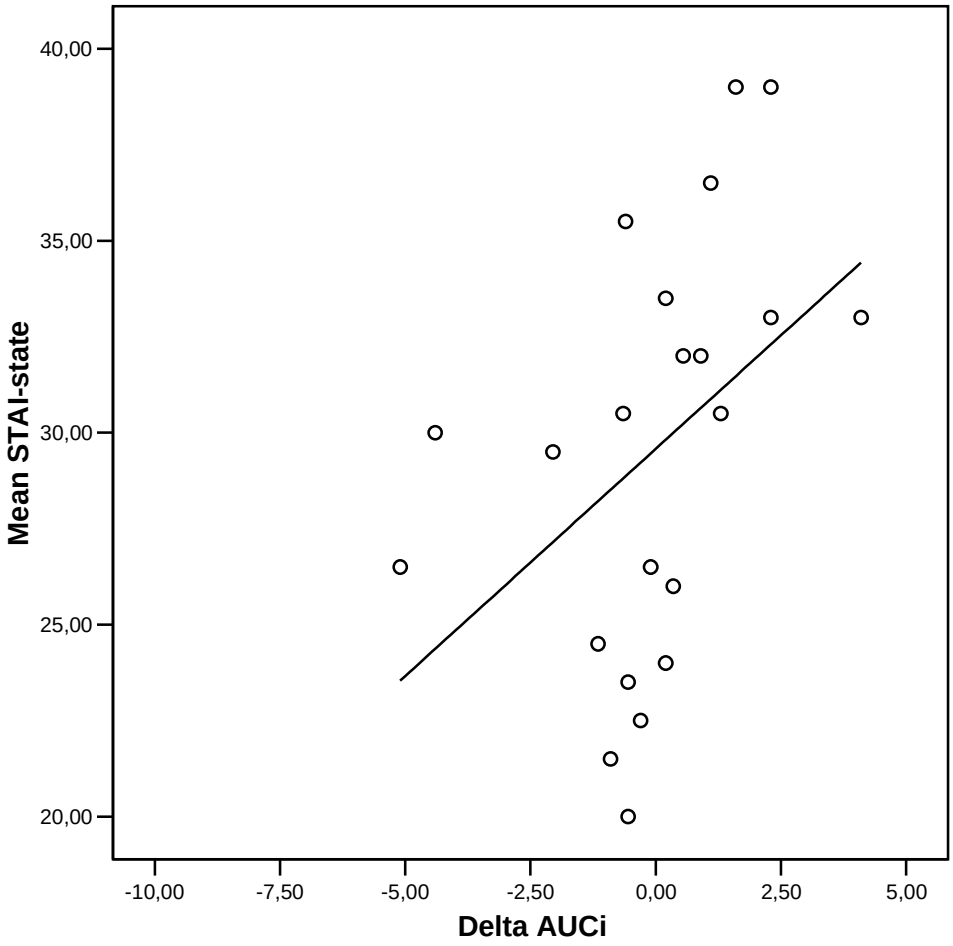

Fig 1: Scatter plot of the delta *AUCi* (real *AUCi* -sham *AUCi*) and mean STAI-state. The presented line represents the least squared fit to the data.

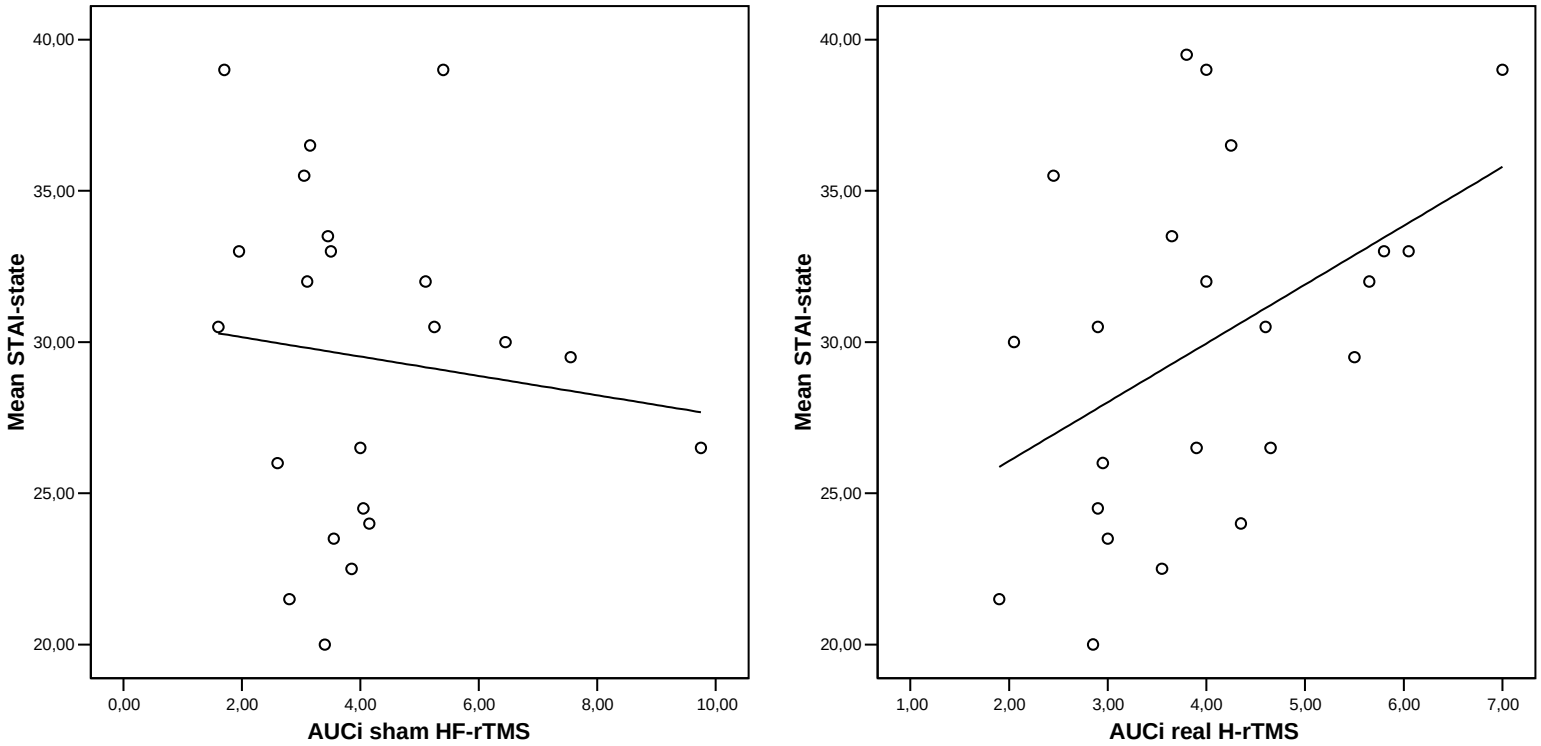

Fig 2: Left: Scatter plot of the sham *AUCi* and sham STAI-state. Right: Scatter plot of real *AUCi* and real STAI-state. The presented lines represent the least squared fit to the data.

	Real HF-rTMS			***Sham HF-rTMS***		
	T1	***T2***	***T3***	***T1***	***T2***	***T3***
Salivary cortisol (µg/L)	5.19 (2.37)	4.54 (1.56)	4.17 (1.36)	5.59 (2.29)	4.80 (1.77)	4.53 (1.64)
POMS Depression	0.57 (1.44)	0.14 (0.47)	0.43 (0.99)	0.43 (0.84)	0.21 (0.63)	0.32 (0.95)
POMS Anger	0.65 (1.40)	0.64 (2.15)	0.35 (0.76)	0.65 (1.53)	0.16 (0.50)	0.14 (0.47)
POMS Tension	1.17 (1.47)	0.64 (1.36)	0.52 (0.95)	0.87 (1.01)	0.42 (1.17)	0.41 (1.05)
POMS Fatigue	3.22 (3.86)	1.68 (2.08)	2.74 (2.67)	3.83 (5.04)	2.37 (1.98)	3.64 (3.63)
POMS Vigor	11.30 (4.63)	10.09 (4.60)	9.00 (4.63)	8.48 (4.28)	8.74 (4.05)	8.32 (5.18)

Table 1. Mean ratings and standard deviations for salivary cortisol and the POMS subscales before (T_1), immediately (T_2) and 30 min after (T_3) HF-rTMS (*real stimulation or sham condition*) on the right DLPFC.