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Short communication

Assessment of multiple salivary biomarkers during repetitive transcranial magnetic stimulation (rTMS) treatment for major depression

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ABSTRACT

Steroid hormones may serve as potential biomarkers of treatment response for major depressive disorder (MDD). Here, we assessed salivary levels of cortisol, dehydroepiandrosterone (DHEA), and DHEA sulfate (DHEA-S), as well as α-amylase activity, across 30 sessions of bilateral repetitive transcranial magnetic stimulation (rTMS) of the dorsolateral prefrontal cortex in MDD patients. While rTMS significantly improved symptoms as measured by three different symptom scales, salivary biomarker levels and their ratios showed no significant changes across sessions. These results do not support the routine clinical use of these biomarkers as reliable indicators of treatment outcome during rTMS administration for MDD.

1. Introduction

Repetitive transcranial magnetic stimulation (rTMS) is safe, easily administered, and able to improve symptoms in a significant number of patients with treatment refractory major depressive disorder (MDD) (McGirr and Berlim, 2018). Different approaches have sought to identify biomarkers that may serve as physiological indices of the clinical effects of rTMS (Fidalgo et al., 2014). Neuroendocrine measurements (e.g., cortisol) offer a relatively easy and low cost method that could be readily applied in community clinical settings. In particular, salivary samples can be readily obtained and reliably analyzed for a variety of potential neuroendocrine markers. Although the exact relationship of neuroendocrine measures to CNS function remains to be fully established, salivary measurement of glucocorticoids can be a potential window into hypothalamic-pituitary-adrenal (HPA) dysfunction (Clow and Smyth, 2020). While the role of steroid hormones in MDD is highly complicated, HPA hormones such as cortisol and dehydroepiandrosterone (DHEA) appear to play important roles in MDD, including hypercortisolemic states in depressed vs non-depressed individuals (Rhebergen et al., 2015). Notably, a recent meta-analysis of multiple biomarkers identified cortisol as the only consistent factor with a predictive effect on the onset, relapse, and recurrence of MDD (Kennis et al., 2020).

The collection of multiple hormones followed by subsequent analyses of the relative ratios of these hormones over time may be critical for assessing changes related to MDD (Kamin and Kertes, 2017). For example, Young et al. (2002) reported that the cortisol:DHEA ratio was elevated in drug-free depressed patients when compared to controls. Such findings are consistent with reports that DHEA and its sulfated ester (DHEA-S) may serve as "antiglucocorticoid" steroid hormones, whereby relatively higher levels confer neuroprotective properties in the face of chronically elevated cortisol (Mocking et al., 2015). In addition to steroid hormones, another promising biomarker is α-amylase, a salivary enzyme that serves as a proxy measure of sympathetic nervous system activity (Granger et al., 2007). Increased α-amylase activity can differentiate MDD from other psychiatric disorders (Bauduin et al., 2018) and higher α-amylase activity and cortisol levels have been associated with the magnitude of depressive symptoms (Booij et al., 2015). Dynamic interactions of α-amylase with cortisol may reflect concurrent dysregulation of sympathetic nervous system and the HPA axis (Ali and Pruessner, 2012).

Based on evidence that specific neuroendocrine measures reflect MDD pathophysiology, the current study assessed their activity over time during daily bilateral rTMS of the dorsolateral prefrontal cortex (DLPFC). We hypothesized that improvement of symptoms across 30 rTMS sessions would be accompanied by declines in both salivary cortisol levels and α-amylase activity relative to DHEA and DHEA-S levels.

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2. Method

2.1. Subjects

Subjects were recruited from among patients accepted for rTMS treatment and enrolled with informed consent. Study protocols were approved by the Western Institutional Review Board (Study #1174617). All subjects had a verified MDD history based on their clinical records and a history of unsatisfactory response to prior treatment before seeking rTMS intervention. We recruited 22 (73%) females and 8 (27%) males, with a mean (±SEM) age of 41.1±3.1 years. Exclusion criteria included a past history of epilepsy, prior neurosurgery, metal or magnetic objects in the brain, hypercortisolism, pregnant women, meeting criteria for alcohol or substance dependence within 6 months of entering the study, acute illness or infection, vaccinations within 4 weeks of entering the study, current antipsychotic drug medication, or current hormone supplements for a minimum of 6 weeks before entry into the study.

2.2. Study protocol

The protocol utilized a within subjects design, whereby each subject had salivary samples collected twice (pre-post collection before and after a single rTMS session) at three separate time points (sessions 1, 6, and 30) for a total of six samples per subject. Prior to treatment, patients underwent evaluation to confirm diagnoses, rule out contraindications, and gather medical history. A 'motor threshold' (MT) procedure was performed in which the magnetic coil was moved along the scalp above the motor cortex until an isolated thumb twitch occurred. Adjustments in stimulation intensity were made until a motor response was observable more than 50% of the time, with subsequent rTMS set at 120% of this intensity. The MT location was used to position the magnetic coil over the DLPFC, located 5.5 to 6.0 cm anterior to the motor cortex. Bilateral magnet placement over the DLPFC was used as bilateral rTMS has been reported to produce greater remission rates when compared to unilateral rTMS (Trevizol et al., 2019). A total of 30 sessions took place, 5 times a week for six weeks using a MagVenture MagPro R30 unit with a standard Cool-B65 coil. Stimulation parameters were as follows: 1) Right DLPFC: 2000 pulses per treatment delivered at 1 Hz (duration of 30-33 min); 2) Left DLPFC: 3000 pulses per treatment delivered at 10 Hz (duration of 15-18 min).

2.3. Symptom rating inventories

We utilized three well validated self-report rating inventories for MDD symptom assessment: the 21 item Beck Depression Inventory (BDI) (Beck et al., 1961), the 7 item Generalized Anxiety Disorders (GAD) assessment (Spitzer et al., 2006), and the 9 item Patient Health Questionnaire (PHQ-9) inventory (Kroenke et al., 2001). All three rating inventories were administered just prior to the treatment session on days 1, 6, and 30 of rTMS.

2.4. Salivary sample collection and analysis

Saliva (1 ml) was collected in the afternoon between 1-5 pm using a standard passive drool procedure and samples were stored at -20°C until analysis. At the time of assay, samples were thawed to room temperature, centrifuged at 1500 x g for 10 minutes, and the supernatant then pipetted into separate vials for subsequent ELISAs. Competitive ELISAs were carried out according to the manufacturer (Salimetrics, Inc.) using ELISA kits for cortisol, DHEA, and DHEA-S, and a kinetic enzyme assay for α-amylase. Plates were read on a plate reader (VersaMax Microplate Reader, Molecular Devices, LLC) with a wavelength setting at 450 nm (cortisol, DHEA, DHEA-S) or 405 nm (α-amylase). The mean inter- and intra-assay coefficients of variation (CV) for analytes were below 7% and 8%, respectively.

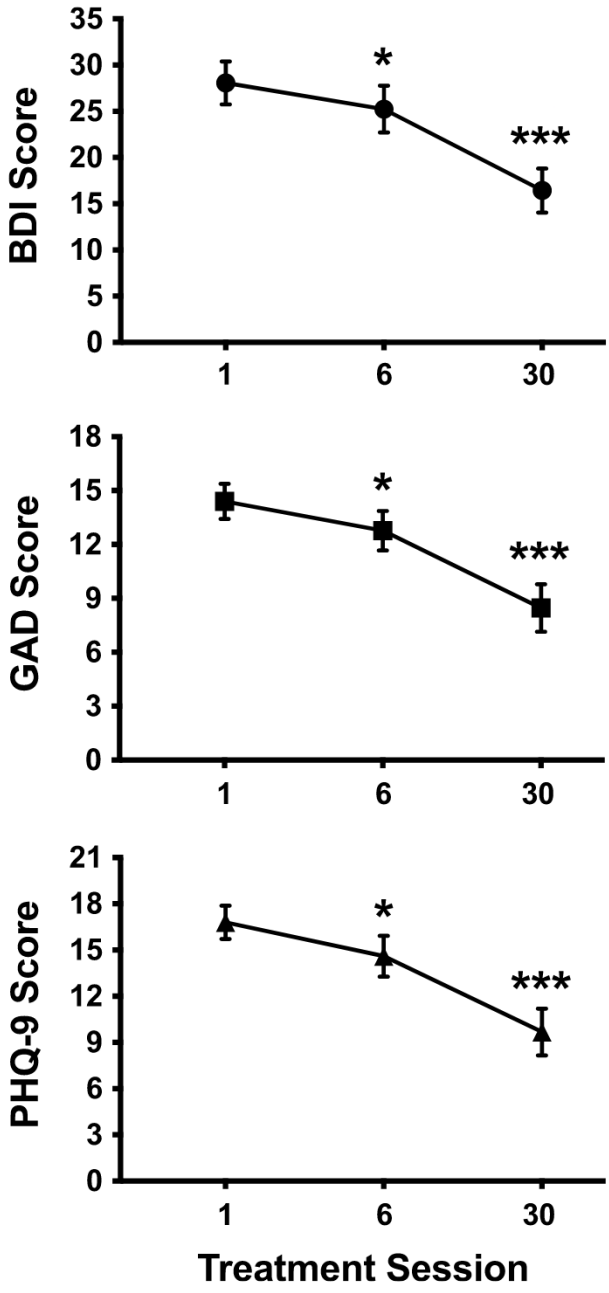

Fig. 1. Symptom rating inventories. Symptom scores over time for the Beck Depression Inventory (BDI), Generalized Anxiety Disorders (GAD), and Patient Health Questionnaire (PHQ-9). Significant differences from session 1 are indicated (*$p<0.05$, ***$p<0.001$, Tukey's multiple comparisons test).

2.5. Data analysis

Symptom scale and analyte values were entered into GraphPad Prism (v 9.0) for statistical analyses. Two subjects did not complete the full treatment course; thus, only the first two timepoints from these subjects were included in the analysis. A few of the salivary data points were unavailable due to inability to obtain enough saliva or due to potential contaminants in the sample. We therefore used a mixed model analysis with the Geisser-Greenhouse correction that allows for missing values. This method provides the same P values and multiple comparison tests as obtained using repeated measures ANOVA. Additional analyses included comparisons of analyte ratios, whereby data were log transformed and ratios analyzed using mixed models analysis. Finally, correlational analyses were conducted to determine any possible

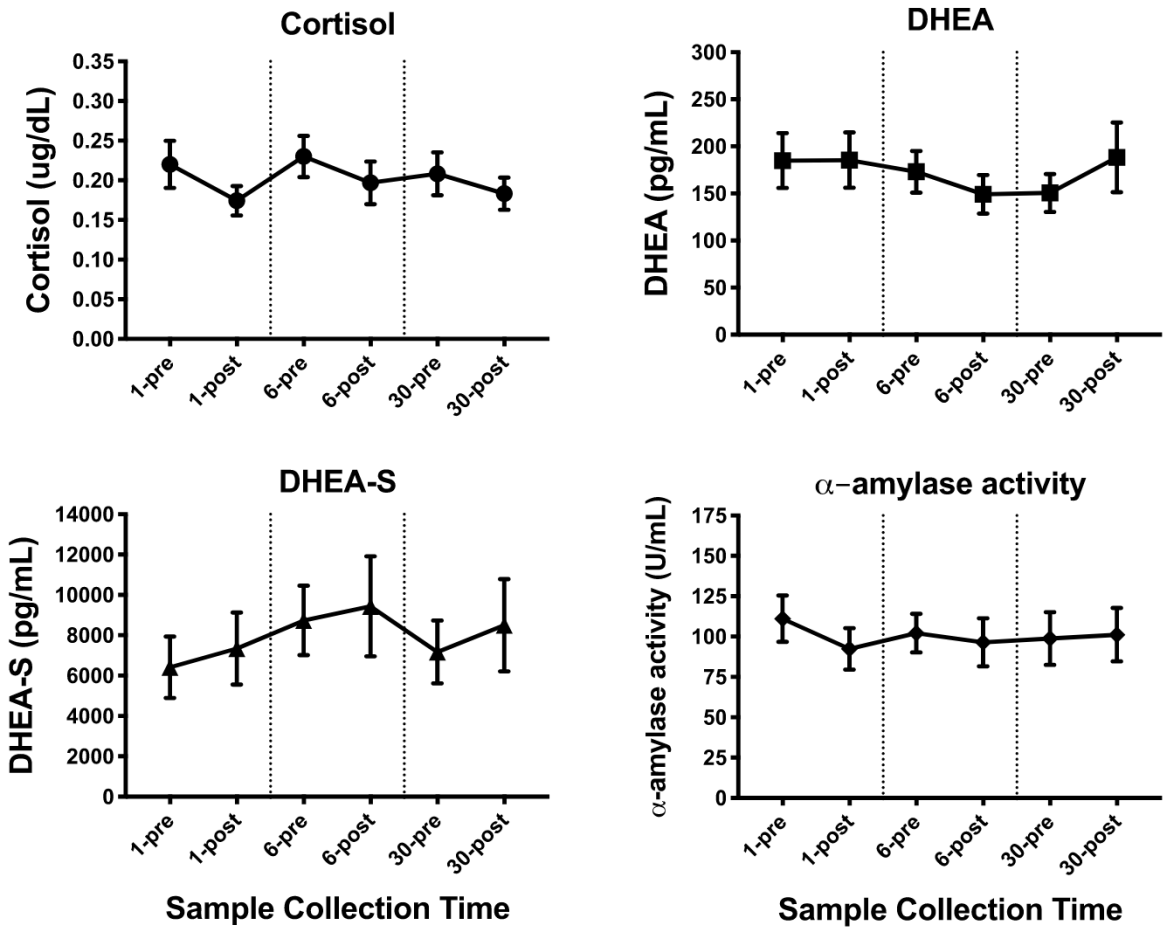

Fig. 2. Salivary analyte levels. Analyte levels over time for cortisol, DHEA, DHEA-S, and α-amylase. Sample collection times are noted for pre and post rTMS at sessions 1, 6, and 30. No significant changes were seen across treatment sessions.

relationships between analyte levels and symptom measures.

3. Results

For all three rating inventories, patients showed significant decreases (11-13% from session 1) in symptom severity after the first week (Fig. 1). Symptom improvement continued over time as seen by substantially lower scores at the end of the 30 sessions of rTMS, with decreases of around 40% from baseline for all three scales. Fixed effects for treatment over time were as follows: BDI, $F_{(1.321,\ 36.98)} = 22.01$, $P<0.001$; GAD, $F_{(1.241,\ 34.76)} = 19.79$, $P<0.001$; PHQ-9, $F_{(1.371,\ 38.39)} = 18.82$, $P<0.001$. Tukey's multiple comparisons tests showed significant differences from session 1 for all three rating inventories ($P<0.05$ at session 6 and $P<0.001$ at session 30).

For ELISA data, all analytes showed values within the predicted ranges. Levels of cortisol, DHEA, and DHEA-S and activity of α-amylase did not significantly change across the treatment sessions (Fig. 2). Fixed effects for treatment over time for each analyte were as follows: cortisol, $F_{(3.292,\ 83.62)} = 1.01$, $P=0.40$; DHEA, $F_{(2.555,\ 59.79)} = 1.11$, $P=0.35$; DHEA-S, $F_{(2.817,\ 64.24)} = 1.19$, $P=0.32$; α-amylase, $F_{(3.287,\ 80.20)} = 0.43$, $P=0.75$. Conversion of analyte values to ratios also failed to show any significant changes over time for analyte ratios (data not shown). Correlation analyses for each inventory score (BDI, GAD, and PHQ-9) with each analyte (cortisol, DHEA, DHEA-S, and α-amylase) failed to show any significant correlations (data not shown).

4. Discussion

In the current study, we found a robust effect of rTMS in treatment refractory MDD patients using a standardized bilateral DLPFC rTMS protocol. Prior studies have consistently shown significant beneficial effects of rTMS for MDD (McGirr and Berlim, 2018). Our results are congruent with earlier findings, with significant symptom reductions as early as one week and further improvement by the end of the 30 treatment sessions. One limitation was the lack of a between subjects comparison using a sham rTMS control group, which was not feasible for ethical reasons. The significant symptom improvement over time thus cannot be fully attributed to rTMS treatment alone, as MDD progression is affected by multiple factors (Kirsch, 2019). Nonetheless, the magnitude of improvement concurs with previous data showing pronounced efficacy of rTMS for MDD patients in clinical practice (Dowling et al., 2020). However, we did not see any significant changes over time in several relevant biomarkers that have been previously linked to depression symptoms.

Only a limited number of studies have measured neuroendocrine factors after rTMS in MDD subjects (reviewed in Perrin and Pariante, 2020). Padberg et al. (2002) reported that plasma DHEA and several other neuroactive steroid levels did not change after 10 sessions of left DLPFC rTMS, even though depression symptoms decreased by around 40%. After a single left DLPFC rTMS session, Baeken et al. (2009) showed an acute decrease in salivary cortisol, but without any change in subjective mood. Mingli et al. (2009) reported that plasma cortisol and ACTH levels decreased after 10 sessions of rTMS of the temporal and

occipital cortices and was accompanied by improved symptoms as measured by the Hamilton Depression Rating Scale. Meille et al. (2017) showed no changes in serum cortisol, prolactin, and thyroid hormone levels after two weeks of right DLPFC rTMS, although they did see a decrease in growth hormone in nonresponsive subjects. Methodological and sample population differences likely account for the variation in findings across these few previous studies.

Prior measurement of salivary α-amylase activity in subjects with MDD has suggested that this putative marker of sympathetic stress reactivity may reflect depressive symptoms (Ishitobi et al., 2010). Our study is the first to measure α-amylase activity after rTMS for MDD and we found no significant impact at any stage of treatment. Further assessment is warranted, as α-amylase activity varies in a cyclical manner (Nater et al., 2007) and elevated α-amylase activity in MDD has been reported relative to controls when sampled in the morning, but not in the evening (Bauduin et al., 2018). In addition, α-amylase activity shows situation-dependent responses, particularly for anxiety related symptoms (Schumacher et al., 2013).

We also performed ratio analyses, as single hormones often function in an interdependent manner with each other (Sollberger and Ehlert, 2016). Such interactions may be of particular importance for cortisol in relation to DHEA (Kamin and Kertes, 2017) and α-amylase (Ali and Pruessner, 2012). We found no significant alterations over time across the various ratio calculations, further indicating that no sustained alterations occurred in the pattern of analyte activity after rTMS in the current population of MDD patients.

Future studies may seek to further explore potential biomarkers of rTMS treatment, including measurement of additional endpoints. One promising area is the assessment of immune related factors, as a recent study reported that rTMS increased brain-derived neurotrophic factor (BDNF) and decreased interleukin (IL)-1β and tumor necrosis factor (TNF)-α over time in patients with refractory MDD (Zhao et al., 2019). In addition, future approaches need to consider the identification of state versus trait biomarkers of MDD before and after rTMS, as Mocking et al. (2015) provided evidence that cortisol and DHEA-S profiles in MDD may better reflect an endophenotypic trait, as opposed to a state effect. By ultimately identifying reliable and consistent biomarkers, future use of rTMS for MDD and other disorders may then be better targeted for subpopulations and specific treatment parameters.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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Author Contributions

RES designed the study, carried out ELISAs and formal data analysis, wrote the original draft, and oversaw all study protocols. BE managed rTMS and saliva collection procedures, organized subject data collection, and assisted with data analysis. DA oversaw subject enrollment, clinical diagnosis, and patient care. MH contributed to study conceptualization and staff management. All authors contributed to the manuscript drafts and approved the final manuscript for submission.

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