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Spinal tDCS and Oxy-Inflammation in patients with Multiple Sclerosis: a pilot study

Dear Editor,

Given the global incidence of multiple sclerosis (MS), with more than 2.8 million people living with the disease worldwide [1], non-invasive neuromodulation as adjuvant therapy [2], and oxidative stress [3], inflammation, and synaptic plasticity as mechanisms involved gained increasing interest. We therefore investigated the clinical effects and action mechanisms of transcutaneous spinal direct current stimulation (tsDCS) assessing Oxyinflammation biomarkers in MS patients by micro-invasive techniques.

In this pilot study, seven MS patients with spasticity (5 men; 42–70 years) received anodal and sham tsDCS (2mA, 20 min/day) for 5 days. Electrodes were placed over the 10th thoracic vertebra (anode) and the right shoulder (cathode). Measures were assessed at baseline (T0), day 5 (T1), one week (T2), and one month (T3) post-treatment (Fig. 1A). Clinical evaluation was based on the: Modified Ashworth Spasticity scale-MAS, Multiple Sclerosis Spasticity Scale-MSSS-88, 12-item Multiple Sclerosis Walking Scale-MSWS-12, Multiple Sclerosis Quality of Life −29 MSQOL29, and 36-item Short Form Health Survey-SF-36. Biomarker assessment consisted in: Reactive Oxygen Species-ROS production and total antioxidant capacity-TAC by capillary blood; transthyretin - TTR by plasma; reduced (red) glutathione by RBC; nitrite/nitrate metabolites-NOx, lipid peroxidation (8-isoprostane), DNA damage-8-OH-2-deoxyguanosine, interleukin-6 - IL-6, Neopterin, and creatinine concentration by urine [4]. TsDCS safety was evaluated via computational modeling [5]. Statistical analysis included classical and Bayesian approaches, the latter being more suitable for small sample sizes [6]. (**Additional file: Materials and methods**).

As shown by computational modeling, current density remained well below harmful thresholds for tissue damage. Due to electrode-target distance, current was largely diverted through surrounding tissues, mainly back muscles and nerve roots. Simulations predicted minimal heating (<1.5 °C) after 20 minutes of tsDCS, insufficient to cause adverse effects in sensitive tissues or to influence neuronal activation (Fig. 1B).

Among the analyzed oxidative stress biomarkers (Fig. 1C), we observed that ROS and 8-ISO showed significantly different changes after anodal and sham stimulation, regardless of the time point: ROS decreased after anodal stimulation (active vs sham: 0.145 ± 0.196 vs 0.049 ± 0.037, ANOVA Stimulation factor $p = 0.018$, Bayesian moderate evidence, BF = 3.34); 8-ISO increased after anodal stimulation (active vs sham, 0.643 ± 0.668 vs −0.153 ± 0.364, ANOVA Stimulation factor $p = 0.003$; Bayesian moderate-to-strong evidence, BF = 6.10). TAC showed an effect that changed in time (ANOVA "Time-*Stimulation"), with a significant increase at T1 after anodal stimulation (anodal tsDCS, T1 vs T3 0.2 ± 0.066 vs 0.05 ± 0.072, post-hoc $p = 0.011$), as confirmed also by Bayesian statistics (moderate-to-strong evidence BF = 4.99). Similarly, DNA damage showed a differential effect of anodal and sham tDCS over time (ANOVA "time * stimulation"), with a significant higher increase at T1 (T1 active vs sham: 10.540 ± 6.499 vs 1.873 ± 2.804, post-hoc $p = 0.009$, Bayesian statistics very-strong evidence, BF = 791.714), but not at the other time points. IL-6 showed a combined effect of stimulation and time with a significant higher increase at T1 (T1 active vs sham: 4.720 ± 1.673 vs 0.831 ± 0.775, post-hoc $p = 0.004$), and at T2 (T2 active vs sham: 3.733 ± 3.026 vs 0.791 ± 0.835, post-hoc $p = 0.045$), but not at T3 (Bayesian statistics very-strong evidence BF = 8277.28). TTR showed a similar pattern of changes with a combined effect of stimulation and time and a significant higher increase at T1 (T1 active vs sham: 17.327 ± 14.573 vs −2.084 ± 2.488, post-hoc $p = 0.031$), and at T2 (T2 active vs sham: 24.677 ± 15.525 vs −1.336 ± 1.354, post-hoc $p < 0.001$) but not at T3, confirmed by Bayesian statistics (very-strong evidence, BF = 112.267). The average values of all biomarkers examined at all time points for anodal and sham tDCS are provided in **Additional file:** Table 1 **results.**

In general, all clinical and psychological scales tended to change after anodal and sham tsDCS, without reaching statistical significance (**Additional file:** Table 2 **results**). Among quality-of-life scales, we could observe a non-significant tendency towards different increase after anodal vs sham stimulation in MSQOL29 cognitive function part and in the SF36 social part. All the scales for SM clinical evaluation did not show differences between anodal and sham stimulation. However, when considering only the Ash score of the left hamstring, we found a significant decrease after anodal vs sham stimulation (Bayesian anecdotic evidence, BF = 1.724) (Fig. 1D).

Considering only the variables showing significant differences between changes induced by anodal and sham stimulation, we run an exploratory correlation analysis to preliminary assess the possible interactions between the changes observed in biomarkers and in clinical and psychological functions. We observed that ROS changes over time significantly correlated with MSQOLcog (inverse correlation $r = -0.356$, $p = 0.033$), SF36 social (inverse correlation, $r = -0.415$, $p = 0.012$), and Ash harm sin (direct correlation, $r = 0.479$, $p = 0.001$) changes over time. In addition, TAC significantly correlated with SF36 social (direct correlation, 0.439, $p = 0.007$), and DNA damage significantly correlated with SF36 social (direct correlation, $r = 0.481$, $p = 0.003$) (Fig. 1E).

This pilot study demonstrated that anodal tsDCS modulated biomarkers of oxidative stress and inflammation, with only modest effects on clinical and psychological scores. Notably, tsDCS reduced ROS levels and increased antioxidant capacity markers such as GSH and TAC, suggesting improved mitochondrial efficiency [7]. However, despite the ROS reduction, oxidative damage to DNA and lipids persisted, possibly due to ongoing MS progression. Anodal tsDCS also induced changes in inflammation-related markers [8], including increased transthyretin and interleukin-6, indicating potential involvement in remyelination

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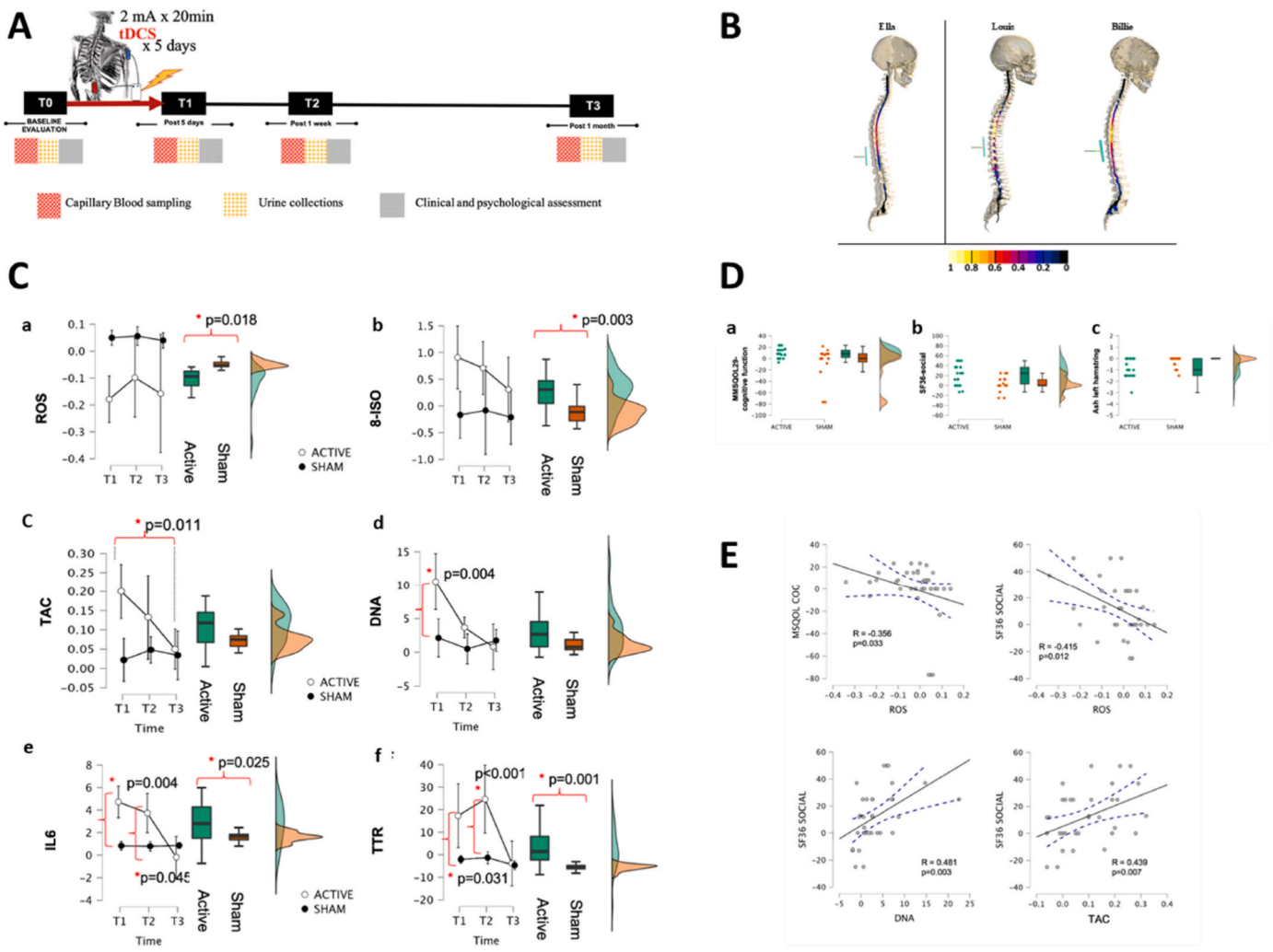

Fig. 1. A. Transcutaneous Spinal Direct **Current Stimulation** (tsDCS**) experimental protocol.** Active tsDCS electrode was placed over the spinal process of the 10th thoracic vertebra and the other (reference) electrode over the right shoulder. 20-minute daily tDCS sessions were applied for 5 consecutive days. Patients were assessed (biological samples - blood and urine- and clinical scales) in the first (T0) and the last day of tsDCS (T1), after 1 week (T2) and 1 month from ended tsDCS (T3). **B. Human models.** Lateral view of the J amplitude distributions over spinal cord, cauda equina and nerves for the three human models, "Ella" (left), "Louis" (middle) and "Billie" (right). The values are normalized with respect to the maximum value found in the spinal cord for each electrode montage. **C. The effect of tsDCS on biomarkers –** The average (N = 7) changes from baseline at T1, T2, and T3 after anodal and sham tsDCS of ROS (a), 8-ISO (b), TAC (c), DNA damage (d), IL6 (e), TTR (f). Each panel is organized with a graph, on the left, superimposing the average changes from baseline at T1, T2, and T3 after sham (black dots) and anodal (white dots) tsDCS. Error bars are 95 % confidence intervals. The central panel represents the box plot of the average changes observed after anodal (green) and sham (orange) tsDCS pooling together all time points. On the right, the corresponding probability distributions. **D. The effect of tsDCS on clinical and psychological scales.** Average (n = 7) changes from baseline after anodal (green) and sham (orange) tsDCS pooling together all time points of MSQOL cognitive part (A), SF-39 social part (B), and ASH left hamstring (C). Each panel is organized with a left panel showing individual values and the box plot of the average changes observed and a right panel showing the corresponding probability distributions. **E. Correlations between biomarkers and clinical/psychological scales.** Scatter plot of the correlation between ROS and MSQOL cognitive part (a), ROS and SF-36 social part (b), DNA damage and SF-36 social part (c), and TAC and SF-36 social part (d). In each panel, each dot represents the change from baseline, occurring at a certain time point (T1, T2, or T3) after sham or active tsDCS. The solid line is the estimated regression line. Dashed lines are 95 % confidence intervals.

and microglial modulation [9]. These changes align with evidence suggesting that tDCS can attenuate neuroinflammation and promote neuroprotection. The significant associations between changes in biomarkers and clinical/psychological functioning highlight the potential of biomarkers profiling as a predictive tool to optimize tsDCS treatment strategies and improve outcomes in MS patients. Preliminary correlations between oxidative and inflammatory biomarkers and improvements in MS-related quality of life scores (MSQOL29 and SF-36) support the hypothesis that tsDCS may influence disease-related symptoms, particularly in physical and social functioning [10]. However, due to the small sample size and variability in responses, statistical significance was not achieved. Further research is needed to elucidate the specific pathways through which tsDCS exerts its therapeutic effects and to validate biomarker signatures that predict response to treatment, and clarify the mechanisms by which tsDCS may modulate neurobiological pathways in MS.

CRediT authorship contribution statement

Simona Mrakic-Sposta: Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Roberta Ferrucci:** Conceptualization, Data curation, Investigation, Writing – original draft, Writing – review & editing. **Sara Marceglia:** Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **Alessandra Vezzoli:** Data curation, Formal analysis, Writing – original draft, Writing – review & editing.

Marta Parazzini: Conceptualization, Data curation, Writing – original draft, Writing – review & editing. **Serena Fiocchi:** Data curation, Investigation, Writing – original draft, Writing – review & editing. **Maurizio Vergari:** Data curation, Investigation, Writing – original draft, Writing – review & editing. **Stefano Floro:** Data curation, Writing – original draft, Writing – review & editing. **Milena Deriz:** Data curation, Writing – original draft, Writing – review & editing. **Alberto Priori:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Sara Marceglia reports a relationship with Newronika SpA that includes: board membership, consulting or advisory, and equity or stocks. Simona Mrakic-Sposta reports a relationship with Newronika SpA that includes: equity or stocks. Roberta Ferrucci reports a relationship with Newronika SpA that includes: equity or stocks. Maurizio Vergari reports a relationship with Newronika SpA that includes: equity or stocks. Alberto Priori reports a relationship with Newronika SpA that includes: board membership, consulting or advisory, and equity or stocks. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brs.2025.05.111.

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