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Transcranial direct current stimulation of the mouse prefrontal cortex modulates serotonergic neural activity of the dorsal raphe nucleus

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To the Editor

Transcranial direct current stimulation (tDCS) is being increasingly considered as a non-pharmacological alternative for various psychiatric and neurological disorders. In major depressive disorder (MDD), anodal tDCS applied over the left dorsolateral prefrontal cortex (DL-PFC) improves symptoms in drug-resistant patients [1]. However, the molecular mechanisms underlying the efficacy of tDCS in MDD are still unknown. One of the main neurotransmitters involved in the pathophysiology and psychopharmacology of MDD is serotonin (5-HT), released by neurons of the dorsal raphe nucleus (DRN), which have widespread projections to cortical, subcortical and brainstem regions. In rodents, the prefrontal cortex directly innervates the DRN [2], and the activation of this pathway by deep brain stimulation (DBS) was shown to have antidepressant activity [3].

We investigated whether tDCS over the PFC in mice affects the activity of DRN 5-HT neurons using single-unit *in-vivo* electrophysiology [4] and analysis of cFos levels (as a measure of cell activation) in the prelimbic cortex (PrL) and the dorsal portion of the DRN (DRD) of adult (2–3 months) anesthetized male C57BL/6 mice. For *in-vivo* electrophysiology, once a spontaneous and stable DRN 5-HT neuron was identified and isolated [4], we recorded the activity for 4-min at rest, for 10 min during 250 μA anodal or cathodal left PFC tDCS and for further 10min after stimulation (1 neuron/mouse; 8 neurons for both anodal and cathodal stimulations). tDCS was applied through a plastic tube filled with saline solution just prior to stimulation. The centre of the active electrode (0.045 cm^2) was positioned over the left frontal areas (1.5 mm lateral and 1.8 mm anterior to bregma). The counter electrode was a saline soaked sponge (5.2 cm^2) adhering to the ventral thorax [5]. Mean firing activity during and after stimulation was measured and calculated as percentage of baseline. For cell activation analysis [6], cFos expression was quantified in 15 mice (5/group: sham, anodal, cathodal). Briefly, 90 minutes after tDCS anesthetized mice were transcardially perfused with PBS followed by PBS-4% paraformaldehyde. Frozen brains were cut into 35-μm sections and incubated in blocking solution for 1h at RT, followed by overnight incubation in the blocking solution containing anti c-Fos rabbit antibodies (Santa Cruz Biotechnology, USA; 1:500) at 4 °C. After washing, the sections were incubated with Alexa Fluor 555-conjugated anti-rabbit IgG antibodies (Thermo Fisher Scientific, USA; 1:1000) at RT for 2 hours. Images were acquired through a 40x objective (5 sections/animal) and c-Fos levels were quantified as described [6] within the sections of interest (PFC: +1.54 to +1.98; DRD: 4.36 to −4.84 from bregma). Briefly, c-Fos^+ cells were counted based on shape and fluorescence intensity (threshold settled with ImageJ software), blinded to experimental conditions All procedures were conducted in accordance with the Italian guidelines of the Ministry of Health.

We found that both anodal and cathodal tDCS acutely decreased DRN 5-HT activity (stimulus: $F(2,28) = 12.99$, $P = 0.0004$; current: $F(1,28) = 8.74$, $P = 0.01$; interaction: $F(2,28) = 0.97$, $P = 0.50$) compared with baseline. DRN 5-HT firing rate remained low even after the stimulus ceased (Fig. 1A–C). Interestingly, Bonferroni post-hoc analysis showed that the acute inhibitory effect of cathodal tDCS was greater than that of the anodic stimulation during both the 10-min stimulus ($P = 0.010$) and the 10-min post-stimulus ($P = 0.023$).

Although it was not possible to perform unbiased stereology, quantitative analysis of the immediate-early gene product cFos (Fig. 1D–E) suggested an increased number of cFos^+ cells in PrL after tDCS in both polarities with respect to the sham group (Fig. 1D; $F(2,12) = 31.25$, $P < 0.001$) and in DRD (Fig. 1E; $F(2,12) = 12.77$, $P = 0.001$). Notably, in the PrL cortex, cathodal tDCS resulted in higher cFos^+ cell activation compared with anodal tDCS ($P = 0.023$).

This study is based on the hypothesis that the modulation of the top–down projections from the PFC to the DRN could be a key mechanism in the antidepressant effects of non-pharmacological treatments. In keeping, we found that acute left PFC-tDCS inhibited DRN 5-HT activity. A similar effect was observed with acute application of current antidepressants, including selective serotonin reuptake inhibitors (SSRIs) [7]. However, this similarity does not imply that the effects of tDCS are mediated by inhibition of serotonin reuptake (see, 8). Indeed, Srejic et al. [3] showed in rats that DBS in the PFC affected the activity of DRN 5-HT neurons likely through the modulation of the local GABAergic tone, that could be the same underlying mechanism we observed with tDCS. Notably, in that work the significant decrease in the firing rate of DRN 5-HT neurons was present only during the stimulation, while we found that acute tDCS inhibitory effects outlasted the stimulation period. The intensity used here corresponds to a charge density of 33.3 kC/m^2, which is higher with respect to typical human studies (33.3 vs. 0.3 kC/m^2).

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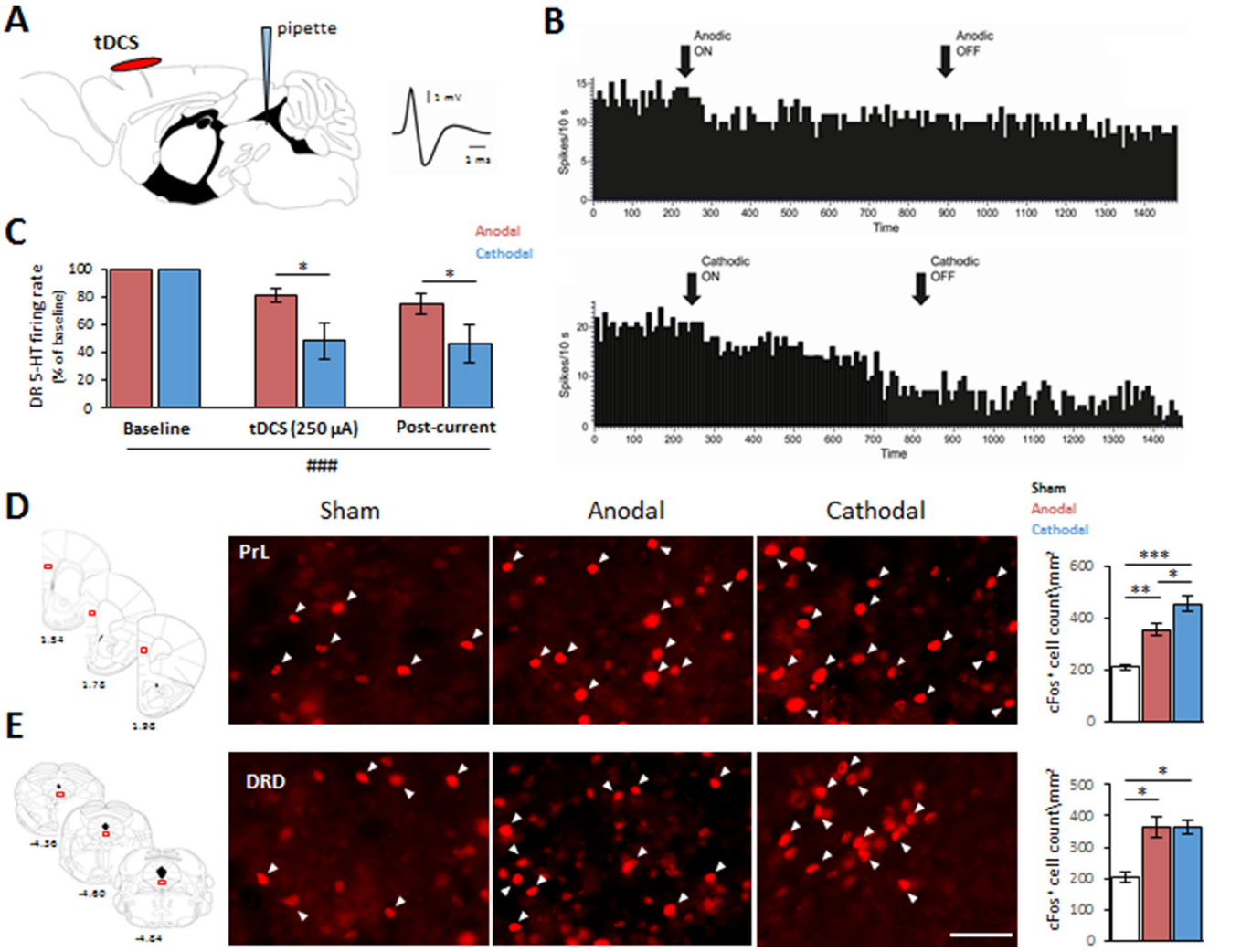

Fig. 1. Left PFC anodal and cathodal tDCS affects DRN activity. A. Midsagittal brain section showing approximate anatomical locations of experimental procedures (left) and representative wave-form of a DRN 5-HT neuron (right). **B.** Representative firing rate histogram showing the acute inhibitory response of a DRN 5-HT neuron to anodal and cathodal tDCS applied over the left PFC. **C.** DRN 5-HT firing activity expressed as percentage of baseline after 10-min of either anodal and cathodal tDCS and 10-min after the stimulus ended. *P < 0.05 anodal vs. cathodal stimulation; ###P < 0.001, effect of stimulation; two-way ANOVA for repeated measures followed by Bonferroni post hoc comparisons. **D-E.** Coronal brain section showing the regions of interest for cFos analysis. Representative images and quantification of the numbers of cFos$^+$ cells in the sham, anodal and cathodal groups in PrL (**D**) and DRD (**E**). Scale bar: 50 µm. Data are given as mean ± S.E.M. *P < 0.05, **P < 0.01, ***P < 0.001; one-way ANOVA followed by Bonferroni post hoc comparisons.

It has been chosen to be consistent with our previous findings in mice [5] and other studies performed in rats [9]. Moreover, these parameters fall within the safety range suggested by similar stimulation condition in animal models [9]. Of note, in addition to the different current density, it has to be taken into account a different electric field direction and magnitude between mice and humans, due to their vast anatomical differences.

In summary, these novel findings show that 10-min anodal or cathodal tDCS over left PFC produces a significant acute inhibition of DRN 5-HT neurons, thus resembling the effects exerted by SSRIs such as fluoxetine or by PFC DBS [3,7,8]. Therefore, it is conceivable that the antidepressant effects deriving from DL-PFC tDCS may occur through the direct or indirect modulation of DRN 5-HT neurons, as also suggested by human studies [10]. Interestingly, although for the treatment of depression in humans anodic tDCS is mostly used, we have found that acute cathodal tDCS induces the activation of PrL cortex neurons and the inhibition of DRN 5-HT neurons at higher magnitude than acute anodal tDCS of the same intensity. As a final remark, it has to be considered that in the clinical setting tDCS has the great advantage of being a non-invasive and side-effect free technique compared with DBS or pharmacological therapies.

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Author contributions

MC and SC conceived and performed experiments, analyzed data, critically review the interpretation of the results and wrote the manuscript; MB and FV critically review the interpretation of the results and wrote the manuscript; LM performed experiments.

Declaration of competing interest

All authors report no disclosures or conflicts of interests.

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