# Early application of cathodal-tDCS in a mouse model of brain ischemia results in functional improvement and perilesional microglia modulation

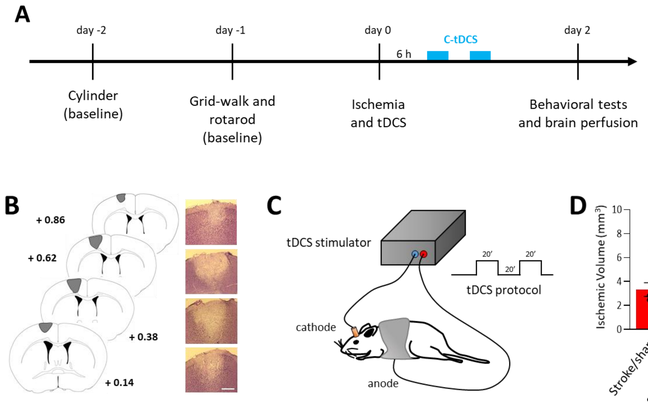
Laura Cherchi, Daniela Anni, Mario Buffelli and Marco Cambiaghi

Department of Neurosciences, Biomedicine and Movement Sciences - University of Verona, Verona, Italy

**Background.** Treatment of acute ischemic stroke is mostly limited to thrombolysis, which unfortunately can be applied only to a minority of cases because of its narrow therapeutic window (<6 hrs). Hence, new therapeutic strategies capable of modulating the ischemic pathophysiology (including the inflammatory response) that can be applied beyond the time window for thrombolysis, are urgently needed. In the last few decades, non-invasive neuromodulatory techniques, such as transcranial direct current stimulation (tDCS), have emerged as an effective treatment for chronic stroke and could be a promising tool for acute/subacute stroke as well. Moreover, in our previous work [*Peruzzotti-Jametti L, Cambiaghi M et al., Stroke 2013*], we showed that application of cathodal but not anodal tDCS during the very early phases of ischemic stroke has neuroprotective and better functional outcomes in a middle cerebral artery occlusion (MCAO) mouse model.

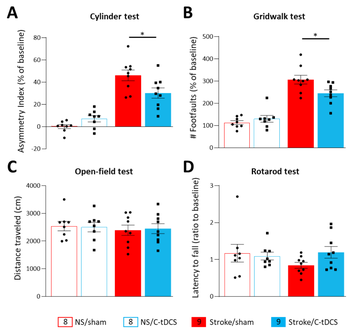
**Aims.** The purpose of this project is to study the effects of early cathodal tDCS (C-tDCS) on motor functionality and microglia activation in a mouse model of ischemic stroke.

**Methods.** Photothrombotic stroke was induced in Cx3CR1GFP+/- mice and C-tDCS applied 6 hours after unilateral M1 ischemia. Motor functionality was tested with the cylinder and foot-fault tests, while the density and activation state of microglia cells were evaluated via 3D imaging quantifications.

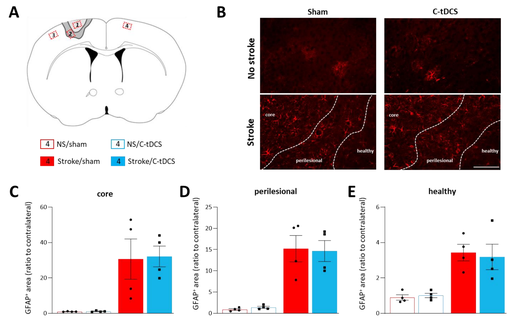



**Early application of cathodal tDCS after photothrombotic ischemia in mice.** (A) Experimental timeline showing the two days of baseline motor tests followed by photothrombotic stroke induction and C-tDCS 6 hours afterward. Motor impairments were reevaluated 48 hours after ischemia. (B) Representative Nissl-stained brain sections showing the lesion in the M1 area (bar=300 µm). (C) Schematic representation of brain stimulation and C-tDCS protocol consisting of 2 series of 20 minutes' stimulation with a 20 minutes interval. (D) Photothrombotic lesion was not different in the sham vs stimulated groups (n=9 per group; p>0.05). Data are given as mean ± SEM (unpaired t-test).

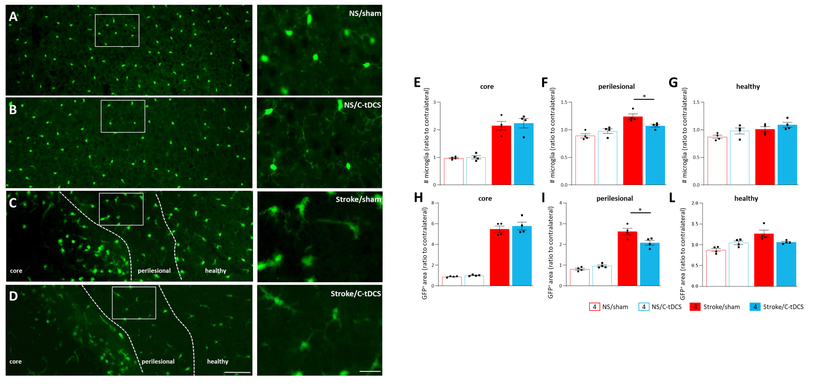
**Results.** Though tDCS application did not decrease the lesion volume at 48 hours, the functionality of the affected forelimb was significantly higher in the C-tDCS group vs sham treated mice. C-tDCS treatment also reduced microglia density in the perilesional area, and induced a resting microglial phenotype with ramified morphology, increased total processes length, process branch points and terminal process branches, vs the sham group.



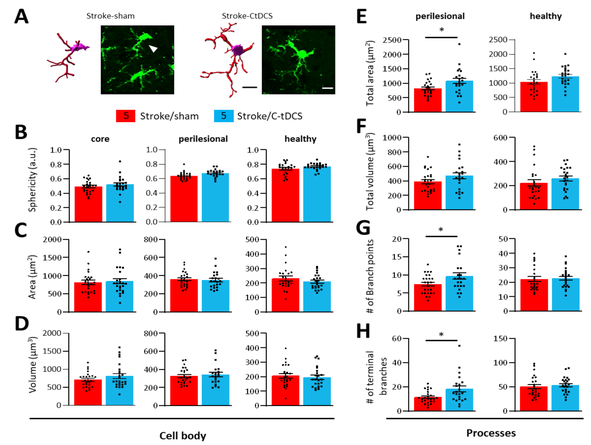

**Cathodal tDCS early after ischemia preserves the affected limb motor activity.** Following photothrombosis mice showed a significant functional deficit of the affected forelimb in the cylinder (A) and the gridwalk test (B) with respect to baseline values. The application of C-tDCS in the stroke groups showed a significant reduction of the asymmetry index (p=0.028) and in the number of footfaults (p=0.046), with respect to non-stimulated stroke mice. Motor tests based on locomotor activity and motor coordination showed no differences among the different groups. The OFT analysis of voluntary locomotion (C) and the mean latency to fall in the rotarod test (D) showed no significant effect of stimulation and photothrombotic ischemia with respect to baseline values. Data are given as mean ± SEM (n=8 for each no-stroke group; n=9 for each stroke group; two-way ANOVA followed by Tukey's).



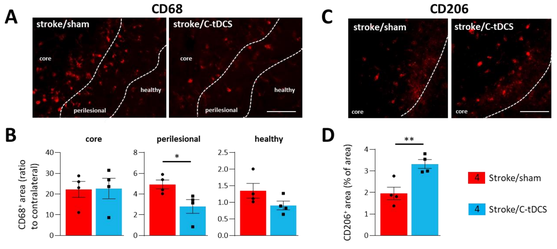

**Astrocytes are not modulated by C-tDCS after cortical ischemia.** (A) Schematic of the areas analyzed with respect to the cortical lesion. Red insets correspond to the analyzed sites (ROIs): 1. Ischemic core; 2. Perilesional region; 3. Healthy tissue in the lesioned hemisphere; 4. Healthy tissue in the contralateral hemisphere. (B) Representative images of the four conditions (bar=100 µm). Quantitative analysis of astrocytes at 48 hours after ischemia showed an increased GFAP+ cells in the (C) core, (D) perilesional area and (E) healthy tissue of the photothrombotic hemisphere; the C-tDCS treatment had no effects in any of these regions. Data are given as mean ± SEM (n=4 per group; two-way ANOVA followed by Tukey's).




**Reduced recruitment of microglia in the perilesional area after early C-tDCS.** (A-D) Representative images showing GFP+ microglia in control (no Stroke, NS) and Stroke mice, sham or stimulated with C-tDCS, with magnifications of the perilesional area (right) (bars=150 and 30 µm, respectively). (E-G) The number of GFP+ microglial cells were higher in the stroke conditions, with no differences in the core and heathy tissue between sham and stimulated mice. In the perilesional region, the number of GFP+ microglial cells in the Stroke/C-tDCS was significantly lower with respect to Stroke/sham mice (p=0.044). Similarly, the total GFP+ area quantification (H-L) showed a significant decrease in the C-tDCS mice exclusively in the perilesional region (0.014). Data are given as mean ± SEM (n=4 per group; two-way ANOVA followed by Tukey's).




**C-tDCS applied after acute stroke induces microglial morphological changes in the perilesional area.** (A) Representative 3D-reconstructed microglia cells of Stroke/sham and Stroke/C-tDCS mice in the perilesional stroke area and their relative confocal images (bar=15 µm). Microglia cells were reconstructed and analyzed from three different areas: ischemic core, perilesional stroke region and contralateral hemisphere (healthy). Quantitative morphometric analysis of microglia cell body (B) sphericity, (C) area and (D) volume) revealed no differences between groups. Analysis of microglial processes showed a significant increase of (E) total area (p=0.015) but not (F) total volume after C-tDCS, that also resulted in increased (G) number of branch point (p=0.035) and (H) terminal branches (p=0.010). All parameters were obtained using Imaris Bit-plane software. 4/5 GFP+ microglial cells per animal (n=5 per group) were reconstructed and analyzed. Data are presented as a mean ± SEM and statistical differences assessed by unpaired-t test *p<0.05.




**Microglia activation changes following ischemia and C-tDCS early application.** (A) CD68 im-munoreactivity was present in the core, perilesional and heal-thy regions (bar=100 µm). (B) Statistical quantification of the CD68+ area showing a lower expression of the CD86 marker in the perilesional region in the stimulated group (p=0.036). No differences were observed in the core and heathy tissue. (C) CD206 expression was evident only in the ischemic core, where a significant increase in the signal was expressed only by C-tDCS mice (D; p=0.008).
Data are presented as a mean ± SEM (n=4 per group; unpaired-t test) *p<0.05; **p<0.01.

**Conclusions.** Taken together, our findings suggest a positive role for early C-tDCS after ischemia applied beyond the conventional time window for thrombolysis, which is able to modulate microglia phenotype and morphology in parallel to motor recovery.

**Cherchi L, Anni D, Buffelli M, Cambiaghi M. Early Application of Ipsilateral Cathodal-tDCS in a Mouse Model of Brain Ischemia Results in Functional Improvement and Perilesional Microglia Modulation. Biomolecules. 2022 Apr 17;12(4):588.**

*This project was founded by a Brain Research Foundation Verona O.N.L.U.S. grant to M.C.*