Transcranial direct current stimulation applied to prevent optic nerve damage and to promote remyelination in experimental autoimmune encephalomyelitis mouse model

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Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease, mediated by immune cells targeting the myelin sheaths that surround nerve axons[1]. Optic neuritis is an acute inflammatory disorder that causes optic nerve demyelination, retinal nerve fiber layer thinning and retinal ganglion cells death[2]. Altered nerve conduction can be modulated by transcranial direct current stimulation (tDCS) which is a non-invasive brain stimulation that has promising clinical outcomes, e.g. MS[3]. TDCS induces polarity-dependent changes in membrane excitability by anodal tDCS, depolarizing, and cathodal tDCS, hyperpolarizing, the membrane potential in neurons of the stimulated areas[4,5]. However, the neurobiological mechanisms underlying tDCS remain poorly understood, impeding its implementation into clinical routine. For this reason, tDCS application on animal models appears fundamental to understand and validate its treatment efficiency. C57BL/6 mice immunized with myelin oligodendrocyte glycoprotein peptide (MOG_{35-55}) exhibit a chronic Experimental Autoimmune Encephalomyelitis (EAE) course[6] with optic nerve abnormalities, consisting in demyelination/axonal loss. Optic nerve and retinal functional alterations can be detectable using non-invasive methods that allowed a follow-up, visual evoked potentials (VEPs), and photopic electroretinogram (pERG). On the other hand, optical coherence tomography (OCT). was involved to detect morphological retinal changes[7].

Objective: The aim was to test multisession tDCS to modulate myelin alteration in different EAE disease phases.

Results – Preventive tDCS

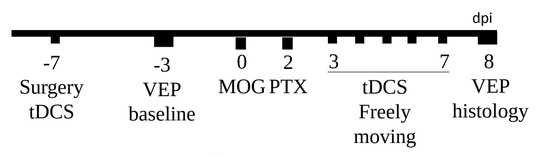
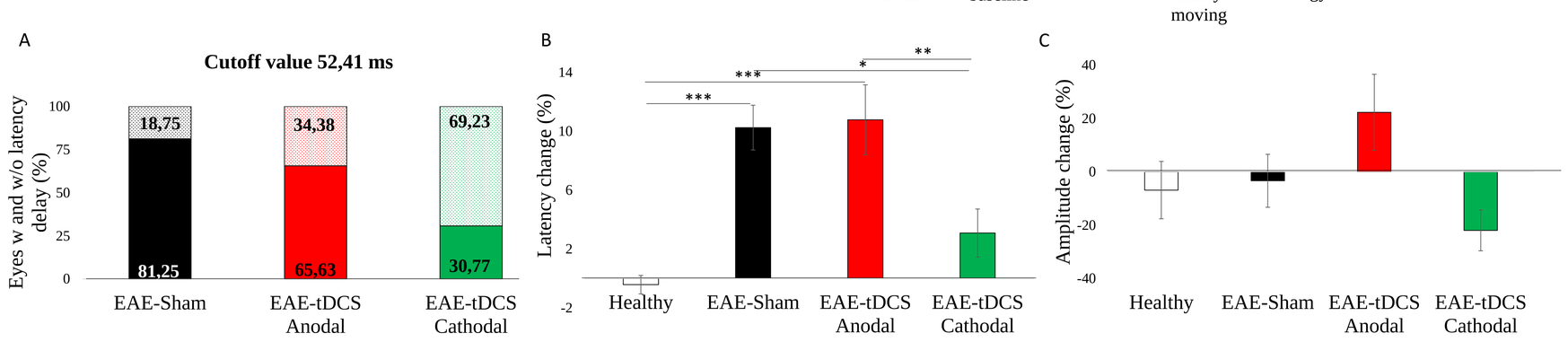


Fig. 1. *Experimental protocol for preventive treatment*

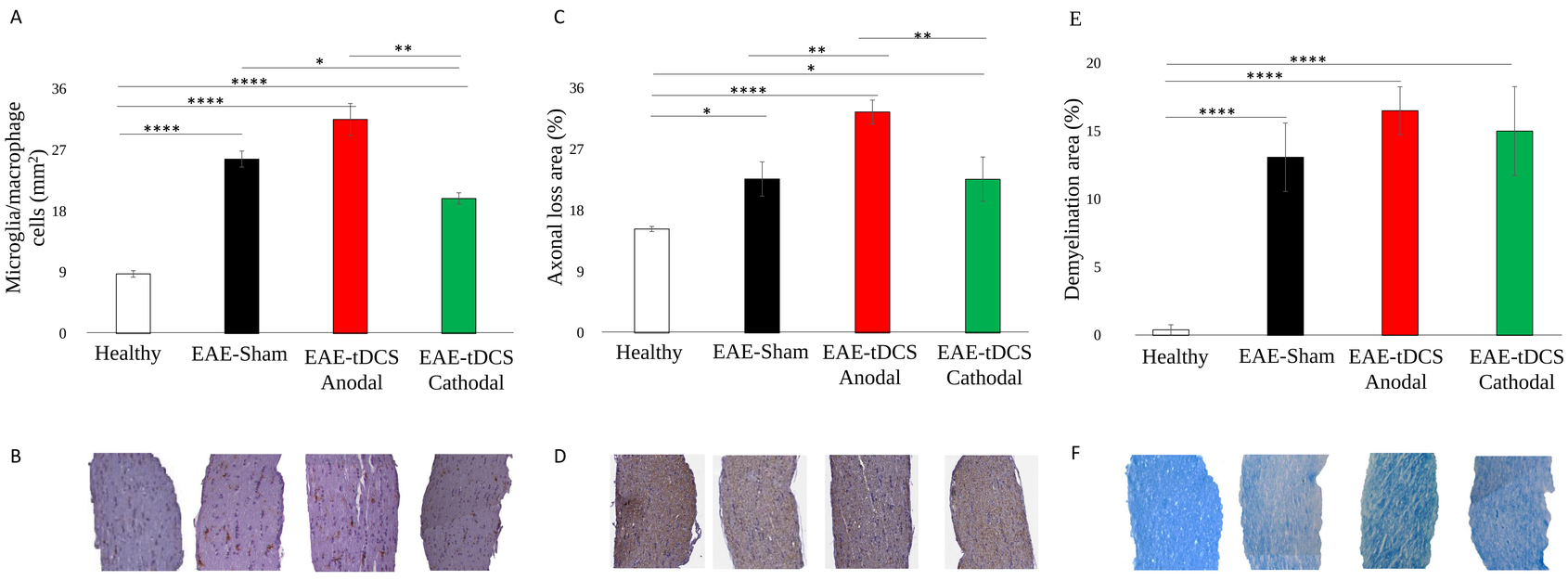


Group	Average baseline (ms)	Average 8 dpi (ms)	vs EAE-Sham p-value	vs EAE-tDCS Anodal p-value	vs EAE-tDCS Cathodal p-value
Healthy	50,31	50,08	0,004	0,002	0,274
EAE-Sham	50,10	55,54		0,825	0,040
EAE-tDCS Anodal	50,19	55,90			0,024
EAE-tDCS Cathodal	50,47	51,85			

Table 1. *Latency (ms) data and statistical analysis*

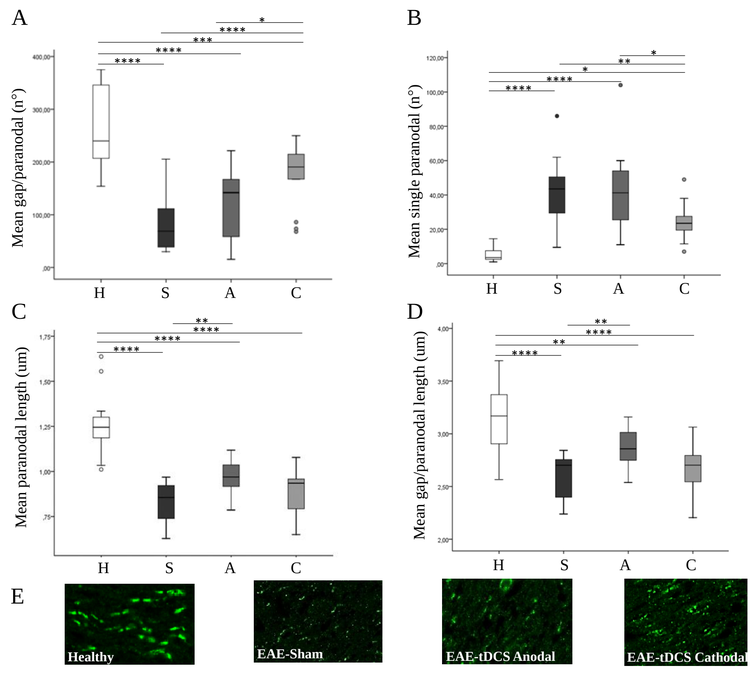
Cathodal tDCS had more effect compared to Anodal stimulation. In particular, Cathodal stimulation significantly decreased the latency delay compared to EAE-Sham and EAE-Anodal groups.

Fig. 2. *VEPs results. A, percentage of eyes with and without latency delay in each group. B, C Latency and amplitude change (%) from baseline to 8 dpi. Healthy (white bars, n = 16 eyes), EAE-Sham (black bars, n = 36 eyes) , EAE-tDCS Anodal (red bars, n=32 eyes) and EAE-tDCS Cathodal (green bars, n = 26 eyes). Error bars represent the SEM. One-way ANOVA followed by LSD post hoc test *p<0.05; **p<0.01; ***p<0.001.*



Cathodal stimulation decreased significant microglia/macrophage cells and axonal loss compared to Anodal tDCS. However, demyelination was compared between EAE groups.

Fig. 3. *Optic nerve immunohistochemistry staining. A, Quantifications of microglia/macrophage cells (mm²), C. axonal loss area (%) E, demyelination area (%) in optic nerve stained by Iba 1, SMI and LFB, respectively. B, D, F, Representative magnification of optic nerves for each group for Iba 1, SMI and LFB staining. Healthy (white bars, n=16 eyes), EAE-Sham (black bars, n=16 eyes), EAE-tDCS Anodal (red bars, n=16 eyes) and EAE-tDCS Cathodal (green bars, n=16 eyes). One-way ANOVA followed by LSD post hoc test *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.*



Both active stimulations had a significant effect. Cathodal tDCS improves numbers of gap/paranode survived and single paranode (define as one green part), while Anodal tDCS increased the paranodal and gap/paranodal length.

Fig. 4. *Optic nerve immunofluorescence staining. A, Quantification of gap/paranodal (n°), B, single paranodal (n°), C, paranodal length (µm), D, gap/paranodal length (um). E, Representative optic nerve sections for Caspr staining in each group. Healthy (white n=16 eyes), EAE-Sham (black,n=16 eyes), EAE-tDCS Anodal (dark grey n=16 eyes) and EAE-tDCS Cathodal (light greyn=16 eyes) One-way ANOVA followed by LSD post hoc test *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.*

Acute tDCS

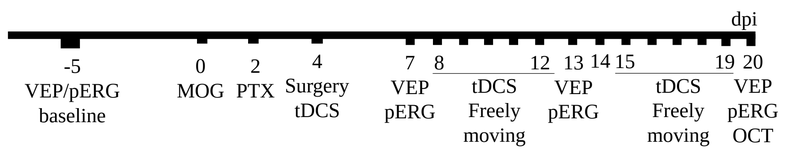
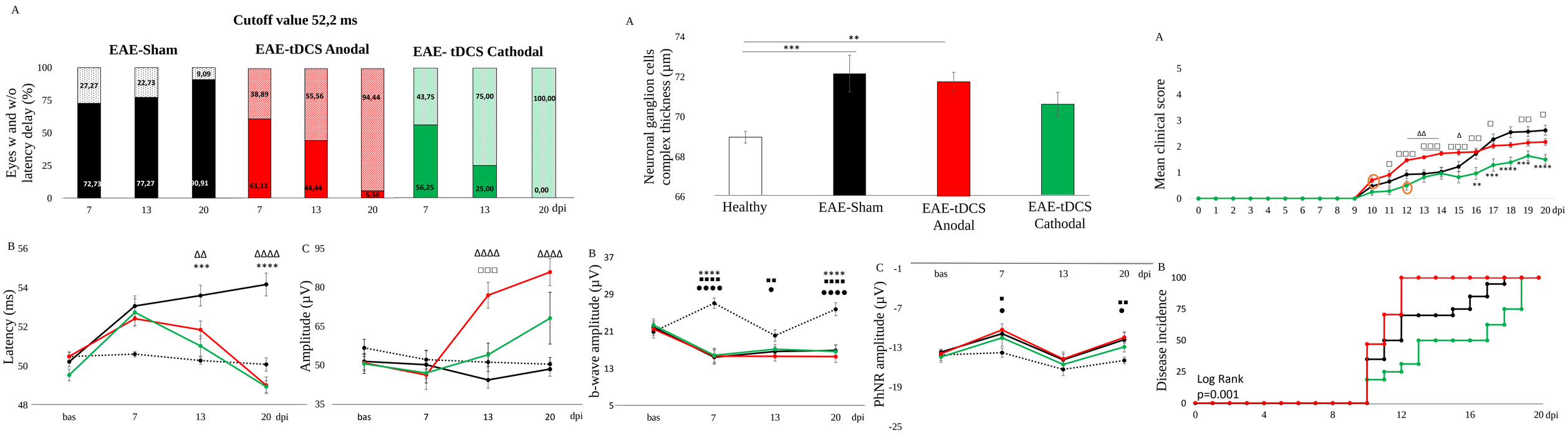


Fig.5. *Experimental protocol for acute treatment*



EAE-tDCS cathodal and anodal mice showed latency recovery 24h after the end treatment.

Fig. 6. *VEPs. A, Percentage of eyes with and without latency delay in each group in follow up. B, C Latency (ms) and amplitude (µV) in follow up. Healthy (dotted lines, n = 20 eyes), EAE-Sham (black bar/lines, n = 22 eyes), EAE-tDCS Anodal (red bar/lines, n = 18 eyes) and EAE-tDCS Cathodal (green bar/lines, n = 16 eyes). Error bars represent the SEM. *significant difference between EAE-Sham vs EAE-tDCS Cathodal, Δsignificant difference between EAE-Sham vs EAE-tDCS Anodal, □represent significant difference between EAE-tDCS Anodal vs EAE–tDCS Cathodal. Mixed ANOVA followed by LSD post hoc was performed **p<0.01;***p<0.001;****p<0.0001.*

Cathodal tDCS mice showed no significant difference in retina thickness compared to healthy. However, both active stimulations did not affect the retina functionality.

Fig. 7. *Retina. A, Neuronal ganglion cells complex thickness (µm) by OCT . B, C b-wave and PhNR amplitude (µV) in follow up. Healthy (white bar/dotted lines, n = 18 eyes), EAE-Sham (black bar/lines, n = 18 eyes), EAE-tDCS Anodal (red bar/lines, n = 14 eyes) and EAE-tDCS Cathodal (green bar/lines, n = 16 eyes). Error bars represent the SEM. *significant difference between Healthy vs EAE-tDCS Anodal; ●significant difference between Healthy vs EAE-Sham, ▪significant difference between Healthy vs EAE-tDCS Cathodal. Two-way ANOVA followed by LSD post hoc test *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.*

Cathodal tDCS decreased and delayed significantly the disease severity and the motor onset.

Fig. 8. *Motor disability. A, Mean clinical score in follow up. B, Disease incidence curve in each group. EAE-Sham (black lines, n = 20 mice), EAE-tDCS Anodal (red lines, n = 17 mice) and EAE-tDCS Cathodal (green lines, n = 14 mice). Error bars represent the SEM. * significant difference between EAE-Sham vs EAE-tDCS Cathodal, Δsignificant difference between EAE-Sham vs EAE-tDCS Anodal, □represent significant difference between EAE-tDCS Anodal vs EAE–tDCS Cathodal. Two-way ANOVA followed by LSD post hoc test *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001*

Conclusion

Results showed that the disease phase in which applied the stimulation is fundamental. Indeed, the preventive results showed that cathodal stimulation improves functional and partially structural recovery, while anodal stimulation seems to have less effect.

Different is the discussion regarding the acute disease phase. Both active stimulations restored the optic nerve functionality, while only cathodal tDCS partially protected from retinal structural damage. Interesting results were found on the clinical score and disease incidence because not only, as already mentioned, cathodal tDCS decreased functional and structural damage in the visual pathway but also the motor disability and the disease severity.

To conclude, the tDCS effects seem dependent on the disease phase. We need to investigate more physiopathological aspects to understand better their respective effects in the acute and post-acute phases of EAE.

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