Optic neuritis detected with visual evoked potentials in experimental autoimmune encephalomyelitis mice model

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Introduction

Background: Multiple sclerosis (MS) is a disease of the central nervous system in which the interplay between inflammatory and neurodegenerative processes typically results in intermittent neurological disorders followed by progressive accumulation of disability[1]. About 10 to 30% of MS patients have a clinical presentation that starts with an attack of optic neuritis (ON). ON is an acute inflammatory disorder that causes demyelination of the optic nerve, thinning of the retinal nerve fiber layer (RNFL), and death of retinal ganglion cells (RGCs)[2]. These clinical symptoms can be observed and studied in the experimental autoimmune encephalomyelitis (EAE) model induced through myelin oligodendrocyte glycoprotein (MOG) injection[3]. Immunized C57BL/6 mice develop chronic EAE[4]. For what concerns the visual system, EAE is characterized by optic nerve abnormalities, consisting in demyelination and/or axonal loss, and retina damage detectable with visual evoked potentials (VEPs) and optical coherence tomography (OCT)[5] respectively.

Objective: The present study aimed to investigating the usefulness of non-invasive visual evoked potential (VEP), optical coherence tomography (OCT) histology and the correlations to detect optic nerve involvement in the EAE model.

Results

Clinical score, VEP, OCT and histology

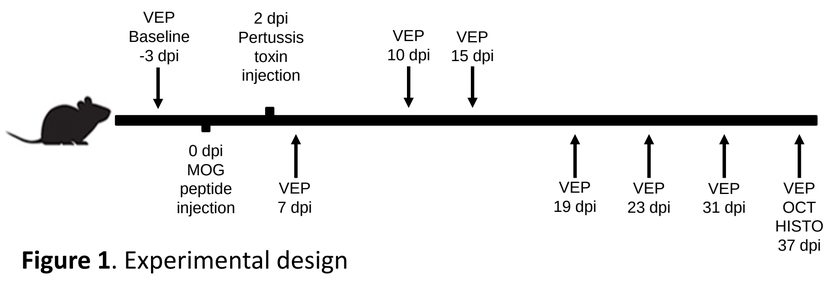


Figure 1. Experimental design

EAE mice were divided in two groups, with optic neuritis (ON, 70%) and without optic neuritis (W/O, 30%), based on cutoff for both latency and amplitude (latency cutoff: 8,12 %; amplitude cutoff: 36%). In both EAE groups, NGCC reductions were not found. While histology showed a significant demyelination (p=0.003) and axonal loss (p=0.001) in EAE ON compared to HEALTHY but was not found in EAE W/O. Pearson's correlation was significant between latency and demyelination and between axonal loss and NGCC reduction. Concerning clinical score, EAE were divided in 3 groups: binocular ON (bON), monocular ON (mON). EAE W/O showed no significant difference compared to healthy. On the other hand, EAE bON and mON both showed a significant increase of clinical score compared to HEALTHY. Interestingly, EAE mON presented a higher motor disability compared to EAE bON (p=0.002).

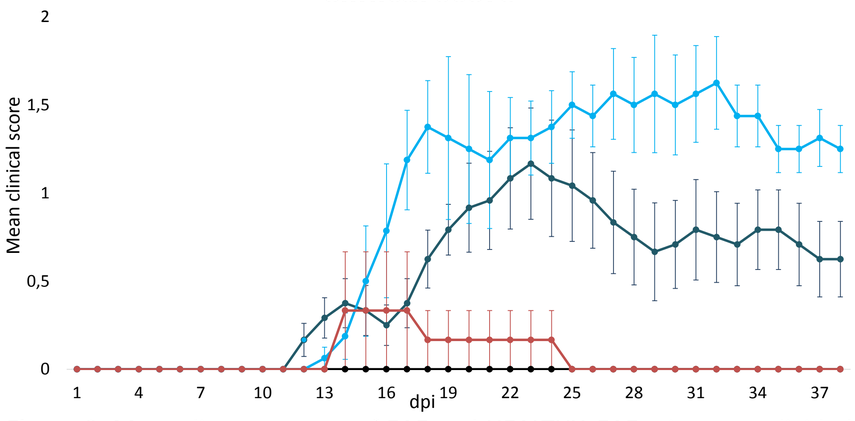


Figure 2. Mean disease course of EAE and HEALTHY. EAE mice were divided in three groups: EAE mice with both eyes without ON (W/O; red line= 3 mice), EAE mice with one eyes with ON (mON; light blue= 8 mice) and EAE mice with both eyes ON (bON; dark blue= 12 mice). HEALTHY mice was represented by black line (n= 12 mice). Two-way ANOVA for repeated measures, showed group effect (p= 0.002).

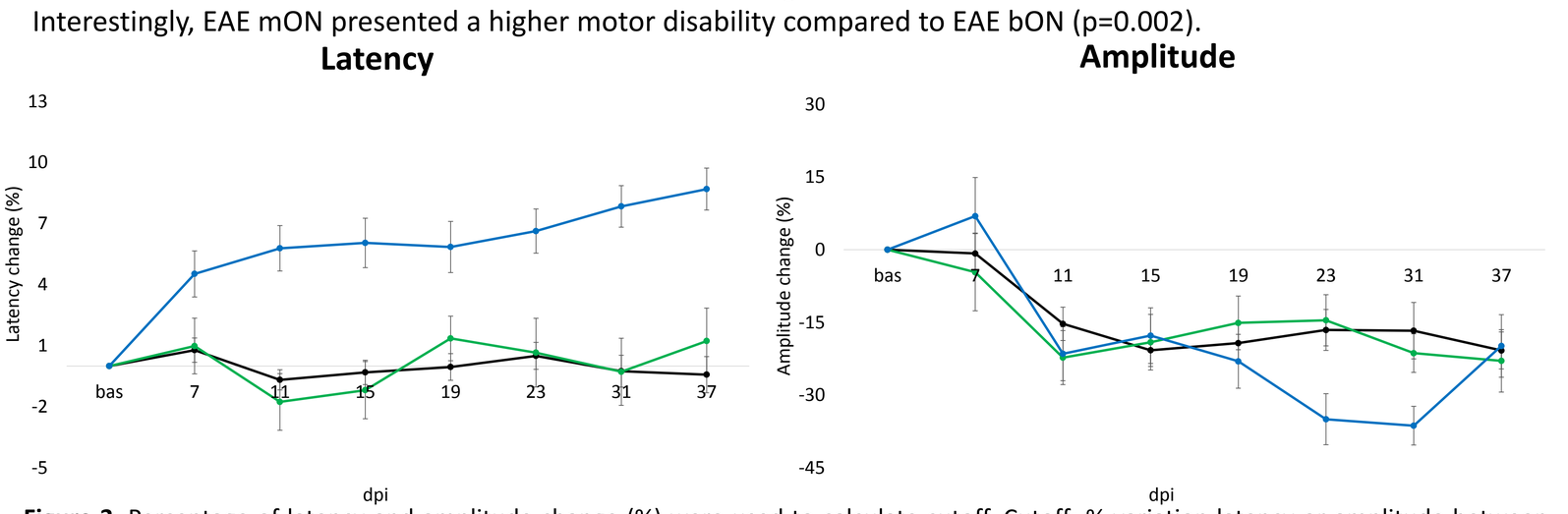


Figure 3. Percentage of latency and amplitude change (%) were used to calculate cutoff. Cutoff**:** % variation latency or amplitude between baseline and each time point and then they were average in a single one value (latency cutoff: 8.12 %; amplitude cutoff: 36%). Statistical analysis were not performed on these two paramters because they were used to devide EAE mice in two groups. Black line represents HEALTHY mice (n= 24 eyes); blue line represents EAE ON (n= 32 eyes); green line represents EAE W/O (n= 14 eyes).

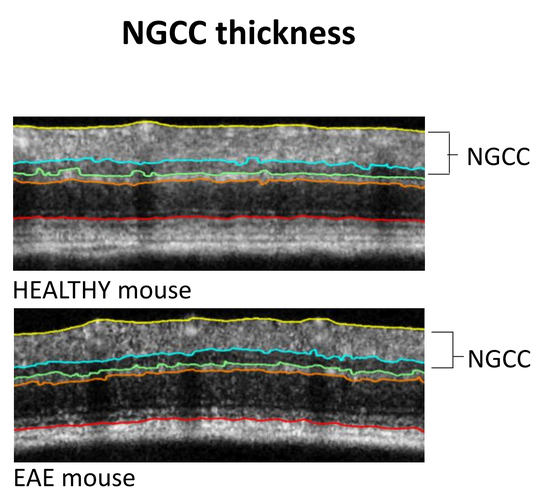


Figure 4. Examples of OCT segmentation in HEALTHY and EAE mice at 37 dpi. Neuronal ganglion cells complex (NGCC) consists of retina nerve fiber layer (RNFL), ganglion cell layer (GCL) and inner plexiform layer (IPL).

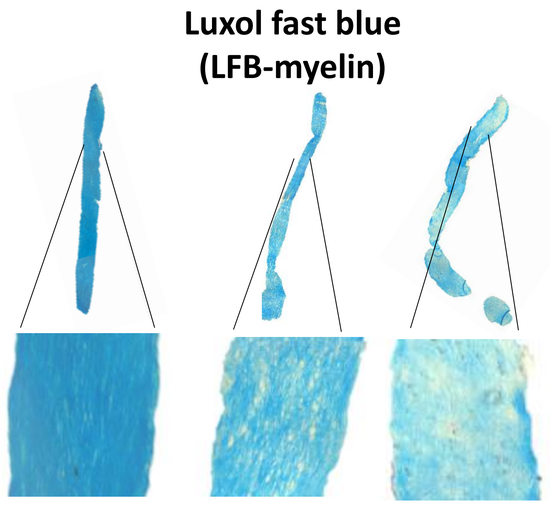


Figure 5. LFB staining on longitudinal optic nerve in HEALTHY, EAE W/O and EAE ON mice at 37 dpi. Images were analyzed by Image J.

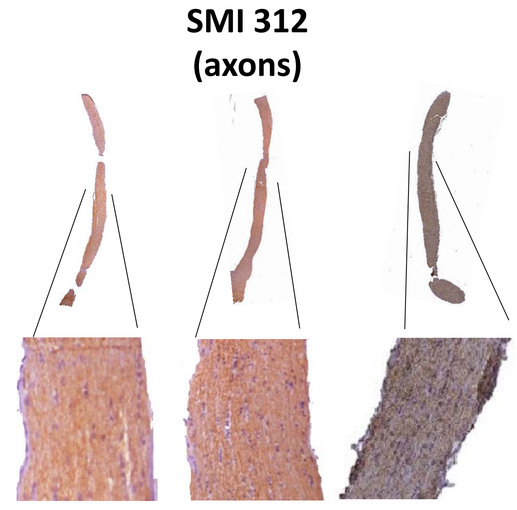


Figure 6. SMI 312 staining on longitudinal optic nerve in HEALTHY, EAE W/O and EAE ON mice at 37 dpi. Images were analyzed by Image J.

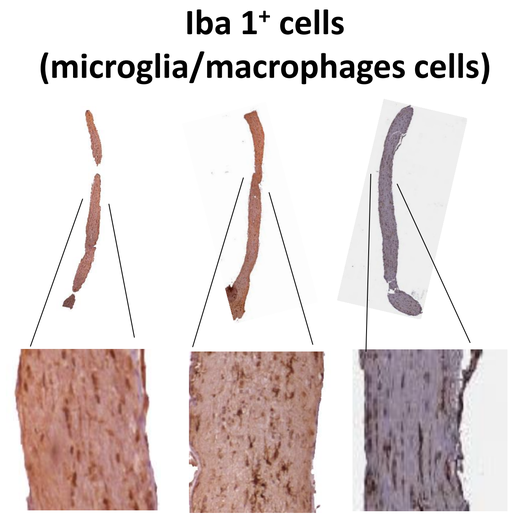


Figure 7. Iba 1⁺ cells staining on longitudinal optic nerve in HEALTHY, EAE W/O and EAE ON mice at 37 dpi. Images were analyzed by Image J.

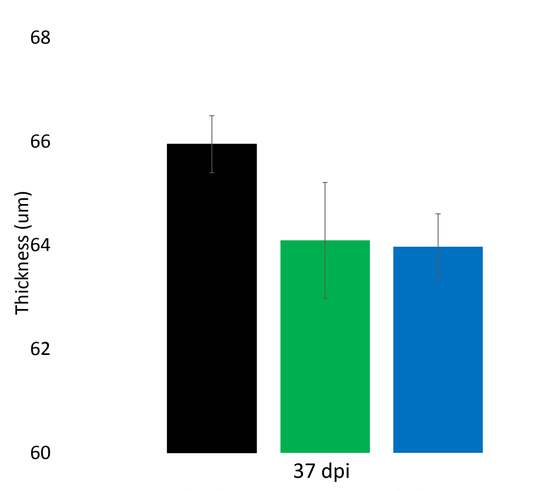


Figure 8. NGCC thickness measured by automatic OCT scan at 37 dpi. Significant difference were not found among groups.

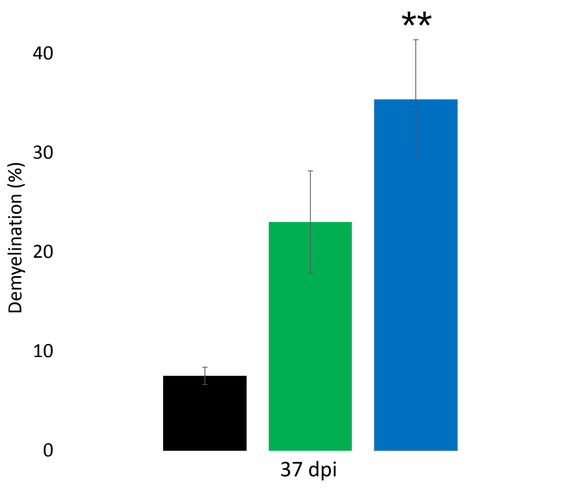


Figure 9. Demyelination measure (%) on longitudinal optic nerve stained by LFB. Statistically significant differences between HEALTHY and EAE ON group was detected at 37 dpi (p= 0.003)

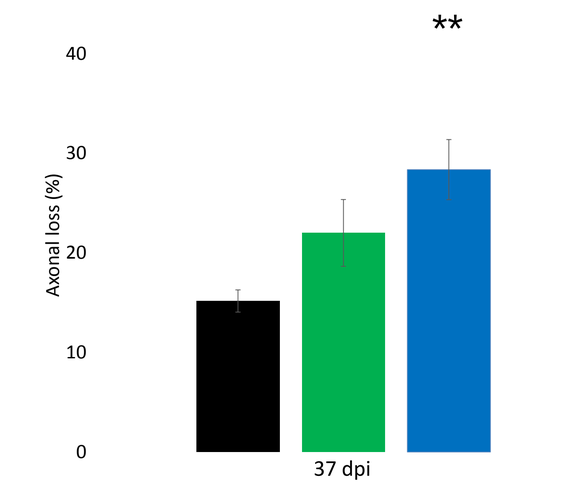


Figure 10. Axonal loss measure (%) on longitudinal optic nerve stained by SMI 312. Statistically significant differences between HEALTHY and EAE ON group was detected at 37 dpi (p= 0.001)

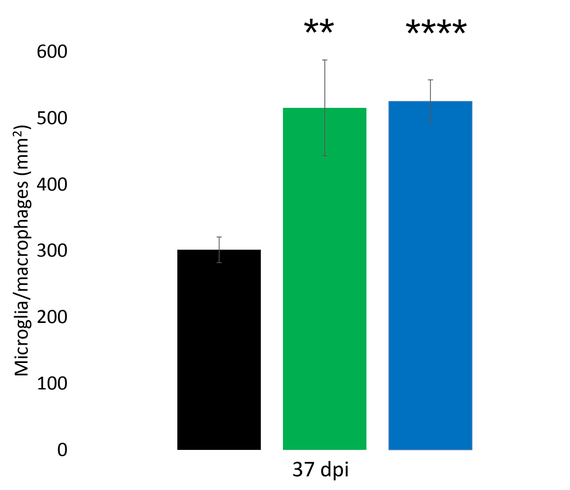
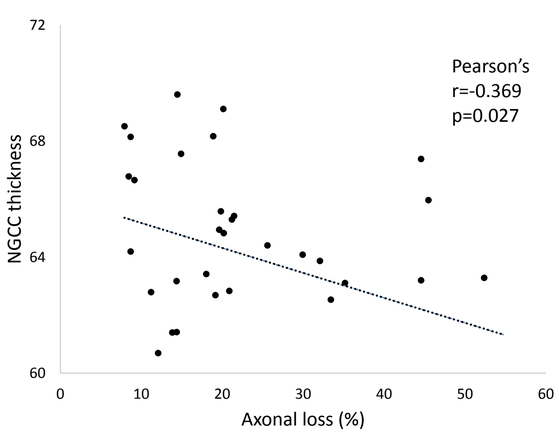
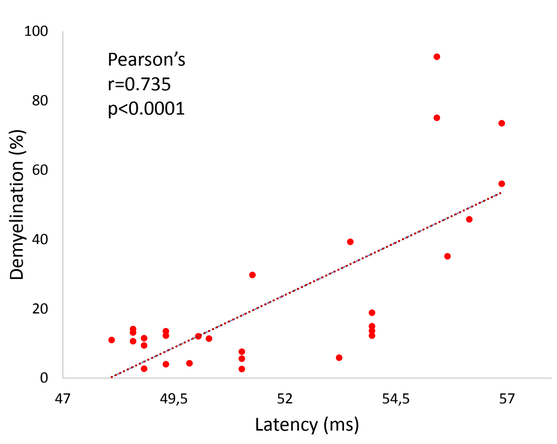
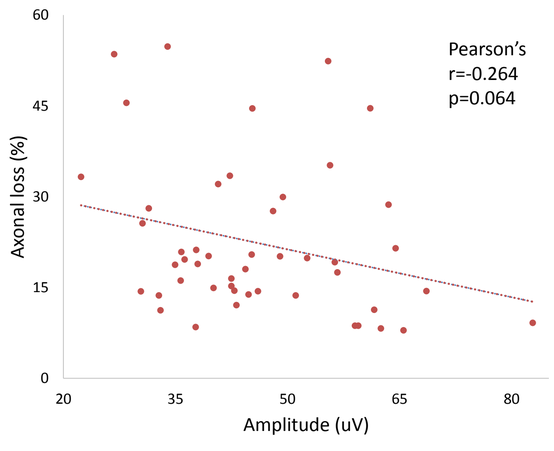
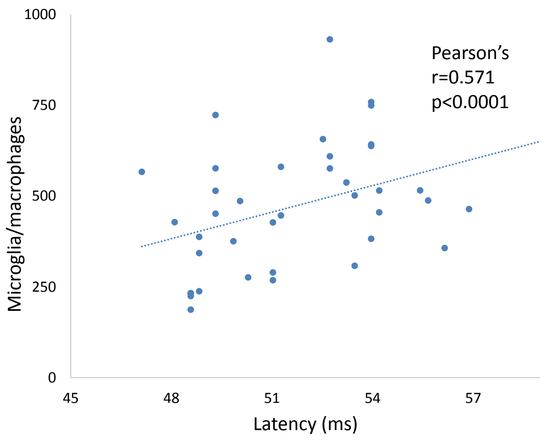


Figure 11. Microglia/macrophages cells measure (mm²) on longitudinal optic nerve stained by Iba 1. Statistically significant differences between HEALTHY and EAE W/O (p= 0.0011) and HEALTHY and EAE ON at 37 dpi (p< 0.00001)









Discussion

✓N1 latencies in EAE mice with ON increased at 7 dpi until 37 dpi. Amplitude decreased only in chronic phase at 23 dpi despite partial variability compared to the latency.

✓NGCC reduction was not significant comparing groups at 37 dpi. The correlation between axonal loss (%) and NGCC thickness was significant.

✓Demyelination correlated with VEP delay while axonal loss did not correlate with amplitude. Moreover, positive correlation was found between inflammation and latency.

✓Non-invasive VEPs can be used to detect optic neuritis and to discriminate between clinical profiles with different degrees of motor disability.

✓These findings suggest that VEPs can be used as an early biomarker of demyelination in EAE to test new remyelinating treatments, while OCT is suitable for monitoring subsequent neuroaxonal loss for testing neuroprotective strategies.

Literature

1. The International Multiple Sclerosis Genetics Consortium & the Wellcome Trust Case Control Consortium. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. 2011
2. Larabee C.M. et al. Loss of Nrf2 exacerbates the visual deficits and optic neuritis elicited by experimental autoimmune encephalomyelitis. Molecular Vision 2016.
3. Guarnieri F.C et al. Synapsin I deletion reduces neuronal damage and ameliorates clinical progression of experimental autoimmune encephalomyelitis. Brain Behav Immun., 2017.
4. Quinn T.A. et al. Optic neuritis and retinal ganglion cell loss in a chronic murine model of multiple sclerosis. Original research article. 2011.
5. Srinivasan V.J. et al. Characterization of Outer Retinal Morphology with High-Speed, Ultrahigh-Resolution Optical Coherence Tomography, 2008.

Conflict of interest

The authors declare no competing financial interest.

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