Functional and structural in vivo assessment of demyelination and axonal loss in experimental autoimmune encephalomyelitis mouse model

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Introduction

Background: Multiple sclerosis (MS) is a disease of the central nervous system in which there is an interplay between inflammatory and neurodegenerative processes[1]. About 20% of MS patients have a clinical presentation that starts with optic neuritis (ON). ON is an acute inflammatory disorder that causes optic nerve demyelination, thinning of the retinal nerve fiber layer, and death of retinal ganglion cells[2]. These clinical symptoms can be studied in the experimental autoimmune encephalomyelitis (EAE) model induced through myelin oligodendrocyte glycoprotein (MOG) injection[3]. Immunized C57BL/6 mice develop chronic EAE[4]. EAE is characterized by optic nerve abnormalities, consisting in demyelination and/or axonal loss, and retina damage.

Objective: The present study aimed to investigating visual pathway alteration with two follow-up studies measuring visual evoked potentials (VEPs), optical coherence tomography (OCT)[5], photopic electroretinogram (pERG) and optic nerve histology

Results

Short follow-up study (pre-onset phase)

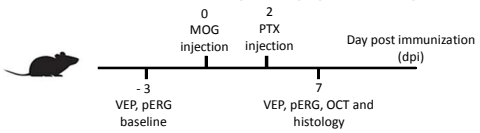


***Fig. 1**. Experimental design of short follow up*

Appling cutoff value, 5.76%, eyes with percentage of latency variation over cutoff were defined "EAE eyes with latency delay (EAE W LD)", while eyes with percentage of latency variation under cutoff were defined "EAE eyes without latency delay (EAE W/O LD)". At 7 dpi the frequency of EAE W LD was 53% (19/36 eyes) and of EAE W/O LD was 47% (17/36 eyes).

VEP

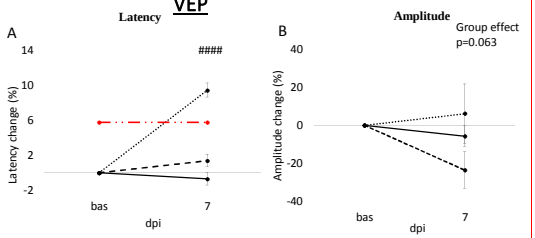


***Fig. 2.** Percentage of VEP change at 7 dpi. Latency (A) and amplitude (B). Red line represents cutoff. Full lines represent healthy (n=20 eyes), dots lines EAE W LD (n=19 eyes) and dashed line EAE W/O LD (17 eyes).*

pERG

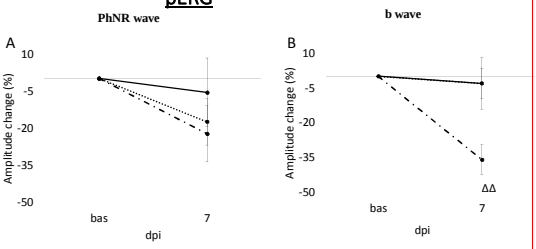


***Fig. 3.** Percentage of photopic ERG change at 7 dpi. PhNR amplitude change (A) and b-wave amplitude change (B). Full lines represent healthy (n=10 eyes), dots lines EAE W LD (n=10 eyes) and dashed lines EAE W/O LD (14 eyes).*

OCT

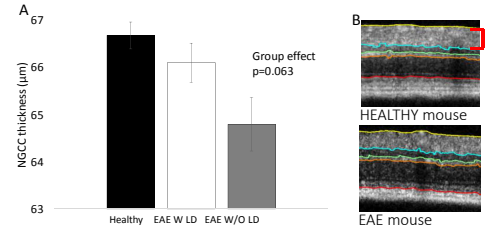


***Fig. 4.** NGCC thickness at 7 dpi. A, Black bar represents healthy (n=20 eyes), white bar represents EAE W LD (n=17 eyes) and grey bar represents EAE W/O LD (n=15 eyes). B, Example of OCT image*

HISTOLOGY

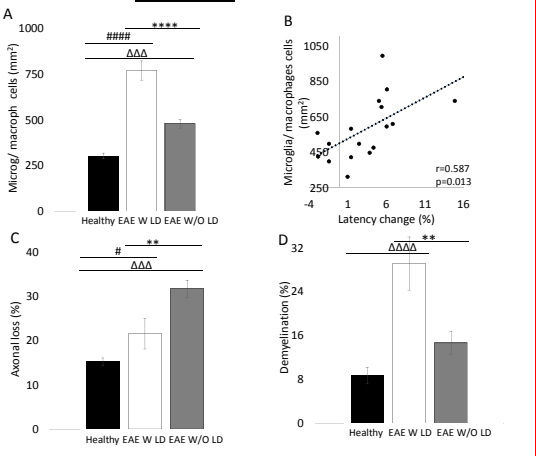


***Fig. 5.** Longitudinal optic nerve histology and correlation at 7 dpi. A and B, Quantifications of microglia and macrophages (mm²) in optic nerves stained with Iba 1 and correlation with percentage of VEP latency.C,Quantifications of axonal loss (%) in optic nerves stained with SMI 312. D, Quantifications of demyelination (%) in optic nerves stained with LFB. A,C,D, black bars represent healthy (n=11 eyes), white bars EAE W LD (n=8 eyes) and grey bars EAE W/O LD (n=13 eyes).*

Long follow-up study (chronic phase)

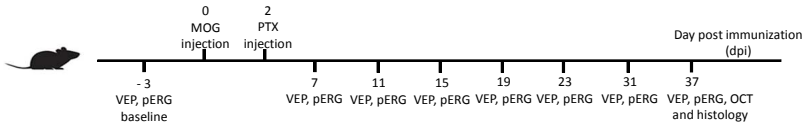


***Fig. 6**. Experimental design of long follow up*

Appling cutoff value, 6.10%, EAE eyes were divided in "EAE eyes with delay before clinical onset (EAE BCO)" and "EAE eyes with delay after clinical onset (EAE ACO)". Eyes frequency in EAE BCO was 50% (23/46 eyes) with a percentage of VEP latency delay at 7 and 11 dpi over cutoff. Eyes in EAE ACO was 37% (17/46 eyes). However, the percentage of latency delay increased at 19 dpi (5.7%), followed by partial decreased at 23 and 31 dpi.

VEP

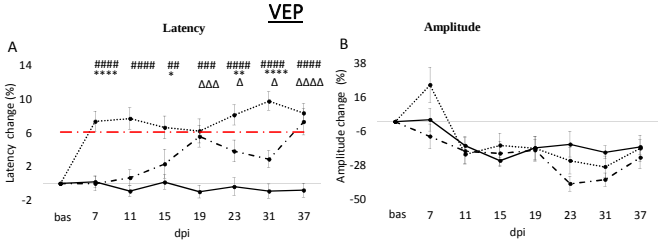


***Fig. 7.** Percentage of VEP change until 37 dpi. Latency (A) and amplitude (B). **Red line represents cutoff.** Full lines in healthy (n=16 eyes), dots lines in EAE BCO (n=23 eyes) and dashed lines EAE ACO (17 eyes).*

pERG

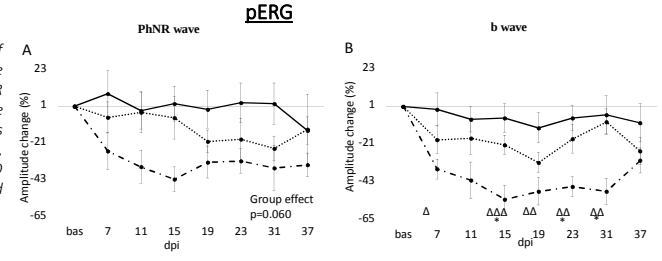
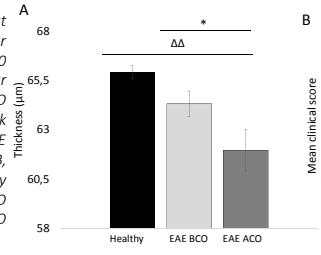


***Fig. 8.** Percentage of photopic ERG change until 37 dpi. PhNR change (A) and b-wave change (B). Full lines healthy (n=14 eyes), dots lines EAE BCO (n=12 eyes) and dashed lines EAE ACO (6 eyes).*

OCT



Clinical score

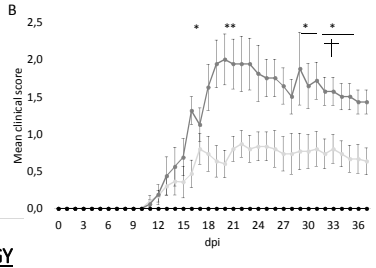


***Fig. 9.** NGCC thickness at 37 dpi. A, Black bar represents healthy (n=20 eyes), light grey bar represents EAE BCO (n=20 eyes) and dark grey bar represents EAE ACO (n=9 eyes). B, Clinical score. Healthy (n=9mice), EAE BCO (n=15mice) EAE ACO (n=8 mice)*

HISTOLOGY

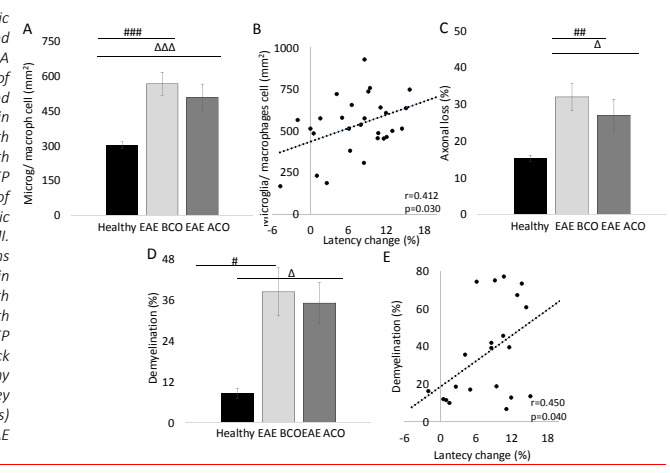


***Fig. 10.** Longitudinal optic nerve histology and correlation at 37 dpi. A and B, Quantifications of microglia and macrophages (mm²) in optic nerves stained with Iba 1 and correlation with percentage of VEP latency.C.Quantificatio of axonal loss (%) in optic nerves stained with SMI. D and E, Quantifications of demyelination (%) in optic nerves stained with LFB and correlation with percentage of VEP latency. A, C, D, black bars represent healthy (n=12 eyes), light grey bars EAE BCO (n=12 eyes) and dark grey bars EAE ACO (n=15 eyes).*

Discussion

- ✓ In the first study, EAE W LD showed a significant VEP latency increase at 7 dpi compared to baseline, while the b-wave component of ERG showed a significant amplitude decrease in EAE W/O LD. Histology showed significant increase of Iba1⁺ cells in both EAE groups, axonal loss only in EAE W/O LD and demyelination in EAE W LD.
- ✓ In the second study, in EAE BCO, a dysfunction seems focused only in the optic nerve. In EAE ACO, ERG b-wave decreased before clinical onset and OCT detected a decrease in thickness of neuronal ganglion cell complex. Optic nerve histology at 37 dpi showed no differences between EAE groups.
- ✓ To conclude, our non-invasive methods can be applied in follow-up to characterize the visual pathway alterations in order to develop more effective treatments.

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Conflict of interest

The authors declare no competing financial interest.

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