Onset of Optic Neuritis Precedes Motor disability in a Preclinical model of Multiple Sclerosis

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Introduction

Experimental autoimmune encephalomyelitis (EAE) is the mostly used preclinical model for multiple sclerosis (MS), it manifests two important phases of MS: neurodegeneration and inflammatory demyelination [1]. The disease course usually evaluated with severity of motor symptoms. Optic neuritis (ON) is the presenting symptom in up to 25% of MS patients [2]. Therefore, visual insults may exist while no motor symptom occurs. Neurodegeneration in retina of rats can be measured as thinning of retinal nerve fiber layer (RNFL) and ganglion cell layer/ inner plexiform layer (GCL-IPL) by optical coherence tomography (OCT), while prolonged latency of visual evoked potential (VEP) indicates inflammation or demyelination[3; 4]. Here we employed OCT and VEP to elucidate the temporal relationship between visual and motor symptom during disease evolution of EAE.

Methods

Ten dark Agouti female rats with EAE induced through myelin oligodendrocyte glycoprotein immunization (MOG) were compared to twelve healthy controls. They all underwent bilateral circular peripapillary OCT scans and flash VEP recordings at baseline, 7, 14, and 21 days post immunization (dpi). RNFL and GCL-IPL were segmented from OCT images and the thicknesses were used to quantify for neuroaxonal loss. Latency of N1 was measured from the N1-P1-N2 wave from VEP. Histology of retina and optic nerve were collected at 21 dpi, retinal ganglion cells (RGCs), myelin, microglia/macrophage, and axons were stained with BRN3, LFB, Iba1, and SMI-312. The EAE rats were separated into w/o and w/ group based on the presence of motor symptoms. Mixed ANOVA (group by time) and post-hoc analysis with Bonferroni correction was used to demonstrate the temporal change among healthy and the two EAE groups.

Results

Five EAE rats did not develop any motor symptoms (w/o group), while the other five had motor symptom onset from 13 dpi (w/ group). Mixed ANOVA of RNFL showed an interaction ($p = 0.0232$). Thicker RNFL in w/ group compared with healthy was found at 7 dpi, probably due to edema. Main effects for both time ($p<0.0001$) and group ($p=0.0225$) was found in GCL-IPL, both EAE groups showed thinning of GCL-IPL at 14 dpi compared with healthy. VEP showed also main effects of time ($p<0.0001$) and group ($p=0.0019$), post-hoc analyses revealed that compared with healthy, delayed VEP was found from 7 to 21 dpi in w/ group, and from 14 to 21 dpi in w/o group. Histology results found no demyelination nor axonal loss. However, significantly higher infiltration of microglia/macrophage and more RGC loss were found in w/ group compared with w/o group. EAE eyes were further separated into 4 groups based on the onset of ON detected with VEP latency. Severe edema of RNFL and continuous GCL-IPL thinning was found only in eyes with delay began at 7 dpi. These eyes also showed most severe inflammation compared with healthy.

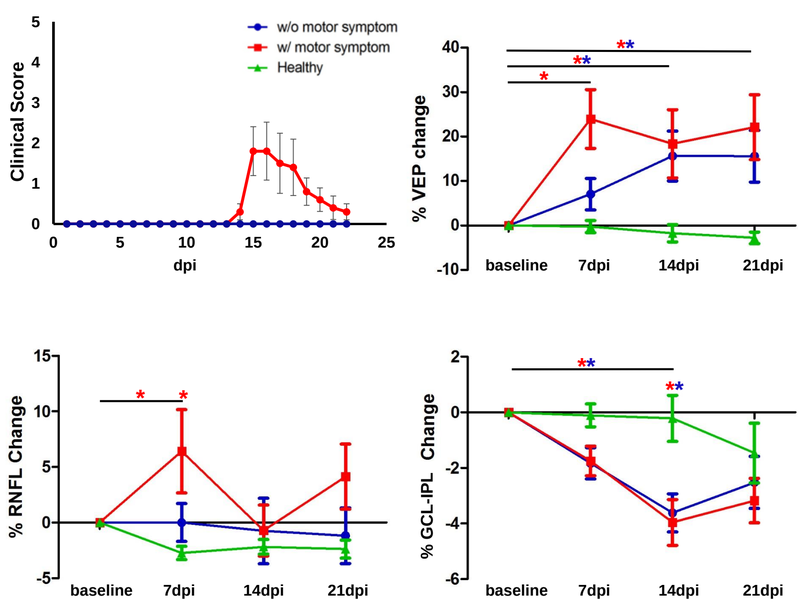

Figure 1. Parameters changing over time. *Blue: rats without motor symptom; Red: rats with motor symptom; Green: healthy. Error bar: SEM. *p<0.05 in post-hoc analysis.*

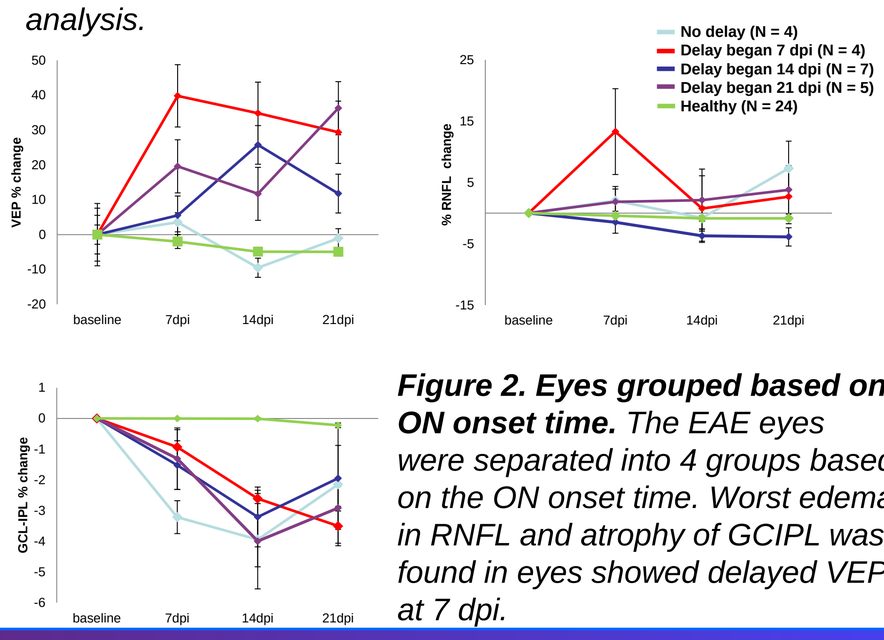

Figure 2. Eyes grouped based on ON onset time. *The EAE eyes were separated into 4 groups based on the ON onset time. Worst edema in RNFL and atrophy of GCIPL was found in eyes showed delayed VEP at 7 dpi.*

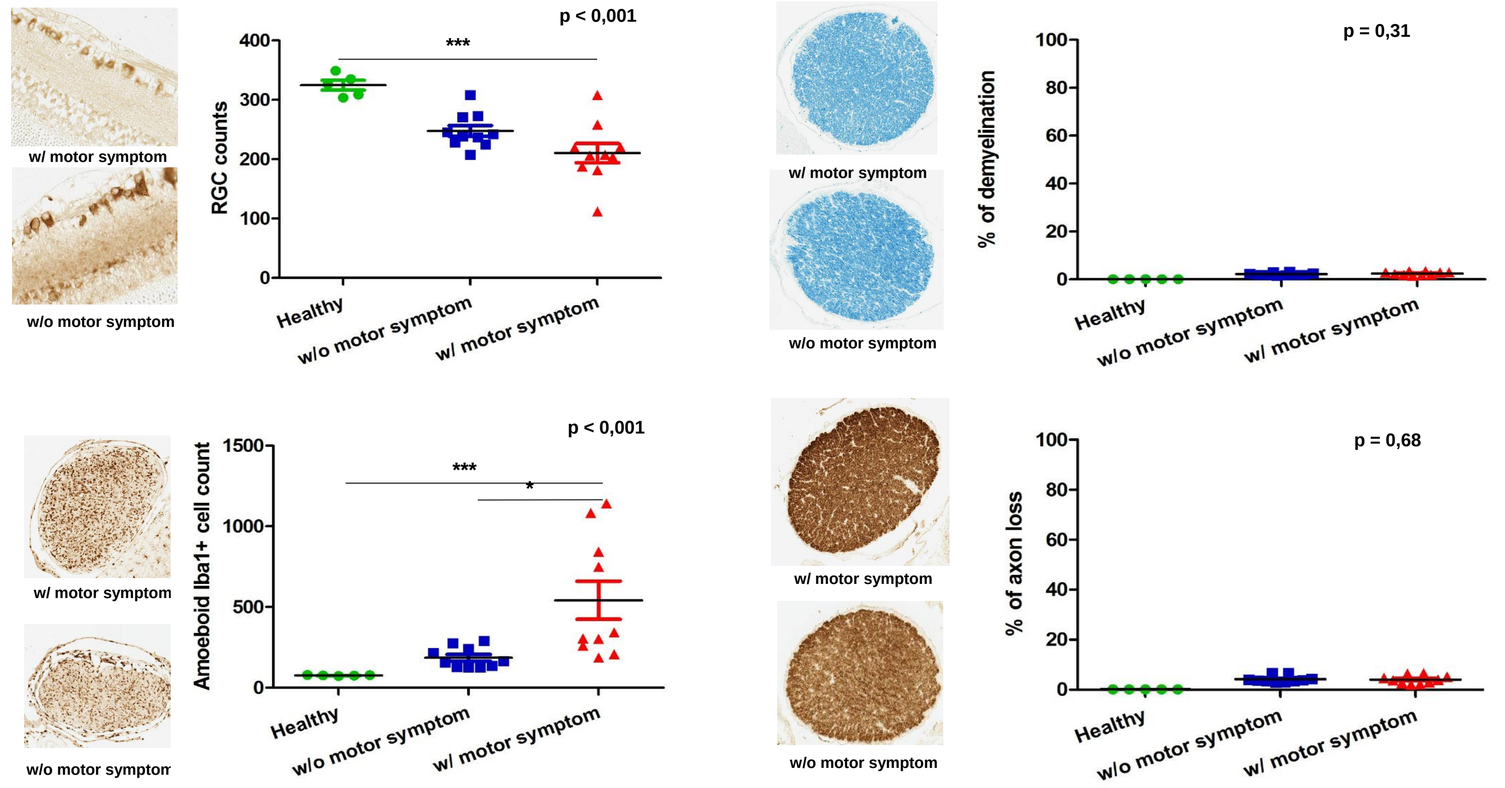

Figure 3. Histology results in the two group. Rats with motor symptom showed more RGC loss and microglia/macrophage infiltration, while no significant demyelination nor axonal loss was presented

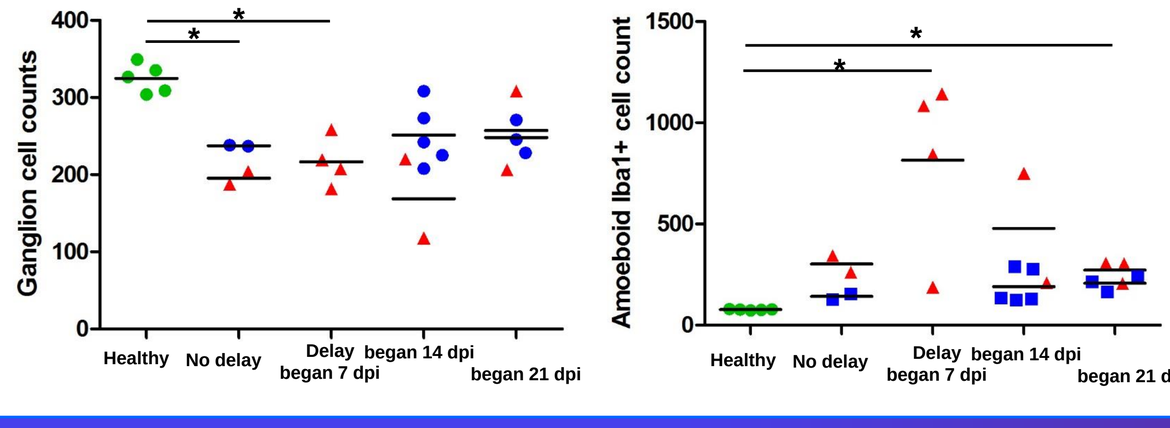

Figure 4. Histologital results based on VEP grouping. Due to small sample size, statistical analyses were performed with eyes between healthy and all EAE eyes regardless of motor symptom. *: $p<0,05$.

Discussions and Conclusions

Our results showed that ON appeared in EAE rats regardless of motor symptom, also, the onset of ON precedes onset of motor symptoms. Therefore in studies focusing on visual involvement of EAE, besides of clinical score, functional exams such as VEP are needed for detecting ON. GCL-IPL thinning was found in both groups, implied that neurodegeneration of RGC happened very early in EAE, and is independent from spinal cord involvement. Histological results showed more severe inflammation and RGC loss in the w/ group, suggesting the observed RGC loss may due to inflammation. The results suggest that in MOG-EAE, ON presence is independent, or even precedes motor symptom, which may benefit in early detection of ongoing inflammation within CNS.

When EAE eyes were grouped based on the onset time of VEP delay, eyes with early VEP delay, which corresponding to severe inflammation by Iba1 staining, showed continuous GCL-IPL thinning from 7 dpi. Also, despite the timing of ON onset, RGC loss was found in all the EAE eyes. Our data also supports that instead of secondary to inflammatory demyelination, neurodegeneration exists from the onset of EAE, mainly driven by inflammation.

References

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Conflict of Interests

No conflict interest to be declared for any of the authors.

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