Pattern ERGs suggest a possible retinal contribution to the visual acuity loss in acute optic neuritis

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KEY WORDS

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Inner Nuclear Layer (INL)

ABSTRACT

Purpose: Macular involvement in optic neuritis (ON) is well-recognised but poorly understood and may be of clinical relevance. This study explores macular structure-function correlates in acute ON.

Methods: This cross-sectional cohort study recruited ON patients within 14 days of symptom onset. Subjects underwent pattern electroretinography (PERG), pattern visual evoked potentials (PVEP) and optical coherence tomography (OCT) imaging. PERG P50 and N95 components were correlated with OCT data.

Results: Twenty-six ON patients were recruited, comprising eleven multiple sclerosis (MSON), six myelin oligodendrocyte glycoprotein associated (MOGON) and nine isolated ON cases. These were compared with 28 healthy controls. PVEPs were undetectable in 11 (42%) of ON cases. When detectable, PVEP P100 was delayed (median 136ms range 110-173ms) and amplitude reduced (median 6μV, range 3-14μV) in ON compared with controls (both $p<0.001$). PERG P50 component amplitudes, largely reflecting macular function, were reduced in affected eyes (median 2.3μV; range 0.8–5.0μV) compared with controls (3.3μV; range 2.8-5.7μV) and compared with fellow eyes ($p<0.001$). There was often associated reduction in N95 but the N95:P50 ratio was below the reference range in the affected eyes of five patients. Eight cases (32%) had subnormal P50 amplitudes (<2.0μV) and these patients had poorer visual acuity ($p=0.020$). P50 amplitudes were positively correlated with inner nuclear layer thickness ($r_s=0.36$; $p=0.009$) and macular ganglion cell and inner plexiform layer (mGCIPL) thickness ($r_s=0.44$, $p=0.022$).

Conclusion: PERG P50 component reduction reveals dysfunction of inner macular layers in acute ON and correlates with structural alterations on OCT. These early macular pathologic processes are likely to contribute to the visual loss.

INTRODUCTION

Optic neuritis (ON) is an inflammatory optic nerve disorder often associated with multiple sclerosis (MSON) [1] but can also occur in association with identified systemic auto-antibodies, such as myelin oligodendrocyte glycoprotein (MOGON) or aquaporin-4 (AQP4). ON can also occur as relapsing (RION) or single-episode isolated ON (SION) with evidence of neither MS nor MOG/AQP4 antibodies[1].

Inflammatory and demyelinating pathophysiological changes [2], with subsequent atrophy of the peripapillary retinal nerve fibre layer (pRNFL) and macular ganglion cell and inner plexiform layer (mGCIPL), occur in ON independent of aetiology [1]. Structural changes in other retinal layers, particularly the inner nuclear layer (INL), have also been reported; with increase in INL thicknesspost-ON being associated with poor visual recovery [3].

Most data on retinal involvement exist for the MSON subtype. INL changes may predict new enhancing radiological lesions and relapses in MS, suggesting an association with inflammatory disease activity [4]. The high incidence of retinal periphlebitis, especially in active disease [5]; uveitis, particularly pars planitis [6]; and activated microglia in MS retina [7] further document retinal inflammatory activity in MS.

INL thickening is likely related to microcystic macular oedema (MMO) [8], present in approximately 10% of ON eyes [3], and slightly more common in MOGON compared with MSON and SION [8]. There is a need to distinguish acute MMO due to inflammation and the chronic change of retrograde maculopathy seen in optic neuropathy of varied aetiology [9]. A better understanding of INL involvement in acute neuro-inflammatory disease is important given the reported associations of INL thickness with poorer clinical recovery after ON [10] and treatment response in MS [11]. There may also be treatment implications ifa maculopathy contributes to visual loss in ON.

The pattern electroretinogram (PERG) is the retinal electrophysiological response to contrast stimulation, conventionally an isoluminant reversing black and white checkerboard. The PERG has two main components: a positivity at approximately 50ms (P50) followed by a larger negativity at 95ms (N95) [12]. The N95 component can be selectively affected in retinal

ganglion cell dysfunction, either primary or consequent upon retrograde degeneration from optic nerve disease, whereas P50 is affected in macular dysfunction. N95 is probably exclusively generated by the retinal ganglion cells (RGCs) [12,13]. P50 origins are not fully ascertained, with approximately 70% being generated by RGCs and 30% more distally [14]. However, P50 is an accepted measure of macular retinal function with a normal response requiring functional integrity of the macular photoreceptors (that "drive" the response), the macular bipolar cells, and the RGCs [12,14]. Thus, the PERG facilitates the electrophysiological differentiation between macular and optic nerve dysfunction [12,13]. The PERG is abnormal in approximately 40% of recovered ON cases [12,15], with most abnormalities (85%) confined to the N95 component [14]. However, P50 abnormalities can occur in *acute* ON [14,16,17]. This P50 reduction typically shows improvement over several weeks, possibly starting as soon as 7 days after symptom onset [14]; while significant N95 component reduction usually remains.

Thus, both structural INL changes and macular dysfunction evolve following ON but the underlying pathophysiological mechanisms are poorly understood. This study investigates the structural correlates of electrophysiologically demonstrable macular abnormalities in acute ON to elucidate further the underlying retinal changes in the early phase of ON.

METHODS

Subjects

Patients with symptomatically unilateral ON, without a previous affected eye episode, were prospectively recruited from Moorfields Eye Hospital, London, UK. Diagnosis was made by a neuro-ophthalmologist (GTP, AP) using international consensus investigation protocols [1].

Patients were recruited within 14 days of onset of visual loss and/or pain on eye movement. Patients were classified as MOG associated ON if seropositive for MOG antibodies (MOGON), as MS associated ON if fulfilling the 2017 MS criteria (MSON) and single episode isolated ON (SION) as described [1].

Controls consisted of cohorts of healthy hospital staff who had undergone PERG (N=28), PVEP (N=29) and OCT testing (N=13), respectively.

Ethics

Approval for the study was obtained from the Queen Square London Ethics committee (number 10/H0716/72) and hospital R&D (FRAC0001). Subjects provided written informed consent. The study complied with the Declaration of Helsinki.

Electrophysiology

PERGs and PVEPs were obtained using the Espion E3 system (Diagnosys LLC) with protocols based on the ISCEV standard recommendations [18]. PERGs were recorded binocularly using gold foil electrodes referred to ipsilateral outer canthus gold-cup surface electrodes with a mid-forehead ground. The stimulus was a high contrast (>95%), isoluminant checkerboard reversal, using 45' checks in 15° x 12° field at 1.52m viewing distance; amplifier bandpass was 1-100 Hz, stimulation rate was 4.2 reversals per second (rps) with white squares of ~100 cd/m^2 luminance. PVEPs were recorded monocularly using gold cup electrodes (Oz-Fz). Responses were elicited by 55' checks, 1.8 rps, using the same field size, amplifier, luminance and contrast parameters as above. Regular calibration and service ensured stability of stimulus parameters. Reference ranges for PVEP and PERG measures were defined as the minimum to maximum ±5% of the control range.

OCT

OCTs were recorded with Spectralis SD-OCT (Heidelberg Engineering, Inc, Heidelberg, Germany) with the eye-tracking function enabled, on acquisition software version 6.7.13.0 [1]. A macular volume scan (1024 A-scans, 25 B-scans volume = 20° × 20°, automatic real-time function [ART] = 25) centred around the fovea and a circular 12° peripapillary scan (1536 A-scans, ART ~100) centred around the optic nerve head were performed. Quality control (QC) was assessed using the OSCAR-IB criteria [19]. One macular OCT scan did not pass QC due to poor fixation.

All B-scans were auto-segmented using Heidelberg Eye Explorer (version 6.15.7.0) followed by manual correction. The pRNFL, ganglion cell layer (GCL), inner plexiform layer (IPL), INL, outer plexiform layer (OPL) and outer nuclear layer (ONL) were segmented. mGCIPL and compound OPL and ONL (OPNL) thicknesses were calculated. Mean layer thickness was computed within

a 3.45 mm diameter circle centred around the fovea. Peripapillary scans were segmented for pRNFL thickness.

Visual acuity testing

Best corrected high-contrast logMAR visual acuity (BCVA) was measured with a retro-illuminated chart at 4m viewing distance [1].

Antibody testing

MOG-IgG and AQP4-IgG Ab status were assessed locally using cell-based assays [1].

Statistics

Data were analysed using "R". Continuous variables were described by median and ranges, and categorical variables by counts and percentages. PVEP data of patients with undetectable responses were excluded from all analyses. An additional interocular difference variable was created for OCT and PERG data by subtracting results in the affected eye from those in the fellow eye. Distributions of continuous variables and dichotomous variables across groups were tested with the Kruskal-Wallis and Fisher exact tests, respectively. Post-hoc continuous variables analysis was performed with Dunn Test, with p-values adjusted for multiple comparisons (Benjamini-Hochberg method). The Wilcoxon rank-sum test was used to compare distributions of continuous parameters between ON eyes and control eyes, and the Wilcoxon signed-rank test to compare PERG parameters from the clinically affected eyes with those from the fellow eye. Correlations were performed by Spearman's rank analysis (r_s=Spearman's rho) and structure-function associations were tested with linear regression. To account for inter-eye correlations, only left eyes of control subjects were analysed. Statistical significance was set at $p<0.05$.

RESULTS

Twenty-six patients with acute ON were recruited (Table 1). Females were overrepresented in the ON group, as expected [1]. Age, gender and BCVA were similar across ON types. Median time from symptom onset was 7 days (range 2–14) at recruitment, with MOGON cases trending towards earlier recruitment than SION cases (p=0.063). Three MSON and one MOGON case had a history of prior ON of the fellow eye. One MSON case had commenced oral steroid therapy two days before trial investigations were performed, 13 others started afterwards. No patients were on maintenance disease modifying treatment. Serological testing for AQP4 was negative in all patients.

Visual acuity

BCVA correlated positively with P100 peak times (r_s=0.58, p=0.003) and negatively with PVEP amplitude (r_s=-0.69, p=0.006). The eight ON cases with P50 amplitudes below the reference limit of 2.0µV had worse median BCVA (1.00; range 0.20–1.15) compared with other cases (0.40; range -0.15–1.10, p=0.020).

PVEP

Representative electrophysiological traces of 2 ON affected patients are shown in Figure 1. PVEPs were undetectable in 11 (42%) affected eyes of ON cases. When detectable, PVEP P100 was delayed (median 136ms, range 110-173ms) and amplitude reduced (median 6µV, range 3-14µV) in affected ON eyes compared with controls (median 11µV, range 6-22µV; both p<0.001). Median peak times and amplitudes did not significantly differ between fellow eyes of ON cases and control eyes (Figure 2) but did differ significantly between affected and fellow eyes (p<0.001 for both) (Supplementary Figure 1). The fellow eye of one MSON patient had uninterpretable PVEPs and was excluded from analysis.

PERG

P50 amplitudes, N95 amplitudes, N95:P50 ratios and P50 peak times fell outside reference limits in 8, 8, 5 and 13 affected, and 4, 3, 2 and 7 fellow eyes, respectively (Figure 2; see Figure 1 for representative traces). For fellow eyes, these were predominantly MS cases with previous ON in that eye. In addition, 3 affected eyes and 1 fellow eye had an N95:P50 ratio above the reference limit. These eyes all had subnormal P50 amplitude results, suggesting

macular dysfunction (supplementary Figure 2). P50 amplitude and N95:P50 ratio differed significantly across ON types (p=0.026 and p=0.032, respectively). Post-hoc analysis showed this related to lower median P50 in MOGON (2.2µV; range 0.8-2.7µV) compared with SION (2.8µV; 1.9-5.0µV), while N95:P50 ratio was higher in MOGON (1.8; 1.3-3.0) compared with SION (1.24; 1.0-1.6) (Table 1).

Affected, but not fellow, eyes of ON cases had lower median P50 amplitudes compared with controls (median 3.3µV; range 2.8-5.7µV, p<0.001). Median P50 amplitude in ON patients was significantly lower in affected eyes (2.3µV; range 0.8-5.0µV) compared with fellow eyes (3.0µV; range 1.0-4.7µV; p<0.001) (Figure 2 and 3). Nine cases (36%) had abnormal relative P50 amplitudes (interocular ratio <0.70) [14]. Eight cases (32%) had subnormal absolute P50 amplitudes (<2.0µV). These cases had a similar time from symptom onset as other cases (p=0.581) at a median of 6 days (range 2-14 days) and individual electrophysiology data are shown in supplementary figure 2.

Median N95 amplitude in control eyes (5.0µV, range 3.1-7.7) was higher compared with affected eyes (p<0.001) but not fellow eyes (p=0.190) of ON patients. N95 amplitudes in ON patients were significantly lower in affected eyes at 3.4µV (range 1.2-5.1µV) compared with fellow eyes at 4.8µV (range 2.1–6.8; p<0.001). Although the group mean data are highly significant, note that there is substantial overlap between the individual data from control and ON cases (Figure 2).

There was no significant mean difference between N95:P50 ratios in controls (median 1.50, range 1.18-2.39) and both affected and fellow eyes of ON patients. There was no significant difference in median N95:P50 ratios between affected and fellow ON eyes at 1.30 (range 0.95-3.00) and 1.56 (range 1.05-2.80), respectively, but there was a trend with eighteen cases (72%) having a lower ratio in the affected compared with the fellow eye.

P50 peak times for affected and fellow ON eyes were shorter than control eyes (median 50ms, range 46-56ms; p<0.001 and p=0.001, respectively). Affected ON eyes had significantly shorter median P50 peak times at 45ms (range 40-55ms) compared with fellow eyes (median 47ms, range 44-56ms; p<0.001).

OCT

The mGCIPL was thinner in both affected and fellow eyes of ON cases (median 0.79 µm and 0.81 µm, respectively) compared with controls (median 0.90 µm; p=0.004 and p=0.014, respectively), while pRNFL thickness was greater in affected compared with fellow ON eyes (median 115 µm and 101 µm, respectively; p=0.002) (Table 1; Supplementary Figure 3). Two MSON patients (7.6% of all ON cases) showed MMO, but INL thickness did not differ significantly between ON cases and controls.

Structure-function associations

Inner retinal layers. Interocular differences of N95 and P50 amplitudes positively correlated with differences in mGCIPL thickness (r_s=0.46, p=0.002 and r_s=0.44, p=0.022, respectively). Results of analysis using absolute measures are shown in supplementary table 1. P50 amplitudes were positively correlated with INL thickness (r_s=0.36, p=0.009) and N95:P50 ratios showed a negative correlation with INL thickness (r_s=-0.52, p=0.015) (Figure 3).

Outer retinal layers. OPNL thickness was not associated with PERG parameters.

P50 peak times and PVEP parameters did not correlate with retinal layer thickness parameters.

Time since symptom onset

Time since symptom onset did not correlate with any electrophysiological measure nor did it differ for cases with subnormal electrophysiology results. As PERG abnormalities in ON may start recovering after 7 days [12,14]. a subgroup analysis of the 18 ON patients that were included ≤7 days from symptom onset was performed. The above-described inter-group differences and structure-function associations remained significant in this subgroup (Supplementary figures 4 and 5).

DISCUSSION

The data demonstrate both structural and functional macular changes in acute ON. PERG P50 amplitudes, reflecting function of macular structures, were reduced in affected eyes and were associated with both mGCIPL and INL thickness as well as impaired visual acuity, consistent with a probable retinal contribution to the visual acuity loss, as suggested by limited earlier data [16,17]. PERG P50 amplitudes showed positive association with INL thickness. There was an expected correlation between the N95 component and mGCIPL thickness.

Our study is the first demonstration that P50 reduction can occur in acute ON of various aetiologies, including MOGON. Although there was some suggestion that P50 amplitudes were more reduced in MOGON compared with SION, MOGON cases tended to present earlier in their disease course, and as P50 reduction in acute ON may start recovering after a week [14,16,17], earlier presentation may be relevant.

It can be presumed in patients with a normal N95:P50 ratio and reduced P50 component amplitude, that the reduction in N95 is associated with the P50 reduction, as has been demonstrated in many maculopathies [20]. However, the N95:P50 ratio was greater than normal in four eyes. These eyes had markedly subnormal P50 components, presumably with intraretinal amplification allowing greater preservation of the RGC generated N95 as occasionally occurs in some maculopathies [20]. Some patients showed N95 reduction without significant P50 involvement, in keeping with the selective loss of N95 that can occur in optic neuropathy [21]. This could reflect previous sub-clinical optic nerve involvement, a well-established occurrence in MS [22]. In general, N95 involvement without P50 involvement occurs in early or mild disease; P50 involvement becomes evident in more severe disease and/or later in the course of the disorder [16,21]. Although P50 origins are not fully ascertained, it is likely in part generated distal to the RGCs [12,14]. The correlation between P50 and INL thickness suggests a probable contribution of the INL to P50 generation.

Importantly, cases with subnormal P50 amplitudes had poorer visual acuities, consistent with earlier data [16]. The data suggest a structure-function relationship between PERG P50 amplitude and INL thickness. The correlation, combined with the identified association of impaired P50 amplitude with visual acuity reduction, suggests probable clinically significant

changes in the INL in the acute stages of ON. INL thickness was not increased in ON eyes, in contrast to some previous reports [10,11]. However, in those previous studies OCT was not performed acutely but 50-100 months after ON; INL thickening later in the disease process is not excluded.

There were associations between mGCIPL thickness and the P50 and N95 components of the PERG. Macular GCL thickness in various pathologies, including advanced MS, has previously shown positive correlation with N95 amplitude, but not P50 amplitude [23]. As most ON patients showed optic disc swelling, no structure-functional association was evident between pRNFL thickness and PERG measures, although seen in chronic MSON [24]. A recent publication [25] addressed multi-focal PERG (mfPERG) in MS patients and suggested foveal axonal dysfunction, with associations between OCT data and mean reduction in the second negative (N2) component of the mfPERG and shorter peak time of the positive P1 component. The relationships between mfPERGs and conventional PERGs are yet to be clinically determined [25].

The data also show that a shortened PERG P50 peak time can occur in acute ON. P50 shortening is associated with significant loss of RGC function [26], and has been reported in recovered ON [12,27]. It also occurs in non-human primates following intravitreal tetrodotoxin injection [13], which blocks spiking cell function but spares the INL and photoreceptors. Shortening of P50 peak time has been reported in advanced but not early Leber Hereditary Optic Neuropathy (LHON) [28] and dominant optic atrophy (DOA)[29]. PERG abnormalities in DOA are confined to the N95 component in the early stages, but with increasing severity there is amplitude reduction and subsequent shortening of P50 peak time [29]. This conforms with current understanding of the origins of the PERG components, with N95 originating in RGCs and P50 originating partly in RGCs and partly from more distal structures [13,16]. As RGC dysfunction advances, those elements of P50 generated anteriorly to the RGCs become more dominant and the P50 component thus appears earlier. The finding of a short peak time P50 in acute ON may indicate that loss of RGC function occurs earlier than in other optic nerve diseases [28–30]. However, retrograde degeneration to the RGCs due to prior subclinical optic nerve involvement cannot be excluded as ON patients overall had thinner mGCIPL thickness than controls.

PVEP findings in clinically affected eyes showed delayed peak times with or without amplitude reduction, but delays were also present in some asymptomatic fellow eyes in keeping with the original observations by Halliday's group [22].

It has previously been demonstrated that increased INL thickness is associated with poorer clinical outcomes in ON and MS [3,4] and that macular involvement in neuroinflammatory disease may extend beyond the pRNFL and RGCs [31]. Although not replicated in this acute cohort, the P50 amplitude reductions observed, consistent with macular dysfunction, were associated with reduced visual acuity and with INL thickness. The mechanisms underlying macular involvement are uncertain. There may be early pathophysiological processes that have functional but not structural consequences. It is unlikely that the data reflect retrograde degeneration, which typically occurs later and does not involve the INL [32]. Different, more acute retrograde effects such as changes in metabolic demand and signalling are not excluded. However, a marked effect on P50 amplitude would necessitate a trans-synaptic effect anterior to the RGCs in the visual pathway and is thus more likely to reflect primary alteration in macular function synchronous with the inflammatory effect on the optic nerve. There may be local inflammatory processes [7,33] or vascular changes [34] that contribute to macular dysfunction.

CONCLUSION

This study documents macular dysfunction in acute ON, as shown by reduction in the PERG P50 component and structure-function correlations between PERG parameters and INL and mGCIPL thickness. This could be due, separately or in combination, to breakdown of the blood-retina barrier at the level of the superior vascular plexus, activation of microglia, or, less likely, the retrograde effects of optic nerve damage. Cases with subnormal P50 amplitudes had poorer visual acuities, suggesting that the macular dysfunction contributes to visual loss in acute ON. This is an important consideration for clinical trials aimed at rescuing optic nerve function in ON and suggests that such clinical trials should consider the inclusion of macular parameters.

FIGURES

FIGURE 1

PERG and PVEP recordings from two study participants

Patient A has a 3-day history of blurred right eye vision accompanied by pain on lateral eye movement. Visual acuity was 6/18 in the affected eye; 6/6 in the left eye. PERG P50 amplitude is significantly reduced in the right eye with preservation of the N95:P50 ratio. Patient B has a 6-day history of a sudden onset of right central scotoma. Visual acuity was 6/60 in the affected eye; 6/6 in the left eye. PERG from the right eye shows marked P50 component reduction with an increased N95:P50 ratio. In both cases the PVEP from the affected eye is severely reduced and delayed, worse for patient B. In both cases the pattern VEP and PERG from the unaffected eye are normal.

FIGURE 2

Electrophysiological data (PERG and PVEP) in acute ON and controls

PERG and PVEP parameters for control eyes (black circles) as well as the affected and fellow eyes of individuals with acute ON (blue=MSON, green=MOGON, red=SION). p-values represent the results of Wilcoxon rank-sum test. The grey horizontal bars represent group medians. (A) PERG P50 amplitudes and (B) PERG N95 amplitudes were significantly lower in affected eyes and fellow eyes (both $p<0.001$) of patients with acute ON patients than in controls. There was no significant difference in (C) the N95:P50 ratio between controls and either affected or fellow eyes of individuals with acute ON suggesting the N95 reduction to be consequent upon the P50 component reduction. (D) P50 peak times were on average shorter in the affected and fellow eyes of acute ON patients compared with controls (both $p<0.001$). (E) and (F) PVEP P100 component peak time and amplitude both differ significantly between control eyes and affected eyes (both $p<0.001$). There was no significant difference in P100 peak time or amplitude between fellow and control eyes. Normative ranges are shown (dashed horizontal reference line). Undetectable PVEPs are shown as “zero” values in the graph, but treated as “missing data” in statistical analyses.

FIGURE 3

Electrophysiological data (PERG and PVEP) on inter-eye differences in acute ON

These figures show interocular differences in PERG and PVEP parameters, with results of the affected eye plotted on the x-axis and of the fellow eye plotted on the y-axis. The central x=y

line is plotted to highlight differences and similarities between eyes. Observations to the upper-left of this line represent an interocular difference with the value higher in the fellow eye compared with the affected eye. p-values represent the results of Wilcoxon signed-rank test for group comparison. MSON, SION and MOGON cases are coloured blue, red and green, respectively. (A) PERG P50 amplitudes and (B) PERG N95 amplitudes were significantly lower in affected eyes compared with fellow eyes of acute ON patients ($p<0.001$). (C) There was no significant interocular difference in N95:P50 ratios suggesting the N95 reduction to be consequent upon the P50 component reduction. P50 peak times were significantly shorter in affected eyes compared to fellow eyes ($p<0.001$). (E) and (F) for both PVEP parameters there was a significant inter-eye difference ($p=0.001$ for both). Undetectable PVEPs are shown as "zero" values in the graph, but treated as "missing data" in statistical analyses.

FIGURE 4

Structure-function correlations between retinal thickness and PERG measures

Relationships between PERG parameters and retinal layer thickness measurements are shown in scatter plots with linear regression lines. r_s is the Spearman's rho, R^2 is the R-squared and p-values represent results of linear regression analysis. MSON, SION and MOGON are coloured blue, red and green, respectively. Symbols representing patients with a prior episode of ON in the fellow eye are filled and are triangular for patients with MMO. (A) Interocular differences for the fellow eye minus the affected eye for the N95 amplitude and (B) P50 amplitude are positively correlated with the interocular difference of mGCIPL thickness in ON cases. (C) Absolute P50 amplitudes were positively correlated with INL thickness and (D) N95:P50 ratios were correlated negatively with INL thickness.

SUPPLEMENTARY FIGURES

SUPPLEMENTARY FIGURE 1

Electrophysiological data (PERG and PVEP) for ON patients with subnormal PERG P50 amplitude

These figures show individually identifiable electrophysiology results for the eight cases with a subnormal PERG P50 amplitude (<2.0μV). Each individual case with an abnormal P50 is

identified through colour-coding, and additional electrophysiological results of these patients are shown.

SUPPLEMENTARY FIGURE 2

Dotplots showing structural (OCT) data in acute ON and controls

Structural data (OCT) on individual eyes and inter-eye differences in acute ON and controls. These figures show the OCT data for (A) mGCIPL, (B) pRNFL, (C) INL and (D) OPNL. Bars represent median observations.

SUPPLEMENTARY FIGURE 3

Inter-eye differences in subgroup ON patients included ≤7 days

These plots show interocular differences in PERG and PVEP parameters, with results of the affected eye on the x-axis and of the fellow eye on the y-axis. I. Observations to the upper-left of the central line represent an interocular difference with the value higher in the fellow eye compared with the affected eye. Cases in these figures can be compared with figure 1 and figure 2 to determine those cases presenting within 7 days. p-values represent the results of Wilcoxon signed-rank test for group comparison. MSON, SION and MOGON cases are coloured blue, red and green, respectively. (A) PERG P50 amplitudes and (B) PERG N95 amplitudes are significantly lower in affected eyes compared with fellow eyes of acute ON patients (p=0.015 and p=0.003 respectively). (C) There was no interocular difference in N95:P50 ratios. (D) P50 peak times were significantly faster in affected eyes compared to fellow eyes (p=0.012). (E) and (F) for both PVEP P100 amplitudes and peak times there was a significant inter-eye difference (p=0.021 and p=0.008). Undetectable PVEPs are shown as “zero” values in the graph, but treated as “missing data” in statistical analyses.

SUPPLEMENTARY FIGURE 4

Structure-functional correlations in subgroup ON patients included ≤7 days

Relationships between PERG parameters and retinal layer thickness measurements are shown in scatter plots with linear regression lines. r_s is the Spearman’s rho, R^2 is the R-squared and p-values represent results of regression analysis. Multiple sclerosis, isolated optic neuritis and MOGON cases are coloured blue, red and green, respectively. (A) Interocular differences (fellow eye – affected eye) of N95 amplitude and (B) P50 amplitude are positively correlated

with the interocular difference of mGCIPL thickness. (C) Absolute P50 amplitude and (D) N95:P50 ratio are positively and negatively correlated with absolute INL thickness, respectively. (E) Interocular difference of N95:P50 amplitude is negatively correlated with interocular difference in INL thickness. For this final analysis one outlier with MMO was excluded.

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COMPLIANCE WITH ETHICAL STANDARDS

DISCLOSURES

IK, LDA, LDP, JG, SL, AR, SAT, GTP and GEH report no disclosures. AP reports personal fees from Novartis, Heidelberg Engineering, Zeiss, grants from Novartis, outside the submitted work; and is part of the steering committee of the OCTiMS study which is sponsored by Novartis and the Angio-OCT steering committee which is sponsored by Zeiss. He does not receive compensation for these activities.

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INFORMED CONSENT

Ethical approval for this study was obtained from the Queen Square London Ethics committee (number 10/H0716/72) and hospital R&D (FRAC0001). Written informed consent for participation and publication was obtained from all individual participants included in the study.