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Original Article – Clinical Science

Multifocal electroretinogram response following subthreshold nanosecond laser intervention in age-related macular degeneration

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Ethics statement: The study was approved by the Human Ethics Committee of the Royal Victorian Eye and Ear Hospital (RVEEH) and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

ABSTRACT

Importance: The effect of subthreshold nanosecond laser (SNL) treatment on retinal function remains unknown.

Background: SNL treatment has been studied as a potential intervention in intermediate age-related macular degeneration AMD (iAMD). This study investigated the longitudinal effect of SNL treatment on retinal function.

Design: This was a sub-study of the LEAD trial; a 36-month, multicenter, randomized, sham-controlled trial.

Participants: Subjects with iAMD.

Methods: Eligible participants were assigned randomly to receive SNL or sham treatment to the study eye at 6 -monthly visits. Multifocal electroretinography (mfERG) was performed at each study visit from a study site. The mfERG responses were grouped into three regions (central, middle and outer rings) and compared between the SNL and sham group.

Main Outcome Measures: mfERG P1 response amplitude and implicit time.

Results: Data were collected from 50 subjects (26 in the SNL group, 24 in the sham group). At baseline, the P1 amplitudes of both the study eyes and the fellow eyes were similar between the groups at all rings. In the sham group, the P1 amplitude gradually decreased over time ($P<0.05$). In the SNL group, there was an improvement in P1 amplitude which became statistically significant at the 36-month visit, detected in both the treated and fellow eyes at the central ($P=0.005$) and middle ring ($P=0.007$) but not at the outer ring ($P=0.070$). No difference in P1 implicit time detected between the groups ($P>0.05$).

Conclusions and relevance: SNL treatment improved electrophysiological function. mfERG could be useful for monitoring AMD progression and evaluating the efficacy of SNL treatment.

Keywords: Age-related macular degeneration (AMD), Electroretinogram, Macular

1. INTRODUCTION

The Laser Intervention in the Early Stages of Age-Related Macular Degeneration (LEAD) study was a multi-center, randomized, sham-controlled trial evaluating the efficacy and safety of a novel sub-threshold nanosecond laser (SNL) intervention for slowing the rate of progression from the early stages to late age-related macular degeneration (AMD) in individuals with bilateral large drusen.[1] In the LEAD study, participants were randomly allocated to receive either SNL or sham treatment at 6-monthly intervals in the study eye with the fellow eye being untreated. The primary efficacy outcome of the study was the rate of progression to late AMD. Details of the study design and baseline characteristics of the participants have been reported previously.[1, 2] Overall, the study did not find a statistical significant effect for slowing the overall rate of progression to late AMD.[2] However, a post-hoc analysis revealed evidence of a significant treatment effect modification based on the baseline coexistence of reticular pseudodrusen (RPD). In this analysis, those iAMD participants without RPD showed reduced progression and those with RPD had a potentially more rapid progression.[2]

Several subjective visual function parameters were also investigated as exploratory outcomes in the LEAD study including best-corrected visual acuity (BCVA), low luminance visual acuity (LLVA) and microperimetric sensitivity.[1, 3] There was a significant difference in the BCVA change from baseline to 36-month visit between the study eye of the SNL and sham treatment groups but not between the non-study eyes of the two groups.[3] The rate of change in BCVA was however similar for the SNL treated, SNL untreated and sham untreated eyes. The difference in BCVA of the study eyes between the two groups was driven by the positive changes in BVCA in the sham group and thus it was likely to be as a result of regression to the mean

and the placebo effect. All other subjective functional parameters showed no significant difference between the groups.[3]

An objective assessment of retinal function was also performed in a sub-set of participants, at one site, in the LEAD study.[1] In this report, we present the findings of the effect of SNL treatment on retinal function, as measured objectively using multifocal electroretinography (mfERG).

2. METHODS

This sub-study assessing the effect of SNL on retinal function was part of the LEAD study, which was approved by the Human Ethics Committee of the Royal Victorian Eye and Ear Hospital (RVEEH) and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

Details of the design of the LEAD study have been reported previously.[1, 2] In brief, the LEAD study was a randomized, sham-controlled clinical trial conducted over 5 sites and registered with the Australian New Zealand Clinical Trials Registry (identifier, ACTRN12612000704897) and clinicaltrials.gov (identifier, NCT01790802). All participants were required to have BCVA of 20/40 or better in both eyes, bilateral large drusen (>125 μm) located centrally (within 1500 μm of the fovea) on colour fundus photographs (CFP). All eyes were required to have no evidence of late AMD on multimodal imaging (MMI), including neovascular AMD (nAMD), geographic atrophy or optical coherence tomography (OCT) imaging defined atrophy including nascent geographic atrophy.[4] The study eye was assigned based on the worse eye by BCVA. Full details about the eligibility criteria, definitions of late AMD and criteria for study eye assignment have been described in the previous reports.[1, 2] Participants were randomized to receive SNL or sham treatment to the study eye in

a 1:1 ratio. Both participants and study staff, except for the treatment clinician, were masked to the treatment allocation throughout the duration of the study. The protocol for the SNL and sham treatment has been described in details previously.[1, 2] In brief, SNL treatment involved delivering subthreshold laser spots of 400 μm in diameter at 12 locations on the retina: 6 in an arc just below the superior vascular arcade and 6 in an arc just above the inferior arcade. For sham treatment, the exact laser procedure was performed, except that short bursts of light from the retinal illumination system on the laser device were used instead of the laser beam. Treatments were performed on the day of randomization and repeated 6-monthly for 30 months if there was no late AMD developed in the study eye (a total of 6 treatments). Participants who developed late AMD in the study eye were deemed ineligible for re-treatment but remained in the study for observation on the potential effect of the treatment on the contralateral eye.

The primary outcome of the LEAD study was the time to development of late AMD in the study eye, and these findings have been reported.[2] The secondary outcome was the time to development of late AMD in the non-study eye due to preclinical and clinical pilot data suggesting a bilateral treatment effect.[1, 5, 6] The pre-specified exploratory outcomes of the LEAD study included drusen volume, participant-reported outcomes (such as Night Vision Questionnaire (NVQ-10) and Impact of Vision Impairment (IVI) questionnaire), subjective visual function (BCVA, LLVA and microperimetric sensitivity) and objective retinal function (mfERG).[1] We have recently presented the findings of the secondary and some exploratory outcomes (subjective visual function and patient reported outcomes) of the LEAD study.[3] The findings of objective retinal function are presented in this report.

2.1 Multifocal Electroretinography

In this sub-study, the first 50 consecutive subjects recruited at the CERA site had additional mfERG assessment in both eyes at baseline and repeated at 6 monthly interval for 36 months or until late AMD developed during the follow-up period.[1] The mfERG data were not collected from the other study sites due to the unavailability of the system and feasibility of the sites.

Retinal function was assessed monocularly using the Visual Evoked Response Imaging System (VERIS Science 6, ElectroDiagnostic Imaging Inc., Redwood City, CA) and Dawson-Trick-Litzkow (DTL) thread electrodes. The details of our mfERG recording protocol have been reported previously.[7-9] In brief, recording was performed for the right eye first followed by the left eye. Pupils were dilated using 1% tropicamide and 2.5% phenylephrine, and recordings commenced only when pupil dilation was at least 7mm. A fixation monitoring system (FMS) delivered the test stimulus consisting of 103 retinal-scaled hexagons (Figure 1A) which alternated between white and black frames on a pseudorandom m-sequence (m = 15) at a rate of 75 Hz. Luminance of the white hexagons was set at 5.33 cd.s/m^2 and the background luminance was set at 200 cd/m^2. The stimuli were adjusted for optimal focus and corrected refractive error by the participant through manual adjustment of the refractor unit. The recorded signals were filtered using a band pass filter between 10 and 100 Hz and was amplified 100,000 times (model 12; Grass NeuroData, Quincy, MA). The duration of the recording was approximately 7 minutes for each eye, which was divided evenly into 16 slightly overlapping segments of approximately 27 seconds each for patients' comfort and to suppress eye movement and blinks. Segments that were contaminated with blinks or eye movements were discarded and re-recorded. The fellow eye that was not tested was occluded with an eye patch.

Figure 1: (**A**) Superimposition of the mfERG stimulus on a fundus photograph to illustrate the retinal area covered by the mfERG recording. The centre hexagon is marked with an "X" sign. (**B**) The mfERG responses were grouped into the central (R1), middle (R2) and outer (R3) ring for the analysis. (**C**) An example of the mfERG response waveform. Double- and single-headed arrows indicate measurements of the first positive response (P1) amplitude and implicit time, respectively.

2.2 Data Analyses

Data of all subjects were included in the analysis. However, those who developed late AMD during the study period, only data prior to the development of late AMD were included in the analysis as mfERG was not performed once late AMD developed. Although mfERG was performed at 6 monthly intervals, only data at baseline, 12, 24 and 36-month visits were presented in this report because there was minimal changes in mfERG responses within 6 months.

The mfERG responses were grouped into three regions of central (R1, 0-4°), middle (R2, 4-12°) and outer ring (R3, 12-22°) (Figure 1B). No spatial averaging was applied to the mfERG analysis. The mfERG P1 response amplitude density and P1 implicit time of the first order-kernel was obtained for each subject. The mfERG P1 response amplitude and implicit time of each ring was calculated for each visit and the changes in the mfERG response over time was analysed using a linear mixed-effects model with age as a covariate. The difference in the mfERG responses between the SNL and sham groups over time was analysed using two-way analysis of variance (ANOVA) and Dunnett's post-hoc test. The analysis was performed initially for all eyes and then separately for eyes with and without RPD. Non-parametric and descriptive statistics were used for eye with RPD due to a small sample size.

3. RESULTS

There were 26 subjects in the SNL group and 24 subjects in the sham group. There was no difference in the average age between the SNL (71.1 ± 7.5 years) and the sham group (69.7 ± 6.5 years, p = 0.420). There was no difference in BCVA at baseline between the groups (SNL: 84.7 ± 3.9 letters; sham: 84.3 ± 5.0 letters; p = 0.663). There were 5 participants in each group, from the initial 50 subjects, who developed late AMD in the study eye during the followed up period.

At baseline, the average P1 amplitude was similar between the SNL and sham groups in both the study and fellow eyes at all concentric rings (Figure 2). In the sham group, the mean P1 amplitude gradually decreased in both eyes over the 36-month follow-up period (Figure 2). The decline in P1 amplitude appeared to be less in the first year and more rapidly in the second and third year, particularly in the central ring.

In the SNL group, there was a slight improvement in P1 amplitude of the treated eyes in ring 1 in the initial 24 months post treatment, and the improvement reached the significance level at the 36-month visit (Figure 2). Similarly in ring 2, the P1 amplitude of the treated eyes appeared to be starting to improve at the 24-month visit and a greater improvement was observed at the 36-month visit. There was also evidence of an improvement in P1 amplitude in the untreated fellow eyes at rings 1 and 2. There was a trend of improvement in P1 amplitude at ring 3 in the SNL group compare to the sham group, however, the difference in P1 amplitude between the groups was not statistically significant.

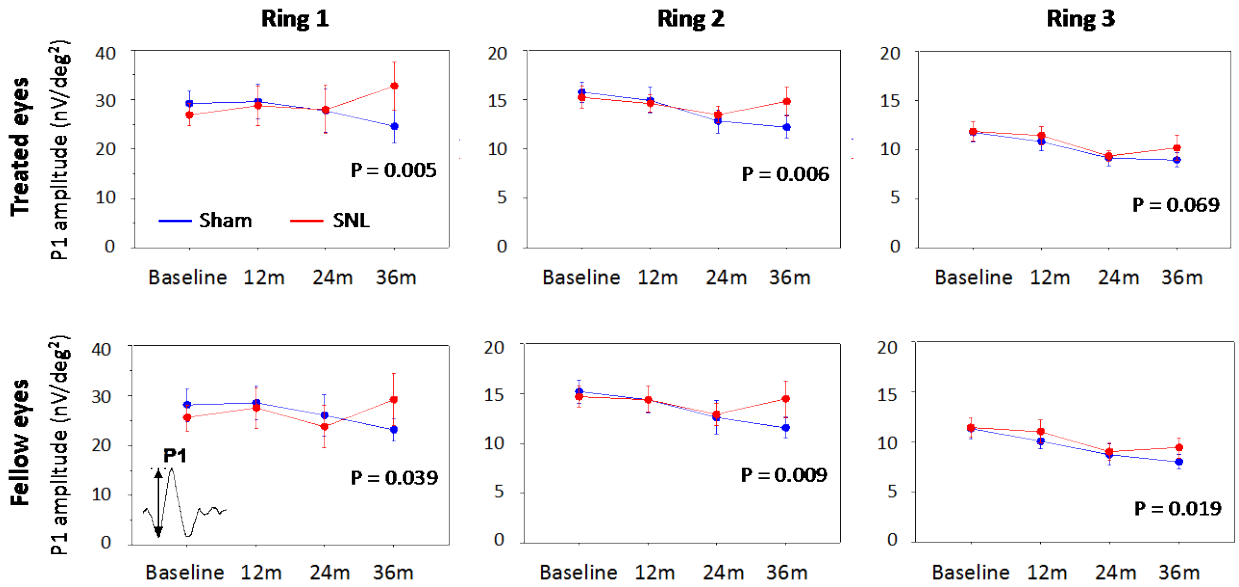

Figure 2: mfERG P1 response amplitude of the treated and untreated fellow eyes at various retinal concentricities over 36 months follow up. In the sham group (blue line), the mean P1 amplitude was gradually decreased in both eyes at all concentricities over the 36-month follow-up period. In the SNL treated group (red line), there was an improvement in P1 response amplitude in both the treated and untreated fellow eye detected in rings 1 and 2 at the 36-month visit.

To examine the effect of RPD on the functional outcomes, the mfERG data were analysed separately for eyes with and without RPD. There were 7 subjects with RPD in the SNL group and 5 subjects in the sham group. When a sub-analysis was performed excluding subjects with RPD, the same findings were obtained in that there was a significant improvement in mfERG responses in the SNL treated group compared to the sham group at the 36-month visit, although only ring 2 data reached the significance level (Figure 3). There was also a trend of mfERG improvement in the SNL treated group compared to the sham group in eyes with RPD (Figure 4).

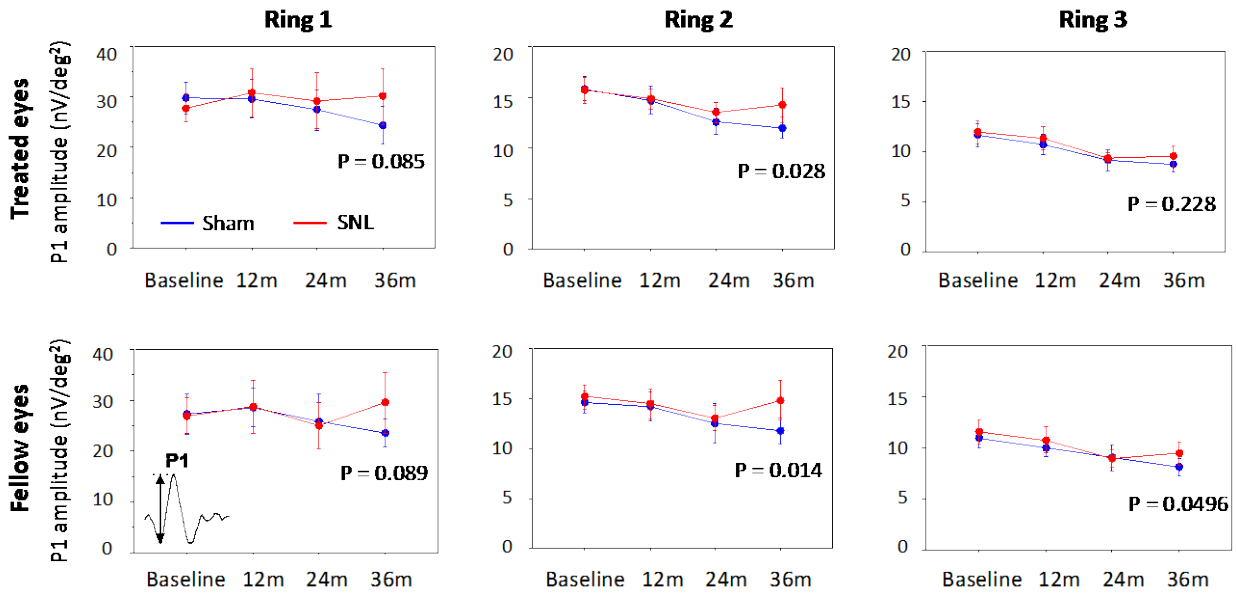

Figure 3: mfERG P1 response amplitude of iAMD eyes without RPD over 36 months follow up. There was an improvement in P1 response amplitude over time in both the treated and untreated fellow eye of the SNL group, particularly in rings 1 and 2.

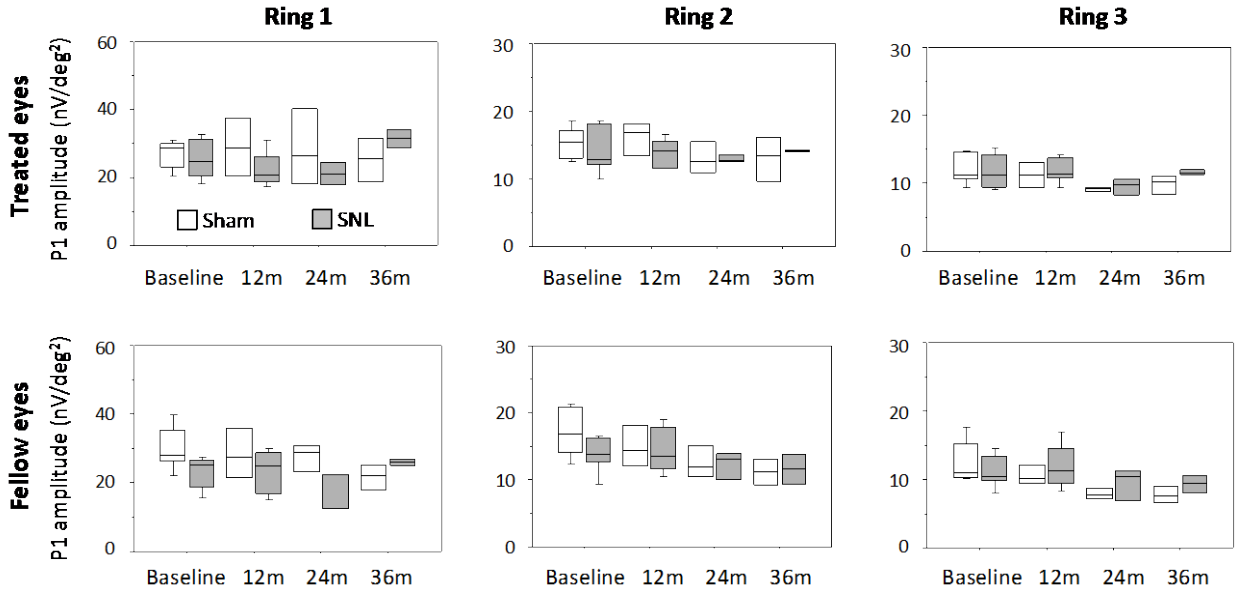

Figure 4: mfERG P1 response amplitude of iAMD eyes with RPD over 36 months follow up. Due to a small sample size the data were presented in box plots. There

was a trend of improvement in P1 response amplitude in both the treated and untreated fellow eye of the SNL group at the 36-month visit, particularly at ring 1.

With regard to the P1 implicit time, there was a slight increase in the P1 implicit time over the course of the study in both the treated and untreated fellow eyes, in both the SNL and sham groups. However, these changes were not significant compared to the baseline value (Figure 5). The P1 implicit was also similar between the SNL and sham groups at all rings and all visits.

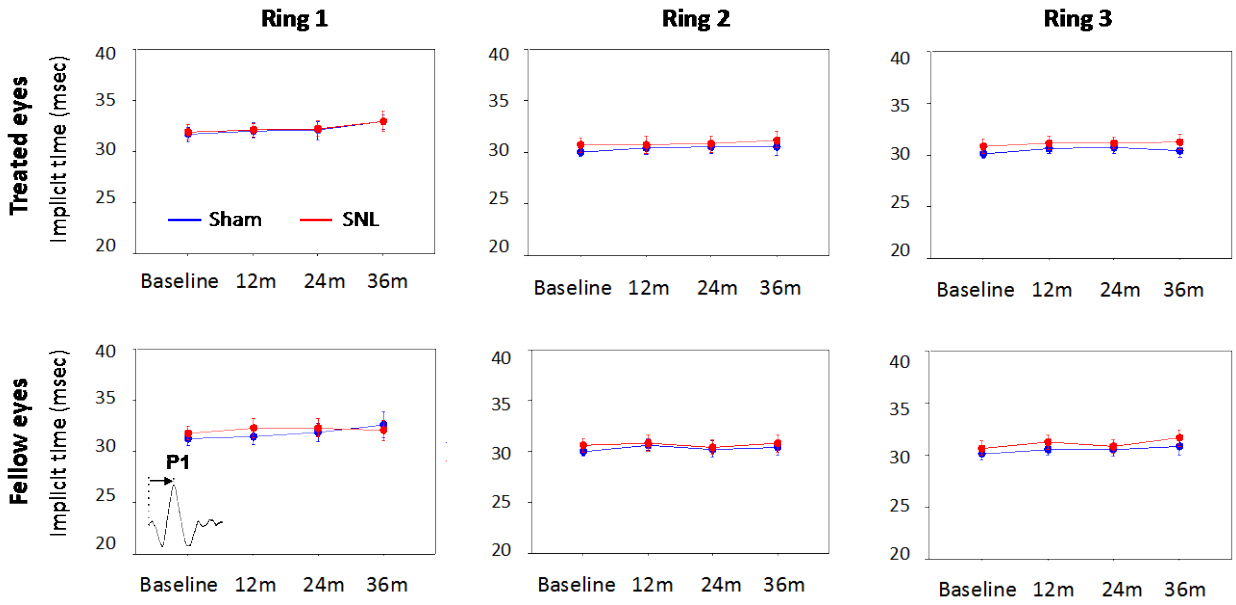

Figure 5: mfERG P1 implicit time of the treated and untreated fellow eyes at various retinal concentricities over 36 months. Overall, there was a slight increase in P1 implicit time over time in both the treated and untreated fellow eyes, in the SNL and sham groups, however the increase in P1 implicit time was not significantly different to the baseline value. There was no significant difference in P1 implicit at any rings or visits between the SNL and sham groups.

4. DISCUSSION

This study examined the effect of SNL treatment on long-term retinal function assessed by mfERG. We found that application of SNL treatment in one eye resulted in an improvement in retinal function in both the treated and untreated fellow eye, and the improvement in retinal function reached the significance level after 24 months from the initial treatment. This is important finding as it may provide clues on the mechanisms of action of the SNL treatment and identify a better approach for evaluating treatment efficacy in future studies of laser intervention for early stages of AMD.

It has been demonstrated in preclinical studies that SNL treatment leads to an improvement in structure and function of the retinal pigment epithelium (RPE) and Bruch's membrane (BM) without causing damage to the underlying neural retina.[5, 10-13] Our group has previously shown that SNL treatment results in a specific alteration in extracellular matrix regulating factors within the RPE.[5] The improvement in retinal function, specifically cone photoreceptor function, demonstrated by the mfERG in this study could potentially relate to the overall improvement in the health of the RPE and BM, which support the photoreceptors, following the SNL treatment. Given that AMD is a chronic disease with debris accumulated over many years, it is reasonable that there be some delay in restoring RPE-BM function which would ultimately be reflected in neuronal function. Thus the delay in improvement in mfERG detected after SNL treatment is not surprising.

Since SNL treatment was delivered every 6 months over 3 years in this trial, it is also possible that the accumulation of SNL treatments over time resulted in the improvement in retinal function observed. It would be interesting to determine whether the improvement in retinal function is associated with the amount and frequency of the SNL treatment in future studies.

The fellow eye effects following SNL treatment have been previously reported. In a preclinical study in which SNL treatment was applied to one eye, we reported that there was thinning in BM and increased genes expression in matrix metalloproteinases in the treated eye as well as in the fellow eye.[5] Thus the bilateral improvement in objective measures of retinal function, observed in this study, is consistent with these anatomical and genes expression changes observed in previous preclinical studies.

The study showed a significant improvement in mfERG response at rings 1 and 2 but not ring 3, although there was a trend towards significance level. There are three possible explanations on the different levels of mfERG improvement seen at different rings. First, the central retina is known to have a greater reduction in function compare to the peripheral retina in AMD.[9] Thus there is much more room for improvement at the central retina compare to the peripheral retina. Second, the magnitude of the mfERG response is greater in the central retina compare to the peripheral retina. Hence, there is a much larger dynamic range and resolution to detect changes in the inner rings compare to the outer rings. Third, the lack of significant difference in P1 amplitude between the groups in ring 3 could due to a relative small sample size in this study.

We have previously shown that there was a gradual decline in subjective visual function over time, such as BCVA and microperimetric sensitivity, in both the SNL and sham treated eyes.[3] We were, however, unable to demonstrate the effect of SNL treatment on visual function using these subjective functional parameters. We believe that the different observations between the mfERG and other subjective functional parameters are likely due to the different nature of each test, which may capture different aspects of retinal function.[7] For example, the mfERG uses a large

stimulus size and provides objective suprathreshold data, whereas the microperimeter uses a much smaller stimulus and provides subjective threshold data.

We do not believe that reticular pseudodrusen (RPD) status had an influence on the mfERG findings. The presence of RPD has been shown to be associated with higher risk of progression to GA[14-17] and abnormal dark adaptation.[18-22] When a sub-analysis was performed separately for eyes with and without RPD, similar findings were obtained in that there was a significant improvement in mfERG responses in the SNL treated group compared to the sham group at the 36-month visit. More importantly, the data did not show a greater reduction in retinal function in the RPD eyes that received the SNL treatment. Furthermore, we and others have previously shown that the presence of RPD has little influence on the mfERG responses.[23, 24] Thus, the presence of RPD does not explain for the improvement in retinal function at the 36-month visit.

The significance of this study is that an improvement in retinal function, as determined objectively by mfERG, following SNL treatment for iAMD is demonstrated in a randomised trial. These findings support a possible beneficial effect of SNL treatment for the early stages of AMD and should be further investigated. It will be important to determine if the improvement in objective measures of retinal function can be reproduced and correlate with slower progression rates to late AMD. It will also be important to determine if the improvement in retinal function is sustained beyond the 3-year follow-up period, when no further treatment was given. Further work on mfERG in SNL will help us understand potential mechanisms of actions of the laser which result in both a study and fellow eye effect.

The strengths of this study include the design that had all mfERG were performed at a single experienced site ensuring uniform data collection and there was a long follow-up on an objective measure of retinal function with a 100% completion rate. The participants were randomised to receive either the SNL or sham treatment, and both the mfERG examiner and the participants were masked to the treatment. The limitations of the study include a relatively small sample and that mfERG data were collected only from the initial 50 subjects at a single site.

In conclusion, application of SNL treatment in one eye resulted in an improvement in electrophysiological function in both the treated and untreated fellow eye, with a significant improvement detectable after the 24-month follow-up time point. The mechanisms of action of the laser that brings about these changes and whether improvement in retinal function is associated with a slower rate of progression to late AMD require further investigation.

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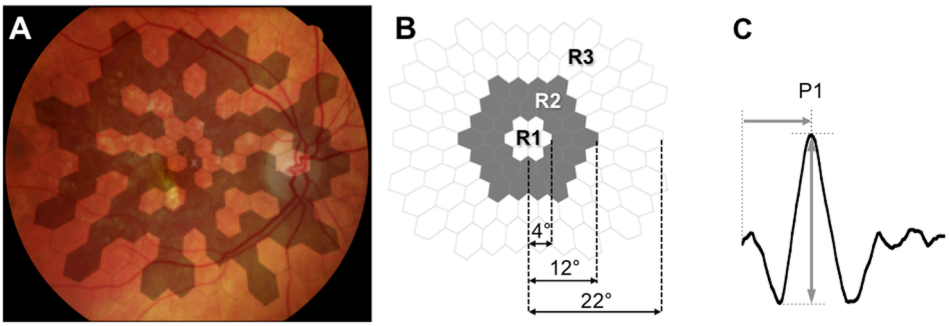

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