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Article

Functional assessment of outer and middle macular layers in multiple sclerosis

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Abstract: The involvement of macular function in its preganglionic elements, during the neurodegenerative process of multiple sclerosis (MS), is controversial. In this case-control observational and retrospective study, we assessed multifocal electroretinogram (mfERG) responses from 41 healthy Controls, 41 relapsing-remitting MS patients without optic neuritis (ON) (MS-noON Group), 47 MS patients with ON: 27 with full recovery of high-contrast best corrected visual acuity (BCVA) (MS-ON-G Group) and 20 with poor recovery of BCVA (MS-ON-P Group). MfERG N1 and P1 implicit times (ITs), and N1-P1 response amplitude densities (RADs) were measured from concentric rings (R) with increasing foveal eccentricity: 0-5° (R1), 5-10° (R2), 10-15° (R3), 15-20° (R4), 20- 25° (R5), and from retinal sectors [superior, nasal, inferior and temporal] between 0-15° and 0- 25°. In MS-ON-P Group, mean mfERG RADs detected from R1 (0-5°) and from the central nasal quadrant (0-15°) were significantly reduced ($p<0.01$) with respect to those of Control, MS-noON and MS-ON-G Groups. No other significant differences between Groups for any mfERG parameters were found. Our results suggest that in MS, exclusively after ON with poor recovery of BCVA, the neurodegenerative process can induce dysfunctional mechanisms involving photoreceptors and bipolar cells of the fovea and of the more central nasal macular area.

Keywords: multiple sclerosis; preganglionic retinal elements; photoreceptors; bipolar cells; multifocal electroretinogram; neurodegeneration.

1. Introduction

Multiple sclerosis (MS) is a neurodegenerative disease, characterized by chronic demyelination of the central nervous system, which can result in visual system involvement including retrobulbar optic neuritis (ON) [1].

The ON event is followed by secondary neurodegenerative processes for retrograde trans-synaptic degeneration [2] that involve retinal ganglion cells (RGCs) and their axons [3] forming the innermost retinal layers (IML). In MS patients, an IML dysfunction has been observed by recording abnormal bioelectrical responses with pattern electroretinogram (P-ERG) [4-6] that is a well-known reliable electrophysiological technique for assessing IML function [7].

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At the present, it is a debated topic to understand whether the neurodegenerative mechanisms occurring in MS, could involve retinal structures beyond the IML towards the preganglionic elements (i.e. photoceptors, bipolar cells) located in the outer and in middle retinal (O-MR) layers.

The function of preganglionic elements can be assessed by ERG recordings [8] that, with its variants, allow to study the bioelectrical activity of photoreceptor and bipolar cells from the whole retina by Full-field ERG (Ff-ERG) [9], from the central retina by focal ERG (F-ERG) [10] and from multiple localized retinal areas by multifocal ERG (mfERG) [11]. In particular, the mfERG technique provides a topographical map of objective bioelectric responses derived from localized retinal areas, which are driven largely by the cone-related preganglionic components. A 'kernel analysis' applied to mfERG responses can be used to assess nonlinear functions of the visual system mainly originating from selected populations of photoreceptors and bipolar cells [12-14].

In MS patients, the Ff-ERG cone a- and b- waves' amplitudes have been found reduced [15-18], thus reflecting post-phototransduction impairment of the photopic system of the whole retina [16], and, by recording F-ERG, impaired photoreceptoral and post-photoreceptoral responses have been found in the macular area [19].

Regarding the mfERG responses in MS, contrasting data have been reported in the recent literature: in fact, mfERG signals have been found either abnormal [18, 20, 21] or normal [22], due to different types of MS patients (with or without history of ON), acquisition systems and analysis of recordings and limited sample size.

All these contrasting electrophysiological evidences led us to consider that there are no conclusive findings on whether there is or not an O-MR layers dysfunction, functional expression of the extended neurodegenerative process beyond IML in MS.

Therefore, to add information on the debated topic of preganglionic functional involvement, or sparing from neurodegeneration, the aim of our work was to assess the function of preganglionic elements in MS. Patients with absence or presence of a history of ON, followed by good or poor recovery of best corrected visual acuity (BCVA), were evaluated. We attempted to determine whether an O-MR dysfunction could be detected in the central macular area, or whether it might affect more peripheral retinal regions. In addition, we investigated whether the possible O-MR involvement could be observed in specific sectors [Superior (S), Nasal (N), Inferior (I), Temporal (T)] of the central macular region (0 to 15 degrees) and/or in more eccentric retinal areas within the vascular arcades (0 to 25 degrees).

2. Results

2.1. Demographic and clinical features

On Table 1 are reported the demographic and clinical features observed in MS-noON, MS-ON-G and MS-ON-P Groups. The descriptive statistics of age, MS-DD and EDSS values were not significantly different between MS-noON, MS-ON-G and MS-ON-P Groups. The descriptive statistics of number of ON and the time elapsed from the ON were not significantly different between MS-ON-G and MS-ON-P Groups.

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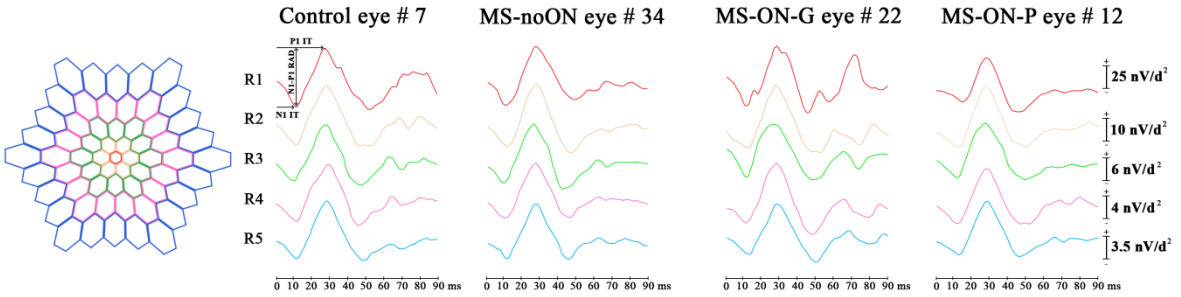
Table 1. Demographic and clinical features in Multiple Sclerosis patients without Optic Neuritis (MS-noON), with Optic Neuritis and good recovery of best corrected visual acuity (MS-ON-G) and with Optic Neuritis and poor recovery of best corrected visual acuity (MS-ON-P)

	MS-noON (N[a]=41) (mean±1SD[b])	**MS-ON-G (N[a]=27) (mean±1SD)**	**MS-ON-P (N[a]=20) (mean ± 1SD)**
Age (years)	41.32±3.72	39.92±4.86[§]	40.38±4.26[§,#]
MS-DD[c] (years)	8.53±4.19	9.06±5.58[§]	9.32±5.64[§,#]
EDSS[d] score	1.43±1.06	1.53±1.22[§]	1.49±1.18[§,#]
ON[e] (N[a])	-	1.00±0.00	1.0 0±0.00[#]
Time elapsed from ON[e] (months)	-	14.12±2.72	13.94±3.12[#]

[a]N= Number of eyes of each Group; [b]SD= one Standard Deviation of the mean; [c]MS-DD = Multiple Sclerosis Disease Duration; [d]EDSS = Expanded Disability Status Scale; [e]ON = optic neuritis; One-way analysis of variance between Groups: [§]p>0.01 vs MS-noON Group, [#]p>0.01 vs MS-ON-G Group.

2.2 Multifocal electroretinogram ring analysis

Examples of averaged mfERG recordings from 5 rings (R1, R2, R3, R4 and R5), obtained in representative Control (#7), MS-noON (#34), MS-ON-G (#22) and MS-ON-P (#12) eyes, are presented in Figure 1.



RING	N1 IT (ms)	P1 IT (ms)	N1-P1 RAD (nV/d²)
1	13.5	28.7	54.3
2	13.5	29.1	22.4
3	11.9	28.5	12.6
4	13.0	29.3	8.4
5	13.5	29.3	7.5

RING	N1 IT (ms)	P1 IT (ms)	N1-P1 RAD (nV/d²)
1	13.5	28.2	54.8
2	14.0	29.5	23.0
3	12.5	29.0	13.2
4	13.0	29.1	8.0
5	13.5	29.6	7.6

RING	N1 IT (ms)	P1 IT (ms)	N1-P1 RAD (nV/d²)
1	13.4	29.1	55.6
2	13.8	28.9	23.6
3	12.3	28.1	11.9
4	12.9	28.8	8.8
5	13.6	28.9	7.0

RING	N1 IT (ms)	P1 IT (ms)	N1-P1 RAD (nV/d²)
1	15.0	29.2	42.2
2	13.6	28.8	22.8
3	13.3	28.6	12.4
4	13.1	28.9	8.2
5	13.5	29.4	7.4

Figure 1. Multifocal electroretinogram averaged recordings obtained in in a Control eye (#7), in a patient with multiple sclerosis (MS) without history of optic neuritis (MS-noON#34), and with history of optic neuritis followed by good or poor visual acuity (MS-ON-G#22 and MS-ON-P#12, respectively) by using ring analysis. For a better comparison, the left eye of representative Control, MS-noON and MS-ON eyes is presented. Ring analysis reports the averaged values of N1 and P1 implicit times (IT, measured in milliseconds -ms-) and of N1-P1 response amplitude density (RAD, measured in nanoVolt/degree2 -nV/d2-) obtained from five concentric annular retinal regions (rings) centred on the fovea: from 0 to 5 degrees (ring 1, R1), from 5 to 10 degrees (ring 2, R2), from 10 to 15 degrees (ring 3, R3), from 15 to 20 degrees (ring 4, R4) and from 20 to 25 degrees (ring 5, R5).

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On Table 2 are reported the mean values of N1 and P1 ITs and of N1-P1 RADs detected in the 5 rings (R1, R2, R3, R4 and R5) in Control, MS-noON, MS-ON-G and MS-ON-P Groups and the relative statistical analysis between Groups.

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Table 2. Multifocal electroretinogram ring analysis in Control (C) eyes and in Multiple Sclerosis patients without Optic Neuritis (MS-noON), with optic neuritis followed by good recovery of best corrected visual acuity (BCVA) (MS-ON-G) or poor recovery of BCVA (MS-ON-P).

		Ring 1: 0-5 Degrees			**Ring 2: 5-10 Degrees**			**Ring 3: 10-15 Degrees**			**Ring 4: 15-20 Degrees**			**Ring 5: 20-25 Degrees**		
		N1 IT[a]	**P1 IT[a]**	**RAD[b]**	**N1 IT[a]**	**P1 IT[a]**	**RAD[b]**	**N1 IT[a]**	**P1 IT[a]**	**RAD[b]**	**N1 IT[a]**	**P1 IT[a]**	**RAD[b]**	**N1 IT[a]**	**P1 IT[a]**	**RAD[b]**
Controls	Mean	14.693	29.785	56.137	13.863	28.793	22.037	12.890	28.110	12.012	12.815	28.168	8.724	13.459	28.944	7.129
N[d]=41	SD[c]	2.666	2.678	10.771	1.712	1.349	4.816	1.312	1.394	3.090	2.396	1.331	2.090	1.247	1.611	1.778
MS-noON	Mean	15.078	30.035	54.273	13.743	28.393	21.505	13.193	27.413	12.525	13.115	28.455	9.050	13.198	28.650	7.535
N[d]=41	SD[c]	2.383	1.958	11.665	2.177	1.661	4.556	2.505	3.076	3.409	1.020	1.285	2.573	1.156	1.115	2.321
A[e] vs C	*f(1,81)*	*0.491*	*0.234*	*0.568*	*0.078*	*1.432*	*0.262*	*0.464*	*0.913*	*0.063*	*0.582*	*1.002*	*0.409*	*0.949*	*0.902*	*0.812*
	P	*0.487*	*0.638*	*0.453*	*0.782*	*0.235*	*0.610*	*0.498*	*0.343*	*0.810*	*0.449*	*0.319*	*0.525*	*0.332*	*0.345*	*0.372*
MS-ON-G	Mean	15.748	30.156	53.467	13.741	29.104	21.081	12.800	28.041	12.344	13.089	28.585	8.926	12.963	28.596	7.511
N[d]=27	SD[c]	2.934	2.739	11.053	1.903	1.761	5.121	1.522	1.831	2.755	1.042	1.484	1.906	0.692	1.196	1.622
A[e] vs C	*f(1,67)*	*2.371*	*0.029*	*1.000*	*0.071*	*0.672*	*0.609*	*0.068*	*0.032*	*0.262*	*0.332*	*1.423*	*0.183*	*3.591*	*0.879*	*0.799*
	P	*0.128*	*0.563*	*0.321*	*0.787*	*0.415*	*0.463*	*0.796*	*0.859*	*0.611*	*0.570*	*0.238*	*0.676*	*0.063*	*0.352*	*0.376*
A[e] vs MS-noON	*f(1,67)*	*1.072*	*0.042*	*0.079*	*0.000*	*2.841*	*0.164*	*0.532*	*0.913*	*0.063*	*0.013*	*0.153*	*0.042*	*0.932*	*0.029*	*0.009*
	P	*0.304*	*0.834*	*0.776*	*1.000*	*0.097*	*0.693*	*0.470*	*0.343*	*0.810*	*0.907*	*0.703*	*0.836*	*0.337*	*0.861*	*0.954*
MS-ON-P	Mean	15.558	29.900	42.183	14.092	29.250	20.058	13.358	28.217	12.492	13.642	28.400	8.525	13.433	27.767	7.225
N[d]=20	SD[c]	2.442	2.733	11.678	1.964	1.653	6.932	1.696	1.338	3.459	2.086	1.717	3.174	1.521	6.326	2.272
A[e] vs C	*f(1,60)*	*1.49*	*0.02*	*2135*	*0.22*	*1.33*	*1.69*	*1.41*	*0.08*	*0.30*	*1.74*	*0.34*	*0.09*	*0.01*	*1.27*	*0.03*
	P	*0.227*	*0.876*	*0.000[f]*	*0.642*	*0.254*	*0.199*	*0.240*	*0.777*	*0.586*	*0.193*	*0.564*	*0.771*	*0.944*	*0.264*	*0.857*
A[e] vs MS-noON	*f(1,60)*	*0.54*	*0.05*	*14.43*	*0.37*	*3.59*	*0.45*	*0.07*	*0.85*	*1.22*	*1.77*	*0.02*	*0.48*	*0.45*	*0.76*	*0.24*
	P	*0.467*	*0.826*	*0.001[f]*	*0.547*	*0.063*	*0.506*	*0.791*	*0.361*	*0.273*	*0.188*	*0.889*	*0.492*	*0.505*	*0.386*	*0.624*
A[e] vs MS-ON-G	*f(1,46)*	*0.06*	*0.10*	*11.41*	*0.38*	*0.08*	*0.34*	*1.40*	*0.13*	*0.03*	*1.43*	*0.16*	*0.29*	*2.02*	*0.45*	*0.25*
	P	*0.815*	*0.753*	*0.002[f]*	*0.540*	*0.774*	*0.563*	*0.243*	*0.718*	*0.871*	*0.239*	*0.695*	*0.592*	*0.162*	*0.508*	*0.617*

[a]IT = Implicit Time (measured in msec); [b]RAD= N1-P1 Response Amplitude Density (measured in ηV/degree^2); [c]SD= one Standard Deviation of the mean; [d]N= Number of eyes of each Group; [e]**A**= one-way analysis of variance. [f]P values <0.01 were considered as statistically significant (in bold) for Group comparisons.

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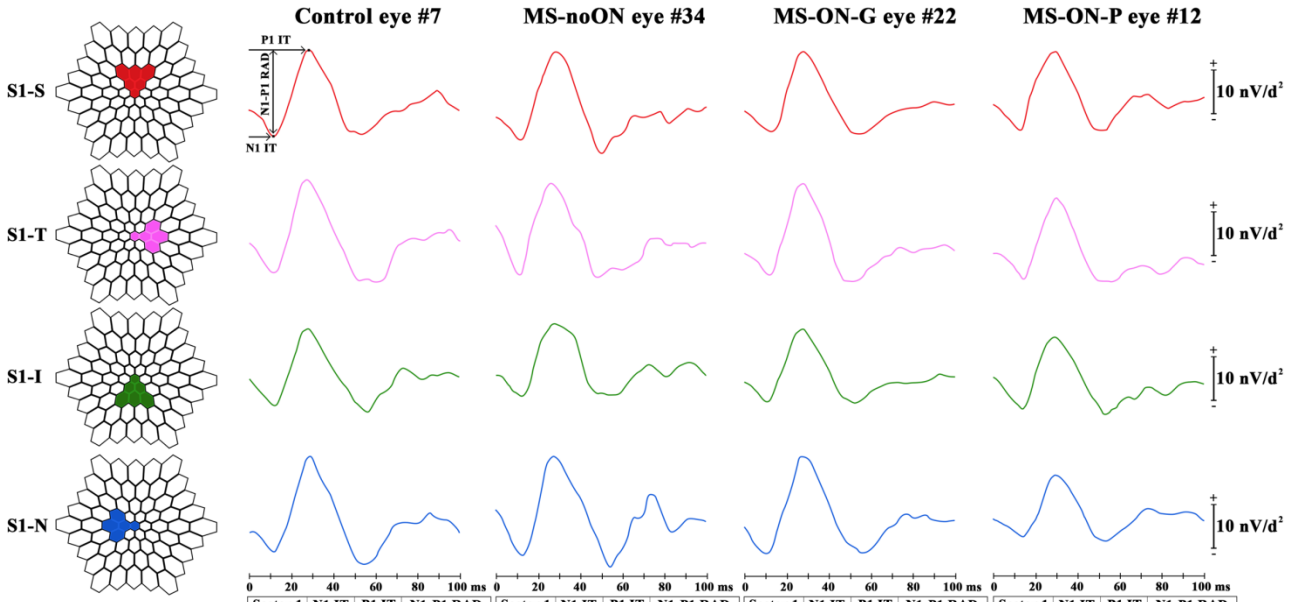
On average, when we considered the mean values of N1 and P1 ITs obtained in the central retinal areas (R1, R2, R3, 0 to15 degrees) and in the more peripheral retinal areas (R4 and R5, 15 to 25 degrees), not statistically significant (p>0.01) differences between all Groups were found.

The mean values of N1-P1 RADs obtained in the most central retinal areas (R1, 0 5 degrees) in MS-noON Group, were not statistically (p>0.01) different with respect to those of controls. In MS-ON-G Group, the mean values of N1-P1 RADs were not significant (p>0.01) different when compared to those of Control and MS-noON Groups; by contrast, in MS-ON-P Group, the mean values of N1-P1 RADs were significantly (p<0.01) reduced whit respect to Control, MS-noON and MS-ON-G Groups ones; the reduction of the individual N1-P1 RADs were not significantly correlated (p>0.01) the corresponding values of BCVA.

In MS-noON, MS-ON-G and MS-ON-P Groups, the mean values of N1-P1 RADs obtained in the other areas (R2, R3, R4 and R5) were not statistically (p>0.01) different with respect to those of Controls; not statistically significant (p>0.01) differences were found between MS Groups.

2.3 Multifocal electroretinogram sector analysis 1 (0-15 degrees)

Examples of averaged mfERG recordings from 4 sectors superior (S1-S), nasal (S1-N), inferior (S1-I) and temporal (S1-T) within 15 degrees of foveal eccentricity, obtained in representative Control (#7), MS-noON (#34), MS-ON-G (#22) and MS-ON-P (#12) eyes, are presented in Figure 2.



Sector 1	N1 IT (ms)	P1 IT (ms)	N1-P1 RAD (nV/d²)
S1-S	12.8	28.6	20.2
S1-T	12.6	28.5	22.4
S1-I	12.8	28.7	17.9
S1-N	12.7	27.9	22.1

Sector 1	N1 IT (ms)	P1 IT (ms)	N1-P1 RAD (nV/d²)
S1-S	12.9	29.1	21.0
S1-T	13.1	28.9	23.2
S1-I	13.0	28.9	16.8
S1-N	12.4	28.4	21.8

Sector 1	N1 IT (ms)	P1 IT (ms)	N1-P1 RAD (nV/d²)
S1-S	13.2	28.9	19.1
S1-T	12.9	28.3	22.6
S1-I	12.7	28.9	16.8
S1-N	13.0	28.4	21.9

Sector 1	N1 IT (ms)	P1 IT (ms)	N1-P1 RAD (nV/d²)
S1-S	13.3	29.2	18.2
S1-T	14.2	29.1	15.0
S1-I	14.0	29.2	16.6
S1-N	14.5	29.6	18.8

Figure 2. Multifocal electroretinogram averaged recordings obtained in a Control eye (#7), in a patient with multiple sclerosis (MS) without history of optic neuritis (MS-noON#34), and with history of optic neuritis followed by good or poor visual acuity (MS-ON-G#22 and MS-ON-P#12, respectively) by using two different sector analyses 1 and 2. For a better comparison the left eye of representative Control, MS-noON and MS-ON eyes is presented.
Sector analysis 1 reports the averaged values of N1 and P1 implicit times (IT, measured in milliseconds -ms-) and of N1-P1 response amplitude density (RAD, measured in nanoVolt/degree^2 -nV/d^2-) obtained from four macular areas from 0 to 15 degrees on the basis of retinal topography: superior (S1-S), nasal (S1-N), inferior (S1-I), temporal (S1-T) with respect to the fovea. bioelectrical responses obtained from the central 0–5 degrees were included in the sector analysis of sector 1.

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On Table 3 are reported the mean values of N1 and P1 ITs and of N1-P1 RADs detected in the 4 central sectors (S1-N, S1-I, S1-T, S1-S) in Control, MS-noON, MS-ON-G and MS-ON-P Groups and the relative statistical analysis between Groups.

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Table 3. Multifocal electroretinogram sector analysis within the 0-15 central degrees in Control (C) eyes and in Multiple Sclerosis patients without Optic Neuritis (MS-noON), with optic neuritis followed by good recovery of best corrected visual acuity (BCVA) (MS-ON-G), or poor recovery of BCVA (MS-ON-P).

		0-15 Central Degrees Superior Sector			0-15 Central Degrees Temporal Sector			0-15 Central Degrees Inferior Sector			0-15 Central Degrees Nasal Sector		
		N1 IT[a]	**P1 IT[a]**	**RAD[b]**	**N1 IT[a]**	**P1 IT[a]**	**RAD[b]**	**N1 IT[a]**	**P1 IT[a]**	**RAD[b]**	**N1 IT[a]**	**P1 IT[a]**	**RAD[b]**
Controls	Mean	13.266	28.800	17.910	13.251	28.917	17.944	13.373	28.327	17.573	13.195	27.698	19.039
N[d]=41	SD[c]	1.712	1.588	4.254	1.659	1.776	4.746	1.747	1.432	4.424	1.742	1.300	4.406
MS-noON	Mean	13.758	28.319	17.442	13.972	28.508	18.119	13.881	27.997	16.489	13.831	27.506	19.369
N[d]=41	SD[c]	2.058	1.438	3.982	1.996	1.677	4.094	2.167	1.525	4.131	1.679	1.587	4.508
A[e] vs C	*f(1,81)*	*1.379*	*2.069*	*0.262*	*3.158*	*1.148*	*0.029*	*1.368*	*1.018*	*1.308*	*2.028*	*0.362*	*0.108*
	P	*0.243*	*0.154*	*0.608*	*0.079*	*0.287*	*0.859*	*0.246*	*0.316*	*0.255*	*0.096*	*0.551*	*0.738*
MS-ON-G	Mean	13.256	28.459	17.241	13.774	28.356	18.278	13.419	28.648	17.007	13.570	28.037	17.933
N[d]=27	SD[c]	1.430	1.554	3.932	2.042	2.237	4.843	2.027	1.289	4.129	1.610	1.806	4.246
A[e] vs C	*f(1,67)*	*0.003*	*0.758*	*0.432*	*1.352*	*1.320*	*0.079*	*0.009*	*0.878*	*0.282*	*0.801*	*0.809*	*1.002*
	P	*0.988*	*0.385*	*0.516*	*0.250*	*0.255*	*0.779*	*0.921*	*0.351*	*0.598*	*0.374*	*0.371*	*0.321*
A[e] vs MS-noON	*f(1,67)*	*1.222*	*0.138*	*0.039*	*0.162*	*0.102*	*0.019*	*0.779*	*3.340*	*0.258*	*0.408*	*1.632*	*1.642*
	P	*0.274*	*0.705*	*0.838*	*0.693*	*0.750*	*0.885*	*0.381*	*0.072*	*0.615*	*0.526*	*0.206*	*0.205*
MS-ON-P	Mean	13.317	28.833	17.342	14.258	29.283	18.200	13.808	28.942	16.567	13.843	28.308	15.008
N[d]=20	SD[c]	2.584	1.646	4.793	2.427	2.551	4.459	2.178	2.199	4.752	1.736	1.769	3.025
A[e] vs C	*f(1,60)*	*0.01*	*0.01*	*0.22*	*3.62*	*0.43*	*0.04*	*0.71*	*1.72*	*0.66*	*1.86*	*2.32*	*13.56*
	P	*0.927*	*0.490*	*0.640*	*0.062*	*0.517*	*0.841*	*0.404*	*0.194*	*0.419*	*0.177*	*0.133*	*0.000[f]*
A[e] vs MS-noON	*f(1,60)*	*0.52*	*1.56*	*0.01*	*0.24*	*2.66*	*0.01*	*0.02*	*3.83*	*0.01*	*0.01*	*3.18*	*15.29*
	P	*0.473*	*0.216*	*0.932*	*0.627*	*0.108*	*0.944*	*0.902*	*0.055*	*0.948*	*0.979*	*0.079*	*0.000[f]*
A[e] vs MS-ON-G	*f(1,46)*	*0.01*	*0.63*	*0.01*	*0.55*	*1.5*	*0.01*	*0.30*	*0.38*	*0.11*	*0.31*	*0.26*	*7.17*
	P	*0.918*	*0.430*	*0.937*	*0.462*	*0.192*	*0.955*	*0.586*	*0.543*	*0.736*	*0.581*	*0.610*	*0.010[f]*

[a]IT = Implicit Time (measured in msec); [b]RAD= N1-P1 Response Amplitude Density (measured in ηV/degree^2); [c]SD= one Standard Deviation of the mean; [d]N= Number of eyes of each Group; [e]A= one-way analysis of variance. [f]P values <0.01 were considered as statistically significant (in bold) for Group comparisons.

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On average, when we considered the mean values of N1 and P1 ITs obtained in the central sectors (S1-S, S1-N, S1-I, S1-T) not statistically significant (p>0.01) differences between all Groups were found.

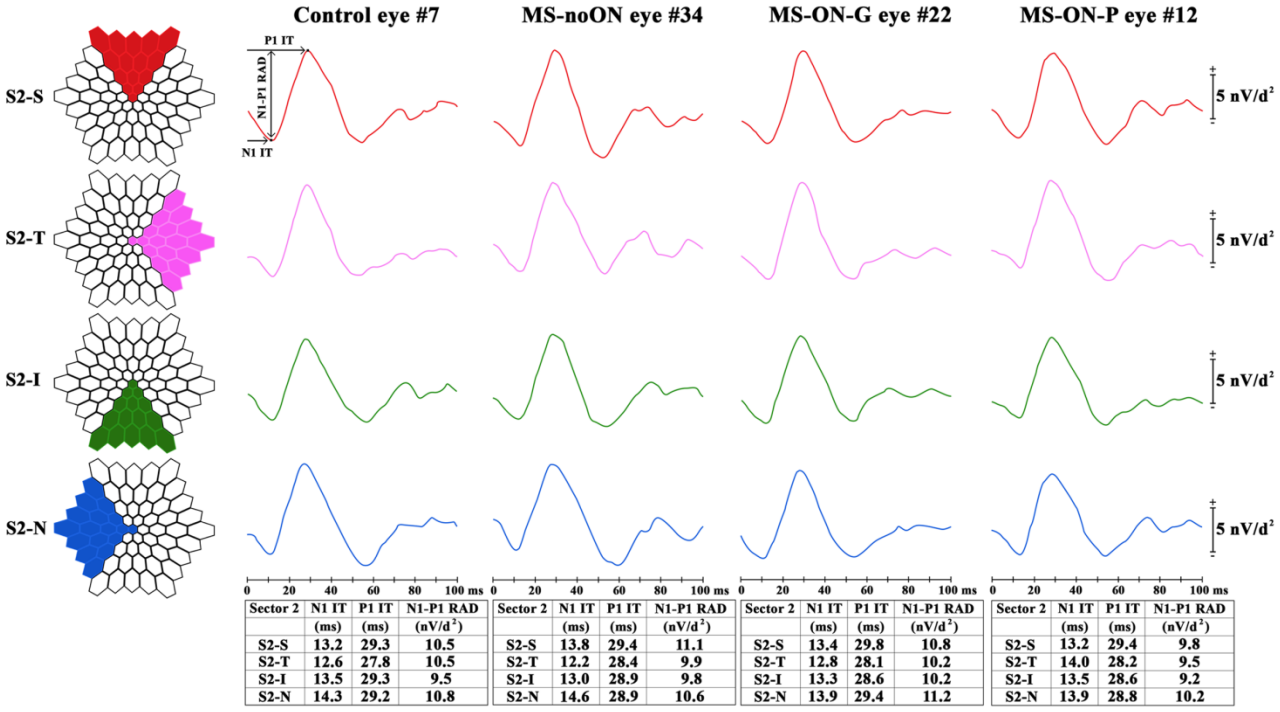
The mean values of N1-P1 RADs obtained in these sectors in MS-noON Group, were not statistically (p>0.01) different with respect to those of Controls.

In MS-ON-G Group, the mean values of N1-P1 RADs from all 4 sectors were not significant (p>0.01) different when compared to those of Control and MS-noON Groups. By contrast, in MS-ON-P Group, while mean values of N1-P1 RADs detected on S1-I, S1-T and S1-S were not significantly (p>0.01) reduced with respect to Control, MS-noON and MS-ON-G ones, it was observed a significant (p<0.01) reduction of N1-P1 RADs in the S1-N sector as compared to Controls, MS-noON and MS-ON-G.

The individual reduced N1-P1 RAD values from S1-N sector in MS-ON-P eyes were not significantly correlated (p>0.01) the corresponding values of BCVA.

2.4 Multifocal electroretinogram sector analysis 2 (0-25 degrees)

Examples of averaged mfERG recordings from 4 sectors (S2-S, S2-N, S2-I, S2-T) within 25 degrees of foveal eccentricity, obtained in representative Control (#7), MS-noON (#34), MS-ON-G (#22) and MS-ON-P (#12) eyes are presented in Figure 3.



Sector 2	N1 IT (ms)	P1 IT (ms)	N1-P1 RAD (nV/d²)
S2-S	13.2	29.3	10.5
S2-T	12.6	27.8	10.5
S2-I	13.5	29.3	9.5
S2-N	14.3	29.2	10.8

Sector 2	N1 IT (ms)	P1 IT (ms)	N1-P1 RAD (nV/d²)
S2-S	13.8	29.4	11.1
S2-T	12.2	28.4	9.9
S2-I	13.0	28.9	9.8
S2-N	14.6	28.9	10.6

Sector 2	N1 IT (ms)	P1 IT (ms)	N1-P1 RAD (nV/d²)
S2-S	13.4	29.8	10.8
S2-T	12.8	28.1	10.2
S2-I	13.3	28.6	10.2
S2-N	13.9	29.4	11.2

Sector 2	N1 IT (ms)	P1 IT (ms)	N1-P1 RAD (nV/d²)
S2-S	13.2	29.4	9.8
S2-T	14.0	28.2	9.5
S2-I	13.5	28.6	9.2
S2-N	13.9	28.8	10.2

Figure 3. Multifocal electroretinogram averaged recordings obtained in a Control eye (#7), in a patient with multiple sclerosis (MS) without history of optic neuritis (MS-noON#34), and with history of optic neuritis followed by good or poor visual acuity (MS-ON-G#22 and MS-ON-P#12, respectively) by using two different sector analyses 1 and 2. For a better comparison the left eye of representative Control, MS-noON and MS-ON eyes is presented.
Sector analysis 2 reports the averaged values of N1 and P1 IT and of N1-P1 RAD obtained from four retinal areas from 0 to 25 degrees based on the retinal topography: superior (S2-S), nasal (S2-N), inferior (S2-I), temporal (S2-T) with respect to the fovea.. The bioelectrical responses obtained from the central 0–5 degrees were included in the sector analysis 2.

The mean values of N1 and P1 ITs and of N1-P1 RADs detected in the 4 sectors (S2-S, S2-N, S2-I, S2-T) in Control, MS-ON and MS-noON Groups and relative statistical analysis between Groups are reported on Table 4.

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Table 4. Multifocal electroretinogram sector analysis within the 0-25 central degrees in Control (C) eyes and in Multiple Sclerosis patients without Optic Neuritis (MS-noON), with optic neuritis followed by good recovery of best corrected visual acuity (BCVA) (MS-ON-G), or poor recovery of BCVA (MS-ON-P).

		0-25 Degrees Superior Sector			**0-25 Degrees Temporal Sector**			**0-25 Degrees Inferior Sector**			**0-25 Degrees Nasal Sector**		
		N1 IT[a]	**P1 IT[a]**	**RAD[b]**	**N1 IT[a]**	**P1 IT[a]**	**RAD[b]**	**N1 IT[a]**	**P1 IT[a]**	**RAD[b]**	**N1 IT[a]**	**P1 IT[a]**	**RAD[b]**
Controls (N[d]=41)	mean	13.090	28.783	9.759	13.402	28.027	9.176	13.283	28.680	8.132	13.268	27.985	9.388
	SD	1.504	1.357	2.463	1.240	1.405	2.793	1.378	1.613	2.217	1.479	1.227	2.197
MS-noON (N[d]=41)	Mean	13.133	28.258	10.192	13.336	28.467	9.181	13.394	28.281	7.994	12.964	27.661	9.994
	SD	1.154	1.240	2.789	1.365	1.132	2.606	1.793	1.640	2.558	0.858	1.217	3.022
A[e] vs C	*f(1,81)*	*0.021*	*3.339*	*0.561*	*0.049*	*2.439*	*0.001*	*0.100*	*1.229*	*0.069*	*1.129*	*1.442*	*1.082*
	P	*0.896*	*0.071*	*0.450*	*0.819*	*0.122*	*0.993*	*0.754*	*0.270*	*0.795*	*0.258*	*0.234*	*0.302*
MS-ON-G (N[d]=27)	Mean	12.904	28.763	10.022	13.333	28.307	9.437	13.296	28.900	8.322	12.793	27.856	9.552
	SD	1.121	1.055	1.857	1.775	1.407	2.224	1.308	1.450	2.246	0.998	1.260	1.963
A[e] vs C	*f(1,67)*	*0.301*	*0.009*	*0.219*	*0.039*	*0.649*	*0.168*	*0.002*	*0.329*	*0.118*	*2.761*	*0.182*	*0.104*
	P	*0.585*	*0.949*	*0.638*	*0.851*	*0.424*	*0.685*	*0.969*	*0.569*	*0.732*	*0.101*	*0.676*	*0.755*
A[e] vs MS-noON	*f(1,67)*	*0.659*	*3.028*	*0.079*	*0.022*	*0.272*	*0.178*	*0.059*	*2.538*	*0.092*	*0.567*	*0.409*	*0.448*
	P	*0.421*	*0.086*	*0.782*	*0.994*	*0.607*	*0.676*	*0.808*	*0.116*	*0.589*	*0.454*	*0.526*	*0.504*
MS-ON-P (N[d]=20)	Mean	13.483	29.017	9.758	13.925	28.650	10.275	13.667	28.492	9.033	13.017	28.033	9.317
	SD	1.113	1.501	2.765	2.132	1.493	3.229	1.390	1.659	2.963	1.700	3.124	2.478
A[e] vs C	*f(1,60)*	*1.07*	*0.37*	*0.00*	*1.50*	*2.54*	*1.88*	*1.04*	*0.18*	*1.77*	*0.35*	*0.01*	*0.01*
	P	*0.304*	*0.544*	*0.999*	*0.225*	*0.117*	*0.176*	*0.312*	*0.674*	*0.188*	*0.556*	*0.932*	*0.910*
A[e] vs MS-noON	*f(1,60)*	*1.26*	*4.38*	*0.33*	*1.71*	*0.28*	*2.02*	*0.36*	*0.22*	*2.00*	*0.03*	*0.45*	*0.35*
	P	*0.265*	*0.04*	*0.569*	*0.196*	*0.596*	*0.160*	*0.552*	*0.640*	*0.163*	*0.871*	*0.506*	*0.389*
A[e] vs MS-ON-G	*f(1,46)*	*3.08*	*0.46*	*0.15*	*1.08*	*0.65*	*1.11*	*0.88*	*0.80*	*0.88*	*0.32*	*0.07*	*0.13*
	P	*0.086*	*0.499*	*0.697*	*0.305*	*0.425*	*0.297*	*0.354*	*0.374*	*0.354*	*0.574*	*0.790*	*0.718*

[a]IT = Implicit Time (measured in msec); [b]RAD= N1-P1 Response Amplitude Density (measured in ηV/degree^2); [c]SD= one Standard Deviation of the mean; [d]N= Number of eyes of each Group; [e]A= one-way analysis of variance.

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On average, the mean values of N1 and P1 ITs and of N1-P1 RADs detected in all sectors (S2-S, S2-N, S2-I, S2-T) in MS-noON, MS-ON-G and MS-ON-P Groups were not statistically ($p>0.01$) modified when compared with those of Controls. In MS-ON-G and MS-ON-P Groups the mean values of N1-P1 RADs from all 4 sectors were not significant ($p>0.01$) different when compared to those of Control and MS-noON Groups. Also, not statistically significant differences ($p>0.01$) were found when mean N1-P1 RADs were compared between MS-ON-G and MS-ON-P Groups in all sectors.

3. Discussion

The purpose of this study was to assess the function of preganglionic elements in MS patients, without and with history of ON, adding information on the debated topic of potential O-MR layers dysfunction, expression of the extension or sparing from neurodegenerative process beyond IML in MS.

We studied by mfERG the function of O-MR elements located in different areas of the central macula (0 to 15 degrees) or more peripheral retina within the arcades (0 to 25 degrees), topographically distinguished in rings or sectors. Our results apply to MS Groups with or without ON highly homogeneous for age, MS-DD, EDSS, and when present for number of ON and for the time elapsed from ON, differently from all previous reported studies in the literature [18,20,21,22].

Our findings showed not statistically significant differences of mfERG N1 and P1 IT values in all Groups (MS-noON, MS-ON-G and MS-ON-P) in any considered central circular areas (R1, R2, R3) or sectors (S1-S, S1-N, S1-I, S1-T) and more peripheral circular areas (R4, R5) or sectors (S2-S, S2-N, S2-I, S2-T) either when responses were compared to Controls or within MS Groups. As for N1-P1 RAD values, we found statistically significant ($p<0.01$) differences in MS-ON-P Group compared to Controls, MS-noON and MS-ON-G only when analyzing responses from Ring 1 (0-5 degrees) and from the S1-N sector, which covers the 0-15 central degrees area. In all other examined central or peripheral rings or sectors, we did not find any significant difference in the values of N1-P1 RAD between Groups. Our results indicate that photoreceptors and bipolar cells of the central fovea, as well as of the more central nasal macular sector within 15 degrees, are functionally impaired in MS only in occurrence of ON and when full recovery of BCVA is not achieved. These results do not apply either to MS-noON nor MS-ON-G Groups, thus confirming that the preganglionic element dysfunction is independent from the event of ON in itself.

As mentioned above, contrasting data are present in literature about the potential functional involvement of O-MR layers in MS degenerative process, depending on MS classification, presence or absence of ON and different mfERG signal analyses. As stated by Hanson et al. [23] “similarities or differences between findings in the central and peripheral retina are yet to be definitively elucidated in MS”, and therefore we thought reasonable to study O-MR function in our patients by applying not only the standard ring analysis, but also the more innovative sector analyses previously used in other neurodegenerative diseases [24, 25].

Our findings in MS-noON Group, showing the functional integrity of O-MR elements are in agreement with the results of a previous mfERG study [22] which, by using the ring analysis, found normal function of preganglionic elements in eyes without ON and normal high-contrast visual acuity. By contrast, our results differ from those by Saidha et al. [20], who found in five MS-noON patients with an abnormal OCT macular thickness and normal visual acuity, normal mfERG latencies with reduced P1 amplitude. As for the comparison of sector analysis results in MS-noON, Boquete et al. [26] studied, by using a more refined mfERG analysis method, a small cohort of newly diagnosed MS patients with less than 6 months from their first symptoms and no ON. They found an impairment of O-MR function exclusively in the supero-temporal quadrant of the macula. In our study, we analysed the mfERG responses sectioning in four sectors (superior, nasal, inferior and temporal) the central macular region up to 15 degrees (0-15 degrees, sector analysis 1) and the whole macular area up to 25 degrees (0-25 degrees, sector analysis 2). By adopting this different way to analyse mfERG sector responses [24, 25], we did not find statistically significant differences between Controls and MS-noON. Because the exact protocol used by Boquete e al. [26] could not be replicated in our study since, as stated by the Authors [26, 27], this method is currently only for research purposes and it is not a commercially available equipment, we could not confirm their data in MS-noON eyes. As for the “primary retinal pathology” process in MS-noON eyes [20], recalled also by Fairless et al. [28], the presence of

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neuro-retinitis phenomena could interfere with the results. This point therefore needs to be confirmed by a large study cohort.

In MS-ON-G Group, when measuring mfERG RADs, we found also absence of O-MR dysfunction either by rings or sectors analyses. Our findings diverge from Hanson et al. [18] who evidenced slight abnormal mfERG responses suggesting inhibitory bipolar cell dysfunction in a mixed cohort of clinically isolated syndrome, primary progressive MS and RR MS eyes, with some cases of ON, and recovery of BCVA. In a very recent study, Filgueiras et al. [21] suggested OR dysfunction based on the exclusive findings of significant shorter mfERG N1 and P1 implicit times in MS with and without ON, and concluding that mfERG may help in differentiating MS-ON from "neuro-myelitis optica" spectrum disorder. In agreement with the commentary by Hanson et al.[23], we also considered as questionable the finding by Filgueiras et al. [21], since "anticipated" N1 and P1 latencies, that were on average 1 msec shorter than Controls, cannot be considered as electrophysiological evidence of supernormal bipolar function in MS patients. In addition, in their work, the decision of not including in the mfERG analysis the R5 areas could have affected latency results. Finally, the Authors [21] did not to correct their p-values for multiple testing, considering the high number of statistical comparisons, thus overestimating the significance of their results.

Our results led us to consider that, when BCVA recovery is poor following ON, the wiring of retinal circuitry in the fovea and the papillo-macular bundle, where the cones and the RGCs have the highest density [27,29], can be severely impaired. Our findings of abnormal mfERG responses specifically in this sector links with Boquete et al. [26] findings (reduced first order kernel RADs in the temporal sectors for their right eyes). The Authors specified that the papillo-macular bundle could be affected earlier in the disease process also in absence of ON, and that this concurs with early Sd-OCT RNFL reduction in the thickest temporal sector in MS [30], as also seen in other neurodegenerative disorders like glaucoma [31], Parkinson's [32] and Alzheimer's [33] diseases.

The biological mechanisms underlying the reduction of RADs in our selected group of MS-ON-P, with no previous or present signs of retinal inflammation, can only be hypothesized.

One hypothesis is that trans-synaptic retrograde degeneration distal to IML could occur in a sub-set of MS-ON patients, in which visual dysfunction can be accounted for outer retinal damage involving photoreceptors and inhibitory bipolar cells. However, animal studies on the retinal changes after optic nerve transection showed that only the innermost retinal layers are impaired at the light and electron microscopy [34]. A full body of evidence supports the fact that trans-synaptic degeneration affects the dorsal lateral geniculate nucleus, but stops at the inner nuclear layer (INL), where the bipolars reside acting as a potential physiological protective barrier against neurodegeneration [35]. The prominent role of INL is also justified by the occurrence at this level of dynamic and transient phenomena also in absence of ON, as the microcystic inner retina edema often seen in MS [36,37]. This fact supports that the homoeostasis of the bipolar system is crucial for neurodegeneration processes in MS. On the other hand, autoimmunity could account for our retinal findings, given that some MS patients with autoantibodies against the retinal protein α-enolase have reduced ERG function [38]. In validated MS mouse models of ON it has been reported early altered synaptic vesicle cycling in ribbon synapses, located between outer and inner retinal layers, which are likely targeted by an auto-reactive immune system process [39]. Two adhesion proteins (CASPR1/CNTN1) [40], present at the level of both the paranodal region of myelinated nerves as well as at retinal ribbon synapses [39], could be the specific targets of the auto-immune response in experimental animal models.

Of course, all previous electrophysiological studies done by recording Ff-ERG or flicker ERG in MS eyes, and almost unanimously finding subnormal cone-driven bipolar cell function [16, 22, 41], are not comparable to our mfERG findings. This is based on the knowledge that mfERG responses are derived from cells localized into the central retina (in our study within the 25 central retinal degrees) [41], whereas Ff-ERG or flicker ERG are generated by the preganglionic elements of the whole retina [9].

4. Materials and Methods

4.1 Study Design and Participants

All research procedures described in this work adhered to the tenets of Declaration of Helsinki. The study protocol was approved by the local Ethical Committee (Comitato Etico Centrale IRCCS Lazio, Sezione

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IFO/Fondazione Bietti, Rome, Italy) and upon recruitment, informed consent after full explanation of the procedure was obtained from each subject enrolled in the study.

Eighty-eight relapsing remitting (RR) MS patients were enrolled at the Visual Neurophysiology and Neurophthalmology Research Unit, IRCCS- Fondazione Bietti referred by the Neurology Department of Tor Vergata Policlinic of Rome, between September 2016 and September 2019.

In order to obtain homogeneous MS Groups (without ON and with ON followed by good or poor recovery of BCVA, see below) the MS patients were selected form a large cohort (n=342) based on the following demographic and clinical characteristics:

1. Age between 28 and 45 years;
2. Diagnosis of RR MS according to validated 2010 McDonald criteria [42];
3. MS disease duration (MS-DD), estimated as the number of years from onset to the most recent assessment of disability, ranging from 5 and 15 years;
4. Expanded Disability Status Scale (EDSS), as ten-point disease severity derived from nine ratings for individual neurological domains [43], ranging from 0 to 3; this score was assessed by two trained [44] neurologists (LaB and MA);
5. Treatment with disease-modifying therapies (DMT) currently approved for preventing MS relapses. DMT considered in our study were Interferon-β-1a, Interferon-β-1b, Peginterferon beta-1a, Glatiramer acetate, Natalizumab, Dimethyl fumarate, Teriflunomide [45];
6. Absence of ON or a single episode of ON without recurrence, that elapsed from the onset of the disease at least 12 months (ranging from 13 to 20 months) before the inclusion in the study. For MS patients with ON, this criterion was chosen, since it is known that the retrograde degeneration following ON occurs over a period of 6 months [46]. When a MS patient was affected by ON in both eyes, we studied the eye affected longer that met the inclusion criteria;
7. Based on the ophthalmological examination, other inclusion criteria were: absence of glaucoma or other diseases involving cornea, lens, uvea, retina; absence of systemic diseases (i.e. diabetes); BCVA between 0.0 and 1.0 LogMAR of the Early Treatment of Diabetic Retinopathy (ETDRS) charts; absence of central visual field defects and ability to maintain a stable fixation that allowed performing multifocal ERG (see below).

A Group of selected 41 age-matched healthy subjects (mean age: 40.64 ± 4.83 years), providing 41 normal eyes, with BCVA of 0.0 LogMAR, served as Controls.

The selected MS patients were divided into two Groups for age, MS-DD, EDSS and for previous history of presence or absence of ON.

- 41 MS patients (mean age 41.32 ± 3.72 years, 27 females and 14 males, mean MS-DD 8.53 ± 4.19 years, range 5-20 years; mean EDSS score 1.43 ± 1.06, range 0-3) without history of unilateral or bilateral clinical signs of ON (i.e. painless reduction of BCVA, contrast sensitivity, color vision and any type of visual field defects) and high-contrast BCVA of 0.0 logMAR. When both eyes met the inclusion criteria, only one eye was randomly chosen for the study. Therefore, we considered 40 eyes from 40 MS patients without ON (MS-noON Group).
- 47 MS patients (mean age 40.23 ± 4.74 years, 29 females and 18 males,) with previous history of unilateral or bilateral ON (i.e. painless reduction of BCVA -between 0.2 and 1 LogMAR-, contrast sensitivity, color vision and visual field defects). They were further divided in to two Groups on the basis of the recovery of BCVA after ON:
- 27 MS patients (mean age 39.92 ± 4.86 years; 17 females and 10 males; mean MS-DD 9.06 ± 5.58 years, range 5-20 years; mean EDSS score 1.53 ± 1.22, range 0-3) with previous history of a single unilateral or bilateral ON and with "good" recovery of high-contrast BCVA (0.0 logMAR) after ON. Therefore, we considered 27 eyes from 27 MS patients with ON (MS-ON-G Group);
- 20 MS patients (mean age 40.38 ± 4.26 years; 12 females and 8 males; mean MS-DD 9.32 ± 5.64 years, range 5-20 years; mean EDSS score 1.49 ± 1.18, range 0-3) with previous history of a single unilateral or bilateral ON with "poor" recovery of high-contrast BCVA (between 0.2 and 1 logMAR) after ON. Therefore, we considered 20 eyes from 20 MS patients with ON (MS-ON-P Group).

Based on the previous mentioned inclusion criteria, the MS Groups with or without ON were homogeneous for age, MS-DD, EDSS, and the MS Groups with ON were homogeneous for number of ON and for the time elapsed from ON (see above, Table 1).

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In all MS patients and Controls, the functional condition of the preganglionic elements, located in the 25 retinal degrees, was evaluated by mfERG recordings.

4.2 Multifocal electroretinogram recordings

The mfERG was recorded by our previously published method [14, 25, 47] by using an Espion system (Diagnosys UK, LTD; Histon, Cambridge, UK) and following the 2011 International Society for Clinical Electrophysiology of Vision (ISCEV) standards [11]. Briefly, the multifocal stimulus, consisting of 61 scaled hexagons, was displayed on a high-resolution, black-and-white monitor (size 30 cm, width and 30 cm height) with a frame rate of 75 Hz. The array of hexagons subtended 25 degrees of VF. Each hexagon was independently alternated between black (1 cd/m^2) and white (200 cd/m^2) according to a binary m sequence. This resulted in a contrast of 99%. The luminance of the monitor screen and the central fixation cross (used as target) was 100 cd/m^2. The m-sequence had 2^{13-1} elements, and total recording time was approximately 8 min. Total recording time was divided into sixteen segments. Between segments, the subject was allowed to rest for a few seconds. Focusing lenses were used when necessary. To maintain a stable fixation, a small red cross target (0.5 degree) was placed in the centre of the stimulation field. At every mfERG reported that he/she could clearly perceive the fixation target. The eye's position was continuously monitored by an in-built video system to track fixation losses.

MfERGs were monocularly recorded in the presence of pupils that were maximally pharmacologically dilated with 1% tropicamide to a diameter of 7–8 mm. Pupil diameter was measured by an observer (LuB) by means of a millimeter ruler and a magnifying lens and stored for each tested eye. The cornea was anaesthetized with Benoxinate eye dropps 0.4%. MfERGs were recorded bipolarly between an active Dawson–Trick–Litzkow (DTL) bipolar contact electrode and a reference electrode (Ag/AgCl electrode placed on the correspondent temporal side of the frontal lobe). A small Ag/AgCl skin ground electrode was placed at the centre of the forehead. Interelectrode resistance was <3 KOhms. After automatic rejection of artefacts, the first-order kernel response was examined. MfERG responses with a signal to noise ≥3 were accepted for the analysis.

In the analysis of mfERG responses, we considered, for each obtained averaged response, the implicit times (ITs) of the first negative peak (N1) and the first positive peak (P1) measured in milliseconds (msec) and the N1-P1 peak-to-peak response amplitude density (RAD) measured in nanoVolt/degree^2 (ηV/degree^2).

We considered three possible retinal topographies to explore the bioelectrical responses derived from specific retinal areas. Data were analysed as follows:

1. Ring analysis: the averaged response obtained from five concentric annular retinal areas (rings) centred on the fovea: from 0 to 5 degrees (ring 1, R1), from 5 to 10 degrees (ring 2, R2), from 10 to 15 degrees (ring 3, R3), from 15 to 20 degrees (ring 4, R4) and from 20 to 25 degrees (ring 5, R5) (Figure 1).
2. Sector analysis 1: the averaged bioelectrical response obtained from the central macular region up to 15 degrees (0-15 degrees) sectioning it in four sectors: superior (S1-S), nasal (S1-N), inferior (S1-I) and temporal (S1-T) with respect to the fovea. In each sector, we included also the responses obtained from the more central macular area (0- 5 degrees) (Figure 2).
3. Sector analysis 2: the averaged bioelectrical response obtained from the retinal area from the fovea up to 25 degrees (0-25 degrees) sectioning it in four sectors: S2-S, S2-N, S2-I and S2-T with respect to the fovea. In each sector, we included also the responses obtained from the more central macular area (0- 5 degrees) (Figure 3).

4.3 Statistical Analysis

We assumed a Gaussian distribution of our data. The normal distribution was assessed by using the Kolmogorov-Smirnov test.

The differences of age, MS-DD, EDSS between MS-noON, MS-ON-G and MS-ON-P Groups were evaluated by the one-way analysis of variance (ANOVA). The differences of the number of ON and the time elapsed from the ON between MS-ON-G and MS-ON-P Groups were evaluated by the ANOVA.

Considering each different mfERG retinal topographies (Ring, Sectors 1 and Sectors 2 analyses), the differences of mfERG N1 and P1 ITs and N1-P1 RADs mean values between Controls, MS-noON, MS-ON-G

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and MS-ON-P Groups were evaluated by ANOVA. Pearson's test was used in MS-ON-P Group to linearly correlate the values of BCVA with those of mfERG parameters.

A p value lower than 0.01, compensating for multiple comparisons, was considered as statistically significant. Minitab 17 (version 1) software was used for statistical analysis.

5. Conclusions

In conclusion, in our study we detected an absence of mfERG abnormalities in MS patients without and with ON followed by full recovery of BCVA. By contrast, the MS neurodegenerative processes in the preganglionic elements of outer and middle retinal layers is clearly evident after an event of ON followed by permanent impairment of visual acuity (poor recovery of BCVA after ON). Our results suggest that in MS the function of preganglionic elements located in the O-MR layers is not modified by the occurrence of ON itself. In order to better understand the role of middle retinal elements in this process, further studies on both experimental [28,39] and clinical sides [20,35] are needed.

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Abbreviations

MS	multiple sclerosis
ON	optic neuritis
MS-noON	multiple sclerosis patients without optic neuritis
MS-ON-G	multiple sclerosis patients with optic neuritis followed by good recovery of best corrected visual acuity
MS-ON-P	multiple sclerosis patients with optic neuritis followed by poor recovery of best corrected visual acuity
BCVA	best corrected visual acuity
MfERG	multifocal electroretinogram
IT	implicit time
RAD	response amplitude density
SD	one standard deviation of the mean
N	number of eyes of each group
A	one-way analysis of variance

References

1. Miller, D.; Barkhof, F.; Montalban, X.; Thompson, A.; Filippi, M. Clinically isolated syndromes suggestive of multiple sclerosis, part 2: non-conventional MRI, recovery processes, and management. *Lancet Neurol.* **2005**, *4*, 341-348.
2. Dinkin, M. Trans-synaptic Retrograde Degeneration in the Human Visual System: Slow, Silent, and Real. *Curr Neurol Neurosci Rep*. **2017**,*17*, 16.
3. Britze, J.; Pihl-Jensen, G.; Frederiksen, J.L. Retinal ganglion cell analysis in multiple sclerosis and optic neuritis: a systematic review and meta-analysis. *J Neurol.* **2017**, *264*,1837-1853.

4. Janáky, M.; Jánossy, Á.; Horváth, G.; Benedek, G.; Braunitzer, G. VEP and PERG in patients with multiple sclerosis, with and without a history of optic neuritis. *Doc Ophthalmol.* **2017**,*134*, 185-193.
5. Parisi, V.; Manni, G.; Spadaro, M.; Colacino, G.; Restuccia, R.; Marchi, S.; Bucci, M.G.; Pierelli, F. Correlation between morphological and functional retinal impairment in multiple sclerosis patients. *Invest Ophthalmol Vis Sci.* **1999**, *40*, 2520-2527.
6. Trip, S.A.; Schlottmann, P.G.; Jones, S.J.; Altmann, D.R; Garway-Heath D.F.; Thompson, A.J.; Plant, G.T.; Miller, D.H. Retinal nerve fiber layer axonal loss and visual dysfunction in optic neuritis. *Ann Neurol.* **2005**, *58*, 383-391.
7. Monsalve, P. Decoding PERG: A neuro-ophthalmic retinal ganglion cell function review. *Curr Ophthalmol Rep*. **2019**, *7*, 51-58.
8. Robson, A.G.; Nilsson, J.; Li, S.; Jalali, S. ; Fulton, A.B. ; Tormene, A.P.; Holder, G.E. ; Brodie, S.E. ISCEV guide to visual electrodiagnostic procedures. *Doc Ophthalmol.* **2018**, *136*, 1-26.
9. McCulloch, D.L.; Marmor, M.F.; Brigell, M.G.; Hamilton, R.; Holder, G.E.; Tzekov, R.; Bach, M.. ISCEV Standard for full-field clinical electroretinography (2015 update). *Doc Ophthalmol*. **2015**, *130*, 1-12.
10. Parisi, V.; Falsini, B. Electrophysiological evaluation of the macular cone system: focal electroretinography and visual evoked potentials after photostress. *Semin Ophthalmol.* **1998**, *13*, 178-188.
11. Hood DC, Bach M, Brigell M, Keating, D.; Kondo, M.; Lyons, J.S.; Marmor, M.F.; McCulloch, D.F.; Palmowski-Wolfe, A.M. International Society For Clinical Electrophysiology of Vision. ISCEV standard for clinical multifocal electroretinography (mfERG) (2011 edition). *Doc Ophthalmol*. **2012**, *124*, 1-13.
12. Bearse, M.A. Jr.; Sutter, E.E. Imaging localized retinal dysfunction with the multifocal electroretinogram. *J Opt Soc Am A Opt Image Sci Vis*. **1996**, *13*, 634-640.
13. Hood, D.C. Assessing retinal function with the multifocal technique. *Prog Retin Eye Res.* **2000**, *19*, 607-646.
14. Parisi, V.; Ziccardi, L.; Stifano, G.; Montrone, L.; Gallinaro, G.; Falsini, B. Impact of regional retinal responses on cortical visually evoked responses: multifocal ERGs and VEPs in the retinitis pigmentosa model. *Clin Neurophysiol*. **2010**, *121*, 380-385.
15. Papakostopoulos, D.; Fotiou, F.; Hart, J.C.; Banerji, N.K. The electroretinogram in multiple sclerosis and demyelinating optic neuritis. *Electroencephalogr Clin Neurophysiol*. **1989**, *74*, 1-10.
16. Hamurcu, M.; Orhan, G.; Sarıcaoğlu, M.S.; Mungan, S.; Duru, Z. Analysis of multiple sclerosis patients with electrophysiological and structural tests. *Int Ophthalmol.* **2017**, *37*, 649-653.
17. Forooghian, F.; Sproule, M.; Westall, C.; Gordon, L.; Jirawuthiworavong, G.; Shimazaki, K.; O'Connor, P. Electroretinographic abnormalities in multiple sclerosis: possible role for retinal autoantibodies. *Doc Ophthalmol*. **2006**, *113*, 123-132.
18. Hanson, J.V.M.; Hediger, M.; Manogaran, P.; Landau, K.; Hagenbuch, N.; Schippling, S. ; Gerth-Kahlert, C. Outer Retinal Dysfunction in the Absence of Structural Abnormalities in Multiple Sclerosis. *Invest Ophthalmol Vis Sci.* **2018**, *59*, 549-560.
19. Falsini, B.; Bardocci, A.; Porciatti, V.; Bolzani, R.; Piccardi, M. Macular dysfunction in multiple sclerosis revealed by steady-state flicker and pattern ERGs. *Electroencephalogr Clin Neurophysiol*. **1992**, *82*, 53-59.
20. Saidha, S.; Syc, S.B.; Ibrahim, M.A.; Eckstein, C.; Warner, C.V.; Farrell, S.K.; Oakley, J.D.; Durbin, M.K.; Meyer, S.A.; Balcer, L.J.; *et al*. Primary retinal pathology in multiple sclerosis as detected by optical coherence tomography. *Brain*. **2011**, *134*, 518-533.
21. Filgueiras, T.G.; Oyamada, M.K.; Preti, R.C.; Apóstolos-Pereira, S.L.; Callegaro, D.; Monteiro, M.L.R. Outer Retinal Dysfunction on Multifocal Electroretinography May Help Differentiating Multiple Sclerosis From Neuromyelitis Optica Spectrum Disorder. *Front Neurol*. **2019**, *10*, 928.

22. Gundogan, F.C.; Demirkaya, S.; Sobaci , G. Is optical coherence tomography really a new biomarker candidate in multiple sclerosis?-A structural and functional evaluation. *Invest Ophthalmol Vis Sci.* **2007**, *48*, 5773-5781.
23. Hanson, J.V.M.; Schippling, S.; Gerth-Kahlert, C. Commentary: Outer retinal dysfunction on multifocal electroretinography may help differentiating multiple sclerosis from neuromyelitis optica spectrum disorder. *Front. Neurol.* **2020**, *11*, 282.
24. Ziccardi, L.; Parisi, V.; Picconi, F.; Di Renzo, A.; Lombardo, M.; Frontoni, S.; Parravano M. Early and localized retinal dysfunction in patients with type 1 diabetes mellitus studied by multifocal electroretinogram. *Acta Diabetol*. **2018**, *55*, 1191-1200.
25. Cascavilla, M.L.; Parisi,V.; Triolo, G.; Ziccardi, L.; Borrelli, E.; Di Renzo, A.; Balducci, N.; Lamperti, C.; Bianchi Marzoli, S.; Darvizeh, F.; *et al.* Retinal dysfunction characterizes subtypes of dominant optic atrophy. *Acta Ophthalmol*. **2018**, *96*, e156-e163.
26. Boquete, L.; López-Guillén, E.; Vilades, E.; Miguel-Jiménez, J.M.; Pablo, L.E.; De Santiago, L.; Ortiz Del Castillo, M. Alonso-Rodríguez, M.C. ; Sánchez Morla, E.M.; López-Dorado, A.; *et al*. Diagnostic ability of multifocal electroretinogram in early multiple sclerosis using a new signal analysis method. *PLoS One*. **2019**, *14*, e0224500.
27. Verdon, W.A.; Haegerstrom-Portnoy, G. Topography of the multifocal electroretinogram. *Doc Ophthalmol.* **1998**, *95*, 73-90.
28. Fairless, R.; Williams, S.K.; Hoffmann, D.B.; Stojic, A.; Hochmeister, S.; Schmitz, F.; Storch, M.K.; Diem, R. Preclinical retinal neurodegeneration in a model of multiple sclerosis. *J Neurosci.* **2012**,32, 5585-5597.
29. McGregor, J.E., Yin, L., Yang, Q.; Godat, T.; Huynh, K.T.; Zhang, G.; Williams, D.G.; Merigan, W.H. Functional architecture of the foveola revealed in the living primate. PLoS One. 2018, 13:e0207102.
30. Garcia-Martin, E.; Pueyo ,V.; Almarcegui, C.; Martin, J.; Ara, J.R.; Sancho, E.; Pablo, L.E., Dolz, I.; Fernandez, J . Risk factors for progressive axonal degeneration of the retinal nerve fibre layer in multiple sclerosis patients. *Br J Ophthalmol.* **2011**, *95*:1577-1582.
31. Mousa, M.F.; Cubbidge, R.P.; Al-Mansouri, F.; Bener, A. Evaluation of hemifield sector analysis protocol in multifocal visual evoked potential objective perimetry for the diagnosis and early detection of glaucomatous field defects. *Korean J Ophthalmol*. **2014**, *28*, 49-65.
32. Satue, M.; Obis, J.; Alarcia, R.; Orduna, E.; Rodrigo, M.J.; Vilades, E.; Gracia, H.; Otin, S.; Fuertes, M.I.; Polo, V.; *et al.* Retinal and Choroidal Changes in Patients with Parkinson's Disease Detected by Swept-Source Optical Coherence Tomography. *Curr Eye Res.* **2018**, *43*,109-115.
33. Polo, V.; Garcia-Martin, E.; Bambo, M.P.; Pinilla, J.; Larrosa, J.M.; Satue, M.; Otin, S.; Pablo, L.E. Reliability and validity of Cirrus and Spectralis optical coherence tomography for detecting retinal atrophy in Alzheimer's disease. *Eye (Lond)*. **2014**, *28*, 680-690.
34. Hollander, H.; Bisti, S.; Maffei, L.; Hebel, R. Electroretinographic responses and retrograde changes of retinal morphology after intracranial optic nerve section. *Exp Brian Res.* **1984**, *55*, 483-494.
35. Petzold, A.; Balcer, L.J.; Calabresi, P.A.; Costello, F.; Frohman, T.C.; Frohman, E.M.; Martinez-Lapiscina, E.H.; Green, A.J.; Kardon, R.; Outteryck, O.; *et al*. Retinal layer segmentation in multiple sclerosis: a systematic review and meta-analysis. *Lancet Neurol* **2017**, *16*, 797-812.
36. Saidha, S.; Sotirchos, E.S.; Ibrahim, M.A.; Crainiceanu, C.M.; Gelfand, J.M; Sepah, Y.J.; Ratchford, J.N.; Oh, J.; Seigo, M.A.; Newsome, S.D.; *et al*. Microcystic macular oedema, thickness of the inner nuclear layer of the retina, and disease characteristics in multiple sclerosis: a retrospective study. L*ancet Neurol.* **2012**, *11*, 963–972.
37. Gelfand, J.M.; Nolan, R.; Schwartz, D.M.; Graves, J.; Green, A.J. Microcystic macular oedema in multiple sclerosis is associated with disease severity. *Brain*. **2012**, *135*, 1786-1793.

38. Gorczyca, W.A.; Ejma, M.; Witkowska, D.; Misiuk-Hojło, M.; Kuropatwa, M.; Mulak, M.; Szymaniec, S. Retinal antigens are recognized by antibodies present in sera of patients with multiple sclerosis. *Ophthalmic Res.* **2004**, 36, 120-123.
39. Dembla, M.; Kesharwani, A.; Natarajan, S.; Fecher-Trost, C.; Fairless, R.; Williams, S.K.; Flockerzi, V.; Diem, R.; Schwarz, K.; Schmitz, F. Early auto-immune targeting of photoreceptor ribbon synapses in mouse models of multiple sclerosis. *EMBO Mol Med*. **2018**, *10*, e8926.
40. Stathopoulos, P.; Alexopoulos, H.; Dalakas, M.C. Autoimmune antigenic targets at the node of Ranvier in demyelinating disorders. *Nat Rev Neurol*. **2015**, *11*, 143-156.
41. Hood, D.C.; Seiple, W.; Holopigian, K.; Greenstein, V. A comparison of the components of the multifocal and full-field ERGs. *Vis Neurosci.* **1997**, *14*, 533-544.
42. Polman, C.H.; Reingold, S.C.; Banwell, B.; Clanet, M.; Cohen, J.A.; Filippi, M.; Fujihara, K.; Havrdova, E.; Hutchinson, M.; Kappos, L.; *et al*. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann Neurol*. **2011**, *69*, 292-302.
43. Kurtzke, J.F. Rating neurologic impairment in multiple sclerosis: an Expanded Disability Status Scale (EDSS). *Neurology*. **1983**, *33*, 1444–1452.
44. Neurostatus.net. Available online: http://www.neurostatus.net/index.php?file=start (accessed on July 6th, 2020)
45. Williams, U.E.; Oparah, S.K.; Philip-Ephraim, E.E. Disease Modifying Therapy in Multiple Sclerosis. *Int Sch Res Notices*. **2014**, 307064.
46. Huang-Link, Y.M.; Al-Hawasi, A.; Lindehammar, H. Acute optic neuritis: retinal ganglion cell loss precedes retinal nerve fiber thinning. *Neurol Sci.* **2015**, *36*, 617-620.
47. Parisi, V.; Ziccardi, L.; Centofanti, M.; Tanga, L., Gallinaro, G., Falsini, B., Bucci, M.G. Macular function in eyes with open-angle glaucoma evaluated by multifocal electroretinogram. *Invest Ophthalmol Vis Sci.* **2012**, *53*, 6973-6980.