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Influence of dopamine deficiency in early Parkinson's disease on the slow stimulation multifocal-ERG

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Abstract

Purpose: In animal studies intravitreal injection of tetrodotoxin (TTX) results in mfERG waveform changes similar to those observed in glaucoma. As TTX blocks amacrine as well as ganglion cells, there is still a question regarding the underlying cell population responsible for these changes in waveform. In an attempt to assess the contribution of the amacrine cells to these changes, a mfERG was obtained from patients with Parkinson's disease as some amacrine cells are mediated by dopamine, a substance lacking in Parkinson's. *Methods*: Eight patients with early Parkinson's disease underwent ophthalmologic examination, testing of contrast sensitivity and electrophysiological examination according to ISCEV standard at least 12 h following their last medication with Dopamine. A slow stimulation mfERG was obtained with a stimulus base interval of 53.3 ms and with a stimulus base interval of 106.6 ms. During MF-ERG recordings 103 hexagons stimulated the central 50 deg of the retina simultaneously and independently (m-sequence 2^{13}, L_{max}: 200 cd/m^2, ~100% contrast). *Results*: Contrast sensitivity and ISCEV standard electrophysiological testing was unremarkable. When the mfERG was analyzed, only four patients had an adequate signal-to-noise ratio to allow further data analysis – one of whom was diagnosed with a multi system atrophy in retrospect. The first order response component was analyzed at a filter setting of 10–300 Hz and at 100–300 Hz (OPs) and compared to mfERGs of a control group. On average, in patients, the amplitude of N1P1 was slightly lower in the central and nasal response averages. When the three OPs at a latency of 72–89 ms were analyzed in the 53.3 ms base interval recording, the most marked difference in amplitude was observed in the superior nasal response average of the first OP. Here a mean amplitude of 1.3 nV/deg^2 in patients compared to a mean amplitude of 1.9 nV/deg^2 in the control group (P: 0.08). *Discussion*: In contrast to our previous findings in NTG, there was a consistent presence of three OPs. Under the stimulus conditions applied, we did not find an influence of dopaminergic amacrine cells on the mfERG in our patients with moderate stages of Parkinsion's. The difficulties in obtaining an adequate signal-to noise ratio due to e.g. muscle artifacts even in Parkinson patients of moderate disease stages render a success of mfERG recording in patients with more advanced stages unlikely. The question of the influence of dopaminergic amacrine cells on the mfERG could possibly be addressed using MPDT in animal research.

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Introduction

The multifocal electroretinogram (mfERG) allows topographic mapping of retinal function. It has been applied to analyze the function of different retinal layers such as the outer [1–5] or inner [6] retina.

Most research applying the mfERG to test inner retinal function has been conducted in glaucoma, a disease that poses a major threat to vision in industrial countries. In glaucoma an inner retinal component, the so called optic nerve head component (ONHC), may be distinguished from a more outer retinal component (RC) [7]. The ONHC is a nonlinear mfERG response component whose propagation seems dependent on the nerve fiber layer and which is attributed mainly to the ganglion cell layer [7–12]. It appears to be diminished in glaucoma [7, 13].

Recently a special slow mfERG stimulus sequence has been suggested to be sensitive in OAG [14, 15]. With an increase in the stimulus base interval to 53.3 ms or more, there is no more overlap between the induced component and the m-sequence response. Under these conditions, oscillatory potentials become apparent in the induced component [16, 17]. These mfOPs, show a marked naso-temporal asymmetry [16, 17] which may be attributed to the misalignment and partial cancellation of the RC with the ONHC in the nasal retina and their relative alignment and enhancement in the temporal retina [16]. Under these conditions the sensitivity to detect normal tension OAG increases to about 85% [15].

The oscillatory potentials of the photopic ERG receive a strong contribution from the inner retinal layers [18]. Glycine, GABA and TTX suppress the function of the inner retina and result in reduced or missing oscillatory potentials of the photopic ERG [19]. In mfERG recordings these substances also affect nonlinear contributions to the mfERG [12, 20].

In animal studies, mfERG waveforms similar to those observed in glaucoma have been reported following intravitreal injection of TTX. In the slow mfERG TTX decreased the Ops and largely eliminated their nasotemporal waveform asymmetries [17]. TTX blocks some amacrine as well as ganglion cells and interplexiform cells. Therefore, the cellular origin of the mfERG changes observed in glaucoma or following TTX are still unclear and cannot be attributed exclusively to ganglion cells [21]. As some amacrine cells are dopaminergic, Parkinson's disease would be a potential model to gain further insight into the cellular origin of these mfERG waveform changes.

Parkinson's disease is not only a motor system disease. There is also a concurrent loss of retinal dopamine [22]. This has resulted in a spatial frequency dependent loss of sensitivity for the pattern ERG (PERG) [23, 24] as well as in abnormal pattern visual evoked potentials (PVEP), presumably secondary to retinal changes [25, 26]. An increase in contrast sensitivity threshold has also been reported in Parkinson's disease [23, 27]. It has been suggested that dopamine has a push–pull effect in the primate retina resulting in a tuned spatial response function [21]. The electrooculogram (EOG) has been reported to show the earliest signs of retinal dysfunction in the very early stages of Parkinson's disease [28] and colour vision deficiencies have been reported to progress during this disease [29].

Thus, in this pilot study an attempt was made to assess the contribution of dopaminergic amacrine cells on the slow stimulation mfERG in patients with Parkinson's disease following at least 12 h dopamine depletion.

Patients and methods

Patients with an early stage of Parkinson's disease (clinical presentation as Hoehn and Yahr stages I to II, that is with uni- or bilateral symptoms without alteration of balance) were recruited from the Department of Neurology. Following informed consent, eight patients volunteered to participate in the testing of visual function following a period of at least 12 h of dopamine deficiency. (Dopamine intake was discontinued for >12 h independent of this study in order to evaluate a patient's response to a one time dose of L-dopa.) Exclusion criteria were a history of ocular surgery, ocular diseases, especially glaucoma or diabetic retinopathy as well as high refractive errors $+/-$ 6 dpt. A morphologic

ophthalmic examination followed the testing of visual function described below, to rule out ophthalmic disorders that may affect the results. The patients' right eye was examined, except in one patient where the right eye had a posterior capsular fibrosis following cataract surgery. In this patient, the left eye was included in the study.

Patients underwent testing of EDTRS visual acuity, testing of contrast sensitivity using the Pelli Robson Chart as well as testing of colour vision with the help of the saturated and desaturated panel D15 test. Goldmann perimetry was obtained to rule out visual field defects. A PERG was obtained with a checkerboard check size of 60′ as well as 90′. Further electrophysiology (Ganzfeld ERG, EOG and PVEP) was performed according to ISCEV standard.

Following these examinations patients underwent mfERG recordings as follows:

In all subjects mfERGs were recorded of one eye, which in control subjects was chosen at random, using VERIS^{TM}. MfERG signals were recorded monocularly using a Burian–Allen bipolar contact lens electrode. Pupils were dilated. The viewing distance was adjusted according to the optimal refraction used at a viewing distance of 40 cm to ensure a constant image size [30].

During recording, the central 50 deg of the retina were stimulated by 103 hexagons where each hexagon flickered according to a slow m-sequence stimulation. Patients underwent two recordings that lasted 7 min 17 s each.

For the first mfERG recording, each m-sequence step (M) with a luminance of either 100 cd/m^2 or <1 cd/m^2 was followed by 7 black frames (B) with a luminance <1 cd/m^2. This eight frame stimulus sequence (MBBBBBBB) reoccurred every 106.6 ms. The length of the m-sequence was 2^{12}–1. The second mfERG recording differed in that only three black frames followed an m-sequence step (MBBB) resulting in a stimulus base interval of 53.3 ms. The length of the m-sequence was 2^{13}–1.

To enhance the signal-quality each recording was split into 16 cycles of about 27.29 s. The raw signals were filtered (10–300 Hz) and amplified (gain = 100 000). A sampling point was obtained every 0.83 ms. An artifact elimination technique [31] was applied once. The first order response component (KI) was analyzed. For each location KI is calculated as the difference between the mean local response to all the bright m-sequence stimuli and the mean focal response to the black m-sequence stimuli occurring in a stimulus cycle and taking into account the entire stimulus base interval.

Results

Mean age of the patients was 62.5 (SD 9.9) years. All patients tested were in an early stage of Parkinson's disease (Schwab scale >80), that is they could still cope with every day aspects of life, taking twice as long as healthy people to perform these tasks and being aware of this handicap.

On the EDTRS visual acuity chart patients could on average read 60 letters (SD 11). This corresponds to an acuity equivalent of 20/32.

Contrast sensitivity as tested with the Pelli Robson Chart was not impaired. On average patient could achieve Pelli Robson Scores of 1.56 (SD 0.2) which compared to 1.67 (SD 0.06) in a healthy control group.

On colour vision testing four patients had significant abnormalities with the desaturated panel D15 colour plates, consisting of at least three confusion lines along the tritan axis.

The electrooculogram was within the range of normal with an Arden ratio of 2.0 (SD 0.6). Amplitudes and latencies of the scotopic and photopic Ganzfeld ERG were normal in Parkinson patients.

Due to a reduced signal-to-noise ratio, the P-ERG could only be analyzed in four patients. In a healthy control group, the N35-P50 amplitude ratio of the 60′ and the 90′ check size was 1.06 μV (SD 0.27). This compared well to Parkinson patients with a mean ratio of 1.03 μV (SD 0.3).

P100 VEP latencies were normal (mean: 110 (SD 8.9 ms). The N75–P100 VEP amplitude was slightly reduced by up to 3 μV below the range of normal in three patients.

A multifocal ERG could be obtained in all eight patients. However, only four patients had an adequate signal-to-noise ratio permitting further analysis of the data, one of whom was later diagnosed with multi system atrophy (MSA). These patients had been treated with L-Dopa 250–500 mg/day which they had discontinued at least 12 h prior to examination.

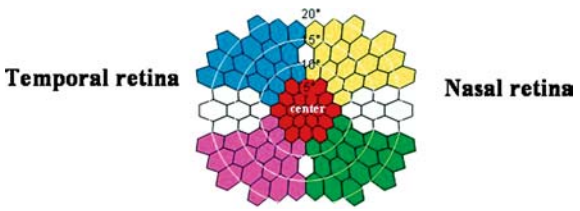

Figure 1. The areas of the response averages that were analyzed from the central 7.5 deg and the four adjoining quadrants.

Figure 1 shows the areas of the response averages that were analyzed from the central 7.5 deg and the four adjoining quadrants. These response averages were chosen, as a naso-temporal asymmetry of the mfOPs has previously been observed [16, 17]. Figure 2 shows the corresponding response averages. On average, the amplitude from the first negative trough (N1) to the first positive peak (P1) was slightly, but not significantly, lower in the central (Parkinson patients' mean: 32 nV/deg^2, control mean: 46 nV/deg^2)) and nasal response averages of Parkinson patients. However, when the Parkinson patient's responses (3 bottom traces) and the patient with MSA (dashed line) were compared to four healthy volunteers (top 4 traces) no significant differences in waveform, amplitudes or latencies were observed. In particular, the oscillatory potentials at a latency of about 70–90 ms were present in all patients.

Figure 3 shows the same response averages shown in Figure 2, but with a different filter setting of 100–300 Hz. The waveforms were filtered offline at 100–300 Hz in order to facilitate analysis of the oscillatory potentials. With this filter setting, OPs resulting from the m-sequence step stimulus can be visualized as well as those OPs resulting from the induced component, that is the stimulus in the sequence following the m-sequence step. Here, the most marked difference was observed in the nasal response average of the first OP. In the upper nasal field a mean amplitude of 1.3 nV/deg^2 in patients compared to a mean amplitude of 1.93 nV/deg^2 in the control group (P: 0.08). In the lower nasal field, the mean amplitude of 0.84 nV/deg^2 in patients compared to a mean amplitude of 1.48 nV/deg^2 in the control group (P: 0.08). Again there was no significant difference between the patients and healthy volunteers. OP-latencies also did not differ from normal. Enhancing the OPs by adding the OPs of the m-sequence step to those of the induced component failed to produce abnormal responses in the Parkinson patients.

Increasing the stimulus base interval to 106.6 ms also did not reveal retinal dysfunction in Parkinson patients other than a slight reduc-

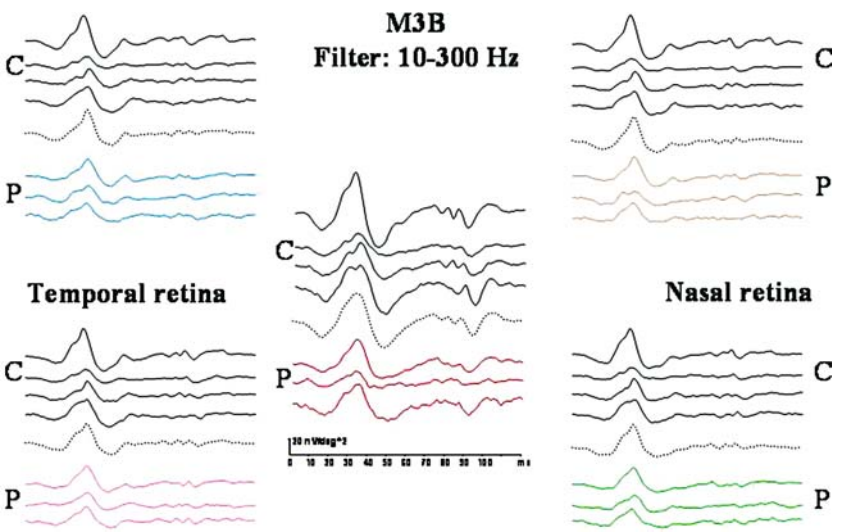

Figure 2. The responses resulting from the group averages shown in Figure 1 are depicted. For each response average, Parkinson patient's responses are shown in the three bottom traces, the patient with MSA is depicted by a dashed line, while for comparison, the waveforms of four healthy volunteers are also shown (top 4 traces). Note, that there are no significant differences in waveform. In particular, mf-oscillatory potentials are present in all patients.

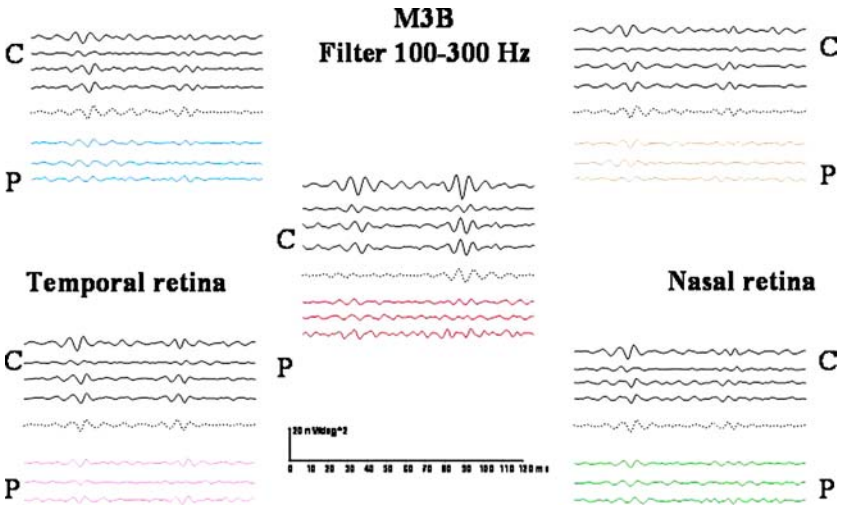

Figure 3. The same response averages shown in Figure 2, but with a different filter setting of 100–300 Hz to facilitate analysis of the oscillatory potentials. With this filter setting, OPs resulting from the M-sequence step stimulus are visualised between 20–50 ms, as well as those OPs resulting from the induced component (70–90 ms).

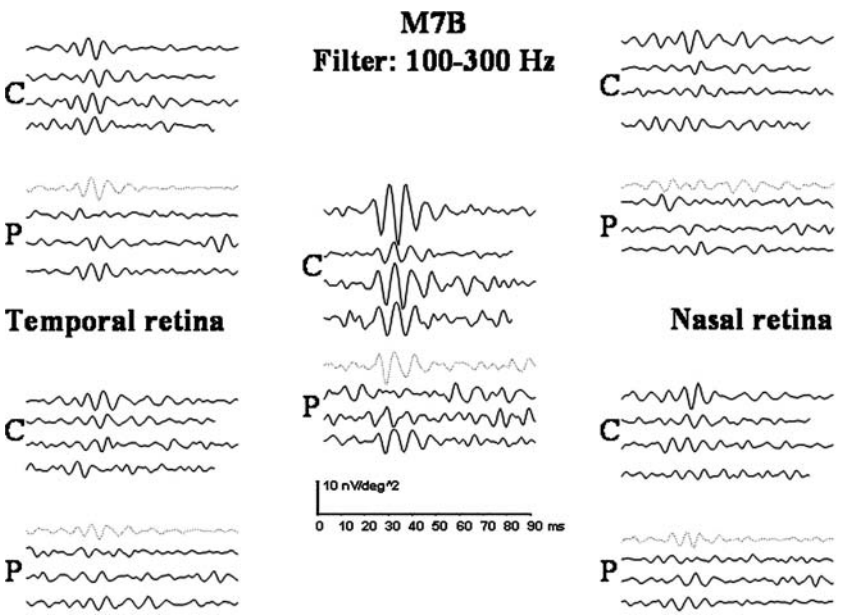

Figure 4. The same response averages for the M7B Stimulus with a filter setting of 100–300 Hz to facilitate analysis of the oscillatory potentials. With this stimulus sequence, an induced component is no longer apparent. For the same recording length, this stimulus sequence contains less signal than the M3B sequence. Central amplitudes of the OPs appear slightly reduced in Parkinson patients.

tion in the central amplitudes of the OPs in Parkinson patients. With this stimulus sequence, an induced component was no longer apparent (Figure 4). For the same recording length, this stimulus sequence contains less signal than the M3B sequence and therefore has a reduced signal-to-noise ratio.

Discussion

Our study shows that is possible to obtain mfERG recordings with a good signal-to-noise ratio in patients with moderate stages of Parkinson's disease. However, even in these early stages of disease, overlapping muscle artifacts resulted

in a bad signal-to-noise ratio preventing further mfERG signal analysis in 50% of the patients.

While this pilot study included only a small number of patients, we nevertheless found a consistent presence of three mfOPs which is in contrast to our previous findings in NTG. Adjusting for the number of tests, electrophysiologic examinations showed no significant abnormalities in Parkinson patients. These negative results do not result from therapeutic dopamine substitution as L-dopa, which has a short half life of 2–3 h, had been discontinued at least 12 h prior to examination and these patients had been and were on no other medication.

Lack of dopamine in these patients with mild to moderate signs of Parkinson's disease seems to have little influence on the mfERG, and especially the mfOPs of the induced component under the stimulus conditions applied. Thus, dopaminergic amacrine cell dysfunction under these conditions may only have a minor influence on the mfERG recorded in moderate stages of Parkinson's disease. We cannot rule out, that differences might have become apparent had we been able to record more patients or more advanced stages of disease. In more advanced stages of Parkinson's disease an increased impairment due to motor symptoms such as tremor can be expected to render testing even more difficult.

Therefore, we feel that in order to further study the influence of dopaminergic amacrine cells on the mfERG, animal studies offer promising alternatives: systemic application of 1-methyl, 4-phenyl,1-2-3-6-tetrahydropyridine (MPTP) has been shown to decrease retinal dopamine content in primates [32]. Thus a possible animal model to examine the effect of dopamine depletion on the mfERG may be the application of MPDT. Indeed, in the primate model, MPDT has been shown to result in spatial frequency dependent changes in the PERG and PVEP [33]. Intravitreal application of this agent may allow for dose-effect analysis with the advantage of not inducing systemic motor symptoms such as tremor.

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