23. Pagon RA (1988) Retinitis pigmentosa. Surv Ophthalmol 33:137–177
24. Potts AM, Inoue J (1968) The electrically evoked response of the visual system (EER). Invest Ophthalmol 7:269–278
25. Potts AM, Inoue J (1969) The electrically evoked response of the visual system (EER) II. Effect of adaptation and retinitis pigmentosa. Invest Ophthalmol 8:605–613
26. Rizzo JF 3rd, Wyatt J, Loewenstein J, Kelly S, Shire D (2003) Perceptual efficacy of electrical stimulation of human retina with a microelectrode array during short-term surgical trials. Invest Ophthalmol Vis Sci 44: 5362–5369
27. Santos A, Humayun MS, de Juan E Jr, Greenberg RJ, Marsh MJ, Klock IB, Milam AH (1997) Preservation of the inner retina in retinitis pigmentosa: a morphometric analysis. Arch Ophthalmol 115:511–515
28. Schwahn HN, Gekeler F, Kohler K, Kobuch K, Sachs HG, Schulmeyer F, Jacob W, Gabel VP, Zrenner E (2001) Studies on the feasibility of a subretinal visual prosthesis: data from Yucatan micropig and rabbit. Graefe's Arch Clin Exp Ophthalmol 239:961–967
29. Stone JL, Barlow WE, Humayun MS, de Juan E Jr, Milam AH (1992) Morphometric analysis of macular photoreceptors and ganglion cells in retinas with retinitis pigmentosa. Arch Ophthalmol 110:1634–1639
30. Tanino T, Kato S, Kawasumi M (1981) Studies on electrically evoked pupillary reflex-Indirect reflex and its frequency characteristics. Jpn J Ophthalmol 25:423–429
31. Tanino T, Kurihara K (1982) Electrically evoked direct and consensual reflexs of the pupil. Jpn J Ophthalmol 26:462–467
32. Tanino T. (1982) Studies on electrically evoked pupillary reaction 1. Indirect electrical reaction and its frequency characteristic. Nippon Ganka Gakkai Zasshi (Japanese) 61:397–402
33. Veraart C, Raftopoulos C, Mortimer JT, Delbeke J, Pins D, Michaux G, Vanlierde A, Parrini S, Wanet-Defalque MC (1998) Visual sensations produced by optic nerve stimulation using an implanted self-sizing spiral cuff electrode. Brain Res 813:181–186
34. Walter P, Heimann K (2000) Evoked cortical potentials after electrical stimulation of the inner retina in rabbits. Graefe's Arch Clin Exp Ophthalmol 238:315–318
35. Yanai D, Lakhanpal RR, Weiland JD, Mahadevappa M, Van Boemel G, Fujii G, Greenberg R, Caffey S, de Juan Jr E (2003) The value of preoperative tests in the selection of blind patients for a permanent microelectronic implant. Trans Am Ophthalmol Soc 101: 223–230
36. Young MJ, Ray J, Whiteley RS, Klassen H, Gage FH (2000) Neuronal differentiation and morphological integration of hippocampal progenitor cells transplanted to the retina of immature and mature dystrophic rats. Mol Cell Neurosci 16:197–205

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5.

CLINICAL INVESTIGATION

Effect of Transcorneal Electrical Stimulation in Patients with Nonarteritic Ischemic Optic Neuropathy or Traumatic Optic Neuropathy

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Abstract

Purpose: To determine whether transcorneal electrical stimulation (TES) can improve the visual function of patients with nonarteritic ischemic optic neuropathy (NAION) or traumatic optic neuropathy (TON).

Methods: Eight consecutive patients at the Osaka University Hospital were studied. TES (600–800 μA, 20 Hz, 30 min) was applied once each to three eyes with NAION and to five eyes with TON, using a contact lens-type stimulating electrode. The primary outcome measurement was the change in visual acuity at 1 to 3 months after TES. An improvement in visual acuity was defined as a change of ≥0.3 log (minimum angle of resolution) (logMAR) units. The side effects of TES were also investigated.

Results: After TES application, the visual acuity improved in two patients with NAION and in four patients with TON. Visual acuity did not worsen in any of the eyes. Only a mild superficial punctuate keratopathy was observed in all eyes immediately after TES, and it healed by the next day.

Conclusions: Visual acuity can be improved after TES without major complications in some patients with NAION or TON. These results suggest that TES should be considered as a new treatment for eyes with optic neuropathy. **Jpn J Ophthalmol** 2006;50:266–273 © Japanese Ophthalmological Society 2006

Key Words: contact lens, electrical stimulation, neuroprotection, nonarteritic anterior ischemic neuropathy, traumatic optic neuropathy

Introduction

Nonarteritic ischemic optic neuropathy (NAION) and traumatic optic neuropathy (TON) are optic nerve diseases accompanied by a sudden decrease in vision.[1] The visual decrease is often severe, and there is no established treatment that can reverse the decrease.[1] The natural course of the changes in visual acuity in eyes with NAION was documented by the Ischemic Optic Neuropathy Decompression Trial (IONDT) study.[2] The percentage of patients with a recovery of ≥3 lines, 0.3 log (minimum angle of resolution) (logMAR) in visual acuity, was 39.7% at 3 months in a carefully followed-up group, but the visual acuity gradually decreased during the remainder of the follow-up period. The natural course of the visual recovery in eyes with TON was documented by the International Optic Nerve Trauma Study (IONTS).[3] The percentage of untreated patients with a recovery of ≥3 lines in visual acuity was 57% at 1 month and 50% at 3 months.

The definitive cause of NAION is unknown, but optic nerve head ischemia secondary to hypoperfusion by the short posterior ciliary arteries is suspected.[4] The IONDT study reported that optic nerve decompression surgery was not effective for treating NAION.[5] Recent studies have

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6. Kelly PJ, Weiter JJ. Resolution of optic disc neovascularization associated with intraocular inflammation. Am J Ophthalmol 1980; 90:545–548.
7. Semple HC, Landers MB, Morse LS. Optic disc neovascularization in juvenile rheumatoid arthritis. Am J Ophthalmol 1990;110:210–212.
8. Pach JM, Herman DC, Garrity JA, Kalina PH. Disc neovascularization in chronic anterior uveitis. Am J Ophthalmol 1991;111:241–243.
9. Kalina PH, Pach JM, Buettner H, Robertson DM. Neovascularization of the disc in pars planitis. Retina 1990;10:269–273.
10. Tugal-Tutkun I, Onal S, Altan-Yaycioglu R, Altunbas HH, Urgancioglu M. Uveitis in Behçet disease: an analysis of 880 patients. Am J Ophthalmol 2004;138:373–380.
11. Atmaca LS, Batioglu F, Idil A. Retinal and disc neovascularization in Behçet's disease and efficacy of laser photocoagulation. Graefes Arch Clin Exp Ophthalmol 1996;234:94–99.
12. Atmaca LS. Fundus changes associated with Behçet's disease. Graefes Arch Clin Exp Ophthalmol 1989;227:340–344.
13. Atmaca LS. Experience with photocoagulation in Behçet's disease. Ophthalmic Surg 1990;21:571–576.
14. International Study Group for Behçet's Disease. Evaluation of diagnostic ('classification') criteria in Behçet's disease: toward internationally agreed criteria. Lancet 1990;335:1078–1080.
15. Tugal-Tutkun I, Urgancioglu M. Childhood-onset uveitis in Behçet disease: a descriptive study of 36 cases. Am J Ophthalmol 2003;136: 1114–1119.
16. Kötter I, Zierhut M, Eckstein AK, et al. Human recombinant interferon α-2a for the treatment of Behçet's disease with sight threatening posterior or panuveitis. Br J Ophthalmol 2003;87: 423–431.
17. De Andrea M, Ravera R, Gioia D, Gariglio M, Landolfo S. The interferon system: an overview. Eur J Paediatr Neurol 2002;6 Suppl A:A41–A46.
18. Ezekowitz AB, Mulliken JB, Folkman J. Interferon α-2a therapy for lifethreatening hemangiomas of infancy. N Engl J Med 1992;326: 1456–1463.
19. Stuebiger N, Koetter I, Zierhut M. Complete regression of retinal neovascularisation after therapy with interferon α in Behçet's disease. Br J Ophthalmol 2000;84:1437–1438.
20. Sfikakis PP, Kaklamanis PH, Elezoglou A, et al. Infliximab for recurrent, sight-threatening ocular inflammation in Adamantiades-Behçet disease. Ann Intern Med 2004;140:404–406.
21. Giansanti F, Barbera ML, Virgili G, Pieri B, Emmi L, Menchini U. Infliximab for the treatment of posterior uveitis with retinal neovascularization in Behçet disease. Eur J Ophthalmol 2004;14:445–448.

shown that treatment with levodopa may improve the vision in patients with recent onset NAION,[6] but the results are not conclusive.[7]

In TON, the optic nerve is indirectly injured by a concussive force to the head. The IONTS results show that no clear benefit is obtained by either corticosteroid therapy or optic canal decompression surgery.[3]

Thus, other treatment modalities are needed to treat the damaged optic nerve in cases of NAION and TON. It was recently reported that electrical stimulation of the spiral ganglion cells is effective in preserving their function in cases of deafness.[8,9] Also, it has been reported that electrical stimulation promotes the speed of motor axonal regeneration in rats.[10] Studies in our laboratory have shown that the survival of retinal ganglion cells in the rat eye after axotomy of the optic nerve is significantly increased if electrical stimulation is applied to the optic nerve just after axotomy in adult rats.[11] In addition, transcorneal electrical stimulation (TES) with a bipolar (concentric rings) contact lens electrode has been shown to stimulate the retinal ganglion cells and/or their axons.[12,13]

In light of these results, we investigated the efficacy and safety of TES as a method of improving and preserving the function of the optic nerve fibers in eyes of patients with NAION or TON.

Methods

Patients

These studies were performed at the Osaka University Medical School, Osaka, Japan. Three consecutive patients with NAION and five consecutive patients with TON were studied between March 2003 and June 2004. The exclusion criteria were visual acuity ≥0.4, a follow-up period of <3 months, use of a cardiac pacemaker, and the presence of corneal or retinal diseases. Patients with NAION who had an erythrocyte sedimentation rate of >30 mm/h or a C-reactive protein (CRP) value of >6 mg/dl were also excluded.

The eight patients were treated by TES after informed consent was obtained, and in full compliance with the regulations of the institutional review board. The procedures used conformed to the tenets of the Declaration of Helsinki.

The pretreatment best-corrected visual acuity (BCVA) ranged from 0.01 to 0.2 (median, 0.2) in the NAION patients, and from hand motion (HM) to 0.2 (median, 0.05) in the TON patients. The fellow eye of all patients was normal except in patient 3. The age of the patients ranged from 57 to 75 years (median, 61 years) in the NAION group, and 14 to 71 years (median, 16 years) in the TON group. The interval between the visual loss and the time of the TES treatment was 4 to 24 months (median, 6 months) in the NAION group, and 3 weeks to 11 months (median, 4 weeks) in the TON group. The follow-up period was 3 to 18 months (median, 7 months) (Table 1).

Figure 1. Schematic diagram of the transcorneal electrical stimulating (TES) system (*left*). Photograph showing the Burian-Allen (B-A) contact lens electrode in place (*right*). The retina was stimulated electrically by the electrodes embedded in the B-A electrode using biphasic pulses.

Interventional Procedures

Patients were treated according to the following protocol. The cornea was anesthetized with 0.4% oxyburocaine hydrochloride, and covered with 3% hyaluronic acid and 4% chondroitin sulfate (Viscoat, Alcon Japan, Tokyo, Japan) to prevent injury from the contact lens electrode. A bipolar contact lens Burian-Allen(B-A) electrode (Hansen Ophthalmic Laboratories, Iowa City, IA, USA) was placed on the cornea, and electric current pulses (20 pulses) were delivered from a stimulator (Nihon Koden, Tokyo, Japan) and a stimulus isolation unit (WPI, Sarasota, FL, USA) through two concentric electrodes embedded in the contact lens (Fig. 1).

Initially, the current of the biphasic pulses (duration, 10 ms; frequency, 20 Hz; number, 20) was increased from 300 μA to 2 mA to determine the threshold current necessary to elicit the phosphenes, which were perceived in both the peripheral and central visual area. The electrical stimuli were delivered continuously for 30 min using biphasic pulses after the phosphenes became visible.

Assessment of Outcome

For functional assessments, BCVA was determined using a standardized Landolt visual acuity chart, and the visual field was determined by kinetic Goldmann perimetry and the value of the critical flicker fusion frequency (CFF). These tests were performed by certified orthoptists who were unaware of the TES protocol. The visual acuity was converted to logMAR units. HM visual acuity was set at 2.9 logMAR units.[4] An improvement of ≥0.3 logMAR units between the pre- and posttreatment visual acuities was considered to be an improvement of visual acuity, while a decrease of >0.3 logMAR units was considered to be a worsening.

For quantitative evaluation of the peripheral visual field, the area of the visual field including the V/4 isopter

Jpn J Ophthalmol
Vol 50: 266–273, 2006

Table 1. Patient characteristics

Patient no.	Age/Sex	Dx	Duration	Time	BCVA	CFF Hz	GP V/4 area	GP Max ISP	Curr of Tx (μA)
1	66/F	NAION	6 m	pre	0.2	14	7.3	I/3	700
				1 m	0.2	16	7.5	I/2	
				3 m	0.5	36	4.5[b]	I/2	
2	57/M	NAION	24 m	pre	0.01	0	4.1	V/4	750
				1 m	0.02	11	14.8[a]	I/4	
				3 m	0.02	11	12.1[a]	I/3	
3	75/F	NAION	4 m	pre	0.2	22	12.2	I/3	600
				1 m	0.3	20	10.8	I/2	
				3 m	0.2	25	13.5	I/2	
4	14/M	TON	3 w	pre	0.05	38	13.7	I/2	650
				1 m	0.07	44	15	I/2	
				3 m	0.08	43	13.3	I/2	
5	24/M	TON	11 m	pre	0.15	30	2.4	I/2	750
				1 m	0.3	33	2.8	I/2	
				3 m	0.2	33	2.2	I/2	
6	71/M	TON	3 m	pre	0.2	18	9.4	I/2	700
				1 m	0.2	16	10.4	I/2	
				3 m	0.2	20	9.6	I/2	
7	14/M	TON	4 w	pre	0.02	19	6.3	I/4	800
				1 m	0.05	27	10.8[a]	I/2	
				3 m	0.05	33	10.8[a]	I/2	
8	16/M	TON	3 w	pre	HM	0	1.4	V/4	800
				1 m	HM	9	1.4	I/4	
				3 m	0.02	10	2.3[a]	I/3	

M, male; F, female; Dx, diagnosis; NAION, nonarteritic ischemic optic neuropathy; TON, traumatic optic neuropathy; Duration, duration from the onset to treatment; Time, time of examination; pre, pretreatment; m, months after treatment; w, weeks after treatment; BCVA, best-corrected visual acuity; GP, Goldmann perimetry; V/4 area, the area of the V/4 isopter (10^3 deg^2); Max ISP, the most sensitive isopter; Curr of Tx, current intensity of treatment; CFF, critical flicker fusion frequency; HM, hand motion.

[a] V/4 area enlarged ≥20% compared with the pretreatment value.

[b] V/4 area decreased ≥20% compared with the pretreatment value.

was determined from the Goldmann perimeter using the Scion Image program (Scion, Frederick, MD, USA). Because the variation in the quantified area of the normal fellow eyes in seven patients (patient 3 excepted) was less than 20% (data not shown), we defined an improvement of the peripheral visual field to have occurred when the area of the poststimulation visual field increased by ≥20%, while a worsening was considered to have occurred when the area decreased by ≥20%. An improvement of the central visual field was also considered to have occurred when a more sensitive isopter was found after the treatment.

The value of CFF was determined by averaging the frequency of flicker appearance and disappearance. Because the variation of CFF in the normal fellow eyes in seven patients (patient 8 excepted) was less than 15% (data not shown), we defined an improvement of the CFF to have occurred when the area of poststimulation CFF increased by ≥15%.

The visual acuity and visual field testing were performed before and 1 and 3 months after TES.

To assess the safety of TES, the anterior segment of the eyes was examined by slit-lamp biomicroscopy to determine whether corneal epithelial defects had been induced, whether there were alterations in the size and symmetry of the pupils, and whether there were cells or flare in the anterior chamber. These examinations were performed immediately after the treatment and on the day following TES, as well as at 1 and 3 months posttreatment. The intraocular pressure (IOP) and fundus were also examined on the day following treatment. Thereafter, the eyes were examined at 1 and 3 months after TES. The number of corneal endothelial cells was counted 3 months after treatment and compared with the pretreatment value.

Results

The BCVA at 3 months after treatment was improved by ≥0.3 logMAR units in six eyes (two NAION eyes and four TON eyes) and was unchanged in the remaining two eyes (one NAION and one TON; Fig. 2). At 3 months after TES, the area of the peripheral visual field improved significantly in three eyes (one NAION and two TON), was unchanged in four eyes (one NAION and three TON), and worsened in one eye (NAION). A more sensitive isopter appeared at 3 months after treatment in five eyes (three NAION and two TON), but did not appear in the other three eyes (zero NAION and three TON). None of the eyes lost the initial isopter (Figs. 3, 4).

The CFF at 3 months after treatment was improved by ≥15% in five eyes (two NAION and three TON) and was

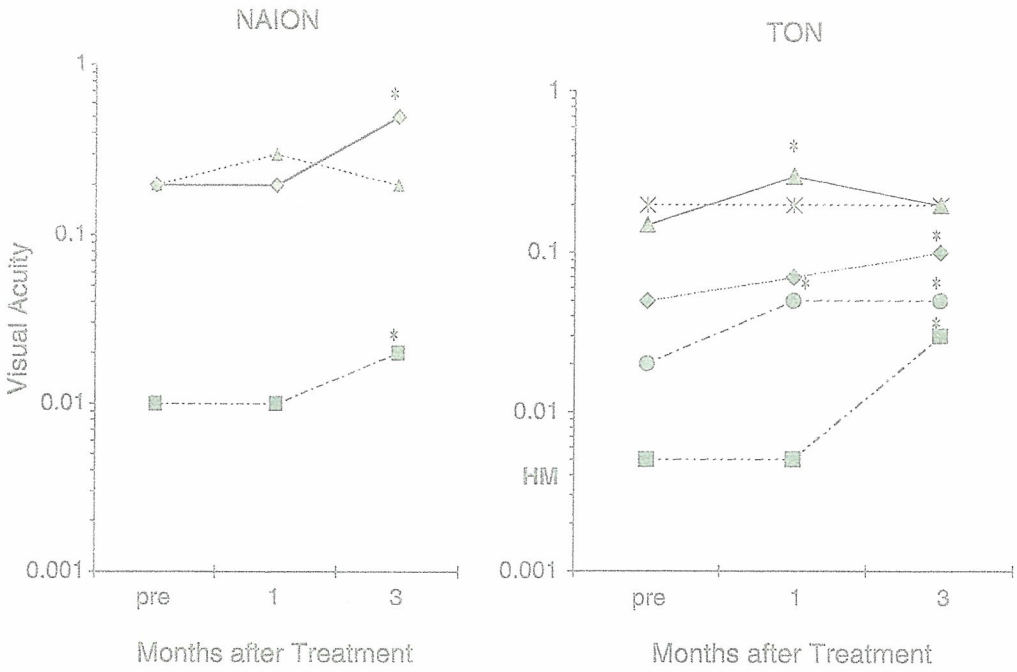

Figure 2. Effect of TES on visual acuity. Change in visual acuity after TES in eyes with nonarteritic ischemic optic neuropathy (*NAION*) (*left*) or traumatic optic neuropathy (*TON*) (*right*). *, improvement of vision by ≥0.3 log minimum angle of resolution(logMAR) units compared with the pretreatment value; *pre*, denotes pretreatment value; *HM*, hand motion. —/◆, Case 1; –·–/■, Case 2; ····/▲, Case 3; –·–·–/◆, Case 4; —/▲, Case 5; ····/✳, Case 6; – · –/●, Case 7; –··–/■, Case 8

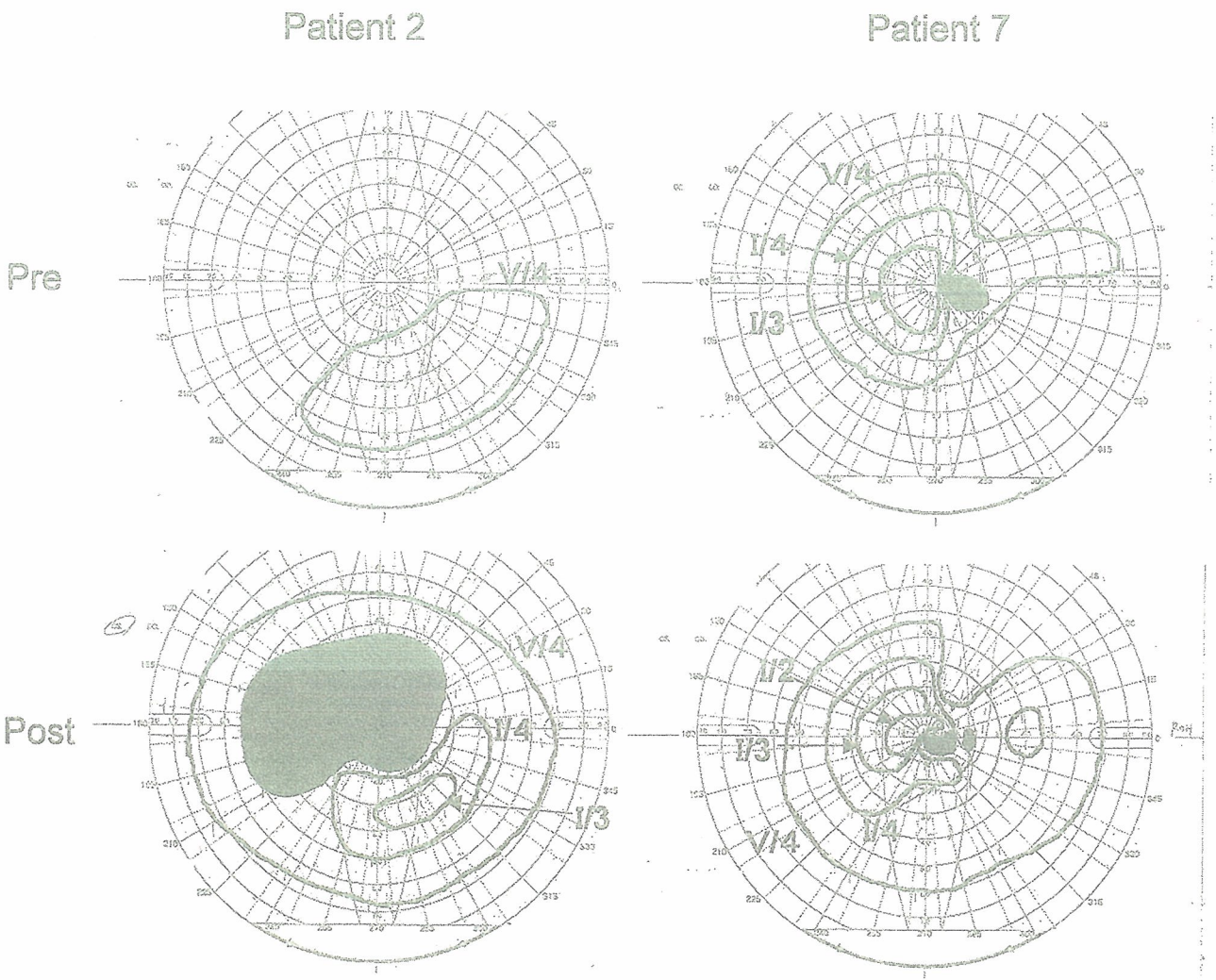

Figure 3. Visual fields. The Goldmann visual fields of patient 2 (NAION, *left*) and patient 7 (TON, *right*) before (*top*) and after (*bottom*) treatment. After treatment, the isopter of the peripheral visual field (V/4) enlarged, and more sensitive isopters (I/4 and I/3 in patient 2 and I/2 in patient 7) were detected.

Jpn J Ophthalmol
Vol 50: 266–273, 2006

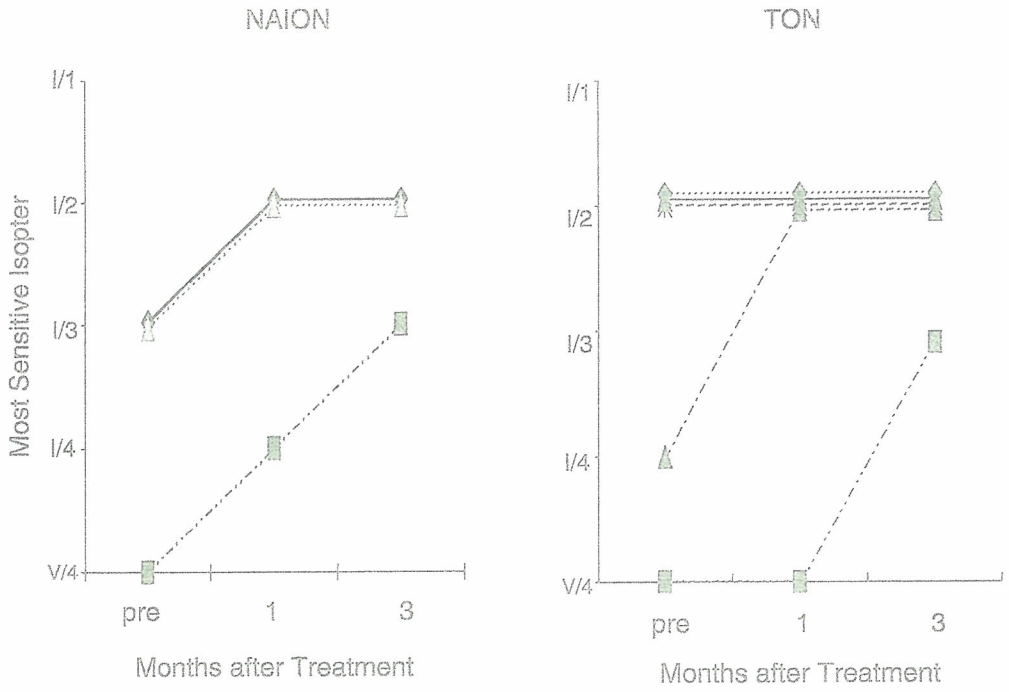

Figure 4. Appearance of more sensitive isopters in the NAION group (*left*) and the TON group (*right*). In all three patients with NAION and in two of the five patients with TON, more sensitive isopters appeared 1 to 3 months after treatment. *I/1* denotes the most sensitive isopter, followed by *I/2*, *I/3*, and *I/4*. *V/4* denotes the isopter of the peripheral visual field. —/◆, Case 1; –·–/■, Case 2; ·····/△, Case 3; —·—/◇, Case 4; – –/▲, Case 5; —/※, Case 6; – · –/▲, Case 7; —··—/■, Case 8

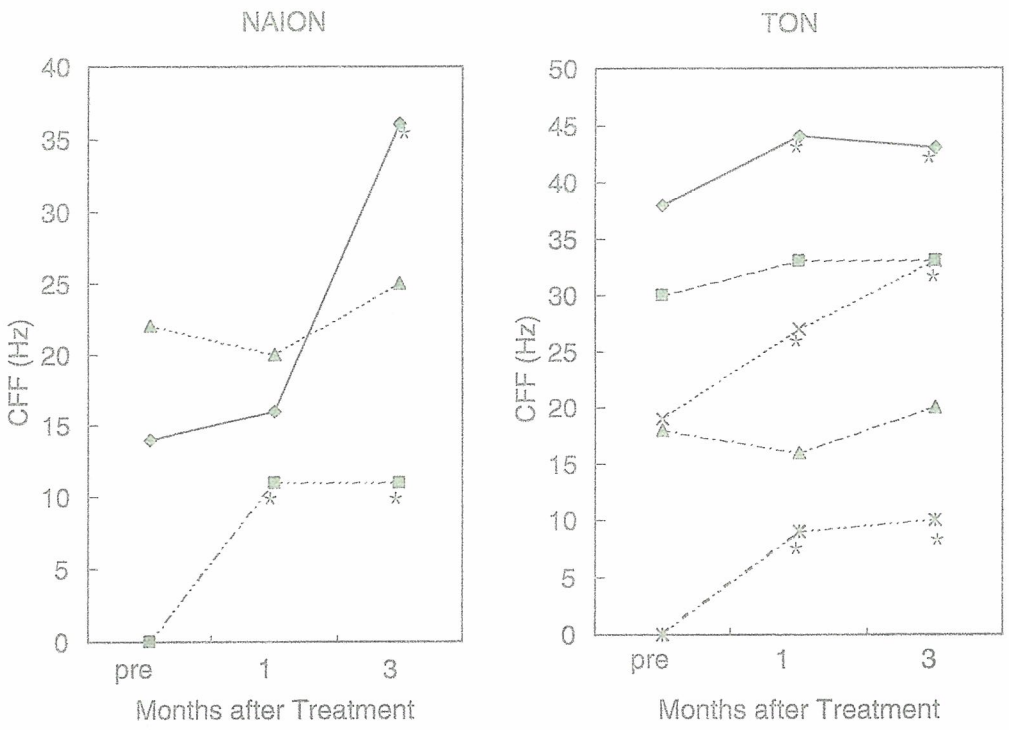

Figure 5. Effect of TES on critical flicker fusion frequency (*CFF*) Change of CFF after TES in eyes with NAION (*left*) and *TON* (*right*). *denotes an improvement of CFF by 15% or more compared with the pretreatment value. —/◆, Case 1; –·–/■, Case 2; ·····/▲, Case 3; —/◆, Case 4; ----/■, Case 5; —··—/▲, Case 6; – · –/×, Case 7; —··—/※, Case 8

unchanged in the remaining three eyes (one NAION and two TON; Fig. 5).

None of the patients reported experiencing pain during the TES treatment. A mild superficial punctuate keratopathy was observed in all eyes after the treatment, which healed by the next day in all eyes (Fig. 6). The number of corneal endothelial cells had not changed significantly in any of the eyes at 3 months, compared with the number before treatment.

The size and shape of the pupil was unchanged in all eyes, and no cells or flare was observed in the anterior chamber of any eyes just after the treatment or on the next

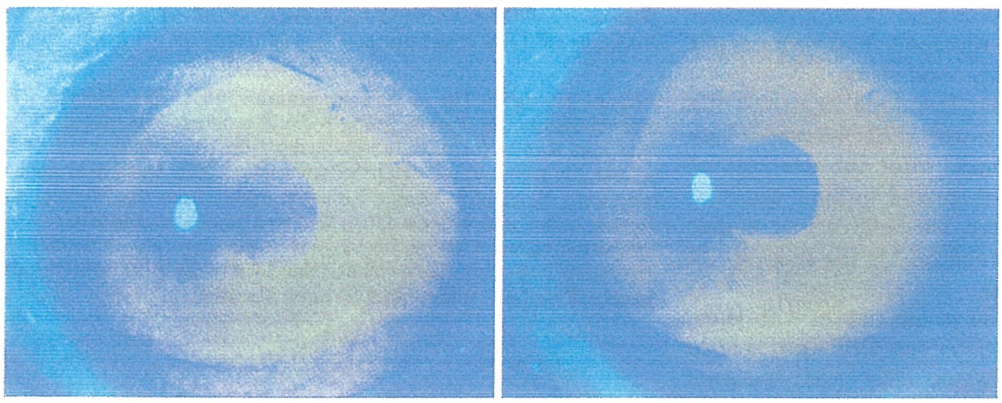

Figure 6. Photographs of the cornea of patient 1 stained with fluorescein dye, taken just after treatment (*left*) and on the following day (*right*). A superficial punctuate keratopathy was observed just after treatment, but was not present the next day.

day. The IOP did not change, and fundus examinations showed no change in the retina or optic disc on the day following treatment.

Case Report

A 64-year-old woman (patient 1) noticed a sudden loss of the inferior visual field in her left eye on 14 November 2002. She was referred to us the next day, and her BCVA was RE, 1.0 and LE, 0.5. A relative afferent pupillary defect was present in her left eye. Ocular fundus examination showed optic disc edema in the left eye (Fig. 7), and fluorescein angiography showed hypoperfusion in the upper temporal disc area (Fig. 6). The CRP value was normal (<2.5 mg/dl). She did not have pain during eye movements, and magnetic resonance imaging did not show changes in the optic nerve. From these results, she was diagnosed with NAION in the left eye. Oral aspirin and vitamin B12 were prescribed.

The BCVA in her left eye decreased to 0.15 in December but recovered to 0.2 in January 2003 and remained unchanged for 5 months. In May 2003, TES was performed on the left eye (600 μA, 20 Hz, 30 min), and the BCVA improved to 0.4 in June and to 0.5 in August. However, the BCVA decreased again to 0.3 in September and to 0.1 in December. TES was performed again in March 2004 (700 μA, 20 Hz, 30 min), and the BCVA improved to 0.2 in April and to 0.3 in June (Fig. 7).

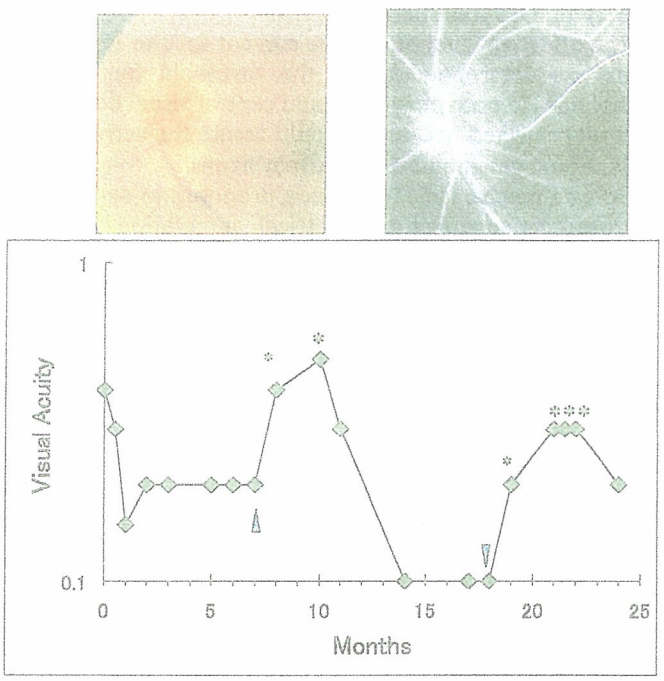

Figure 7. Fundus photograph (*top left*) and early-phase fluorescein angiogram (FA) (*top right*) of the left eye of patient 1 at the first visit. Papilledema of moderate degree can be seen. FA shows hypoperfusion in the upper temporal disc area. The time course of the best corrected visual acuity (BCVA) in the left eye (*bottom*). *Arrowhead* denotes the day of TES treatment. *denotes the improvement of BCVA ≥0.3 logMAR units from the pretreatment value.

Discussion

The visual acuity and CFF improved in two of the three eyes in the NAION group, while the other eye showed no change. In these three eyes, a more sensitive isopter was detected in the visual field after TES (Figs. 2, 4, 5). The IONDT study showed that the mean visual acuity improved up to 3 months after the onset of NAION,[2] and did not improve thereafter during the 24-month follow-up period. Thus, we conclude that TES treatment was effective in some eyes with NAION even if the TES treatment was performed more than 4 months after the onset of NAION.

Our case report shows that in a patient whose visual acuity was stable for more than 5 months, TES can still improve the visual acuity at 3 months after the treatment. Although the visual acuity decreased 6 months later, after a second TES treatment, the vision improved again (Fig. 7). These findings strongly suggest a causal relationship between the treatment and the visual improvement.

Our case report also shows the limitation of a single TES treatment, because the recovery of vision was obtained for only 3 months, suggesting that repeated treatments might be necessary to maintain the improved vision.

Johnson et al.[6,14] showed that levodopa improved the visual function in 30% of patients with NAION, even if they were treated more than 6 months after the decrease of vision,[14] and in 75% of patients if treated within 2 weeks of the onset of NAION.[6] Therefore, we believe that earlier TES treatment will be more beneficial for the recovery of visual acuity in NAION patients.

This theory is supported by the results in eyes with TON. Three months after TES, the BCVA and CFF improved in all three eyes treated within 4 weeks after onset (patients 4,7,8) but did not improve in the two eyes treated 3 months or more after onset (patients 5, 6; Fig. 2). However, the possibility of spontaneous recovery cannot be ruled out.[15] A randomized controlled study is necessary to confirm the efficacy of TES treatment for TON.

The effective level of electric current for the TES treatment was determined to be the threshold for eliciting phosphenes in both peripheral and central visual fields. This level was selected because it would assure the activation of most retinal ganglion cells and their axons.

Several mechanisms have been proposed to explain the neuroprotective effect of electrical stimulation.[10,16,17] We have demonstrated that insulin-like growth factor 1, a neurotrophic factor, is gradually upregulated in the rat retina up to 2 weeks after TES.[18] This may explain why a single TES treatment is effective in improving vision for up to 3 months. However, additional laboratory studies are necessary to determine more conclusively the mode of action of the electrical stimulation.

To the best of our knowledge, this is the first application of TES for the treatment of optic neuropathy, and we considered it very important to test its safety. The effects of using the Burian-Allen contact lens for the TES treatment are comparable to those following its use for electroretinographic recordings. Only mild corneal punctuate keratopathy was observed after TES treatment in all eyes, and the keratopathy healed by the next day (Fig. 6). The safety of TES on the corneal epithelium may stem from the protective effect of hyaluronic acid and chondroitin sulfate, as well as the balanced charge stimulation using biphasic pulses.

Because the current delivered by the contact lens electrode also stimulated the ciliary body, we carefully checked the size and shape of the pupils, and whether cells or flare was present in the anterior chamber. No significant alterations were observed. The electrical current may also penetrate the cornea, so we confirmed that there were no changes in the corneal endothelium. In particular, no changes were observed in the number of corneal endothelial cells. These observations suggest that TES is a safe method for stimulating the retinal ganglion cells and their axons.

In conclusion, TES led to the improvement of the visual acuity in eyes of some patients with NAION or TON. However, a larger prospective, randomized clinical trial with controls is necessary to confirm conclusively the effectiveness of TES treatment.

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References

1. Trobe JD. The neurology of vision. New York: Oxford University Press; 2001. p. 215–218.
2. The Ischemic Optic Neuropathy Decompression Trial Research Group. Ischemic optic neuropathy decompression trial. Twenty-four-month Update. Arch Ophthalmol 2000:118;793–798.
3. Levin AL, Beck RW, Joseph MP, Seiff S, Kraker R, and the International Optic Nerve Trauma Study Group. The treatment of traumatic optic neuropathy. Ophthalmology 1999;106:1268–1277.
4. Lessel S. Nonarteritic anterior ischemic optic neuropathy. Enigma variations. Arch Ophthalmol 1999;117:386–388.
5. The Ischemic Optic Neuropathy Decompression Trial Research Group. Optic nerve decompression surgery for nonarteritic anterior ischemic optic neuropathy (NAION) is not effective and may be harmful. JAMA 1995;273:625–632.
6. Johnson LN, Guy ME, Krohel GB, Madsen RW. Levodopa may improve vision loss in recent-onset, nonarteritic anterior ischemic optic neuropathy. Ophthalmology 2000;107:521–526.
7. Lee AG, Brazis PW. Clinical pathways in neuro-ophthalmology. An evidence-based approach. 2nd ed. New York: Thieme; 2003. p. 82–83.
8. Miller JM, Altschuler RA. Effectiveness of different electrical stimulation conditions in preservation of spiral ganglion cells following deafness. Ann Otol Rhinol Laryngol 1995;166:57–60.
9. Leake PA, Hradek GT, Snyder RL. Chronic electrical stimulation by a cochlear implant promotes survival of spiral ganglion neurons after neonatal deafness. J Comp Neurol 1999;412:543–562.
10. Al-Majed AA, Brushart TM, Gordon T. Electrical stimulation accelerates and increases expression of BDNF and trkB mRNA in regenerating rat femoral motorneurons. Eur J Neurosci 2000; 12:4381–4390.
11. Morimoto T, Miyoshi T, Fujikado T, Tano Y, Fukuda Y. Electrical stimulation enhances the survival of axotomized retinal ganglion cells in vivo. Neuroreport 2002;13:227–230.
12. Potts AM, Inoue J. The electrically evoked response of the visual system (EER). Invest Ophthalmol 1968;7:269–278.
13. Miyake Y, Yanagida K, Yagasaki K. Clinical application of EER (electrically evoked response). (1) Analysis of EER in normal subjects. Nippon Ganka Gakkai Zasshi (Acta Soc Ophthalmol Jpn) 1980;84:354–360.
14. Johnson LN, Gould TJ, Krohel GB. Effect of levodopa and carbidopa on recovery of visual function in patients with nonarteritic anterior ischemic optic neuropathy of longer than six months' duration. Am J Ophthalmol 1996;121:77–83.
15. Berestka JS, Rizzo JF. Controversy in the management of traumatic optic neuropathy. Int Ophthalmol Clin 1994;34:87–96.
16. Franklin JL, Sanz-Rodriguez C, Juhasz A, Deckwerth TL, Johnson EM. Chronic depolarization prevents programmed cell death of

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BRIEF COMMUNICATION

Prevalence of Glaucoma in Adults with Down's Syndrome

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Abstract

Purpose: To compare the prevalence of glaucoma in adults with Down's syndrome (DS) to that in non-DS control adults.

Methods: Twenty-six patients (14 men and 12 women) with DS and 188 control subjects (105 men and 83 women) were studied. The mean age was 35.1 ± 6.9 (± SD) years in the DS group and 36.9 ± 5.2 years in the control group. There were no significant differences in age or sex distribution between the two groups. Glaucoma was diagnosed by two glaucoma specialists based on the optic disc findings obtained through dilated pupils.

Results: The prevalence of patients with glaucoma in the DS group was 11.5%, significantly higher ($P = 0.014$) than that in the control group, 1.1%. There was no significant difference in intraocular pressure between glaucomatous eyes (12.2 ± 3.2 mmHg) and nonglaucomatous eyes (11.1 ± 4.1 mmHg) in the DS group ($P = 0.465$).

Conclusions: The prevalence of glaucoma in adult patients with DS was significantly higher than that in age-matched control subjects. **Jpn J Ophthalmol** 2006;50:274–276 © Japanese Ophthalmological Society 2006

Key Words: Down's syndrome, glaucoma, neurodegenerative disease, prevalence

Introduction

Glaucoma is a degenerative disease of the optic nerve caused by various factors. Recently, glaucoma has been reported to be associated with systemic neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease; the prevalence of glaucoma in patients with Alzheimer's disease is 24.5%, and that in patients with Parkinson's disease is 23.7%.[1]

Patients over the age of 30 years with Down's syndrome (DS) tend to develop neuropathological changes similar to those in Alzheimer's disease.[2] The prevalence of glaucoma in children with DS has been reported to be somewhat low by several studies.[3,4] Recently, the life expectancy of patients with DS has been extended; however, the prevalence of glaucoma in adults with DS has not been reported. Therefore, we evaluated the prevalence of glaucoma in adult patients with DS and compared the results with age-matched control subjects.

Subjects and Methods

Twenty-six adult patients with DS (14 men, 12 women), who were recruited from six institutions in Hiroshima, Japan, underwent ophthalmologic examinations in June and July 2003. The ages of the patients ranged from 22 to 56 years (mean, 35.1 years). Patients who had had retinal surgery or vitreous surgery were excluded, but those who had had cataract surgery were included.

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sympathetic neurons in vitro but does not support growth: requirement for CA2+ influx but not Trk activation. J Neurosci 1995; 15:643–664.
17. Miller AL, Prieskorn DM, Altschuler RA, Miller JM. Mechanism of electrical stimulation-induced neuroprotection: effects of verapamil on protection of primary auditory afferents. Brain Res 2003;966:218–230.
18. Morimoto T, Miyoshi T, Matsuda S, et al. Transcorneal electrical stimulation rescues axotomized retinal ganglion cells by activating endogenous retinal IGF-1 system. Invest Ophthalmol Vis Sci 2005;46:2147–2155.

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Contribution of retinal neurons to d-wave of primate photopic electroretinograms

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Abstract

The purpose of this study was to determine the contribution of different types of retinal neurons to the d-wave of the primate electroretinogram using pharmacological agents. NMDA + TTX was used to suppress inner retinal activity, and APB and PDA to block the activity of the ON- and OFF-pathways, respectively. Results indicated that the inner retinal neurons had a small but certain contribution to the d-wave. The initial rapid phase of the d-wave originates from the activity of the cone OFF-pathway nearly exclusively, and the later slow phase is shaped by the cone photoreceptors. The cone ON-pathway acts in a direction opposite to that of the other components.

Keywords: Electroretinogram (ERG); Photopic; d-wave; Off; Monkey

1. Introduction

The d-wave of the electroretinogram (ERG) is a positive-going wave which is recorded at the termination of a light stimulus and is best recorded under photopic conditions. Whereas the origin of the b-wave, which is a positive wave at light onset, has been comprehensively studied (Heynen & van Norren, 1985a, 1985b; Massey, Redburn, & Crawford, 1983; Miller & Dowling, 1970; Newman, 1980; Robson & Frishman, 1995; Sieving, Murayama, & Naarendorp, 1994; Xu & Karwoski, 1994), the d-wave has been studied less extensively.

There is, however, some information on the origin of the d-wave. In the amphibian retina, there is evidence that cone photoreceptors are the major contributor to the d-wave (Yanagida, Koshimizu, Kawasaki, & Yonemura, 1986), although later pharmacological results demonstrated that cells post-synaptic to the photoreceptors make a major contribution to the d-wave (Awatramani, Wang, & Slaughter, 2001; Stockton & Slaughter, 1989; Xu & Karwoski, 1995). In the mammalian retina, it has been long believed that the d-wave is largely produced by the positive-going wave of the late receptor potential (Brown, 1968). However, recent pharmacological studies in monkeys strongly suggest that proximal retinal neurons also participate in producing the d-wave. Sieving et al. (1994) demonstrated that the d-wave represented the activity of the OFF-bipolar cells together with the offset of the cone photoreceptor response. They also suggested that the waveform of the d-wave was modulated by the activity of ON-bipolar cells, because the d-wave was enhanced after blocking the activity of ON-bipolar cells. However, it was not clear how each type of retinal neurons or pathways contributes to each phase of the d-wave.

Therefore, the purpose of this study was to determine the contribution of the various types of retinal neurons to the d-wave of the ERG in primates. To accomplish

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this, we used pharmacological agents to suppress specific retinal neurons or pathways in monkey, and compared the ERGs before and after the drugs.

2. Materials and methods

2.1. Animals

Five eyes of five rhesus monkeys (*Macaca mulata*) were studied. The animals were sedated with an intramuscular injection of ketamine hydrochloride (7 mg/kg initial dose; 5–10 mg/kg/h maintenance dose) and xylazine (0.6 mg/kg). The respiration and heart rate were monitored, and hydration was maintained with slow subcutaneous infusion of lactated Ringer solution. The cornea was anesthesized with topical 1% tetracaine, and the pupils dilated with topical 0.5% tropicamide, 0.5% phenylephrine HCl, and 1% atropine. Experiments were conducted in accordance with guidelines of the American NIH regarding the care and use of animal for experimental procedures.

2.2. Visual stimulus

ERGs were elicited using a densely packed array of 102 green LEDs (525 nm peak wavelength; 50 nm width at half-amplitude) placed at the top of a Ganzfeld dome and presented through a diffuser. The stimulus was presented as 200 ms square wave flashes or a 1 Hz saw-toothed ("rapid off") flicker stimulus to elicit the d-wave. The LEDs were controlled by a digital function generator (WF1945, NF Corporation, Tokyo, Japan). The maximum stimulus intensity measured in the dome was 2.5 log cd/m^2, and the dome also housed a rod-suppressing white background light of 40 cd/m^2. The stimulus intensity was attenuated by neutral density filters (Wratten, Kodak, Rochester, NY) in 0.2 log unit steps over a range of 1.4 log units.

2.3. ERG recordings and analysis

Following 10 min of light adaptation to 40 cd/m^2, ERGs were recorded with a Burian-Allen bipolar contact lens electrode (Hansen Ophthalmic Development Labs, Iowa City, IA). The ground electrode was attached to the ipsilateral ear. Responses were amplified and the bandpass filters were set at 0.3–1000 Hz. The ERGs were digitized at 4.3 kHz, and a 60 Hz notch filter was used to reduce line noise. Twenty to thirty responses were averaged for each recording (Power Lab, AD Instruments, Castle Hill, Australia). The amplitude and implicit time of the d-wave were measured from the stimulus offset to the peak of the d-wave. The subtracted data were obtained from the original digital data using Excel (Microsoft Corporation, WA, USA).

2.4. Drug application

The drugs and intravitreal injection techniques have been described in detail (Bush & Sieving, 1994; Hood, Frishman, Saszik, & Viswanathan, 2002; Kondo & Sieving, 2001; Sieving et al., 1994; Ueno, Kondo, Niwa, Terasaki, & Miyake, 2004). The drugs were injected into the vitreous with a 30 gauge needle inserted through the pars plana approximately 4 mm posterior to the limbus. The drugs (Sigma Chemical, St. Louis, MO and Sankyo, Tokyo, Japan) were dissolved in sterile saline and injected in amounts of 0.05–0.07 ml. The intravitreous concentrations were: 1 mM for L-2-amino-4-phosphonobutyric acid (APB); 3–5 mM for *cis*-2,3-piperidine dicarboxylic acid (PDA); 4 μM for tetrodotoxin citrate (TTX); and 4 mM for *N*-methyl-D-aspartic acid (NMDA).

Recordings were begun about 60–90 min after the drug injections, and studies were completed within 5 h. Although the effects of these drugs are mostly reversible after a recovery period of several weeks, the results presented were recorded from eyes not previously treated.

3. Results

3.1. Square and saw-toothed stimuli

Two stimulus patterns were used to elicit the d-wave: first was a long duration square stimulus, and the second was a rapid saw-toothed flicker stimulus (Alexander, Fishman, Barnes, & Grover, 2001; Barnes, Alexander, & Fishman, 2002). Both stimuli were presented on a rod-suppressing background. The waveforms of the d-wave using these two stimuli for four different stimulus intensities are shown in Fig. 1. A 200 ms duration square wave flash was used because this stimulus duration is often used in the clinical setting.

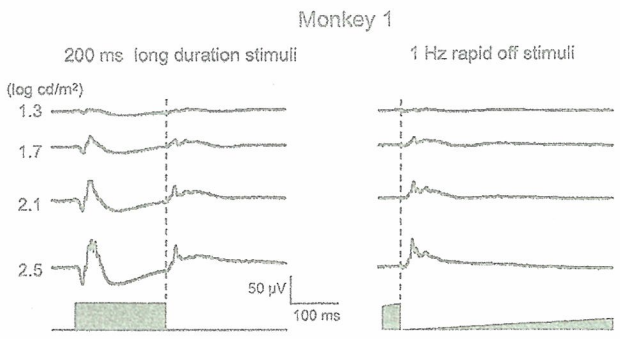

Fig. 1. Photopic ERGs of a monkey recorded by two different shaped stimuli. The ERGs in the left column were elicited by 200 ms square wave flashes, and those in the right column by 1 Hz rapid saw-toothed stimuli. Responses were recorded over a range of stimulus intensities (log cd/m^2) as indicated on the left.

The amplitude of the d-wave increased with increasing stimulus intensities for both stimulus patterns, and the waveforms of the d-wave were essentially the same for the two types of stimuli. We used a 1 Hz saw-toothed stimulus to record the d-wave because the waveforms were less noisy, the baseline was more stable even in anesthetized monkeys, and there are recent evidences that saw-toothed on and off stimuli are useful for quantitative assessment of the properties of the on and off responses in retinal diseases (Alexander et al., 2001; Barnes et al., 2002). However, it has been reported that even using saw-toothed on and off stimuli, it is not possible to separate the neural activities from ON- and OFF-pathways completely (Khan et al., 2005).

3.2. Effect of TTX and NMDA

To examine what the inner retinal neurons contribute to the d-wave, we injected TTX and NMDA into the vitreous of two eyes (monkeys 2 and 3). TTX blocks voltage-gated sodium channels and blocks the generation of action potentials by ganglion cells and some types of amacrine cells (Narahashi, Moore, & Scott, 1964; Narahashi, 1974; Bloomfield, 1996). NMDA suppresses synaptic transmission by depolarizing neurons with NMDA receptors that are located primarily on third-order neurons (Massey & Miller, 1990; Slaughter & Miller, 1983). It is generally believed that the intravitreous injection of TTX + NMDA can suppress most, if not all, of the electrical activities from inner retinal neurons (Hood et al., 2002; Rangaswamy, Hood, & Frishman, 2003; Robson & Frishman, 1995).

The changes in the d-waves after TTX + NMDA are shown in Fig. 2. In the right column, the ERGs recorded after the drugs (black lines) are superimposed on the pre-drug control waveforms (gray lines). After TTX + NMDA, the amplitude of the d-wave was slightly reduced to 75–90% of control, and the reductions occurred at all stimulus intensities. The implicit time and initial slope of the d-wave did not change. The oscillatory components riding on the descending limb of the d-wave were also reduced after TTX + NMDA. The later slow phase of the d-wave was larger (arrowhead) after TTX + NMDA. These results indicate that the activity of both spiking and non-spiking inner retinal neurons contribute to the d-wave (Viswanathan, Frishman, Robson, Harwerth, & Smith, 1999, 2002).

3.3. Effect of blocking ON-pathway by APB

We injected APB into the vitreous of two eyes (monkey 1 and 4). APB is a glutamate analog that blocks transmission from the photoreceptors to the ON-bipolar cells, and thus eliminates the neural activities of the ON-pathway, i.e., the ON-bipolar cells and inner retinal neurons driven by ON-bipolar cells (Slaughter & Miller, 1981). In the upper middle column of Fig. 3, the waveforms after APB (black traces) are superimposed on the pre-drug control waveforms (gray traces). After blocking the ON-pathway activity by APB, the amplitude of the d-wave increased as reported (Evers & Gouras, 1986; Sieving et al., 1994). The increase in the d-wave was about 200% in both animals, and this degree of enhancement was nearly the same for all stimulus intensities (Fig. 3, lower traces).

The slope of the initial positive-going phase of the d-wave did not change after APB, and the initial waveforms overlapped completely. We also noted that the oscillatory components, which were recorded on the peak of the control d-wave were replaced by two large positive peaks after APB.

The component removed by APB was isolated by subtracting the post-APB responses from the control responses (Fig. 4, left half). The component removed by APB had two negative peaks, and the amplitudes increased with increasing stimulus intensities. The amplitude of this component was as large as that of pre-drug d-waves. These results indicated clearly that the

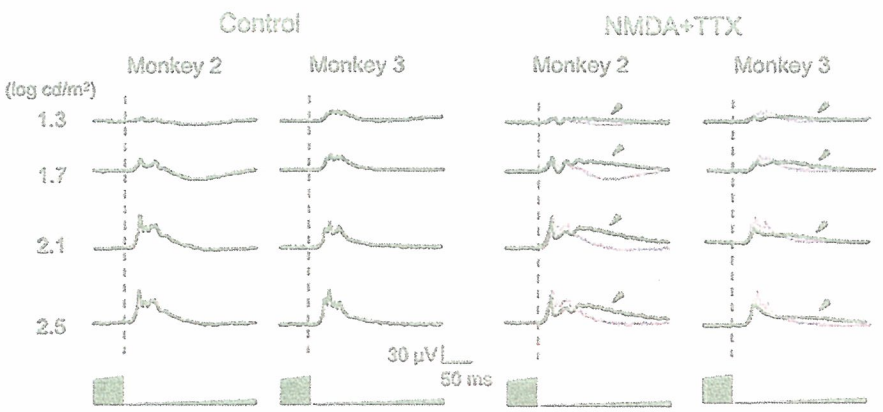

Fig. 2. ERGs to off stimuli of four different intensities before and after intravitreal injection of TTX + NMDA to the same eyes. The ERGs recorded after administration of drugs (black trace) are superimposed on the pre-drug control ERGs (gray trace). Arrowhead shows an increase of the later slow phase of the d-wave after TTX + NMDA.

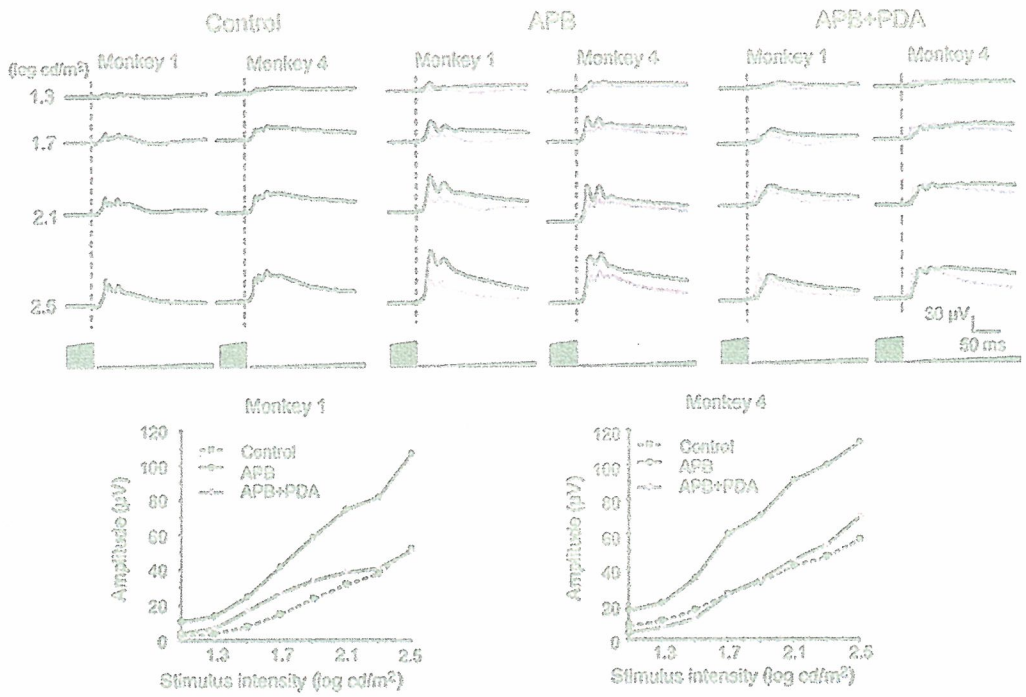

Fig. 3. Effect of APB and PDA on the d-waves. Top traces: photopic off responses elicited by four stimulus intensities before and after APB and APB + PDA treatment. ERGs recorded after administration of drugs (black trace) are superimposed on the pre-drug control ERGs (gray trace). Bottom traces: intensity–response curves of the peak amplitudes of the d-wave before and after drug application of two monkeys (monkey 1 and 4) (■ control, ◦ after APB, ○ after APB + PDA).

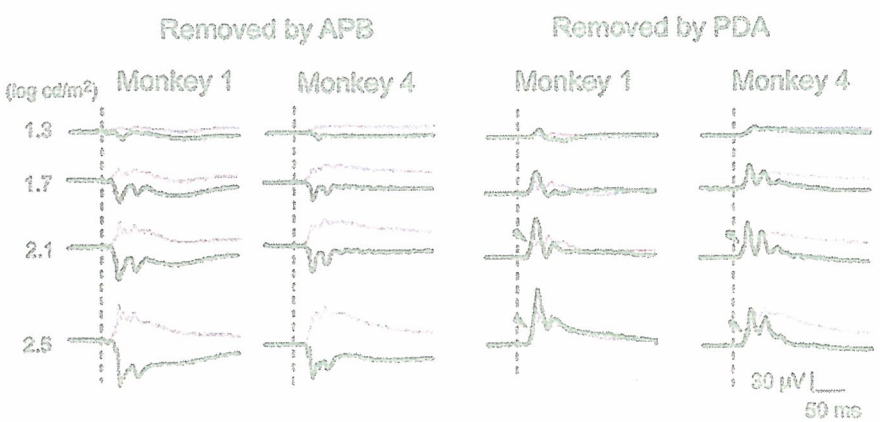

Fig. 4. Subtracted ON-pathway components (removed by APB) and subtracted OFF-pathway components (removed by PDA) at four different stimulus intensities. ON-pathway component was obtained by subtracting the post-APB records from the pre-drug control records. OFF-pathway component was obtained by subtracting the post-APB + PDA records from the post-APB records. Results from the two eyes (monkey 1 and 4) are shown. Subtracted responses (black trace) are superimposed on the pre-drug control ERGs (gray trace). Arrowhead points to the slope of initial rapid portion of OFF-pathway component (removed by PDA) and is consistent to that of control d-wave.

activity of post-synaptic ON-pathway contributes considerably to the d-wave.

3.4. Injection of APB and PDA to isolate the cone photoreceptor

We next examined what the activity of the cone OFF-pathway (OFF-bipolar cells and inner retinal neurons driven by OFF-bipolar cells) contributed to the d-wave. PDA is a glutamate analog that blocks transmission to the OFF-bipolar and horizontal cells to inner retinal neurons (Sieving et al., 1994; Slaughter & Miller, 1983). We injected PDA after APB in the same two eyes (monkey 1 and 4). This also allowed us to see how the cone photoreceptors contributed to the d-wave.

The ERGs after APB + PDA are shown in the upper right trace of Fig. 3. The waveforms after APB + PDA (black traces) are superimposed on the pre-drug control waveforms (gray traces). After the addition of PDA, the positive d-wave became smaller and smoother than after APB only, but still remained as large as the control d-waves. The implicit time of the d-wave was delayed by 14–25 ms compared to that of controls, and the slope of the rising phase of the d-wave became less steep. These results also indicated that the cone photoreceptors contributed to the later, slow phase of the d-wave rather than the initial rapid phase.

We also isolated the component removed by PDA by subtracting the post-(APB + PDA) responses from the post-APB responses (right half of Fig. 4). The component removed by PDA had two positive peaks, and the slope of the initial rapid portion was similar to that of control d-wave (arrowhead). These results indicated that the initial rapid phase of the d-wave originate mainly from the neural activity of the OFF-pathway. We also noted that the waveform of the component removed by PDA resembled the inverted image of the component removed by APB. Interestingly, the amplitudes of the component removed by PDA were nearly the same as the component removed by APB (Fig. 4).

3.5. Effect of PDA alone

Finally, we wanted to see how the d-wave is altered after application of PDA alone (Fig. 5). The positive d-wave of the ERGs was replaced by a smooth negative wave after PDA alone as reported (Sieving et al., 1994). Subtraction of the before and after waveforms showed that the component removed by PDA was a large positive deflection whose amplitude was larger than that of control d-waves.

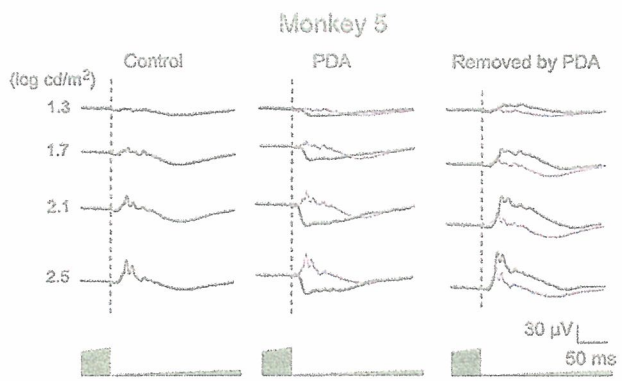

Fig. 5. ERG responses to off stimuli of four different intensities before and after injection of PDA alone in the same eye and the removed component by PDA. ERGs recorded after administration of drugs (black trace) and (black trace) removed component by PDA are superimposed on the pre-drug control ERGs (gray trace).

4. Discussion

Our results clearly demonstrated that the origin of the d-wave of primate photopic ERG is very complex, and the activities from several types of retinal neurons/pathways participated in the shaping the d-wave. We found that not only the cone photoreceptors, but also post-receptoral components of the ON- and OFF-pathways and the inner retinal neurons affected by TTX and NMDA, contributed to the d-wave.

Our results indicated that although the cone photoreceptors are one of the major sources of the d-wave (Brown, 1968), their contribution is mainly in the later phases. As shown in Fig. 3, a large positive component still remained even after blocking the post-receptoral components of the ON- and OFF-pathways by APB and PDA, and its amplitude was as large as that of the control d-wave. However, the slope of the rising edge of the d-wave became less steep, and the implicit time was delayed after APB + PDA. These results suggested that the neurons other than the cone photoreceptors participated in the initial rapid phase of the d-wave.

Our results demonstrated that the initial rapid phase of the d-wave is nearly exclusively shaped by the activity of cone OFF-pathway. As shown in Fig. 4, the component removed by PDA had a steep positive component, whose slope completely overlapped that of the control d-wave. In addition, after suppressing the activity of the OFF-pathway by PDA alone, the positive d-wave was no longer seen, and only a slow, negative deflection remained (Fig. 5). These results suggest that the cone OFF-pathway, presumably cone OFF-bipolar cells, contributed to the initial rapid phase of the d-wave nearly exclusively.

We also confirmed the earlier hypothesis that the d-wave can be influenced by cone ON-pathway activity, and its contribution was opposite to the activity from OFF-pathway (Evers & Gouras, 1986; Khan et al., 2005; Sieving et al., 1994). As shown in Fig. 4, the waveforms of the component removed by PDA resembled the inverted images of the component removed by APB, and the amplitudes of the component removed by APB were nearly the same as the component removed by PDA.

We have summarized the contribution of the photoreceptors (waveform after APB + PDA), ON-pathway (component removed by APB), and OFF-pathway (component removed by PDA), based on the results from monkey 1 in Fig. 6. Higher amplification of the beginning part of ERG is also shown in the lower trace. The waveform of the ON-pathway component (blue) was close to an inverted image of the OFF-pathway component (red), and these two components seemed to cancel each other. However, the rising edge of the OFF-pathway component starts 2–3 ms earlier than the descending edge of the ON-pathway

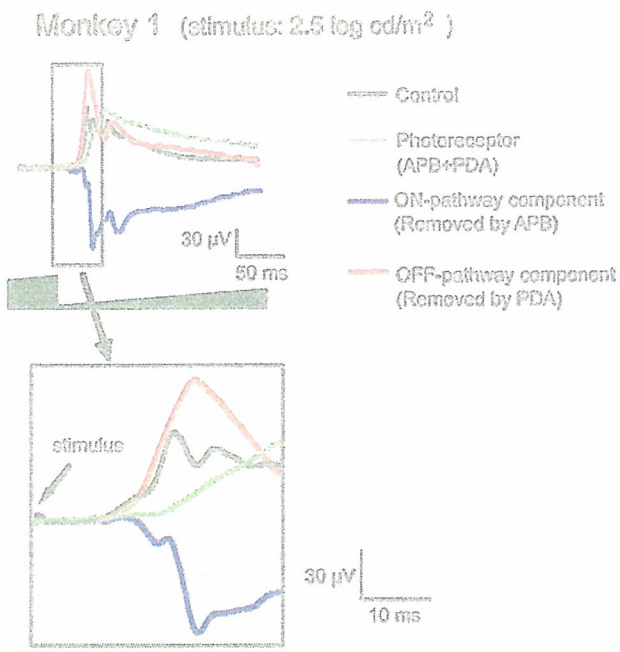

Fig. 6. The photoreceptoral component (after APB + PDA, green), the ON-pathway component (removed by APB, blue) and OFF-pathway component (removed by PDA, red) of the ERG d-wave to 2.5 log cd/m^2 off stimulus of monkey 1. High magnification of the beginning part of ERG is shown in the lower trace.

component, and this timing lag produced the initial positive part of d-wave. The contribution of the cone photoreceptors (green) to the d-wave was mainly at the later phase.

We also noted that inner retinal neurons, both spiking and non-spiking affected by TTX + NMDA, contributed to the d-wave, even though their contribution were not so large. After TTX + NMDA, the amplitude of the d-wave was reduced to 75–90% of the control d-wave. Other studies also showed a reduction of the d-wave amplitude after TTX in monkey (Viswanathan et al., 1999, Viswanathan, Frishman, & Robson, 2002). The oscillatory components were also affected after TTX + NMDA. One interesting finding was that after TTX + NMDA, the later part of the d-wave became larger (Fig. 2). This suggests that the inner retinal neurons affected by TTX + NMDA also contributed to the slow, negative component of the primate d-wave. This component is probably the photopic negative response (Viswanathan et al., 1999).

In conclusion, our results indicated that: (1) the initial rapid phase of the d-wave originates from the activity of the cone OFF-pathway; (2) cone photoreceptors contribute to the later slow phase of the d-wave; (3) the d-wave is also affected by the activity of the cone ON-pathway in opposite polarity; and (4) there is a small, but significant, contribution from inner retinal neurons to the d-wave.

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References

Alexander, K. R., Fishman, G. A., Barnes, C. S., & Grover, S. (2001). On-response deficit in the electroretinogram of the cone system in X-linked retinoschisis. *Investigative Ophthalmology and Visual Science, 42*, 453–459.

Awatramani, G., Wang, J., & Slaughter, M. M. (2001). Amacrine and ganglion cell contributions to the electroretinogram in amphibian retina. *Visual Neuroscience, 18*, 147–156.

Barnes, C. S., Alexander, K. R., & Fishman, G. A. (2002). A distinctive form of congenital stationary night blindness with cone ON-pathway dysfunction. *Ophthalmology, 109*, 575–583.

Bloomfield, S. A. (1996). Effect of spike blockade on the receptive-field size of amacrine and ganglion cells in the rabbit retina. *Journal of Neurophysiology, 75*, 1878–1893.

Brown, K. T. (1968). The electroretinogram: Its components and their origins. *Vision Research, 8*, 633–677.

Bush, R. A., & Sieving, P. A. (1994). A proximal retinal component in the primate photopic ERG a-wave. *Investigative Ophthalmology and Visual Science, 35*, 635–645.

Evers, H. U., & Gouras, P. (1986). Three cone mechanisms in the primate electroretinogram: Two with, one without off-center bipolar responses. *Vision Research, 26*, 245–254.

Heynen, H., & van Norren, D. (1985a). Origin of the electroretinogram in the intact macaque eye-I. Principal component analysis. *Vision Research, 25*, 697–707.

Heynen, H., & van Norren, D. (1985b). Origin of the electroretinogram in the intact macaque eye- II. Current source-density analysis. *Vision Research, 25*, 709–715.

Hood, D. C., Frishman, L. J., Saszik, S., & Viswanathan, S. (2002). Retinal origins of the primate multifocal ERG: Implications for the human response. *Investigative Ophthalmology & Visual Science, 43*, 1673–1685.

Khan, N. W., Kondo, M., Hiriyanna, K. T., Jamison, J. A., Bush, R. A., & Sieving, P. A. (2005). Primate retinal signaling pathways: Suppressing ON-pathway activity in monkey with glutamate analogues mimics human CSNB1-NYX genetic night blindness. *Journal of Neurophysiology, 93*, 481–492.

Kondo, M., & Sieving, P. A. (2001). Primate photopic sine-wave flicker ERG: Vector modeling analysis of component origins using glutamate analogs. *Investigative Ophthalmology and Visual Science, 42*, 305–312.

Massey, S. C., & Miller, R. F. (1990). *N*-Methyl-D-aspartate receptors of ganglion cells in rabbit retina. *Journal of Neurophysiology, 63*, 16–30.

Massey, S. C., Redburn, D. A., & Crawford, M. L. (1983). The effects of 2-amino-4-phosphonobutyric acid (APB) on the ERG and ganglion cell discharge of rabbit retina. *Vision Research, 23*, 1607–1613.

Miller, R. F., & Dowling, J. E. (1970). Intracellular responses of the Muller (glial) cells of mudpuppy retina: Their relation to b-wave of the electroretinogram. *Journal of Neurophysiology, 33*, 323–341.

Narahashi, T. (1974). Chemicals as tools in the study of excitable membranes. *Physiological Reviews, 54*, 813–889.

Narahashi, T., Moore, J. W., & Scott, W. R. (1964). Tetrodotoxin blockage of sodium conductance increase in lobster giant axons. *Journal of General Physiology, 47*, 965–974.

Newman, E. A. (1980). Current source-density analysis of the b-wave of frog retina. *Journal of Neurophysiology, 43*, 1355–1366.

Rangaswamy, N. V., Hood, D. C., & Frishman, L. J. (2003). Regional variations in local contributions to the primate photopic flash ERG: Revealed using the slow-sequence mfERG. *Investigative Ophthalmology and Visual Science, 44*, 3233–3247.

Robson, J. G., & Frishman, L. J. (1995). Response linearity and kinetics of the cat retina: The bipolar cell component of the dark-adapted electroretinogram. *Visual Neuroscience, 12*, 837–850.

Sieving, P. A., Murayama, K., & Naarendorp, F. (1994). Push–pull model of the primate photopic electroretinogram: A role for hyperpolarizing neurons in shaping the b-wave. *Visual Neuroscience, 11*, 519–532.

Slaughter, M. M., & Miller, R. F. (1981). 2-Amino-4-phosphonobutyric acid: A new pharmacological tool for retina research. *Science, 211*, 182–185.

Slaughter, M. M., & Miller, R. F. (1983). An excitatory amino acid antagonist blocks cone input to sign-conserving second-order retinal neurons. *Science, 219*, 1230–1232.

Stockton, R. A., & Slaughter, M. M. (1989). B-wave of the electroretinogram. A reflection of ON bipolar cell activity. *Journal of General Physiology, 93*, 101–122.

Ueno, S., Kondo, M., Niwa, Y., Terasaki, H., & Miyake, Y. (2004). Luminance dependence of neural components that underlies the primate photopic electroretinogram. *Investigative Ophthalmology and Visual Science, 45*, 1033–1040.

Viswanathan, S., Frishman, L. J., Robson, J. G., Harwerth, R. S., & Smith, E. L. (1999). The photopic negative response of the macaque electroretinogram: Reduction by experimental glaucoma. *Investigative Ophthalmology & Visual Science, 40*, 1124–1136.

Viswanathan, S., Frishman, L. J., & Robson, J. G. (2002). Inner-retinal contributions to the photopic sinusoidal flicker electroretinogram of macaques. *Macaque photopic sinusoidal flicker ERG. Documenta Ophthalmologica, 105*, 223–242.

Xu, X., & Karwoski, C. (1994). Current source density analysis of retinal field potentials. II. Pharmacological analysis of the b-wave and M-wave. *Journal of Neurophysiology, 72*, 96–105.

Xu, X., & Karwoski, C. (1995). Current source density analysis of the electroretinographic d wave of frog retina. *Journal of Neurophysiology, 73*, 2459–2469.

Yanagida, T., Koshimizu, M., Kawasaki, K., & Yonemura, D. (1986). Microelectrode depth study of electroretinographic b- and d-waves in frog retina. *Japanese Journal of Ophthalmology, 30*, 298–305.

Reduction of Oscillatory Potentials and Photopic Negative Response in Patients with Autosomal Dominant Optic Atrophy with *OPA1* Mutations

Kentaro Miyata, Makoto Nakamura, Mineo Kondo, Jian Lin, Shinji Ueno, Yozo Miyake, and Hiroko Terasaki

PURPOSE. To study the electroretinographic (ERG) findings in patients with autosomal dominant optic atrophy (ADOA) with *OPA1* mutations.

METHODS. Eight ADOA patients (age range, 24–55 years; mean, 41 years) with *OPA1* mutations were studied. In addition to routine ophthalmological tests, full-field ERGs including the rod response, mixed rod-cone response, oscillatory potentials (OPs), single-flash cone response, and photopic negative response (PhNR) were recorded and compared with those from 25 age-matched controls. The correlation between the ERG data and averaged retinal nerve fiber layer (RNFL) thickness around the optic disk measured by optical coherent tomography, mean deviation of the static perimetry (Humphrey 30-2 program), or corrected visual acuity was also examined.

RESULTS. Amplitudes of the PhNR and OPs, both of which are believed to originate from inner retinal layers, were significantly smaller in ADOA patients than in control subjects ($P < 0.01$). Amplitudes of other ERG components were not statistically different in the two groups. OP amplitude was inversely correlated with the patient's age. The RNFL was thinner and the retinal sensitivities obtained by static perimetry were lower in ADOA patients, but these values were not correlated with the amplitude of PhNR or OPs.

CONCLUSIONS. These results suggested that there are functional impairments not only in the ganglion cell layer but also in the inner nuclear and plexiform layers, including the amacrine cells of ADOA patients with *OPA1* mutations. (*Invest Ophthalmol Vis Sci.* 2007;48:820–824) DOI:10.1167/iovs.06-0845

Autosomal dominant optic atrophy (ADOA) is the most common form of hereditary optic neuropathy. This disease is characterized by symmetrical bilateral optic atrophy associated with a decrease of visual acuity and color vision defect for blue hues.[1–6] Visual impairments usually progress slowly, and phenotypic severity varies considerably among patients even within the same family.[3–5] Histopathologic studies of donor eyes of patients with ADOA suggest that the fundamental pathologic condition is a degeneration of the retinal ganglion cells leading to optic atrophy.[7,8]

ADOA is genetically heterogeneous, and mutations of the *OPA1* gene are one of the causative genetic alterations.[9–17] The OPA1 protein is a mitochondrial dynamin-related guanosine triphosphatase (GTPase) located in the mitochondrial inner membrane space mainly anchored to the cristae of the inner membrane.[16] This protein is considered to be involved in mitochondrial fusion and in maintenance of the mitochondrial genome and network.[19–21]

It was shown that the *OPA1* gene is ubiquitously expressed in several tissues but is most abundant in the retina and brain.[10,22] Recent immunohistochemical studies in rat and mice retinas showed that the OPA1 protein was expressed predominantly in ganglion cell layer but was also expressed in the inner plexiform layer, the inner nuclear layer including the amacrine cells, and the outer plexiform layer.[20,23–25]

It is generally believed that full-field ERG findings in patients with ADOA are normal.[26,27] However, Holder et al.[28] reported that some ADOA patients had a reduction of the P50 component of the pattern ERGs thought to originate distal to the retinal ganglion cells. Because of the results of these immunohistochemical and physiological studies, we thought that a more comprehensive functional examination with the use of electroretinography should be conducted on ADOA patients with *OPA1* mutations.

We show here that the amplitudes of the photopic negative response (PhNR) and the oscillatory potential (OP), each of which is thought to originate from the inner retinal layer, were significantly reduced in the ADOA patients. Interestingly, the reduction of OPs was inversely correlated with patients' ages. These results indicated that the functions not only of the ganglion cell layer but also of the inner nuclear and inner plexiform layers are altered in the human retina with *OPA1* mutations.

PATIENTS AND METHODS

Patients

Among our patients with *OPA1* gene mutations,[16] eight Japanese patients (five men and three women) from six families underwent electroretinographic examination and were recruited for this study. Detailed information on the *OPA1* gene mutations and clinical characteristics in the eight patients have been reported.[16] All the patients had typical characteristics of ADOA; one patient with an apparently atypical *OPA1* gene mutation associated with a negative ERG finding was excluded from this study.[17] The protocol of the study adhered to the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of Nagoya University. Informed consent was obtained from all patients after full explanation of this study.

Clinical Examination

Ophthalmic examination included best-corrected visual acuity, slit lamp biomicroscopy, indirect ophthalmoscopy, fundus photography,

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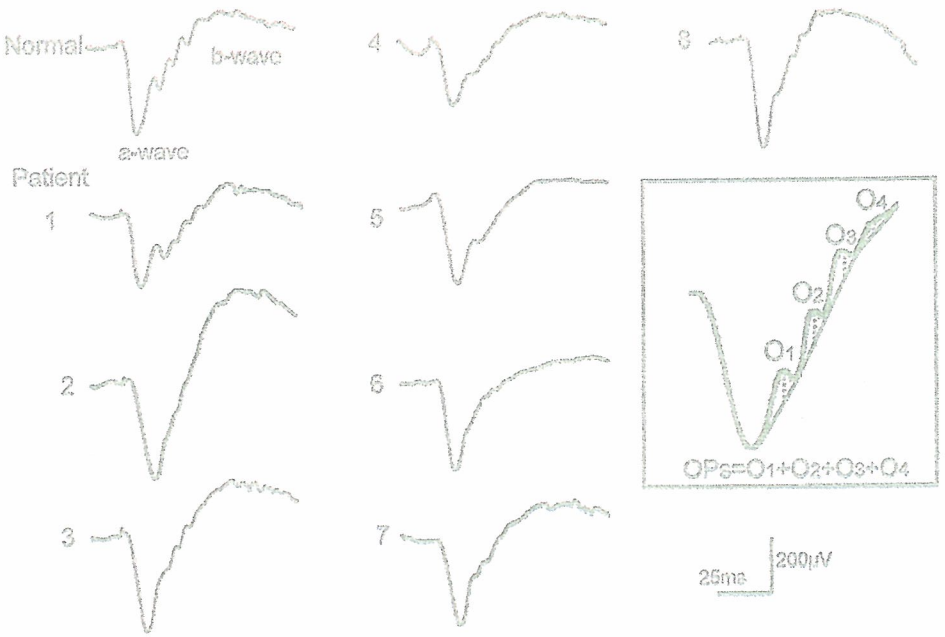

FIGURE 1. Mixed rod-cone maximal ERGs recorded from a control subject and eight ADOA patients with *OPA1* mutations. *Inset*: method used to measure OP amplitudes, expressed as the sum of the first four positive wavelets on the ascending limb of the b-wave.

visual field testing by kinetic and static perimetry, color vision testing with Farnsworth panel D-15 plates, retinal nerve fiber layer (RNFL) thickness analysis, and full-field electroretinography. Static perimetry was performed using the standard 30–2 program (size V target; Humphrey Field Analyzer; Carl Zeiss Meditec, Dublin, CA), and the mean visual field sensitivity (dB) within 30° borders of the visual field was determined. RNFL thickness was measured by optical coherence tomography (OCT-3000; Carl Zeiss Meditec) by calculating the mean RNFL thickness from 512 points around the optic disk.

Electroretinograms

Pupils were fully dilated with a combination of 0.5% tropicamide and 0.5% phenylephrine hydrochloride. Corneas were anesthetized by topical 0.4% oxybuprocaine hydrochloride before contact lens electrodes were inserted. Full-field electroretinograms (ERGs) were recorded with a Burian-Allen bipolar contact lens electrode (Hansen Ophthalmic Development Laboratories, Iowa City, IA) and Ganzfeld ERG recording system (model GS2000; LACE, Pisa, Italy). A time constant of 0.1 second and a 500-Hz high-cut filter were used.

After 30 minutes of dark adaptation, the rod response was recorded with a dim blue light at an intensity of 5.2×10^{-3} cd · s/m^2. A mixed rod-cone maximal ERG was elicited by a white flash at an intensity of 44.2 cd · s/m^2. After 10 minutes of light adaptation, a single-flash cone ERG was elicited by a white stimulus of 1.9 cd · s/m^2 on a white background of 18 cd · s/m^2.

Methods used to measure the amplitudes of the OPs and photopic negative response (PhNR) are shown in the insets of Figures 1 and 2, respectively. OP amplitudes were calculated by adding the first four positive wavelets on the ascending limb of the b-wave (Fig. 1, inset). The amplitude of the PhNR was measured from the baseline to the first negative trough after the b-wave of the single-flash cone ERG (Fig. 2, inset).

RESULTS

Clinical Findings

Clinical characteristics of the patients with *OPA1* mutations are summarized in Table 1. Visual acuities of the eight patients ranged from 0.7 to 0.01. Changes in the optic disks were symmetrical in all patients. Three patients had temporal pallor only, and in one it was subtle. The other four patients had diffuse atrophy of the optic disk. Visual field tests by Goldmann kinetic perimetry showed central scotoma in three patients (patients 1–3), concentric constriction in one patient (patient

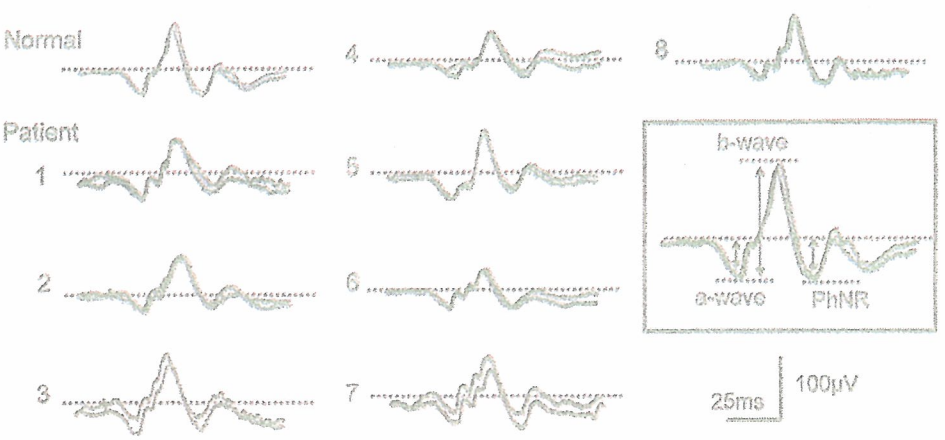

FIGURE 2. Single-flash cone ERGs recorded from a control subject and eight ADOA patients with *OPA1* mutations. *Dotted line*: baseline of the ERG response. *Inset*: method used to measure the amplitude of PhNR and the a- and b-waves.