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Electroretinographic findings in a patient with congenital stationary night blindness due to a novel *NYX* mutation

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Abstract

Purpose—To document a novel *NYX* gene mutation in a patient with X-linked complete congenital stationary night blindness and to describe this patient's electroretinogram (ERG) characteristics.

Methods—ERGs were recorded from a 17-year-old male with a previously unreported *NYX* mutation (819G>A) that results in a missense codon change (Trp237Ter). ERGs were recorded in response to brief-flash stimuli, 6.33 Hz sawtooth flicker, and sinusoidal flicker ranging from 6.33 to 100 Hz. The omitted stimulus response (OSR) of the flicker ERG, which is thought to be generated within the ON-pathway, was also assessed.

Results—The patient's single-flash responses were consistent with previously documented *NYX* ERG characteristics, including a high-luminance flash response that was electronegative under dark-adapted conditions and a square-like a-wave followed by an abnormally shaped positive potential under light-adapted conditions, both of which are consistent with an ON-pathway deficit. Further evidence for an ON-pathway deficit included: 1) ERGs to rapid-on sawtooth flicker in which b-wave amplitude was reduced more than a-wave amplitude, and 2) responses to sinusoidal flicker that lacked the normal amplitude minimum and phase inflection near 12 Hz, ERG characteristics that are like those of patients with other *NYX* mutations. Novel findings included a pronounced amplitude attenuation for sinusoidal flicker at frequencies above approximately 50 Hz and an absent OSR, suggesting ON-pathway dysfunction at high frequencies.

Conclusion—The substantial loss of ERG amplitude and apparent ON-pathway dysfunction at high temporal frequencies distinguish this patient with a Trp237Ter *NYX* mutation from those with other previously reported *NYX* mutations.

INTRODUCTION

Congenital stationary night blindness (CSNB) refers to a group of inherited retinal disorders characterized by nyctalopia that is present from birth. One form of the complete type of CSNB, with X-linked inheritance, is due to mutations in the *NYX* gene (CSNB1-*NYX*).[1,2] Several different mutations in the *NYX* gene have been identified, with most mutations occurring in exon 3.[3] The *NYX* gene encodes nyctalopin, a small leucine-rich repeat (LRR) protein that is expressed in both the inner and outer plexiform layers.[4] Although the function of nyctalopin is not entirely understood, it appears to be essential for localizing the transient receptor potential melastatin 1 (TRPM1) channel to the dendritic tips of ON bipolar cells.[5]

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The TRPM1 channel is the nonselective cation channel that mediates the light response of ON bipolar cells.

The standard clinical ERG characteristics of patients with *NYX* mutations includes: (1) an "electronegative" response to a high-luminance flash under dark-adapted conditions, in which b-wave amplitude is reduced substantially more than a-wave amplitude; (2) an abnormal response to a brief flash under light-adapted conditions that has an a-wave with a square-like appearance followed by an abnormally shaped positive potential; and (3) a normal or slightly subnormal amplitude in response to 30-Hz flicker. These ERG characteristics are generally considered to represent a defect in signal transmission from photoreceptors to ON bipolar cells,[6] which is consistent with the essential role of nyctalopin in maintaining the integrity of the retinal ON pathway.[5]

Further evidence of an ON-pathway deficit in CSNB1-*NYX* was provided by Khan et al.,[7] who examined the ERGs of four patients with three different *NYX* mutations in response to sawtooth and sinusoidal flicker across a broad range of temporal frequencies. The patients' ERG waveforms in response to low-frequency rapid-on sawtooth flicker were abnormal in shape, but the ERG waveforms for rapid-off sawtooth flicker were normal in shape, indicating an ON-pathway deficit. Additionally, the patients' ERG responses to sinusoidal flicker lacked the normal amplitude minimum and phase inflection seen at approximately 12 Hz. Because an interaction between the ON and OFF pathways is thought to underlie the amplitude minimum and phase inflection at 12 Hz, the absence of these characteristic features in patients with *NYX* mutations is consistent with an ON-pathway defect[7]. In contrast to the abnormalities at low temporal frequencies, ERG amplitudes for both sawtooth and sinusoidal flicker were normal at high temporal frequencies in these patients. The finding of abnormal low frequency flicker responses but normal responses for high frequencies led Khan et al.[7] to conclude that nyctalopin is of primary importance for the function of a subset of ON bipolar cells that are tuned to relatively low temporal frequencies.

The present report describes the ERG characteristics of a patient with a novel *NYX* gene mutation who had both similarities to and differences from the *NYX* patients reported by Khan et al.[7] Single-flash and 32-Hz flicker responses were recorded to determine how this patient's clinical ERG compares to previously reported CSNB1-*NYX* ERG waveforms.[1] The nature and extent of ON-pathway dysfunction was evaluated using an approach similar to that of Khan et al.[7] Specifically, ERGs were recorded in response to rapid-on and rapid-off sawtooth flicker and to sinusoidal flicker presented at a range of temporal frequencies.

The omitted stimulus response (OSR), a newly reported component of the human flicker ERG,[8] was also evaluated as an index of ON-pathway function. The OSR refers to an extra response cycle that occurs when a flicker train is interrupted or terminated.[9] Based on single unit recordings from mouse and salamander retina, it has been proposed that the OSR arises from a resonant oscillation within ON bipolar cells.[10] In the present study, the OSR of the flicker ERG was evaluated by determining whether the ERG waveforms of the *NYX* patient contained an additional response following flicker train offset.

MATERIALS AND METHODS

The patient is a 17-year-old Asian male who initially presented with poor night vision and has a family history that includes a maternal aunt and uncle who also had poor night vision. He is highly myopic (OD: -13.50 + 0.75 × 120; OS: -14.25 + 1.00 × 100) and has reduced visual acuity (OD: $20/60^{-2}$; OS: 20/50), horizontal nystagmus, normal color vision (Ishihara plates), and normal intraocular pressures of 13 mm Hg (OD) and 14 mm Hg (OS). Slit lamp

examination of the anterior segment did not show any abnormalities. A fundus exam showed normal but tilted optic discs and a prominent choroidal pattern in both eyes, characteristic of high myopia.

Molecular genetic analysis was performed using a blood sample. DNA was purified from leukocytes (GenElute mini prep kit, Sigma) and the 3 exons of the *NYX* gene were amplified using PCR and previously described primer sequences[11] that were synthesized by Integrated DNA Technologies. Products of the PCR were sequenced with the primers used for amplification. The patient was found to have a mutation in the *NYX* gene that, to our knowledge, has not been reported previously. This mutation is a single nucleotide change in exon 3 (819G>A), which causes a nonsense codon change (Trp237Ter) and occurs within the LRR-9 motif.

ERGs were recorded from the patient's right eye using a Burian-Allen bipolar contact lens electrode. The left eye was patched. Prior to all ERG recordings, the pupil of the tested eye was dilated with 2.5% phenylephrine hydrochloride and 1% tropicamide drops.

Full-field single-flash and 32-Hz flicker ERGs were obtained in response to ganzfeld stimuli produced by xenon flashes of 5.1 cd•s/m^2 that were presented within a Nicolet diffusing sphere (Nicolet Instrument Technologies). The single-flash stimuli were presented under both dark- and light-adapted conditions; the 32-Hz flicker was presented under light-adapted conditions. ERG responses were acquired with a Nicolet Viking IV signal-averaging system. The patient's single-flash and 32-Hz flicker ERG responses were compared to those from a database of normally sighted controls tested under identical conditions, available through the UIC Inherited Retinal Disease and Electrophysiology Service.

In a separate testing session, ERGs were recorded in response to sawtooth and sinusoidal flicker trains. The full-field flicker stimuli were generated by LED arrays with peak wavelengths of 516 nm and 640 nm and were presented in a desktop ColorDome (Diagnosys, LLC). The mean luminance of the flicker trains was 200 cd/m^2, consisting of 100 cd/m^2 of each of the 516-nm and 640-nm lights. A rod-saturating adapting field of 12.3 cd/m^2, generated by LEDs with a peak wavelength of 464 nm, was presented continuously. During the flicker train, the combined 516-nm and 640-nm light was modulated either as rapid-on sawtooth flicker, rapid-off sawtooth flicker, or sinusoidal flicker. The sawtooth stimuli were presented at 6.33 Hz and the sinusoidally modulated stimuli were presented at temporal frequencies ranging from 6.33 to 100 Hz. The sawtooth and sinusoidal flicker trains were approximately 1 s in duration, with an even number of cycles. Between presentations of the flicker stimuli, unmodulated 516-nm plus 640-nm light was presented at the mean level, along with the rod-saturating adapting field.

The responses to the sawtooth and sinusoidal stimuli were acquired with a Diagnosys E^2 system. A minimum of three responses were obtained for both the sawtooth flicker and for each temporal frequency of sinusoidal flicker. Analyses were based on the average of the three responses that had the fewest eye movement artifacts. Fundamental amplitudes and phases of the ERG responses to sinusoidal flicker were derived from Fast Fourier Transforms (FFT). The initial and final few cycles of the waveform were omitted from the analysis to avoid onset and offset transients.

The patient's ERG responses to sinusoidal flicker were compared to those of 12 visually normal subjects (8 female and 4 male), ranging in age from 23 to 55 years, obtained under identical conditions. For 7 of these 12 subjects, ERGs were also obtained in response to 6.33 Hz rapid-on and rapid-off sawtooth flicker. The tested eye (selected at random) of the control subjects had best-corrected visual acuity of 20/20 or better as measured with the Lighthouse Distance Visual Acuity Chart, normal letter contrast sensitivity as measured with

the Pelli-Robson Contrast Sensitivity Chart, clear ocular media, and normal-appearing fundi on ophthalmologic examination. The spherical refractive error of the tested eye of the control subjects ranged from 0.00 D to -11.75 D.

Written informed consent was obtained from the *NYX* patient and from the control subjects. The research protocol was approved by an institutional review board at the University of Illinois at Chicago and conformed to the tenants of the Declaration of Helsinki.

RESULTS

Figure 1 illustrates ERG responses from a representative control subject (first column) and the *NYX* patient (second column). Each waveform represents the average of at least two responses. For the *NYX* patient, the dark-adapted response to a high-luminance flash (first row) had an electronegative waveform, in which the b-wave amplitude was more attenuated than the a-wave amplitude. The a-wave amplitude was approximately 100 μV below the lower limit of the normal range, which is consistent with previous reports of a reduced a-wave amplitude in some patients with *NYX* gene mutations.[1,12] The patient's ERG response to a high luminance flash under light-adapted conditions (second row) had an a-wave amplitude that was within the normal range, but the waveform had a prolonged negativity with a late positive-going potential. The amplitude of the patient's response to 32-Hz flicker (third row) was approximately 30 μV below the lower limit of the normal range and the implicit time was delayed by approximately 3 ms compared to the upper limit of the normal implicit time range.

Figure 2 shows the mean of three responses to rapid-on (top) and rapid-off (bottom) sawtooth flicker for one of the 7 control subjects (first column) and the *NYX* patient (second column). The stimulus waveforms are plotted along the abscissas in each panel. The response of the control subject to the rapid-on stimulus contained an a-wave followed by a b-wave, and the response to the rapid-off stimulus showed a d-wave, consistent with previous reports.[7,13,14] The shape of the patient's response to the rapid-on stimulus was markedly different from that of the control subject, in that b-wave amplitude was reduced substantially more than a-wave amplitude. In comparison, a normally shaped d-wave was present in response to the rapid-off stimulus.

Figure 3 shows the log amplitude (top) and phase (bottom) of the FFT-derived fundamental response to sinusoidal flicker as a function of stimulus temporal frequency for the *NYX* patient (circles) compared to the range of values from the 12 normally sighted control subjects (shaded region). The amplitude function for the control subjects had a peak near 32 Hz and a minimum near 12 Hz, as expected from previous studies.[7,15] This minimum was not present in the amplitude function of the patient, a finding that is consistent with an attenuated ON bipolar cell response,[7] as noted in the Introduction. For frequencies above approximately 50 Hz, the response of the patient was reduced by at least 0.7 log units below the lower limit of normal.

The phase function of the 12 control subjects (Figure 3, bottom) was non-monotonic, with a phase inflection near 12 Hz and a systematic phase decrease for frequencies above approximately 20 Hz, as is typical of visually normal subjects.[15] In comparison, the phase function of the patient decreased monotonically as stimulus frequency increased, the normal phase inflection at 12 Hz was absent, and there was a phase lag for frequencies above 12 Hz. These phase characteristics are similar to those of patients with other *NYX* mutations and are presumed to reflect ON-pathway dysfunction.[7]

The results for the OSR are presented in Figure 4. This figure shows the ends of ERG recordings obtained in response to sinusoidal flicker at the stimulus frequencies indicated to

the right of the plots. The left column presents the results for the same control subject shown in Figure 2 and the right column shows the waveforms for the *NYX* patient. Each x-axis is plotted in terms of cycles rather than time in order to facilitate comparisons of the waveforms across the different stimulus temporal frequencies. The last two stimulus cycles are shown at the bottom of each column. The dotted cycle represents the omitted stimulus, which refers to the next stimulus cycle had the flicker train continued.

The control subject showed no OSR at 31.25 Hz (top row), which is typical at this frequency.[8] The small positive-going response component that is apparent at the end of the 31.25-Hz waveform of this control subject is the response to the rising portion of the last stimulus cycle. On the other hand, for stimulus frequencies between 38.46 Hz to 62.50 Hz, the ERG waveforms of this control subject contained an extra response, indicated by the arrows. The amplitude of this response was maximal at approximately 50 Hz, as described previously.[8] This additional response following flicker train offset represents the OSR, and it was present in the waveforms of all 12 control subjects across the frequency range of 38.46 Hz to 62.50 Hz. By comparison, no OSR was present in the flicker ERG of the *NYX* patient at any stimulus frequency. Instead, the patient's waveforms across the frequency range of 38.46 Hz to 62.50 Hz were similar to the waveform recorded at 31.25 Hz.

DISCUSSION

This report documents the ERG characteristics of a patient with CSNB1 who has a novel *NYX* gene mutation. This mutation was identified as a single nucleotide change in exon 3, which causes a nonsense codon change (Trp237Ter) within the LRR-9 motif. Based on the location of the mutation within the reported structure of the *NYX* gene,[2] a substantial truncation of the nyctalopin protein is expected to occur, with the loss of the GPI anchor and at least one of the LRRs. This mutation is identified as disease-causing by Mutation Taster,[16] but its specific effect on protein properties is presently unknown.

The single-flash ERG responses of our *NYX* patient are consistent with previously documented *NYX* ERG characteristics.[1] That is, his dark-adapted response to a high-luminance flash was electronegative and the cone response featured an a-wave with a square-like appearance followed by an abnormally shaped positive potential. These ERG abnormalities are generally considered to represent a defect in signal transmission from photoreceptors to ON bipolar cells.[6] Moreover, our patient had sawtooth and sinusoidal flicker ERGs that were similar to those of the *NYX* patients reported by Khan et al.[7] Specifically, there was a marked attenuation of b-wave amplitude in response to rapid-on sawtooth flicker but a relatively preserved d-wave response to rapid-off sawtooth flicker, as well as a temporal response function for sinusoidal flicker that lacked the normal amplitude minimum and phase inflection near 12 Hz. These sawtooth and sinusoidal flicker data provide evidence of ON-pathway dysfunction at low temporal frequencies.

At high temporal frequencies, the *NYX* patients reported by Khan et al.[7] had sinusoidal flicker ERG responses that were within the normal range. Based on the selective low-frequency ERG abnormalities of their *NYX* patients, Khan et al.[7] proposed that nyctalopin may preferentially affect signal transmission in a subset of ON bipolar cells that are tuned to low temporal frequencies. In contrast, our *NYX* patient had an attenuation of ERG amplitude over the entire high frequency range. This amplitude attenuation is unlikely to be due to the patient's high myopia, because the myopic control subjects (who had spherical refractive errors ranging up to -11.75 D) did not have a similar high-frequency amplitude attenuation. Instead, we propose that the high-frequency amplitude attenuation is related to ON-pathway dysfunction. In support of this conjecture, there is evidence for a large ON

bipolar cell contribution to the high-frequency flicker ERG,[17] so that an ERG amplitude attenuation would be expected to occur if the ON bipolar cell contribution is reduced.

Further support for ON-pathway dysfunction at high temporal frequencies is that our *NYX* patient showed no OSR in his high-frequency flicker ERG waveforms. The OSR is presumed to be generated by retinal ON bipolar cells,[10] so its absence in our *NYX* patient is consistent with a deficient ON pathway. Of note, the OSR was absent at frequencies that showed a relatively small loss of fundamental amplitude. For example, the fundamental amplitude was reduced by less than a factor of 2 at 38.46 and 41.67 Hz, but the OSR was completely absent. Therefore, it is unlikely that the absence of the OSR can be attributed to an overall reduction in the ERG amplitude.

The explanation for the difference between the abnormal high-frequency flicker ERGs of the *NYX* patient of the present study and the normal flicker ERGs of the *NYX* patients of Khan et al.[7] is presently unclear, but it may be related to the specific gene mutations of the patients and the effect on nyctalopin. Further work with a larger sample of *NYX* patients is needed to determine the exact relationship between the nature of the *NYX* mutation and the temporal frequency range over which ON-pathway function may be affected.

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References

1. Bech-Hansen NT, Naylor MJ, Maybaum TA, Sparkes RL, Koop B, Birch DG, Bergen AA, Prinsen CF, Polomeno RC, Gal A, Drack AV, Musarella MA, Jacobson SG, Young RS, Weleber RG. Mutations in NYX, encoding the leucine-rich proteoglycan nyctalopin, cause X-linked complete congenital stationary night blindness. Nat Genet. 2000; 26:319–323. [PubMed: 11062471]
2. Pusch CM, Zeitz C, Brandau O, Pesch K, Achatz H, Feil S, Scharfe C, Maurer J, Jacobi FK, Pinckers A, Andreasson S, Hardcastle A, Wissinger B, Berger W, Meindl A. The complete form of X-linked congenital stationary night blindness is caused by mutations in a gene encoding a leucine-rich repeat protein. Nat Genet. 2000; 26:324–327. [PubMed: 11062472]
3. Zeitz C, Labs S, Lorenz B, Forster U, Uksti J, Kroes HY, De Baere E, Leroy BP, Cremers FP, Wittmer M, van Genderen MM, Sahel JA, Audo I, Poloschek CM, Mohand-Saïd S, Fleischhauer JC, Hüffmeier U, Moskova-Doumanova V, Levin AV, Hamel CP, Leifert D, Munier FL, Schorderet DF, Zrenner E, Friedburg C, Wissinger B, Kohl S, Berger W. Genotyping microarray for CSNB-associated genes. Invest Ophthalmol Vis Sci. 2009; 50:5919–5926. [PubMed: 19578023]
4. Morgans CW, Ren G, Akileswaran L. Localization of nyctalopin in the mammalian retina. Eur J Neurosci. 2006; 23:1163–1171. [PubMed: 16553780]
5. Pearring JN, Bojang P Jr, Shen Y, Koike C, Furukawa T, Nawy S, Gregg RG. A role for nyctalopin, a small leucine-rich repeat protein, in localizing the TRP melastatin 1 channel to retinal depolarizing bipolar cell dendrites. J Neurosci. 2011; 31:10060–10066. [PubMed: 21734298]
6. Miyake, Y.; Horiguchi, M.; Suzuki, S.; Kondo, M.; Tanikawa, A. Complete and incomplete type congenital stationary night blindness (CSNB) as a model of “off-retina” and “on-retina”. In: LaVail, M., editor. Degenerative Retinal Diseases. New York: Plenum Press; 1997. p. 31-41.
7. Khan NW, Kondo M, Hiriyanna KT, Jamison JA, Bush RA, Sieving PA. Primate Retinal Signaling Pathways: Suppressing ON-Pathway Activity in Monkey With Glutamate Analogues Mimics Human CSNB1-NYX Genetic Night Blindness. J Neurophysiol. 2005; 93:481–92. [PubMed: 15331616]
8. McAnany JJ, Alexander KR. 2009 Is there an omitted stimulus response in the human cone flicker electroretinogram? Vis Neurosci. 26:189–194. [PubMed: 19272196]

9. Schwartz G, Harris R, Shrom D, Berry MJ. Detection and prediction of periodic patterns by the retina. Nat Neurosci. 2007; 10:552–554. [PubMed: 17450138]
10. Schwartz G, Berry MJ. Sophisticated temporal pattern recognition in retinal ganglion cells. J Neurophysiol. 2008; 99:1787–1798. [PubMed: 18272878]
11. Zeitz C, Minotti R, Feil S, Mátyás G, Cremers FP, Hoyng CB, Berger W. Novel mutations in CACNA1F and NYX in Dutch families with X-linked congenital stationary night blindness. Mol Vis. 2005; 11:179–183. [PubMed: 15761389]
12. Leroy BP, Budde BS, Wittmer M, De Baere E, Berger W, Zeitz C. A common NYX mutation in Flemish patients with X linked CSNB. Br J Ophthalmol. 2009; 93:692–696. [PubMed: 18617546]
13. Alexander KR, Barnes CS, Fishman GA. High-frequency attenuation of the cone ERG and ON-response deficits in X-linked retinoschisis. Invest Ophthalmol Vis Sci. 2001; 42:2094–2101. [PubMed: 11481277]
14. Alexander KR, Fishman GA, Barnes CS, Grover S. On-response deficit in the electroretinogram of the cone system in X-linked retinoschisis. Invest Ophthalmol Vis Sci. 2001; 42:453–459. [PubMed: 11157882]
15. Burns SA, Elsner AE, Kreitz MR. Analysis of nonlinearities in the flicker ERG. Optom Vis Sci. 1992; 69:95–105. [PubMed: 1584559]
16. Schwarz JM, Rödelsperger C, Schuelke M, Seelow D. MutationTaster evaluates disease-causing potential of sequence alterations. Nat Methods. 2010; 7:575–576. [PubMed: 20676075]
17. Kondo M, Sieving PA. Primate photopic sinewave flicker ERG: vector modeling analysis of component origins using glutamate analogs. Invest Ophthalmol Vis Sci. 2001; 42:305–312. [PubMed: 11133883]

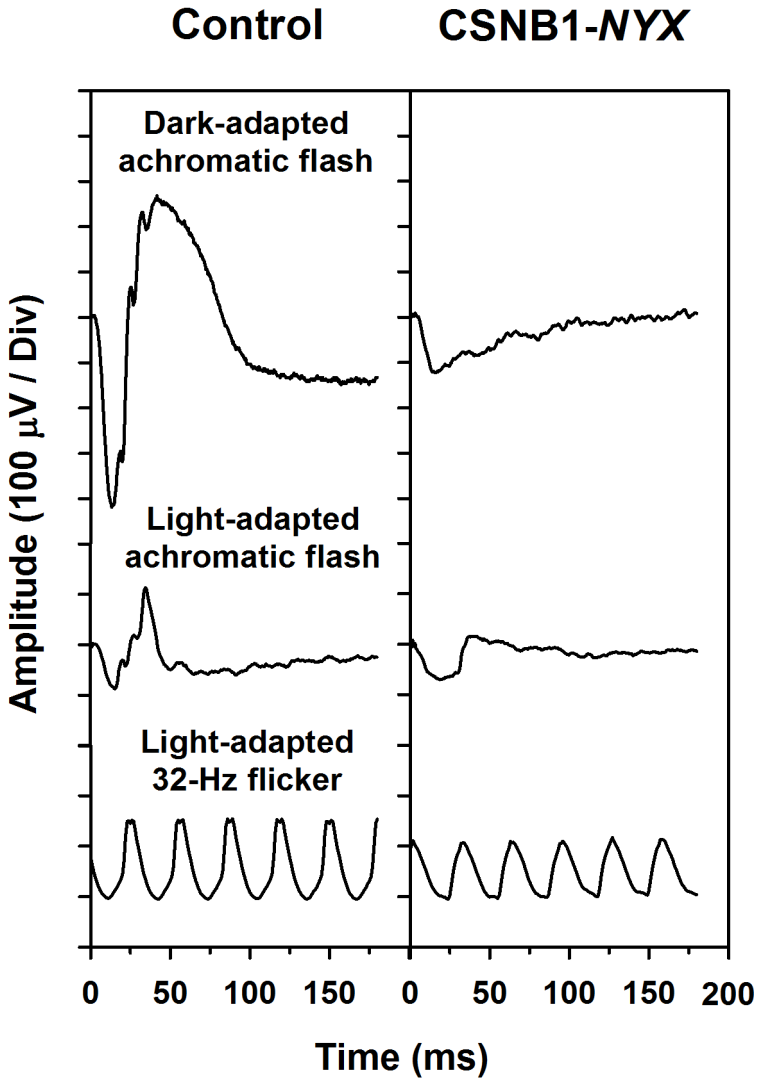

Figure 1.
ERG responses of a representative control subject (left) and the patient (right) to an achromatic flash under dark-adapted conditions (first row), light-adapted conditions (second row), and to 32-Hz achromatic flicker under light-adapted conditions (third row).

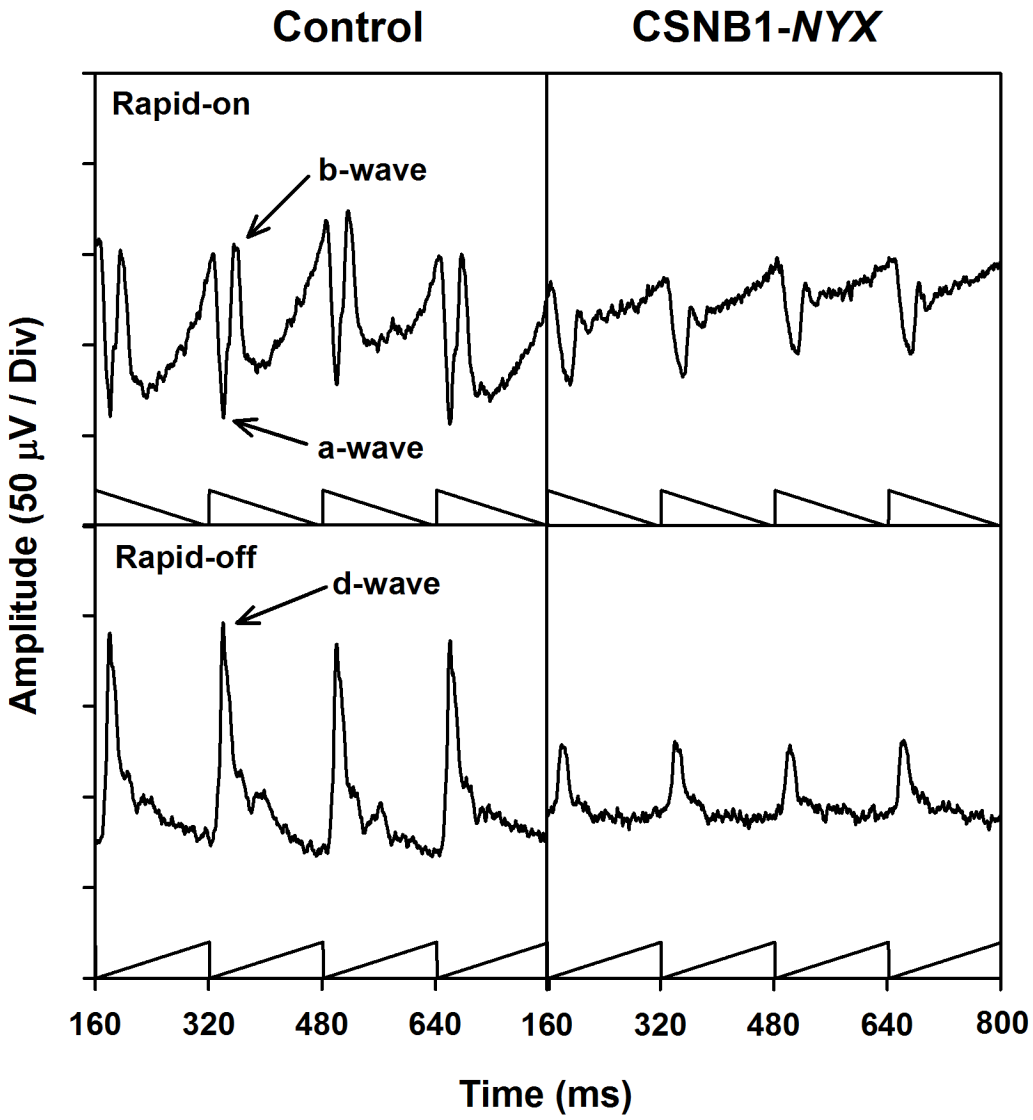

Figure 2.
ERG waveforms in response to rapid-on (top) and rapid-off (bottom) sawtooth flicker for one representative control subject (left) and the patient (right). The ERG waveforms illustrate the middle 4 response cycles elicited by the 1-s sawtooth flicker. The stimulus waveforms are plotted along the abscissas.

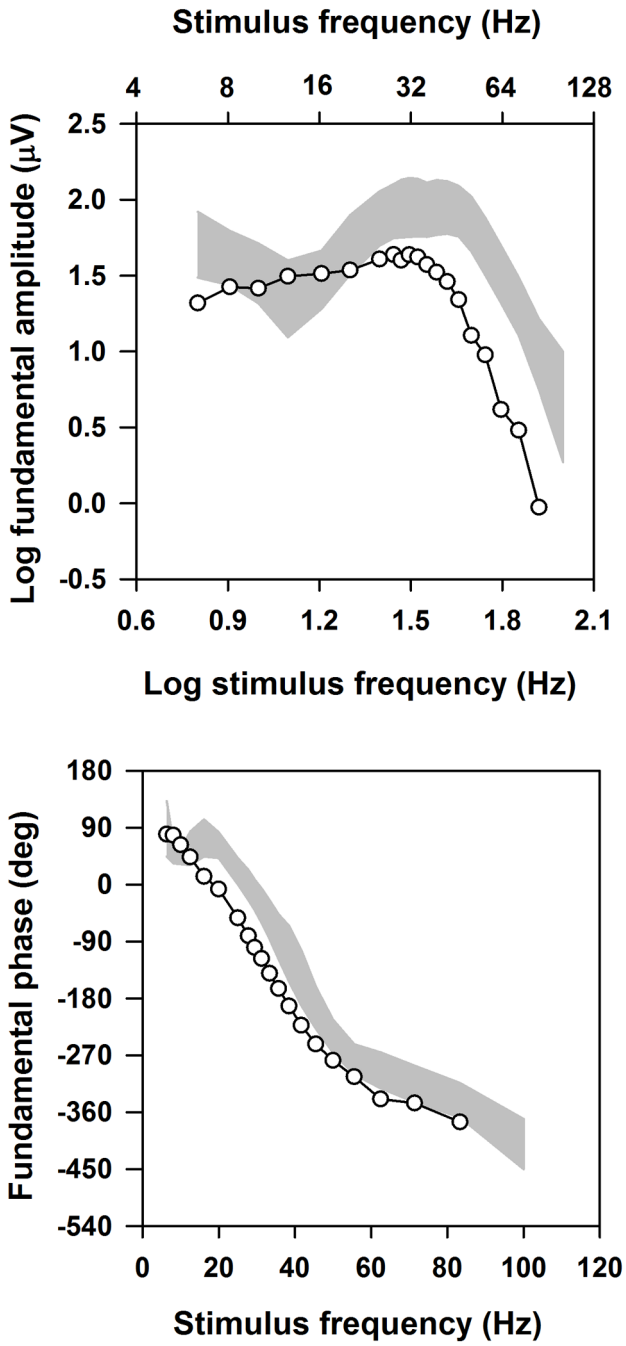

Figure 3.

Log fundamental amplitude (upper panel) and phase (lower panel) of the flicker ERG response as a function of stimulus frequency on log coordinates and linear coordinates (upper and lower panels, respectively). Data for the patient (circles) are compared to the range of normal (shaded region). No data point for the patient is plotted at 100 Hz because the response at this frequency did not exceed the noise level, which was defined as the mean of the fundamental amplitudes at the two frequencies on either side of the stimulus frequency.

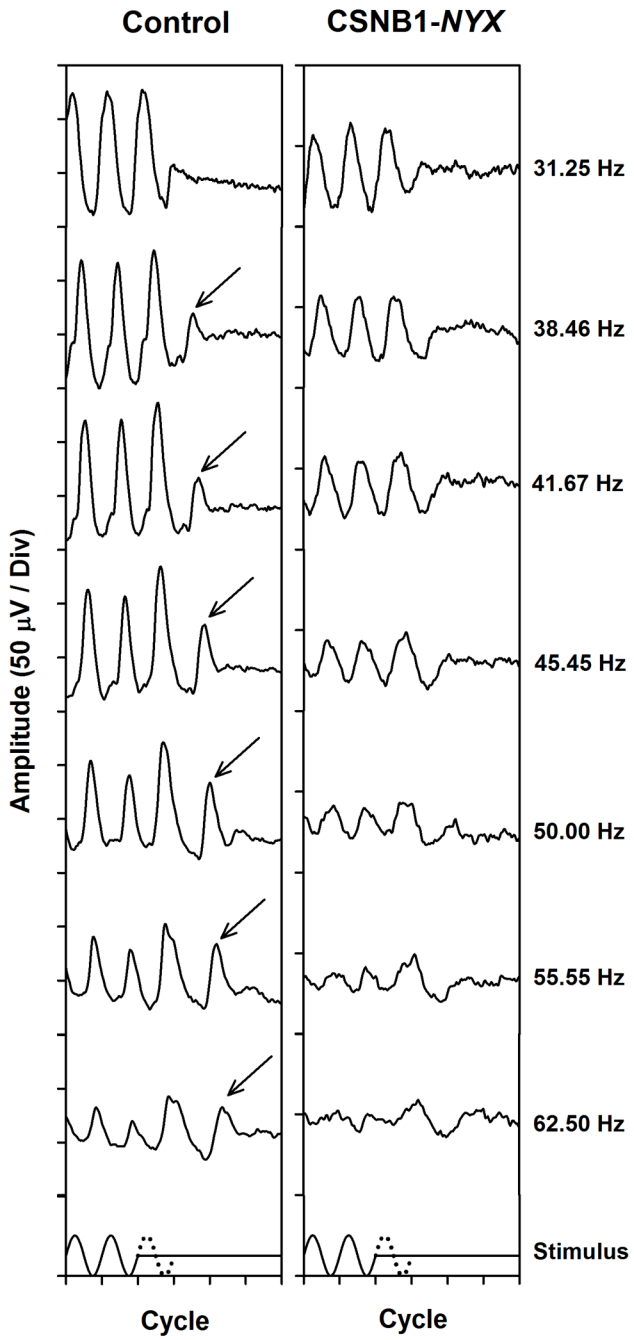

Figure 4.

ERG waveforms for a representative control subject (left) and the patient (right). Only the last few cycles of the flicker ERG waveforms are shown. Stimulus temporal frequencies are indicated to the right of the waveforms. Arrows indicate the OSR. The ERG traces are plotted in terms of stimulus cycle, with the stimulus waveform given below the responses. The dotted cycle represents the omitted stimulus, which refers to the next stimulus cycle had the flicker train continued. The y-axis amplitude scales differ between the patient and control, given the patient's relative attenuation of ERG amplitude over this frequency range.