ON and OFF Electroretinography and Contrast Sensitivity in Duchenne Muscular Dystrophy

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Purpose. The study investigated possible asymmetric dysfunction of the ON and OFF visual mechanisms in DMD (Duchenne muscular dystrophy) patients associated with specific genetic alterations.

Methods. Nineteen DMD patients and 7 heterozygous DMD carriers were tested, as well as 19 age-matched controls. Full-field ERGs were recorded using mesopic (1 cd/m^2) and photopic (250 cd/m^2) sawtooth luminance modulations as stimuli: rapid increase and ramping decrease (to isolate ON responses) or rapid decrease and ramping increase (for OFF responses). In addition, a psychophysical study comprised contrast sensitivity tests using two checkerboard stimuli at either higher (ON) or lower (OFF) luminance relative to the background: 0.3 cycles per degree (cpd) presented for 33 ms (low spatial frequency, short duration) and 2 cpd presented for 1500 ms (high spatial frequency, long duration).

Results. A significant ERG amplitude reduction, relative to controls, was detected in the DMD patients in the mesopic positive peaks for both ON and OFF stimuli, as well as for the photopic ON stimulus ($P < 0.05$). Contrast sensitivity was significantly lower in the DMD patients ($P < 0.05$) relative to controls for the ON stimuli. Neither the ERG nor the contrast sensitivities were altered in the carriers.

Conclusions. This study suggests that there are ON and OFF ERG alterations when both rods and cones contribute to the ERG responses in DMD patients. When only cones are activated there is an asymmetrical ERG alteration, also revealed by the contrast sensitivity measurements.

Keywords: dystrophin, electroretinogram, contrast sensitivity, retina, ON OFF pathways

Duchenne muscular dystrophy (DMD) is an X-linked recessive genetic disease caused by transcription alterations in dystrophin, a 427-kDa protein (Dp427), that lead to a severe muscular impairment.[1,2] Shorter isoforms of the dystrophin protein, also transcribed by the dystrophin gene, are present in different tissues.[3] The 71-kDa isoform (Dp71) is expressed in different organs such as liver, lung, kidney, and central nervous system.[4] The 116- and 140-kDa isoforms (Dp116 and Dp140) are mainly expressed in the peripheral and central nervous system, respectively.[5,6] Finally, the largest isoform, the 260-kDa dystrophin (Dp260) isoform, is mainly found in the outer plexiform layer (OPL) of the retina and is considered to be the retinal isoform of dystrophin.[7]

The existence of dystrophin in the retina has been shown by several groups. In addition to the Dp260 isoform, Dp427, and Dp140 and Dp71 isoforms were also shown to be present in the mouse retina.[7–18] However, the specific role dystrophin plays in the retina is still not completely known, and there is also a lack of information on the physiological changes resulting from a deficiency of the different isoforms. The Dp71 isoform is associated with the Müller glial cells and with the astrocytes of the retina,[11,17,19] and its dysfunction does not affect the electroretinogram (ERG) of Dp71-null mice.[20]

The presence of abnormal ERG responses in DMD patients showed that a deficit in the dystrophin isoforms leads to functional alterations.[21–27] The b-wave of the scotopic full-field ERG is reduced in DMD patients[22,27] as well as in the mdx^{Cv3} mouse model of DMD[28] with alterations in Dp427 and the smaller isoforms.[29] In addition to the studies using full-field short-duration flash ERGs,[22,30,31] DMD patients have been tested with photopic long-duration flash stimuli in order to separate ON and OFF contributions to the ERG. A positive wave can be found at onset of the stimulus (the b-wave) and another positive deflection at stimulus offset (the d-wave). The long-flash paradigm has been used to measure selective retinal alterations in the ON and OFF responses in different retinal disorders such as retinal dystrophies[32] and glaucoma.[33,34] In DMD patients, the ON response under photopic stimulation (b-wave) was found to be impaired, whereas the OFF response (d-wave) was preserved.[24,35] Moreover, heterozygous female DMD

carriers displayed a reduced b-wave amplitude in the ON response to the long-duration flash stimulus,[36] but no ERG response alterations were found in the short-duration flash responses.[36,37] In addition, our group has recently reported color vision and contrast sensitivity losses in patients with deletions of the dystrophin gene downstream of exon 30, thus affecting both the Dp260 and the Dp427. In patients with gene alterations upstream of exon 30, which affect only the Dp427, these losses are either absent or less severe.[38,39]

The intersubject variability in the responses to long-duration flash stimuli is large; these are exclusively driven by the cones since the measurements are normally performed under light-adapted conditions. One alternative ERG protocol to evaluate ON and OFF retinal responses is the flicker ERG using sawtooth waveforms.[40–45] The advantage of the use of sawtooth stimuli to evaluate ON and OFF retinal responses over the long-duration flash is the fact that they provide a constant state of adaptation. Additionally, the temporal frequency can be changed in order to investigate the temporal properties of the ON and the OFF activity.

In summary, the long-duration flash ERG data suggest that the ON and OFF pathways are selectively impaired in DMD patients and female carriers.[24,36] However, it is not known if this asymmetry is confined to the cone-driven pathway or if the rod-driven pathway is also involved. This question is important because the rods contact a specific type of bipolar cells, rod bipolar cells, that are exclusively depolarized by light and thus of the ON type.[46] A description of the dark-adapted ON and OFF responses might enable a stricter localization of the visual deficits in DMD patients. For instance, if the mesopic ON and OFF ERG is impaired in a manner similar to the photopic ON and OFF ERG, it can be concluded that the genetic changes in dystrophin affect mainly ON-type (depolarizing) bipolar cells in general.

Asymmetrical alterations in ON and OFF mechanisms found in the ERG, with greater losses in the ON pathway responses, may be revealed in psychophysical thresholds to stimuli designed to probe the respective pathways. Measurement of visual thresholds to positive (ON) and negative (OFF) contrasts relative to a background provides the possibility of studying ON and OFF mechanisms separately[47] and might be useful for revealing if retinal changes in ON and OFF pathway responses in DMD patients have consequences for visual perception.

It was the purpose of the present study to examine ON and OFF pathway responses in male DMD patients and female DMD carriers through the measurement of full-field ERGs using flicker stimulation with sawtooth waveforms. In addition, we aimed to evaluate if ON and OFF ERG changes are reflected in psychophysical thresholds to stimuli designed to probe these two pathways.

Methods

Subjects

The experiments adhered to the tenets of the Declaration of Helsinki and were approved by the institutional ethics committee (CEP-HU/USP 642/06). Signed informed consent was obtained from the subjects and, when applicable, from their parents after explanation of the nature and possible consequences of the study.

Participants were 19 DMD patients (all males, mean age 15 ± 3 years) and 7 DMD carriers (all females, mean age 39 ± 6 years; mothers of patients who participated in the study). The controls were 19 male (mean age 15 ± 7 years) and 7 female (mean age 37 ± 8 years) healthy subjects. All subjects underwent a complete ophthalmological examination. The inclusion criteria for the DMD patients and carriers were best-corrected visual acuity 20/30 or better, absence of cataract, and normal appearance of the fundus. The inclusion criteria for the controls were best-corrected visual acuity 20/30 or better, absence of ophthalmological diseases, and absence of any disease that could affect the visual system.

The Table shows the demographic information of the participants. The column labeled "DNA" gives the exon(s) in which the genetic alteration was found. DMD 1 and 2 as well as DMD carriers 1 and 2 have gene alterations before exon 30 (which is the promoter exon for Dp260). As a result, Dp427 is defective, but the other dystrophin isoforms are normal. This genotype is called "upstream 30." All the other patients and carriers have gene alterations after exon 30 and are called "downstream 30." Thus, the Dp427 is defective. However, they also have dysfunctions of Dp260 and Dp140 isoforms, but their Dp71 is normal. Only one patient (number 5) and one carrier (number 6) show a dysfunction of the Dp427 and the Dp260 isoforms, but not of Dp140 and Dp71 isoforms (because the gene alteration is downstream of exon 30 but upstream of exon 45, which is the promoter exon for Dp140).

ERG Measurements

The ERGs were obtained from one randomly chosen dilated eye of 10 DMD patients, 12 male controls, and all female subjects using the Super Color Ganzfeld (Q450 SC) stimulator with the RETIport System (Roland Consult, Brandenburg, Germany). The Ganzfeld's white LED (light-emitting diode) array with CIE (Commission Internationale de l'Éclairage) 1931 coordinates (0.37, 0.42) was used to generate the 4-Hz flicker stimulation. Four ERG protocols were applied: mesopic (1 cd/m^2 mean luminance) rapid-ON sawtooth; mesopic rapid-OFF sawtooth; photopic (250 cd/m^2 mean luminance) rapid-ON sawtooth; and photopic rapid-OFF sawtooth. All stimuli had 100% contrast. One DTL (Dawson, Trick, & Litzkow)[48] and two gold cup electrodes were used as active, reference (ipsilateral temple), and ground (forehead) electrodes, respectively. The signals were acquired through a preamplifier, digitized by the RETIport system with a sampling rate of 1024 Hz, and imported afterward into MatLab (The MathWorks, Natick, MA) for detailed analysis. The signals were band-pass filtered with 1-Hz and 300-Hz cutoff frequencies. At least forty 1-second episodes were averaged to obtain large signal-to-noise ratios for all subjects and all conditions. As shown in Figure 1, the negative (N; homologue to the a-wave) and positive (P; homologue to the b-wave) components were analyzed in the time domain for the ON response, and the positive (P; homologue to the d-wave) component was analyzed in the time domain for the OFF responses. The N and P amplitudes (μV) were measured from the baseline to the trough and to the peak of the signal, respectively. The baseline was the average of the potentials during 5 ms prior to the rapid change in the stimulus. The implicit times (ms) were measured from 0 ms (when the rapid change in the stimulus occurred) to the peak of the respective component. Figure 1 also presents a sketch of the luminance modulation of the stimuli below each signal. A single response amplitude value was obtained by averaging the values measured in each of the four cycles in the 1-second episode at all ERG protocols; for the implicit time, only the first cycle was considered.

Contrast Sensitivity Measurements

The stimuli for the psychophysical contrast sensitivity measurements were displayed in a Sony FD Trinitron CPD–G420 monitor (Sony, Tokyo, Japan) with 100-Hz refresh rate and 600 × 800 pixels spatial resolution. The monitor was driven by a

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TABLE. Demographic Information of the Participants: DMD Patients, Female Carriers, M Controls, and F Controls

Group	Age	DNA	Type	Eye Tested	ERG	Group	Age	Eye Tested	ERG
DMD						**M control**			
1	12	3–7	Duplication	OD	X	1	6	OS	X
2	13	3–7	Duplication	OD	X	2	8	OD	
3	23	46–55	Deletion	OS	X	3	8	OS	
4	14	47–52	Deletion	OD	X	4	15	OS	
5	16	43	Deletion	OS	X	5	13	OS	
6	19	51	Deletion	OD		6	12	OD	
7	14	45	Deletion	OS		7	7	OD	X
8	11	47–48	Deletion	OD	X	8	12	OD	X
9	11	45–52	Deletion	OS	X	9	7	OD	X
10	20	43–45	Deletion	OS	X	10	12	OS	X
11	19	50	Deletion	OS	X	11	11	OS	X
12	19	45–52	Deletion	OS	X	12	21	OD	X
13	14	44–47	Deletion	OS		13	22	OS	
14	17	46–49	Duplication	OS		14	23	OS	
15	15	47–51	Deletion	OD		15	26	OD	X
16	12	55	Point mutation	OD		16	21	OD	X
17	12	46–49	Duplication	OD		17	25	OD	X
18	14	55	Point mutation	OS		18	18	OD	X
19	13	51–52	Deletion	OD		19	25	OD	X
Average	**15**				$N = 10$	**Average**	**15**		$N = 12$
SD	**3**					**SD**	**7**		
Carrier						**F control**			
1	41	3–7	Duplication	OD	X	1	47	OS	X
2	38	3–7	Duplication	OS	X	2	31	OD	X
3	28	47–48	Deletion	OD	X	3	50	OD	X
4	34	45–52	Deletion	OS	X	4	35	OD	X
5	46	47–52	Deletion	OD	X	5	33	OS	X
6	41	43	Deletion	OS	X	6	31	OD	X
7	45	43–45	Deletion	OS	X	7	29	OD	X
Average	**39**				$N = 7$	**Average**	**37**		$N = 7$
SD	**6**					**SD**	**8**		

DNA, exon at which the mutation is found; F, female; M, male; OD, oculus diestrum; OS, oculus sinistrum; SD, standard deviation; X, participants who underwent the ERG tests.

VSG 2/4 graphics board (Cambridge Research System, Rochester, UK) and controlled by the PSYCHO for Windows version 2.3 software (Cambridge Research System, Rochester, UK) with which the experiment was performed. The stimuli consisted of a 17 cm × 17 cm checkerboard (approximately 10° of visual angle from 1 m distance) presented on a background with mean chromaticity of $x = 0.34$ and $y = 0.35$ (CIE 1931) and mean luminance of 56.7 cd/m^2. Half the checks had the same luminance as the background. The remaining checks had either more or less luminance than the background. The checks were presented at two spatial frequencies: 0.3 (6 × 6 checks) and 2 (40 × 40 checks) cycles per degree (cpd). At 0.3 cpd, the stimulus was presented for a short period of time (33 ms); the 2-cpd stimulus was presented for a longer duration (1500 ms). The stimuli were designed to favor the magnocellular pathway with the large checks and short duration of the presentation. Conversely, the small checks and long-duration stimulus were designed to favor parvocellular pathway activity. The stimulus was replaced by the background for 0.5 seconds between each presentation. Figure 2 shows a sketch of the four types of stimuli used for the psychophysical measurements. The stimulus was monocularly viewed by the same eye as tested with the ERG. The subject sat in a dark room 1 m away from the monitor. The experimenter determined the time of stimulus presentation. The task of the observer was to fixate the center of the monitor and to indicate verbally, after a tone presentation, if the stimulus was detected or not. The psychophysical procedure was a two-alternative forced-choice (2AFC) staircase with logarithmic changes of the positive or negative contrasts. The stimulus was presented at all trials (35 times for each of the four protocols). The contrast decreased 3 dB when the observer indicated that the stimulus was perceived and increased 1.5 dB when the stimulus was not detected. The last positive response at the lowest contrast value was used to determine a reversal value. The threshold (in Michelson contrast) was calculated by the software using the mean of the last six reversal values. The contrast sensitivity was estimated using the inverse of the contrast threshold.

Statistical Analysis

The Shapiro-Wilk statistical test indicated that the data were not normally distributed. Therefore, a nonparametric statistical test (Mann-Whitney U test) was used to compare results between the groups. To reduce the possibility of Type I error that can occur in multiple statistical comparisons, the Holm-Bonferroni correction was used to treat the dataset families. A P value less than 0.05 was defined to indicate a significant difference. The software used for the statistical analysis was SPSS (Statistical Package for the Social Sciences, Hong Kong, China). The two DMD upstream 30 patients as well as the two carriers of the upstream 30 gene were not included in the statistical analysis.

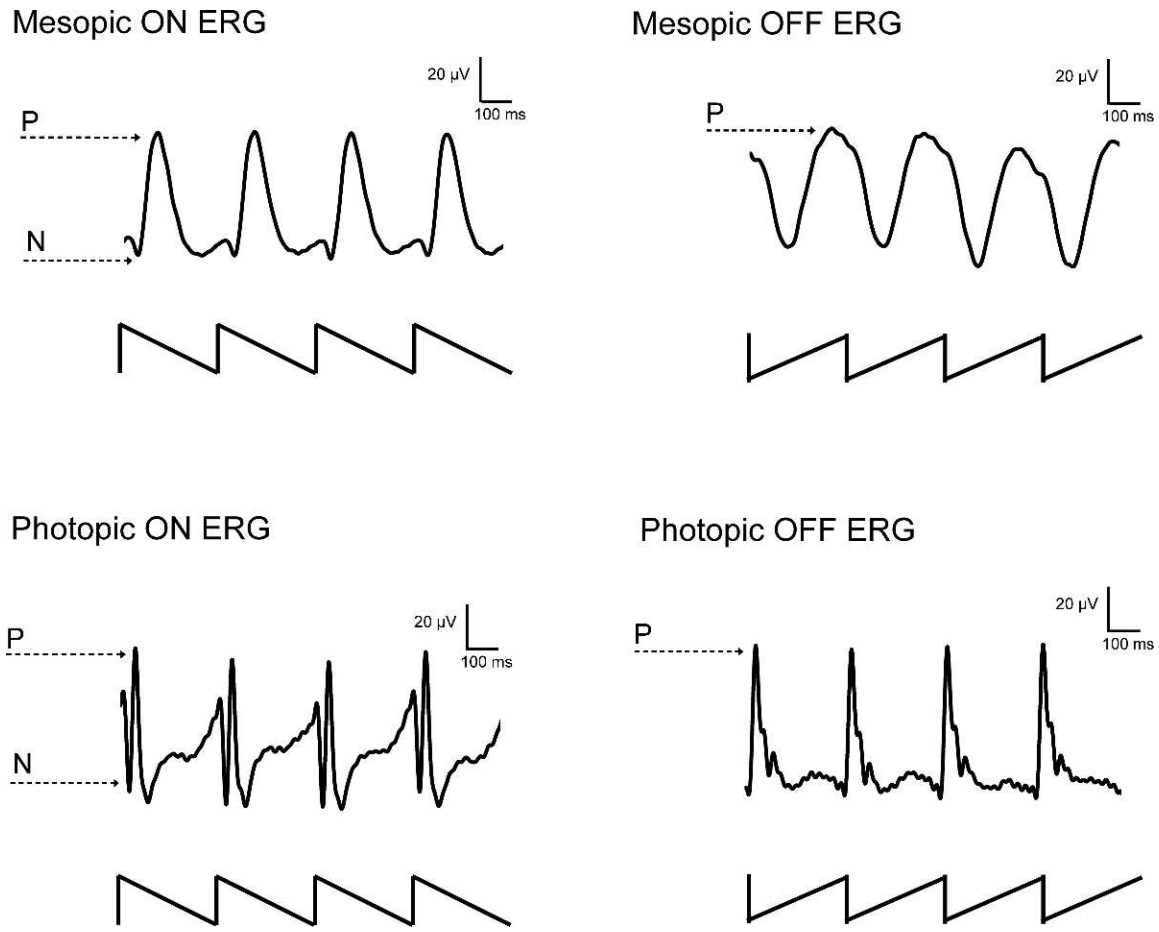

FIGURE 1. Original ERG responses of one subject of the control group. A sketch of the (sawtooth) luminance modulation of the stimuli is given below each response. The negative (N) and positive (P) components were analyzed in the ON response, and the positive (P) component was analyzed in the OFF responses. The N and P amplitudes (μV) were measured from the baseline to the trough and to the peak of the signal, respectively. The baseline was the first 5 seconds of the signal averaged. The implicit times (ms) were measured from the time at which the sudden luminance change in the stimulus occurred to the peak of the respective component.

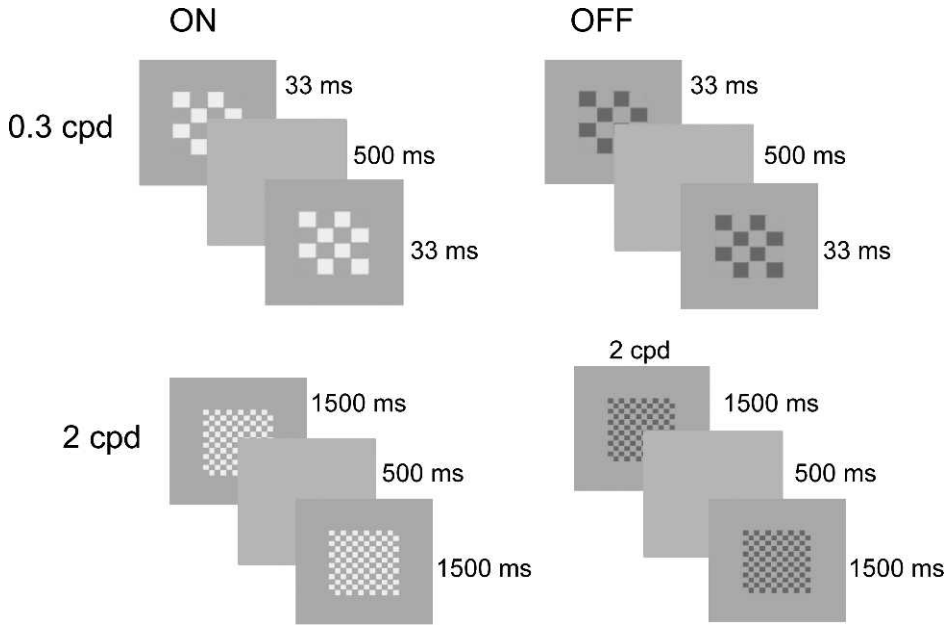

FIGURE 2. Sketch of the four types of stimuli used in the psychophysical measurements. The following check sizes and spatial frequencies were used: 0.3 (6 × 6 checks) and 2 (40 × 40 checks) cycles per degree (cpd). The 0.3-cpd stimulus was presented for a short duration (0.033 seconds); the 2-cpd stimulus was presented for a longer duration (1.5 seconds).

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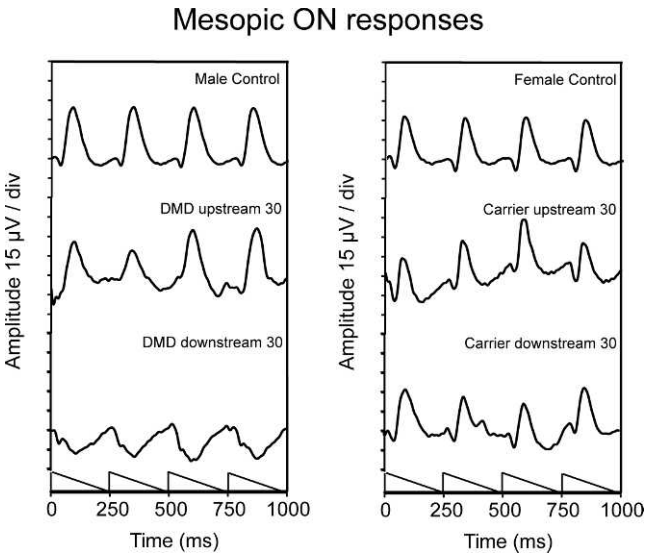

FIGURE 3. Mesopic ON responses from representative individuals: one male control and two DMD patients (*left graph*); one female control and two DMD carriers (*right graph*).

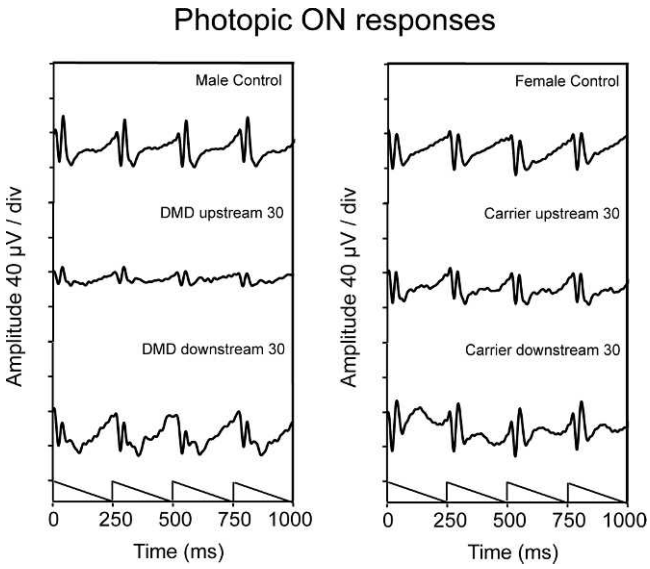

FIGURE 5. Photopic ON responses from representative individuals: one male control and two DMD patients (*left graph*); one female control and two DMD carriers (*right graph*).

RESULTS

ON and OFF Electroretinograms

Original ERG responses from representative individuals are shown in Figures 3 through 6. Figure 3 shows the mesopic ON responses from a male control and from two DMD patients (left graph). Note that there is only a slight change in the responses of the DMD upstream 30 patient relative to the control's response. The DMD downstream 30 patient, on the other hand, shows a severe change in the response. The right graph shows responses of one female control and two DMD carriers (the mothers of the patients whose responses are shown in the left graphs). These responses were very similar to each other.

The mesopic OFF responses measured in controls, patients, and carriers (Fig. 4) revealed a pattern of response change similar to that described for the mesopic ON responses: changes of the responses in the DMD patients compared to the control that were more severe in the downstream 30 than in the upstream 30 patient (left graph), and similar responses of the female control and the two carriers (right graph).

The photopic ON responses (Fig. 5) are strongly altered in both up- and downstream 30 DMD patients compared to controls (left graph), with the greater amplitude reduction for the DMD upstream 30 patient. The female carriers had reduced photopic ON responses compared to the female controls (right graph). The photopic OFF responses (Fig. 6) were altered in the upstream 30 patient and to a lesser extent in the

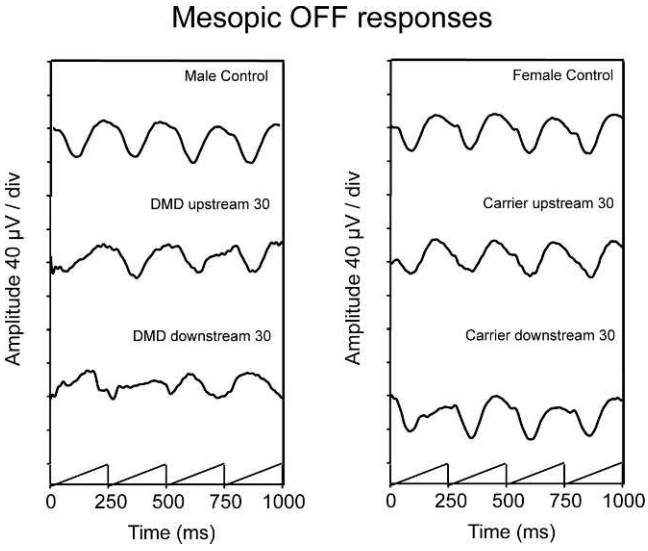

FIGURE 4. Mesopic OFF responses from representative individuals: one male control and two DMD patients (*left graph*); one female control and two DMD carriers (*right graph*).

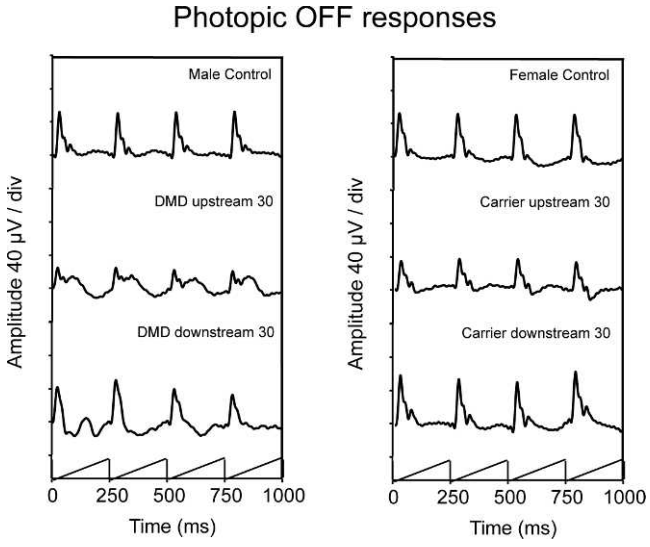

FIGURE 6. Photopic OFF responses from representative individuals: one male control and two DMD patients (*left graph*); one female control and two DMD carriers (*right graph*).

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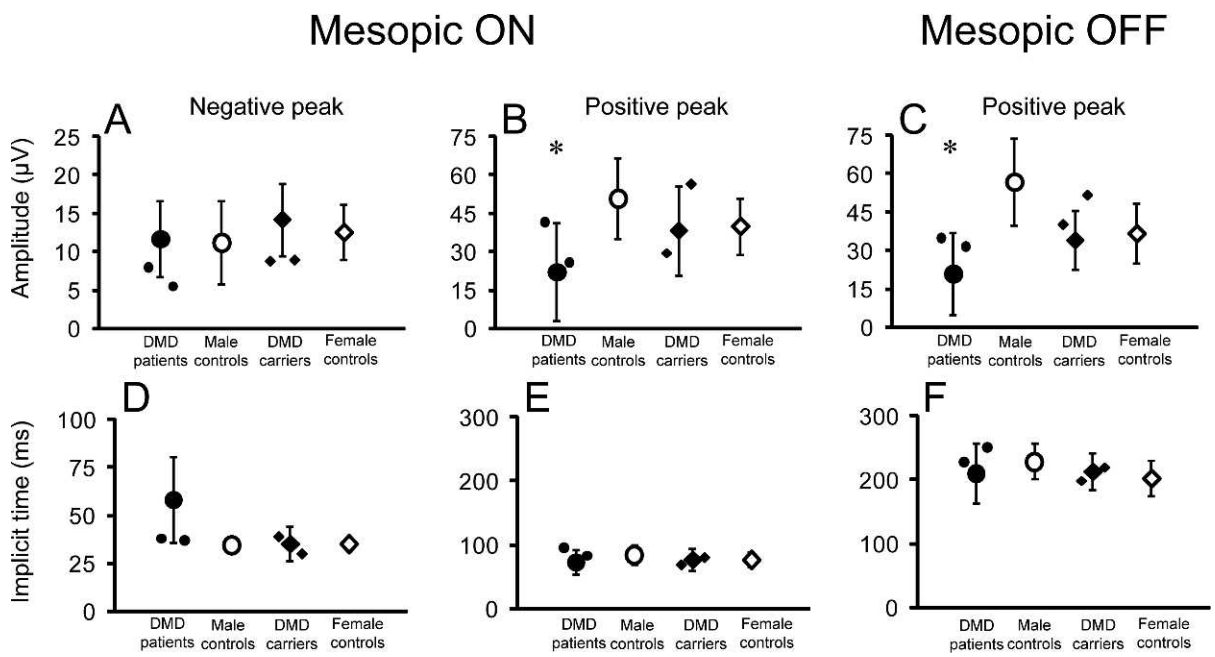

FIGURE 7. Mesopic response amplitudes (**A–C**) and implicit times (**D–F**) of the different ERG components. The *large symbols* represent the averaged results (±1 SD) for downstream 30 and controls: downstream 30 DMD (*filled circles*; $n = 8$), male controls (*open circles*; $n = 12$), DMD carriers (*filled diamonds*; $n = 5$), and female controls (*open diamonds*; $n = 7$). The *smaller symbols* represent the individual results of the two upstream 30 DMD patients (*circles*) and the two carriers of the upstream 30 gene (*diamonds*). $^*P < 0.05$, comparison between downstream 30 DMD patients and male controls. The amplitudes of the positive peaks were significantly reduced in the DMD patients compared to the male controls.

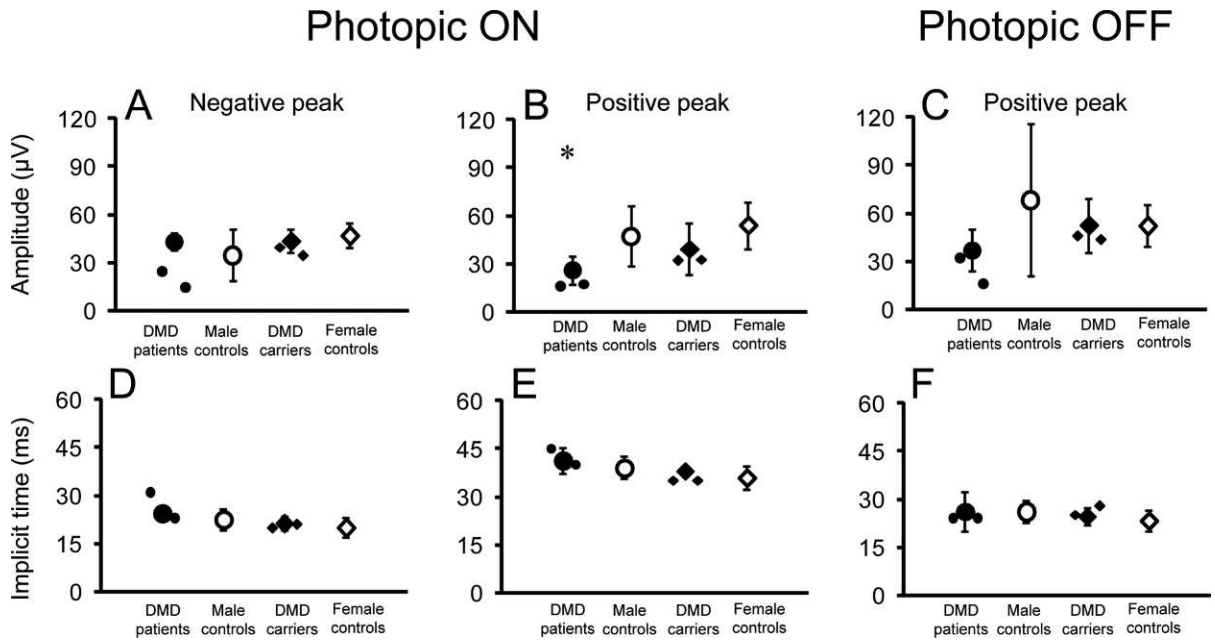

FIGURE 8. Photopic response amplitudes (**A–C**) and implicit times (**D–F**) of the different ERG components. The *large symbols* represent the averaged results (±1 SD) for downstream 30 and controls: downstream 30 DMD (*filled circles*; $n = 8$), male controls (*open circles*; $n = 12$), DMD carriers (*filled diamonds*; $n = 5$), and female controls (*open diamonds*; $n = 7$). The *smaller symbols* represent the individual results of the two upstream 30 DMD patients (*circles*) and the two carriers of the upstream 30 gene (*diamonds*). $^*P < 0.05$, comparison between downstream 30 DMD patients and male controls. The amplitudes of the positive peak in the ON responses in the downstream 30 DMD patients were significantly reduced compared to the male controls. There was no significant decrease in the amplitudes of the photopic OFF responses.

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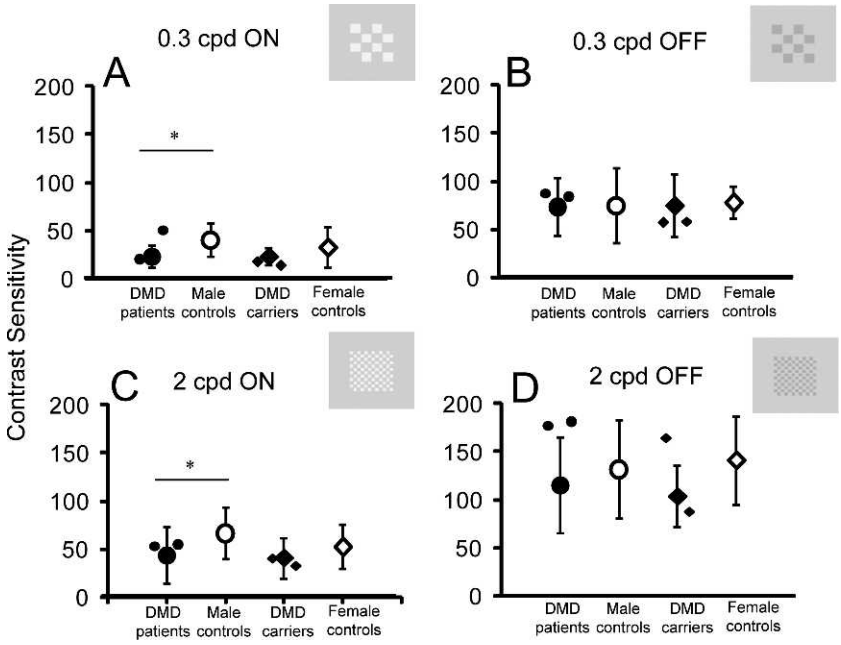

Figure 9. Results of the psychophysical contrast sensitivity measurements for the ON (**A**, **C**) and for the OFF (**B**, **D**) protocol. The *large symbols* represent the averaged results (±1 SD) for downstream 30 and controls: downstream 30 DMD (*filled circles*; $n = 17$), male controls (*open circles*; $n = 19$), DMD carriers (*filled diamonds*; $n = 5$), and female controls (*open diamonds*; $n = 7$). The *smaller symbols* represent the individual results of the two upstream 30 DMD patients (*circles*) and the two carriers of the upstream 30 gene (*diamonds*). $^*P < 0.05$, comparison between downstream 30 DMD patients and male controls. There were significant differences between the sensitivities of DMD patients and those of male controls for the ON stimuli in both protocols.

downstream 30 patient (left graph). The responses were similar for the female carriers and controls (right graph).

In Figures 7 and 8, the response amplitudes (upper graphs) and implicit times (lower graphs) are shown for the different ERG components. The smaller symbols represent individual results from the two upstream 30 DMD patients (circles) and the two carriers of the upstream 30 gene (diamonds). The larger symbols represent the averaged results (±1 SD) for each group: downstream 30 DMD (filled circles), male controls (open circles), DMD carriers (filled diamonds), and female controls (open diamonds).

The mesopic ON data showed that the negative peak had similar amplitudes for all groups (Fig. 7A). The amplitudes in the upstream 30 patients were small but still in the normal range. The implicit time was not significantly different in the DMD patients compared to controls (Fig. 7D). The positive peaks of the mesopic ON ($P < 0.02$) and the mesopic OFF ($P < 0.03$) responses were significantly reduced in the DMD patients compared to the male controls. The female carriers displayed amplitudes similar to those of the female controls. The implicit times of the positive peaks were similar in the four groups.

Photopic response amplitudes of the positive peak in the ON responses (Fig. 8) were significantly reduced (Fig. 8B) in the patients ($P < 0.05$), but not in the female carriers. There was no significant decrease in the amplitudes of the photopic OFF responses of DMD patients (Fig. 8C). The implicit times of all photopic responses were similar in the four groups (Figs. 8D–F).

ON and OFF Psychophysical Thresholds

The results of the psychophysical contrast sensitivity measurements are shown in Figure 9. The contrast sensitivities were higher for the OFF protocols compared to the ON protocols for all subjects. Moreover, in the OFF protocols at 2 cpd ("parvo") stimulus, the sensitivities were higher than at 0.3 cpd ("magno"). On the other hand, in the ON protocols the contrast sensitivities were similar for magno and parvo.

There were significant differences between the sensitivities of DMD patients and those of male controls for the ON stimuli in both protocols ($P < 0.01$). On the other hand, the data from the DMD carriers were similar to those of the female controls. There was no difference in the OFF data among the four groups ($P > 0.05$).

Discussion

Our data confirm that mutations, deletions, or duplications in the dystrophin gene that affect the retinal expression of the full protein and its isoforms in DMD patients cause changes in the full-field ERG. To our knowledge, we show here for the first time that under mesopic conditions, the positive peaks of ERG responses to ON and OFF sawtooth stimuli are reduced to a similar extent in DMD patients with both types of gene alteration: downstream 30 and upstream 30. The more severe changes at the mesopic level were found in the downstream 30 DMD patients, suggesting that the Dp260 is mainly responsible for the response change. At photopic levels, the ON responses seem to be more affected than the OFF responses, to a similar extent in the downstream and the upstream 30 DMD patients, suggesting the important role of Dp427 in this response. We found small alterations of the photopic ON responses of the DMD carriers that were not statistically significant when compared to those of the control group.

The presence of dystrophin in the retina has been previously described in humans,[21] and it is known that genetic alterations in dystrophin, such as those causing DMD, are associated with alterations in the ERG. These alterations are

more conspicuous in DMD patients with gene changes that prevent the expression of the Dp260, in agreement with the finding that the absence of this isoform in mdx^{Cv3} mice is related to ERG alterations.

In the OPL, the photoreceptors (rods and cones) make synaptic contact with depolarizing (ON bipolar or rod bipolar) and hyperpolarizing (OFF bipolar) cells[49] that give rise to the ON and OFF visual responses. In photopic conditions, the negative peak in the ON response and the positive peak in the OFF response are homologous to the a- and d-wave, respectively, and originate in the photoreceptors with some contribution of the OFF bipolar cells. The fact that they have similar implicit times reinforces the assumption that they have similar origins (cf. the lower graphs of Fig. 8). The ON response positive peak is probably homologous with the b-wave of the long-flash ERG and originates mainly in the activity of ON bipolar cells.[50] Our photopic ERG data reveal a change in the positive peak of the ON responses of the DMD patients. This agrees with the notion that the signal transmission in the OPL is disturbed in DMD. In the mesopic conditions, the positive peaks of both ON and OFF responses are affected in DMD patients. This indicates that the OFF response has different origins in mesopic and photopic conditions. An obvious explanation may be that rods exclusively contact ON bipolar cells.[46,51]

It is known that rod-driven retinal responses are slower and therefore the OFF response seems to be suppressed by the ON response with use of the long-flash-duration protocol.[52] The present study showed that retinal responses elicited by rapid-ON and rapid-OFF modulation of the mean luminance at mesopic levels are very different from each other, including waveforms, amplitudes, and implicit times (see Fig. 1). Therefore we believe that their retinal origins are also different. To our knowledge there are no studies showing ERGs in animal models using drugs for blocking specific groups of cells and testing with the conditions used in this study. Moreover, it is not completely known whether the rods send information only to the rod bipolar (depolarizing) cells in the OPL or to other cells as well. The rods may signal other circuits. For example, they are able to stimulate cones through electrical synapses; thus they can use the ON and the OFF pathways of the cones.[53] In addition, the rods can directly stimulate, by excitatory chemical synapses, one type of OFF bipolar (hyperpolarizing) cell from the cone pathway.[54] We believe our mesopic OFF ERG may be the result of activation of the indirect (rod to ON bipolar) and direct circuits in the OFF rod mechanism (in addition to the contribution of the cone activity to the mesopic OFF responses).

How would these circuits operate in DMD patients? Recent accounts of the distribution of dystrophin isoforms[17] have confirmed that the Dp260 is present in the OPL, along with the Dp140 and Dp427 isoforms, which are present in smaller amounts than the Dp260. These three isoforms together were found more in rod than in cone synapses, while the Dp427 is more expressed by the cones. These findings may explain why both downstream 30 DMDs (with alteration of Dp427, Dp260, and Dp140) and upstream 30 DMDs (with alteration of only Dp427) have different changes in their ERGs. The dysfunction of Dp427, Dp260, and Dp140 in our downstream 30 DMD patients is likely to lead to alterations in the dark-adapted (rod driven) ERG responses, while the upstream 30 DMD patients would have alterations in their photopic ERGs. These data suggest that the different isoforms of dystrophin have distinct functions in the signal transmission: While Dp427 is active at all light levels, Dp260 and Dp140 seem to be active mainly in mesopic light levels and are possibly present in the rods.

Benoff and collaborators[55] found a selective deficit in the ON pathway measured by visual evoked potentials in DMD patients. Our psychophysical data show that the contrast sensitivity in DMD patients is reduced using stimuli with luminance increments (ON stimuli) but not to the same extent for luminance decrements (OFF stimuli). This result agrees with the previous data.

In conclusion, the cone- and rod-driven ERG responses are altered in DMD patients. These alterations are different for rod- and cone-driven ERG signals. Furthermore, the data indicate a functional deficit in the synaptic transmission between photoreceptors and bipolar cells. Possibly, the ON and OFF cone-driven mechanisms are also differently affected. Accordingly, an asymmetry between ON and OFF pathway responses is also revealed in psychophysical contrast sensitivities.

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