

# Transcorneal Electrical Stimulation Shows Neuroprotective Effects in Retinas of Light-Exposed Rats

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**PURPOSE.** To examine the effects of transcorneal electrical stimulation (TES) on retinal degeneration of light-exposed rats.

**METHODS.** Thirty-three Sprague Dawley albino rats were divided into three groups: STIM ( $n = 15$ ) received 60 minutes of TES, whereas SHAM ( $n = 15$ ) received identical sham stimulation 2 hours before exposure to bright light with 16,000 lux; healthy animals ( $n = 3$ ) served as controls for histology. At baseline and weekly for 3 consecutive weeks, dark- and light-adapted electroretinography was used to assess retinal function. Analysis of the response versus luminance function retrieved the parameters  $V_{max}$  (saturation amplitude) and  $k$  (luminance to reach  $\frac{1}{2}V_{max}$ ). Retinal morphology was assessed by histology (hematoxylin-eosin [HE] staining; TUNEL assay) and immunohistochemistry (rhodopsin staining).

**RESULTS.**  $V_{max}$  was higher in the STIM group compared with SHAM 1 week after light damage (mean intra-individual difference between groups 116.06  $\mu$ V;  $P = 0.046$ ). The b-wave implicit time for the rod response (0.01 cd.s/m<sup>2</sup>) was lower in the STIM group compared with the SHAM group 2 weeks after light damage (mean intra-individual difference between groups 5.78 ms;  $P = 0.023$ ); no other significant differences were found. Histological analyses showed photoreceptor cell death (TUNEL and HE) in SHAM, most pronounced in the superior hemiretina. STIM showed complete outer nuclear layer thickness preservation, reduced photoreceptor cell death, and preserved outer segment length compared with SHAM (HE and rhodopsin).

**CONCLUSIONS.** This sham-controlled study shows that TES can protect retinal cells against mild light-induced degeneration in Sprague Dawley rats. These findings could help to establish TES as a treatment in human forms of retinal degenerative disease. (*Invest Ophthalmol Vis Sci.* 2012;53:5552-5561) DOI: 10.1167/iovs.12-10037

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Electrical stimulation is a therapeutic approach in ophthalmology described as early as 1873.<sup>1</sup> More recently, it has regained attention through reports of patients carrying passive subretinal implants who have experienced visual improvements in retinal areas beyond the implant location.<sup>2-5</sup>

The application of transcorneal electrical stimulation (TES) has demonstrated effects on various neurons, including retinal and optic nerve cells, improving function and/or morphology in different disorders.<sup>6-10</sup>

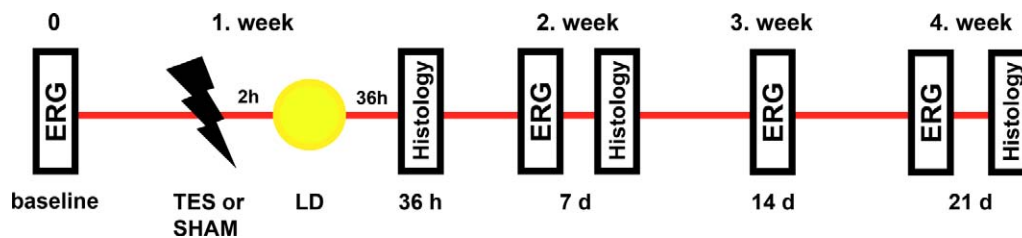
In recent studies, TES has been reported to improve visual function in diseases such as traumatic optic neuropathy,<sup>11</sup> nonarteritic anterior optic neuropathy,<sup>11</sup> retinal artery occlusion,<sup>12</sup> branch retinal artery occlusion,<sup>13</sup> and more recently in retinitis pigmentosa (RP).<sup>14</sup> In addition, TES was able to induce increased chorioretinal blood flow in healthy patients.<sup>15</sup>

In animals, a protective effect of TES has been described for ischemic rat retinas by Wang et al.<sup>16</sup> Moreover, Morimoto et al.<sup>17</sup> have shown an increased survival of axotomized retinal ganglion cells, which has been confirmed by Okazaki et al.<sup>18</sup>

The effect of TES has been attributed to the release of neurotrophic factors,<sup>5</sup> such as an increased expression of insulin-like growth factor (IGF-1),<sup>19-21</sup> brain-derived neurotrophic factor (BDNF),<sup>6,22,23</sup> FGF2,<sup>24</sup> and B-cell lymphoma 2 protein.<sup>25</sup> Protective effects of TES have also been linked to the downregulation of pro-apoptotic factors, such as BAX and tumor necrosis factors,<sup>25</sup> which are involved in the apoptosis cascade of retinal cells.<sup>26</sup>

A common animal model in the study of retinal degenerations and potential therapeutic approaches is the Royal College of Surgeons (RCS) rat, where photoreceptor cell loss occurs due to a mutation of *MERTK*.<sup>27</sup> Neuroprotective effects of electrical stimulation in RCS rats have been demonstrated by several groups.<sup>5,24,28-30</sup> This animal model has, however, specific disadvantages that make it difficult to compare with the condition found in most patients with RP. Importantly, the degeneration of retinal cells is severe, sets in early, and progresses very rapidly with large interindividual differences.<sup>31-34</sup> An alternative model to study the effects of novel therapeutic strategies for retinal degenerative diseases is retinal light-induced damage, where bright light induces photoreceptor degeneration as, for example, described by Wenzel et al.,<sup>35</sup> Remé et al.,<sup>36</sup> and Noell et al.<sup>37</sup> This disease model is now well described,<sup>38-43</sup> and onset as well as severity of degeneration can be planned prospectively and respective treatment regimens are reproducible.<sup>36,37</sup> But despite the etiological mechanisms (e.g., gene mutation, environmental insult [oxygen, light], or age-related deterioration), all these factors tend to converge to cell death. The last convergent mechanism in all these degenerations may be targeted by the procedure of TES.

Recently, Ni et al.<sup>22</sup> found a neuroprotective effect of TES on light-induced retinal degeneration in Sprague Dawley rats. Because their findings may have considerable impact on



**FIGURE 1.** Experimental procedure of the study: Baseline ERG measurements were performed 1 week before TES or sham stimulation (sham) and light damage (LD). The first histological examination of treated animals was performed 36 hours after treatment. Thereafter, ERG was performed weekly (7 d (days), 14 d, and 21 d) and histology at the first week after treatment (7 d) as well as at the third week after treatment (21 d). Healthy and untreated animals ( $n = 3$ ) served as controls for histology and are not included in the figure.

potential treatment options of human diseases such as RP, the goal of our study was to investigate the neuroprotective effect of TES on a mild light-induced retinal degeneration using detailed electrophysiological protocols with extended analyses of several functional outcome measurements confirmed by histology. A mild light damage protocol was used, in order to investigate the effects of TES on mild retinal degeneration. In addition and in contrast to previous studies our outcome measures were studied using a sham-stimulated control group.

## METHODS

### Animals

Thirty-three healthy Sprague Dawley rats (mean  $\pm$  SD weight at baseline = 210.19 g  $\pm$  14.63; Charles River Laboratories GmbH, Sulzfeld, Germany) were included. All experimental procedures were performed following the recommendations of the ARVO statement for use of animals in ophthalmic and vision research and in accordance with the local committee for animal welfare. Rats were housed under standard laboratory conditions with light-dark cycles of 12/12 hours, and divided into three groups. The first group (STIM;  $n = 15$ ) received 60 minutes of TES 2 hours before exposure to bright light, and the second group (SHAM;  $n = 15$ ) received sham stimulation 2 hours before exposure to bright light. Healthy animals ( $n = 3$ ) served as controls for histology. All rats of the STIM and the SHAM groups were measured by electroretinography at the beginning of the study as baseline (Fig. 1). Electroretinography (ERG) measurements were then performed every week for 3 consecutive weeks after exposure to bright light. Animals were used for histology at baseline (before light damage as healthy animals;  $n = 3$ ), 36 hours after light damage (36 h;  $n = 3$  for each group), 1 week thereafter (7 d;  $n = 3$  for each group) and at conclusion of the study (3 weeks after light damage; 21 d;  $n = 3$  for each group).

### Electroretinography

ERG was performed using an *Espion* (Diagnosys LLC, Cambridge, UK). Rats received anesthesia by intraperitoneal injection of ketamine 100 mg/kg and xylazine 5 mg/kg (WDT eG, Garbsen, Germany). After 12 hours of dark adaptation, 1 drop of tropicamide 0.5% (Mydriatikum-Stulln, Stulln, Germany) was applied 20 minutes before ERG measurement for pupil dilation. Animals were kept on a warming plate during the entire ERG procedure. A self-developed ERG electrode-holder using a DTL fiber (Dawson et al.<sup>44</sup>) was used as active electrode as previously described.<sup>45</sup> Methocel (2% methylcellulose; Omnivision GmbH, Puchheim, Germany) was applied regularly to the cornea to avoid exposure keratopathy. Two subcutaneous needle electrodes (Ambu Neuroline Twisted Pair Subdermal, Bad Nauheim, Germany), one inserted between the eyes and the other in the tail served as reference and ground electrode, respectively. Measurements were performed only when acceptable impedance levels of less than 10 k $\Omega$  at 25 Hz (using the machine's built-in algorithm) were reached.

The dark-adapted ERG protocol consisted of 10 steps with increasing stimulus strengths from 0.000003 to 3 cd.s/m<sup>2</sup> with a mixed white light (white 6500K) produced by a Ganzfeld stimulator (ColorDome; Diagnosys LLC, Cambridge, UK). All flashes were presented without background illumination and constant interstimulus intervals of 1 second for dim flashes up to 30 seconds for bright flashes to maintain dark adaptation. Flash duration was 4 ms in all steps. Band-pass filtering was applied from 1.25 to 300 Hz. Averages ranged from 20 trials for dim flashes to 2 trials for bright flashes.

Light-adapted ERGs were recorded after light adaptation with a background illumination of 30 cd/m<sup>2</sup> (white 6500 K) for 10 minutes. Stimulus strength of 3 cd.s/m<sup>2</sup> was chosen for single flash and 20 Hz flicker responses. Twenty trials were averaged for single flash responses and 30 trials for flicker stimulation.

### Histology

Animals were killed with a CO<sub>2</sub> overdose; eyes were enucleated 36 hours after light exposure and the anterior parts and lenses removed; for paraffin sectioning, fixed eyecups (4% paraformaldehyde in 0.1 M phosphate buffer [pH 7.4] for 1 hour at 4°C) were dehydrated in EtOH, infiltrated in chloroform, and embedded in paraffin. Radial 5- $\mu$ m sections were stored at 4°C.

Histology included quantification of outer nuclear layer (ONL) thickness and inner/outer segments (IS/OS) length, which was measured at 4 equidistant positions from one another, 300  $\mu$ m apart, starting next to the optic nerve along the superior and inferior hemiretina.

### Immunohistochemistry

Tissue sections were deparaffinized, rehydrated, and blocked with endogenous peroxidase with H<sub>2</sub>O<sub>2</sub> for 30 minutes. Antigen retrieval was achieved by pressure cooking in 0.1 M citrate buffer, pH 6, for 10 minutes followed by cooling at room temperature before incubation with the antibodies. Radial sections were pre-incubated with PBS (50 mM, pH 7.4) containing 20% normal goat serum and 0.03% Triton X-100 (Sigma-Aldrich, Hamburg, Germany) for 2 hours at room temperature so as to block nonspecific antibody binding. Subsequently, the sections were incubated overnight at 4°C with specific primary antibodies. The following antibodies were used: rhodopsin clone RET-P1 (Mouse, mAb, 1:400; Millipore Chemicon, Billerica, MA). The immunoreaction was visualized with Alexa Fluor 488 anti-rabbit antibody (Rockland Immunochemicals Inc., Gilbertsville, PA) diluted 1:750. Controls were carried out by omitting the first antibody. All showed micrographs were taken from the superior region of the retina.

### TUNEL Assay

TUNEL staining<sup>46</sup> was performed using an in situ cell death detection kit as described by the manufacturer (Fluorescein or TMR; Roche Diagnostics GmbH, Mannheim, Germany).

## Treatment

TES was applied 1 week after performing ERG baseline testing (Fig. 1). A prior dark adaptation of 12 hours was performed; all treatment procedures were performed under dim red light. DTL electrodes were attached using our newly developed electrode holder.<sup>45</sup> A commercially available, custom-modified neurostimulator (Twister DN; Dr Langer Medical GmbH, Waldkirch, Germany) produced rectangular, biphasic electrical stimuli with a duration of 2 ms (1 ms positive followed immediately by 1 ms negative pulse) with 200  $\mu$ A at a frequency of 20 Hz for 1 hour.<sup>47</sup> Only right eyes were stimulated in the STIM group. The SHAM group received the exact same treatment for 1 hour, but the stimulator was not switched on.

After the treatment, all rats were kept in darkness for 2 hours to awake from anesthesia, received one drop of atropine and thereafter light exposure for 2 hours under a mean brightness of 16,000 lux (4 Philips TLD 965 lamps; Philips, Hamburg, Germany). The cages were lined with aluminum foil to reach an environmental illumination. During light exposure, the temperature in the rectangular cages was checked every 15 minutes and animals were continually checked by one of the authors to ensure that they did not close their eyes and move freely in the cage without leaning against the wall of the cage to ensure equal and reproducible light exposure in all animals. Subsequently, all animals were again kept in darkness for 12 hours. This procedure was performed equally for each animal in both groups.

## Data Analysis

Statistical analyses were performed using *JMP* (version 8.0.2; SAS Institute Inc., Cary, NC).

Data analysis was performed on results of the right eyes of the animals. For ERG, the difference between parameters measured after light damage and baseline was calculated subtracting baseline from postexposure values (3 follow-up weeks). This difference was used for the comparison between experimental groups for 3 follow-up measurements. For between-group analyses a two-tailed unpaired *t*-test was used to compare intra-individual differences in both groups. Histological results were compared by a two-tailed unpaired *t*-test and all results were shown represent the mean and SD.

ERG wave amplitudes and implicit time was determined using a semi-automatic software, as previously described.<sup>48</sup> For dark-adapted flashes (0.00003 - 0.03 cd.s/m<sup>2</sup>), rod sensitivity and saturation were estimated using a hyperbolic function (Naka and Rushton -formula 1<sup>49</sup> where *V*<sub>max</sub> is the saturated response; *k* as intensity needed for 50% of *V*<sub>max</sub>) were extracted (*n* as gradient of fit).

$$V(I) = \frac{V_{max} \times I^n}{I^n + k^n} \quad (1)$$

Additionally, a- and b-wave amplitudes and implicit times of different luminance flashes (0.01 and 3 cd.s/ms) of rod, of combined responses and light-adapted responses were analyzed as recommended in the International Society for Clinical Electrophysiology of Vision standard.<sup>50</sup>

Oscillatory potentials (OP) were analyzed after offline band-pass filtering with 75 to 300 Hz of ERG responses elicited by combined responses. OPs were calculated as area under the curve between the a-wave trough and the b-wave peak.

## RESULTS

Electrophysiological data are summarized in the Table.

### ERG: Dark-Adapted Findings

No significant differences were observed between groups at baseline measurements under dark-adapted or light-adapted conditions (the Table).

*V*<sub>max</sub> showed a statistically significant difference between groups (*P* = 0.046) in the first week after treatment, with higher amplitudes for STIM animals (Fig. 2), whereas in the second and third week these significant differences disappeared (Fig. 2).

The b-wave amplitude under dark-adapted conditions for rod (0.01 cd.s/m<sup>2</sup>) or combined response (3 cd.s/m<sup>2</sup>) stimulus showed no significant differences between TES-treated animals in comparison with SHAM, but in the second week after exposure, the implicit time of the rod response revealed a significantly (*P* = 0.023) shorter time for STIM rats compared with SHAM (Fig. 2). The a-wave amplitudes and implicit times of the combined response showed no significant differences (Fig. 2).

Analysis of oscillatory potentials (Fig. 3) showed higher amplitudes for the STIM group in comparison with the SHAM group in the first week after treatment without being significant. This was also observed in the second week and at the end point of the study in week 3 after treatment.

### ERG: Light-Adapted Findings

No significant differences were detected in analyses of the light-adapted ERG (Fig. 3). Neither the amplitudes nor the implicit times of the light-adapted standard flash (3 cd.s/m<sup>2</sup>) and the 20-Hz flicker responses differed between STIM and SHAM groups (Fig. 3).

### HE Staining

After light damage and TES (Figs. 4A, 4B; 36 hours) retinal cross-sections revealed a pronounced decrease in the length of photoreceptor IS and OS in the superior and to a lesser extent in the inferior retina in the SHAM group (Fig. 5A). TES-treated retinas showed a significant preservation (position two of the histological measurements in the first week after treatment *P* < 0.001; Fig. 5) of the IS/OS length at three of four positions in the superior retina and partly in the inferior retina (Figs. 4A-D; Figs. 5A, 5C). In week 3 after treatment (Figs. 4E-F, respectively), the IS/OS returned to their normal appearance in SHAM, whereas values in the STIM group had remained stable. Increase of the IS/OS length was observed in the superior and the inferior retina, reaching values between 35 and 45  $\mu$ m in the SHAM group and 40 to 50  $\mu$ m in STIM group (Fig. 5E).

In the SHAM group, a reduction in ONL thickness was observed, primarily in the superior retina (Fig. 5B), and in the first week after light exposure, in the inferior retina (Fig. 5D). In the STIM group, normal values in the ONL thickness were observed in the superior and the inferior retina at all time points (Figs. 5B, 5D, 5F).

### Rhodopsin Staining

Rhodopsin immunolabeling showed normal distribution and was restricted to photoreceptor OS in STIM and SHAM groups (Figs. 4G-L). However, shortening of OS in the SHAM group was clearly recognizable in comparison with the TES-treated animals, especially during the first week after light exposure (Figs. 4G-L).

### TUNEL Staining

No TUNEL-positive cells were present in the ONL of STIM animals (Figs. 4M, 4O, 4Q). In contrast, apoptotic cells in the ONL were frequently found in the SHAM group directly (Fig. 4N) after light exposure, decreasing from the first week (Fig.

TABLE. Summary of all ERG Amplitudes (amp) and Implicit Times (imp) during the Study Procedure

	cd.s/m <sup>2</sup>	Baseline		7 Days after Treatment		14 Days after Treatment		21 Days after Treatment	
		STIM	SHAM	STIM	SHAM	STIM	SHAM	STIM	SHAM
Scot. function									
Vmax (μV)	0.000003-0.03	477.71 ± 112.75	520.35 ± 67.91	<b>616.96 ± 123.13</b>	<b>543.54 ± 115.21</b>	606.83 ± 154.08	608.05 ± 117.29	586.88 ± 120.75	559.13 ± 99.60
k (log I)		-3.24 ± 0.12	-3.16 ± 0.11	-3.30 ± 0.14	-3.32 ± 0.09	-3.35 ± 0.09	-3.30 ± 0.10	-3.37 ± 0.08	-3.35 ± 0.11
Dark-adapted									
b-wave									
amp. (μV)	0.01	443.01 ± 111.57	480.53 ± 62.10	578.13 ± 121.27	510.36 ± 112.34	572.00 ± 148.55	569.96 ± 111.68	551.85 ± 118.59	523.22 ± 94.62
imp. (ms)		88.00 ± 4.50	86.22 ± 4.41	82.78 ± 6.22	83.11 ± 3.98	<b>77.33 ± 2.00</b>	<b>81.33 ± 3.08</b>	78.56 ± 1.67	79.22 ± 6.14
amp. (μV)	3	778.08 ± 138.38	791.64 ± 89.16	924.03 ± 136.19	833.04 ± 165.98	894.70 ± 191.85	910.46 ± 133.96	907.87 ± 167.49	858.31 ± 146.14
imp. (ms)		80.11 ± 7.37	80.44 ± 7.45	76.22 ± 6.48	78.00 ± 7.19	72.44 ± 1.42	74.89 ± 2.67	74.78 ± 4.55	76.11 ± 7.30
a-wave									
amp. (μV)	3	-273.97 ± 45.60	-267.55 ± 26.46	-311.44 ± 42.36	-291.41 ± 53.36	-296.75 ± 62.21	-302.73 ± 44.83	-291.56 ± 47.86	-282.59 ± 55.00
imp. (μV)		13.67 ± 0.71	14.22 ± 1.09	13.67 ± 1.58	13.67 ± 0.71	13.11 ± 0.78	13.89 ± 0.93	13.22 ± 0.44	13.11 ± 0.60
OP									
AUC (nV.s)	3	1770.46 ± 595.63	1888.35 ± 416.16	1884.12 ± 565.61	1796.07 ± 464.06	1757.29 ± 319.21	1671.66 ± 435.60	1977.47 ± 592.79	1884.24 ± 481.06
Light-adapted									
b-wave									
amp. (μV)	3	133.18 ± 26.60	125.87 ± 21.47	141.51 ± 37.03	132.73 ± 47.81	150.88 ± 34.58	146.21 ± 26.98	133.17 ± 35.31	140.60 ± 30.08
imp. (ms)		47.67 ± 2.45	48.78 ± 1.71	45.67 ± 2.69	46.33 ± 2.78	45.00 ± 1.22	46.89 ± 1.54	46.56 ± 1.88	45.89 ± 2.80
20 Hz amp. (μV)	3	21.54 ± 5.55	17.09 ± 4.32	31.03 ± 11.91	24.76 ± 12.26	35.83 ± 15.60	25.86 ± 8.82	31.29 ± 15.60	32.02 ± 11.95
20 Hz imp. (ms)		44.56 ± 3.21	44.22 ± 5.70	41.56 ± 3.43	41.33 ± 1.50	40.33 ± 1.94	44.11 ± 3.33	43.11 ± 3.82	42.89 ± 3.26

Significant differences between groups ( $P < 0.05$ ) are marked in bold. Data are shown as mean and SD.



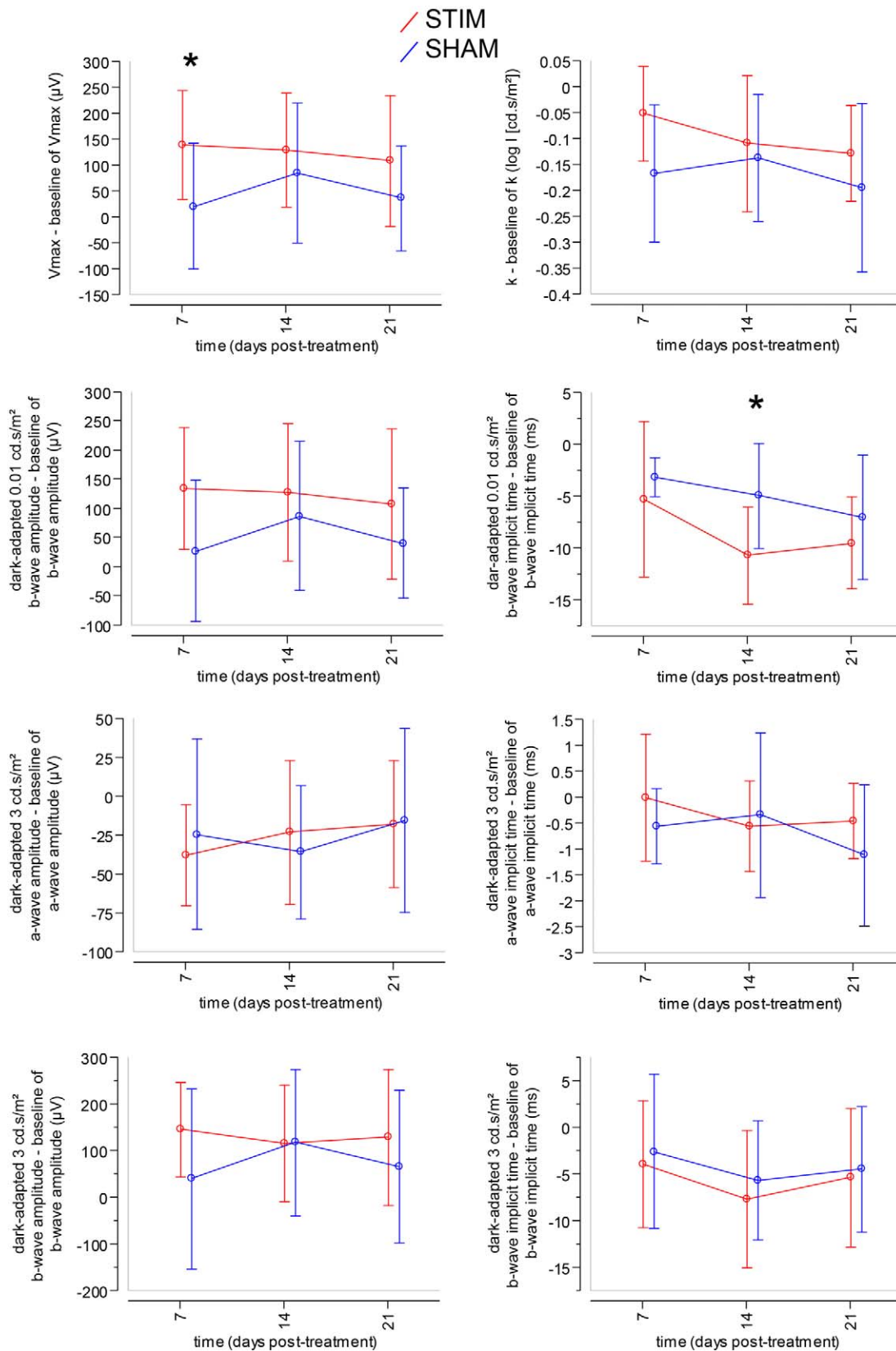


FIGURE 2. Comparison of intra-individual differences between both groups (STIM and SHAM) for dark-adapted ERG measurements shows significant differences ( $P < 0.05$ ) of  $V_{max}$  1 week after treatment (7 days) and of b-wave implicit time 2 weeks after treatment (14 days). Rings indicate mean of intra-individual differences; whiskers indicate SD; number of animals are  $n_{7 \text{ days}} = 12$ ,  $n_{14 \text{ days}} = 9$ , and  $n_{21 \text{ days}} = 9$ .

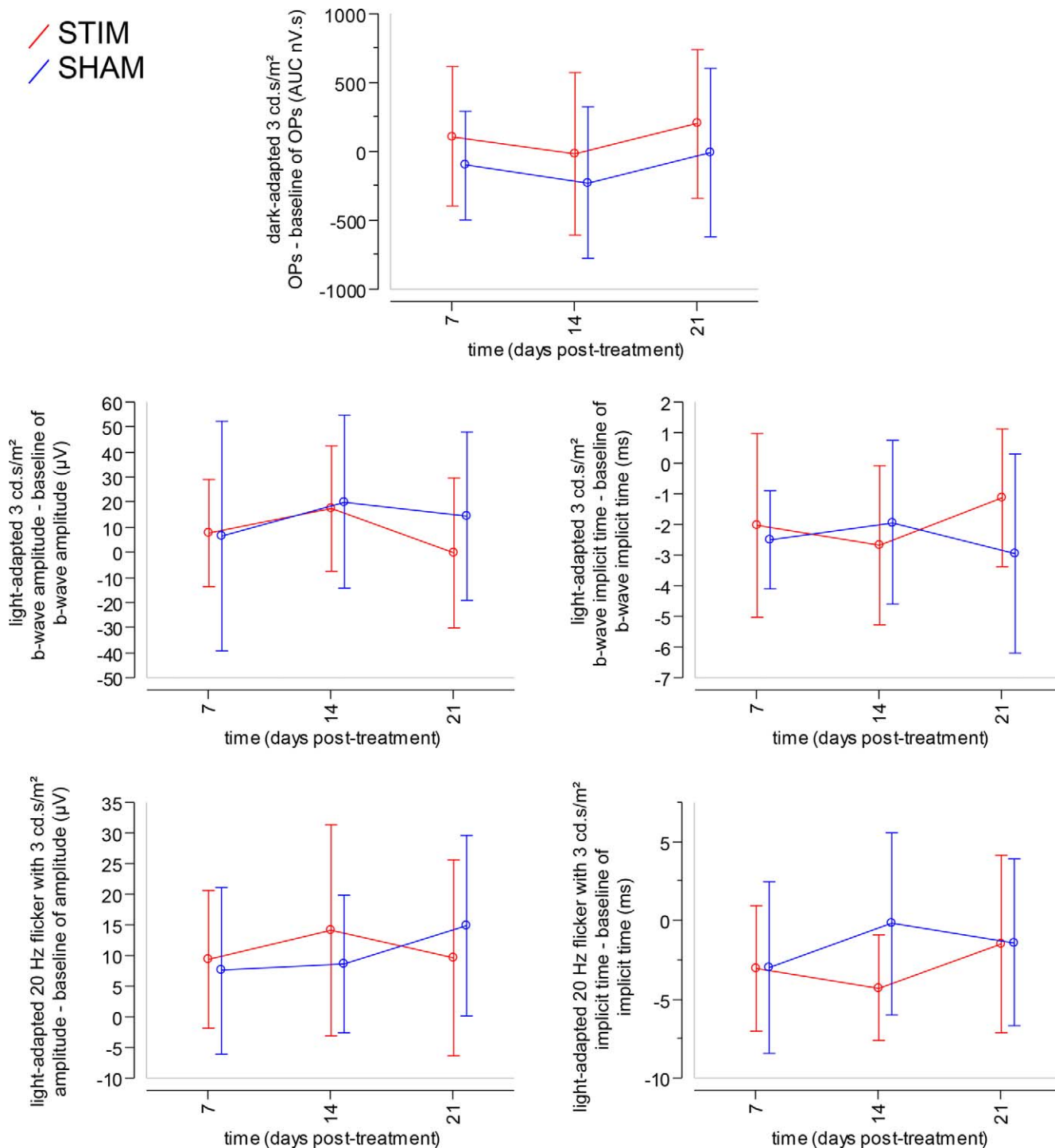


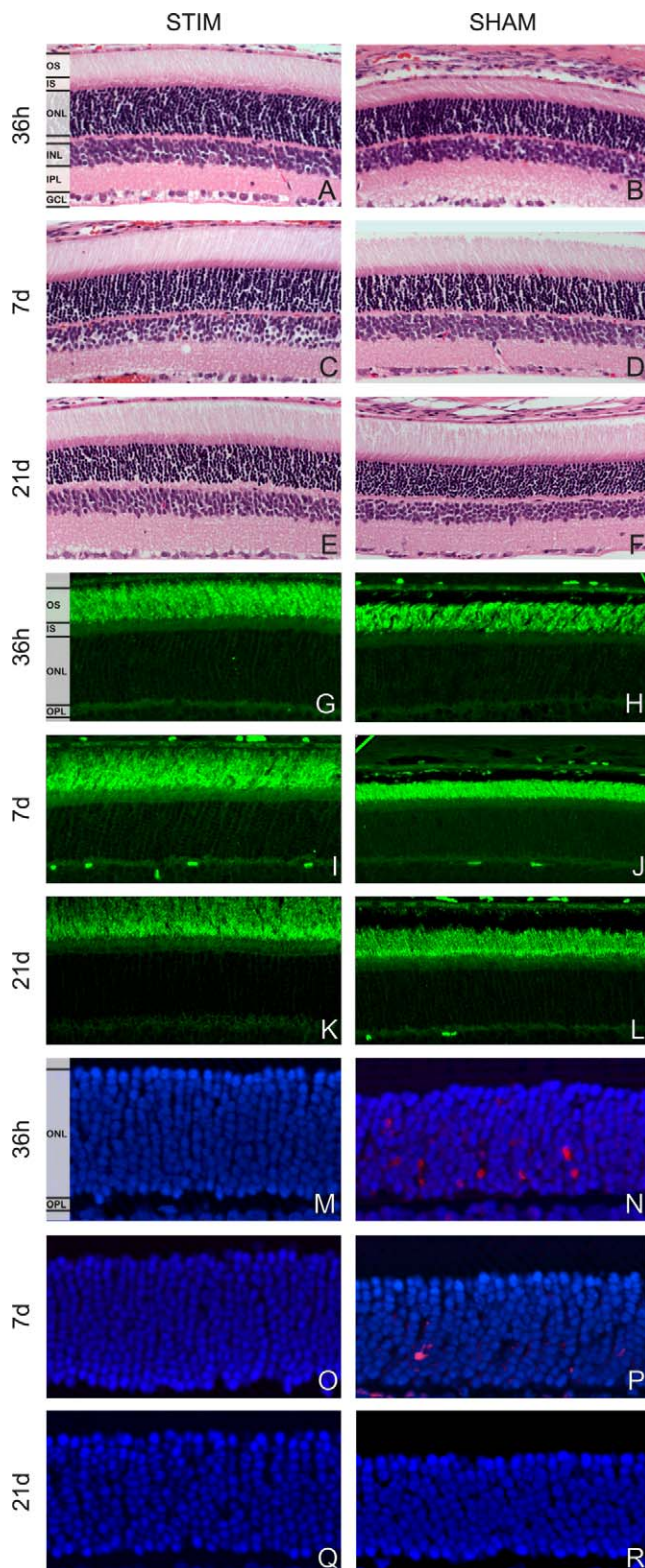
FIGURE 3. Comparison of intra-individual differences between both groups (STIM and SHAM) for dark-adapted OPs and light-adapted ERG measurements shows no significant difference (at  $P = 0.05$ ) for all parameters. Rings indicate mean of intra-individual differences; whiskers indicate SD; number of animals are  $n_{7 \text{ days}} = 12$ ,  $n_{14 \text{ days}} = 9$ , and  $n_{21 \text{ days}} = 9$ .

4P). In the third week, no TUNEL-positive cells were observed in any group (Figs. 4Q, 4R).

**DISCUSSION**

Support for beneficial effects of electrical stimulation in various forms of retinal degenerations and optic neuropathies have previously been published,<sup>11-13,16-18,51,52</sup> including data from

RCS rats,<sup>4,5,28-30</sup> and more recently from light-induced retinal degeneration.<sup>22</sup> In our study, we aimed at investigating the neuroprotective effect of TES in a defined experimental set-up including a sham-stimulated control group. Extended electrophysiological and histological methods were used with a protocol of mild light-induced damage to simulate a mild retinal degeneration. To assess a potential preventive protective effect, TES was applied before light damage. Our rearing



**FIGURE 4.** (A–F) HE staining of retinal sections 36 hours (A, B) and 7 days (C, D) and 21 days (E, F) after light exposure from STIM- and SHAM-treated animals. A decreased OS and IS length can be noticed in the SHAM group after light exposure. The STIM group shows no influences by light exposure on the photoreceptor layer. In the first (7 days) and third week (21 days) after light exposure, the IS/OS and ONL in the SHAM group return to normal appearance. (G–L) Expression of rhodopsin in rat retina 36 hours (G, H) and 7 (I, J) and 21 (K, L) days after light exposure from STIM and SHAM groups. Staining is restricted

protocol with light-dark cycles of 12/12 hours contributes to the mild retinal degeneration as postulated by Noell et al.<sup>53</sup>

### Functional Measurements

Using a previously described light-exposure protocol<sup>42,54</sup> we were able to induce a localized retinal damage pattern and avoid profound retinal degeneration, as demonstrated by histology with a clearly visible photoreceptor OS shortening and ONL thickness reduction in the SHAM group, most pronounced in the superior hemiretina. As a consequence, to this pattern of degeneration only a mild decrease in retinal function was confirmed by ERG. The results indicate a mild degree of retinal degeneration, noticeable in  $V_{max}$  and rod implicit time (Fig. 2).

An increase of the b-wave amplitude, as well as the dark-adapted sensitivity, has been previously described until the age of 60 days for the SD rats.<sup>55</sup> Because we included rats at the age of 50 days ( $210.19 \pm 14.63$  g), at least a small increase of the b-wave amplitude can reflect the normal growth of animals for the 2 weeks later at the first follow-up examination, rather than a true increase under TES treatment.  $V_{max}$  of the Naka-Rushton function was smaller in the SHAM than in the STIM group without any differences on the a-waves of dark-adapted and light-adapted responses. This substantiates an effect of TES on inner retinal layers and was supported by the findings on implicit times of the rod b-wave.

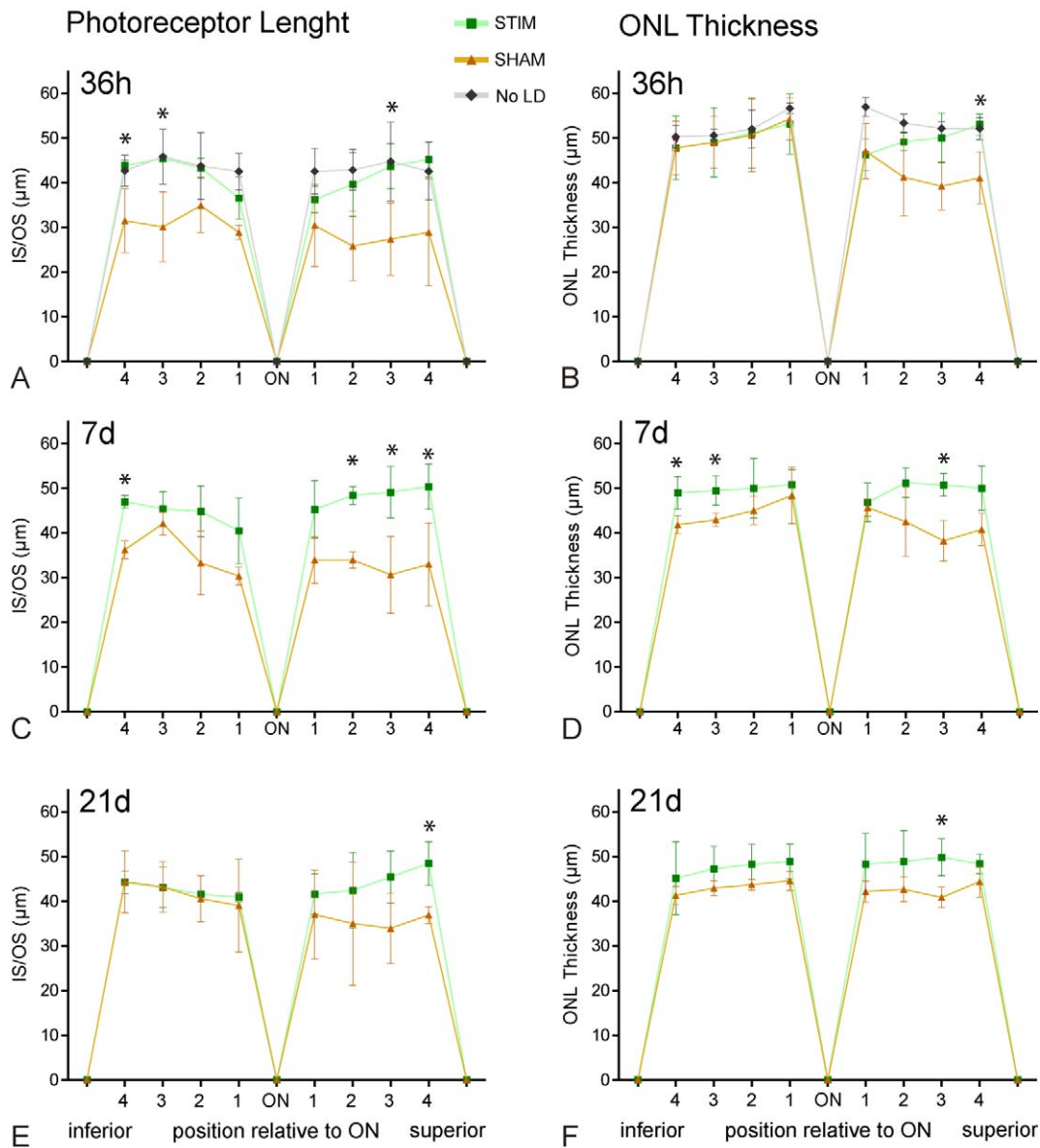
The significant differences in  $V_{max}$  indicate a loss of potentials generated by mainly rod-bipolar and to a lower extent by Müller cells. Although speculative, the effect of light damage to the Müller cells, as reported by Iandiev et al.,<sup>56</sup> may explain the significant difference of rod b-wave amplitudes between SHAM and STIM.

### Morphological Findings

The histological results clearly demonstrate degeneration of the photoreceptor cells expressed in a shortening of photoreceptor OS length and reduction of ONL thickness in the SHAM group (Figs. 4, 5), which was validated by rhodopsin staining and the TUNEL assay. In line with previous studies,<sup>37,40,43,57,58</sup> our data also show a predilection of the superior hemiretina for cell damage after light exposure (Fig. 5). Differences of the localization of light-induced retinal degeneration were first shown by Rapp and Williams in 1980.<sup>59</sup> The reason for this predilection of the superior retina is not entirely understood.<sup>38</sup> It may be related to the greater rhodopsin levels and outer segment lengths of the photoreceptor in the superior hemisphere of the rat eye as postulated by other research groups.<sup>60–62</sup> Further assumptions are a better intraretinal circulation and higher expression of neuroprotective factors (e.g., bFGF) in the inferior retina.<sup>63–65</sup> Another reason for the greater susceptibility to light damage in the superior hemisphere is most likely that the light entering the eye is from reflections from the floor of the cage. A protection of retinal cells was detected in the STIM group and measurements of retinal layer thickness were equivalent to normal untreated eyes. The retinal degeneration induced by our

to rod photoreceptor OS in both groups. Note that the SHAM group presents shorter OS. (M–R) TUNEL assay for dying cells in rat retina. Directly 36 hours (M, N) and 7 (O, P) and 21 (Q, R) days after light exposure from the STIM and SHAM groups. TUNEL-positive cells are observed in the SHAM group directly 36 hours and 7 days after light exposure. STIM treatment results in absence of apoptotic cells. INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer; OPL, outer plexiform layer.





**FIGURE 5.** Comparison of photoreceptor IS/OS length and ONL thickness between STIM and SHAM retinas 36 hours (A, B) and 7 days (C, D) and 21 days (E, F) after light exposure. The equidistant positions (1–4 inferior and superior) of retinal sections are analyzed around the optic nerve (ON). Measurements are shown as mean (*point*) and SD (*wiskers*). For descriptive analysis, a healthy group of untreated animals is added to the examination 36 hours after light exposure. A decrease in IS/OS can be noticed in the SHAM group 36 hours and 7 days after light damage. In the third week (21 days), a reversal of IS/OS length can be noticed. The STIM group shows significantly higher IS/OS and ONL length at different points of time ( $*P < 0.05$ ) and area. The resulted length of IS/OS and ONL are comparable with those measured in healthy untreated animals. The quantification of the ONL layer shows a reduction in SHAM group directly 36 hours and 7 days after treatment in comparison with the STIM group, which remains unaffected. The damage caused by light exposure is mostly apparent in the superior areas of the retina.

light-damage protocol and the effects of TES are not completely detectable by electrophysiological recordings, most likely due to the histologically shown focal degeneration of retinas after light damage,<sup>66–68</sup> which does not yield changes in Ganzfeld ERG recordings.

**Protective Mechanisms**

Protective effects of TES in this study were found in inner retinal layers, as shown by the electrophysiological results, as well as on the photoreceptor layer, as noticed by histology. The mechanisms underlying these findings are still not entirely clear, but as shown by Sato et al.,<sup>23</sup> an expression of BDNF has been shown directly after electrical stimulation. BDNF is also one of

the neuroprotective factors that has been shown to protect retinal cells against light damage.<sup>57</sup> Another pathway may be via the increased expression of IGF-1 after TES.<sup>19–21</sup> Also, a potential effect of TES on activator protein 1 regulation, which has been shown to be an initiator of photoreceptor degeneration in light damage,<sup>35,69</sup> may be involved. It seems likely that TES induces a variety of beneficent neuroprotective regulators, but further studies are still needed to clarify definite protective pathways.

**CONCLUSIONS**

In summary, our histological and electrophysiological data support the concept that TES provides protection against light-



induced retinal degeneration. In addition to previous studies,<sup>22</sup> the current work presents a detailed functional investigation of the influence of TES on light-damaged retinal cells and is the first sham-controlled study with a follow-up period of 3 weeks after treatment using established paradigms for light damage and electrical stimulation.<sup>42,47,70</sup> However, the neuroprotective effect of TES was, compared with previous studies, smaller than expected. Nevertheless, the mild retinal damage introduced may simulate a mild retinal degeneration and better reflect the clinical findings in patients who consider this kind of treatment.

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