Blockade of Retinal Oscillations by Benzodiazepines Improves Efficiency of Electrical Stimulation in the Mouse Model of RP, *rd10*

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Purpose. In RP, photoreceptors degenerate. Retinal prostheses are considered a suitable strategy to restore vision. In animal models of RP, a pathologic rhythmic activity seems to compromise the efficiency of retinal ganglion cell stimulation by an electrical prosthesis. We, therefore, strove to eliminate this pathologic activity.

Methods. Electrophysiologic recordings of local field potentials and spike activity of retinal ganglion cells were obtained *in vitro* from retinae of wild-type and *rd10* mice using multielectrode arrays. Retinae were stimulated electrically.

Results. The efficiency of electrical stimulation was lower in *rd10* retina than in wild-type retina and this was highly correlated with the presence of oscillations in retinal activity. Glycine and GABA, as well as the benzodiazepines diazepam, lorazepam, and flunitrazepam, abolished retinal oscillations and, most important, increased the efficiency of electrical stimulation to values similar to those in wild-type retina.

Conclusions. Treatment of patients with these benzodiazepines may offer a way to improve the performance of retinal implants in cases with poor implant proficiency. This study may open the way to a therapy that supports electrical stimulation by prostheses with pharmacologic treatment.

Keywords: rd10 retina, multi electrode array, retinal degeneration, electrical stimulation, benzodiazepines

RP leads to photoreceptor death and, ultimately, blindness while the inner retina persists. One commonly used model for RP is the *rd10* mouse.[1–3] In *rd10* mouse, rods start to degenerate after postnatal day 16 (P16). Rod death peaks between P21 and P25. By P60, only cones have survived, yet most disappear at 2 months of age with few cones left at 9 months. Loss of inner retinal cells is moderate, mostly concerning rod bipolar (approximately 20%) and horizontal cells (approximately 29%) and nearly completed or far advanced at 3 to 4 months. Compared to the *rd1* mice, *rd10* mice mimic the disease process in human RP more accurately as they display delayed onset and slower degeneration pace.[1–3]

One approach in the treatment of RP is driving activity in the inner retina via electrical stimulation by neural prostheses. Devices, such as Argus II or Alpha AMS, were marketed to provide visual percepts in RP subjects. They have been used in approximately 400 patients worldwide with a considerable number of patients experiencing improvements in visual tasks in their daily living. However, in many cases visual performance was poor and remained within the range of legal blindness.[4–7]

When restoring vision with prostheses, success crucially depends on the functional integrity of the remaining retinal ganglion cells (RGC). Two pathologic features reported in models of RP might have deleterious effects on prosthesis-driven RP therapy. First, oscillations were recorded in RGC spiking and in local field potentials (LFP) using multielectrode arrays (MEAs). In *rd10* retina, the frequency of this oscillation is in the range of 3 to 6 Hz.[8–10] Second, the efficiency of electrical stimulation was reported to be lower in RP models than in wild-type (wt) retina (*rd1*[11–15]; *rd10*[10]; human[16]).

Several models are discussed to explain the origin of the oscillations. One involves the electrically coupled cone ON-bipolar cells and AII amacrine cells.[17–20] Choi et al.[21] showed that oscillations only occurred when the membrane potential of the AII amacrine cells was found to be within a range suitable to open voltage-activated sodium channels expressed in these cells. In this model, oscillations are relayed via bipolar cells. In contrast, Yee et al.[22] proposed an intrinsic oscillator in amacrine cells as source for rhythmic activity.

The blockade of oscillations is expected to have beneficial effects on the visual perception of patients with RP, because oscillations may compromise the performance of retinal implants in several ways. First, oscillatory activity may decrease the clarity of information transmission from the eye to the brain. Abolishing retinal oscillations with blockers of gap junctions[23] increased the signal-to-noise ratio of optogenetically triggered RGC responses. Second, it seems conceivable that, by an as-yet unknown mechanism, oscillations may interfere with exogenous stimulation, decreasing stimulation efficiency. Given the strong correlation we observed between oscillations and decreased stimulation efficiency,[10] we hypothesized that abolishing oscillations might lead to an increase in stimulation efficiency, that is, in the number of elicited spikes per stimulus. Hence, blockade of retinal oscillations should improve visual performance by increasing simultaneously signal-to-noise ratio and efficiency of electrical stimulation.

First, we set out to corroborate the correlation between oscillations and low stimulation efficiency in *rd10* retina. Second, we extended the palette of pharmacologic agents that block oscillations. Inner retinal neurons express receptors for the inhibitory transmitters glycine and γ-aminobutyric acid (GABA, for review[24]), among them the AII amacrine cells.[25] In the AII model, hyperpolarizing AII amacrine cells below the critical membrane potential should abolish oscillations.[21] In the amacrine oscillator model,[22] hyperpolarizing amacrine cells should decrease oscillatory input into the retinal network. We show that treatment of *rd10* retina with glycine and GABA indeed abolished oscillations and, most important, increased stimulation efficiency to levels observed in wt retina. To capitalize on this effect in patients with RP with retinal implants requires a suitable pharmacologic approach. In patients, benzodiazepines are used to modulate activity of $GABA_A$ receptors.[26] We show that the three most commonly used benzodiazepines—diazepam, flunitrazepam, and lorazepam—also abolished retinal oscillations and improved stimulation efficiency. It is tempting to speculate that treatment with these benzodiazepines could improve the performance of retinal implants in patients with RP with poor implant proficiency.

Methods

Animals

Wt animals of the strain C57BL/6 were obtained from Charles Rivers Laboratories (Wilmington, MA). *Rd10* mice were bred locally from breeding pairs obtained from Jackson (B6.CXB1-*Pde6b*^{rd10}/J). In this line, the *rd10* mutation was backcrossed onto the C57BL/6J background for five generations before intercrossing to homozygosity. Animals were kept on a 12-hour light/dark cycle with food and water ad libitum. Experiments were performed in accordance with “ARVO Statement for the Use of Animals in Ophthalmic and Vision Research,” the German Law for the Protection of Animals and after approval was obtained by the regulatory authorities.

MEA Recording and Electrical Stimulation

MEAs containing 60 titanium nitride electrodes (diameter 30 µm, spacing 200 µm, impedance 50 kΩ at 1 kHz) on a glass substrate (Multi Channel Systems MCS GmbH, Reutlingen, Germany) were used. The data acquisition system (MC_Card, Multi Channel Systems) consisted of a USB MEA60-Up System, an integrated preamplifier and filter, stimulus generator STG 4002-1.6 mA, and a PC. Signals were sampled at 25 kHz/channel. For electrical stimulation (single biphasic current pulses with cathodic phase first; phase duration, 400 µs; amplitude, 100 µA), several electrodes were chosen as stimulation electrodes and the other surrounding electrodes were used for recording.

Tissue Preparation

Briefly, mice (3–4 months of age) were anesthetized deeply with isoflurane and humanely killed by decapitation. Eyeballs were enucleated and retinae isolated. Retinae were cut into two halves and one-half was mounted with RGCs towards the electrode side of the MEA. MEAs were pretreated in a plasma cleaner (Diener Electronic GmbH + Co. KG, Ebhausen, Germany) and coated with 0.5 mg/mL of poly-D-lysine hydrobromide (Sigma, St Louis, MO) overnight. The retinal preparation was maintained in carbonate-buffered AMES solution (pH of approximately 7.4), bubbled with 95% O_2 + 5% CO_2. Drugs were dissolved in the same solution and delivered to the retina by continuous perfusion at a flow rate of 3 mL/min at room temperature.

Pharmacology

MEA recordings were compared under physiologic and pharmacologic conditions in wt and *rd10* retinae. Retinae were stimulated electrically during all three phases of the experiment: (1) AMES for 20 to 30 minutes, (2) drug for 5 to 30 minutes, and (3) wash-out with AMES. Drugs given are as follows: GABA, 100 to 500 µM; glycine, 40 to 100 µM; diazepam, 5 to 100 µM; flunitrazepam, 100 µM; and lorazepam, 50 µM.

Data Analysis

Data were either used unfiltered, low-pass filtered (50 Hz) for LFPs or high-pass filtered (200 Hz) to analyze action potentials (AP). Unfiltered data were converted to ASCII files by the software MC-Data Tool to analyze them in MATLAB (MathWorks, Natick, MA). Using a custom-made script, Fast Fourier Transformation was used to analyze the LFPs. Stimulation efficiency was measured as the spike rate ratio, which is calculated by dividing the poststimulus AP rate (determined over 0.4 seconds after the stimulus pulse) by the prestimulus AP rate (determined over 8 seconds before stimulus pulse). Differences in stimulation efficiency were compared using a two-tailed Student *t*-test, or, if the values were not normally distributed, using the Mann–Whitney rank sum test. Asterisks indicate the level of significance (***$P \leq 0.001$; **$P \leq 0.01$; *$P \leq 0.05$). All values are given as mean ± standard error of the mean.

Results

Rhythmic Activity in Retinae of *rd10* Mice Can Wax and Wane

Experiments were performed on 3- to 4-month-old wt and *rd10* animals. In agreement with other studies,[1–3] we found that in our *rd10* animals at the age of 3 to 4 months, only a minute fraction of photoreceptors was left, similar to the situation of human patients with RP who would receive an implant. At 3 to 4 months of age, oscillations were robust and reproducible to allow for the time-consuming application and wash-out of the drugs. Figure 1 shows three typical firing patterns of RGCs recorded in *rd10* retina with raw data (Figs. 1Ai–Ci), high-pass filtered to show

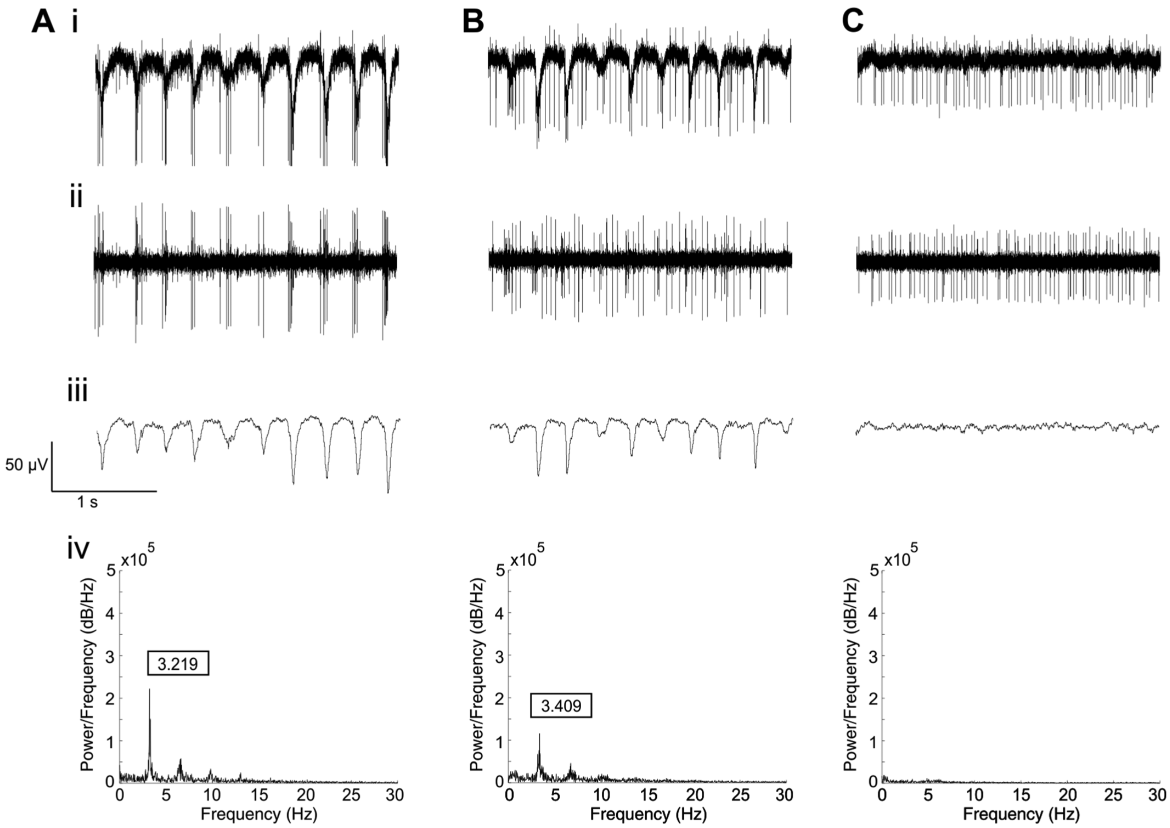

Figure 1. Spontaneous activity of different RGCs in *rd10* retina. (**A–Ci**) Raw data of MEA recordings at different electrodes. (**A–Cii**) High-pass filtered to show the APs. (**A–Ciii**) Low-pass filtered to isolate the LFP. (**A–Civ**) Frequency analysis of the oscillations using Fast Fourier Transformation. In most of the recordings, oscillations could be observed at a frequency of approximately 3 Hz (**Aiv** + **Biv**), but in some cases no oscillations (**Ciii**) or dominant frequency were observed (**Civ**). The age of the *rd10* mouse was 3.5 months.

the APs (Figs. 1Aii–Cii) or low-pass filtered to display the LFP (Figs. 1Aiii–Ciii). Large negative deflections in the LFP were observed in *rd10* retina. Analysis of the raw data by the Fast Fourier Transformation revealed a frequency range for the oscillations of 3 to 6 Hz, confirming previous results.[8–10] Normally, a clear main peak could be observed as well as peaks at higher frequencies, mostly appearing as second and third harmonics. Cells could be divided into three groups based on their firing behavior. In one type, spike bursts were phase locked to the negative deflections of the oscillations (high-amplitude spikes in Fig. 1A). On the same electrode, a second cell type (small-amplitude spikes) displayed spontaneous stochastic activity. In the third type, bursts of APs (high-amplitude spikes in Fig. 1B) were phase locked to the positive going phase or the flat component of the LFP (note that small-amplitude spikes from another RGC again phase locked to the negative deflection).

In most of the retinal pieces, we observed oscillatory activity in the majority of the electrodes (60%–80%). In clusters of electrodes, we recorded spiking activity but no oscillations (Fig. 1C), similar to wt retina. Oscillations could also be present for some time in the recording, disappear and come back over time. This switch from an oscillatory state to a nonoscillatory state has also been described by Biswas et al.[8]

Efficiency of Electrical Stimulation Is Strongly Decreased in Regions With Oscillations

For all animal models for RP, efficiency of stimulating the retina with electrical pulses was reported to be lower than in wt. In Figure 2, the response of wt and *rd10* retina to biphasic current pulses is displayed. In wt retina, a burst of APs was typically observed directly after the stimulation pulse (Fig. 2A, pulse marked by an asterisk). In the case of *rd10* retina showing oscillations, clear bursts of APs were barely observed (Fig. 2B). In Figure 2C, a recording of one *rd10* RGC in a region without oscillations shows a burst of APs comparable with the bursts in wt cells. We determined the stimulation efficiency in form of the spike rate ratio (Fig. 2D). Stimulation efficiency was 2.64 ± 0.08 in wt retina and reached a slightly lower value in *rd10* retina without oscillations with 2.17 ± 0.05. In *rd10* retina showing oscillations, the stimulation efficiency was significantly lower: 1.48 ± 0.02 (***$P \leq 0.001$).

GABA and Glycine Abolish Oscillations and Increase Stimulation Efficiency

We tested whether the blockade of oscillations would result in an increase in stimulation efficiency. To hyperpolarize retinal cells, we applied glycine (Fig. 3B) and GABA (Fig. 3E) to

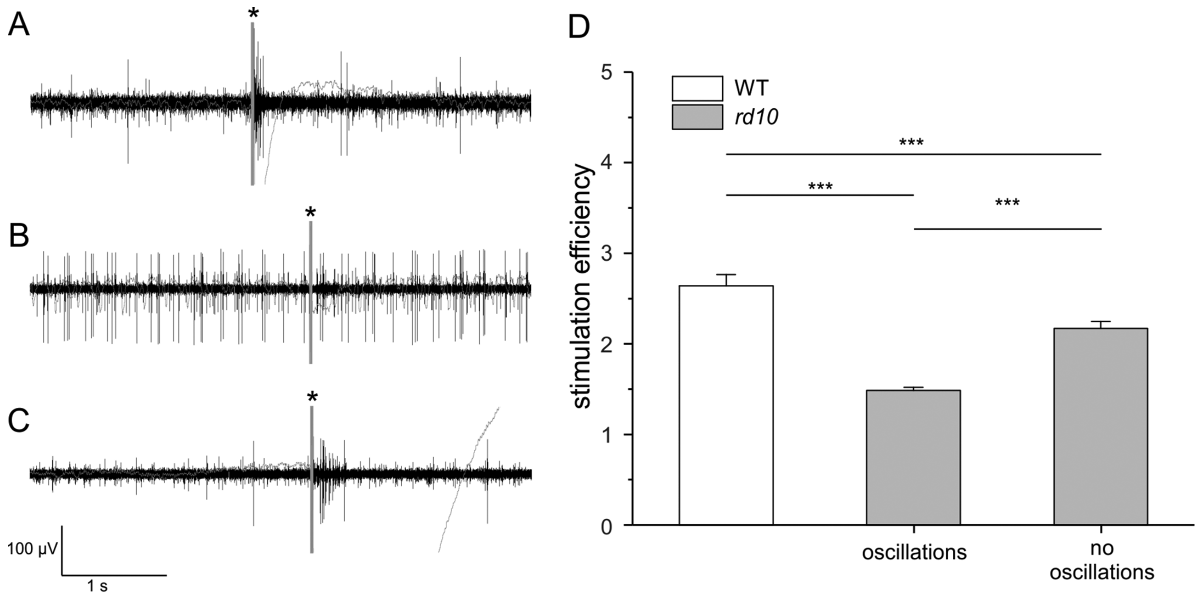

Figure 2. Responses of wt and *rd10* retina to a biphasic current pulse. (**A**) Example of a typical reaction of wt retina (age, 3.5 months; pulse amplitude of ±100 µA; phase duration of 400 µs). A burst of APs could be observed as response to the stimulus. *Grey bar with asterisk* represents the point in time of electrical stimulation. LFP is shown in grey and APs are shown in *black*. (**B**) Example of a typical reaction to electrical stimulation of *rd10* retina showing oscillations (age: 3 months). No clear burst as response to the stimulus could be observed. (**C**) Example of a typical reaction to the electrical pulse of *rd10* retina without oscillations (age: 4 months). A burst of APs could be observed as a response to the stimulus. (**D**) Comparison of the stimulation efficiency in wt and *rd10* retina with or without oscillations. Analyzing the stimulation efficiency, the poststimulus AP rate was divided by the prestimulus AP rate. The bar chart depicts the mean ± standard error of the mean. The stimulation efficiency in wt was 2.64 ± 0.08 ($n_{\text{cells}} = 513$), in *rd10* showing oscillations 1.48 ± 0.02 ($n_{\text{cells}} = 698$) and in *rd10* without oscillations 2.17 ± 0.05 ($n_{\text{cells}} = 946$). In total, the stimulation efficiency was evaluated in 27 retinae of 17 wt mice and in 52 retinae of 31 *rd10* mice. The Mann–Whitney rank sum test was used to test for significance. Differences were considered as highly significant at a *P* value of ≤0.001***.

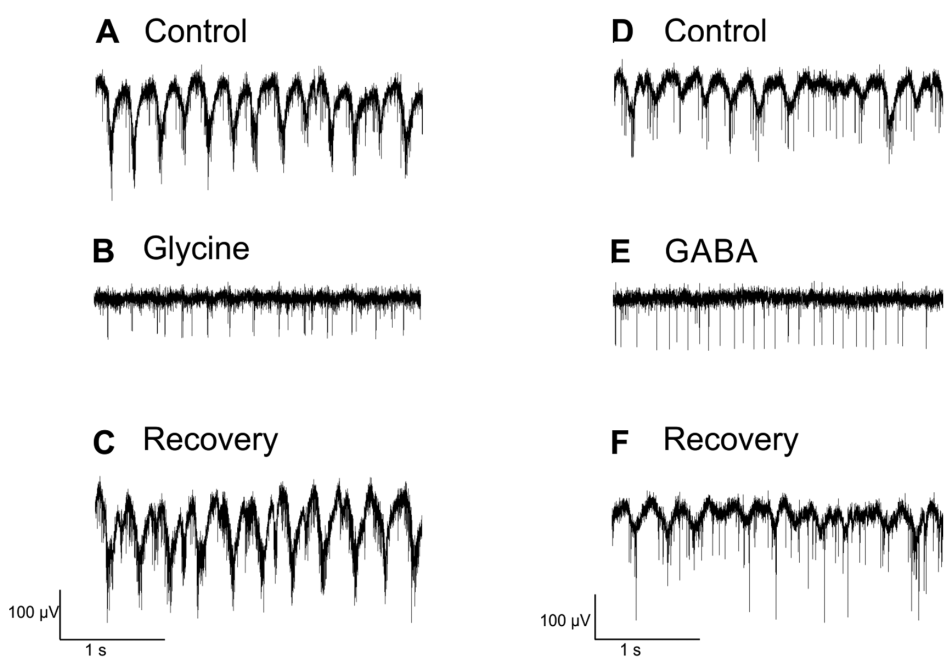

Figure 3. Effect of the inhibitory neurotransmitters glycine and GABA on rhythmic electrical activity in *rd10* retina. (**A**, **D**) Recording under control condition (unfiltered raw data) displayed oscillations in the LFP. (**B**) Application of 100 µM glycine for 7 minutes nearly abolished oscillations. Oscillations were also abolished by 40 µM glycine; however, a wash-in time of 20 to 30 minutes was required (data not shown). (**C**) The effect was reversible during wash-out (10 minutes). (**E**) Application of 500 µM GABA for 5 minutes completely abolished oscillations. With 100 µM GABA (data not shown), oscillations vanished on most electrodes and amplitudes of oscillations were decreased on remaining electrodes after wash-in time of 20 to 30 minutes. (**F**) The effect was reversible during wash-out. The age of the *rd10* mice was 3.5 months.

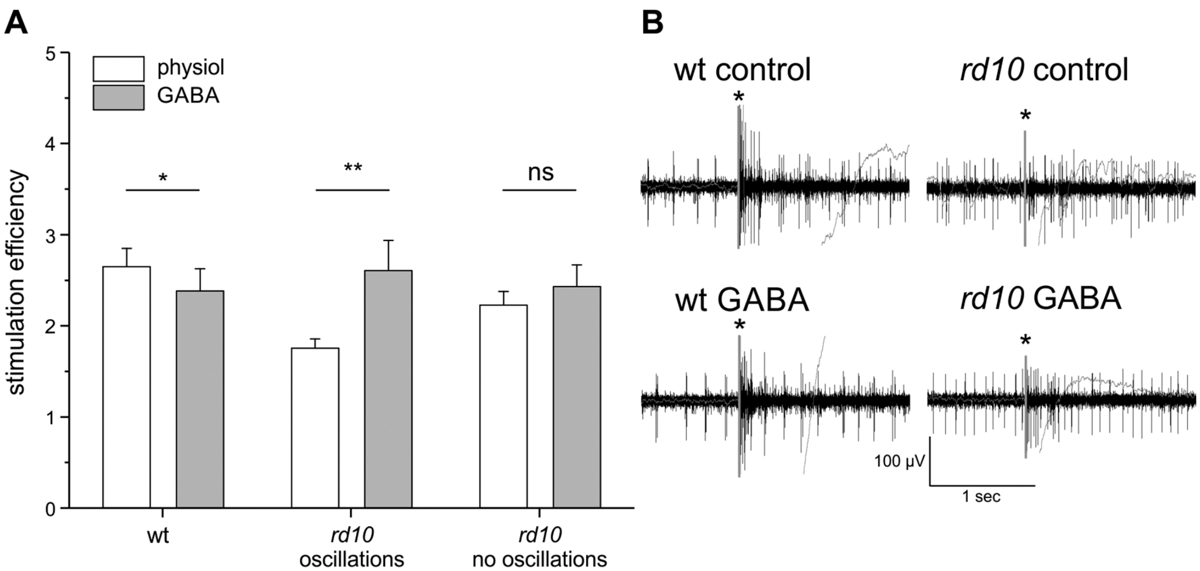

Figure 4. Effect of GABA on electrical stimulation in wt and *rd10* retina. (**A**) Comparison of the stimulation efficiency in wt and *rd10* retina showing oscillations or showing no oscillations under control condition and during GABA application. Analyzing the stimulation efficiency, the poststimulus AP rate was divided by the prestimulus AP rate. The bar chart depicts the mean ± standard error of the mean. The stimulation efficiency was: wt, control 2.65 ± 0.13 ($n_{\text{cells}} = 162$), GABA 2.38 ± 0.16 (Mann–Whitney rank sum test; *$P = 0.031$); *rd10* showing oscillations, control 1.75 ± 0.07 ($n_{\text{cells}} = 124$), GABA 2.61 ± 0.22 (Mann-Whitney rank sum rest; **$P = 0.010$); *rd10* without oscillations, control 2.23 ± 0.10 ($n_{\text{cells}} = 350$), GABA 2.43 ± 0.16 (Mann-Whitney rank sum test, $P = 0.319$ [not significant]). In total, the stimulation efficiency was evaluated in 12 retinae of 8 wt mice and in 15 retinae of 9 *rd10* mice. (**B**) (*Top row, left*): Typical response of a wt retina (age, 3 months) to a biphasic current pulse showing a clear burst of APs after the stimulation pulse (*bar with asterisk*). (*Right*) Example of the reaction of *rd10* retina showing oscillations (age, 3.5 months) to the same biphasic current pulse. No clear burst was observed (the small burst after the stimulus is part of the intrinsic bursting activity of *rd10*). (*Bottom row*) Response behavior of the same cells during GABA. A prominent burst of APs was triggered by the stimulus also in the *rd10* cell (*grey bar*, 46 cells in 11 retinae of 7 mice).

rd10 retina. Both substances abolished retinal oscillations in an effective and reversible way.

Most important, the blockade of oscillations by GABA (Fig. 4) or glycine (data not shown) was concomitant with the increase in stimulation efficiency. In *rd10* retina with oscillations, stimulation efficiency increased from 1.75 ± 0.07 to 2.61 ± 0.22 during GABA application, that is, to levels found in wt retina (Fig. 4A). No effect could be observed in *rd10* retina without oscillations. In wt retina, there was a slight decrease in the stimulation efficiency during GABA application (Mann–Whitney rank sum test, *$P = 0.031$). The stimulation efficiency is determined by dividing the poststimulus AP rate by the prestimulus AP rate, that is, the spontaneous activity. Because GABA caused a slight decrease in the spontaneous activity of the RGCs (Fig. 3), one might argue that the spike rate ratio was artificially increased by decreasing the denominator. Figure 4B compares the different response behaviors of wt and *rd10* retina with our standard stimulus pulse. In the absence of GABA, a typical burst of APs was evoked by the stimulus in wt retina but not in *rd10* retina showing oscillations (top row). However, a clear burst could be elicited in the same RGC when the oscillations were blocked by GABA (bottom row). These findings provide proof that the increase in stimulation efficiency does not simply rest on a mathematical artifact, but reflects an increase in the number of APs to electrical stimulation.

Next, we tested the effect of the three $GABA_A$ receptor modulators diazepam, flunitrazepam, and lorazepam. Figure 5 shows that all three benzodiazepines evoked similar effects as GABA: (1) oscillations were effectively and reversibly abolished, (2) stimulation efficiency was strongly increased, and (3) clear bursts of APs were elicited by electrical stimulation in *rd10* retina in the presence of benzodiazepines but barely in their absence (only depicted for diazepam, not shown for the other benzodiazepines). Compared with GABA, the wash-in and wash-out times for all three benzodiazepines was longer (wash-in for 100 µM diazepam, 10–15 minutes; 20 µM diazepam, 25–30 minutes; and 10 µM diazepam, 40–50 minutes). In most cases, oscillations came back after 30 minutes of wash-out time.

Discussion

Two major physiologic changes have been described in models of RP: rhythmic activity and low efficiency of electrical stimulation. Our study shows that these features are closely correlated. First, stimulation efficiency was strongly decreased in RGCs showing oscillations, but much less or not at all in RGCs without oscillations. Second, blocking oscillations increased the efficiency of electrical stimulation to values observed in wt retina.

Several mechanisms were suggested to induce oscillations in the retinal network, among them remodeling processes, including the formation of new synaptic connections that might induce pacemaker activity.[3,27–32] Abnormal spontaneous activity in the outer retina of *rd1* mouse was mediated by pathologic synaptic connections between cones and rod bipolar cells as a consequence of retinal remodeling.[33] Using the rod bipolar cell as route, this activity might also be relayed to the inner retina and affect RGC firing. However, with 1 to 3 Hz, the frequency of these oscillations was lower than the frequency of oscillations recorded from

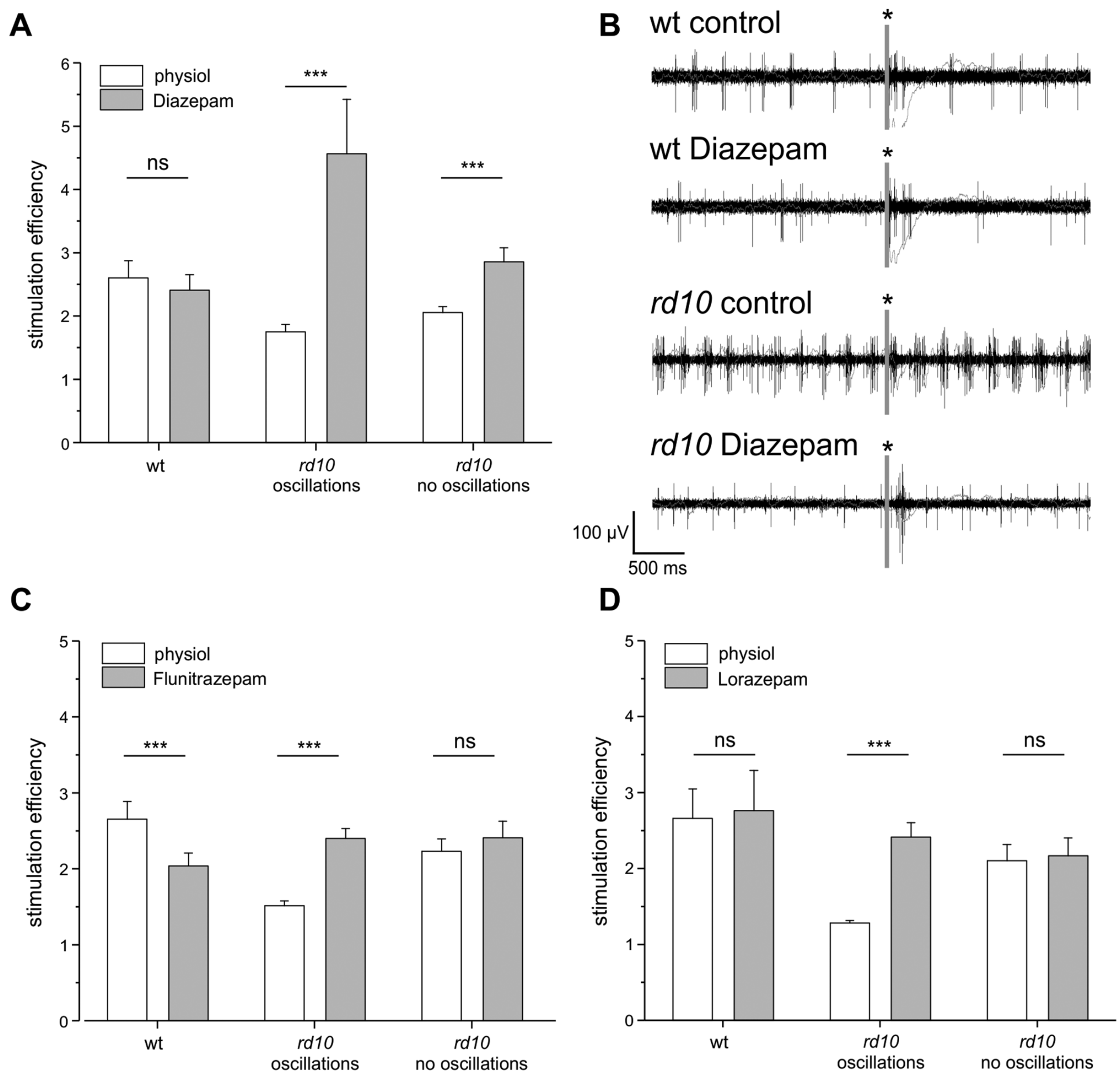

Figure 5. Effect of different benzodiazepines on electrical stimulation in wt and *rd10* retina. (**A**) Diazepam. The stimulation efficiency was: wt, control 2.6 ± 0.18 (n_{cells} = 135), diazepam 2.4 ± 0.16 (Mann-Whitney rank sum test, $P = 0.098$ [not significant]); *rd10* showing oscillations, control 1.75 ± 0.08 (n_{cells} = 52), diazepam 4.56 ± 0.57 (Mann-Whitney rank sum test, $^{***}P \leq 0.001$); *rd10* without oscillations, control 2.06 ± 0.06 (n_{cells} = 251), diazepam 2.85 ± 0.15 (Mann-Whitney rank sum test, $^{***}P = 0.001$). (**B**) (*Upper two rows*) Example of the reaction of an RGC in wt retina (age, 3 months) under control conditions and in the presence of diazepam. Stimulus-elicited burst is similar in both cases. (*Lower two rows*) Example of the reaction of an RGC in *rd10* retina showing oscillations (age, 3.5 months). Clear bursts could barely be observed under control condition (note that bursts reflect oscillatory activity), but after oscillations were blocked by diazepam. (**C**) Flunitrazepam. The stimulation efficiency was: wt, control 2.65 ± 0.15 (n_{cells} = 167), flunitrazepam 2.04 ± 0.11 (Mann-Whitney rank sum test, $^{***}P \leq 0.001$); *rd10* showing oscillations, control 1.51 ± 0.04 (n_{cells} = 254), flunitrazepam 2.4 ± 0.09 (Mann-Whitney rank sum test, $^{***}P \leq 0.001$); *rd10* without oscillations, control 2.23 ± 0.11 (n_{cells} = 244), flunitrazepam 2.41 ± 0.15 (Mann-Whitney rank sum test, $P = 0.984$ [not significant]). (**D**) Lorazepam. The stimulation efficiency was: wt, control 2.66 ± 0.26 (n_{cells} = 49), lorazepam 2.76 ± 0.35 (Mann-Whitney rank sum test, $P = 0.649$ [not significant]); *rd10* showing oscillations, control 1.28 ±0 .02 (n_{cells} = 268), lorazepam 2.41 ± 0.13 (Mann-Whitney rank sum test, $^{***}P \leq 0.001$); *rd10* without oscillations, control 2.1 ± 0.14 (n_{cells} = 101), lorazepam 2.17 ± 0.16 (Mann-Whitney rank sum test, $P = 0.711$ [not significant]). In total, the stimulation efficiency was evaluated in 5 retinae of 3 wt mice and in 12 retinae of 7 *rd10* mice for diazepam application, in 7 retinae of 4 wt mice and in 14 retinae of 8 *rd10* mice for flunitrazepam application, and in 3 retinae of 2 wt mice and in 11 retinae of 7 *rd10* mice for lorazepam application.

rd1 (9–16 Hz[9,17,18,34]). In contrast, it was suggested that oscillations may simply arise from the loss of photoreceptor input without the necessity of remodeling processes. This idea is supported by studies in wt retina, in which the blockade of signal transmission from photoreceptors to bipolar cells[18] or bleaching of photopigments[35] led to oscillations similar to those in RP models. However, oscillations were described in 2-week-old *rd10* retina before photoreceptor degeneration and major remodeling starts.[36]

Upon the loss of photoreceptors in *rd10* retina, the decrease in glutamatergic input in conjunction with changes in the expression and distribution of the glutamate receptor mGluR6[2,29,37,38] may lead to a change in the membrane potential of cone ON-bipolar cells and the electrically

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coupled AII amacrine cells. Oscillations were reported to only appear when the membrane potential of AII amacrine cells was found within the range suitable to activate sodium channels in these cells.[21] Rhythmic activity in AII amacrine cells could trigger rhythmic release of glutamate by the bipolar cells and, thus, oscillatory activity in RGCs.[18] This model is supported by studies showing that oscillations were abolished by gap junction blockers[8,18,34,39] (however, note that also oscillations in the outer retina were dependent on electrical synapses[33]) and by glutamate receptor blockers.[8,14,17,34] In contrast, the model published by Yee et al.[22] proposes intrinsic oscillators in amacrine cells as source of oscillations, whereas oscillations in bipolar cells were considered to be irrelevant. Our data can be reconciled with both models. We showed that the application of glycine, GABA, and benzodiazepines had the same effects on *rd10* retina: (1) Spontaneous RGC spiking was decreased, in agreement with the overall inhibitory effect of these substances. (2) Oscillations were blocked in agreement with a possible shift of AII amacrine cell membrane potential in the AII model[21] or hyperpolarization of the amacrine cell oscillators[22] concomitant with reduced oscillatory input into the retinal network. (3) The signal-to-noise ratio of electrically evoked activity was improved. (4) Most important, in agreement with the central hypothesis of our study, stimulation efficiency was strongly enhanced as the number of APs elicited per stimulus increased.

In previous studies in degenerated retina, the gap junction blocker MFA blocked oscillatory activity[8,18,34] and improved the signal-to-noise ratio of RGC activity when stimulated electrically or optogenetically.[23,39,40] We observed an increase in the signal-to-noise ratio in two steps. In *rd10* retina, regular bursts of typically 4.26 ± 0.01 APs generated a high level in background activity. With oscillations blocked, bursting and background noise disappeared. At the same time, the number of APs elicited by each stimulus increased to 13.5 ± 0.65 APs, similar to wt retina (12.8 ± 0.64 APs). Our results suggest that the decrease in the background noise and the increase in the number of APs per stimulus would synergistically and strongly improve the visual percept elicited by a retinal implant. Because basically all cells express inhibitory receptors,[24] targeting these receptors is expected to generally dampen activity rather than induce complex changes in RGC firing in agreement with our results. Importantly, our data show that, in *rd10* retina, even strong stimulation of these receptors did not totally abolish activity, but rather enabled an increase in stimulation efficiency.

In ERG measurements of patients with RP, oscillations have not yet been described. However, because ERG recordings average over the entire retina, even slight differences in the frequency at different retinal sites or phase shifts between retinal areas (as shown by Biswas et al.[8]) would average oscillations out. Several lines of evidence argue that oscillations are not confined to *rd1* and *rd10* retina. First, rhythmic activity has been also reported in models of pharmacologically[10] or optically[41] induced photoreceptor degeneration, as well as in minipig models of RP.[42] Oscillations, therefore, seem to be a common feature in degenerated retina. Second, patients with RP have reported the presence of phosphenes[43] that could reflect spontaneous retinal activity. It is, therefore, conceivable that oscillatory activity also exists in patients with RP. Electrical prostheses can partially restore vision, for example, enabling the differentiation of large geometric forms or letters. However, in many instances the benefit of retinal prostheses was smaller than anticipated. Two likely explanations should be considered: (1) the signal elicited by the implant might be buried in a high background noise of activity, making it difficult for the brain to interpret the data or (2) the efficiency of electrical stimulation might be suboptimal. This may be particularly pronounced if the contact between implant and retina is poor.[44,45]

Our results show that benzodiazepines might yield a strong improvement in the performance of retinal implants by increasing signal-to-noise ratio and stimulation efficiency at the same time. Two scenarios can be envisioned that allow replication of these results *in vivo* in animal models and capitalizing on the beneficial effect in human RP therapy. First, inhibitory amino acids or benzodiazepines could be delivered specifically and continuously to the retina in a targeted manner. Ophthalmic systems are already applied for a controlled delivery of medicines to the posterior segment of the eye, for example, the nonbiodegradable fluocinolone acetonide intravitreal implant Iluvien (Alimera Sciences, Alpharetta, GA) or the biodegradable dexamethasone intravitreal implant Ozurdex (Allergan, Dublin, Ireland) (for review[46]). A recent study has described the development of a nanofluidic microsystem that represents a potential platform for long-term intraocular delivery of therapeutics.[47] Such devices could deliver benzodiazepines at high retinal concentrations without eliciting unwanted systemic side effects.

Second, benzodiazepines can be taken orally—a well-established procedure in human pharmacology. The plasma concentration of diazepam in patients in typical dosage is around 100 to 1500 ng/mL or 0.5 to 5.0 µM.[48–50] The values in the brain were reported to be around three times higher.[51] However, this value was obtained from brain samples and, therefore, averaged over both intra- and extracellular spaces. Hence, it can be expected that the concentration in the extracellular space is higher than the average value. The lowest concentration of diazepam that reliably blocked oscillations in our experiments was 10 µM; however, the wash-in time was significantly longer than with 20 or 100 µM. Patients receiving diazepam at regular intervals might have a sustained basal diazepam concentration that could overlap with the concentration range used in our experiments. It is, therefore, tempting to speculate that benzodiazepines used in typical dosage might show similar beneficial effects as in our *in vitro* experiments.

In summary, we show that the three most commonly used benzodiazepines in human therapy decrease background noise, increase the signal-to-noise ratio, and considerably improve the efficiency of electrical stimulation. Using benzodiazepines or related substances with fewer side effects may improve the performance of retinal implants and may lead the way to a combined electropharmacologic therapy in the treatment of RP.

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