Temporal Response Properties of Retinal Ganglion Cells in *rd1* Mice Evoked by Amplitude-Modulated Electrical Pulse Trains

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**Purp**ose. The electrophysiological properties of degenerated retinas responding to amplitude-modulated electrical pulse trains were investigated to provide a guideline for the development of a stimulation strategy for retinal prostheses.

**Methods**. The activities of retinal ganglion cells (RGCs) in response to amplitude-modulated pulse trains were recorded from an in vitro model of retinal prosthesis, which consisted of an *rd1* mouse retinal patch attached to a planar multielectrode array. The ability of the population activities of RGCs to effectively represent, or encode, the information on the visual intensity time series, when the intensity of visual input is transformed to pulse amplitudes, was investigated.

**Results**. An optimal pulse amplitude range was selected so that RGC firing rates increased monotonically and linearly. An approximately 10-Hz rhythm was observed in the field potentials from degenerated retinas, which resulted in a rhythmic burst of spontaneous spikes. Multiple peaks were present in post-stimulus time histograms, with interpulse intervals corresponding to the oscillation frequency of the field potentials. Phase resetting of the field potential oscillation by stimulation was consistently observed. Despite a prominent alteration of the properties of electrically evoked firing with respect to normal retinas, RGC response strengths could be modulated by pulse amplitude. Accordingly, the temporal information of stimulation could be faithfully represented in the RGC firing patterns by an amplitude-modulated pulse train.

**Conclusions**. The results suggest that pulse amplitude modulation is a feasible means of implementing a stimulation strategy for retinal prostheses, despite the marked change in the physiological properties of RGCs in degenerated retinas. (Invest Ophthalmol Vis Sci. 2010;51:6762–6769) DOI: 10.1167/iovs.10-5577

Many retinal ganglion cells (RGCs), and following central visual nervous system, may remain functional despite the photoreceptor degeneration caused by retinitis pigmentosa or age-related macular degeneration. Thus, the restoration of vision by electrical stimulation may be possible. Visual prosthetic devices, including retinal prostheses, have been actively pursued for this purpose. Current research has been focused primarily on hardware development, surgical techniques, and human trials for crude visual perception. For successful vision, neural responses should convey sufficient information on the spatiotemporal patterns of visual input. Determining a mechanism to generate electrical stimulation is crucial to the development of visual prosthetic devices. Unlike the attention given cochlear implants, stimulation strategies for visual prosthesis have been given little attention. Because neural responses strongly depend on specific stimulation parameters such as the rate, intensity, and duration of pulses, valuable information that can be used to determine optimal parameters can be obtained by observing the changes in RGCs in response to these parameters. We recently showed the feasibility of this approach by analyzing the multiunit RGC responses to temporally patterned pulse trains using a planar multielectrode array (MEA) and normal retinas.

The neurophysiological properties of photoreceptor-degenerated retinas are significantly altered compared with those of normal retinas, as has been demonstrated by recent studies using retinal degeneration animal models such as *rd1* mice. The most significant alteration of spontaneous neural activities of the *rd1* retina is the presence of an oscillatory rhythm with an 8- to 10-Hz frequency. These abnormal rhythms were observed from both spontaneous RGC spikes and field potentials. Accordingly, the electrically evoked RGC activities are also expected to be considerably altered, and the RGC activities evoked by electrical stimulation should be investigated using a degenerated retina. There have only been a few studies on electrically evoked *rd1* RGC responses, and none have examined the effect of the abnormal rhythm.

In this study, the electrophysiological properties of the degenerated retinas of *rd1* mice responding to amplitude-modulated pulse trains were investigated to provide a guideline for the development of a stimulation strategy for retinal prostheses. Assuming that visual input intensity can be encoded by pulse amplitudes in retinal prostheses, the ability of RGC activity to effectively represent, or encode, the temporal pattern of visual intensity was studied. The results herein were compared with those from our previous study on RGC activities in the normal retina evoked by amplitude-modulated pulses.

**Materials and Methods**

**Retinal Tissue Preparation**

Animal use protocols were approved by the institutional animal care committee of Chungbuk National University. The *rd1* mice (C3H/HeJ)
strain, postnatal day [P] 56) were used as animal models of retinal degeneration. The animals were anesthetized with an intramuscular injection of 30 mg/kg zolazepam hydroxide ( Zoletil 50; Virbac, São Paulo, Brazil) and 10 mg/kg xylazine hydroxide (Rumpun; Bayer, Shawnee Mission, KS) sufficient to extinguish forefoot withdrawal reflexes. Retinal patches were prepared according to the method of Stett et al. Briefly, the eyeball was enucleated, and the retina was isolated and cut into approximately 3 mm × 3 mm patches. After placement in artificial cerebrospinal fluid bubbled with 95% O2 and 5% CO2 at 32°C, the retinal patches were mounted onto a planar MEA, with the ganglion cell layer facing downward onto the MEA.

Recoding of Retinal Field Potential and Single-Unit RGC Activity

A planar MEA (Multichannel Systems GmbH, Reutlingen, Germany) was used to record field potentials and RGC spiking activities. The MEA contained 64 TiN electrodes (circular shape; diameter, 30 μm) on a glass substrate in an 8 × 8 square-type grid layout, with 200-μm interelectrode spacings. The four electrodes at the vertices were inactive. Impedances of the electrodes were approximately 50 kΩ.

Electrical Stimulation

Before stimulation, spontaneous activities were recorded for approximately 5 minutes. Amplitude-modulated current pulse trains were generated and applied to the retina by 1 of 60 electrodes on the MEA. An electrode at the center was selected for stimulation, and the others were used for the recording. The stimuli consisted of symmetric, charge-balanced biphasic pulses (Fig. 1A). Pulse durations were fixed at 500 μs per phase. Pulse trains were applied to the stimulation electrode using a stimulus generator (STG 1004; Multichannel Systems GmbH).

For the characterization of the modulation behavior of RGC responses by pulse amplitude, trains of 20 identical pulses were applied at 1-second interpulse intervals while the pulse amplitude was increased. The pulse rate was fixed at 1 Hz, and the order of presenting pulse trains with different amplitudes (2, 5, 10, 20, 30, 40, 50, or 60 μA) was randomized. Pulse amplitudes were then modulated according to predetermined temporal patterns, such as the triangular and sawtooth waveforms shown in Figures 1B and 1C, respectively, to determine whether the temporal pattern of RGC responses could be made to follow the temporal pattern of pulse amplitude variation.

Pulse train amplitudes were also modulated by a random time series to quantify the accuracy of the temporal pattern encoding the RGC responses. Random time series with Gaussian distributions were generated and low-pass filtered using a fourth-order Butterworth filter with a 0.1-Hz cutoff frequency. Because the spectral power of the low-pass-filtered Gaussian time series decreases as a function of frequency, it provides a good model of natural scenes. The filtered time series were transformed into the amplitudes of the electrical pulse trains within a range of 2 to 20 μA with a 1-μA resolution. The temporal pattern of Gaussian random amplitude modulation is shown in Figure 1D. The repetition rate of the pulse trains varied from 1 to 6 Hz, and the stimulus presentation time was fixed at 150 seconds.

Statistical Analysis

The raw waveforms were separated into field potentials and spike trains using low- and high-pass filtering (for field potential: second-order Butterworth low-pass filter, 20-Hz cutoff frequency; for spikes: second-order high-pass filter, 100-Hz cutoff frequency).

Because the recordings within approximately 5 ms of the stimulus onset were usually obscured by stimulus artifacts, an artifact removal technique based on average subtraction was applied to recover early-phase (short-latency) RGC spike responses. Two distinct classes of recorded waveforms near the stimulus onset, with or without evoked RGC action potentials, could be visually identified. The average waveforms of the stimulus artifact could be estimated from multiple waveforms that did not contain the evoked action potentials. The RGC spike waveforms within approximately 5 ms of the stimulus onset could be recovered by subtracting the averaged stimulus artifact obtained from single trial waveforms containing action potentials.

Spike sorting software (Offline Sorter; Plexon Inc, Dallas, TX) was then used to transform the waveforms containing multiunit activities into multiple single-unit spike trains. The number of poststimulus RGC spikes was counted to measure the RGC response strength and to analyze the modulation of the RGC response strength by pulse amplitude. To quantify the accuracy of the input information represented by the RGC responses, similarities between the pulse amplitude variation time series and the RGC response strength time series were computed by coding fraction. The coding fraction, γ, is defined as follows:

$$\gamma = 1 - \frac{\epsilon}{\sigma_\epsilon}$$

where $\epsilon$ and $\sigma_\epsilon$ denote the root mean square error of estimating pulse amplitude time series from firing rate time series and standard deviation of pulse amplitude time series, respectively. $\epsilon$ can be calculated from the difference between pulse amplitude time series and firing rate time series. When the firing rate is not at all correlated to the input (i.e., pulse amplitude), the mean square error becomes equal to the variance of stimulus $\sigma^2$, and thus, $\gamma$ becomes 0. Conversely, when the two time series are perfectly correlated, $\epsilon$ is zero and, thus, $\gamma$ becomes 1. Hence, the coding fraction provides a convenient method to quantify the accuracy of stimulus encoding. We evaluated the accuracy of temporal information on the input that is encoded in RGC responses by the coding fraction.

**FIGURE 1.** Generation of biphasic pulse trains for stimulation. (A) Amplitude modulation of biphasic pulse trains based on a linearly increasing waveform. (B) Triangular waveform (period = 10 seconds, 1 pulse/s). (C) Sawtooth waveform (period = 18 seconds, 1 pulse/s). (D) Band-limited Gaussian random waveform. The pulse rate was fixed to 1 pulse/s (150 seconds).
RESULTS

Single-unit spiking activities were found from 334 RGCs of seven retinal patches obtained from seven mice. The average number of single-unit RGCs per patch was 47.7 ± 4.39. Among these, 135 RGCs (40.4%) showed consistent and evident modulation of evoked responses according to pulse amplitude. For these cells, the correlation coefficients between the number of poststimulus spikes and pulse amplitudes were greater than 0.8. For another 58 RGCs (17.4%), the response modulation by pulse amplitude did not exhibit monotonic behavior, though evoked responses were observed. The remaining 141 RGCs (42.2%) did not show any reliable evoked responses but did show spontaneous firings.

Rhythmic Local Field Potential and Spontaneous RGC Activities

Rhythmic oscillatory behaviors in field potential waveforms were clearly observed (Figs. 2A, 2B), similar to those in our previous study.14 The spectral peak of the field potential was at approximately 8 Hz (Fig. 2B, right panel). The spontaneous RGC firing pattern also showed a consistent temporal structure with a rhythmic burst of spikes (Fig. 2C). The interspike interval histogram (ISIH; Fig. 2C, right panel) demonstrates the temporal structure of spontaneous RGC spike trains in detail. There were two distinct peaks in the ISIHs. The first peak, at approximately 15 ms, was the result of an interspike interval within a single burst of spikes. The second peak, at approximately 120 ms, roughly corresponded to the interburst interval. These results are consistent with the recent results of Stasheff,17 which showed a rhythmic burst of spikes, and with those of Margolis et al.,16 which showed an oscillatory synaptic input and a rhythmic burst of spikes.

Comparison of the field potential and spike waveforms shown in Figures 2B and 2C revealed a close relationship between the phase of the oscillatory rhythm and the firing time of the spontaneous RGC action potential. The spontaneous RGC spike bursts occurred at the troughs of the oscillatory rhythm (Fig. 2C, arrows). Taken together, the temporal structure of the field potential and the spontaneous spike train suggest that the oscillatory waveform of the field potential reflects synaptic input, which produces the rhythmic burst of spontaneous RGC spikes that is phase-locked with the oscillatory field potential.

RGC Responses Evoked by Electrical Pulses

Typical waveforms of the responses of a representative RGC to biphasic pulse stimulation (five trials) are plotted in Figure 3A. Initially, the waveforms seemed to be similar to the spontaneous waveform (Fig. 2A) because they included an oscillatory rhythm in the background and a rhythmic burst-type firing of action potentials. The number of spikes, however, increased primarily within approximately 100 ms of stimulus onset (Fig. 3A, arrows), which roughly corresponded to the first burst of spikes. The temporal structure of the RGC responses, which was investigated by the poststimulus-time histogram (PSTH; Fig. 3B), was drastically different from that of normal retinal RGCs, which show a single-peak profile.12,19 Multiple peaks were repeatedly present, with interpeak intervals that were close to the interburst interval (120 ms). The heights of the first peak, which occurred at approximately 50 ms, were much higher than those of later peaks, and later peaks faded over poststimulus time. Typically, there were four or five distinct peaks in the PSTHs (Fig. 3B).

Overlapped waveforms of the RGC responses to pulses at various amplitudes (2–60 µA) are shown in Figure 4A. RGC response strength was quantified by counting the number of poststimulus spikes within 400 ms after stimulation onset, which corresponded to the first three peaks in the PSTHs. Response strengths increased as a function of pulse amplitude (10 representative RGCs; Fig. 4B). The number of poststimulus RGC spikes generally increased monotonically when the pulse amplitude was increased up to approximately 20 µA and was saturated thereafter.

The amplitude modulation behavior of the RGC spikes within each peak of the PSTH was also investigated. The temporal epochs of the first three peaks were defined by

FIGURE 2. Recorded waveforms of spontaneous retinal activity. (A) Typical raw waveform. (B, left) Field potential waveform isolated by low-pass filtering (second-order Butterworth low-pass filter; cutoff frequency = 20 Hz) showing clear rhythmic oscillatory behavior. Right: power spectral density of the field potential shown (estimated by Burg algorithm).31 with the spectral peak, arrow; at approximately 8 Hz. (C, left) Spontaneous spike trains extracted by highpass filtering (second-order Butterworth highpass filter; cutoff frequency = 100 Hz). Arrows: rhythmic burst of spikes at approximately 8 Hz is obvious from the temporal structure of the waveform. Right: interspike interval histogram of the spontaneous spike train shown at the left. The ISIH contains two distinct peaks. The first peak, at approximately 15 ms, seemed to be caused by the interspike interval within one burst of spikes. The second peak, at approximately 120 ms, roughly corresponded to the interburst interval.
visual inspection of the PSTHs. For the RGC shown in Figure 3, for example, the three peaks were defined as 0 to 100 ms, 110 to 250 ms, and 280 to 420 ms. The spikes in the first and second peak could be effectively modulated by pulse amplitudes (Figs. 4C, 4D). Optimal modulation of the RGC response strength was found to be possible when the pulse amplitude was varied within a 0- to 20-μA range because the behavior of the response strength with respect to the pulse amplitude was close to linear and increased monotonically. The behavior of the response strength modulation was best for the spikes within the first peak, though those in the second and third peaks could also be modulated by the pulse amplitude.

**Phase Resetting of Oscillatory Field Potential**

Field potentials were significantly affected by stimulation. Overlapped field potential waveforms recorded during 20 repetitive stimulations with four different pulse amplitudes (30, 40, 50, and 60 μA) are shown in Figure 5A. The phases of the oscillatory field potentials were randomly distributed over trials before stimulation (Fig. 5A, thin traces), and the amplitudes of the intertrial average waveforms (Fig. 5A, thick traces) were accordingly much smaller than those of single-trial waveforms before stimulation. Conversely, the phases of the oscillatory rhythms were reset by the stimulation pulse, and each trial turned into synchronization by stimulation. This pattern is reflected by the significant amplitude increase in the intertrial average waveforms after stimulation (Fig. 5B). In addition, the intertrial average waveforms at different pulse amplitudes were very similar after the stimulation onset (Fig. 5B).

**Representation of Pulse Amplitude Time Series by Evoked RGC Activities**

The temporal patterns of the RGC response strengths closely replicated the pulse train amplitudes, which were modulated based on triangular and sawtooth waveforms. Pulse amplitude and RGC response strength time series, measured by the number of poststimulus spikes within 0 to 100 ms, were very similar to each other, suggesting that the RGC responses were a faithful representation of the temporal information on pulse amplitude variation. Typical examples of pulse amplitude and RGC response time series are shown in Figures 6A and 6B. Optimal modulation of the pulse amplitude was observed as the number of poststimulus spikes occurring within 200 ms post-stimulus were included in the determination of the response strength. The similarity between the pulse amplitude and the response strength time series was much higher when the poststimulus spikes within the 0 to 200-ms range (Fig. 6A; coding fraction, 0.84) were considered rather than those within the 0 to 100-ms range (Fig. 6B; coding fraction, 0.92). The difference between the two cases was statistically significant (Fig. 7C; 27 repeated trials, t-test, P < 10⁻⁶).

**DISCUSSION**

Spontaneous and stimulated activities of RGCs from rd1 mice were investigated in this study. An observed approximately 10-Hz rhythm in field potential, which had been previously recognized, was shown to result in a rhythmic burst of spontaneous spikes with the same frequency. Although the properties of electrically evoked RGC responses in the degenerated retinas were markedly altered from those of normal retinas, the RGC response strengths could still be modulated by the pulse amplitude. Accordingly, the temporal information of input could be faithfully encoded in the RGC firing patterns. These results may provide useful information to help design a stimulation strategy for retinal prostheses.

**Aberrant Oscillatory Rhythm**

RGCs in degenerated retinas have been demonstrated to be structurally stable, but the neuronal activity in these degenerated retinas were remarkably modified with respect to those in normal retinas. An aberrant, approximately 10-Hz rhythm was observed in both the spontaneous RGC spiking and the field potential in this study. These results are consistent with the recent findings that showed an oscillation in synaptic currents and a rhythmic burst of spontaneous spikes using intracellular recordings. The rhythmic firing pattern of the rd1 spontaneous spikes was also recently reported in extracellular recordings obtained using MEA.

The oscillatory field potential is expected to result from the fluctuation of synaptic currents because of abnormally strong excitatory glutamate signaling from a bipolar cell to the RGC based on several factors: the observation that glutamate antagonists cause the rhythm to disappear; the consistency with recent findings that demonstrated elevated glutamate signaling...
in degenerated retinas, and the potential effect of decreased inhibitory inputs caused by the degeneration. The strong oscillatory rhythm induced hyperactivity in spontaneous rd1 RGC spikes in a recent study by Stasheff. The shapes of the ISIHs are similar in both the present study and that of Stasheff, with both containing secondary peaks that reflected the rhythmic burst of spikes at an approximately 10-Hz frequency.

This aberrant rhythmic activity has a significant influence on the electrically evoked RGC responses of the degenerated retina, subsequently resulting in significantly different RGC responses than those of normal retinas. Several studies on the electrically evoked RGC responses of degenerated retinas can be found in the literature. Using direct current injection and patch-clamp recording, Margolis et al. observed that the intrinsic firing properties of RGCs are maintained in the face of retinal degeneration. Although the temporal structure of electrically evoked RGC spikes revealed by PSTH was drastically altered from that of normal retina, our results support the stability of the intrinsic firing property given that the primary response peaks occurred primarily at approximately 50 ms poststimulus. Secondary and later peaks seemed to be

![Figure 4](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932969/)
caused by the phase resetting of synaptic inputs, which resulted in the realignment of spontaneous spikes by stimulation.

The phase of the oscillatory rhythm was reset by stimulation, with strong phase locking occurring after stimulation over multiple trials. This effect was also expected to cause phase resetting of the RGC spike bursts. The timing of the spike bursts became synchronized by the phase resetting of the field potential, with each trial turning into a synchronized rhythm, as reflected by the significant amplitude increase of the intertrial average waveform subsequent to the stimulation. (B) Overlapped plot of the average waveforms of different stimulation pulse amplitudes. This plot clearly demonstrates that the field potential phases were reset by stimulation caused by the phase resetting of synaptic inputs, which resulted in the realignment of spontaneous spikes by stimulation.

As discussed, a few studies on electrically evoked RGC spikes in the degenerated retina have been reported in the literature, but none mention the oscillatory field potential and resultant rhythmic burst of spikes. The failure to detect the oscillatory field potential may be explained by the bandpass filter being set for extracellular recording, which is usually tuned for the extraction of spike waveforms (e.g., cutoff frequencies of 100-5000 Hz were used in Jensen and Rizzo18). Jensen and Rizzo15,18 observed that the spikes from some RGCs occurred in two or three bursts, which may reflect the effects of the oscillatory synaptic input as described in Margolis et al.16 Although O’Hearn et al.15 stated that there was no dramatic change in the response properties of rd1 RGCs, direct comparison between their and our studies seems to be inappropriate given that they only monitored the early-phase responses, occurring at approximately 2 ms after stimulation.22,28 These early-phase responses occurred only once per pulse and, thus, account for only a small portion of the overall evoked responses.

**Pulse Amplitude Modulation of rd1 RGC Responses**

In normal retinas, evoked RGC responses are concentrated in the 20- to 50-ms period after stimulation12,19 and form a single peak in PSTHs. Considering that the first peak of the PSTHs of degenerated retinas occurred at approximately 50 ms and that the amplitudes of the first peak were considerably larger than those of later peaks, the evoked responses within the first peak of PSTH were considered to include the direct consequences of stimulation in addition to the effect of phase resetting described. The timing of the evoked RGC firing in the degenerated retina was similar to that of the normal retina, which is in agreement with the recent observation by Margolis et al.16 that showed that the intrinsic firing property of RGCs was not significantly altered in the rd1 mouse compared with the wild-type mouse.

In spite of a noticeable modification in functional characteristics, the response strength of rd1 RGCs could be consistently modulated by pulse amplitude. The responses within the first peak of PSTH could be modulated efficiently. The number of poststimulus spikes could be increased by approximately 4 spikes/s when the pulse amplitude was increased by 20 μA. This result is comparable to the response characteristics of normal RGCs that we recently reported.12 We reported that the RGC activities could be modulated to track a temporal pattern of pulse amplitude variations, which implies that the amplitude modulation is an effective method to enable encoding of temporal visual patterns by retina prosthesis.12 In the present study, we have verified this for the degenerated retina. It should be noted that successful encoding was possible only when the pulse amplitudes were modulated within the range in which response strength increased monotonically and linearly according to the pulse amplitude, as shown in Figure 4 of this study and Figure 2 of Ryu et al.12

**FIGURE 5.** Phase resetting of the rhythmic oscillatory field potential by stimulation. (A, thin black traces) Overlapped field potential waveforms recorded before and after 20 stimulations using various amplitudes. Thick gray traces: average waveforms. Before stimulation (marked above the waveforms), the phase of oscillatory field potential was randomly distributed over trials, resulting in the smaller amplitudes of the intertrial average waveforms compared with those of the single-trial waveforms. In contrast, based on the stimulation, the phases of the oscillatory rhythms could be reset, with each trial turning into a synchronized rhythm, as reflected by the significant amplitude increase of the intertrial average waveform subsequent to the stimulation. (B) Overlapped plot of the average waveforms of different stimulation pulse amplitudes. This plot clearly demonstrates that the field potential phases were reset by stimulation caused by the phase resetting of synaptic inputs, which resulted in the realignment of spontaneous spikes by stimulation.

**FIGURE 6.** Representation of a pulse amplitude time series by RGC responses. RGC response strength after temporal patterns of (A) sawtooth or (B) triangular pulse amplitude modulations. Period = (A) 10 seconds, (B) 18 seconds. Pulse rate = 1 pulses/s. The coding fractions were as high as (A) 0.93 and (B) 0.92.
The evoked spikes in the secondary peaks of the PSTH are expected to be useful for encoding relevant information on stimulus pulses because pulse amplitude can modulate the response strengths in this epoch, even though these peaks are considered to be byproducts of stimulation through phase resetting of the field potential. The response strengths in the second peak were modulated fairly well (by approximately 1.5 spikes/s when the pulse amplitude was increased by 20 \( \mu \text{A} \)). The responses in the third peak could also be modulated, though in a less effective fashion.

As expected from the response characteristics based on pulse amplitude modulation, the RGC response strengths could closely follow the temporal patterns of pulse train amplitudes when the pulse amplitudes were varied within the range of 0 to 20 \( \mu \text{A} \). Considering a recent study showing that the perceived brightness of phosphenes increased as a function of current amplitude, these results imply that the temporal information on the light intensity of visual input may be represented in RGC spike trains by the amplitude modulation of pulses. The similarity between pulse amplitude and RGC response time series increased when the spikes within the secondary peaks in the PSTHs were considered in the quantification of response strength. In the design of a stimulation strategy for a visual prosthesis, exploitation of these later spikes might be advantageous if a high stimulation rate is not necessary. This design, however, may not be adequate for the perception of a rapidly changing visual scene.

Additional Studies

In this study, we focused on the proper representation of temporal information by RGC responses, and a spatially uniform light intensity was implied. Spatial information should, however, also be accurately encoded to achieve successful visual perception by prosthetic stimulation. For this purpose, the response properties of RGCs in the degenerated retina should be studied using spatiotemporally patterned multichannel stimulation. The optimal pulse amplitude ranges should be different for the multichannel stimulation and may be determined using the strategy suggested in this study. Using mul-

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**Figure 7.** More accurate representation, or encoding, of a pulse amplitude time-series was generally possible (A) when the poststimulus spikes occurred within 0 to 200 ms after the stimulus onset was included to determine the response strength (coding fraction, 0.92) compared with (B) including the spikes within 0 to 100 ms epoch (coding fraction, 0.84). (C) Comparison of the similarities between the response strength and the pulse amplitude time series for the two different amplitude ranges. The difference between two cases was statistically significant (27 RGCs; \( t \)-test = \( P < 10^{-16} \)).
tichannel recording by MEA, it was observed that the responses of the RGCs close to or distant from the stimulation site were simultaneously evoked, though response strength decreased as a function of the distance between stimulation and recording electrodes. This is consistent with our recent results and those of Stett et al. and implies that the amplitude range must be optimized according to the intended spatial resolution because unnecessarily large pulse amplitudes may result in low spatial resolution from inadvertent stimulation of distant RGCs. This is a topic for further study.

The neuronal signal response to natural vision represents specific visual features, such as the speed and direction of motion and local contrast. The ability to encode these features and light intensity in the RGC responses by electrical stimulation should be another area of study.

Because current retinal prostheses make use of large stimulation electrodes (up to approximately 500 μm in diameter) and pulse amplitudes and durations that are non-specific bulky stimulation is expected. The reduction of pulse amplitude, duration, and electrode size should be pursued for better spatiotemporal resolution. Selective stimulation of RGCs in close proximity can be achieved by the use of a stimulation electrode as small as 25 μm. A smaller pulse amplitude/duration may be able to evoke a single spike by one pulse, enabling the precise control of spiking, as shown by Fried et al. The reduction of pulse duration has been beneficial to the excitement of smaller regions with a higher dynamic range. Whether such precise control of RGC firing time and the spatial extent of the stimulation are also feasible for degenerated retinas should be further investigated. In these future studies, the approach presented herein should be applied for minute, specific stimulation.

References