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Comparison of Basal Oscillatory Rhythm of Retinal Activities in *rd1* and *rd10* Mice

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Abstract— **Among the many animal models of retinitis pigmentosa (RP), the most extensively characterized animal is the *rd1* mouse. Recent studies showed that the neurophysiological properties of *rd1* retinas differ significantly from those of normal retina; the presence of an oscillatory rhythmic activity (~10 Hz) both in retinal ganglion cell (RGC) spikes and field potentials (slow wave component, SWC). However, lesser studies have been done regarding electrical characteristics of *rd10* retina, carrying the mutation of same rod-PDE gene and showing a later onset degeneration of photoreceptors. Therefore, in this study, we compared the oscillatory rhythm in RGC spike and SWC between *rd1* and *rd10* mice in different postnatal ages to understand neural code used by two diseased retinas to communicate with the brain. Extracellular action potentials are recorded by 8 × 8 MEA from the RGC in the *in vitro* whole mount retina. 4 and 8 weeks in *rd1* mice and 4, 10, 15, and 20 weeks in *rd10* mice were used (n=3 for each postnatal age). From the raw waveform of retinal recording, RGC Spikes and SWC were isolated by using 200 Hz high-pass filter and 20 Hz low-pass filter, respectively. Fourier transform was performed for detection of oscillatory rhythm in RGC spikes and SWC. In *rd1* mice, there is no statistical difference between the frequency of SWC and spike in 4 weeks [p>0.05; spike 9.3 ± 0.9 Hz (n=40), SWC 9.3 ± 1.5 Hz (n=25)] and 8 weeks [p>0.05; spike 10.0 ± 1.3 Hz (n=87), SWC 10.9 ± 1.7 Hz (n=25)]. While in *rd10* mice there is no statistical differences among the SWC through 4 ~ 20 weeks, significant differences were observed between the frequency of RGC spike and SWC and also among RGC spikes [4 weeks (p<0.001): spike 5.5 ± 1.3 Hz (n=59), SWC 10.8 ± 3.1 Hz (n=14); 10 weeks (p<0.001): spike 6.8 ± 3.8 Hz (n=79), SWC 10.3 ± 2.6 Hz (n=25); 15 weeks (p<0.05): spike 3.9 ± 0.7 Hz (n=33), SWC 9.9 ± 1.2 Hz (n=25); 20 weeks (p<0.05): spike 4.4 ± 1.2 Hz (n=53), SWC 9.8 ± 1.2 Hz (n=25)].**

Keywords: oscillatory rhythm, retinal ganglion cell spike, slow wave component, rd1 mice, and rd10 mice

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I. Introduction

RETINAL prostheses are being developed to restore vision for the blind with retinal diseases such as retinitis pigmentosa (RP) or age-related macular degeneration (AMD) [1]-[3]. While the retinal degenerations result in photoreceptor loss, significant numbers of bipolar and ganglion cells remain for many years. The preservation of the remaining neural network in patients with RP and AMD provides the opportunity to restore vision by means of an electronic retinal prosthesis.

Among the many animal models of RP, the most extensively characterized animal is the *rd1* (formerly *rd*, now *Pde6b*^{rd1}) mouse [4]. A major limitation of this mutant is that rod photoreceptor degeneration begins before normal synaptogenesis is complete [5]. This fact makes it difficult to distinguish the cause of blindness resulting from rod degeneration or from abnormal synaptic formation.

The more recently identified *Pde6b*^{rd10} *(rd10)* mouse, which carries a mis-sense mutation in the same gene, has a later onset and slower rate of photoreceptor degeneration than the *rd1* mouse [10]. The slower degenerative time course makes *rd10* a more appropriate model of human RP, and presents a broader window of opportunity to test therapies for photoreceptor rescue [7]-[8].

In recent studies including ours using *rd1* mice, it is known that the neurophysiological properties of photoreceptor degenerated retinas differ significantly from those of normal retina [9]-[12]. The most significant alteration of spontaneous activities of retinal networks in *rd1* mice is the presence of an oscillatory rhythmic activity with ~10 Hz frequency both in RGC spikes and field potentials [11]-[12]. We named oscillatory rhythm in field potential as slow wave component (SWC) and we proposed the mechanism of this SWC as postsynaptic potential [12]. However, only few studies have been done regarding electrical characteristics of retinal waveform in *rd10* mice, and most of them are focused on *in vivo* ERG study [13-14].

Neuronal oscillations appear throughout the nervous system, in structures as diverse as the cerebral cortex, hippocampus, subcortical nuclei and sense organs [15]. High frequency oscillations (20 to 120 Hz), those generated by the internal dynamics of the system, have been found at all stages of visual processing, from the retina to the cortex [16]. However, ~10 Hz oscillatory rhythmic activity in adult retina has hardly been reported. Whether neuronal oscillations contribute to normal function, are merely epiphenomena, or

even interfere with physiological processing are topics of vigorous debate [15]. Whatever the role is, other than spike rate it is one way of neural code of retina to communicate with the brain.

Therefore, in this study, we investigated whether there is oscillatory rhythm in RGC spikes and field potential in *rd10* mice. Also, we compared the oscillatory rhythm in RGC spikes and field potential in two photoreceptor degeneration mice; *rd1* and *rd10* mice to understand the neural code used by the two diseased retinas to communicate with the brain. This understanding will facilitate to set disease specific targeting for treatment. Some of the preliminary results have been reported in Ye *et al* [17].

II. Methods

A. In Vitro Recording of Retinal Activity

Animal use protocols were approved by the institutional animal care committee of Chungbuk National University (approval number: CBNURA-042-0902-1). Postnatal 4 weeks and 8 weeks *rd1* mice (*Pde6*^{brd1} mutation) and 4, 10, 15 and 20 weeks *rd10* mice (*Pde6*^{brd10} mutation) were used. For each different postnatal ages, 3 retinal patches out of 3 mice were used. The retinal patches were prepared following the method of Stett *et al.* [13]. The eyeball was enucleated, and then, the retina was isolated and cut to the patches of ~3 mm × 3 mm sizes. The retinal patches were carried out under moderate illumination in an artificial cerebrospinal fluid (ACSF) solution (124 mM NaCl, 10 mM Glucose, 1.15 mM KH_2PO_4, 25 mM $NaHCO_3$, 1.15 mM $MgSO_4$, 2.5 mM $CaCl_2$, and 5 mM KCl) bubbled with 95 % O_2, 5 % CO_2 with a pH of 7.3 ~ 7.4 and a temperature of 32 °C and then mounted onto a planar microelectrode array (MEA). They were placed ganglion cell layer down onto the MEA.

B. Electrode and Data Recording System

The MEA60 system (Multi Channel Systems GmbH, Germany) included electrode array, stimulator (STG1004), amplifier (MEA1060), temperature control units, data acquisition hardware (Mc_Card) and software (Mc_Rack). The electrode array had 60 active electrodes in an 8 x 8 grid layout with electrode diameters of 30 μm and inter-electrode distances of 200 μm and coated with porous titanium nitride (TiN) to minimize electrical impedance. Multi-electrode recordings of the retinal activity were obtained from 60 electrode channels with a bandwidth ranging from 10 to 3000 Hz at a gain of 1200. The data sampling rate was 25 kHz/channel. From the raw waveform of retinal recording, retinal ganglion cell (RGC) Spikes and SWC were isolated by using 200 Hz high-pass filter and 20 Hz low-pass filter, respectively. No light or electric stimulation was applied for this experiment and the spontaneous retinal activity was recorded.

C. Data Analysis

Stored data were processed off-line by a spike sorting software (Offline Sorter^{TM}) to transform the waveforms containing multiunit activities into multiple single unit spike trains. The analyzer function of the Mc_rack software was used to identify the amplitude and frequency of RGC spike and the slow wave component. With Matlab, fourier transform was performed for detection of the frequency of SWC and oscillatory rhythm of RGC spikes. For the statistical analysis, student t-test was used.

III. Results

A. Oscillatory Rhythm in Retinal Waveform in normal and degenerate retina

Figure 1a depicts typical recordings of spontaneous retinal activity from a postnatal day (P) 28 wild-type and *rd1* mouse and P70 *rd10* mouse by MEA. Wild-type mice only shows spikes while retinal degeneration mice (*rd1, rd10* mice) shows RGC spikes and slow wave component (Fig. 1).

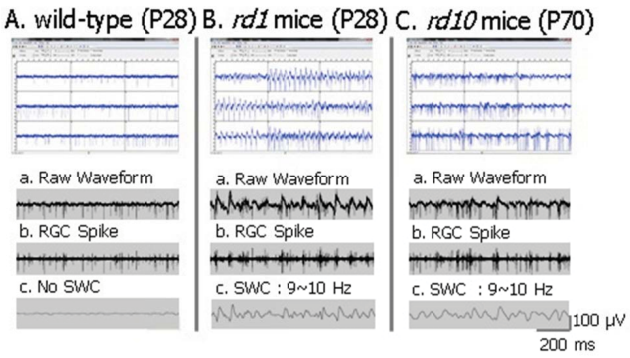

Figure 1. Retinal waveform in wild-type and two retinal degeneration mice (*rd1, rd10* mice). Wild-type and rd1 mice at postnatal 28 days, rd10 mice at postnatal 70 days. Top: display window of data acquisition software (MC_Rack). a. Raw waveform: MEA recording with a bandwidth of 10 to 3000 Hz b. Isolation of spikes from raw waveform using a high pass filter with a 200 Hz cut-off frequency. The bandwidth ranges from 200 to 3000 Hz. c. Isolation of the slow wave component (SWC) from raw waveform using a low pass filter with a 20 Hz cut-off frequency. The bandwidth ranges from 10 to 20 Hz.

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B. Peak to peak amplitude of RGC spikes and SWC

In *rd1* mice, peak to peak amplitude of spikes and SWC was 405.02±87.37 μV and 75.40±12.04 μV (retinal preparation number, n=7, channel number n=47). In *rd10* mice, peak to peak amplitude of spikes and SWC was 249.62±75.67 μV and 74.56±12.33 μV (retinal preparation number, n=3, channel number, n=9). The amplitude of spikes in *rd1* mice is significantly bigger than that in *rd10* mice ($p<0.001$). The amplitude of SWC shows no difference in *rd1* and *rd10* mice.

C. Frequency of RGC spikes vs. SWC in rd1 mice and rd10 mice

In P28 and P56 *rd1* mice, frequency of RGC spikes and

SWC was compared. In *rd1* mice, regardless of postnatal ages, there is no statistical difference between the frequency of SWC and spike in 4 weeks [spike 9.3 ± 0.9 Hz (n=40), SWC 9.3 ± 1.5 Hz (n=25); p>0.05] and 8 weeks [spike 10.0 ± 1.3 Hz (n=87), SWC 10.9 ± 1.7 Hz (n=25); p>0.05].

In *rd10* mice, frequency of RGC spikes and SWC was compared at P28, P70, P105, and P140. There is no statistical differences among the SWC through 4 ~ 20 weeks, significant differences were observed between the frequency of RGC spike and SWC and also among RGC spikes [P28: spike 5.5 ± 1.3 Hz (n=59), SWC 10.8 ± 3.1 Hz (n=14); (p<0.001)], [P70: spike 6.8 ± 3.8 Hz (n=79), SWC 10.3 ± 2.6 Hz (n=25); (p<0.001)], [P105: spike 3.9 ± 0.7 Hz (n=33), SWC 9.9 ± 1.2 Hz (n=25); (p<0.05)], [P140: spike 4.4 ± 1.2 Hz (n=53), SWC 9.8 ± 1.2 Hz (n=25); (p<0.05)] (Fig. 2).

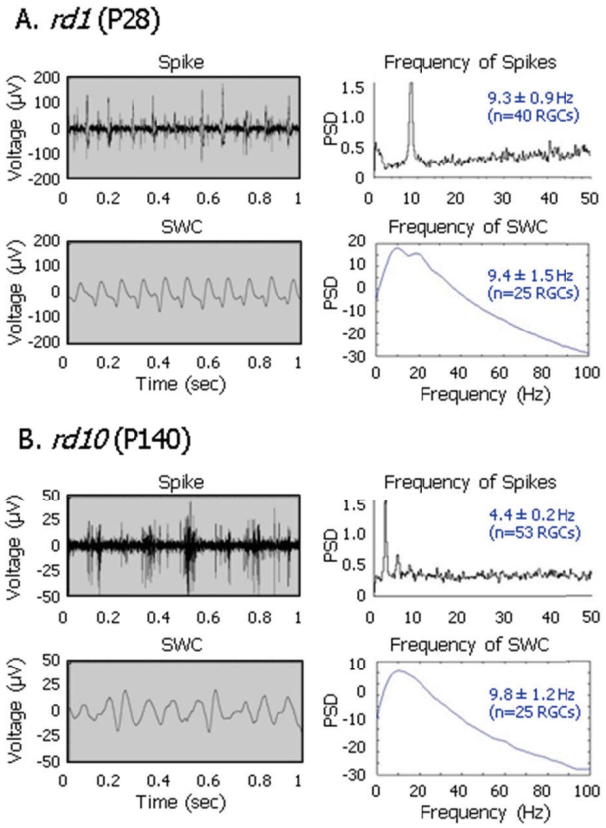

Figure 2. Frequency of oscillatory rhythm in bursting retinal ganglion cell (RGC) spikes and slow wave component (SWC) in *rd1* of postnatal 28 days (A) and in *rd10* mice of postnatal 140 days (B) (A) Frequency of RGC spike and SWC shows no significant difference. (B) Frequency of RGC spike (4.4 ± 0.2 Hz) and SWC (9.8 ± 1.2 Hz) differs significantly (p<0.01).

IV. Discussion

We show that in degenerative retina, ~10 Hz oscillatory rhythm is observed not only in *rd1* but also in *rd10* mice. In *rd1* mice, this oscillatory rhythm in bursting RGC spikes and SWC preserves same regardless of postnatal ages. However, in *rd10* mice, the oscillatory rhythm found in bursting activity of RGC spikes and SWC appear different in different postnatal ages. There is no statistical difference among the frequencies of SWC from P28 up to P140, while the frequency of RGC spike in different postnatal age shows statistical difference. The frequency of RGC spike (~5 Hz) is about half of that of SWC through all different postnatal ages.

Aberrant ~10 Hz oscillatory rhythm is observed not only in rd1 but also in rd10 mice retina.

Degeneration occurs rapidly, with onset of rod loss at P8 and near complete loss of rods by P21 in *rd1* mice [18]. Since horizontal cells degenerate by 8 weeks in *rd1* mice [19], we used P28 and P56 *rd1* mice, and observed if there is any possible change regarding frequency of oscillatory rhythm in RGC spike and SWC. About 10 Hz rhythmic activities were observed from both spontaneous spikes of RGCs and slow wave component and this rhythm preserved up to P56 when the degeneration of horizontal cell completed. Our result can be interpreted that even if further degeneration occurs in second order neuron after P28, retinal waveforms recorded with MEA does not change.

While in *rd10* mice, rod degeneration starts around P18, and photoreceptor death is accompanied and followed by dendritic retraction in bipolar and horizontal cells [14]. Recent study of Phillips *et al.* using *rd10* mice at various stages of degeneration ranging from P30 to postnatal month 9.5 (PNM9.5) showed that horizontal cells and rod and cone bipolar cells underwent morphological remodeling including loss of dendrites, cell body migration, and the sprouting of ectopic processes [20]. Despite these changes, the laminar organization of bipolar and amacrine cells and the ON-OFF organization in the inner plexiform layer was largely preserved. Surviving cone and bipolar cell terminals continued to express the appropriate cell-specific presynaptic proteins needed for synaptic function up to PNM9.5 [20].

If the synaptic function is preserved up to PNM9.5 in *rd10* mice, our finding of ~5 Hz oscillatory rhythm in RGC spikes and ~10 Hz oscillatory rhythm in SWC in *rd10* mice may not be related with any synaptic change occurring in *rd10* mice.

Different oscillatory rhythm of RGC spikes in rd1 and rd10 mice retina (~10 Hz vs.~5 Hz)

Koepsell *et al.* reported that individual RGCs multiplex two streams of information; One stream encodes visual information by changes in firing rate time-locked to external visual stimuli, the other stream encodes information using spike timing relative to intrinsic retinal oscillations [21]. Since the oscillatory rhythm of bursting RGC spikes differ in *rd1* and *rd10* mice in our study, this might be related with the intrinsic property difference encoding the visual information both in *rd1* and *rd10* mice.

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