Electrical Stimulation Parameters in Normal and Degenerate Rabbit Retina

Gye-Hwan Jin*, Yong Sook Goo†

*Department of Radiology, Nambu University, Gwangju, †Department of Physiology, Chungbuk National University Medical School, Cheongju, Korea

Retinal prosthesis is regarded as the most feasible method for the blind caused by retinal diseases such as retinitis pigmentosa (RP) or age related macular degeneration (AMD). Recently Korean consortium launched for developing retinal prosthesis. One of the prerequisites for the success of retinal prosthesis is the optimization of the electrical stimuli applied through the prosthesis. Since electrical characteristics of degenerate retina are expected to differ from those of normal retina, we performed voltage stimulation experiment both in normal and degenerate retina to provide a guideline for the optimization of electrical stimulation for the upcoming prosthesis. After isolation of retina, retinal patch was attached with the ganglion cell side facing the surface of microelectrode arrays (MEA). 8×8 grid layout MEA (electrode diameter: 30 μm, electrode spacing: 200 μm, and impedance: 50 kΩ at 1 kHz) was used to record in-vitro retinal ganglion cell activity. Mono-polar electrical stimulation was applied through one of the 60 MEA channel, and the remaining channels were used for recording. The electrical stimulus was a constant voltage, charge-balanced biphasic, anodic-first square wave pulse without interphase delay, and 50 trains of pulse was applied with a period of 2 sec. Different electrical stimuli were applied. First, pulse amplitude was varied (voltage: 0.5~3.0 V). Second, pulse duration was varied (100~1,200 μs). Evoked responses were analyzed by PSTH from averaged data with 50 trials. Charge density was calculated with Ohm's and Coulomb's law. In normal retina, by varying the pulse amplitude from 0.5 to 3 V with fixed duration of 500 μs, the threshold level for reliable ganglion cell response was found at 1.5 V. The calculated threshold of charge density was 2.123 mC/cm^2. By varying the pulse duration from 100 to 1,200 μs with fixed amplitude of 2 V, the threshold level was found at 300 μs. The calculated threhold of charge density was 1.698 mC/cm^2. Even after the block of ON-pathway with L-(1)-2-amino-4-phosphonobutyric acid (APB), electrical stimulus evoked ganglion cell activities. In this APB-induced degenerate retina, by varying the pulse duration from 100 to 1200 μs with fixed voltage of 2 V, the threshold level was found at 300 μs, which is the same with normal retina. More experiment with APB-induced degenerate retina is needed to make a clear comparison of threshold of charge density between normal and degenerate retina.

Key Words: Degenerate retina, Retinal prosthesis, Microelectrode array (MEA), Ganglion cell, Charge density

INTRODUCTION

Photoreceptor loss as a result of retinal degenerative diseases, such as retinitis pigmentosa (RP) and age related macular degeneration (AMD) are the leading causes of blindness in adults.[1] While the majority of cell death occurs in the outer nuclear layer (ONL) containing the photoreceptors, the inner nuclear and ganglion cell layers in the macula survive at fairly high rates in patients afflicted with RP[2] and ARMD.[3]

Despite a report stating that degenerating mammalian retinas can effectively incorporate the rod photoreceptor precursor cells into ONL,[4] treatment modalities for the degenerate retina such as, gene therapy,[5] and retinal transplantation[6] achieved only limited success. Retinal prosthesis, by electrical sti-

This work was supported by the research grant of the Chungbuk National University in 2006.
Submitted December 13, 2007, Accepted February 27, 2008
Corresponding Author: Yong Sook Goo, Department of Physiology, Chungbuk National University Medical School, 12 Gaeshin-dong, Hungduk-gu, Cheongju 361-763, Korea.
Tel: 043)261-2870, Fax: 043)272-1603
E-mail: ysgoo@chungbuk.ac.kr

mulation of the remaining retinal neurons, could be a feasible treatment which could restore useful vision in blind individuals. This is supported by clinical studies which have shown that controlled electrical signals applied to a small area of the retina of a blind volunteer via a microelectrode resulted in the perception of a small spot of light.[7]

For the retinal prosthesis to succeed, several prerequisites must be satisfied, such as selection of electric stimulation parameters, selection of biocompatible device materials, development of surgical methods for implantation and fixation of the device, electronic design of intra- and extra-ocular components, and design of schemes for transmission of power and signal to the intra-ocular electronics. One of the most important is optimization of the electrical stimuli applied through the prosthesis.[8,9]

Recently Korean consortium launched for developing retinal prosthesis. This paper presents experimental results regarding the threshold of charge density on voltage stimulation of *in vitro* normal and chemically degenerate rabbit retina.

MATERIAL AND METHODS

1. Preparation of in vitro retina

We used the New Zealand white rabbits (n=5), weighing 2~2.5 kg. The method used in Stett *et al*[10] was modified for retinal preparation. Briefly, the eyeball was enucleated and the retina was isolated. From the isolated rabbit retina (number of retinas: n=10), a retinal patch (~5×5 mm) was attached to the ganglion cell side facing the surface of the multi-electrode arrays (MEA) (Fig. 1a). The retinal preparation was 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. All pharmacological agents were dissolved in an oxygenated ACSF solution and delivered to the retina by continuous perfusion of the ACSF at a rate of 1 mL/min.

To mimic degenerate retina in which normal light response is impaired, first we blocked ON-pathway with L-(1)-2-amino-4-phosphonobutyric acid (APB). The glutamate analog, APB acts selectively on metabotropic glutamate receptors, and thus blocks transmission from photoreceptors to ON bipolar cells.[11]

2. Electrode and data acquisition system

The MEA60 system (Multi Channel Systems GmbH, Germany) included an 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 8×8 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. The impedance level

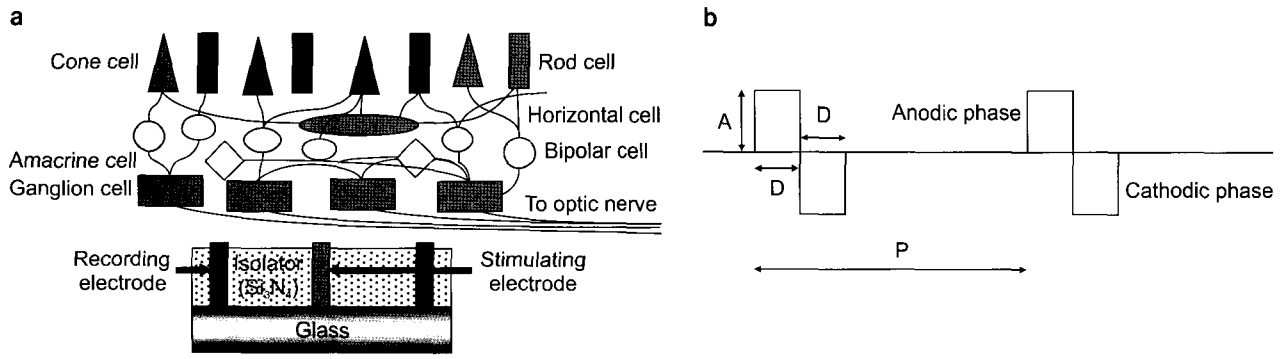

Fig. 1. (a) In-vitro stimulation and recording of retinal preparation with MEA. A retinal patch (top) is attached with the ganglion cell side facing the surface of MEA (bottom). Electrical stimulation was applied through one of the 60 MEA channel, and the remaining channels were used for recording. (b) The electrical stimulus used for this experiment. The stimulus was a constant voltage, charge-balanced biphasic, anodic-first square wave pulse without interphase delay, and 50 trains of pulse was applied with a period of 2 sec (A: Amplitude, D: duration, P: period). Stimulus amplitude and duration were varied in this experiment.

of the MEA was 50 kΩ at 1 kHz. The amplifier was placed in a Faraday cage with a laboratory-made ground system. Microelectrode recordings of the retinal activity were obtained from up to 60 electrode channels with a MEA60 system with a bandwidth ranging from 10 to 3,000 Hz with a gain of 1,200. The data sampling rate was 25 kHz/channel. We used one channel of MEA (out of 60 channels) for stimulus and remained channels for recording. The electrical stimulus was a constant voltage, charge-balanced biphasic, anodic-first square wave pulse without interphase delay, and 50 trains of pulse was applied with a period of 2 sec (Fig. 1b). Different electrical stimuli were applied. First, pulse amplitude was varied (voltage: 0.5~3.0 V). Second, pulse duration was varied (100~1,200 μs).

3. Data analysis

Stored data were processed off-line by a spike extraction program (Offline sorter™) using a threshold algorithm. From these data, raster plots and poststimulus time histograms (PSTH) of 50 trials were obtained. In PSTH, evoked responses were counted during a 10~20 ms time span after the stimulation. From the PSTH, we obtained normalized response curve vs. voltage duration or voltage amplitude, respectively.

The charge delivery (Q) and charge density (D) were calculated with Ohm's and Coulomb's law.

$$I = \frac{V}{R} \quad (1)$$

where I is the calculated current, V is the threshold voltage which evokes ganglion cell response (1.5 V), and R is the resistance of the electrode (50 kΩ).

$$Q = I \cdot T \quad (2)$$

where T is the threshold of pulse duration which evokes ganglion cell response.

Charge density (D) was calculated with equation 3.

$$D = \frac{Q}{\pi r^2} \quad (3)$$

where Q is the calculated charge delivery and r is the radius of the electrode (15 μm).

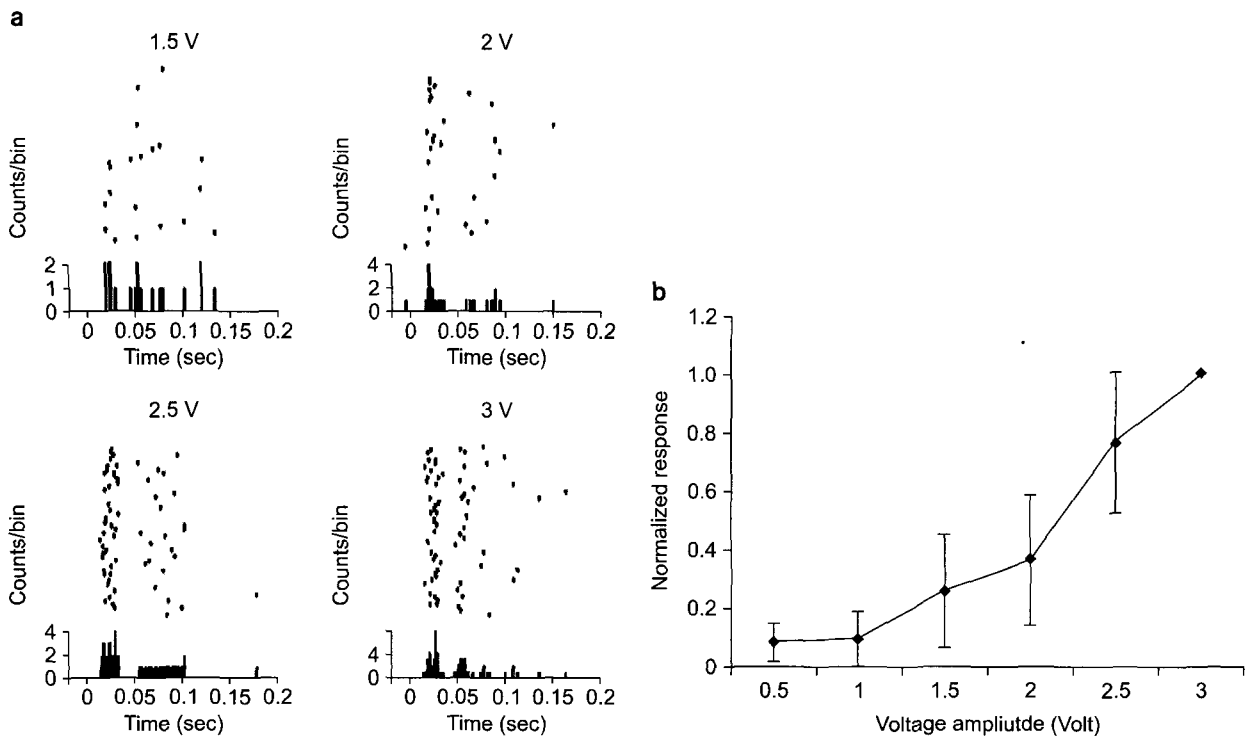

Fig. 2. Response of retinal ganglion cell evoked by voltage pulses (amplitudes as indicated, fixed pulse duration of 500 μs). (a) Raster plots (upper trace) and PSTHs (lower trace) of 50 trials with 1.5, 2, 2.5, and 3 V stimuli. (b) Normalized response of retinal ganglion cells to different voltage amplitude (number of ganglion cells: n=3). Mean±S.D. was shown.

RESULTS

1. Evoked ganglion cell activities to electrical stimulation

1) Ganglion cell responses to different voltage amplitudes (Fig. 2): Ganglion cell activities were recorded with voltage stimulation. The voltage amplitude were varied from 0.5 to 3 V with fixed duration of 500 μs biphasic square wave pulse. Threshold voltage of 1.5 V had to be surpassed in order to elicit a reliable response of ganglion cell spike. Up to 3 V, the higher the voltage level, the more ganglion cell spikes were evoked. The calculated threshold of charge density and charge delivery was 2.123 mC/cm^2 and 15 nC/phase, respectively.

2) Ganglion cell responses to different voltage durations (Fig. 3): The voltage duration were varied from 100 to 1,200 μs with fixed voltage intensity of 2 V. Reliable response was evoked with the threshold duration of 300 μs and the saturation was observed with the duration over 700 μs. The calculated threshold of charge density and charge delivery was 1.698 mC/cm^2 and 12 nC/phase, respectively.

2. Ganglion cell activities were evoked with electrical stimulation after the ON-pathway block with APB (Fig. 4)

To mimic degenerate retina in which normal light response is impaired, first we blocked ON-pathway with L-(1)-2-amino-4-phosphonobutyric acid (APB). The glutamate analog, APB acts selectively on metabotropic glutamate receptors, and thus blocks transmission from photoreceptors to On bipolar cells.[11]

The light responses of ganglion cells were tested with full field illumination with white light turned on (2 sec) and off (5 sec) in a square wave fashion. The light intensity was kept in high range that activates most cones (1.45 μW/cm^2). Many ganglion cells responded with a brief increase in the firing rate at light onset (ON cells: 35.0±4.4%), at light offset (OFF

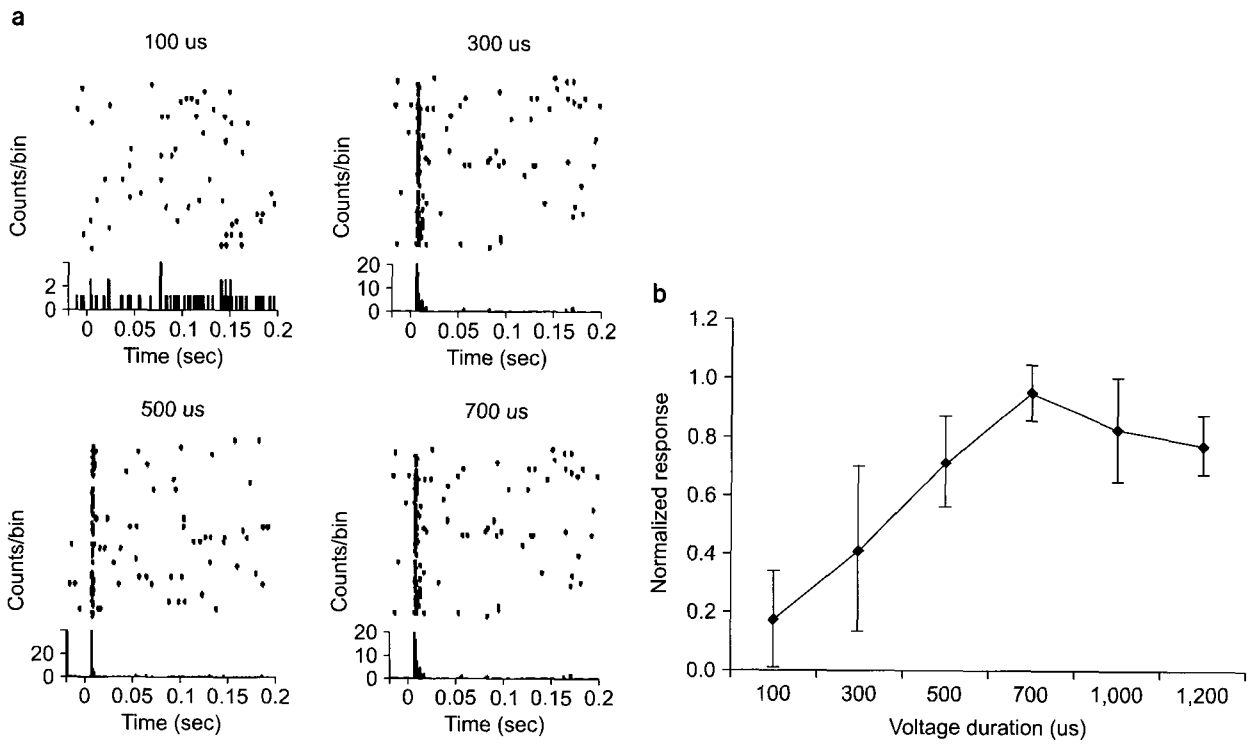

Fig. 3. Response of retinal ganglion cell evoked by voltage pulses (pulse duration as indicated, fixed voltage amplitude of 2 V). (a) Raster plots (upper trace) and PSTHs (lower trace) of 50 trials with 100, 300, 500, and 700 μs stimuli. (b) Normalized response of retinal ganglion cells to different voltage duration (number of ganglion cells: n=9). Mean±S.D. was shown.

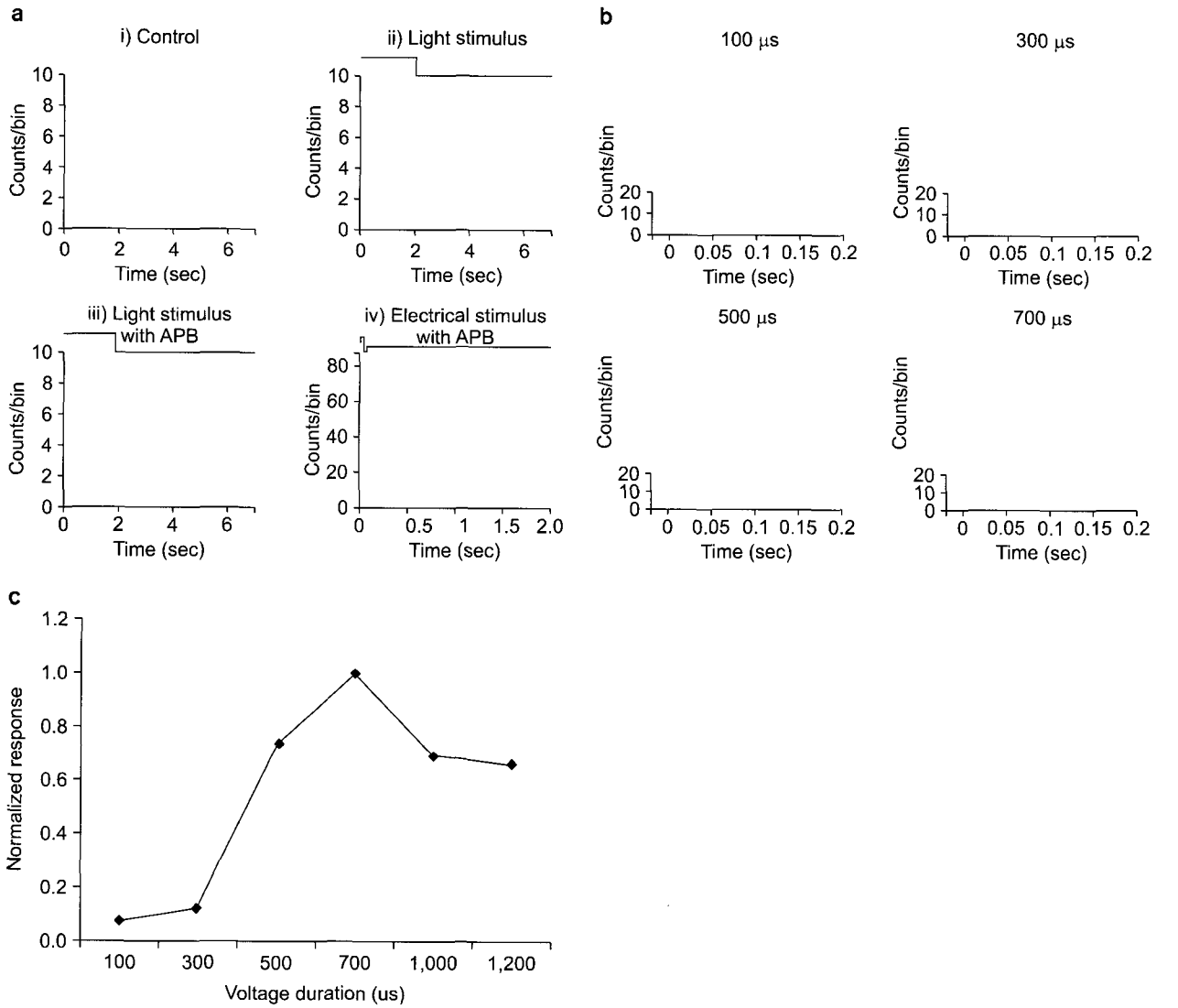

Fig. 4. (a) Spontaneous and evoked activity of retinal ganglion cell. i). Ganglion cell activity without any stimulus, ii) Evoked response of ganglion cell to light stimulus, iii) Evoked response of ganglion cell to light stimulus after addition of 100 mM APB, iv) Evoked response of ganglion cell to electrical stimulus after addition of 100 mM APB. (b) Raster plots (upper trace) and PSTHs (lower trace) of 50 trials with 100, 300, 500, and 700 μs stimuli with fixed voltage of 2 V in the presence of APB. (c) Normalized response of retinal ganglion cell to different voltage duration (number of ganglion cells: n = 1) in the presence of APB.

cells: 30.4±1.9%), or following both transitions (ON/OFF cells: 34.6±5.3% (number of total retinal pieces=8)). After finding the ON/OFF ganglion cell with light stimulation, we applied 100 μM APB to eliminate On responses to light. Even with APB, electrical stimulus evoked ganglion cell activities. When the voltage duration from 100 to 1,200 μs were varied with fixed voltage intensity of 2 V, reliable response of ganglion cell was evoked with the threshold duration of 300 μs and the saturation was observed with the duration over 700 μs, which is the same with normal retina.

DISCUSSION

Stimulation thresholds have been studied in a wide variety of models and species with very different methods, making direct comparisons difficult. The threshold of charge density

required to produce a response is a critical variable of electrical stimulation. As we can expect, thresholds vary significantly with stimulus conditions.

Using platinum wires for retinal stimulation, Humayun et al found charge densities of 8.92~14.8 μC/cm^2 to elicit a cortical response.[12] Chow et al performed subretinal electrical stimulation and recorded cortical evoked potential in rabbits and reported charge densities of 2.8 nC/cm^2.[13]

Since our recording is not from the visual cortex but from the retina, direct comparison of our data with Humayun's or Chow's would not be meaningful. Since Stett et al[10] performed retinal stimulation with MEA and recorded evoked ganglion cell activity with glass electrode, comparison with their data would provide more meaning. Comparing with the charge density of Stett's (500 μC/cm^2), our result of about 2,000 μC/cm^2 is reasonable if we consider the distance of recording electrode from the stimulus is minimum of 200 μm while in Stett's experiment it was less than 100 μm.

The latency of ganglion cell activities with electrical stimulus (5~30 ms) is shorter than that with light stimulus (100~150 ms), which could be explained by the fact that with light stimulus phototransduction cascade and synaptic transmission from photoreceptors to ganglion cell is needed while electrical stimulation might directly apply to the ganglion cell. Because retinal prosthesis is for the patients with degenerate retina, the next step is selecting optimal electrical stimulus in degenerate retina. Therefore, we are planning to make retinal degeneration model. For this attempt, we applied 100 μM APB to block ON-pathway and different voltage duration (100 ~1,200 μs) of stimuli were applied (trial number: n=1). Against our expectation, the threshold level was observed at 300 μs, which is the same with normal retina. But to make a clear comparison of threshold of charge density between normal and degenerate retina, more experiment should be performed with APB-induced degenerate retina. Since APB blocks only ON-pathway, simultaneous block of OFF-pathway with strychnine[11] will be needed, and finally animal model of RP should be used for future experiment. Our data will provide a guideline for optimization of stimulation parameters especially for epiretinal prosthesis,[8] since in our retinal preparation the ganglion cell layer is mostly stimulated.

REFERENCES

1. **Hyman L**: Epidemiology of eye disease in the elderly. Eye 1 (Pt 2):330-341 (1987)
2. **Humayun MS, Prince M, de Juan Jr E, et al**: Morphometric analysis of the extramacular retina from postmortem eyes with retinitis pigmentosa. Invest Ophthalmol Vis Sci 40:143-148 (1999)
3. **Medeiros NE, Curcio CA**: Preservation of ganglion cell layer neurons in age-related macular degeneration. Invest Ophthalmol Vis Sci 42:795-803 (2001)
4. **MacLaren RE, Pearson RA, MacNeil A, et al**: Retinal repair by transplantation of photoreceptor precursors. Nature 444:203-207 (2006)
5. **Acland GM, Aguirre GD, Ray J, et al**: Gene therapy restores vision in a canine model of childhood blindness. Nat Genet 28:92-95 (2001)
6. **del Cerro M, Gash DM, Rao GN, et al**: Retinal transplants into the anterior chamber of the rat eye. Neuroscience 21:707-723 (1987)
7. **Humayun MS, de Juan Jr E, Dagnelie G, et al**: Visual perception elicited by electrical stimulation of retina in blind humans. Arch Ophthalmol 114:40-46 (1996)
8. **Margalit E, Maia M, Weiland JD, et al**: Retinal prosthesis for the blind. Surv Opthalmol 47:335-336 (2002)
9. **Lowenstein JI, Montezuma SR, Rizzo III JF**: Outer retinal degeneration: an electronic retinal prosthesis as a treatment strategy. Arch Ophthalmol 122:588-596 (2004)
10. **Stett A, Barth W, Weiss S, et al**: Electrical multisite stimulation of the isolated chicken retina. Vision Res 40: 1785-1795 (2000)
11. **Soucy E, Wang Y, Nirenberg S, et al**: A novel signaling pathway from rod receptors to ganglion cells in mammalian retina. Neuron 21:481-493 (1998)
12. **Humayun M, Probst R, deJuan E, et al**: Bipolar surface electrical stimulation of vertebrate retina. Arch Ophthalmol 112:110-116 (1994)
13. **Chow AY, Chow VY**: Subretinal electrical stimulation of the rabbit retina. Neurosci Lett 225:13-16 (1997)

정상 망막과 변성 망막을 위한 전기자극 파라미터

*남부대학교 방사선학과, †충북대학교 의과대학 생리학교실

진 계 환*·구 용 숙†

망막색소변성(retinitis pigmentosa: RP)과 연령관련 황반변성(age-related Macular Degeneration: AMD)은 망막변성으로 인해 실명에 이르는 대표적인 질환이며 망막이식장치의 개발을 통해 치료될 수 있다고 간주되고 있다. 최근에 국내에서도 망막이식장치 개발을 위한 연구팀이 조직되었다. 성공적인 망막이식장치 개발을 위하여 여러 가지 선결요소가 필요하지만 그 중 한 가지가 이식장치에 인가할 전기자극을 최적화하는 것이다. 변성망막의 전기적 특성은 정상 망막과 다르리라 예측되므로 우리는 장차 개발될 망막 이식장치에 인가할 전기자극 최적화를 위한 가이드라인을 제공하기 위해 정상 망막과 변성 망막의 전압자극 파라미터에 관한 실험을 하였다. 망막을 분리한 후 망막절편을 신경절세포 층이 다채널전극의 표면을 향하게 하여 전극에 붙인다. in-vitro 상태에서 망막 신경절세포의 전기신호를 기록하기 위해 전극 직경: 30 μm, 전극간 거리: 200 μm , 전극 임피던스 1 kHz 에서 50 kΩ 인 8행 8열의 다채널전극을 사용하였다. 다채널전극의 60채널 중 두 채널을 자극전극과 접지로 사용하여 단극전기자극을 인가하였고 나머지 전극을 기록전극으로 사용하였다. 가한 전기자극은 전압자극으로 전하균형을 맞춘 이상성자극을 아노딕 사각파를 먼저 주고 캐쏘딕 사각파가 나중에 나오는 형태로 두 사각파간의 지체는 없도록 하였으며 동일한 자극을 2초 간격으로 50회 반복하여 인가하였다. 다양한 전기자극을 사용하였는 바 첫째는 사각파의 크기를 달리하였다 (0.5~3 V). 둘째는 사각파의 시간을 달리하였다 (100~1,200 μs). 전하밀도는 옴의 법칙과 쿨롱의 법칙을 이용하여 계산하였다. 전기자극으로 유발된 반응은 50회 자극에 대한 평균치를 얻은 후 자극 후 히스토그램(PSTH)을 그려 분석하였다. 전압자극의 크기를 0.5 μ3 V로 달리하였을 때 믿을 만한 망막신경절세포가 유발되는 자극은 1.5 V이었고 이때 계산된 전하밀도의 역치는 2.123 mC/cm^{2}이었다. 전압의 크기를 2 V로 고정하고 자극 지속시간을 100~1,200 μs로 달리하였을 때 믿을 만한 망막신경절세포가 유발되는 자극의 역치는 300 μs에서 관찰되었다. 이때 계산된 전하밀도의 역치는 1.698 mC/cm^{2}이었다. L-(1)-2-amino-4-phosphonobutyric acid (APB)을 사용하여 ON-경로를 차단한 후에 전기자극을 인가하였을 때도 자극에 의해 망막신경절 세포의 반응이 유발되는 것을 확인하였다. APB-변성망막에서 전압의 크기를 2 V로 고정하고 자극 지속시간을 100~1200 μs로 달리하였을 때 믿을 만한 망막신경절세포가 유발되는 자극의 역치는 300 μs에서 관찰되었으며 이는 정상망막의 결과와 같았다. 추후 APB-변성망막을 가지고 좀더 실험이 진행되어야 정상망막과 변성망막의 전하밀도에 관한 명료한 비교가 가능할 것이다.

중심단어: 변성망막, 망막이식장치, 다채널전극, 망막신경절세포, 전하밀도