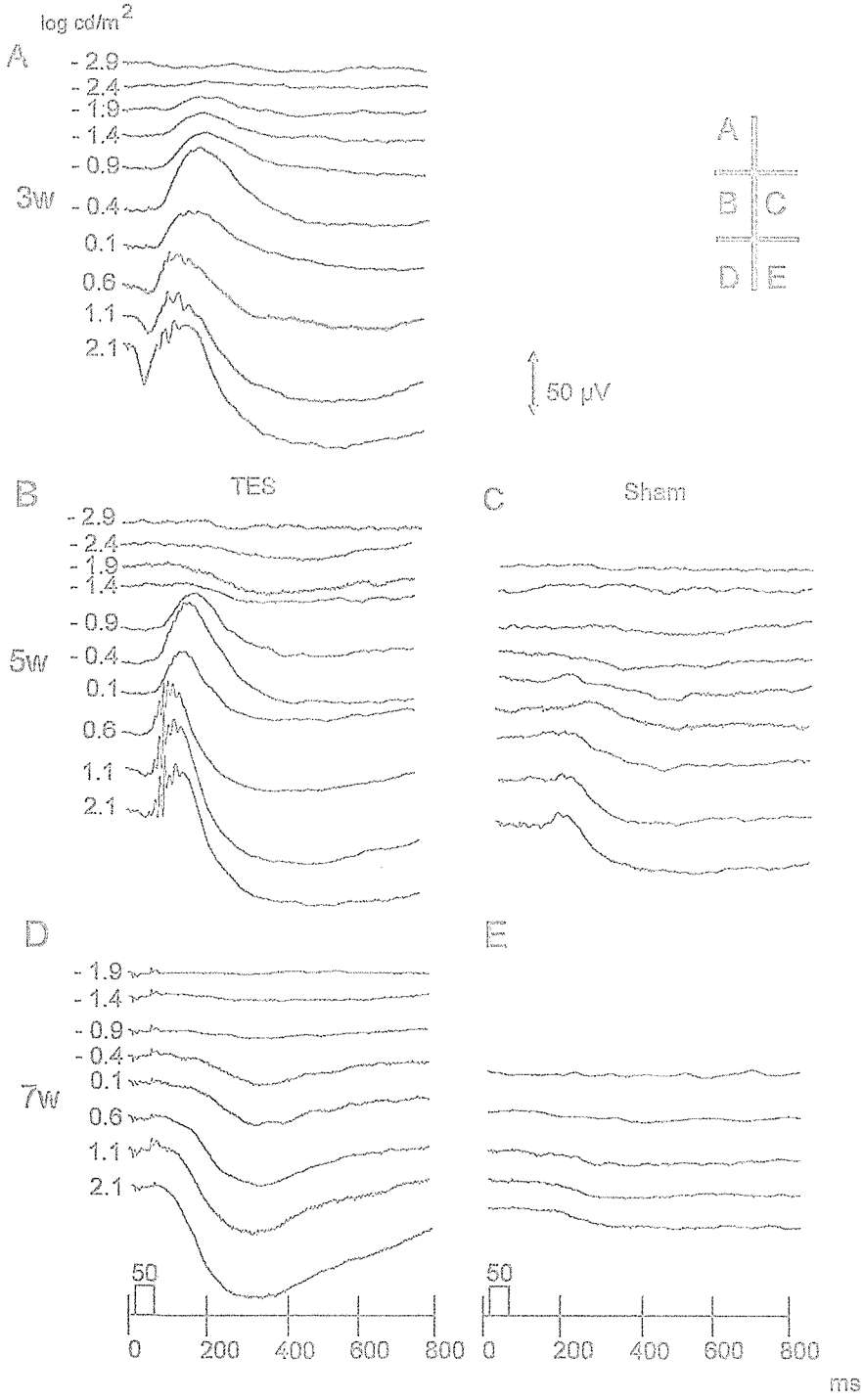
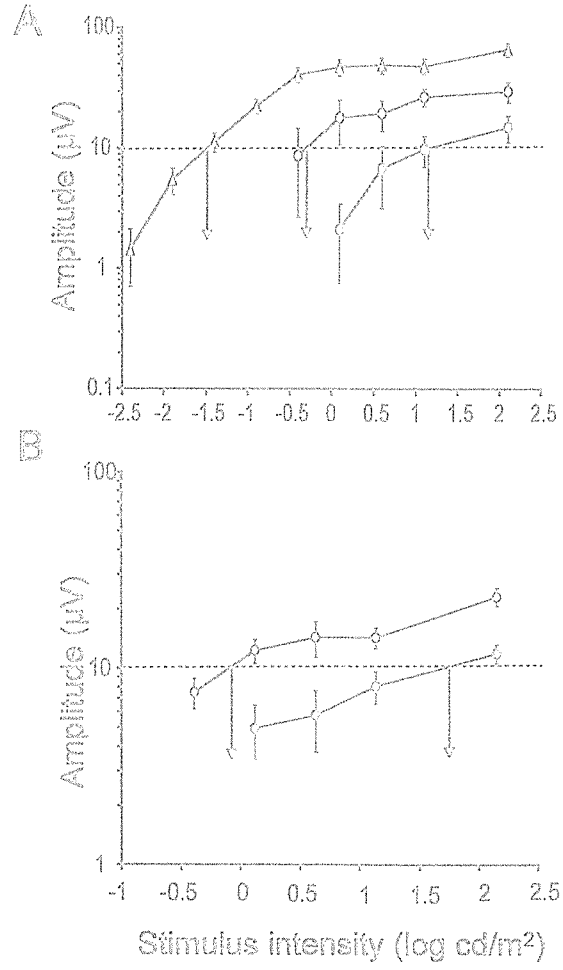
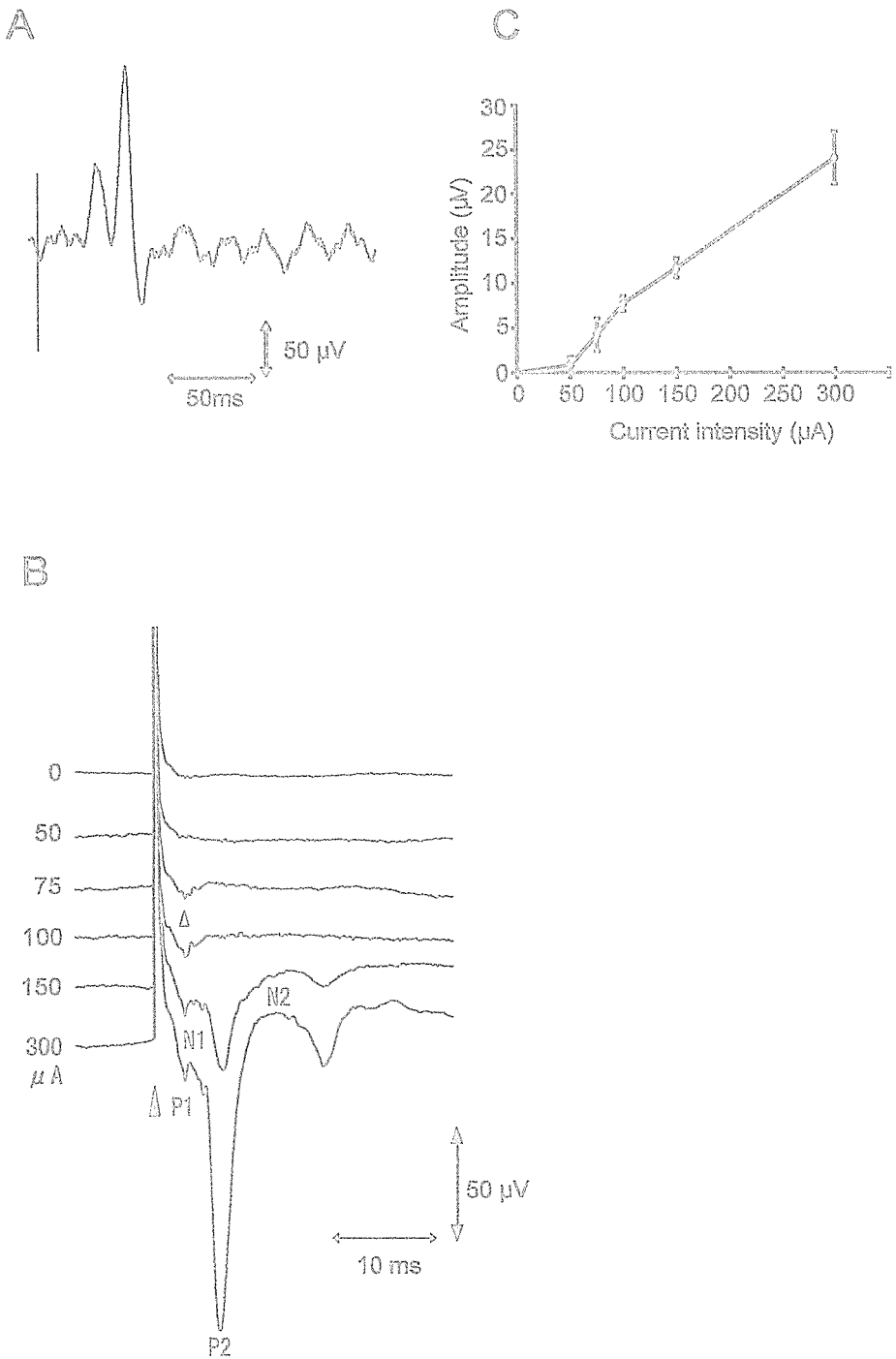
A
ONL Thickness (µm)
50
45
40
35
30
25
20
15
10
5
0
3w
5w
9w
*
**
B
ONL Thickness (µm)
Ora 9 8 7 6 5 4 3 2 1 ONH 1 2 3 4 5 6 7 8 9 Ora
Superior hemisphere
Inferior hemisphere
C
INL Thickness (µm)
60
50
40
30
20
10
0
NS
NS
3w
5w
9w

A
B
C
D
E
RPE
ROS + RIS
+ debris
ONL
OPL
INL
IPL
GCL
ILM


log cd/m2
A
- 2.9
- 2.4
- 1.9
- 1.4
- 0.9
3w
- 0.4
0.1
0.6
1.1
2.1
A
B
C
D
E
50 µV
B
TES
- 2.9
- 2.4
- 1.9
- 1.4
- 0.9
5w
- 0.4
0.1
0.6
1.1
2.1
C
Sham
D
- 1.9
- 1.4
- 0.9
- 0.4
0.1
7w
0.6
1.1
2.1
50
0
200
400
600
800
E
50
0
200
400
600
800
ms


A
100
10
1
0.1
Amplitude (µV)
-2.5 -2 -1.5 -1 -0.5 0 0.5 1 1.5 2 2.5
B
100
10
1
Amplitude (µV)
-1 -0.5 0 0.5 1 1.5 2 2.5
Stimulus intensity (log cd/m2)


A
50 µV
50ms
C
Amplitude (µV)
30
25
20
15
10
5
0
0 50 100 150 200 250 300
Current intensity (µA)
B
0
50
75
100
150
300
µA
Δ
N1
N2
Δ P1
P2
50 µV
10 ms

Artificial Vision: Vision of a Newcomer

Takashi Fujikado[1], Hajime Sawai[2], and Yasuo Tano[3]

Department of Applied Visual Science[1], *Department of Physiology*[2], *and Department of Ophthalmology*[3], *Osaka University Medical School*

Correspondence to: Yasuo Tano, M.D.

Department of Ophthalmology, Osaka University Medical School
Rm E-7, 2-2 Yamadaoka, Suita, 565-0871, Japan

Abstract:

The Japanese Consortium for an Artificial Retina has developed a new stimulating method named Suprachoroidal Transretinal Stimulation (STS). Using STS, Electrically evoked potentials (EEPs) were effectively elicited in Royal College of Surgeons rats and in rabbits and cats with normal vision, using relatively small stimulus currents, such that the spatial resolution appeared to be adequate for a visual prosthesis. The histological analysis showed no damage to the rabbit retina when electrical currents sufficient to elicit distinct EEPs were applied. It was also shown that trans-corneal electrical stimulation to the retina prevented the death of retinal ganglion cells. STS, which is less invasive than other retinal prostheses, could be one choice to achieve artificial vision, and the optimal parameters of electrical stimulation may also be effective for the neuroprotection of residual retinal ganglion cells.

Key Words: artificial retina, suprachoroidal-transretinal stimulation, RCS rat, neuroprotection.

1. INTRODUCTION

In 2001, the Japanese Consortium for Artificial Retina (Principal Investigator, Yasuo Tano, Osaka University) was organized with supports from the Ministry of Economy, Trade and Industry and the Ministry of Health, Welfare and Labor in Japan.

Because our consortium was a newcomer to the field of artificial retinal and we had to play catch-up with several pioneering groups in USA, Germany and other countries, we sought our original approach and developed a new stimulation method named Suprachoroidal-Transretinal Stimulation (STS).

We also investigated the effect of electrical stimulation on the retina and obtained results suggesting the neuroprotective effect of electrical stimulation on retinal ganglion cells (RGCs). In this article, we summarize the progress during the first 3 years of our consortium with emphasis on the evaluation of STS method and the electrically induced neuroprotection.

2. Overall Research Goals of Japanese Consortium for Artificial Retina

The goal of our research is to develop within 5 years in animal models a prosthesis for artificial vision that provides visual acuity sufficient for finger counting (the level of visual acuity that allows the patient to identify the number of fingers held at a distance of 30cm).

The first candidate patients for the artificial retina would be those with advanced retinitis pigmentosa (RP) or autoimmune retinopathy, but in the next step, patients with age-related macular degeneration could be included as candidates.

3. The Concept of Suprachoroidal-Transretinal Stimulation (STS)

Many research groups are currently developing electrode arrays that can be attached directly to the retina and used to stimulate the retina in an attempt to restore vision to patients with retinal degeneration.[1-3]

In general, there are two types of retinal prostheses: an epi-retinal[4-8] and a sub-retinal.[9-12] Epiretinal stimulating prostheses are inserted into the vitreous cavity and are attached or placed against the inner retinal surface. The potential risk of this approach is the possible permanent attachment of the device to the retina. Epiretinal implantation can cause damage around the area of the retinal tack,[6, 13] or may be unstable without the use of special methods to fix the electrodes.[3]

Subretinal prostheses are placed between the pigment epithelial layer and the outer layer of the retina. These arrays are more stable but chronic implantation in this space might lead to a proliferation of glial tissue around the electrode array.[12] Although improvements in surgical and electronic technologies may solve some of the problems of implanting retinal stimulating prostheses, there is the potential risk of significant damage to the eye after inserting

an electrode intraocularly. To avoid the risks of implanting a stimulating prosthesis into the epi- or subretinal space, we have developed a new approach for electrode implantation, namely, suprachoroidal-transretinal stimulation (STS), which avoids direct contact of electrodes with the retina.[14]

In STS, the stimulating electrodes were placed on the fenestrated sclera or in the suprachoroidal space and the counter electrode was inserted intravitreally (Figure 1). The merits of STS method are that (1) Surgical technique is less complicated because vitreous surgery is not required for this method (2) Electrodes are not in direct contact with the retina because they are placed in the scleral pocket, (3) Electrodes are easy to be removed or replaced because they don't contact with the retina (4) A wide area of the retina can be stimulated because electrodes are placed in the outer-coat of the eyeball. On the other hand, the potential disadvantages are (1) The spatial resolution of STS could be limited because the electrodes are situated far away from the retina, and (2) Electrical current needed to attain artificial vision by STS could be higher than that by epi- or sub-retinal electrode because electrodes do not contact the retina. In order to examine the efficacy and safety of STS method, we performed experiments on small animal (rats) and medium-sized animals (rabbits and cats).

The procedures used on all animals conformed to the Institutional Guidelines of Osaka University and the ARVO Resolution on the Use of Animals in Research.

4. The Effectiveness of STS in Animal Model.

4.1 Artificial vision in Royal College of Surgeons (RCS) rats.

Since it has been reported in patients with RP that phosphenes can be obtained by electrical stimulation with electrodes that are not in direct contact with the retina, we expected that STS would be able to excite RGCs. Thus we tested our expectations regarding the feasibility of STS for an artificial retina, by acute electrophysiological experiments in retinal dystrophic rats (Royal College of Surgeons rats, RCS rats). RCS rats are recognized as one of the best animal models for retinitis pigmentosa. In adult RCS rats (25~30 weeks of age) that we used, the outer retinal layers, including the photoreceptors, were always completely degenerated, as shown in Fig 2 A, whereas the laminar organization of the inner retina was well preserved. Owing to such degeneration of the outer retina, flashing light stimuli elicited no detectable responses in the electroretinogram, and no evoked field potentials could be recorded from the superior colliculus (SC) (the upper and the middle traces in Fig 2B, respectively).

We first addressed the following issues: can a focal STS to the retina with degenerated photoreceptors excite the residual neurons, especially RGCs, and can their excitation be transmitted via the optic nerve to the visual areas of the brain?

A single STS, that is, a brief (0.5 ms) monophasic pulse of electrical current was applied to the one of the eye balls between an anodic suprachoroidal electrode placed on fenestrated sclera and a cathodic electrode inserted into the vitreous (Fig. 1, 2C). The responses to the STS were recorded from the surface of the SC contralateral to the stimulated eye (Fig. 2C). The threshold intensity of these STS-evoked responses was about 10~15 nC of electrical charge (20~30 μA, 0.5 msec). Although the electrodes were apart from the retina, the threshold in STS was comparable degree to that in epiretinal or subretinal stimulation with the electrodes directly attached to the retina.

The evoked potentials (EPs) consisted of a fast positive wave (P1) followed by a slow negative wave (N1) , as shown in Fig 2B. The peak latencies of the P1 and the N1 were 7.1 +/- 1.5 ms and 13.0 +/- 2.8 ms, respectively (mean +/- SD, n=11). The peak amplitude of these two components was dependent on stimulus intensity. With high-intensity stimulus, N1 was occasionally followed by a negative peak (N2). The mean +/- SD of the N2 was 25.6 +/- 5.0 ms (n=7). The same EPs to STS were recorded in normal hooded rats, and no statistical difference was seen between the two strains, in terms of shape, and peak latencies of P0, N1 and P1, as well as threshold.

It was interesting that inverting the polarity of STS greatly reduced the collicular evoked responses. Moreover, the latency of EPs to STS was slightly but consistently longer than that of the EP evoked in response to stimulation at the optic chiasm. The EPs to STS was completely abolished after

transection of the optic nerve just behind the eye ball. These results demonstrated that STS induced excitation of RGCs and the excitation was transmitted to the SC via the ON. These results indicate that STS is potentially suitable for retinal prostheses and artificial vision.

Then, we further studied the feasibility of STS, with attention to the spatial resolution that could be achieved with STS-based artificial vision. Because RGC axons are known to project to the SC in a precise topographical order, the N1-P1 amplitude of EPs to supra-threshold intensity of STS was surveyed on the SC at intervals of 0.25 mm along the mediolateral and rostrocaudal axis, in order to evaluate the localization of the retinal excitation induced by STS.

We found that the smallest responsive area was less than a square with sides 200 μm, and the mean (+/- SE) of the area was 0.24 +/- 0.12 mm^2 (n=5). A topographical correspondence was also found between the points beneath the suprachoroidal electrodes and the sites where the maximal responses were evoked in the SC. Finally, the suprachoroidal electrodes were moved 1 mm dorsally. Such relocation of the electrode slightly but obviously shifted the localization of collicular EPs to STS from medial to lateral, as shown in Fig. 2D, suggesting that phosphens induced by two suprachoroidal electrodes of a STS-based multi-electrode array would be distinguishable from each other if these electrodes are at least 1 mm apart.

These observations in RCS rats strongly suggest that a STS can provide a focal excitation of the RGCs under the suprachoroidal electrodes. Thus, one can expect that patterned STS via an array of suprachoroidal electrodes will provide patterned phosphenes to RP patients.

4.2 Artificial vision in rabbit by STS

We used rabbits to develop the surgical procedure for implanting the electrode and for the functional assessment of STS method.[15]

4.2.1 Surgical procedure to insert STS electrode in rabbit eyes

A scleral pocket (3 × 5 mm) was created just over the area of visual streak, which was about 12 mm posterior to the limbus (Figure 3 A). The multichannel electrode array was then implanted into the scleral pocket and sutured with 5-0 Dacron onto the sclera just above the pocket. The counter electrode was a platinum (Pt) wire coated with polyurethane resin and exposed at the tip. It was inserted 4mm into the vitreous cavity and was fixed 2 mm from the limbus with 5-0 Dacron suture. This surgical procedure was not complicated and can is applicable to human patients.

4.2.2 The stimulating electrode and electrically evoked potential (EEP)

The stimulating electrode consisted of eight dome-shaped electrodes (fabricated by NIDEK CO., Gamagori, Japan). These are mounted in a polyimide strip 3 mm wide, 4 mm long, and 180 μm thick. The dimensions of each electrode was; height above the surface = 120-130 μm; diameter = 250 μm; distance between electrodes = 500 μm. Their impedance in saline was approximately 10 kΩ at 1 kHz.

The bundle of insulated leads from the microarray was also sutured at the limbus with 5-0 Dacron. A stimulator (SEN-7203,NIHON KOHDEN, Shinjyuku, JAPAN) was then connected through an isolator (A-395R , World Precision Instruments INC., Sarasota FL USA) to the microelectrode array. The Recording electrode was a screw in the skull bone above the visual cortex. The EEPs were elicited by monophasic electrical pulses of 0.5 ms duration. The direction of the current was set for inward flowing currents (electrode array was positive and reference electrode was negative). Fifty responses were averaged. A band-pass filter of 5 Hz to 1 kHz was used

4.2.3 Threshold current by STS in rabbit

In order to determine the minimum threshold of the EEP, each of the 8 electrodes was stimulated with a current of 500 μA, and the amplitudes of EEP waves were compared (Figure 3 B). The electrode that elicited the largest EEP was selected to determine the threshold current. The electric current was decreased in steps, and the minimum electric current that elicited the first or second positive peaks of the EEP was defined as the threshold current. The relationship between the second and third peak amplitude of the EEPs and the inward current was examined in the 6 rabbits

(Fig.3). This regression curve showed that the EEP amplitude increased almost linearly at lower stimulus currents and tended to be saturated with higher stimulus currents. The mean threshold current that elicited a small EEP was 55.0 ± μA for the six rabbits (27.5 ± 5.0 nC or 56.0 ± 10.2 μC/ cm^2).

Humayun et al reported that a charge density of 8.92-11.9 μC/cm^2 was required to elicit EEPs from rabbits with epi-retinal electrodes.[5] In experiments on cats, Dawson and Radtke found a threshold charge density of 30.5 μC/cm^2.[16] Chow et al implanted their electrodes subretinally in rabbits, and reported a threshold charge density of 2.8 nC/cm^2 to elicit EEPs.[3] Although the distance from the electrodes to the retina was farther in our transcleral electrodes than that of the epi- or subretinal electrodes, the threshold charge density to elicit EEPs was comparable with the other electrode placements.

McCreery, et al found that the threshold for stimulation-induced neural damages is determined by a synergetic interaction between charge density and charge per phase, and the limit for safe charge density decreased as charge density increased. With our electrodes, 50 NC/Phase would give a charge density of approximately 110 μC/cm^2 and the work of McCreery, et al suggests that this combination would not be injurious.

Our electrodes were not in direct contact with the neural tissue. Therefore, the limit of electrical charge density may be expected to be higher than that with epi- retinal stimulation. Indeed, Nakauchi K, et al reported that for φ100μm Platinum electrode by STS, retinal tissue was not damaged by a current up to 1mA (2100 μC/cm^2) using biphasic pulses (anodic first, duration; 0.5 msec, frequency; 20 Hz) continuously for an hour (Chapter@ in this text). These data suggest that our newly developed STS method could safely elicit the percepts necessary for artificial vision.

4.2.4 What we learned from the rabbit STS experiment

As a preclinical study of artificial vision by the STS method, we investigated the surgical procedure, functional analysis, and tissue effects. The surgical procedure to insert electrode into a scleral pocket was less complicated than the implantation of epi- or sub-retinal electrode and could be applied clinically, although the anatomical difference between rabbit and human should be carefully considered.

The EEP was elicited by electrical stimulation using a combination of charge and charge density that we expect will not be damaging to the retina, suggesting the clinical applicability of the STS method. However, the spatial resolution of STS could not be analyzed by EEP recordings in the rabbit. For this purpose, the cat is more suited as a medium-sized animal, in which the retinotopic mapping onto visual cortex has been more thoroughly investigated.

4.3 Artificial vision in cats by STS

Although we demonstrate that a single STS induced a localized excitation of RGCs under a suprachoroidal electrode even in retinal dystrophic rats, there still remains the question as to the spatial resolution of STS-based artificial vision. To investigate the question further, a rat eye is too small to give a focal STS to multiple sites on the retina, and the rat visual system, especially the retino-geniculo-cortical system involved in pattern recognition, is not well developed. Therefore, we used adult cats and recorded unitary discharges from a relay cell of the lateral geniculate nucleus (LGN) for analyzing the spatial properties of STS-evoked excitation more precisely (in preparation).

A multi-channel electrode array with nine platinum electrodes was set on a fenestrated sclera and a single charge-balanced biphasic pulse of electrical current was delivered as a STS between one of the nine electrodes and a counter electrode inserted into the vitreous. After identification of the receptive field center of a LGN neuron by mapping on a tangent screen in front of the eye, spike responses of the neuron to STS through each stimulating electrode were investigated. A higher stimulus intensity of STS was required to elicit spike bursts from the LGN neurons, when STS was applied through an electrode that was further from their receptive field.

The threshold current for inducing spike response to STS with a probability of 50 percent was analyzed for every neuron in relation to the distance from a stimulating electrode to the central point of its receptive

field. This analysis revealed that the threshold tended to become lower as the distance decreased. For some neurons, the distance was within a visual angle less than 2 degrees with a threshold current less than 0.1 mA (50 nC). Therefore, retinal excitation induced by minimal STS seems to be sufficiently localized, ensuring that a STS-based artificial retina can provide spatial resolution that is adequate for finger counting.

5. Neuroprotection by electrical stimulation

Electrical stimulation of the nervous system has profound biological effects on neurons in addition to the induction of neural activities. Recent studies have shown that electrical activity of neurons can modify neurons themselves, such as neurite extension, dendritic reorganization and synaptic connectivity in both adulthood and developmental stages.[18]

These drastic neuronal modifications by electrical activity are mediated by the changes in extracellular signal networks among the cells in the neural tissues and intracellular signaling systems of the neurons themselves. From the standpoint of treatments for pathologies of the nervous system, it is reasonable to imagine these cellular effects of electrical activity can also affect the viability of damaged neurons and the functionality of pathological neural systems. In fact, there are several studies that electrical stimulation through the cochlear implant can prevent the secondary degeneration of spiral ganglion neurons after the loss of hair cells.[19, 20]

Injury to the optic nerve (ON) is one of experimental models for the study of the pathology of retinal ganglion cells (RGCs). Axonal injury of RGCs such as ON transection or crush causes their rapid retrograde death. Many models have been developed to investigate protection of ganglion cells after damage to the optic nerve. Most of these studies were extrinsic supplements of the various trophic factors which activate or inhibit intracellular signaling systems involved in cell death and/or survival.[21-23] As another approach, we investigated neuroprotection by electrical activation of the damaged retina.

First, we investigated electrical stimulation of the transected optic nerve stump.[24] After prelabeling of RGCs with fluorescent dye bilaterally-applied to the superior colliculi, the left ON was completely transected at 3 mm behind the eyeball, and the transected stump was stimulated for 2 hours with monophasic pulses (50μA-50μsec, 20Hz) via a pair of silver ball electrodes immediately after the transection. One week after the transection, the survival rate of RGCs in the stimulated group was 83% of that of the intact retina, whereas the survival rate of RGCs with transection only was 54%. The intensity of the stimulus current was varied from 20μA to 70μA. Stimulation at 50 μA had the maximum survival-promoting effect. This proved that the electrical stimulation has a neuroprotective effect on the damaged RGCs in vivo.

Although the ON is easy to access surgically and can be stimulated in experimental animals, electrical stimulation of the optic nerve is unlikely to be useful for clinical purpose. Therefore we developed another stimulating method, transcorneal electrical stimulation (TES), for neuroprotection (Fig. 5).[25] In TES, electrical stimulation is applied via a contact lens electrode attached to the cornea. This simulation method has already proved to be able to evoke the sensation of light, phosphene.[26] This makes it possible to reduce the invasiveness and to treat the patient repeatedly only with the surface anesthesia. As shown in Fig. 5, we examined the neuroprotective effect of TES on the axotomized RGCs. The TES was applied immediately after optic nerve transection, and the survival rate of axotomized RGCs was examined 7 days later. When the TES of 1 ms/phase duration and 100 μA intensity was applied for an hour, the survival rate of axotomized RGCs was 85%, which is equivalent to the protective effect of electrical stimulation applied to the transected optic nerve.

We hypothesize that the mechanism of the neuroprotective effect of TES involves neurotrophic factors in the retina, because it was reported that expression of neurotrophic factors are upregulated by electrical stimulation.[27] The changes after TES of four major neurotrophic factors and their receptors, brain derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), basic fibroblast growth factor (bFGF), insulin-like growth factor-1 (IGF-1), trkB,

CNTF-R alpha, FGF-R1 and IGF-1R, were analyzed by reverse transcriptase-polymerase chain reaction (RT-PCR) after TES.

Amongst these, IGF-1 mRNA increased gradually and showed prominent expression on day 7 after TES (Fig. 6). Northern and Western blotting also confirmed the remarkable upregulation of IGF-1 in the retina treated by TES. Moreover, the intraperitoneal administration of the antagonistic peptide of IGF-1R blocked the effect of TES on survival of axotomized RGCs. These results indicate that IGF-1 plays a key role in the TES-induced neuroprotection of the axotomized RGCs.

To investigate in more detail the mechanism of the neural protection, the localization of IGF-1 in the retina after TES was examined immunohistologically (Fig. 7). In the normal retina, IGF-1 was present only in the inner limiting membrane (ILM) and nerve fiber layer. After TES, the IGF-1 immunoreactivity expanded from the ILM to the inner nuclear layer (INL) on day 7. An especially intense signal was observed from the radial structure extending from INL to ILM. The double staining of IGF-1 and glutamine synthetase, a marker of Müller cells, showed that the IGF-1 signal was strong on the endfeet and processes of the Müller cells, in addition to the diffuse signals in the inner retina. These immunohistological results showed that Müller cells produced IGF-1 after TES and release it into the extracellular space. Although it remains to be resolved whether activation of RGCs itself is required for neuroprotection by TES and the mechanism of activation of the Müller cells by TES, these series of experiments indicate that the TES activates the intrinsic retinal IGF-1 system and prevents the death of the RGCs.

Our findings are consistent with the concept of electrical stimulation therapy, which activates the intrinsic neuroprotective system. Electrical stimulation can be applied to any nervous system non-invasively, repeatedly and chronically if an adequate system or devise is developed. TES is simple and non-invasive therapy, and may have a therapeutic potential in other diseases of retinal neurons. In fact, we reported that TES also prolonged the survival of photoreceptors and delayed the loss of retinal function in RCS rats.[28]

We also hypothesize that STS-based retinal implants for artificial vision can work as a kind of neuroprotective device for various retinal dystrophic diseases through electrical stimulation. This idea was supported by a recent report that subretinal implant had a neuroprotective effect for photoreceptors of RCS rats, although the neuroprotective contribution of electrical stimulation itself was observed only in the ERG, not in the morphology.[29] In the future, the retinal electrode may act not only function as a retinal prosthesis but also may help to prevent the degeneration of retinal cells.

References

1. Humayun MS (2001) Intraocular retinal prosthesis. Trans Am Ophthalmol Soc, 99:271-300.

2. Margalit E, Maia M, Weiland JD, Greenberg RJ, Fujii GY, Torres G, Piyathaisere DV, O'Hearn TM, Liu W, Lazzi G, Dagnelie G, Scribner DA, de Juan E ,Jr, Humayun MS (2002) Retinal prosthesis for the blind. Survey of ophthalmology 47:335-356

3. Zrenner E (2002) Will retinal implants restore vision?. Science 295:1022-1025.

4. Eckmiller R (1997). Learning retina implants with epiretinal contacts. Opthalmic Res 29:281-289.

5. Humayun MS, Probst R, de Juan E ,Jr, McCormick K, Hickingbotham D (1994). Bipolar surface electrical stimulation of the vertebrate retina. Arch Ophthalmol 114:40-46.

6. Majji AB, Humayun MS, Weiland JD, Suzuki S, D'anna SA, de Juan E ,Jr (1999). Long-term histological and electrophysiological results of an inactive epiretinal electrode array implantation in dogs. Invest Ophthalmol Vis Sci 40:2073-2081.

7. Nadig MN (1999). Development of a silicon retinal implant: cortical evoked potentials following focal stimulation of the rabbit retina with light and electricity. Clinical Neurophysiology 110:1545-1553.

8. Walter P, Heimann K (2000). Evoked cortical potential after electrical stimulation of the inner retina in rabbits. Graefes Arch Clin Exp ophthalmol 238:315-318.

9. Chow AY, Chow VY (1997) Subretinal electrical Stimulation of the rabbit retina. Neuroscience letters 225:13-16.

10. Chow AY, Pardue MT, Perlman JI, Ball SL, Chow VY, Helting JR, Peyman GA, Liang C, Stubbs EB Jr., Peachey NS (2002) Subretinal implantation of semiconductor-based photodiodes: Durability of novel implant designs. J Rehabilitation Res Develop 39:313-322.

11. Schwahn HN, Gekeler F, Kohler K, Kobuch K, Sachs HG, Schulmeyer F, Jakob W, Gabel VP, Zrenner E (2001). Studies on the feasibility of a subretinal visual prosthesis: data from Yucatan micropig and rabbit. Graefes Arch Clin Exp Ophthalmol 239:961-967.

12. Zrenner E, Stett A, Weiss S, Aramant RB, Guenther E, Kohler K, Miliczek KD, Seiler MJ, Haemmerle H (1999) Can subretinal microphotodiodes successfully replace degenerated photoreceptors? Vision Res 39:2555-2567.

13. Walter P, Szurman P, Viobig M, Berk H, Ludtke-Handjery H-C, Deng HR, Mittermayer C, Heimann K, Sellhaus B (1999). Successful long term implantation of electrically inactive epiretinal microelectrode arrays in rabbits. Retina 19:546-552.

14. Kanda H, Morimoto T, Fujikado T, Tano Y, Fukuda Y, Sawai H (2004). Electrophysiological Studies on the Feasibility of Suprachoroidal-Transretinal Stimulation for Artificial Vision in Normal and RCS Rats. Invest Ophthalmol Vis Sci 45:560-566.

15. Nakauchi K, Fujikado T, Kanda H, Morimoto T, Choi JS, Ikuno Y, Sakaguchi H, Kamei M, Ohji M, Yagi T, Nishimura S, Sawai H, Fukuda Y, Tano Y. (2005) Transretinal electrical stimulation by an intrascleral multichannel electrode array in rabbit eyes. Graefes Arch Clin Exp Ophthalmol., 243:169-174.

16. Dawson WW, Radtke ND. (1977). The electrical stimulation of the retina by indwelling electrodes. Invest Ophthalmol Vis Sci 16:249-252.

17. McCreery DB, Agnew WF, Yuen TG, Bullara L. (1990). Charge density and charge per phase as cofacters in neural injury induced by electrical stimulation. IEEE Trans Biomed Eng 37:996-1001.
18. Zhang LI, Poo MM (2001) Electrical activity and development of neural circuits Nat. Neurosci. 4(Suppl): 1207-1214.
19. Leake PA, Hradek GT, Rebscher SJ, Snyder RL (1991) Chronic intracochlear electrical stimulation induces selective survival of spiral ganglion neurons in neonatally deafened cats. Hear Res 54:251-257.
20. Leake PA, Hradek GT, Snyder RL (1999) Chronic electrical stimulation by cochlear inplant promotes survival of spiral ganglion neurons after neonatal deafness. J Comp Neurol 412:543-562.
21. Mansour-Robaey S, Clarke DB, Wang YC, Bray GM, Aguayo AJ (1994) Effects of ocular injury and administration of brain-derived neurotrophic factor on survival and regrowth of axotomized retinal ganglion cells. Proc Natl Acad Sci USA 91:1632-1636.
22. Kermer P, Kloecker N, Labes M, Baehr M (1998) Inhibition of CPP32-like proteases rescues axotomized retinal ganglion cells from secondary cell death *in vivo*. J Neurosci 18:4656-4662.
23. Shen S, Wiemelt AP, McMorris FA, Barres BA (1999) Retinal ganglion cells lose trophic responsiveness after axotomy. Neuron 23:285-295.
24. Morimoto T, Miyoshi T, Fujikado T. Tano Y, Fukuda Y (2002) Electrical stimulation enhanced the survival of axotomized retinal ganglion cells in vivo. Neuroreport, 2002:13:227-230.
25. Morimoto T, Miyoshi T, Matsuda S, Tano Y, Fujikado T, Fukuda Y (2005) Transcorneal electrical stimulation rescues axotomized retinal ganglion cells by activating endogeneous retinal IGF-1 system. Inv. Ophthal. Vis. Sci., 2005:46;2147-2155
26. Potts AM, Inoue J, Buffum D (1968) The electrically evoked response of the visual system (EER). Invest Ophthalmol 7:269-278.
27. Al-Majed AA, Brushart TM, Gordon T (2000) Electrical stimulation accelerates and increases expression of BDNF and trkB mRNA in regenerating rat femoral motoneurons. Eur J. Neurosci. 12:4381-4390.
28. Morimoto T, Choi JS, Miyoshi T, Fujikado T, Fukuda Y Tano Y, Fujikado T.(2005) Effects of transcorneal electrical stimulation on the survival of photoreceptors in RCS rats, ARVO 2005 46: E-Abstract.
29. Paedue MT, Phillips MJ, Yin H, Sippy BD, Webb-Wood S, Chow AY, Ball SL (2005)
Neuroprotective effect of subretinal implants in the RCS rat. Inv. Ophthal. Vis. Sci., 46:674-682.

Figure Legends

Figure 1

A new approach for the artificial retina 'suprachoroidal transretinal stimulation (STS)'

In this approach, the stimulating electrode was placed in the suprachoroidal space or in the fenestrated sclera, while the counter electrode was placed in the vitreous space The key features of this approach are that the electrodes do not contact the retina.

Figure 2.

STS in normal and RCS rats

(A) A photomicrograph of a retinal section of an adult RCS rat stained by hematoxylin-eosin. GCL:ganglion cell layer, IPL: inner plexiform layer, INL: inner nuclear layer.

(B) A design of electrophysiological experiment in rats. SE: suprachoroidal electrode (anode), VE: vitreous electrode (cathodic)

(C) Neuronal responses evoked by flashing light and STS in a RCS rat. Upper trace: electroretinogram (ERG) to flashing light stimulus, showing that the retina is unresponsive to the light stimulus. Middle trace: Averaged visual evoked potentials (VEP) recorded from the SC, also showing no reponse. Bottom trace, Averaged electrically evoked potentials (EEP) to STS (10, 30, 60, 80 and 100 μA) recoded from the SC.

(D) Differential distribution of STS-evoked responses via two separated electrodes. Left: two crosses indicates retinal positions of STS applied via a suprachoroidal elecrode, separated 1 mm from each other (SE1 and SE2) . A filled circle indicates the optic nerve head. D: dorsal, T: temporal, V: ventral, N:nasal Right: distribution of collicular EPs in response to a single STS of 100 μA to SE1 (the lower), and SE2 (the upper). Recording of EPs was done at every distance of 250 μm. Center and radius of each Circle indicate the recording site of EPs and the relative amplitude of N1-P1 component, respectively. R: rostral, L: lateral

Figure 3. Surgical procedure and EEPs by STS in normal rabbit

(A). Surgical procedure. A scleral pocket was created 12mm from the limbus and a.multichannel electrode. array. was. inserted just beneath the visual streak. The counter electrode was inserted in the vitreous space and transretinal electrical current with monophasic pulse was applied. (B). EEPs by STS. EEPs with different amplitude were recorded by stimulating different electrode.

Figure 4. Amplitude of the EEP induced by STS in rabbit

A typical EEP waveform evoked by 500uA stimulation.

P2-N2 amplitude plotted in relation to the stimulus intensity in rabbit (n=6)

The mean threshold current was 55.0 ± 10.0 μA. The increase in response amplitude was approximately linear with low stimulus intensities.

Figure. 5. Protection of retinal ganglion cells (RGCs) from by transcorneal electrical stimulation (TES).

The RGCs were labeled by fluorescent dye retrogradely from bilateral superior colliculi before optic nerve transection. A biphasic pulse of 100μA was applied through corneal lens-type electrode for an hour after the transection of rat optic nerve. With this current, the EEP was effectively recorded from the SC of the intact rat in other experiment, suggesting that retina was stimulated enough by the current through the contact lens electrode. One week after optic nerve transection, the surviving RGCs were counted. The photomicrographs of the flat-mounted retinas shows more RGCs survived in 'cut+TES' retina than 'cut' retina with transection only. In the sham operated group, 53% of RGCs survived, while 'cut+TES' group showed 70 to 85% survival. This

proved that the electrical stimulation on the retina was effective to promote the survival of RGCs.

Intact, the intact retina; cut, the retina with optic nerve transection; sham, the retina with optic nerve transection and attachment of TES electrode but without current through it; cut+TES, the retina with optic nerve transection and TES.

Figure 6. Expression of mRNA of neurotrophic factors and their receptors after TES.

The expression was analyzed by reverse transcriptase-polymerase chain reaction (RT-PCR) at different time points ranging from 1 hour to 7 days after TES without optic nerve transection. The expression of only IGF-1 increased up to day 7. C, control intact retina without optic nerve transection; IGF-1, insulin-like growth factor-1; BDNF, brain derived neurotrophic factor; bFGF, basic fibroblast growth factor; CNTF, ciliary neurotrophic factor.

Figure 7. Immunohistrological analysis of IGF-1 in the retina after TES.

Upper panels show the localization of IGF-1 at different time points from day 1 to day 14 after TES. In the control retina (no TES), there is very weak IGF-1 immunoreactivity in the inner limiting membrane (ILM) and in the nerve fiber layer (NFL). After TES, the IGF-1 signal increased and extended to inner plexiform layer diffusely. On day 4 and 7, the radial structure from inner nuclear layer (INL) to ILM was also observed. After the maximum on day 7, the signal decreased on day 14. Lower panels show the double staining of IGF-1 and glutamine synthetase (GS), a marker of Müller cells. In the control retina, weak IGF-1 signals in the ILM and NFL were seen on the endfeet of Müller cells. On day 7 after TES, strong immunoreactivity appeared in the endfeet and processes of Müller cells. Scale; 100μm (upper panels), 50μm (lower panels).

A new approach for the artificial retina

Suprachoroidal-Transretinal Stimulation (STS)

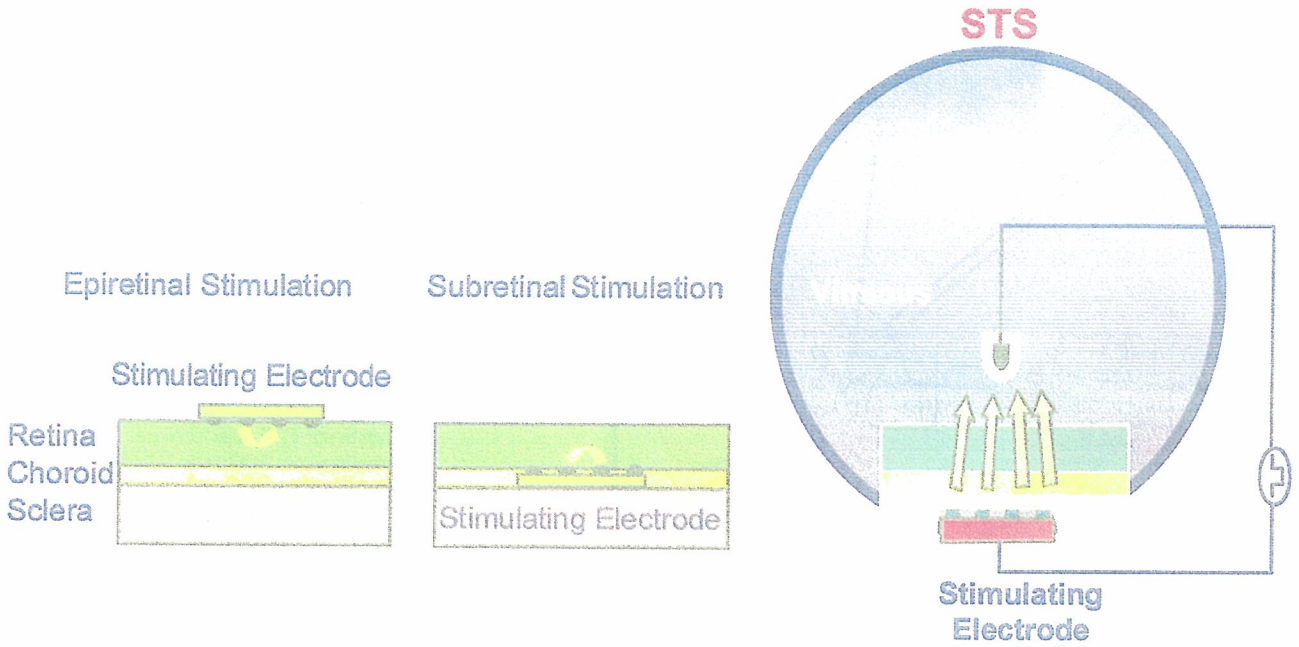


Fig.1

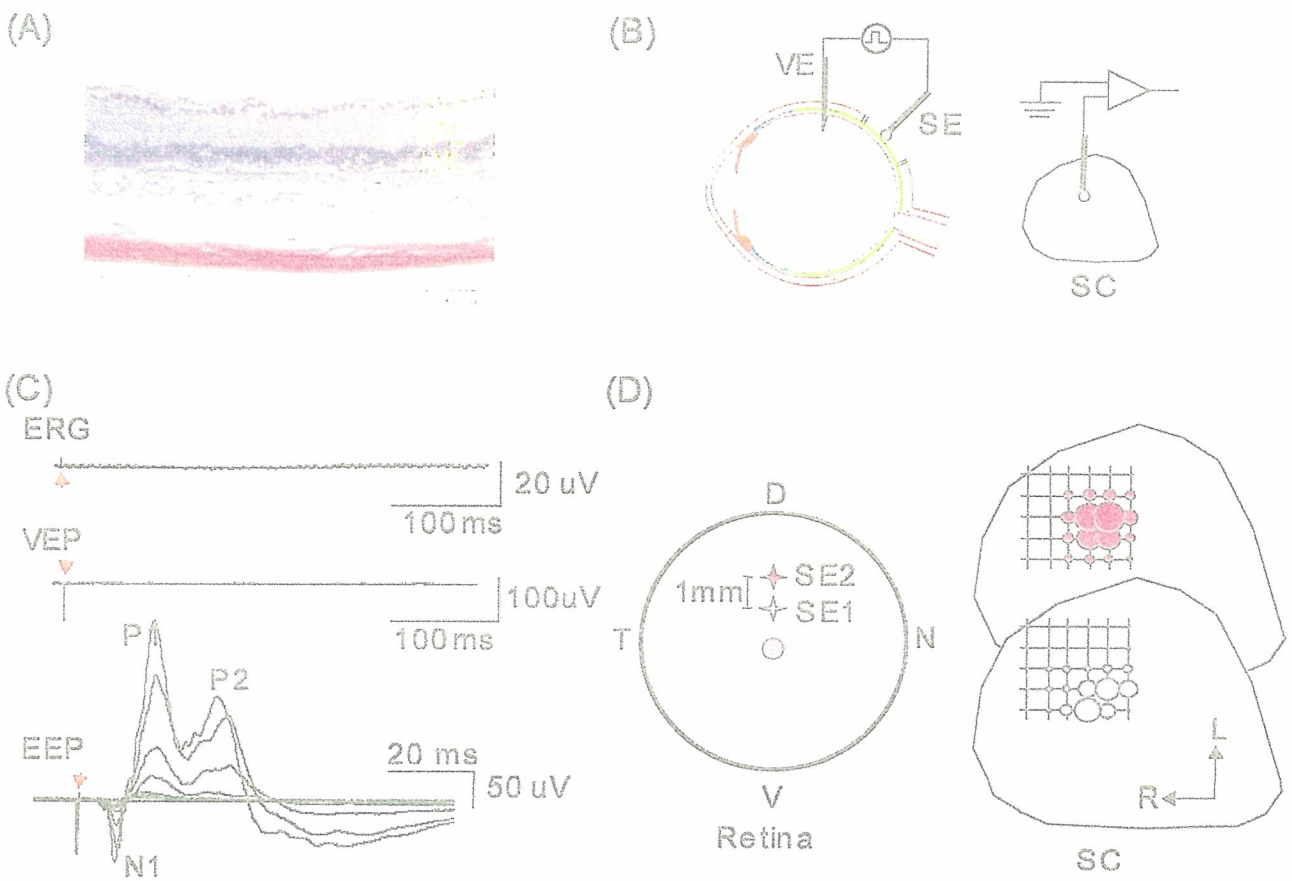


Fig. 2

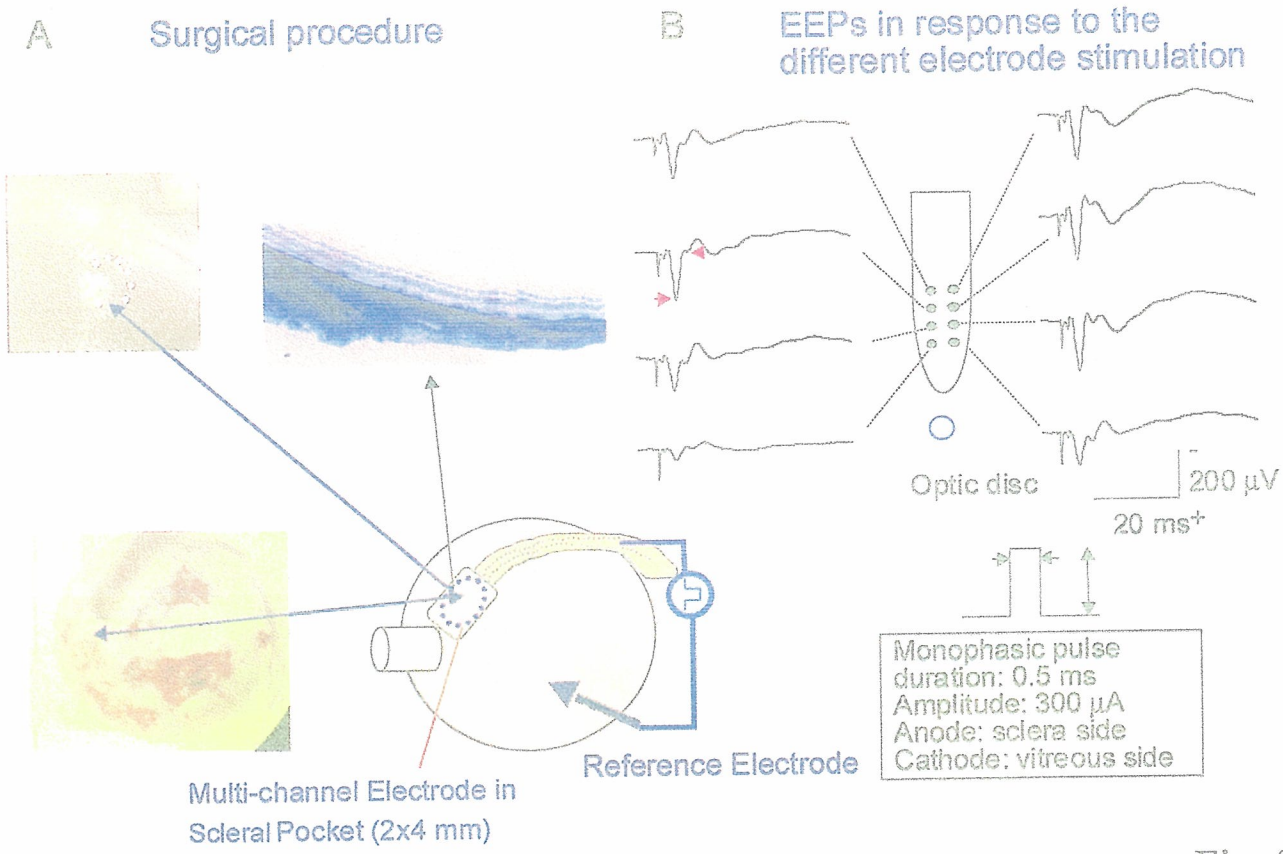

A
Surgical procedure
B
EEPs in response to the different electrode stimulation
Optic disc
200 μV
20 ms
Monophasic pulse
duration: 0.5 ms
Amplitude: 300 μA
Anode: sclera side
Cathode: vitreous side
Reference Electrode
Multi-channel Electrode in
Scleral Pocket (2x4 mm)

Fig. 3

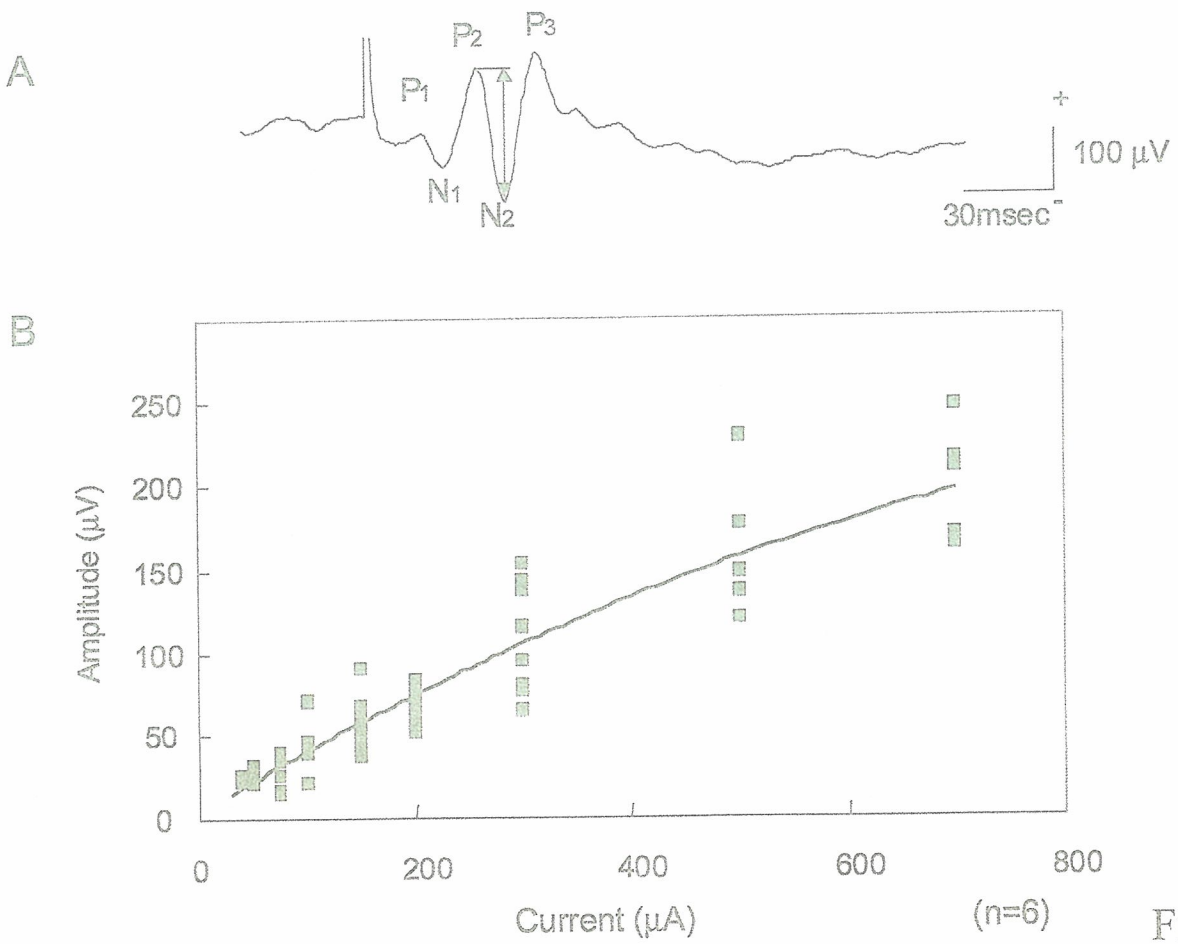

A
P1
P2
P3
N1
N2
+
100 μV
30msec
B
Amplitude (μV)
250
200
150
100
50
0
0
200
400
600
800
Current (μA)
(n=6)

Fig. 4

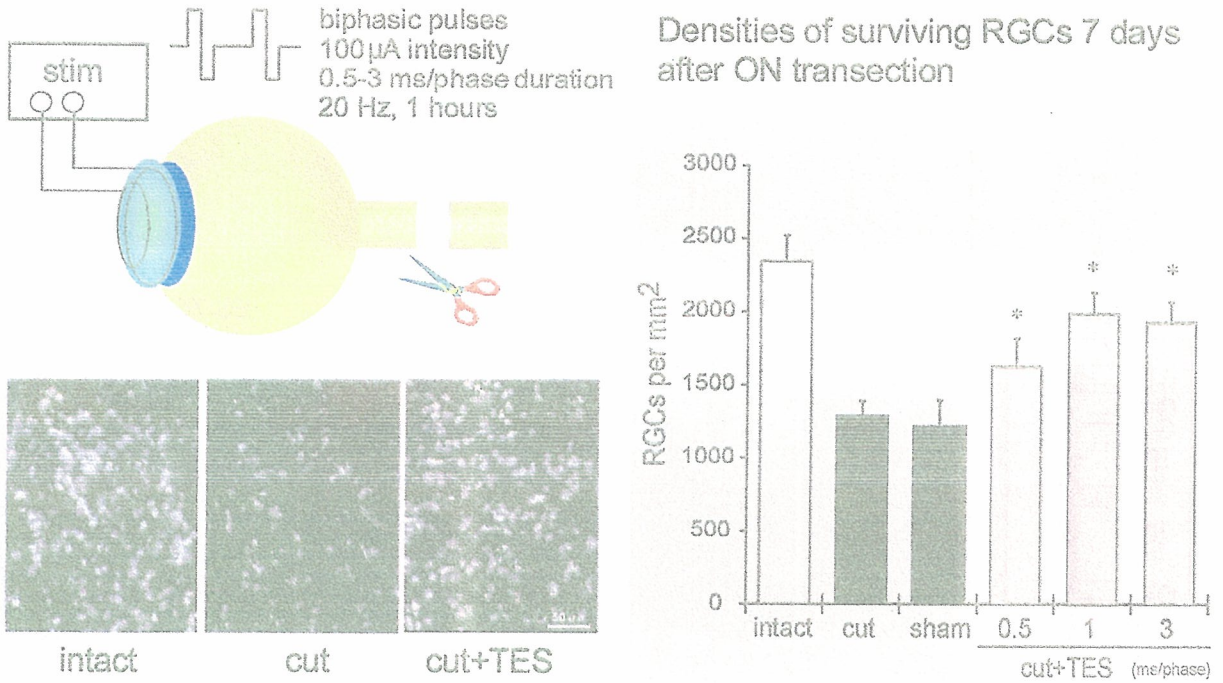

stim
biphasic pulses
100 µA intensity
0.5-3 ms/phase duration
20 Hz, 1 hours
intact
cut
cut+TES
Densities of surviving RGCs 7 days after ON transection
RGCs per mm2
3000
2500
2000
1500
1000
500
0
intact
cut
sham
0.5
1
3
cut+TES (ms/phase)

Fig. 5

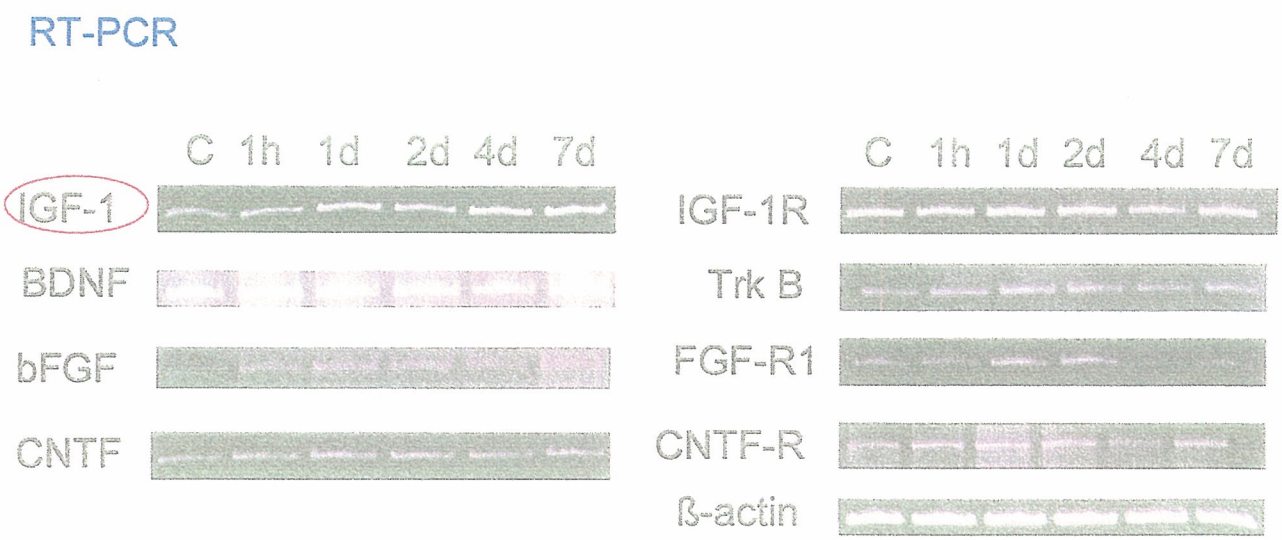

RT-PCR
C 1h 1d 2d 4d 7d
IGF-1
BDNF
bFGF
CNTF
C 1h 1d 2d 4d 7d
IGF-1R
Trk B
FGF-R1
CNTF-R
ß-actin

Fig. 6

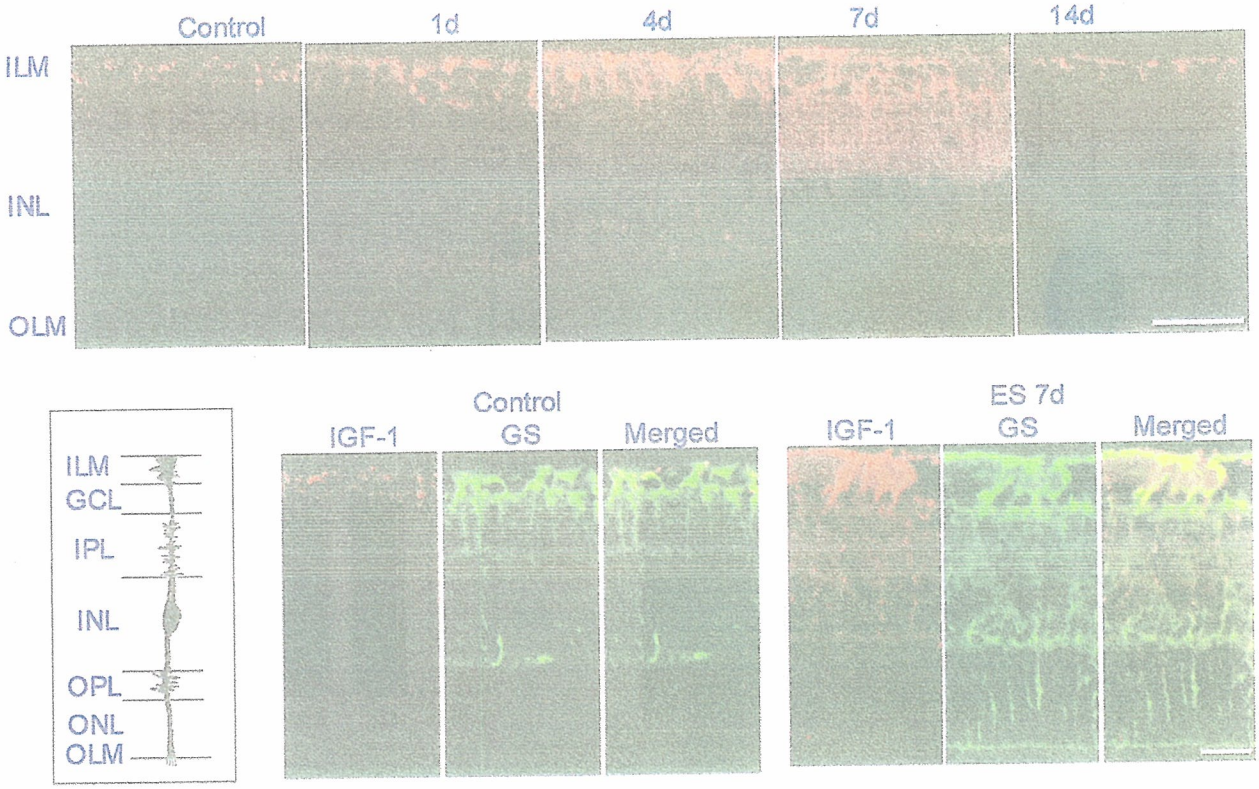

Control
1d
4d
7d
14d
ILM
INL
OLM
ILM
GCL
IPL
INL
OPL
ONL
OLM
Control
IGF-1
GS
Merged
ES 7d
IGF-1
GS
Merged

Fig. 7

総説

視覚再生

田野保雄

〔要　約〕

近年の眼科学の進歩は目覚しく，診断精度の著しい改善とともに新たな治療法が次々と開発され，かつての難治性眼疾患の多くが治療可能となってきている。しかし，未だに網膜色素変性をはじめとする網膜変性疾患に対する有効な治療法は皆無といわざるを得ない状態である。最近になって，移植医療や再生医療などとともに新たな治療手段として人工視覚による復明を実現しようとする努力が各国で続けられている。本稿では人工視覚による視覚再生の概要を中心に述べる。

はじめに

増殖糖尿病網膜症やスティーブンスジョンソン症候群などかつて難治とされた眼科疾患の多くは硝子体手術や培養上皮移植術などで治療できるようになった。加齢黄斑変性や近視性新生血管黄斑症の治療法も種々の抗新生血管物質の登場によって大きく変わろうとしている。一方，網膜色素変性をはじめとする網膜変性疾患に対する有効な治療法は未だに皆無といってよい状態であるが，移植医療や再生医療などとともに新たな治療手段として人工視覚による復明を実現しようとする努力が各国で続けられている。本邦においても2001年度から経済産業省（NEDO）と厚生労働省との両省連携国家プロジェクトとして人工視覚システムの研究開発計画が始まった。本プロジェクトでは，大阪大学，株式会社ニデック，奈良先端技術大学院大学，杏林大学，名古屋大学，滋賀医科大学，九州大学によってコンソーシアムが形成された。当面の目標は実験動物における眼前指数弁程度の視力，すなわち約0.01の人工視覚獲得を目指している。本年3月第一段階のNEDO側プロジェクトは一旦終了し，新たに本年から産業技術実用化開発助成事業に採択され，いよいよ人工視覚システム実用化のための研究開発の段階に入っている。本稿では人工視覚による視覚再生への取り組みを中心に述べる。

Ⅰ．擬似光覚

網膜や視神経に何らかの鈍的外力やX線あるいは電流などの物理的刺激を与えることによってフォスフェンと呼ばれる擬似光覚が得られる。この現象は古くから知られており，正確な眼軸長測定法としてX線によるフォスフェン現象が用いられていたほどである[1]。1970年代初頭にBrindleyらが実験的に大脳皮質への電気刺激によってフォスフェンが起こることを報告したことに引き続き[2]，Dobelleらが大脳皮質に刺激電極をおき，全盲の患者がフォスフェンを感じたことを報告した[3]。Rizzoらは網膜表面に小型電極を

田野　保雄：大阪大学大学院医学系研究科脳神経感覚器外科学

設置し，残存する網膜を電気刺激することによって人工視覚が得られる可能性があることを提唱した[2]。Humayun らは網膜色素変性で失明したボランティアに対して，局所麻酔下で硝子体手術を行い，手術中に電極を眼内に挿入して網膜表面を電気刺激し，視細胞が機能していない眼でもフォスフェンを感じることを証明して[3]，網膜電極による人工視覚の現実性を高めた。一方，コンピュータ制御の小型信号変換装置を導入することによって，かつて大脳皮質に刺激電極を移植した患者の一人で独歩可能な人工視覚が得られたことを Dobelle らがメディアに公開し，大きな反響を呼んだことは記憶に新しい[4]。現在，開発されている人工視覚の大半は，大脳皮質，視神経，網膜上，網膜下のいずれかで電気刺激を行うことによって，フォスフェンを生じさせ，物の形状や動きを認知させようとするものである。

Ⅱ．人工視覚の原理と種類

極く単純に言えば，視覚は光を介した情報により視細胞が刺激されて生じた局所的な電気現象が視路を介して視覚野まで伝達されることによって生じる。視細胞以降，第2ニューロンである網膜双極細胞，第3ニューロンである網膜神経節細胞とその軸索である網膜神経線維，さらには外側膝状体から大脳皮質視覚野に到るまでの視路は電気的現象によって情報を伝達する電気回路である，ともいえる。したがって，有用な視力を喪失した重症の網膜色素変性であっても，障害部位が視細胞と網膜色素上皮細胞であり，網膜双極細胞以降の機能が残存しているのであれば，たとえ全く視細胞が機能していない場合でも，理論的には視路のどこかで電気信号が発生するような刺激を与えれば人工視覚が生じ得ることになる。実際，進行した網膜色素変性の網膜を組織学的に検討した Santos ら[5]によれば，網膜双極細胞は78%，網膜神経節細胞は30%残存しており，これらに対する電気刺激によって全盲の患者が人工視覚を得る可能性は十分にあると考えられる。

現在開発中の人工視覚システムを刺激部位によって分類すると，大脳皮質刺激電極によるもの，視神経刺激電極によるもの，網膜下刺激電極によるもの，網膜上刺激電極によるものの4つがある。また，特殊な刺激装置としてウィスコンシンやスタンフォードのグループが目指している神経伝達物質微量放出装置による刺激法がある。今のところこれらのうちで，実用に耐えるとされるヒトでの人工視覚を実現したのは前述の Dobelle らによる大脳皮質刺激電極による装置である。カメラからの情報を画像処理し，物体の輪郭のみを認識するようにしているため，対象が高コントラスト物体でなければならない制約があるものの，位置情報を熟知した通勤経路のような道であれば独歩できる人工視覚が達成されたとされる。しかも，この方式であれば外傷などによる全盲例においても復明を期待することができる。ただし，この方式は開頭手術を要し，癲癇や感染症などの重篤な障害発生率が他の方式に比して高いことが大きな問題点であるとされている。また，ベルギーのグループが研究している視神経周囲への電気刺激方式は，刺激パターンの変化と組み合わせによって種々の空間的位置にフォスフェンが得られることを利用して像を再構築する方式であるため認識に時間がかかる点や，大脳皮質刺激方式と同様に刺激装置埋植に関連する障害発生率が高いと考えられている。

Ⅲ．人工網膜

一方，眼内で刺激する網膜下刺激電極あるいは網膜上刺激電極を用いる方式は網膜神経節細胞以降視中枢までの機能がある程度残存していることが絶対条件となるものの，大脳皮質刺激電極や視神経刺激電極に比較すれば，刺激電極埋設に関わる医学的安全性は高いと考えられる。また大脳皮質刺激方式や視神経刺激方式に比較すると，分解能を上げやすい可能性があることから，現在開発途上にある人工視覚開発計画の多くは人工網膜を目指したものである（図1）。

人工網膜のうち，網膜下刺激電極方式は光電素子によって光エネルギーを電気エネルギーに変換し網膜を裏面から刺激しようとするものであるが，対象となる疾患では網膜視細胞は機能していないので，網膜双極細胞または神経節細胞を刺激することになる。当初提唱された方式では光電素子，