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ORIGINAL ARTICLE

Others

Electrophysiological evaluation of a chronically implanted electrode for suprachoroidal transretinal stimulation in rabbit eyes

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Abstract

In this study, we aimed to determine the electrophysiological efficacy, safety, and electrical stability of a chronically implanted electrode for suprachoroidal transretinal stimulation (STS) in rabbit eyes. A platinum microelectrode was implanted into the scleral pocket of rabbit eyes ($n=5$) and followed-up for 6 months. To evaluate the electrophysiological efficacy, electrically evoked potentials (EEPs) were measured every month after implantation. To evaluate safety, fundus examinations, fluorescein angiograms, electroretinograms (ERGs), and visually evoked potentials (VEPs) were measured before and every month after the implantation. At the end of the experiment, histological examination of retinal tissue beneath the site of the electrode was performed. To evaluate electrical stability, the resistance of the circuit was measured every month after implantation. EEPs could be elicited from the STS electrodes at all testing times. The mean threshold current to evoke EEPs was 186.4 ± 47.0 μA at 6 months after implantation. There was no significant change in the threshold over the follow-up period. The resistance of the circuit was significantly increased at 1 months after implantation, with no further increase at 6 months. There was no statistically significant change in the relative amplitudes and implicit times of *a*- and *b*-waves of ERGs and VEPs. No intraocular infection, inflammation, or vitreoretinal proliferation was observed in any eye. Histological examination revealed no retinal damage beneath the electrode. We conclude that chronically implanted electrodes for STS appear to be effective, safe, and electrically stable.

Keywords Suprachoroidal transretinal stimulation · Electrically evoked potentials · Electroretinograms · Visually evoked potentials · Resistance

Introduction

Retinitis pigmentosa (RP) and various other ocular conditions cause gradual loss of vision, for which currently there is no treatment. Several research groups have attempted to develop artificial vision systems to restore vision. Targets of electrical stimulation in artificial vision systems include the visual cortex, lateral geniculate nucleus, the optic nerve, and the retina [1–12].

Our research group has developed two types of artificial vision systems, each with its own stimulation target. One such system is the suprachoroidal transretinal stimulation (STS) system, a retinal prosthesis that stimulates the retina via an electrode implanted in the scleral pocket [8, 9]. The other type stimulates the optic nerve fibers via a direct optic nerve electrode (AV-DONE) [10, 11]. We have previously performed clinical trials for both artificial vision systems [9–11].

Although a clinical trial of chronic implantation has been performed, an animal experiment is crucial because the measurement of electrical evoked potentials (EEPs) from animals has advantages. We can drill the skull and implant the cortical recording and reference electrode. This allows the recording of EEPs with higher signal-to-noise ratio. Measuring EEPs from humans is the best way to investigate

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the efficacy of artificial vision objectively. However, EEPs measurements are typically associated with low signal-to-noise ratios [12], given that the cortical recording electrode is typically positioned on the surface of the skull. Furthermore, histological evaluations are impossible when human patients are involved. Therefore, in the present study, we evaluated the electrophysiological efficacy, safety, and electrical stability of a chronically implanted electrode for STS in rabbits over a period of 6 months.

Methods

Animals

Five eyes of 5 Dutch-belted rabbits(Biotech, Saga, Japan; weighing 2.7–2.9 kg) were used. All procedures were done in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research, and the policies stated in the Guide to the Care and Use of Laboratory Animals issued by the NIH.

Each rabbit was anesthetized with an intramuscular injection of ketamine hydrochloride (40 mg/kg body weight) and xylazine hydrochloride (4 mg/kg). The pupils were dilated with 0.5% phenylephrine hydrochloride and 0.5% tropicamide, and the eyes were draped.

Stimulating electrodes

Stimulating electrode implantation was performed as previously described [8]. Briefly, a single stimulating electrode was used. The wire (90% platinum, 10% iridium; diameter: 60 µm) was insulated with PDMS (polydimethylsiloxane) and embedded in a 2 mm horizontal × 5.5 mm vertical × 50 µm-thick parylene® plate. The tip of the wire was connected to a single stimulating platinum electrode (diameter, 500 µm) (Fig. 1a-1). A scleral pocket (3 × 5 mm) was created just over the visual streak. The electrode plate was then implanted into the scleral pocket and sutured with 5-0 Dacron® onto the sclera just above the pocket. The insulated strand lead from the electrode was sutured at the limbus with 5-0 Dacron®. As the return electrode, 7-mm long platinum wire was used. The electrode was inserted into the vitreous cavity and was fixed 1 mm posterior to the limbus with 5-0 Dacron®.

Cortical recording electrodes

Under deep general anesthesia(details described before), the top of the skull was exposed and 1-mm holes were drilled

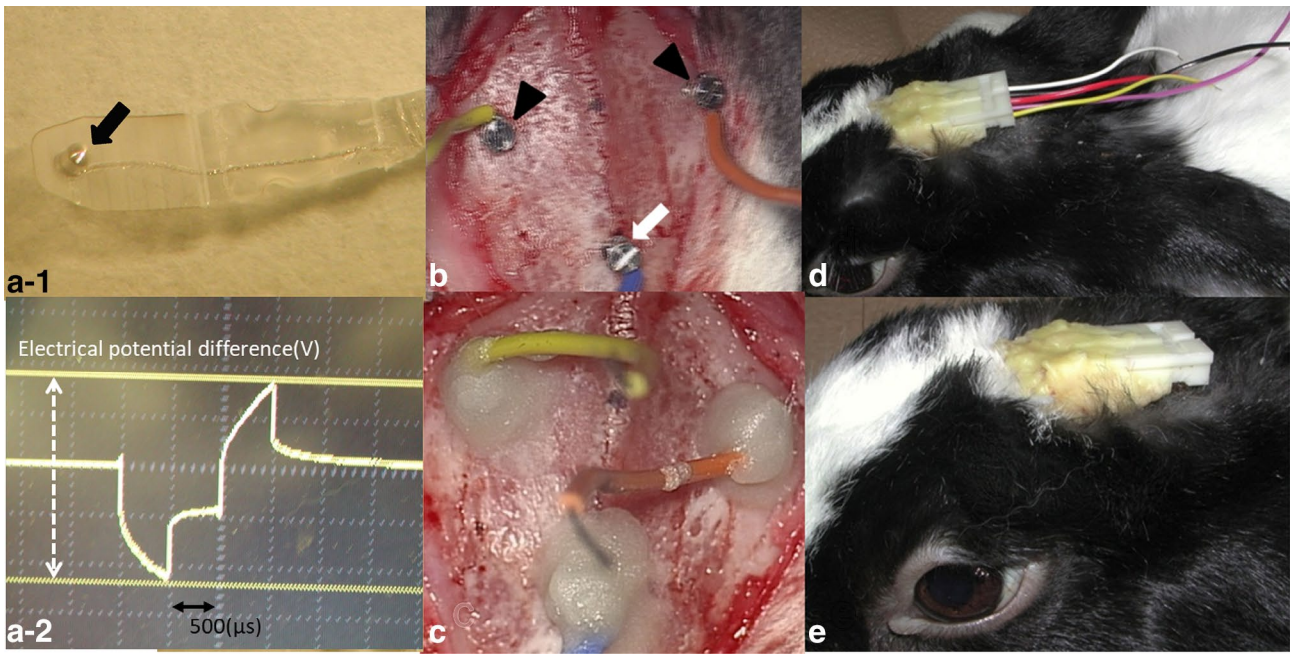


Fig. 1 STS stimulating electrode, the resistance of the circuit and cortical recording electrodes. **a**-1, A single stimulating platinum electrode(diameter, 500 µm, Black arrow) **a**-2, The electrical potential difference between stimulating electrode and return electrode was measured by an oscilloscope. The resistance of the circuit was calculated by dividing the number of the electrical potential difference by the arrow. **b** Recording electrode (Black arrow head) and return electrode (Black arrow). **c** The screw-type stainless steel electrodes were protected by dental cement. **d** All wires from the stimulating electrode, the vitreal electrode and cortical electrodes were leaded to the connector. **e** The connector was protected by plastic cap

through the skull 8 mm anterior to the lambdoid suture and 7 mm right and left of the midline. Then, screw-type stainless steel recording electrodes coated with silver were screwed into the skull to make electrical contact with the dura mater. The reference electrode was then screwed into the skull at the bregma. Those electrodes were protected by dental cement. All wires from the stimulating electrode, the reference electrode and the cortical electrodes were leaded to the connector on the head, which was protected by a plastic cap (Fig. 1b–e).

Eliciting EEPs

EEPs were recorded at the implantation day and every month after the implantation. An electronic stimulator (SEN-7203; Nihon Kohden, Shinjuku, Japan) was connected through a stimulus isolation unit(A-395R; World Precision Instruments, Sarasota, FL, USA) to the STS electrode during the stimulation. The electrical stimulating current was changed from 50 to 1000 μA, and biphasic pulses were used for the electrical stimulation. We used cathodic-first (CF) biphasic pulse. The duration of both phases was 500 μs (CF500) or 1000 μs (CF1000), and interpulse (500 μs) was located between biphasic pulses. The threshold current necessary to elicite an EEP was determined by decreasing the electric current in steps. The minimum electric current that elicited the first or second positive peak of the EEP (P1 or P2) was defined as the threshold current.

Resistance of the circuit

The electrical potential difference between stimulating electrode and return electrode was measured(Figs. 1a, 2) using an oscilloscope (TPS2014; Tektronix, Beaverton, OR, USA) after applying a constant current (500 μA, 20 Hz) for more than 5 min. The resistance of the circuit was calculated by dividing the number of the electrical potential difference by the number of current. The electrical potential difference was measured at the implantation day and every month after the implantation.

Slit lamp examinations, fundus examinations, and fluorescein angiography (FA)

Baseline and follow-up examinations were performed before and every month after the implantation by slit-lamp

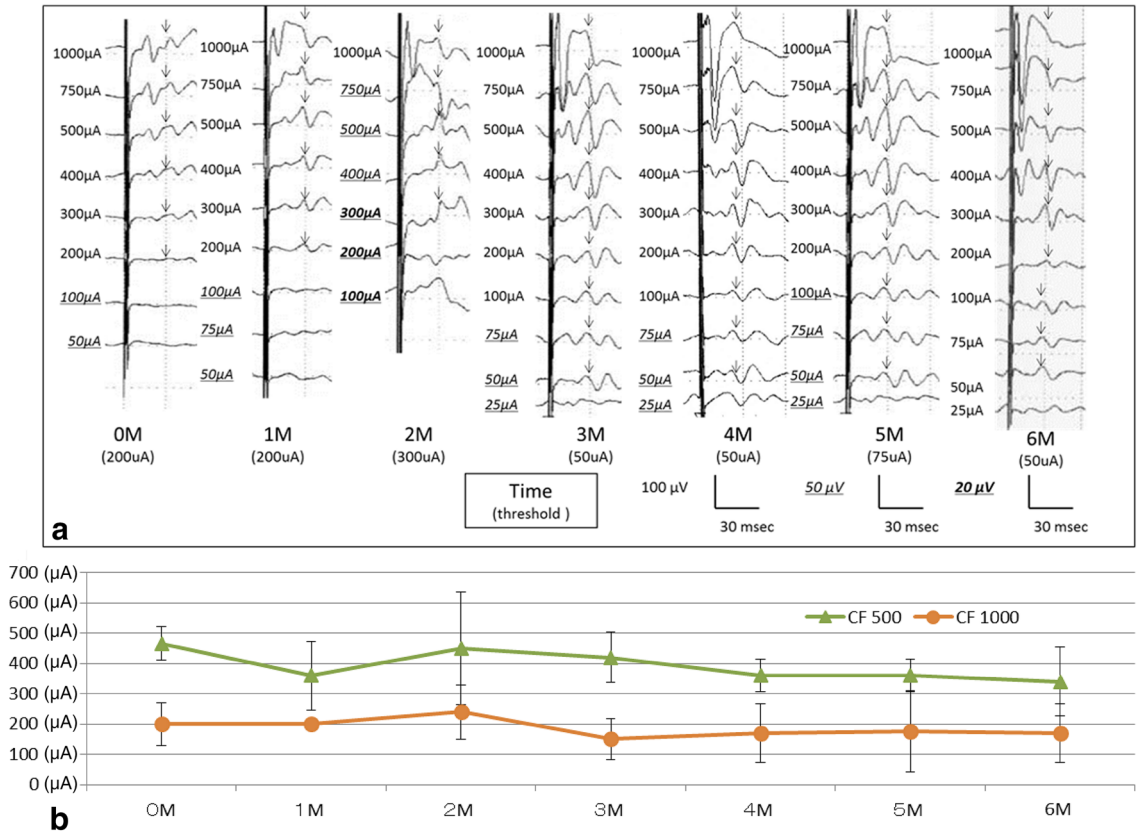


Fig. 2 Time course of thresholds of EEPs. a Representative consecutive EEPs(Black arrows) of one rabbit. The threshold of EEPs were obtained during following-up period using CF1000. **b** No significant difference was found between time-points for the thresholds of CF500 and CF1000

microscopy, indirect ophthalmoscopy, and FA. The anterior and posterior segments of the eye were evaluated. All of the fundi were photographed with a fundus camera (TRC-50IX; TOPCON, Tokyo, Japan).

Electroretinograms (ERGs)

ERGs were recorded before and every month after the implantation. The rabbits were anesthetized and the pupils were dilated and the cornea was anesthetized. Then, a bipolar contact lens electrode (8017BFPW-4 ERG; Mayo, Nagoya, Japan) was placed on the cornea. After dark adaptation, ERGs were elicited by white flashes that were driven by an electronic stimulus generator (LS-704B, SLS-3100; Nihon Kohden, Tokyo, Japan). The strobe flash unit was placed 15 cm in front of the rabbit's eye, and the energy of the light was set at 1.2 J, and the stimulus duration was 32 μs. Ten ERGs were averaged by a computer (Neuropack μ. Model MEB-9104; Nihon Kohden) with an inter-stimulus interval of 10 s. The a-wave amplitude was measured from the baseline to the first negative trough, and the *b*-wave from the negative trough to the positive peak. Mean *a*- and *b*- waves' implicit times and amplitudes of the ERGs measured from the implanted eye were analyzed and compared with those from the control eye, at each time-point. The *a*- and *b*-wave implicit times and amplitudes were expressed as relative values, i.e., the values in the experimental eye as percentages of those in the control eye. The averaged recordings of control eye at each time point were taken as 100%.

Visually evoked potentials (VEPs)

VEPs were recorded at the implantation day and every month after the implantation. All VEPs recording sessions were performed in the dark. The rabbits were anesthetized and the pupils were dilated. Cortical responses were recorded between the contralateral cortical electrode and the reference electrode in response to flash stimuli to the implanted or control eyes separately. Flash stimuli were performed as described above.

Ten VEPs were averaged by a computer (Neuropack μ. Model MEB-9104; Nihon Kohden) with an inter-stimulus interval of 10 s. The amplitude of VEPs was measured from the baseline to the first positive trough (P1). The means of the implicit times and amplitudes of the major positive deflection (P1) from the implanted eye were analyzed and compared with those from the control eye at each time-point. The mean implicit time and amplitude of P1 from the implanted eye were expressed relative to those of the control eye.

Histological analyses

After the final examination, the rabbits were euthanized. Then, the eyes were fixed in 4% paraformaldehyde and embedded in paraffin. Paraffin sections of 7-μm thickness were cut at the center of the visual streak, which corresponded to the site of the electrode, and stained with hematoxylin and eosin. The slides were examined by light microscopy, and the images were analyzed with Axio Vision 2.0 (Carl Zeiss Japan) software on a Windows computer.

Statistical analyses

One-way repeated measure analysis of variance (ANOVA) was used to calculate the significant differences in the threshold of EEPs by each stimulating pattern, means of relative amplitude and implicit time in ERGs, VEP, and EEPs and the resistance of the circuit between the implantation day and at 6 months after implantation. $P < 0.05$ was considered statistically significant. All statistical analyses were performed using JMP® Pro 14.0.0 (SAS Institute, Cary, NC, USA).

Results

Electrically evoked potentials (EEPs)

EEPs were successfully elicited by STS from all eyes over the course of the 6-month follow-up. Representative waveforms of the EEPs and the means ± standard deviations (SDs) of the threshold of the EEPs are shown in Fig. 2 and Table 1.

The mean ± SD of the threshold of CF500 or CF1000 from the implantation day to 6 months after implantation were 392.9 ± 71.8 μA or 186.4 ± 47.0 μA respectively. The average current density of CF500 or CF1000 were 17.8 μC/cm^2 or 8.4 μC/cm^2 from the implantation day to 6 months after implantation. No significant difference was found between time-points for the thresholds of CF500 and CF1000.

Resistance of the circuit

The resistance of the circuit was significantly increased from the implantation day to 1 months after implantation day [$F(6,32) = 6.4103$, $p < 0.001$], but there was no significant change of the resistance of the circuit between 2 and 6 months after implantation, i.e., the resistance of the circuit reached a plateau at 2 months after implantation. The means ± SDs of resistance of the circuit at the implantation day and at 1 and 6 months after implantation were 6.6 ± 0.29 (kΩ), 8.8 ± 0.47 (kΩ), and 10.0 ± 0.87 (kΩ),

Table 1 Summary of ERGs, VEPs and EEPs results

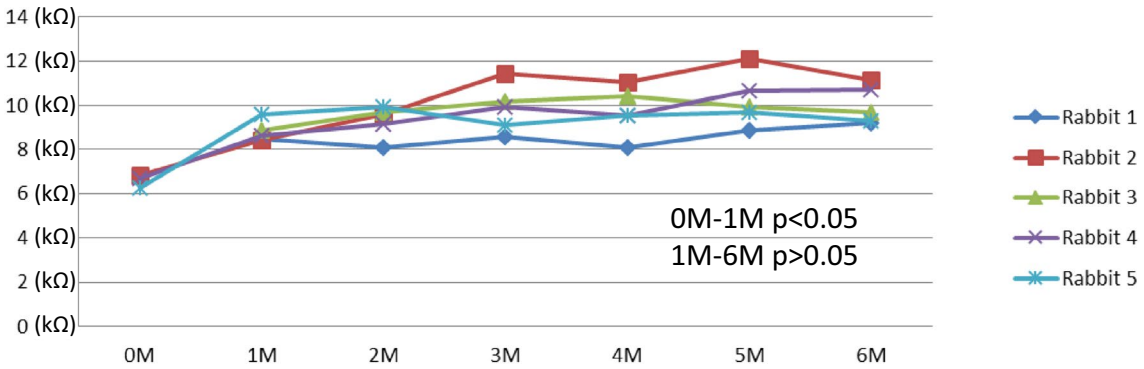
Time	ERGs				VEPs		EEPs	
	a-wave		*b*-wave		P1		Threshold	
	Amplitude	Implicit time	Amplitude	Implicit time	Amplitude	Implicit time	CF500	CF1000
	(experimental/control eye, mean ± standard deviation, %)						μA(μC/cm^2)	
Before or on day of implantation	80.8 ± 4.4	100.6 ± 3.9	96.0 ± 7.7	100.5 ± 3.0	109.7 ± 10.7	100.5 ± 4.2	460.0 ± 54.8	200.0 ± 70.7
1M	96.5 ± 8.8	101.9 ± 3.7	96.8 ± 2.7	96.0 ± 4.1	92.9 ± 23.5	95.3 ± 2.2	360.0 ± 114.0	200.0 ± 0.0
2M	119.1 ± 39.2	97.7 ± 5.5	111.1 ± 22.4	98.0 ± 2.3	87.7 ± 8.9	96.4 ± 0.8	450.0 ± 187.1	240.0 ± 89.4
3M	112.4 ± 18.7	99.5 ± 3.1	101.7 ± 7.5	98.0 ± 1.4	110.0 ± 15.0	96.3 ± 5.0	420.0 ± 83.7	150.0 ± 68.5
4M	108.9 ± 14.0	99.6 ± 4.3	102.7 ± 15.0	98.6 ± 4.3	103.7 ± 20.5	96.2 ± 2.8	360.0 ± 54.8	170.0 ± 97.5
5M	117.2 ± 7.3	97.7 ± 2.3	114.3 ± 11.0	98.4 ± 3.4	107.0 ± 22.3	93.4 ± 2.3	360.0 ± 54.8	175.0 ± 134.6
6M	119.3 ± 25.4	95.4 ± 4.9	107.1 ± 20.3	95.5 ± 3.7	100.2 ± 26.1	95.5 ± 6.0	340.0 ± 114.0	170.0 ± 97.5
P*	0.140	0.237	*0.325*	0.323	0.323	0.168	0.386	0.760

The relative amplitudes and implicit times of the *a*- and *b*-waves of the ERGs and of P1 of the VEPs did not change significantly and the mean threshold currents to evoke EEPs did not change significantly throughout the post-implantation period

respectively. The resistance of the circuit at 6 months after implantation was about 150% of the resistance of the circuit at the implantation day(Fig. 3).

Slit lamp examinations, fundus examinations, and FA

In two eyes, a subretinal hemorrhage was found by indirect ophthalmoscopy at the implantation day; at the end of the experiment, the subretinal hemorrhage was identified as



Time	Rabbit 1(kΩ)	Rabbit 2(kΩ)	Rabbit 3(kΩ)	Rabbit 4(kΩ)	Rabbit 5(kΩ)	Ave.	SD
0M	N.A.	6.82	N.A.	6.68	6.26	6.6	0.29
1M	8.5	8.42	8.84	8.64	9.6	8.8	0.47
2M	8.08	9.6	9.68	9.14	9.94	9.3	0.73
3M	8.56	11.44	10.16	9.92	9.12	9.8	1.1
4M	8.08	11.04	10.4	9.52	9.52	9.7	1.12
5M	8.88	12.08	9.92	10.64	9.68	10.2	1.21
6M	9.2	11.12	9.68	10.72	9.28	10	0.87

Fig. 3 Time course of resistance of the circuit. The resistance of the circuit was significantly increased from the implantation day to 1 months after implantation day, but there was no significant change of the resistance of circuit between 2 and 6 months after implantation *One-way-repeated measure ANOVA

chorioretinal atrophy and the location of the lesion was found to be beneath the suture of the cable (Fig. 4). Those regions did not spread or show any sign of inflammation on indirect ophthalmoscopy or FA.

In three eyes, signs of suture thread infection, including discharge and focal conjunctival injection, were detected around the return electrode; those sutures were exposed outside the conjunctiva. However, no vitreous opacity, cells, or hypopyon were detected in the anterior chamber. After the sutures were removed and antibiotic ointment and eye drop were applied, all eyes recovered soon. There was no other significant finding on slit lamp examination, fundus examination, and FA.

ERGs

Representative waveforms of the dark-adapted, full-field ERGs are shown in Fig. 4a. Mean relative amplitudes and implicit times of the *a*- and *b*-waves (implanted/control) prior to implantation and at every month after implantation are shown in Table. 1. There was no significant difference in the mean relative amplitudes and implicit times of the *a*- and *b*- waves over the experiment period. ($P=0.140$, $P=0.237$, $P=0.325$, and $P=0.323$, respectively).

VEPs

Representative waveforms of the VEPs are shown in Fig. 4b. Relative amplitudes and implicit times of the P1 (implanted/

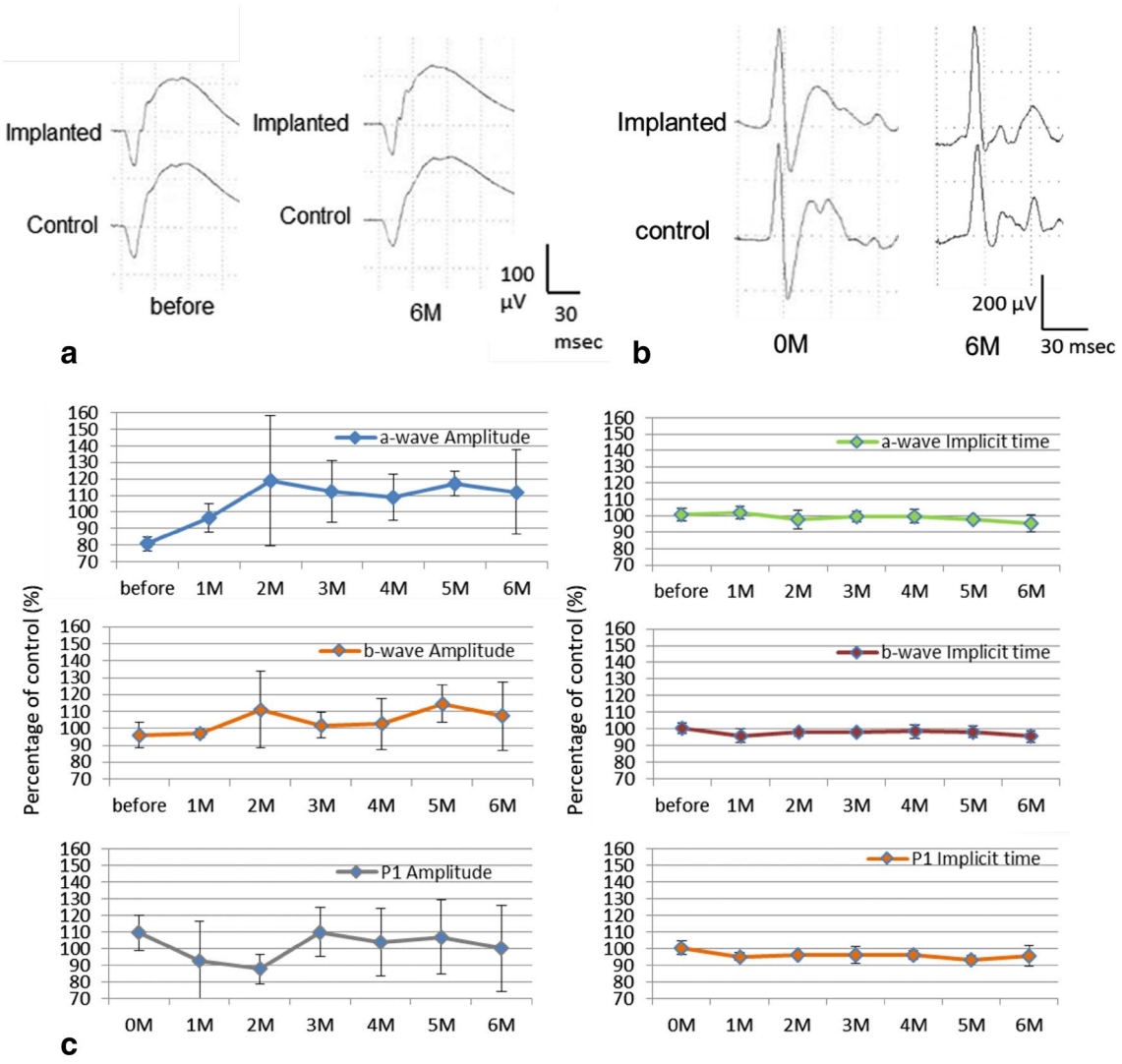


Fig. 4 Time course of EEPs and VEPs. Representative waveforms of the ERGs (**a**) and the VEPs (**b**). **c** Time course of parameters of the ERGs and VEPs. The mean amplitude and implicit times of *a*- and *b*-waves of ERGs and P1 of VEPs did not change significantly. The values are expressed as percentages of those in the control eye of the same rabbit. Values are given as means ± standard deviations

control) on the day of implantation and at every month after implantation are shown in Table 1. There was no significant difference in the mean relative amplitudes or implicit times of P1 ($P=0.386$ and $P=0.760$) (Table 1).

Histological examinations

There was no obvious histological change in the retinal tissue beneath the stimulating electrode (Fig. 5g–h).

Discussion

To our knowledge, this is the first report on the long-term electrophysiological efficacy, safety, and electrical stability of an STS electrode. Our data demonstrated the electrophysiological efficacy and safety of a chronically implanted STS electrode, which could elicit EEPs for 6 months without any significant increase in the threshold or ocular adverse effects.

Our data also demonstrated the electrical stability of a chronically implanted STS electrode. The resistance of the circuit was significantly increased at 1 months after implantation, when it reached a plateau at a value that was approximately 150% of that on the day of implantation. The stability of the circuit resistance is important, because an increase in the resistance is associated with the need for a higher electric power to apply the same current.

One research group [13] has previously shown increases of impedance of the circuit in a chronically implanted brain cortex-stimulating electrode.The increased impedance after implantation may be attributed to the fibrosis and gliosis around the stimulating electrode [14–18]. The difference in the increase in these parameters between our study and the previous one [13] may be attributed to the different locations of stimulating electrode implantation. When the stimulating electrode is directly attached to the neuron, gliosis may occur around the stimulating electrode [14, 15]. Other groups placed the electrode on the retina [3, 12], under the retina [4–6], in the optic nerve [9–11], on the visual cortex [1], or in the lateral geniculate nucleus [2]. In these cases, gliosis must be induced around the electrodes [14, 15] and the impedance of the circuit must be increased. However, there are few reports about the increase in the impedance [18].

In STS, even though the stimulating electrode does not directly attach to the retina, fibrosis may occur around the stimulating electrode and increase the resistance of the circuit. In the present study, the resistance of the circuit increased about 1.5 times from the day of implantation. The increase in the resistance of an STS electrode is acceptable, and the influence of fibrosis seems to be limited.

Subretinal hemorrhage (2/5) and local suture thread infection (3/5) were observed in our study. Subretinal hemorrhage changed to chorioretinal atrophy over time, and it was detected immediately after implantation in both cases

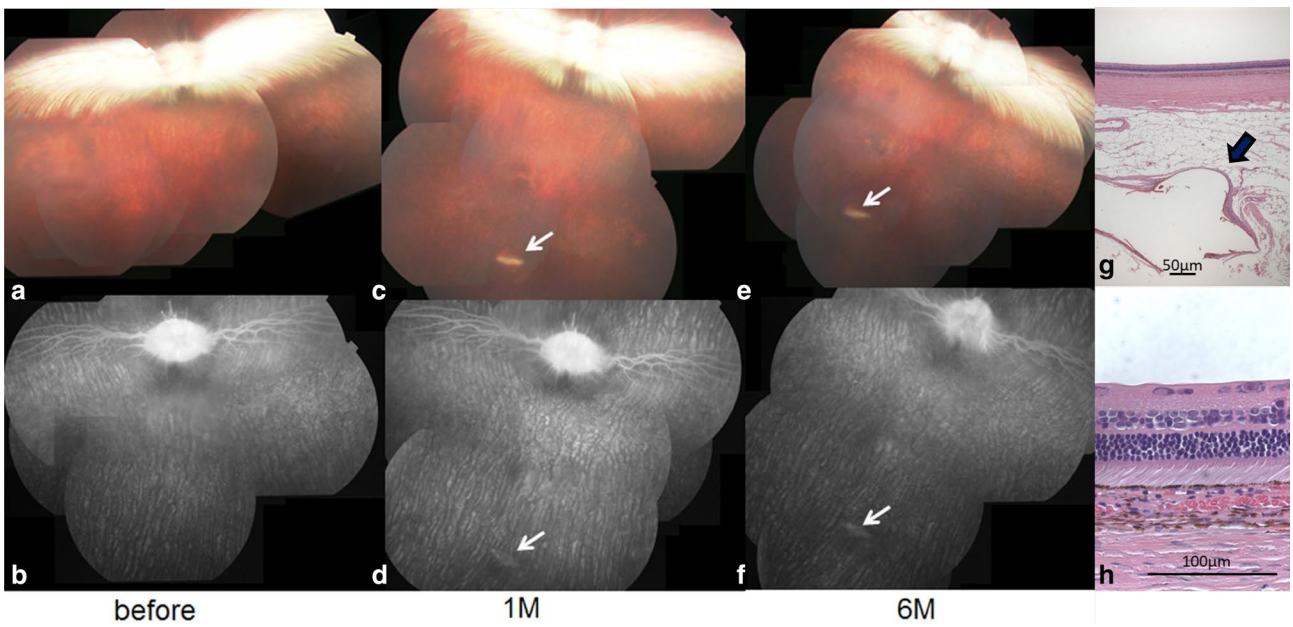


Fig. 5 Representative fundus photographs and FAs of implanted eye. Subretinal hemorrhage that corresponded to the cable was found by indirect ophthalmoscopy at the implantation day and at the same lesion chorioretinal atrophy was found (White arrow) after 1 month implantation (**c, d**). The lesion was not changed after 6 month implantation (**e, f**). Histology of the retina beneath the stimulating electrode (Arrow) There was no obvious histological change in the retinal tissue beneath the stimulating electrode (**g, h**)

and did not spread. The location of the lesion was beneath the suture of the cable, which may have been caused by the suture. This was not a consequence of STS; rather, it can be explained by the fact that the thickness of the rabbit sclera [19] is lesser than that of the human sclera [20].

Sutures were placed to fix the cable of the electrodes and were covered by the conjunctiva. Local suture thread infection was observed in three eyes and resolved soon after removal of the infected thread and treatment with an antibiotic eye drop and ointment for a few days. The infected thread was exposed outside the conjunctiva in each case, which possibly caused the infection [21]. In the present study, we used material (electrodes and cable) developed for humans, which was large for the rabbits' eyes. Therefore, it was difficult to cover the implants with the conjunctiva after implantation. Complete coverage of the implants was finally achieved; however, this generated conjunctival tension. After the implantation, the cable was exposed outside the conjunctiva because of the conjunctival tension, ultimately causing thread infection. However, thread infection is considered to be a type of complication specific to rabbits.

In our previous study [8] using the same type of stimulating and return electrodes in rabbits, the threshold values for the control eyes was 360.0 ± 114.0 μA with CF500, respectively which were consistent with this study. With CF1000, the threshold decreased to 186.4 ± 47.0 μA; thus, CF1000 could be used in patients who exhibit a high threshold with CF500.

Previous studies have investigated electrophysiological responses to surprachoroidal stimulation. One study [22] used rabbits to test the effects of continuous stimulation with a wireless activation system; however, the results reflected measurements of EEPs only 6 weeks after implantation and included only a single rabbit. Another study [18] performed long term electrophysiological and safety evaluations. However, information on the threshold of EEPs was only available for 3 months after implantation. Moreover, longitudinal follow-up data of the waves of EEPs within the same animal were not available. In this study, the authors used dogs, which are an expensive and laborious experimental model. In our study, we decided to use rabbits, since they are a less expensive and easier to handle. We have, thus, established the feasibility of using rabbits as a model that can be used for long-term physiological evaluation of other visual prostheses as well.

A limitation of this study is that the results cannot be readily extrapolated to humans. Nevertheless, this study characterized the long-term electrophysiological efficacy, safety, and electrical stability of STS, information that is difficult to evaluate during clinical trials in humans.

Compliance with ethical standards

Conflict of interest Ke. Nishida, None; H. Sakaguchi, None; M. Kamei, None; T. Saito, NIDEK Co., Ltd, Employment; T. Fujikado, None、Ko. Nishida, None.

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