Electrophysiological Studies of the Feasibility of Suprachoroidal-Transretinal Stimulation for Artificial Vision in Normal and RCS Rats

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PURPOSE. Assessment of a novel method of retinal stimulation, known as suprachoroidal-transretinal stimulation (STS), which was designed to minimize insult to the retina by implantation of stimulating electrodes for artificial vision.

METHODS. In 17 normal hooded rats and 12 Royal College of Surgeons (RCS) rats, a small area of the retina was focally stimulated with electric currents through an anode placed on the fenestrated sclera and a cathode inserted into the vitreous chamber. Evoked potentials (EPs) in response to STS were recorded from the surface of the superior colliculus (SC) with a silver-ball electrode, and their physiological properties and localization were studied.

RESULTS. In both normal and RCS rats, STS elicited triphasic EPs that were vastly diminished by changing polarity of stimulating electrodes and abolished by transecting the optic nerve. The threshold intensity (C) of the EP response to STS was approximately 7.2 ± 2.8 nC in normal and 12.9 ± 7.7 nC in RCS rats. The responses to minimal STS were localized in an area on the SC surface measuring 0.12 ± 0.07 mm² in normal rats and 0.24 ± 0.12 mm² in RCS rats. The responsive area corresponded retinotopically to the retinal region immediately beneath the anodic stimulating electrode.

CONCLUSIONS. STS is less invasive in the retina than stimulation through epiretinal or subretinal implants. STS can generate focal excitation in retinal ganglion cells in normal animals and in those with degenerated photoreceptors, which suggests that this method of retinal stimulation is suitable for artificial vision.

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Since it was first proposed by Tasssicker in 1956,1 a retinal prosthesis for artificial vision has remained conceptual. During the past decade, however, a retinal prosthesis has become much more realistic and has been supported by recent developments of implantable materials and microelectronics (see reviews2–5). Previous in vivo experiments have indicated that impaired parts of the retinal network can be bypassed by electrical stimulation to the retina by micro electrode arrays implanted in the eye.6–10 Results obtained in animal experiments suggest that retinal implantation of stimulating devices hold great promise for restoration of the vision of people with outer retinal degeneration, such as retinitis pigmentosa (RP) and age-related macular degeneration (AMD). Although there have been a few optimistic reports of retinal implants for human patients,11–13 there is room for improvement of electrode arrays for clinical application.

Two ways of stimulating the retina for artificial vision are currently well developed. One is subretinal stimulation (SRS), in which a sheet containing a microphotodiode array is inserted into the subretinal space to compensate for lost photoreceptor function and stimulate the outer retinal network.6,7,14 The other is epiretinal stimulation (ERS), in which retinal ganglion cells (RGCs) and their axons are stimulated with a multielectrode array attached to the vitreous side of the retina.6–12 Either type of retinal stimulation has its own advantages and disadvantages.3 For example, fixing the electrode array is easier with SRS than ERS. In contrast, SRS requires intact optics, whereas ERS does not, and SRS needs a great deal more electrical power than does ERS. Whereas SRS can use retinal circuitry, ERS requires the processing of visual information into specific patterns for the stimulation of RGCs. One common drawback of both types of implants is that implanted electrodes are directly attached to the retina, so that the risk of retinal damage at implantation is inevitable. Although some reports claim that there is no detectable damage to the retina after long-term implantation of microelectrode arrays,14–16 surgical difficulties remain if removal or replacement is necessary, both of which are possible. From a clinical viewpoint, it is therefore preferable to have the stimulating electrodes of implants placed away from the retina.

To meet this clinical need, we designed a novel type of transretinal stimulation, with electrodes unattached to the retina. We named it “suprachoroidal-transretinal stimulation” (STS), in which the anodic stimulating electrode is positioned on the choroidal membrane and the cathode is placed in the vitreous body. Because it has been demonstrated that various types of transretinal stimulation can induce field responses in central visual areas,17,18,19,20 STS can be expected to activate the retinal network. Our major concern regarding STS is that, because the suprachoroidally placed electrodes are not in contact with the retina, the following disadvantages for artificial vision may result. First, STS may be much less effective than ERS and SRS, resulting in a high threshold for stimulation of the retinal circuitry. The strong electrical stimulation of STS may damage the retina and increase the power load on implanted electronic devices. Second, STS may stimulate a much broader area of the retina than SRS and ERS do, resulting in such a low resolution that artificial vision is impossible.

To test the feasibility of STS for artificial vision, we addressed a critical question in the study reported herein—namely, whether local application of STS can evoke definite responses in the primary visual center that receives direct input from the retina. If this is the case, the localization and threshold of the evoked response are also important issues to
examine. To answer this question, we performed acute electrophysiological experiments using the rat retinocollicular system. The superior colliculus (SC) in rats is easily accessible for recording responses to retinal stimulation. Because of the precise retinotopic organization of the SC, localization of the collicular response could tell us the extent of the retinal area stimulated by STS. From a clinical perspective for further development of STS for human RP, we used Royal College of Surgeons (RCS) rats and normal hooded rats. RCS rats, with inherited retinal degeneration that seems to resemble human RP, are well established as one of the best animal models for retinal degeneration. With this retinal dystrophic rat we were able to investigate whether STS can stimulate the residual circuitry of the inner retina and can be used for artificial vision in human RP.

METHODS

Animals
As experimental animals, male hooded rats (Long-Evans; SLJ, Hamamatsu, Japan; n = 17) from 8 to 14 weeks old and RCS rats (inbred at the Department of Ophthalmology, Osaka University; n = 12) from 25 to 30 weeks old were used. All animals were housed under a 12-hour light–dark cycle. All procedures conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Every effort was made to minimize animal discomfort and to use only the number of animals necessary to produce reliable scientific data.

Surgical Preparation
The animals were anesthetized initially with urethane (1.75 g/kg, intraperitoneally). The same anesthetic was administered (0.5 g/kg, intraperitoneally) every 3 hours to keep them anesthetized during the surgical preparations and electrophysiological experiments. A heating pad was used to maintain body temperature at approximately 37°C. Heart rate and electrocardiogram (ECG) were monitored throughout the experiments. The pupils were dilated with a mydriatic (Midorin P; Santen Pharmaceutical Co., Ltd., Osaka, Japan), and the corneas were covered with contact lenses.

After tracheal cannulation, the heads of the animals were fixed stereotaxically. Cerebrospinal fluid was drained through a small incision made into the dura on the occipital bone. Cranioptomy was performed on the right side of the temporal bone, and the underlying occipital cortex was removed by gentle aspiration to expose the dorsal surface of the SC. The surface of the SC was filled with mineral oil during the experiments to prevent drying and leakage of current from the recording electrodes. For stimulation of the retina or the optic nerve (ON), the sciera and the ON of the left eye were exposed intraorbitally. After incision of the surrounding tissue and the dura mater, the ON was exposed very carefully to avoid retinal ischemia.

Photic and Electrical Stimulation
The light source, a photic and sonic stimulator (Nihon Kohden, Tokyo, Japan), was positioned 5 cm in front of the left eye. Stimuli consisting of single flashes were given at intervals of 3 to 5 seconds, and electroretinogram (ERG) or collicular EP was recorded.

For electrical stimulation, a silver-ball–stimulating electrode (suprachoroidal electrode or S-electrode, diameter: 0.2–0.3 mm) was inserted into a small lamellar scleral resection (diameter: approximately 0.5 mm) made at a distance of 1.5 to 2.5 mm from the ON in the upper temporal part of the sclera. The stimulating electrode, electrically isolated from the surrounding tissue with mineral oil, was used as an anode. A cathodic electrode of epoxy-coated stainless wire (vitreous body electrode or V-electrode, diameter: 0.2 mm) with approximately 2 mm of the tip exposed was inserted approximately 4 mm into the vitreous. A single monophasic pulse of electrical current was applied between these two electrodes through an isolator (SS-202J; Nihon Kohden) for 0.05, 0.2, or 0.5 ms, depending on the level of the EP threshold. The range of current amplitude was 5 to 300 μA. Inward stimulation between the anodic S-electrode and the cathodic V-electrode was used as the standard, and outward stimulation was tested when necessary. The ON was also stimulated with a pair of silver-ball electrodes placed in the intraorbital section, approximately 3 mm behind the globe.

Electrophysiological Recordings
A silver-ball recording electrode (Ag/AgCl, 0.2–0.3 mm in diameter) was moved systematically by means of a hydraulic three-dimensional micromanipulator (MMW; Narishige Science Institute Laboratory, Tokyo, Japan) and placed on the exposed SC surface. A stainless-steel screw was implanted into the occipital bone approximately 1 mm behind the lambda and used as a reference electrode. Responses from the SC were amplified 10,000 times with a band-pass of 15 Hz to 3 kHz. Approximately 50 evoked responses were averaged with a signal processor (LEG-1000; Nihon Kohden).

A flash ERG was also recorded from the left eye using a contact lens electrode (Kyoto Contact Lens, Kyoto, Japan) after a 20-minute scotopic adaptation. ERG responses were amplified 10,000 times with a band-pass of 1 Hz to 3 kHz.

Histologic Analysis
After electrophysiological recordings were obtained from the right SC, a small incision was made with a fine needle at the site where the largest EPs were recorded, as a reference point for reconstruction of the recording sites on the SC. A suture was made at the dorsal pole of the stimulated eye as a marker for retinal orientation. The animals were then deeply anesthetized with a lethal dose of pentobarbionate sodium and perfused intracardially with 0.1 M phosphate-buffered saline (PBS) followed by a fixative (4% paraformaldehyde in PBS). The fixed eye and midbrain were resected for histologic examination under a binocular microscope.

The distance was measured between the optic disc and the position of the S-electrode, which was easily identified by the resection scar on the sclera, and no adjustment was made for shrinkage after fixation. Micrographs of the posterior view of the eye with the optic nerve fixed in the sclera, and the suture at the dorsal pole were taken with a digital camera attached to the microscope. Micrographs were also taken of the dorsal view of the midbrain for reconstruction of the recording sites. Because of shrinkage of the midbrain after fixation, the digital images were sufficiently enlarged that reference incisions for recording sites could be adjusted with their stereotaxic coordinates. The outlines of the SC and the recording sites were then extracted from these adjusted images.

After identification of the position of the stimulating electrode, the eyes were hemisected to make eye cups, which were postfixed and then cryoprotected with 25% sucrose in PBS. Vertical cryosections of the retinal cups were made by means of a freezing microtome (CM1900; Leica, Solms, Germany). These sections were then mounted on gelatinized glass slides and stained with hematoxylin and eosin. Finally, the stained specimens were dehydrated, cleared, and coverslipped for microscopic analysis of the cytoarchitecture of the retinas.

RESULTS

Field Responses to STS in Normal Hooded Rats
We examined first whether focal STS evokes collicular responses. With a brief pulse of a constant current (0.5 ms, 100 μA) applied transretinally, evoked potentials (EPs) were consistently obtained from the contralateral SC. Because the amplitudes of the responses depended on the recording site within the SC and the intensity of the current, we first identified the center of the responsive area (CRA) where the largest EPs were recorded for a given intensity of STS. Next, the
stimulus intensity dependence of the EPs at CRA was examined.

Figure 1A shows a typical series of EPs at the CRA for various intensities of stimulation in a normal hooded rat. With suprathreshold level of STS, the EPs were composed of a sharp positive deflection (P1) followed by a large negative wave (N1) and a small, long-lasting positive wave (P2). The N1 wave was occasionally accompanied by another small negative deflection (N2; see Figs. 2E, 2F). The ranges of the peak latencies of the P1, N1, and P2 components were 6.7 ± 1.1 ms (mean ± SD; n = 14), 15.3 ± 4.3 ms (n = 14), and 34.6 ± 6.5 ms (n = 10), respectively. The amplitude of these three components was reduced by tetanus stimulation (at a frequency of approximately 50 Hz), indicating that these responses were postsynaptic (data not shown). At a medium intensity of stimulation (20–80 μA, 0.5 ms: 10–40 nC), a small positive wave (P0) was

**Figure 1.** EPs recorded from the SC to inward STS (A, B), outward STS (C) and ON stimulation (D) in a hooded rat. Stimulus intensity (in microamps) is indicated at the left of each trace. (A) The EPs consisted of a fast positive wave and a slow negative potential (N1) with a P0 (△) that was identified in the second to the fourth trace. The threshold currents for the activation of P1 were 10 to 20 μA. (B) The EPs to STS disappeared after the ON was severed. (C) In outward STS, the amplitude of EPs decreased. (D) The EPs to the ON also consisted of P0, P1, and P2. *Arrow*: onset of stimulus. Calibration: (A, B, C) 50 μV, 10 ms; (D) 100 μV, 10 ms.

**Figure 2.** Comparison of retinal cytoarchitecture and collicular EPs of hooded and RCS rats. Photomicrographs of hematoxylin-eosin-stained retinal sections of hooded (A) and RCS rats (B) show complete loss of the outer retinal layers in the RCS rat. Flashing-light stimuli generated EPs from the SC of hooded (C) but not RCS (D) rats. The inward STS generated EPs from both hooded (E) and RCS (F) rats. OS, outer segment; ONL, outer nuclear layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer. Stimulus intensities were 10, 30, 50, 80, 100, 150, 200, and 300 μA in (E) and 30, 50, 80, 100, 150, 200, and 300 μA in (F). *Arrow*: onset of stimulus. Calibration: (C, D) 100 μV, 50 ms; (E, F) 100 μV; 10 ms. Scale bar: (A, B) 100 μm.
often recorded before the P1 wave at a peak latency of 4 to 5 ms, with a peak amplitude of 2 to 10 μV (Fig. 1A; small open triangle). At higher intensities of stimulation, P0 was engulfed by P1 and remained as a notch in the rising phase of the P1 wave. The amplitude did not change with high-frequency stimulations (up to 100 Hz), indicating that the wave reflects the response of the retinal axon terminals.24 At lower stimulus intensities, the EPs became smaller, and only P0 and P1 remained at 20 μA (10 nC), whereas no response was observed below 10 μA (5 nC). The mean (±SD) threshold of STS for evoking EPs was 7.2 ± 2.8 nC (n = 6). The EPs were completely abolished after transection of the ON just behind the eyeball (Fig. 1B). Thus, it is unlikely that retinal stimulation directly stimulated the ON and/or the SC.

The threshold of the EPs also depended on the polarity of stimulation. When the stimulating current passed from the V-electrode to the S-electrode (outward stimulation through the retinas), the threshold of the EP dramatically increased from 10 to 60 μA, and, even at 100 μA, only a small P1 was observed (Fig. 1C). In all six cases we examined, switching to outward STS reduced the P1 to N1 amplitude to an average of one tenth. Moreover, this change in stimulus polarity slightly prolonged the peak latency of EPs in two thirds of the examined cases. Figure 1C shows the extension of the P1 latency from 7.0 to 9.5 ms.

**Field Responses to ON Stimulation in Normal Hooded Rats**

To determine whether STS directly activates the ON, we electrically stimulated the ON to record EPs from the CRA of the SC, and compared these EPs with those in response to the retinal stimulation. The EPs for the supramaximal ON stimulation consisted of two positive deflections (P0, P1) followed by a negative deflection (N1), as shown in Figure 1D. The first positive deflection (P0) and the other two components (P1, N1) were identified as presumably ON fiber terminals-derived and postsynaptic, respectively. Because P1 and N1 were completely eliminated by tetanic stimulation, but P0 was unaffected. These three components corresponded well to T1, C1, and C2, as designated by Sefton in 1969.25 The mean peak latencies of these components (P0, P1, and N1) were 2.5 ± 0.1, 4.3 ± 0.4, and 9.5 ± 0.5 ms, respectively, thus consistently shorter than those of the EPs elicited by the retinal stimulation (P1: P = 0.029, N1: P = 0.035, Student’s paired t-test). The statistically significant differences in peak latency of EPs between STS and the ON stimulation rule out the possibility that STS stimulated the ON directly.

**Field Responses to STS in RCS Rats**

RCS rats have been used as one of the most effective animal models for human retinal dystrophy, especially RP.25 To evaluate the suitability of an STS-based artificial retina for clinical application in patients with RP, we used RCS rats to investigate whether STS can bypass their degenerated outer retina and directly activate the residual retinal circuitry.

Figure 2 shows typical results for RCS rats. Histologic examination of RCS rats confirmed severe loss of the outer part of the retinas. The photoreceptor layer completely disappeared in an RCS rat (Fig. 2D) but not in a normal hooded rat (Fig. 2A). Although the inner nuclear layer of the RCS rat was slightly thinner than that of the normal hooded rat, cytoarchitecture of the inner half of the RCS retina seemed to be normal. Some cells with large somata, suggestive of RGCs, were identified in the ganglion cell layer. Moreover, immunostaining for PKC, known as a reliable marker of rod bipolar cells,26 resulted in marked preservation of the bipolar cells in the inner nuclear layer of the RCS rats (data not shown).

As could be expected from these results, neither ERG (data not shown) nor collicular EP (Fig. 2D) was recorded in response to the flashing-light stimulus in the RCS rat, whereas the same photic stimulus generated conspicuous EPs in the SC of the normal hooded rat (Fig. 2C). The STS (≥20 μA for a 0.5-ms pulse) evoked clear field responses on the SC of the RCS rats (Fig. 2F). The EPs consisted of a fast positive wave (P1) followed by a slow negative wave (N1). The peak latencies of the P1 and the N1 were 7.1 ± 1.5 and 13.0 ± 2.8 ms, respectively (mean ± SD, n = 11). When electric current was increased up to 100 μA, the two peaks became very clear. The N1 was occasionally followed by a negative peak (N2) as seen in the case of the high-intensity stimulus. The mean ± SD of the N2 peak latency in normal hooded rats was 24.6 ± 4.8 ms (n = 7) and 25.6 ± 5.0 ms (n = 7) in RCS rats. The EPs to STS in the RCS rats were identical with those in the normal rats (Fig. 2E) in terms of shape an peak latencies of P0, P1 and N1, as well as threshold. The average threshold intensity of the EP in response to STS was approximately 12.9 ± 7.7 nC (n = 7) in RCS rats. It was slightly higher than that in normal hooded rats, but the difference was not statistically significant. The effect of changing the polarity of stimulating electrodes was confirmed in RCS rats. The amplitude of EPs decreased up to 8% by outward STS.

**Topological Correspondence of Field Responses to the Retinal Stimulation Sites**

Because it is well established that retinocollicular projections are topographically ordered,21 the collicular positions of the CRA should correspond to the retinal regions in which RGC discharges were elicited by STS. To know whether RGCs below anodic S-electrodes were excited by STS, we examined topological relationship between positions of CRA in the SC and those of S-electrodes. Figure 3 illustrates the results obtained from five normal and five dystrophic rats. In Figures 3A and 3C, individually numbered dots indicate the position of the S-electrode at the dorsoventral retina at a distance of 1.7 to 2.8 mm from the optic disc. The corresponding CRAs of these animals are plotted as numbered black dots in the outline of...
their SCs as based on photographs of the midbrain after fixation (see the Methods section). In Figures 3B and 3D the drawings of these outlines have been placed on top of one other along the midline and posterior edge of the SC. The CRAs were consistently confined to the lateral part of the central SC, which is known to receive inputs exclusively from the upper retina (see Fig. 3 in Siminoff et al.21). Thus, there seems to be a topological relationship between the positions of the CRA and the S-electrodes, but not the V-electrodes.

Spatial Extent of Field Responses to the STS

To define the extent of the area that responded electrophysiologically to STS, the EPs were recorded at sites separated by 100 to 200 μm along the rostrocaudal and mediolateral axis of the SC. Because the extent of the responsive area varied with the intensity of STS, it was placed just above the threshold of the EPs at CRA. Figure 4 shows the smallest, intermediate, and largest areas responding to the minimum STS in normal (Fig. 4A) and dystrophic (Fig. 4B) rats. Filled circles indicate recording sites where an EP was obtained and are surrounded by unresponsive sites (horizontal bars). The smallest responsive area was less than a square with sides of 100 μm (Figs. 2, 4A), and the mean ± SE of the area was 0.12 ± 0.08 mm² (n = 5) in the normal rats. In dystrophic rats, the former was less than a square with sides of 200 μm (Figs. 3, 4B), and the latter area was 0.24 ± 0.12 mm² (n = 5). Thus, the method of retinal stimulation used in this study can excite a very small area of the SC, indicating that the retina is focally stimulated.

Resolution of EP to STS at Two Separate Sites

Last, we investigated whether STS of two adjoining areas of the retina can evoke two discrete field responses in the SC of a normal hooded rat. A pair of S-electrodes, S1 and S2, were placed on the eyeball at a distance of approximately 0.7 mm from each other, and these two points were stimulated with an interval of 400 ms. The CRA to STS with the S1 electrode (STS1) was different from that with the S2 electrode (STS2). On the line between these two CRAs, EPs to STS1 and STS2 recorded at intervals of 0.1 mm showed a stimulus intensity of 52 nC (260 μA at a duration of 0.2 ms). Figure 5 illustrates the relative amplitudes of the EPs at each recording site, demonstrating that the response profile to STS1 was spatially differentiated by 0.5 to 0.6 mm from that to STS2. This means that spatial resolution of STS for artificial vision would be 0.7 mm at most.

DISCUSSION

Several studies have demonstrated that ERS or SRS with microelectrode arrays elicits responses from visual centers.6–10 In the present study, we developed a novel means of retinal stimulation for artificial vision (i.e., STS) and demonstrated that focal STS successfully produced localized excitation of the rat SC. This STS was applied through the anode on the fenestrated sclera and the cathode placed in the vitreous body, thus avoiding direct contact of stimulating devices with the retina. Localized EPs were recorded from a small and defined area of the contralateral SC, which corresponds retinotopically to the position of the anode. As with ERS and SRS it can thus be expected that spatially patterned STS through anodes arranged in an orderly manner on the sclera can provide visual centers with essential features of images on the retina.

Suitability of STS for Artificial Vision

By comparison with ERS and SRS, we assessed the suitability of STS for an artificial retina in terms of stimulus efficacy and spatial resolution. High efficacy of stimulation is very important in artificial vision because it results in localizing retinal excitation, preventing retinal damage from electricity, and reducing energy consumption. Efficacy of STS can be assessed by means of the threshold of the stimulation that elicits certain responses in visual centers. The threshold of the collicular response to STS in our study was as low as 5 to 8 nC. Although the threshold of STS is not as low as that of ERS or SRS (for example, 1–36 nC for SRS,6 and 14 nC8 or 0.1–0.3 nC9, or 0.5–6.0 nC10 for ERS), it is still low enough for the design of an STS-based artificial retina. In fact, the threshold of STS was quite low when it is taken into account that neither of the stimulating electrodes was in contact with the retina. Probably because electrical resistance of the sclera is much higher than that of the retina and the vitreous, scleral fenestration under the anode may play a role in a highly conductive path, thus contributing to the reduction in the threshold of STS. In fact, we often found during preparation of the animals that the threshold of EPs to STS decreased by up to one half after the fenestration.

Another important question is how a localized area can be stimulated by STS, in that this is crucial for spatial resolution in artificial vision. With minimum stimulus intensity, the area of the threshold of the stimulation that elicits certain responses in visual centers. The threshold of the collicular response to STS in our study was as low as 5 to 8 nC. Although the threshold of STS is not as low as that of ERS or SRS (for example, 1–36 nC for SRS,6 and 14 nC8 or 0.1–0.3 nC9, or 0.5–6.0 nC10 for ERS), it is still low enough for the design of an STS-based artificial retina. In fact, the threshold of STS was quite low when it is taken into account that neither of the stimulating electrodes was in contact with the retina. Probably because electrical resistance of the sclera is much higher than that of the retina and the vitreous, scleral fenestration under the anode may play a role in a highly conductive path, thus contributing to the reduction in the threshold of STS. In fact, we often found during preparation of the animals that the threshold of EPs to STS decreased by up to one half after the fenestration.

Another important question is how a localized area can be stimulated by STS, in that this is crucial for spatial resolution in artificial vision. With minimum stimulus intensity, the area of the stimulated retina can be calculated as 1.1 mm², assuming that the whole retinal surface area is 57 mm² in rats.27 and without
STS is potentially suitable for retinal prostheses and artificial retinas. This conclusion is ultimately supported by the results obtained in RCS rats, a well-established model of human RP. To the best of our knowledge, this is the first study using RCS rats to demonstrate that localized electrical stimulation to the retina can bypass the degenerated outer retinal circuitry and provide excitation of the visual center, which receives inputs from RGCs.

The EPs to focal STS in the RCS rats were highly similar to those in the normal hooded rats in terms of waveform, peak latencies of the components, and threshold. This similarity suggests that the essential electrophysiological function of the inner retinal circuitry may be maintained even after the degeneration of photoreceptors. Histologic analysis proved that the organization of the inner nuclear layer, the inner plexiform layer, and the ganglion cell layer was normal, which agrees with previous findings that indicate that no transneuronal changes occur after the loss of photoreceptor cells. On the contrary, there are reports of some abnormalities in horizontal, Müller, and bipolar cells. Furthermore, it has been found in aged animals that secondary degeneration of RGCs occurs probably due to contraction of intraretinal vessels. Preservation of STS for artificial vision must therefore also be tested in aged RCS rats.

Preservation of normal retinofugal projection after the loss of photoreceptors is one of the prerequisites for an artificial retina. No information is available about the topographic organization of retinofugal projection in case of complete dysfunc-
tion of phototransduction, other than an anterograde tracing study that demonstrated that the projection patterns in RCS rats appeared similar to those in their congenic controls, although terminal density was somewhat reduced in dystrophic rats. In the present study, EP-recording sites roughly showed topological correspondence to the retinal points stimulated at the anode in the RCS rats as well as in the normal rats. No significant difference was seen between the two strains in the extent of the retinal area activated by STS. These results do not necessarily imply, however, that the topological order of retinofugal projection survives in RCS rats after loss of photoreceptors. Further examination of this point is clearly needed.

**Transretinally Stimulated Cells**

What types of retinal neurons were stimulated by STS? Photoreceptor cells can be excluded because of our finding that STS evoked collicular responses, not only in normal hooded rats but also in RCS rats with degenerated photoreceptors. Exclusion of photoreceptors as a target of transretinal stimulation was also confirmed by Poits and Inoue, who reported that a change in potential was recorded from the cortex of rats with hereditary photoreceptor degeneration on electrical stimulation applied to the globe. Other lines of evidence about electrically evoked potentials (EEPs) in response to various types of transretinal stimulation also indicate that photoreceptors are not involved in generation of the EEPs. Therefore, STS must stimulate the inner retinal cells, such as bipolar cells, amacrines, cells, and RGCs, to elicit collicular EPs.

According to electrophysiological principles, the inward transretinal current of STS has a depolarizing effect on the vitreous and a hyperpolarizing effect on the scleral side of every radially oriented retinal cell. Among the inner retinal cells, involvement of bipolar cells and their related synaptic sites in transretinal stimulation was indicated in a few reports using the intact retina. Although the transretinal stimulation in these studies is different from STS, it is likely that STS could also stimulate bipolar cells and their related synaptic sites. The possibility of the RGC as a target for stimulation was excluded by the authors in those studies. For example, Stett et al. demonstrated in vitro that ganglion cell discharge in response to transretinal stimulation in the chick retina was reduced or eliminated after the application of agents for blocking synaptic transmission. However, when nine neighboring points were stimulated simultaneously (so-called box stimulation), the fast spike and delayed response remained, even after application of a high concentration of Mg2+ which suggests that direct stimulation of RGCs may occur when the stimulated area extends within a square with sides of 200 μm. Because this size is comparable to that of the fenestrated area used in our study, it is possible that STS stimulates RGCs directly. Moreover, using isolated frog retinas, Li et al. have provided evidence of direct excitation of RGCs by transretinal stimulation (Li et al. *IOVS* 2002;43:ARVO E-Abstract

**FIGURE 5.** Spatial separation of amplitude profiles of EPs in response to inward STS between two different anodic electrodes, S1 (○) and S2 (●). Relative amplitudes of EPs are plotted on the ordinate and positions of the recording electrode on the abscissa. Distance between the two peaks on the surface of SC was 0.5 to 0.6 mm. *Arrows:* peak response sites to S1 and S2.
References