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Can subretinal microphotodiodes successfully replace degenerated photoreceptors?

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Abstract

The idea of implanting microphotodiode arrays as visual prostheses has aroused controversy on its feasibility from the moment it appeared in print. We now present results which basically support the concept of replacing damaged photoreceptors with subretinally implanted stimulation devices. Network activity in degenerated rat retinae could be modulated through local electrical stimulation in vitro. We also investigated the long term stability and biocompatibility of the subretinal implants and their impact on retinal physiology in rats. Ganzfeld electroretinograms and histology showed no significant side effect of subretinal implants on retinal function or the architecture of the inner retina. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Visual prosthesis; Subretinal implant; Subretinal microphotodiodes; Photoreceptor replacement; Retinal degeneration

1. Introduction

The feasibility of visual prostheses for the blind has been discussed for many decades. Early concepts of retinal and cortical implants (Tassiker, 1956; Brindley & Lewin, 1968; Dobelle, Mladejovsky & Girvin, 1974; Dawson & Radtke, 1977; Michelson, 1986), some of which were even tested in human volunteers, proved unsuccessful due to technical and microsurgical limitations at that time. In the meantime however, amazing advances in microelectronics have made it possible to partly replace the inner ear with a quite useful technical prosthesis: the cochlear implant (Loeb, 1989). This leads naturally to the question whether corresponding prostheses which can stimulate retinal neurons electrically and perhaps even restore sight might be feasible with today's technology.

New attempts to develop retinal prostheses have taken different approaches: Some research groups try to stimulate the ganglion cells and their axons with *epiretinal* stimulators which receive signals generated by an external camera and an external or implanted data processing system (Narayanan, Rizzo, Edell & Wyatt, 1994; Humayun, de Juan, Dagnelie, Greenberg, Propst & Phillips, 1996; Wyatt & Rizzo, 1996; Eckmiller, 1997; Rizzo & Wyatt, 1997). Others have undertaken to replace lost photoreceptor function with implants for *subretinal* stimulation of the retinal network (Chow, 1993; Chow & Chow, 1997; Zrenner, Miliczek, Gabel, Graf, Guenther & Haemmerle, 1997; Chow, Chow, Pardue, Perlman & Peachey, 1998; Peyman, Chow, Liang, Chow, Perlman & Peachey, 1998). Recently, a new study has lent credibility to the feasibility of a visual prosthesis for intracortical microstimulation (Schmidt, Bak, Hambrecht, Kufta, O'Rourke & Vallabhanath, 1996), and new types of electrodes which are suitable for cortical stimulation have been developed (Normann, Maynard, Guillory & Warren, 1996).

Common to all these approaches is a functional, electrical multisite stimulation of specific neurons and neuronal networks. Neuronal activity which is useful for vision must be evoked under conditions of safe, chronic charge injection. This requires both knowledge

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of appropriate stimulation parameters and their effects on neuronal integrity and a familiarity with minimally invasive implantation techniques and biocompatible materials which are suitable for long-term implantation in the eye.

Our group has developed a silicon chip with an embedded microphotodiode array (MPDA) similar to that which has been described in several patents by Chow and associates. When implanted under the retina, it stimulates adjacent retinal neurons through multisite injection of photocurrents generated by locally absorbed light (Fig. 1A).

Histological examination of human donor eyes has indicated that *retinitis pigmentosa* retinae still possess a neuronal network with relatively intact morphology (Santos, Humayun, de Juan, Greenberg, Marsh, Klock et al., 1997; Zrenner et al., 1997). We therefore investigated whether the remaining cells of this retinal network can be electrically stimulated in such a way that useful information will be transmitted to the visual centers of the brain. Although the answer to this question is crucial for the success of any retinal prosthesis, it clearly cannot be answered by simply implanting a prototype into the eye of a healthy animal. We therefore chose to test the usefulness of MPDA prototypes for electrical retinal stimulation in vitro by using degenerated retinae from Royal College of Surgeons (RCS) rats. The RCS rat is a well-established animal model for the study of human retinal degeneration (Sheedlo, Gaur, Li, Seaton & Turner, 1991a; Kohler, Guenther & Zrenner, 1997a).

This paper describes the present status of the authors' work on the crucial issue of subretinal prosthesis function and its effect on retinal tissue. While the report focuses primarily on in vitro stimulation experiments with RCS rat retinae, it also presents the results of our in vitro biocompatibility tests with cell cultures from retinae of Brown Norway rats. The long-term tolerance of implanted chip prototypes was evaluated with Ganzfeld electroretinograms and histology.

Some of the results described here have been presented elsewhere in abstract form (Kohler, Hartmann, Fischer & Zrenner, 1997b; Stett, Weiss, Gnauck, Stelzle, Nisch, Haemmerle et al., 1997; Troeger, Guenther, Schlosshauer, Hoff & Zrenner, 1997; Miliczek, Scholz, Aramant, Seiler, Tornow, Kohler et al., 1998; Stett, Kohler, Weiss, Haemmerle & Zrenner, 1998; Weiss, Herrmann, Kohler, Stett & Haemmerle, 1998).

2. Methods

2.1. Chip technology

Implantable silicon chip prototypes with embedded MPDAs (Fig. 1B) were fabricated with standard semiconductor technology as described previously (Zrenner et al., 1997; see also the similar technique described by Peyman et al., 1998). The MPDAs were then supplied with metallic stimulation electrodes and cut into small rectangular chips with an area of 0.48–0.8 mm^2 and a thickness of about 50 μm. Details of the chip fabrication technique will be published elsewhere.

2.2. Functional electrical multisite stimulation in vitro

Retinae were dissected free from the eye cups of RCS rats and cut into 5 × 5 mm segments. Each segment was then attached either on the ganglion cell side (Fig. 2A, B) or the photoreceptor side (Fig. 2C) to a microelectrode array (MEA). The MEA consisted of a glass plate with 60 substrate integrated planar metallic electrodes of gold [Au] or titanium nitride [TiN]; these were 10 μm

Fig. 1. (*Opposite*, *left*) The subretinal approach of a retinal prosthesis. (A) A silicon chip containing an array of individual microphotodiodes (MPDA, microphotodiode array) is implanted in the subretinal space in an area with degenerated rods and cones. Incident light (arrows from the left) is absorbed in the photodiodes and generates current in direct proportion to the local intensity of the light. Via the metallic stimulation sites the current is injected into the retinal tissue (small bundles of arrows from the right). Therefore at the distal side of the retina a spatiotemporal charge injection pattern is generated by multisite light-to-current-conversion. (B) Light-microscopic view of a small area of the chip. The light sensitive area of a single microphotodiode is 625 μm^2. Each diode has its own stimulation electrode on its center (gold, square size: 8 × 8 μm).

Fig. 2. (*Opposite*, *right*) Functional electrical retina stimulation in vitro. (A) Sandwich preparation technique: pieces of whole mount retinae are attached to a microelectrode array (MEA) with the ganglion cell side facing the transparent glass plate and its embedded planar electrodes (*). The MPDA prototype chips are then placed onto the retina and illuminated with flashes of light (arrow from bottom). Multi-unit ganglion cell activity (spikes) evoked by the light generated photodiode current (arrows from top) is recorded with several MEA electrodes in parallel. (B) Monofocal distal current injection: a tungsten electrode is lowered into the distal side of the retina. Monopolar charge balanced current pulses of different duration and strength are applied. (C) Multisite charge injection: with the ganglion cell side up, multifocal stimulation of the distal retina side is obtained by applying voltage pulses to a variable number of electrodes of the MEA. The retinal response is recorded from ganglion cell bodies with a glass pipette. (D) Histological cross section of a retina piece of a 194-day-old RCS rat that had previously been put on a MEA and electrically stimulated for about 6 h. At that age the entire photoreceptor layer has disappeared from the RCS rat retina and only a debris layer (*) with some scattered cell bodies can be found proximal to the inner retina. In contrast, the architecture of the inner retina is well preserved and all layers, i.e. INL, IPL, GCL, are present. (E) Spontaneous activity of a ganglion cell in a 185-day-old RCS rat retina (top: rasterplot of 20 subsequent recordings; center: spike histogram of 50 recordings, bin width 10 ms; bottom: interspike frequencies [ISF]).

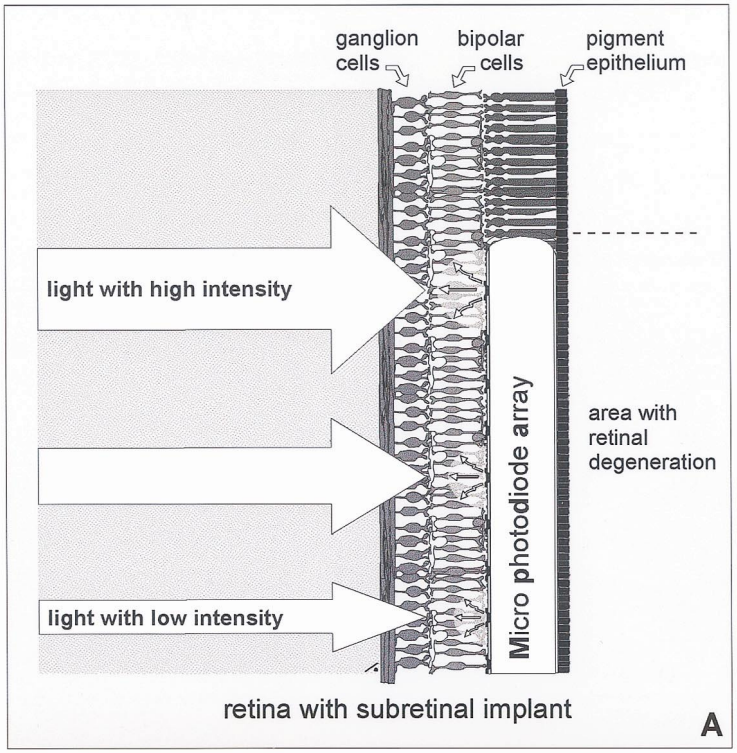


Fig. 1

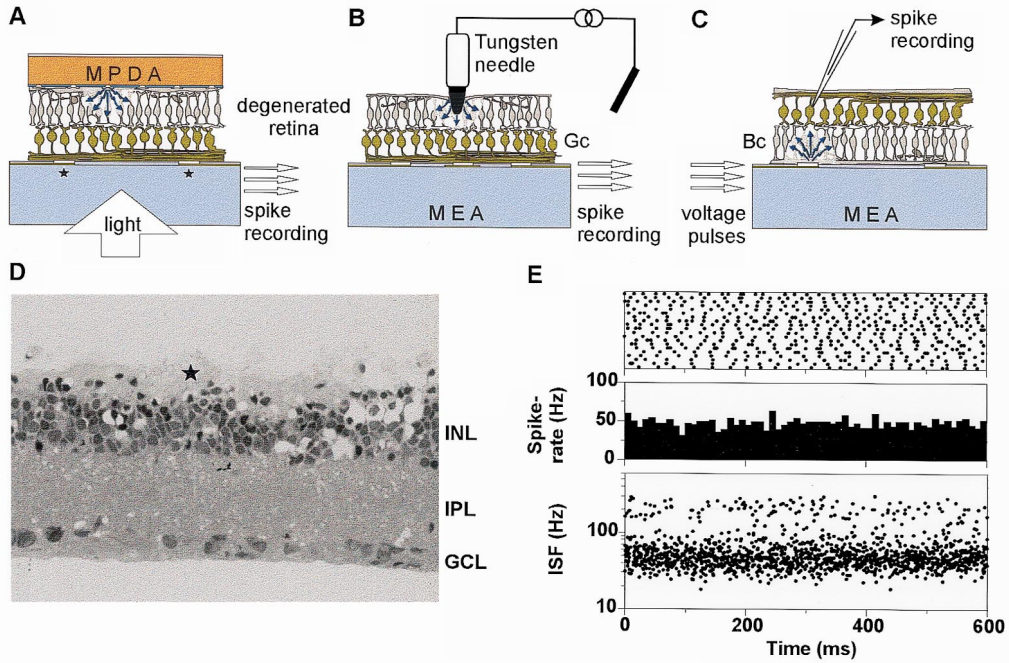


Fig. 2

Fig. 1. *Caption opposite.*

Fig. 2. *Caption opposite.*

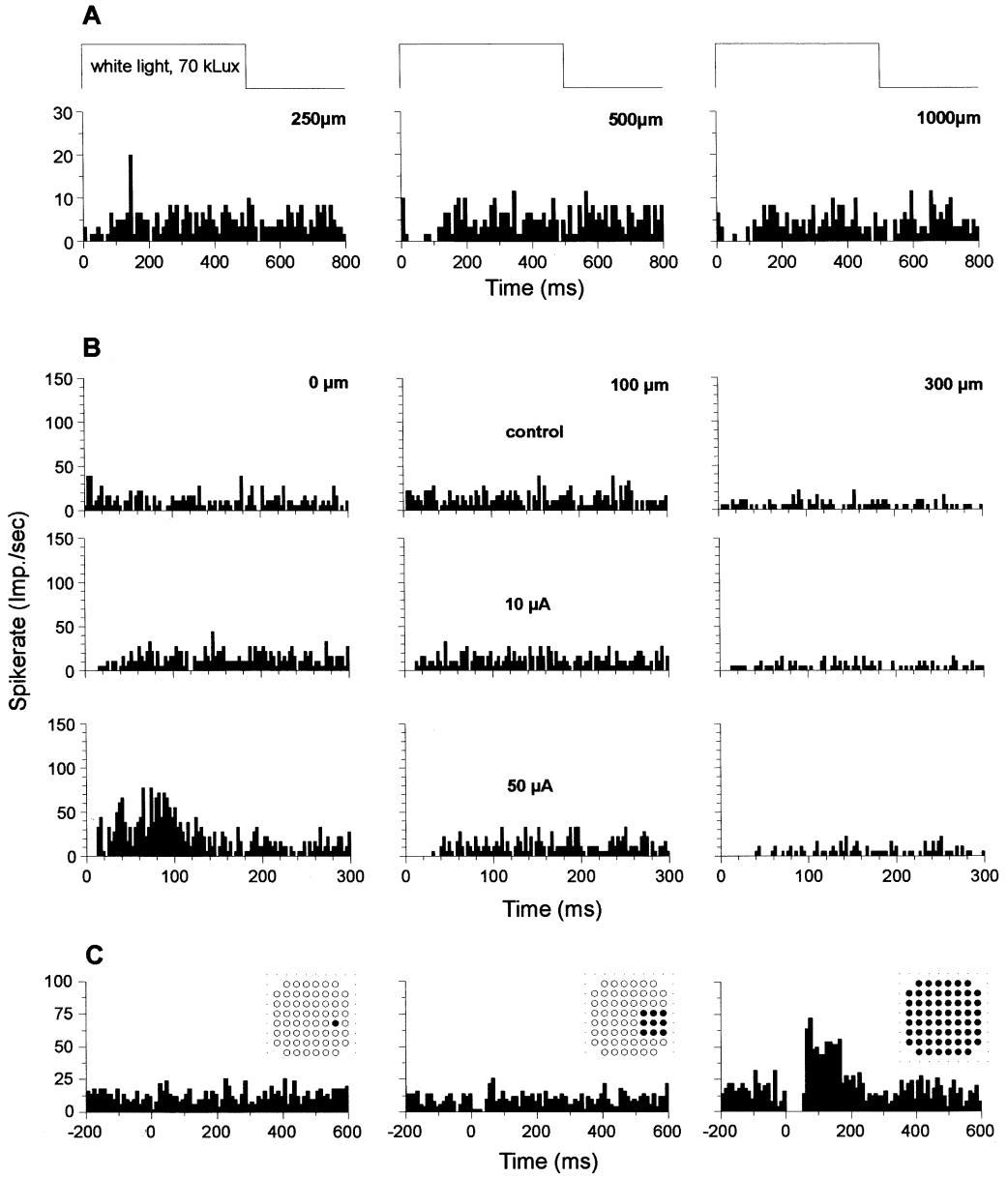


Fig. 3. Response histograms of electrically evoked spike activity of ganglion cells in RCS rat retinae. (A) Sandwich test: the MPDA (monopolar pin diodes, size 20×20 µm^2) was illuminated with pulses of white light (retinal illuminance: 70 kLux, pulse duration 500 ms, pulse onset at time 0 ms, spot diameter 250, 500, 1000 µm). Histograms were obtained from single unit recordings of 60 subsequent trials, bin width 10 ms; retina from a 229-day-old animal. (B) Monofocal current stimulation with charge balanced current pulses against ground electrode (rectangular pulse at time 0 ms, duration 1 ms, followed by an exponential discharging phase, amplitude as indicated). Shown are histograms (60 trials, bin width 3 ms) from unsorted multi unit recordings underneath the stimulation site (left column, 0 µm), and 100 µm (middle column) and 300 µm (right column) apart from the current injection site; retina from a 81-day-old animal (details see text). (C) Multisite charge injection with monophasic voltage pulses against ground electrode (1 V, duration 500 µs) applied at time 0 ms to the MEA electrodes indicated by filled circles in the inserted maps; histograms (50 trials, binwidth 10 ms) obtained from spike recordings of a ganglion cell body located above the marked electrode in the left map; retina from a 132-day-old animal.

in diameter and were spaced 100 and 200 µm apart, respectively. A circular chamber was centered on the recording area as described by Nisch, Böck, Haemmerle and Mohr (1994). These chambers containing the respective retinal preparation were constantly perfused with oxygenated standard perfusate at 35°C.

The so-called Sandwich preparation technique (Fig. 2A) was used to test MPDA prototypes in vitro as described previously (Zrenner et al., 1997). In this test, the distal retina was electrically stimulated by sending flashes of either white or infrared light through the glass substrate of the MEA onto a MPDA located on the distal retina. In a second set of experiments with the ganglion cell side down, monopolar current pulses were applied to the distal retina via a needle microelectrode positioned on the receptor layer, and the spike activity

of retinal ganglion cells was recorded with the MEA electrodes (Fig. 2B). In a variation of this technique with the receptor side down, the distal retina was stimulated by applying voltage pulses via selected MEA electrodes (Fig. 2C). The spike activity evoked by these spatial stimulus patterns in individual ganglion cell bodies was recorded extracellularly by means of a glass electrode.

2.3. Biocompatibility of chip materials

Retinal cell cultures were prepared from enzymatically and mechanically disrupted retinae of 4–12 day old Brown Norway rats as described elsewhere (Guenther, Rothe, Taschenberger & Grantyn, 1994). The cells were plated with a density of 3500 cells/mm^2 either directly on different technical substrates or on glass cover slips for comparison and were then incubated at 37°C and 5% CO_2 for up to 4 weeks. The substrates were coated with poly-L-lysine. ARAC (arabinosylcytosine-hydrochloride, 1 mM) was added after 4 days to stop mitosis. The cell cultures were fixed in 4% paraformaldehyde after 1, 7, 21, and 28 days in vitro. Cell nuclei were labeled by DAPI staining, and the actin containing cytoskeleton was labeled with Phalloidin-TRITC. Neurones can be separated from glia cells by this method due to the smaller size of their nuclei (Fig. 4A). Viable cells from the different cell types were recorded under a fluorescence microscope as the mean values of ten randomly selected areas. The toxicity of different materials and diffusible factors were tested by registering either the percentage of viable cells in direct contact with the technical materials or the percentage of viable cells in a culture dish containing the different materials.

2.4. Implantation techniques

For implantation into rat eyes a different tool and procedure were developed than those described previously (Zrenner et al., 1997). The implant was no longer injected or pushed into place (Seiler & Aramant, 1999). Rats (10 Sprague-Dawley and 15 Long-Evans rats) were anesthetized with intraperitoneal injections of sodium pentobarbital (38–40 mg/kg) and atropine (0.4 mg/kg), followed by xylazine (3–7 mg/kg) 10 min later. A small incision (0.5–1.0 mm) was made just through the sclera, choroid and retina behind the pars plana and parallel to the limbus. Microchips were loaded into the custom-made implantation tool, which was then used to insert the microchip into the subretinal space (Fig. 5A). Most of the implants were placed in the superior quadrant of the rat eye (Fig. 5B). After implantation, the incision was closed with 10–0 sutures. The animals were treated according to the regulations in the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.5. Electrophysiological function testing in vivo

Rats were anesthetized with intraperitoneal injections of ketamine (100 mg/kg) and xylazine (15 mg/kg). A *TOENNIES MULTILINER* with a Ganzfeld stimulator (white light) or custom-made LED stimulator (white or infrared light, respectively) was used. Corneal electrodes made of gold wire were used for electroretinogram (ERG) recordings. Subcutaneous steel needle electrodes were used as reference and ground electrodes. High frequency (low pass) filters were set to maximum frequencies (5 or 20 kHz) to detect the fast potentials created by illuminated MPDAs.

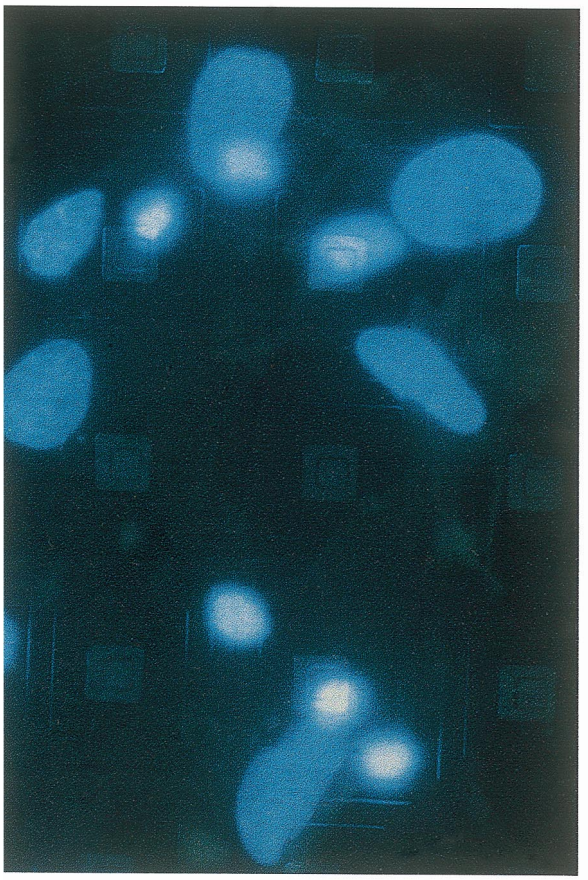
2.6. Histology

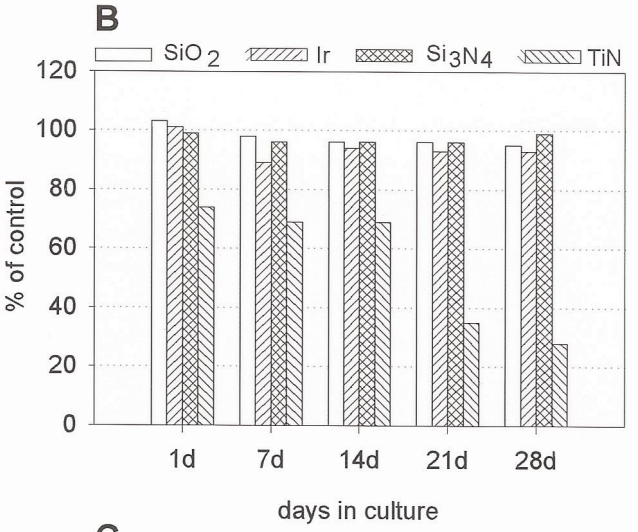
The eyes were enucleated after decapitation of the rats. Subsequently they were opened along the ora serrata, and the posterior eye cups were immediately fixed for 2 h in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. To examine the retina overlying an implanted device, the tissue was carefully removed from the implant and either embedded in methacrylate (Technovit 7100, Heraeus, Germany) or prepared for immunohistology. From the methacrylate blocks 2 μm sections were cut, mounted on slides, stained with toluidine blue and microscopically examined. For the immunostaining the fixed retina was immersed in 30% sucrose in phosphate buffered saline, embedded in mounting medium, and frozen. Cryo-sections of 12 μm were collected on gelatin coated slides. After pre-incubation in a solution of 20% normal goat serum, standard immunohistochemistry was carried out with an antibody against glia fibrillary acidic protein (GFAP; Sigma, Germany) diluted 1:500 in 0.05 M phosphate buffered saline, 0.03% Triton X-100; the immunoreaction was visualized with a fluorescent chromogen. Cell nuclei were occasionally counterstained with DAPI (4,6-diamidino-2-phenylindole).

3. Results

3.1. Functional electrical stimulation of degenerated retinae in vitro

Spontaneous activity in the retinal networks of the degenerated retinae was determined by means of the arrangements shown in Fig. 2A–B. Regardless of the stage of degeneration, the ganglion cells displayed a relatively high spontaneous activity of up to 48 Hz as shown in Fig. 2E. This was true of all retinae examined so far ($n = 20$, postnatal age from 80 to 230 days). None





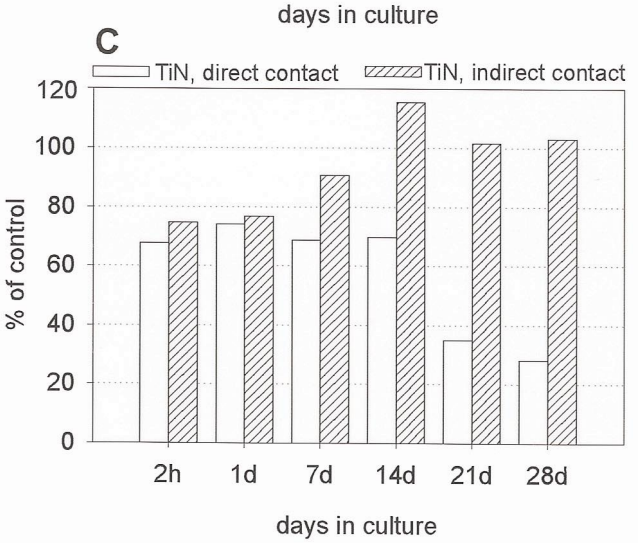
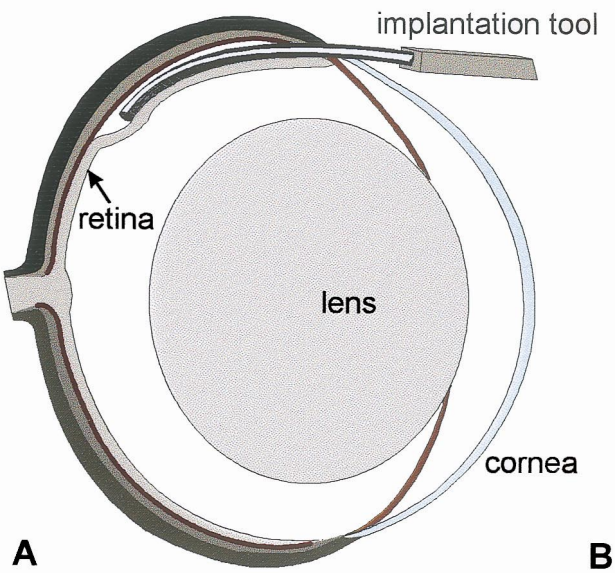


Fig. 4



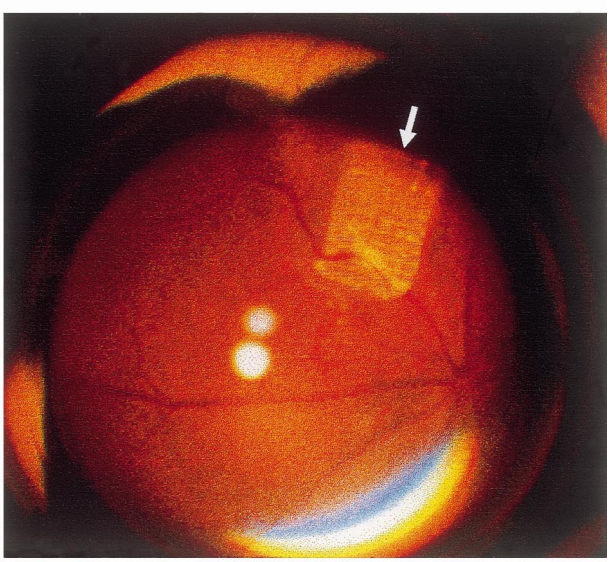


Fig. 5

Fig. 4. *Caption opposite.*

Fig. 5. *Caption opposite.*

of the retinae exhibited any response in relation to stimulation with visible light.

Beams of white light were flashed onto MPDAs on the distal side of degenerated retinae in order to test for ganglion cell activity under electrical stimulation of retinal cells with photocurrents from an MPDA prototype (Fig. 2A). Following the onset of light incidence, an inhibition of spontaneous ganglion cell activity was observed in the peristimulus time histograms at retinal illuminances between 10 and 100 kLux (Fig. 3A). This inhibition increased to saturation level as the diameter of the stimulating light spot widened from 250 to 1000 μm. Even at exceptionally high illuminances of 70 kLux, no modulation of activity was detectable under small light spots which illuminated only a few microphotodiodes of the array.

Local electrical excitability of the retinae was tested with focal current injection using a needle electrode lowered onto the distal retina as shown in Fig. 2B. Multichannel recordings of spike activity revealed a clear correlation between the strength of stimulation and the local retinal response at distances of 0, 100 and 300 μm from the stimulation site, as shown in Fig. 3B. Control measurements without electrical stimulation are shown in the upper row of Fig. 3B, while the middle row and lower row present the data recorded after stimulation with low (10 μA) and high (50 μA) current amplitude, respectively. A current pulse of 10 μA amplitude and 1 ms duration beneath the site of current injection (0 μm) provided a clear suprathreshold stimulus, resulting in a short but clear transient inhibition of spontaneous activity. A further increase in current amplitude to 50 μA increased the firing rate at the site of stimulation (0 μm). The same current pulse of 50 μA also resulted in a clear inhibition at recording sites 100 and 300 μm from the location of charge injection (Fig. 3B, third row).

In a further series of experiments we used the MEAs for distal multifocal charge injection. This arrangement (see Fig. 2C) corresponded very well to multisite stimulation with the individual planar stimulation electrodes of the MPDA. Applying voltage pulses to the metallic electrodes resulted in a time course of the injected current very similar to that obtained by MPDAs flashed with light. Again, the application of a single voltage pulse elicited a temporal inhibition of cell firing

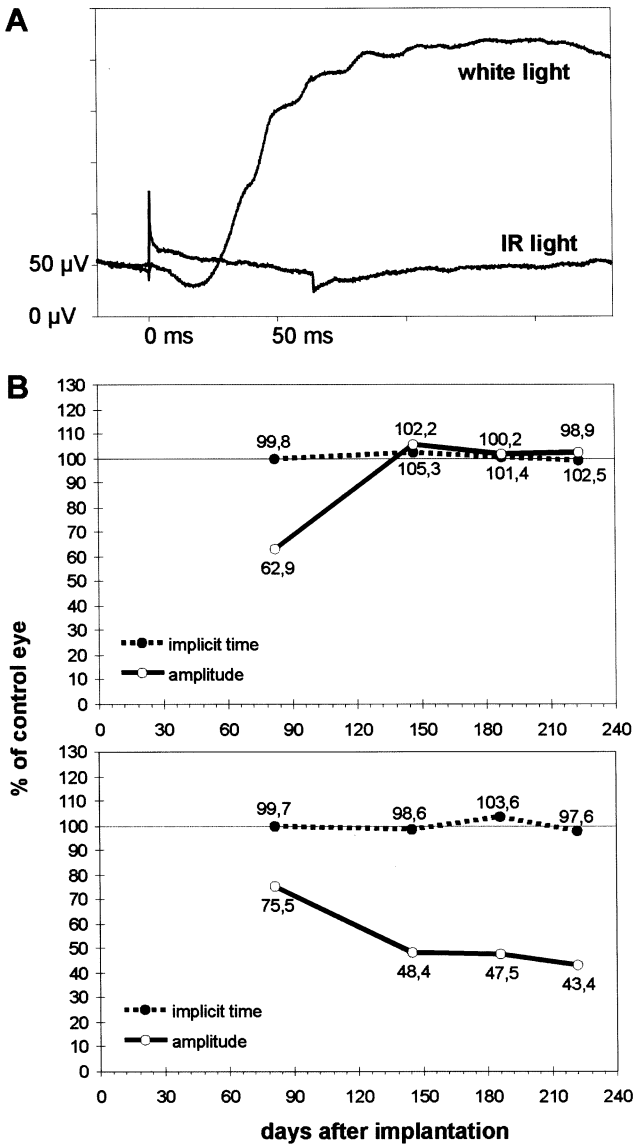


Fig. 6. Long term functional stability and tolerance of implanted MPDAs. (A) Electroretinogram (ERG) of an implanted eye 14 months after implantation of a MPDA. The upper curve shows a normal ERG waveform after full field stimulation with white light (8 mW/cm^2) from a LED. After stimulation with an infrared LED (300 mW/cm^2) the chip response is recorded which corresponds to the duration of the stimulus in both cases. (B) Time courses of amplitudes and latencies of Ganzfeld-ERGs of implanted eyes with reference to the values obtained from the eye without implant (top: implant size: 0.8 × 1.0 mm, location see corresponding fundus photograph in Fig. 5; bottom: implant size: 0.65 × 1.0 mm).

Fig. 4. (*Opposite*, *top*) Biocompatibility of four different chip materials: silicon oxide (SiO_2), iridium (Ir), silicon nitride (Si_3N_4) and titanium nitride (TiN). (A) Retinal cell culture on a MPDA followed up for 28 days: cells were labelled by a combined cytoskeleton/cell nuclei staining. Large, faint blue cell nuclei are those of glia cells; small, white blue nuclei those of retinal neurons. (B) Cell survival of retinal cells cultured on different implant materials. (C) Test for toxicity of diffusible factors of TiN. A reduced cell survival can only be seen for retinal cells in direct contact with TiN material.

Fig. 5. (*Opposite*, *bottom*) Rat implantation technique. (A) A small incision (0.5–1.0 mm) is cut in the pars plana or just behind, parallel to the limbus. MPDAs are loaded into a custom-made implantation tool, which inserts the chip into the subretinal space. (B) Fundus photograph 229 days after implantation. The implant (arrow) is located perfectly in the subretinal space.

rates, followed by a time interval with increased spike activity when the amplitude of the applied pulse was increased up to 2 V (data not shown). As shown in Fig. 3C this behaviour of the retinal network was also evoked by increasing the stimulated retinal area surrounding the recording site, and by applying the voltage pulses to an increasing number of electrodes and was most pronounced when large areas were stimulated (Fig. 3C, right).

3.2. Biocompatibility of implant materials

The survival of retinal neurons cultured on or in close vicinity to different substrates of a MPDA was tested as a means of evaluating the biocompatibility of potential chip materials. Fig. 4 shows the results of such an analysis of SiO_2 and Si_3N_4 (used as insulators) and Ir and TiN (used as electrode materials). Most of the materials tested had good biocompatibility (Fig. 4B), and there was no significant difference in cell survival from that of the control group ($P = 0.001$, data not shown here). TiN, however, showed a different biocompatibility. After 4 weeks, only $30 \pm 5\%$ of the control cells had survived in direct contact with this material (Fig. 4C, white bars) whereas no significant difference from the control groups was found in retinal cells cultured in a dish containing TiN. Thus, no soluble factor of the TiN material is responsible for this effect.

3.3. Surgical procedures

Due to the smallness of the rat eye and its large lens (Fig. 5A), implantation of the MPDAs was difficult with conventional methods. We therefore chose to use the elegant implantation technique devised by Aramant and Seiler (see Section 2), which makes it possible to insert relatively large MPDAs as shown in Fig. 5B.

3.4. Long-term function and tolerance of the implant

Long-term function the MPDAs were measured electrophysiologically at different times after implantation. An original response to white light and infrared light (which alone is seen by the MPDA) is shown in Fig. 6A for comparison. After a total of 20 months after implantation the chip response to illumination still remained essentially stable. No electrically evoked retinal potential in vivo was unambiguously due to postreceptoral neurons, despite external infrared light intensities up to 180 mW/cm^2. This was possibly due to the fact that the area of the retina stimulated by the MPDA is very small and thereby the postreceptoral neuronal response itself is disguised by the high discharge response of the MPDA. To test the impact of the implanted MPDA on retinal physiology, ERGs were recorded simultaneously in both eyes up to 229 days after implantation in five normally pigmented rats (Long Evans) which had received a microphotodiode array in one eye. Two examples are shown in Fig. 6B. Amplitudes (solid lines) and implicit times (dotted lines) of the b-waves of the implanted eyes were compared to those of control eyes which had not received an implant. Although the b-wave amplitudes were reduced in some cases (3/5), the implicit time was never affected (Fig. 6B). This indicates that the number of photoreceptors was slightly reduced, but that the function of photoreceptors was not affected.

3.5. Histology after implantation

More than 4 months after the implantation of MPDAs with an area of 0.48–0.8 mm^2 and a thickness of 50 μm, the architecture of the rat retina directly above the MPDA was still intact (Fig. 7A). Only a few remaining cell bodies of the outer nuclear layer (ONL) were found between the implant and the retina. Parts of the outer plexiform layer (OPL) were still detectable with the light microscope, even though the OPL had become atrophic after the photoreceptors completely disappeared. The thickness of the inner nuclear layer (INL) and the inner plexiform layer (IPL) was unchanged, and the number of cells in the INL and the ganglion cell layer (GCL) was essentially the same outside and above the implant. We do not know at present whether all neuronal cell types are preserved in a similar way and whether single neurons in the INL may be replaced by glia cells. However, the undisturbed stratification of the inner retinal layers makes proliferation of Müller glia rather unlikely. After an implantation period of 8 months, the longest period examined until now in a rat retina, immunoreactivity for GFAP (glia fibrillary acidic protein) was enhanced in the Müller cells, indicating that alterations in protein expression occur even when standard histology is normal (Fig. 7B). In addition, enhanced numbers of cells were observed in the GCL and the ganglion cell fiber layer in some areas of the retina; this was probably due to the proliferation of astrocytes. The pattern of proliferation at the vitreal side of the retina was irregular, and proliferation was restricted to a few areas 40–100 μm in diameter. Proliferation on the vitreal side also corresponded to an intense GFAP-staining at the level of the former OPL. It is unclear whether this glial proliferation was caused by an injury during surgery, by an irregularity on the surface of the microphotodiode, due e.g. to handling during implantation, by a specific morphological or physiological situation of the retina, e.g. a large epiretinal vessel, or whether it represented gliotic hot spots with a tendency to progressive growth. However, no glia encapsulation of the implant was observed.

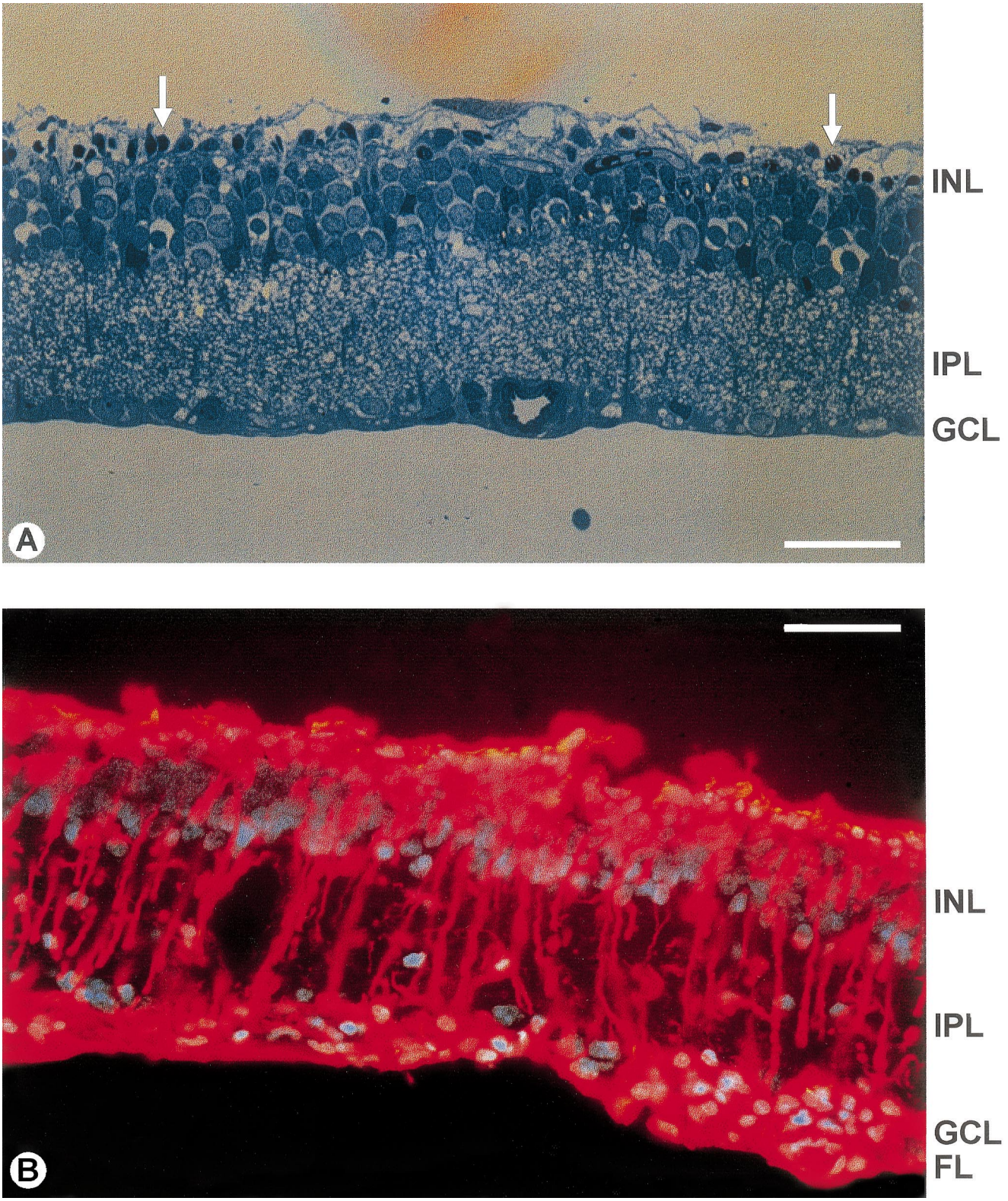


Fig. 7. Histologic cross sections of retinal implantation sites. (A) Rat retina overlaying a subretinally implanted MPDA after more than four months of implantation. The former outer nuclear layer is reduced to a single row of cell bodies (arrows) but the architecture of the inner retina is well preserved. (B) GFAP immunoreactivity in a rat retina overlying a subretinally implanted MPDA after eight months of implantation. There is a massive upregulation of GFAP immunoreactivity (red) in the Müller cells. Note the enhanced number of cell nuclei (blue; DAPI staining) in the ganglion cell layer and the optic fiber layer which is probably due to the proliferation of astrocytes. INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer; FL, optic fiber layer. Scale bar 25 μm.

4. Discussion

4.1. Functional electrical stimulation of degenerated retinae in vitro

Several groups of researchers have shown that it is possible to evoke phosphenes by electrical stimulation of degenerated retinae (Potts, Inoue & Buffum, 1968; Miyake, Yanagida & Yagasaki, 1981; Kato, Saito & Tanino, 1983). In these experiments, contact lens electrodes were used to elicit something like a full-field electrical response of the visual system. Although these results are highly intriguing because they demonstrate basic possibility of eliciting visual sensation by means of electrically mediated retinal responses in blind subjects, it remains unknown whether spatial resolution can be achieved by focal electrical stimulation of either the distal or proximal side of the retina. Humayun et al. were the first to use focal electrical stimulation of degenerated retinae of human volunteers to investigate the feasibility of a retinal prosthesis with pixelized visual input (Humayun et al., 1996). They inserted stimulating probes with two or three electrodes, respectively, through the sclera to deliver current to the retinal surface. Although the subjects were able to localize and resolve evoked phosphenes, such experiments have drawbacks. First, the exact distance between the electrodes and the retinal surface is unknown. Second, intraoperative retinal stimulation experiments in human volunteers involves risks such as that of intraocular infections. To circumvent these problems, we have developed in-vitro methods for investigating basic issues related to functional electrical multisite stimulation of normal and degenerated retinal networks.

We found that intrinsic spontaneous ganglion cell activity is present even in a highly degenerated retinal network and that it can be modulated by local electrical stimulation. This is important for all approaches to a visual prosthesis, because it is now certain that output neurons of the retina are still able to transmit information to higher visual centers. This intrinsic activity also constitutes an important noise background against which electrically evoked signals carried by the cell must be detected. This is also true of visual signals transmitted in intact retinae, as Troy et al. have emphasized (Troy & Robson, 1992; Troy & Lee, 1994).

Our results clearly indicate that stimulus-related activity can be evoked even in highly degenerated retinal networks, thus permitting modulation of firing rate of ganglion cells depending on the amount and spatial extent of the distally injected charge. The geometry of the stimulation electrode (needle vs. planar electrode) is apparently unimportant for the stimulation of retinal cells. Excitation thresholds in this study, typically up to 10×10^{-9} C per balanced pulse and monopolar electrode injected into the subretinal space (Fig. 3B), were far below those reported by other authors who stimulated degenerated retinae from the vitreal side (Humayun et al., 1996; Katona, Humayun, de Juan, Suzuki, Weiland & Greenberg, 1998). This might be due to the fact that we used a much shorter distance between the stimulation electrode and the excited neuronal tissue. The authors just mentioned also point out that they stimulated cells of the inner nuclear layer from the epiretinal side (Greenberg, Humayun & de Juan, 1998); in comparison to direct subretinal stimulation of these cells, this may also have resulted in an increase of stimulation thresholds.

4.2. Biocompatibility of implant materials

Our results showed that retinal cells can be cultured on materials intended for use in MPDA implants. Although most of implant materials used in this study had good biocompatibility, cells which had direct contact with TiN had a shorter survival time. This is unfortunate, since TiN electrodes have the greatest safe charge injection capacity of all electrode materials examined so far (Janders, Egert, Stelzle & Nisch, 1996), because of their exceptionally high capacity and columnar surface design. Since relatively high charge densities are needed for threshold stimulation of retinal cells, and the largest possible operating range below the safe charge injection limit is desirable for a retinal implant, a compromise between the needs for the best stimulation electrodes and the biocompatibility of electrode materials had to be found. However, it is clear that no soluble factor is responsible for the decreased cell survival. Hence, no release of toxic substrates is expected from subretinally implanted chips made of the materials tested so far.

4.3. Histology after implantation

An implanted microchip in the subretinal space forms a diffusion barrier between the retinal pigment epithelium (RPE) and the retina. This reduces metabolic interactions between the RPE and the neuronal retina, and degeneration may be the result within the neuronal network. In species with well vascularized retinae an appropriate supply of the inner parts of the retina can be maintained without diffusion via the RPE, such retinae should be therefore less affected by an implant. Similar to the human retina, the rat retina has epiretinal as well as intra-retinal vessels which run through all retinal layers terminating below the photoreceptor synapses in the outer plexiform layer.

Histology showed that the implants were well tolerated for eight months and did not alter the neuronal architecture of the inner retina. However, immunohistochemistry for GFAP clearly showed that the Müller glia was affected by the implant. Müller cells do not normally express GFAP, but upregulation of GFAP expression is

a common event during degeneration of the retina irrespective of the etiology of the degeneration. Increased GFAP immunoreactivity in Müller cells has been observed in response to retinal injury, including retinal light damage (Eisenfeld, Bunt-Milam & Sarthy, 1984; Burns & Robles, 1990), inherited retinal degeneration (Ekström, Sanyal, Narfström, Chader & van Veen, 1988; Sarthy & Fu, 1989; Smith, Brodjian, Desai & Sarthy, 1997) and induced retinal injury (Seiler & Turner, 1988; Tyler & Burns, 1991). Therefore, if a device is implanted into a degenerated retina a reactive Müller glia will already be present. For a proper function, the implant has to deal with this phenomenon.

Conclusive information about the histological situation at the implant-tissue interfaces can only be obtained when the silicone chip is examined in situ. To this end we are currently developing new techniques for sectioning the technical device together with the biological tissue which adheres to it.

4.4. Long-term tolerance and function of the implant

Because of the high retinal illuminances required to evoke recordable network activity with MPDAs (cf. Fig. 3A), we concentrated our electrophysiological in vivo experiments on the question whether passive MPDAs maintain a good long-term function in the silicon-hostile environment of the subretinal space and are tolerated by the neuronal network. Others have addressed this question as well (Chow & Chow, 1997; Peyman et al., 1998) and have found results comparable to ours, although different animal models and surgical methods were used in these investigations.

The slightly reduced ERG b-wave amplitudes in some preparations may have been due in part to the implantation technique. Despite extreme caution some retinal lesions may have occurred, since the surgeon was unable to observe his manipulations and therefore could not precisely assess final localization of the implant during the operation. The implanted chips were 50 μm thick, rigid, and did not adapt to the curvature of the eye. Injury to the host retina might be reduced by implanting thinner, flexible chips like those presently being developed by Schubert, Hierzenberger, Wanka, Graf, Graf and Nisch (1997). Another reason might be degeneration of the photoreceptors underneath the implant as revealed by histology. The photoreceptor degeneration was most likely caused by the fact that the chip was not perforated; this reduced the transport of nutrients from the choroid to the outer retina. Thinner, flexible and better designed chips with openings to allow diffusion should alleviate these problems.

Thus far it has not been possible to record retinal potentials which were electrically evoked by the MPDA in rats. Electrically evoked cortical potentials have been recorded, however, in rabbits by infrared stimulation of the MPDA. Unfortunately such experiments can not be performed in rats with presently available technology.

4.5. The future of subretinal microphotodiodes as visual prostheses

Both in vitro and in vivo, we found that retinal illuminance above naturally occurring levels is needed to generate local photocurrents in high enough quantities to successfully stimulate the retinal network by means of the MPDA. Various concepts are now being developed to provide additional energy to subretinally implanted chips from an external power supply.

Is it really possible to replace degenerated photoreceptors with subretinally implanted microphotodiodes? Our experiments with degenerated retinae clearly indicate that the answer is yes. We think that basic questions regarding functional electrical multisite stimulation of degenerated retinae, the biocompatibility of implant materials, surgical procedures, and the long term function of implants have now basically been answered. This opens up promising perspectives for future work in this field. Future experiments will need to implant chips in animal models with retinal degeneration, such as the RCS rat, and then address the question whether the degenerated retina can respond to the chip in vivo as well as has been shown in vitro in the present paper. However, much work remains to be done in fields like the development of a MPDA with external power supply before the implantation of MPDAs in patients suffering from photoreceptor loss seems appropriate.

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