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Implantation of stimulation electrodes in the subretinal space to demonstrate cortical responses in Yucatan minipig in the course of visual prosthesis development

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PURPOSE. During the course of the development of visual prostheses, subretinal stimulation films were implanted in micropigs in order to prove the feasibility of subretinal electrical stimulation with subsequent cortical response. One aim was to demonstrate that epidural recording of visual evoked potentials is possible in the micropig.

METHODS. Film-bound stimulation electrode arrays were placed in the subretinal space of micropigs. This enabled the retina to be stimulated subretinally. Since conventional visual evoked potential (VEP) measuring is virtually impossible in the pig from the neurosurgical point of view, epidural recording electrode arrays were positioned over the visual cortex as permanent electrodes.

RESULTS. The feasibility of temporary implantation of film-bound stimulation electrode arrays was successfully demonstrated in the micropig model. On stimulation with monopolar voltage pulses (1000 to 3000 mV), reproducible epidural VEP measurements (5 to 10 µV) were detected.

CONCLUSIONS. The feasibility of subretinal stimulation of the retina was demonstrated in a retinal model that is similar to the human retina. This animal model therefore offers a suitable means of studying the tolerability of stimulation situations in the course of visual prosthesis development. (Eur J Ophthalmol 2005; 15: 493-9)

KEY WORDS. Animal model, Retinal implant, Visual prosthesis

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INTRODUCTION

For many years, attempts have been made to develop visual prostheses for the blind (1-7). The considerable advances made in the field of microelectronics led to a marked increase in research activity on this subject. For the study groups attempting to inject an artificial visual stimulus into the visual system, demonstrating the feasibility of their particular method was crucial (8-12). A wide variety of questions needed to be answered in order to achieve a workable implant with long-term stability (13). Various animal models have been employed to address these questions. As well as the rabbit model, which has mainly been used for biocompatibility studies, the cat model has greatly helped to answer electrophysiologic questions. We found the micropig to be the most suitable

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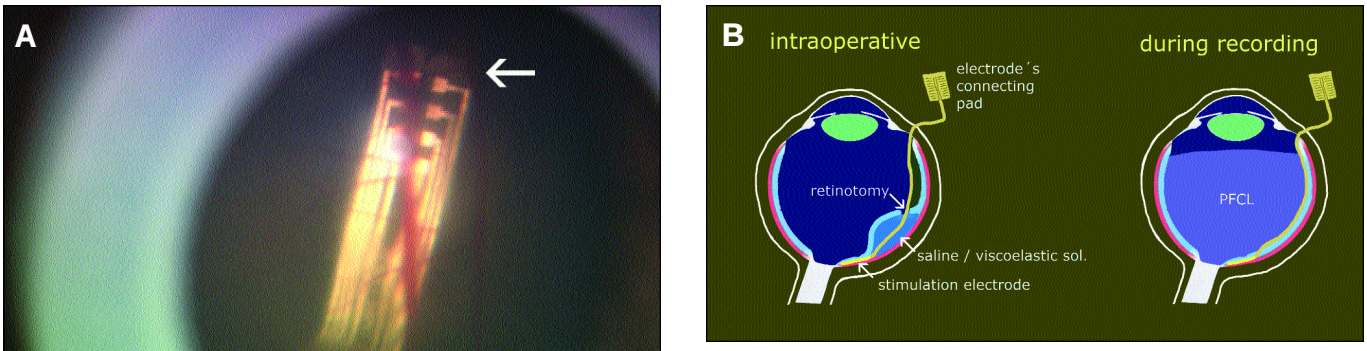

Fig. 1 - (A) Stimulation film implanted in the subretinal space of a Yucatan minipig (intraoperative - arrow showing single electrodes). **(B)** Scheme of subretinal stimulation film implantation.

model for the development of a surgical procedure for retinal implants and suitability was demonstrated by electrophysiologic data. It was thus possible to obtain electrophysiologic data from a model similar to humans.

How does the subretinal method differ from the other stimulation variants?

Subretinal stimulation differs fundamentally from other methods currently under investigation, at least in theory. Unlike the other approaches, in which stimuli are applied more proximal (epiretinal approach (14-15), stimulation of the optic nerve (16), stimulation of the visual cortex (16-17), stimulation in the subretinal space theoretically does not require any pre-processing of the stimuli.

This is because, at the site where stimulation in the visual system is physiologically generated by the photoreceptors, this is merely replaced by an artificially produced stimulus. Unlike the other approaches, natural eye movements can still be harnessed by the subretinal stimulation method.

This factor greatly helps to enhance image quality (18). In the evolution of sight, different strategies for improving image quality can be seen in the animal kingdom, which employ head movements or movable eyes as suitable mechanisms.

The analogy with the approaches studied by the various research groups is unmistakable: the subretinal approach can exploit the mobility of the eyes in order to gain a visual impression and register objects, in contrast to other stimulation methods involving a camera fixed to a pair of spectacles, for example, where a moving object would inevitably result in a corresponding head movement.

Why experiment with film-bound stimulators?

There are no fixation problems with a completely subretinal implant. This was demonstrated with the microphotodiode arrays (MPDAs) initially used, in long-term cat and micropig studies over 14 months (18, 19).

The MPDAs initially implanted in the subretinal implantation experiments comprised an array of numerous microphotodiodes on a silicone disk, approximately 50 µm thick and 2 to 3 mm in diameter. Under normal ambient light conditions, however, these implants were too weak to stimulate the retina sufficiently.

Therefore, stimulation experiments were performed with film-bound stimulators (19), where the stimulation electrodes had an extraocular connection out of the subretinal space.

By this method, markedly more energy for stimulating the retina from the subretinal space could be made available locally and it was possible to vary the electrical stimulation parameters.

The micropig, with its holoangiotic retinal supply and an eye very similar to that of humans from a surgical point of view, is readily available in comparison with primates.

This model presents considerable problems, however, in terms of the electrophysiologic diagnosis (visual evoked potentials (VEP)) that is necessary for objectifying the visual impression in an animal model.

The signal that may be recorded in the micropig is attenuated by a covering of bone up to 5 cm thick over the occipital part of the brain. In order to obtain adequate VEP signals for diagnosis, the cortical recording electrodes need to be placed close to the visual cortex and this requires a neurosurgical procedure.

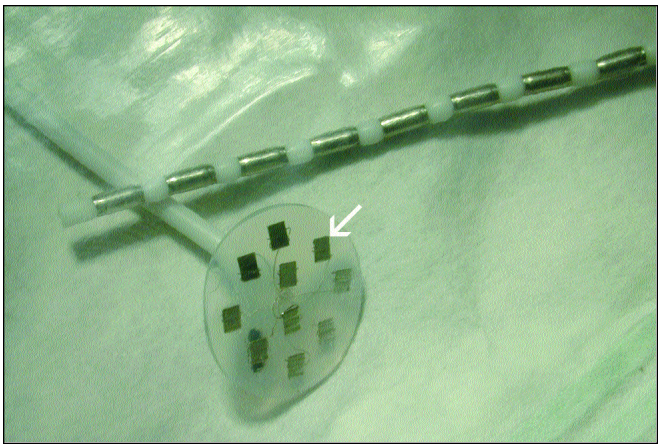

Fig. 2 - Epidural recording electrode (arrow) with contact plug.

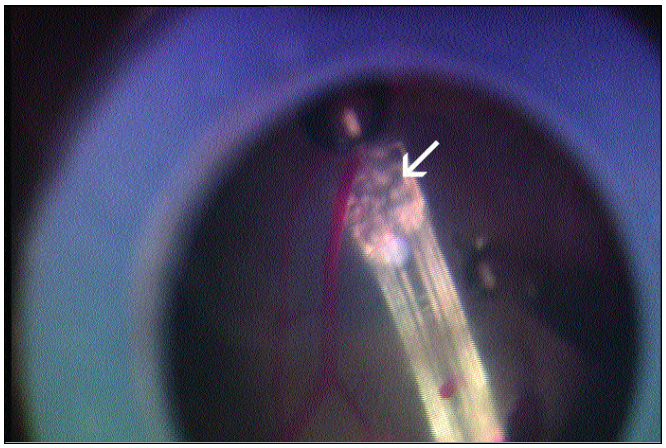

Fig. 3 - Subretinal electrolysis: gaseous bubbles above each single stimulation electrode (arrow).

METHODS

In order to prove that the visual system could be stimulated from the subretinal space, film-bound stimulation electrode arrays were implanted in the subretinal space of anesthetized micropigs in acute experiments. These retinal contact structures applied to polyamide film were prepared by the Fraunhofer Institute of Biomedical Technology (IBMT), St. Ingbert. At the end of the 12-µm-thick film, which is partly placed under the retina, there are a total of eight platinum electrodes in two rows. The distance between electrodes is 330 µm and the size of the electrodes 100 x 100 µm. With a total length of 5 cm, the film with the electrode-covered section can be placed under the retina and connected to a stimulus generator via a miniplug located outside the eye. The stimulator used was an STG 1008 eight-channel stimulus generator for current and voltage signals manufactured by Multi Channel Systems in Reutlingen. Stimulation took the form of monopolar positive voltage pulses of between 1000 and 3000 mV, a pulse duration of 400 µs, at a frequency of 0.8 to 1.0 Hz.

Surgery

All surgical and electrophysiologic procedures were performed in Regensburg with anesthetized Yucatan micropigs. The procedures were in accordance with the guidelines of the European Communities Council Directive (86/609/EEC) and were approved by an official German Animal Care and Use Committee. In addition we followed the NIH Principles of Laboratory Animal Care (Publication No. 85-23, revised 1985), the OPRR Public Health Service

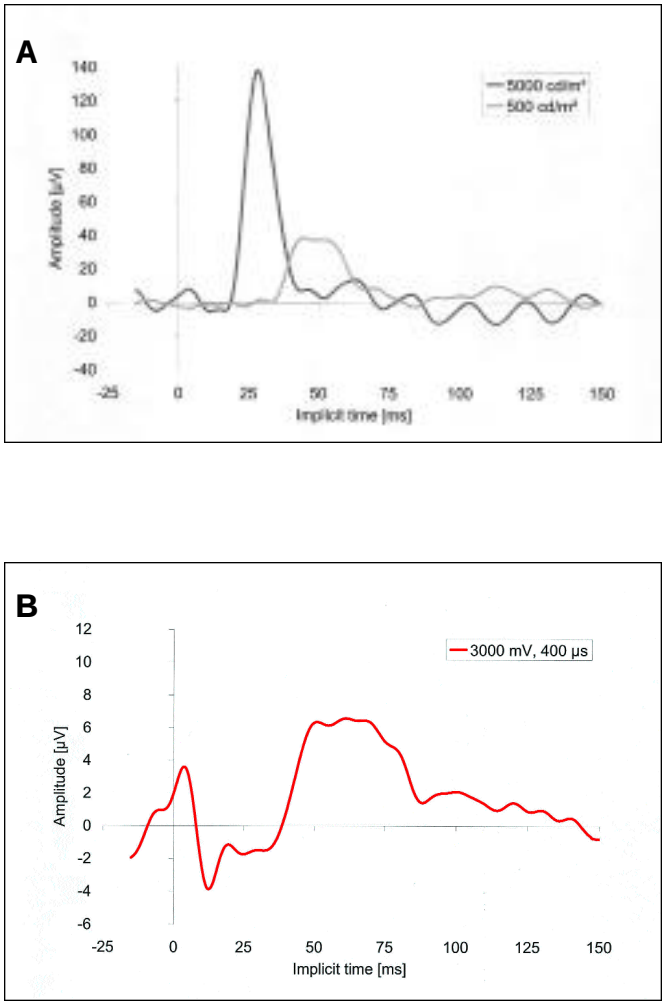

Fig. 4 - Cortical response (epidural visual evoked potentials). **(A)** Response from light stimulation; **(B)** response from subretinal electrical stimulation.

Policy on the Human Care and Use of Laboratory Animals (revised 1986), the US Animal Welfare Act, and the Association for Research in Vision & Ophthalmology (ARVO) guidelines.

Anesthesia

The test animals were premedicated with ketamine hydrochloride (Ketanest 15 mg/kg body weight) and midazolam (1 mg/kg body weight) given by intramuscular injection. A deep paravertebral site of injection at the back of the neck was used. A commercially available cannula with two infusion lines was connected up and joined to a three-way tap. The appropriate amount of midazolam and ketamine hydrochloride can be administered via this three-way tap at a distance from the animal, which usually starts running around the pen after puncture. This means the animal does not have to be restrained for this purpose. After 4 to 10 minutes, the experimental animal was adequately sedated to allow a venous access to be placed in its ear vein. The animal was then taken to the mobile operating table where monitoring started by means of ECG and pulse oximeter. The pulse oximeter was clamped onto the pig's tail. Using a special porcine mask (custom-made by the Medical Technology Department of Großhadern University Clinic in Munich) allowed interim ventilation, largely preventing hypoxia on the way to the operation or during intubation. A size 4 Miller spatula was used for intubation. The tubes used were generally 6.0 microlaryngeal tubes (MLT). Depending on intubation and circumstances, these were advanced through a bite wedge with a hole and connected to the ventilator (Servo 900, Siemens Germany). Ventilation was pressure-controlled (PCV).

The animals were turned on their sides so that one eye could be placed under the operating microscope. Anesthesia was induced with remifentanil 1 mg/kg body weight and propofol 3 to 5 mg/kg body weight. Cisatracurium 0.1 mg/kg body weight was used as a muscle relaxant. Remifentanil 200 to 400 mg/h and propofol 100 to 600 mg/h were administered by continuous infusion in order to maintain anesthesia. Additional cisatracurium was given, as required. For experiments lasting more than 3 hours, animals were wrapped in heat-reflecting survival film to prevent heat loss. After the end of the experiment and anesthesia, spontaneous breathing by the animal was awaited and, once circulation was stable, the animal was extubated and returned to its pen.

Implantation of the stimulation film

Platinum polyimide film electrodes were implanted in the right eyes of micropigs in a modified standard three-port vitrectomy procedure. Owing to the limited conditions for surgical access to the eye in the micropig, a nasal access was mandatory. The nasal access allowed ease of manipulation in the pars plana region. A small surgical speculum proved helpful in widening the canthotomy and gaining enough space for the required sclerostomies. The conjunctiva was widely opened to obtain sufficient space for the infusion port, two sclerostomies, and an additional sclerostomy for the stimulation film. The infusion port was fixed to the sclera in the 9 o'clock position between the access for the vitrectomy probe and the required intraocular light source and the access for the stimulation film. A fiberoptic intraocular light source was necessary to illuminate the intraocular surgical field. The vitrectomy was carried out using standard surgical parameters. A BIOM II (Ocular Instruments, CA) was used for intraocular observation. It was difficult to access the lateral and anterior parts of the vitreous cavity through this nasal port, while avoiding touching the lens. We did not apply any indenting procedures to completely remove the vitreous. After partial vitreous removal, the retina was prepared for film implantation. Therefore a retinotomy in an inferior nasal area about 1 to 2 mm below the papilla was carried out by injecting balanced salt solution (BSS) via a Teflon cannula into the subretinal space. The retina was opened conventionally with vitreous scissors in the bleb area created. A quantity of 0.1 to 0.3 mL viscoelastic solution (Healon) was introduced via the retinotomy into the subretinal space to ease the subretinal introduction of the stimulation film from the vitreous cavity. To stiffen the highly flexible stimulation film, two less flexible guide films were used. They resemble the stimulation film in shape and are used to introduce the stimulation film into the vitreous cavity. The stimulation film was sandwiched between the guide films during scleral passage. The guide films were withdrawn when the stimulation film was grasped in the central region above the papilla with vitreous forceps. These forceps were used to advance the stimulation film through the retinotomy into the subretinal space (Fig. 1, a and b) until the desired macular area was reached. Viscoelastic solution and subretinal fluid in the bleb area were aspirated and perfluorocarbon liquid (PFCL) was carefully injected. The film portion running out of the vitreous cavity was sutured onto the sclera in

accordance with conventional buckle surgery and the sclerostomies were sealed using 7.0 Vicryl sutures for both purposes.

Recording site

Unlike humans, the micropig's occipital brain lies beneath a layer of bone nearly 5 cm thick with overlying fat and muscle tissue, which makes conventional VEP recording far more difficult, if not virtually impossible. The recording electrodes need to be placed close to the visual cortex in order to obtain low-noise VEP signals. Owing to the lack of functional data about the pig brain, the visual cortex was identified beforehand under light stimulation with the aid of single photon emission computed tomography (SPECT) so as to optimize the neurosurgical access and thus minimize the bone defect. In order to record the cortical VEP response potentials, neurosurgery colleagues placed a 10-pole recording electrode (Fig. 2) from Research Surgical Products Corporation (Racine, WI) over the visual cortex of the micropig on the dura mater. The nuchal surgical access for the neurosurgeons lay in the midline, from where the occipital craniotomy was then performed.

The nuchal muscle and fatty tissue in the midline was then dissected down to the bony cranium and this was opened in the area of the occipital brain. The connection between the recording electrode and the measuring unit can be established via an adapter (TECH-ATTACH connector) at the free end of the electrode. Measurements of cortical potentials were taken using a modified Espion standard electrophysiology system from Diagnosys LLC (Littleton, MA). After implantation, the free end of the electrode was placed in a pocket of skin in the micropig's nuchal area. Later it could then be freed from the subcutaneous tissue at an easily accessible site.

RESULTS

Implantation of the epidural recording electrodes by the neurosurgeons was successful in each of the five micropigs operated on. Repeated, consecutive recording of low-noise light VEPs could be readily carried out with the permanent epidural electrodes placed by neurosurgeons after the nuchal pocket of skin, in which the microplug was placed, had been opened. Precise placement of the recording electrodes over the visual cortex was made possible by electrophysiologists directing changes to the position of the recording electrode in response to light stimulation during the neurosurgical operation, until the response to stimuli reached a maximum amplitude. It was thus possible to operate on the right eye of the micropigs at later stages (between 1 week and 6 months) after cortical electrode implantation and to implant a subretinal stimulation film and record VEP in the same diagnostic session. Successful, reproducible, subretinal stimulation could only be detected in two pigs, although a light response in the VEP was clearly detectable in all cases. Increasing the stimulation amplitudes to over 3000 mV, at which macroscopic retinal changes were already noticeable, did not yield any cortical response to the subretinal electrostimulation. Biomicroscopic studies during the experiment revealed evidence of subretinal electrolysis. On closer observation, gas bubbles were seen directly above the particular stimulation electrode in individual cases (Fig. 3). Subretinal gas bubbles were only seen with higher stimulation currents (from approximately 3 V) but a threshold for the phenomenon could not be clearly identified because of the difficult observation conditions. A marked decrease in the evoked responses through to complete cessation was noted, as expected, as soon as this phenomenon was detectable.

The recording after focal light stimulation, which was carried out at a stimulus intensity of 5000 cd/m^2 (48 ms duration for a visual angle of approximately 12°), elicited cortical amplitudes of approximately 200 µV (Fig. 4a). Reducing the light intensity led to lower amplitudes and increasing implicit times. A typical, recordable, successful response is shown in Fig. 4b. In this, electrical stimulation with 3000 mV was applied for a duration of 400 µs. The size of the electrically stimulated area corresponds to an equally large area of focal light stimulation, which is shown for comparison.

At 3000 mV electrostimulation, which was equivalent to a charge transfer of 50 nC in our experiments per individual electrode, amplitudes of less than 10 µV were detected at the cortex. That is comparable to a light intensity of approximately 10 to 20 cd/m^2 on focal stimulation. No cortical potentials could be demonstrated below a stimulation amplitude of 2000 mV.

It was shown in five micropigs that subretinally placed stimulation electrode arrays can be implanted during the course of a pars plana vitrectomy. It was possible to stimulate the retina reproducibly in an acute study by means of the stimulation films used. After subretinal stimulation with

monopolar voltage pulses in the range of 2000 to 3000 mV, electrically evoked potentials of around 5 to 10 µV over the visual cortex were measured with low noise and reproducibly using the cortical recording electrodes implanted by us.

The cortical electrodes implanted by neurosurgeons allowed low-noise recording of epidural cortical potentials over the 6-month test period selected by us.

DISCUSSION

The development of visual prostheses relies on suitable models for evaluating the required stimulation parameters. One disadvantage of the micropig as an animal model is that the physiology of the visual process in this model has been little researched.

However, it is very close to humans in terms of eye anatomy and hence surgery. Furthermore, this model is readily available. Other animal models similar to humans, such as primates, are virtually unusable because of animal protection laws.

In the experimental animals on which the surgical methods were developed, cortical readings could be demonstrated in two out of five subretinally placed stimulation films. We assume that the direct trauma associated with subretinal implantation is a possible explanation for the low success rates (50%) in terms of electrostimulation, because cortical readings were detectable in every case by means of light stimulation.

The surgical trauma also might be an explanation for the relative high stimulation thresholds. The possibility of a fluid barrier between the subretinal electrode and the back of the retina should be considered. Whether it was a matter of irrigation fluid entering the subretinal space during the implantation process or residues of the viscoelastic solution, which was used at the retinotomy site to ease implantation, could not be ascertained in this instance for reasons of methodology.

The ophthalmoscopy performed to investigate this did not reveal any evidence of fluid infiltrating the area of electrode implantation. Subretinal PFCL, which was employed to reattach the retina and might also act as an insulator, was ruled out as a cause by ophthalmoscopy.

Another possible explanation is that the retina could no longer be stimulated because of a relevant acute trauma. A successful stimulation in an intact retina may have lower thresholds or lower thresholds might be assumed if the retina were allowed to recover for a longer period. When comparing the experimental situation with observations made after retinal surgery, lifting the retina then reattaching it shortly afterwards is bound to lead to a reduction in its sensitivity.

This is reflected in the slow improvement of visual acuity after successful surgery for retinal detachment. Lower amplitudes being accompanied by successful cortical recording would be likely in such an experimental situation. Thus dangerous stimulation currents leading to subretinal gas bubble formation could be avoided.

This problem might be alleviated by chronic implantation of the stimulation film, giving the retina time to recover after the surgical trauma. It is also not known whether a retina has the same or a different response to electrostimulation once it has been lifted then reattached shortly afterwards.

The fundamental difficulties experienced with electrostimulation here are a serious problem, not merely for the subretinal approach, and they warrant proper consideration. Completely subretinal implants consisting of MPDAs are obviously not able to stimulate the retina under ambient light conditions (19, 20). The data from our successful stimulation film experiments reveal that there might be a lack of stimulation energy when using implanted microphotodiodes only.

Proving that the retina can be stimulated and cortical potentials can be consecutively recorded after subretinal implantation of stimulation electrodes in micropigs did present certain methodologic difficulties, but these can be overcome. The micropig offers the opportunity of carrying out the necessary ongoing studies with an informative model that is transferable to humans in many respects. The micropig is therefore a valuable animal model for research in physiology of vision. In the form proposed by us, it allows electrophysiologic tests to be performed that are necessary for high-quality recording of VEPs with a comparable longitudinal pattern. This model is also of great value from a surgical point of view.

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