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Efficacy and reliability of long-term implantation of multi-channel microelectrode arrays in the optical nerve sheath of rabbit eyes

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ABSTRACT

In addition to epiretinal and subretinal areas, the optic nerve (ON) is also a candidate location for implanting visual prosthesis to restore vision of patients with retinitis pigmentosa (RP). Since the ON receives all the signals from the retina, stimulating the ON may potentially evoke phosphenes over a wider range of visual field. In this study, we designed a 9-channel microelectrode array and implanted it between the dura mater and pia mater of rabbit ONs by lateral orbitotomy. We recorded the current thresholds and evaluated the efficacy of the array using electrically evoked potentials (EEPs). Spatial discrimination of approximately 20° was verified by EEP maps over visual cortex. A large area of the visual field (over 130° along horizontal meridian) could be activated by this microelectrode array. Visual evoked potentials (VEPs) and different pathological examinations were used to examine potential damage of ONs. One year post implantation, we did not notice significant damages to either the ONs or the microelectrode arrays. EEPs were successfully recorded up to 6 months post implantations. However, further studies are still needed to reduce fibrous encapsulation of the microelectrode array, which resulted in a gradual elevation of current thresholds to elicit EEPs.

1. Introduction

Previous reports (Bosnjak & Benedicic, 2008; Brelen, Vince, Gerard, Veraart, & Delbeke, 2010; Cai et al., 2009; Wang, Li, Jiang, & Dong, 2007) have demonstrated that the optic nerve (ON) is a potential site for implanting artificial prosthesis to restore vision of patients with retinitis pigmentosa (RP). Although physiological and morphologic changes occur in the inner retinas of the affected patients (Fariss, Li, & Milam, 2000; Santos et al., 1997; Strettoi, Porciatti, Falsini, Pignatelli, & Rossi, 2002), the opportunity exists for direct electrical excitation of ON as a means of restoring vision. Brelen et al. (2010) examined visual evoked potentials (VEPs) and electroretinograms (ERG) generated during electrical stimulations of the human ON in two RP volunteers. Sakaguchi et al. (2009) implanted three wire electrodes into the optic disk of a RP patient with no light perception, and confirmed the efficacy of the electrode. Since 2005, researches concerning ON prosthesis have also been carried out by our Chinese *C-Sight Group*, which have demonstrated the efficacy of a multi-channel penetrating microelectrode arrays (Cai et al., 2009). Although the efficacy of different types of ON prostheses has been confirmed by these reports, more studies are needed to address several important issues, such as microelectrode array design, surgical approach, spatial discrimination, range of activated visual field, microelectrode array durability, and long-term pathological changes of ON after implantation.

Electrical stimulations on either pia mater or dura mater of ON could elicit electrically evoked potentials (EEPs). However, direct electrical stimulation on pia mater of ON is more efficient and requires lower current intensity and charge density (Wang et al., 2007). In general, there are two ON prosthesis designs: penetrating microelectrode arrays and contact microelectrode arrays. Since a needle-type microelectrode inserted into the pia mater of ON may cause damage to axons (Wang et al., 2007), the penetrating microelectrode array was not adopted in this research. Instead, a contact multi-channel microelectrode array made by platinum embedded in polymide was designed and implanted into the sheath of ON to contact pia mater. The purposes of this study were to evaluate the efficacy and durability of this newly designed microelectrode array and to examine long-term pathological changes of ON after the implantation.

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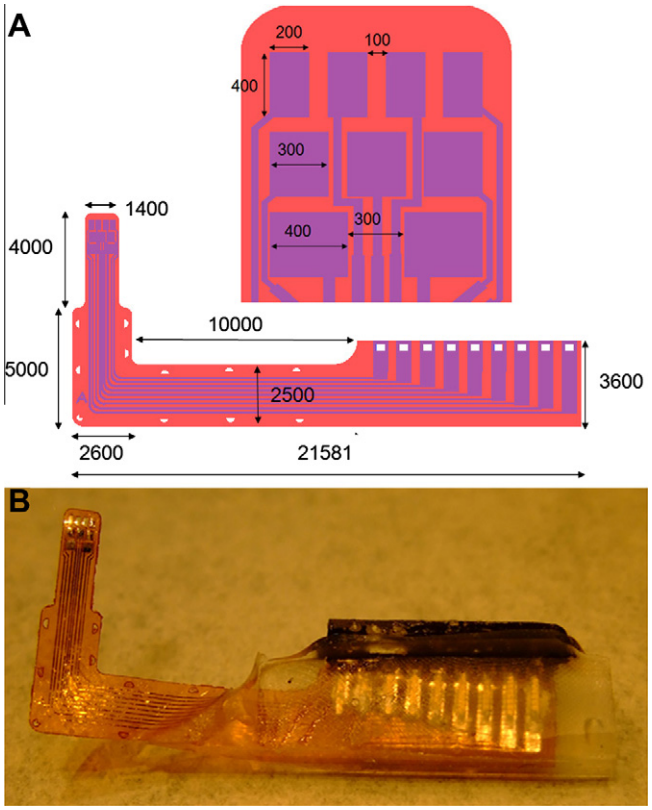

Fig. 1. Microelectrode array used in this study. (A) The design of the microelectrode array. The unit of numbers is micron. (B) A real microelectrode array sealed in silica gel.

2. Material and methods

2.1. Multi-channel microelectrode array

The microelectrode array used in this research was a 9-channel microelectrode array shown in Fig. 1. The circuit was embedded in insulating polymide, and each microelectrode and all wires within the circuit were made by platinum. The plug, which was sealed in silica gel and fixed outside the body, connected fingers of the microelectrode array using conductive rubber. Impedance of each channel was measured at different frequencies in an electrochemistry work station (Chen Hua Ltd., China), as shown in Fig. 2.

2.2. Animals and surgical approach to implant microelectrode arrays

Fifty-six adult New Zealand white rabbits (2.5–3.0 kg) were used in this study. The animals were treated according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, the Public Health Service Policy on the Humane Care and Use of Laboratory Animals (revised 1986), and the US Animal Welfare Act. Only the right eye was used in this study. All experiments were approved by and carried out under supervision of the Animal Care Committee of Peking University Health Science Centre.

Of all the 56 rabbits, eight were used to verify spatial discrimination of the microelectrode array and the other 48 were randomly assigned to six groups with eight rabbits in each for long-term observation. Of the 48 rabbits, eight rabbits served as normal control (Group 1) and the other forty rabbits were implanted with the microelectrode arrays and divided into five experimental groups (Group 2–Group 6), which represented 2 weeks, 1 month, 2 months, 6 months and 1 year after the implantations, respectively. During surgeries (implantation of microelectrode arrays or recording electrodes), the rabbits were anesthetized with an intramuscular injection of ketamine hydrochloride (32 mg/kg body weight) and xylazine hydrochloride (4 mg/kg body weight).

In order to minimize any potential damage to the ON, a superior temporal orbitotomy was performed to clearly expose the ON before putting the microelectrode array into the ON sheath. After clearance of soft tissues around the ON, a 15-degree cornea knife was used to make an incision perpendicular to the course of the ON to penetrate the dura mater, followed by separation of the dura mater and the pia mater of the ON. Then the microelectrode array was inserted beneath the dura mater to contact the pia mater. All rabbits in the experimental groups underwent this surgery. After that, the microelectrode array was fixed on the sclera of the eye ball by suturing with a 10–0 non-absorbable suture (Alcon Ltd., USA) through the holes within the circuit. Then the plug of the microelectrode array was sutured on the skin next to the outer canthus and fixed by self-curing denture acrylic (Heraeus Kulzer Dental Ltd., Germany) (Fig. 3).

2.3. Parameters for long-term electrical stimulations and electrically evoked potentials

Biphasic rectangular pulse trains with initial cathodic pulse generated by a calibrated stimulator (Master-8 vp, AMPI Ltd., Israel) were used in this study. The reference electrode used for long-term stimulation was the first microelectrode (Fig. 4E5) in the second row within the microelectrode array. The stimulation electrode was the last microelectrode (Fig. 4E7) in the second row. The number of pulses in each train was designated as 2, 4, or 6. Durations of each pulse were set at 64 μs, 128 μs, 256 μs, or 512 μs. The interval between pulse trains was 769 ms. For long-term observation, repeated electrical stimulation was carried out for 6 h on alternate days up to 1 year, and the current intensity was set at 150 μA (duration: 256 μs, number of pulses: 2). Before stimulation, electrical pulses generated by the stimulator were measured by an oscilloscope.

The cortical recording electrodes were placed over the rabbit visual cortex to contact the dura mater, which was similar to what we reported before (Wang et al., 2007). In brief, the skull was exposed at the top of the head along the midline, and five holes with a diameter of 1.25 mm were drilled into the skull. One hole, 26 mm anterior to the lambdoid suture on the midline, was used to house the reference electrode, two holes were positioned 3.5 mm anterior to the lambdoid suture and 2 mm lateral to the midline to house recording electrodes, and two more holes positioned 4.5 mm anterior to the lambdoid suture and 5 mm lateral to the midline over the visual cortex, were also used to house recording electrodes. Five silver-coated electrodes were screwed into these holes to contact the dura mater. The ground electrode (a needle type electrode) was inserted subcutaneously into the forelimb. The recording amplifier pass band was 50–300 Hz. Before electrical stimulations, the impedances of the recording electrodes were measured and the values were all less than 5 kΩ. With a 4-channel Roland RETI system (Roland Consult Ltd., Germany), one hundred cortical responses were recorded and averaged to record EEPs, and the current thresholds were recorded at different time points after the microelectrode array implantations.

2.4. Spatial correspondence of EEPs to electrical stimulation delivered by different microelectrode pairs

Spatial resolution of the microelectrode array is an important issue concerning visual prosthesis. In this research, we chose