Spatiotemporal Properties of Multipeaked Electrically Evoked Potentials Elicited by Penetrative Optic Nerve Stimulation in Rabbits

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PURPOSE. To investigate the spatiotemporal properties of the cortical responses elicited by intraorbital optic nerve (ON) stimulation with penetrating electrodes as means of designing optimal stimulation strategies for an ON visual prosthesis.

METHODS. The ON of rabbits was exposed by orbital surgery for electrical stimulation. Craniotomy was performed to expose the visual cortex contralateral to the operated eye. Electrically evoked potentials (EEPs) were recorded by an electrode array positioned on the visual cortex.

RESULTS. There were primarily four components (N1, P1, P2, P3) in EEPs with implicit times of 8.0 ± 0.6, 11.3 ± 1.3, 20.5 ± 1.4, and 26.9 ± 1.5 ms, respectively, when the ON was stimulated by penetrating electrodes. The thresholds to elicit these components were different, and the higher thresholds were seen with slower cortical components. The corresponding thresholds were 13.8 ± 3.1 μA for N1, 21.8 ± 4.7 μA for P1, 36.4 ± 11.4 μA for P2, and 68.4 ± 17.2 μA for P3. The time courses of the EEP components were also distinct. The locations of EEPs with the maximum P1 amplitude showed a spatial correspondence to the ON stimulation sites. Different profiles of cortical responses could be discriminated when the ON stimulation sites were separated by 150 μm.

CONCLUSIONS. Multiple components with different properties were elicited in EEPs when the ON was stimulated by penetrating electrodes. Retinotopic and localized stimulation could be achieved with this stimulating approach. (Invest Ophthalmol Vis Sci. 2011;52:146–154) DOI:10.1167/iovs.09-4024

The rapid development of biological microelectro-mechanical systems and biomaterials have attracted worldwide research interests in the fabrication of visual prostheses as a potential approach to restore functional vision. It is hoped that by electrically stimulating the intact parts of the visual systems of blind patients, a usable, albeit initially only partial, image may be restored at the cortical level and may be behaviorally relevant. Initially, a cortical prosthesis was put forward.1–3 However, because a large percentage of the inner retinal neurons remain intact even in patients with severe retinal degenerative diseases, such as retinitis pigmentosa (RP) and age-related macular degeneration (AMD),4,5 many considered that retinal prosthesis holds greater promise as a treatment of option.6–12 These retinal devices have been developed for epiretinal and subretinal locations. Visual prostheses based on suprachoroid,13–15 intrapapilla,16–18 extraocular,17,18 and thalamic19 electrical stimulation have also been proposed. Preliminary clinical trials by several groups8–11,20 have reported some degree of success.

Veraart et al.21 first proposed using the optic nerve (ON) and electrical stimulation as a viable alternative target in the pursuit of visual rehabilitation. A four-contact, self-sizing spiral cuff electrode was implanted around the ON of a 59-year-old blind volunteer with RP. Phosphenes and simple patterns and orientations could be discriminated after encoded electrical stimulation was delivered to the ON.22,23 Thus, the ON visual prosthesis is a potentially feasible approach for recovering partial vision.

Compared with surface electrical stimulation, penetrating electrodes have a lower threshold and better spatial resolution.24 Recently, our group proposed a new visual prosthesis based on ON stimulation with a penetrating multielectrode array.25 In this approach, the penetrating array acts as a neural interface to couple the encoded electrical stimuli to the axons of the ganglion cells local to each electrode. The ON was chosen as the stimulus target in our approach because it is relatively spared by the most prevalent degenerative retinal diseases and because phosphenes can be elicited over a large visual field area using the array. Furthermore, the ON can be accessed intraorbitally by a minimally invasive procedure without disturbing the already diseased retinal tissue.

To design optimal stimulating strategies and parameters for an ON prosthesis based on penetrative stimulation, it is crucial to investigate the spatiotemporal response properties of the visual cortex in response to ON stimulation. The response properties of the primary positive peak (P1) from electrically evoked potentials (EEPs) after ON stimulation were extensively examined in our previous study.26,27 We showed that the P1 threshold for ON stimulation was much lower for penetrating...
electrodes than for surface electrodes, and short-duration symmetrical cathode-first low-frequency biphasic current pulses were more efficacious in eliciting P1 responses.\textsuperscript{26,27} However, ON stimulation generates not only a P1 response; it also generates multiplets in the EEPs and indicates the involvement of more fibers or cortical areas during ON stimulation. We studied the spatiotemporal properties of multiplet EEPs to further refine the ON stimulus parameters that will be necessary to provide functional restoration of vision.

**METHODS**

**Animals**

Twenty-one healthy adult Chinese albino rabbits (Fengxian, Shanghai, China), each weighing 2.0 to 2.5 kg, were used in the experiments. All the experimental procedures were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the policies in the Guide to the Care and Use of Laboratory Animals issued by the US National Institutes of Health and were approved by the Ethics Committee of Shanghai Jiao Tong University.

The methods for stimulation of the intraorbital ON and recording from the primary visual cortex (V1) in rabbits have been described previously\textsuperscript{27} and will be briefly summarized.

**Surgical Procedures**

Each rabbit was anesthetized with a 5% pentobarbital sodium intravenous injection at an initial dose of 5 mg/kg, and anesthesia was maintained at 15 mg/kg/h. Body temperature was monitored and kept at approximately 39°C by a heating pad (HKWDY-III; Xinxiaoyuan Biotech Ltd., Nanjing, China). Heart rates and electrocardiograms were monitored throughout the experiments (MPA 2000; ALCBIO Ltd., Shanghai, China).

The rabbit was mounted in a stereotactic frame (model for rabbit and cat; ALCBIO Ltd.), and the scalp was partially anesthetized with 1% lidocaine before reflecting the scalp and making a craniotomy over the visual cortex contralateral to the stimulated eye. The opened area

**FIGURE 1.** Placement of stimulating and recording electrodes and theoretical estimation of the stimulated ON areas by the penetrating electrodes. (A) For cortical recording, a 4 × 4 silver ball recording electrode array with central interelectrode spacing of 3 mm was positioned epidermally to cover the contralateral visual cortex, 1 to 10.5 mm lateral to the midline and from 5 to 14.5 mm posterior to the bregma suture. The visuo-topic map of Thompson et al.\textsuperscript{30} has been modified and superimposed on the cortex and recording array. (B) The linearly arranged stimulating electrodes were inserted into the intraorbital ON of the right eye, with the first electrode approximately 1 mm behind the globe. (C) The ON fiber density map of Vaney and Hughes\textsuperscript{44} has been modified to illustrate the insertion position of the electrode. The dark gray shaded region represents the estimated ON area stimulated by the penetrating electrodes in this study. (D) Retinal ganglion cell density map of Vaney and Hughes\textsuperscript{44} has been modified with the retinal map of Thompson et al.\textsuperscript{50} The gray shaded region represents the theoretically estimated maximum retinal area corresponding to the ON area stimulated by the penetrating electrodes in this study. (E) Biphasic charge-balanced rectangular stimuli with cathode-first pulse were used to stimulate the ON. (A, D) Parts of these illustrations were modified from Thompson JM, Woolsey CN, Talbot SA. Visual areas I and II of cerebral cortex of rabbit. J Neurophysiol. 1950;13:277–288. (C, D) Parts of these illustrations were modified with permission from Vaney DI, Hughes A. The rabbit optic nerve: fiber diameter spectrum, fiber count, and comparison with a retinal ganglion cell count. J Comp Neurol. 1976;170:241–251. © 1976 The Wistar Institute Press. http://onlineibrary.wiley.com/journal/10.1002/(ISSN)1096-9861.
along the ON axis or separated by 0.15 and 0.5 mm perpendicular to the ON axis, and the position of return electrode was in the sclera. Inner and outer diameters (insulation) of each electrode measured 80 μm and 90 μm, respectively, with an uncoated sharpened tip length of 100 μm (area approximately $2.7 \times 10^{-4}$ cm$^2$). The impedance of the electrode ranged from 5 to 10 kΩ measured by a precision LCR meter (E4980A; Agilent Technologies, Santa Clara, CA) using a 100-μA, 1-kHz current.

Electrical current–stimulating pulses were generated by an isolated and programmable current source stimulator (MS16; Tucker-Davis Technologies) and were applied to the stimulating electrodes. Biphasic charge-balanced rectangular stimuli with a cathode-first pulse were used (Fig. 1E), and the pulse amplitude varied from 5 to 100 μA with the pulse duration fixed at 0.5 ms and frequency at 1 Hz. Charge density was calculated by dividing the charge per phase by the exposed surface area of the stimulating electrode.

**Electrophysiological Recordings**

A recording electrode array made up of 4 × 4 silver ball electrodes 0.3 to 0.4 mm in diameter with an interelectrode distance of 3 mm (from the electrode center) was moved by a three-dimensional micromanipulator (MPA 2000; ALCBIO Ltd.) and was placed on the exposed visual cortex (Fig. 1A). The impedance of each silver ball electrode ranged from 500 to 800 Ω, measured as described. A stainless steel needle reference electrode was inserted into the scalp of the forehead ipsilateral to the stimulated eye, and a ground electrode was placed subcutaneously in the ear tip.

V1 responses to photic and electrical stimulation were recorded on 16-channels by a TDT system (System3; Tucker-Davis Technologies, Alachua, FL). The threshold and effects of stimulating current strength on different components of the EEPs were examined and the amplitudes of first negative peak (N1) and three positive peaks (P1, P2 and P3) measured as a representation of the intensity of each component. The 16-channel EEP responses were compared to find the array channel recording the maximal N1, P1, P2 and P3 amplitude (named as the M-channel) to determine the threshold for each component.27

**Data Preprocess and Statistical Analysis**

Multichannel signals were recorded by the TDT system at a 6 kHz sampling rate for each channel, amplified, and filtered with a band-pass filter of 1 to 2000 Hz. Fifty consecutive responses were averaged. To analyze the temporal properties of EEPs with varied current strength, the amplitude, implicit time, rise time, and width-at-half-amplitude of each component at its M-channel were analyzed. To determine the spatial distribution of EEP responses, we plotted the M-channel position in relation to the recording array and superimposed it on a visual field map of the rabbit visual cortex. For analysis of the ability to spatially discriminate EEPs in response to ON stimulation, color-coded maps were drawn to compare the P1 amplitudes on the 16 channels. Results were analyzed for statistical significance by one-way ANOVA and t-test. $P < 0.05$ was considered significant for both tests.

**RESULTS**

**Electrically Evoked Potentials with Multipeaks**

Multipeaked EEP waveforms recorded from the contralateral V1 after electrical ON stimulation with the penetrating electrodes and VEP waveforms after photic stimulation are illustrated in Figure 2. The EEP waveforms consisted of several different implicit time peaks and an N1 peak followed by P1, P2, and P3 components. The implicit times of the N1, P1, P2, and P3 components were 8.0 ± 0.6, 11.3 ± 1.3, 20.5 ± 1.4, and 26.9 ± 1.5 ms, respectively, at the stimulating current of 100 μA with 0.5-ms pulse duration (mean ± SD, 15 stimulating electrode pairs, 5 rabbits). VEPs had a wave pattern similar to that seen with EEPs (Fig. 2B), though, as expected because of retinal processing, the implicit times of the corresponding components of VEPs were longer (N1, 18.2 ± 2.3 ms; P1, 26.2 ± 1.5 ms; P2, 35.3 ± 1.9 ms; P3, 46.4 ± 2.3 ms; 4 rabbits).

**Thresholds for Different EEP Components**

EEP components with different implicit times have different thresholds such that a shorter implicit time component had a lower threshold. Figure 3 illustrates a series of recorded EEPs from one animal as the stimulating current intensity was increased from 5 to 100 μA. The N1 component was first recorded with a current of 7.5 μA, and with further increases the positive components, P1 and P2, were added to the recording. In some cases, another positive wave (P0) before the N1 was sometimes observed, such as that seen in Figure 3. However, not all EEP components were seen across experiments. For example, the clear P3 component seen in Figure 2 is not seen in Figure 3. Thresholds for the P0, N1, P1, P2, and P3 components were 13.0 ± 1.1, 13.8 ± 3.1, 21.8 ± 4.7, 34.6 ± 11.4, and 68.4 ± 17.2 μA, respectively (22 stimulating electrode pairs, 9 rabbits), and the corresponding charge thresholds were 65 ± 0.5, 6.9 ± 1.5, 10.9 ± 2.4, 18.2 ± 5.7, and 34.2 ± 8.6 nC, respectively. Given the uncoated area of the stimulating electrode ($2.7 \times 10^{-4}$ cm$^2$), the corresponding charge density thresholds were 24.1 ± 1.9, 25.6 ± 5.6, 40.4 ± 8.9, 67.4 ± 21.1, and 126.7 ± 51.9 μC/cm$^2$ for P0, N1, P1, P2, and P3, respectively.

**Time Courses of Different EEP Components**

Differences among components of multipeaked EEPs included not only the thresholds and implicit times but also the time course of each component for which the rise time and width were considerably different. The definitions used to calculate rise time and width are illustrated in Figures 4A and 4B. The rise time of N1 was defined as the time difference between N1 peak and the immediately preceding positive one, and that of P1, P2, or P3 was defined as the time difference between the corresponding peak and its immediately preceding negative one (Fig. 4A). The width of N1 was defined as the duration from the point at half-amplitude of the descending phase to the corresponding point of the rising phase, and that of P1, P2, or P3 was defined as the duration from the point at half-amplitude of the rising phase to the corresponding point of the descending phase (Fig. 4B). P3 was only analyzed at current strengths of 75 and 100 μA because of its higher threshold (i.e., >50 μA). The rise times of N1 versus P1, P1 versus P2 and P2 versus P3 were all significantly different ($P < 0.05$), wherein N1 had the shortest rise time. The rise times of N1, P1, and P2 all decreased with incremental changes in
current strength between 25 and 100 μA, whereas those of P3 varied little between 75 and 100 μA (Fig. 4C; 15 stimulating electrode pairs, 6 rabbits).

Comparisons of the width for the N1, P1, and P2 components at different stimulus currents showed these to be significantly different, where N1 had the shortest duration \((P < 0.05)\). The width of P3 showed no significant difference from that of P2 between 75 and 100 μA \((P > 0.05)\). The width of the N1 response decreased slightly with increases in current strength, in contrast to P1 and P2, which first increased (25–50 μA stimulus current) but then decreased with further increases in stimulating intensity (Fig. 4D). The width of P3 showed a slight decrease from 75 to 100 μA.

**Strength-Response Curves of Different EEP Components**

To compare the dynamic variation in the amplitudes of different EEP components, strength-response curves of N1, P1, and P2 were calculated (Fig. 5). The amplitude of N1 was measured as the difference between the first negative peak and the immediately preceding positive peak or the immediately preceding positive peak of each component to reduce the variations among rabbits. All data were from 15 stimulating electrode pairs in five rabbits. Positions of data points of P1 and P2 were adjusted in the leftward/rightward direction slightly to make a clear recognition for error bar. Bars, ±SD.
ceding point with the phase jump if there was no obvious immediately preceding positive peak. The amplitude of P1 or P2 was measured as the difference between the corresponding positive peak and the immediately preceding trough. The amplitude of each component was measured at its corresponding M-channel and was normalized by the maximal response of each component to reduce variation among animals. The amplitudes of N1, P1, and P2 increased nearly linearly with incremental increases in current strength from 25 μA (12.5 nC) to 100 μA (50 nC); no significant differences were found among components at each current strength (P > 0.05; Fig. 5; 0.5-ms pulses; 15 stimulating electrode pairs, 5 rabbits). These results indicated that the dynamic ranges of N1, P1, and P2 were at least 0.6 log units (12.5–50 nC), which were in a similar range with subretinal stimulation used by Gekeler et al.29 and epiretinal stimulation used by Humayun et al.11

As shown in Figure 5, there was no obvious evidence for saturation of any response component at the 100-μA (50-nC) stimulus level. It is likely that the axons in the densest region of the stimulus current gradient have already reached saturation at much lower levels of stimulus current. However, more fibers are recruited with the incremental current. Saturation for P1 was observed at a stimulus level of 60 nC in our previous study.26

Spatial Correspondence of EEP Responses to ON Stimulation Sites

The spatial correspondence of EEP responses to ON stimulation sites was examined in this study. The faster component (P1) of the EEP responses most likely reflected the synaptic inputs generated by the faster conducting axons. The later components, such as the P2 and P3, were not as readily explained as the P1 component. Their longer implicit times and higher thresholds suggest that, in part, these may be potentials generated by slower conducting axons. The postsynaptic corticocortical connections may also be influencing these potentials. It is also unclear what effect collicular input by the thalamus might have had on these later cortical waves. Therefore, P1 was the most reliable component on which to focus the analysis of spatial correspondence. The M-channel for P1 was plotted according to its location within the recording array, which superimposed on the visuotopic representation of V1 redrawn from Thompson et al.30 (Fig. 6; 17 stimulating electrode pairs, 7 rabbits). Note that the maximal response of the P1 component was primarily along the representation of the horizontal meridian and lower visual field, as predicted by the electrode placement (Figs. 1, 6).

Spatial Discrimination of EEP Responses to ON Stimulation at Two Separate Sites

Localization of the ON stimulation is a crucial issue for a visual prosthesis to restore functional vision. We examined shifts in the location of the M-channel of P1 in response to monopolar ON stimulation from adjacent electrodes separated by 0.15, 0.5, or 1.0 mm along the ON axis or separated by 0.15 and 0.5 mm perpendicularly to the ON axis. Our results showed that two different spatial distributions of EEP responses were elicited, even with an ON electrode separation of 0.15 mm along or perpendicular to the ON axis (4 rabbits with the electrodes along the ON axis; 3 rabbits with the electrodes perpendicular to the ON axis). Figure 7 illustrates an example of EEP responses when the ON was stimulated by electrodes separated by 150 μm. The distance between the M-channel of the P1 response profiles (Figs. 7A2, 7B2) was 3 mm, corresponding to a shift of approximately 20° along the horizontal meridian and 5° along the vertical meridian of the rabbit visual field, when the ON was stimulated by two electrodes (e1 and e2) separated by 0.15 mm along the ON at the threshold (20 μA). The distance between the M-channel of the P1 response profiles (Figs. 7C2, 7D2) was 4.2 mm, corresponding to a shift of approximately 10° along the horizontal and vertical meridians, when the ON was stimulated by two electrodes (e3 and e4) separated by 0.15 mm perpendicular to the ON at the threshold (27.5 μA). The spatial extent of P1 responses increased, and the M-channel location varied sometimes as the stimulating current increased (Figs. 7A2–4, 7B2–4, 7C2–4, 7D2–4). Experimental results that the M-channel location of P1 response could not be discriminated from each other were also observed when the ON was stimulated by two adjacent electrodes of 0.15 mm along the ON (4 rabbits), which was probably due to the excitation of similar axons by this arrangement of stimulating electrodes.

Discussion

In our study, multipeaked EEPs were often recorded in the rabbit V1 when the ON was electrically stimulated with penetrating electrodes. The waveforms of EEPs were comparable to those of VEPs recorded using full-field photic stimulation, albeit with a shorter implicit time attributed to bypassing the signal processing time of the retinal neural network. Wang et al.32 also recorded EEPs with several components when the rabbit ON was electrically stimulated inside the pia mater, with implicit times of 4.36 ± 0.52 ms for P1 and 6.75 ± 0.71 ms for N1, which compared favorably with P0 and N1 implicit times of 5.0 ± 0.5 ms and 8.0 ± 0.6 ms, respectively, in the present study. However, reported waveform and implicit times show some variation, and P1 implicit times have been reported between 8 and 12 ms for intrapapilla stimulation15,16,32 and between 7 to 13 ms for intraorbital ON stimulation.26,27 Variations in EEP waveforms and implicit times between our and other studies might have resulted from the locations of stimulating electrodes and fiber populations, stimulus parameters, and recording methods.
Spatial discrimination of P1 responses in V1 elicited by two electrodes along or perpendicular to the ON with an inter electrode distance of 150 \( \mu \)m (monopolar stimulation). \( \text{(A1, B1)} \) Schematic illustration showing the two penetrating electrodes \((e1\) and \(e2)\) arranged along the ON and a return electrode in the sclera. \( \text{(C1, D1)} \) Schematic illustration showing the two penetrating electrodes \((e3\) and \(e4)\) arranged perpendicularly to the ON and a return electrode in the sclera. \( \text{(A2, B2, C2, D2)} \) The color-coded spatial distribution maps of P1 elicited by \(e1\), \(e2\), \(e3\), and \(e4\), respectively, with the current strength at thresholds \((20\ \mu\text{A})\) for \(\text{A2 and B2}\), \(27.5\ \mu\text{A}\) for \(\text{C2 and D2}\) and superimposed on the same modified visual field map as that in Figure 6, modified from Thompson JM, Woolsey CN, Talbot SA. Visual areas I and II of cerebral cortex of rabbit. J Neuropath. 1950;15:277–288. The color of each subunit substitutes the P1 amplitude on each recording channel, and the colors from \text{red to blue} stand for amplitudes from large to small. The \text{white} \(M\) in each map represents the M-channel of P1. \( \text{(A3, B3, C3, D3)} \) Color-coded spatial distribution maps of P1 with stimulating current of 50 \( \mu\text{A}\). \( \text{(A4, B4, C4, D4)} \) Color-coded spatial distribution maps of P1 with stimulating current of 100 \( \mu\text{A}\). \( \text{(A5, B5, C5, D5)} \) Estimated retinal sites from the M-channel locations elicited by \(e1\), \(e2\), \(e3\), and \(e4\), respectively, at their corresponding thresholds. \( \text{(A, B)} \) Series from one rabbit. \( \text{(C, D)} \) Series from another rabbit.

Multipeaked EEPs were also observed in response to subretinal, epiretinal, and suprachoroidal transretinal stimulation.\(^{34,35,36}\) Chow et al.\(^{35}\) recorded a negative-positive complex of EEPs with an implicit time of approximately 20 ms (extrapolated from Fig. 2) in response to subretinal stimulation in rabbits, whereas Nakauchi et al.\(^{36}\) recorded EEPs composed of three to four positive peaks with implicit times longer than 15 ms when the retina was stimulated transretinally with suprachoroidal electrodes in rabbits. Wilms and Eckhorn\(^{34,35}\) stimulated the epiretina of the cat and recorded multipeaked EEPs in visual cortex, with an implicit time of approximately 15 ms for the first positive wave. The thresholds of different EEP components varied in our study (Fig. 3) such that the component with a shorter implicit time had a lower threshold than components with longer implicit times and was in agreement with similar observations after epiretinal electrical stimulation in the cat\(^{34}\) and transretinal stimulation in the rat,\(^{44}\) although the mechanism underlying this phenomenon may be different because these studies stimulated different targets. It has been shown previously that large-diameter fibers with higher conduction velocities can be activated by a lower stimulation current than small-diameter fibers with lower conduction velocities.\(^{37}\) In the rabbit ON, at least four, and occasionally five, classes of fibers are found according to their conduction velocities.\(^{38,39}\) and although we often observe four peaks (and occasionally a fifth peak if the P0 is included) in the EEPs, not all correspond to ON velocity groups. The ON conduction velocity classes can be approximately related to the several different retinal ganglion cell types found in the rabbit retina.\(^{40–44}\) However, not all ganglion cell classes project to V1 through the dorsal lateral geniculate nucleus, and, as discussed, the later EEP components with longer implicit times are also influenced by intracortical signal- ing and by collicular input through thalamic connections.

Previous work in the cat has shown clearly that in the ON the first component (t1) is related to the large-diameter Y (\(\alpha\)) fibers and t2 is related to the medium-sized X (\(\beta\)) fibers. A t3 component, consisting of the response of medium to small W (\(\gamma\)) fibers, is sometimes seen, although more usually it is obscured by the larger overlapping t2 response.\(^{45}\) In cortical recordings after pressure blocking of the t1 ON component, Burke et al.\(^{46}\) indicated that the earliest positive wave component (C1) in V1 was caused by the presynaptic volley from Y fibers in the optic radiation (r1), and this obscured a slightly later r2 response from the X cell class (C2). They concluded that their second positive wave (C3 in their figures) is the result of postsynaptic events related to the r1 (C1) component. They also noted that the cortical response to t2 did not appear to be as large as that to the t1 and might have been mixed with responses to slower fibers or a later cortical response (C4).\(^{46}\) VEP analysis in humans has shown similar results in which the early C1 component is related to the M pathway (fast conduc- tion, Y) and the C2 component is related to the P pathway (roughly equivalent to X cells).\(^{47}\) Retinotopic correspondence and localized electrical stimulation are crucial issues for a visual prosthesis to restore pattern vision. Our results show a rough agreement between the position of the ON stimulation site and the visuotopic distribution of the M-channel of the P1 response, which was the most reliable peak component. Although we have not directly ex- amined the fiber organization of the ON, based on previous work in a number of species, the ON closest to the globe is most likely to be organized on a rough retinotopic plan such that centro-peripheral areas around the retina will project to areas around the ON (i.e., temporal retina to temporal ON) but without a clear centro-peripheral organization.\(^{48,49}\) The placement of the electrode on the temporal-dorsal side of the ON corresponded to an area that could extend from the disc to the temporal-superior periphery and was strongly biased to the horizontal meridian in the lower visual field. This appears to be in agreement with the data shown in Figure 6. The short implicit time of the P1 suggests that this may be a population.
of large Y fibers (see also the density map in Fig. 1). Indeed, the distribution of the P1 response is more restricted than would be predicted based on the maximum possible retinotopic distribution of stimulated fibers (Fig. 1), which suggests localized bundle stimulation in the ON.

The ON in humans contains approximately 1 million ganglion cell axons, which are packed into a cylinder approximately 3 to 4 mm in diameter; therefore, localization of ON stimulation is considered a challenge. Our experimental results showed that two penetrating electrodes spaced 0.15 mm apart could evoke responses in rabbit V1 with different spatial distribution when the ON was stimulated appropriately. When the stimulating electrodes were positioned perpendicularly to the ON, the spatial profiles of P1 responses could be distinguished from each other very well because of the stimulation of different regions of the cross-sectional plane of the ON. When the stimulating electrodes were placed along the ON, the M-channel location could be discriminated from each other in half the cases and could not be distinguished in the other half. Increasing the current strength increased the probability of stimulating the same groups of ON fibers (Fig. 7A2–4). However, the discriminative stimulation of different fibers could be expected by varied penetrating depths of the electrodes to avoid the stimulation of the same groups of fibers when the electrodes were positioned along the ON. Occasionally, a large shift of P1 M-channel over 100° was observed in our experiments, when the stimulating electrodes were placed as closely as 0.15 mm (data not shown). This might have resulted because of stimulating a mixture of fibers coming from central and peripheral areas of the retina (see also Ref. 49). Our experimental data indicate that ON stimulation with penetrating electrodes can achieve relatively localized stimulation and that cross-talk between two adjacent electrodes spaced as closely as 150 μm apart does not appear to be a problem. The spatial discrimination for ON stimulation with this approach could be further increased by refining the electrodes in future studies. Although the spatial resolution with ON stimulation may not be as good as that for subretinal and epiretinal stimulation, including a technique named anode block and stimulus waveform modulation.57,55,59–61 However, the selective stimulation of ON may prove a more complex task than that in the motor or sensor system given the different functionality of the ganglion cell population. How to achieve this selective stimulation of ON fibers requires further investigation.

A visual prosthesis based on ON stimulation supplies an alternative approach to restore vision for blind patients with untreatable degenerative retinal diseases such as RP and AMD.21,22,62,63 A visual prosthesis based on penetrative ON stimulation proposed by our group is expected to have a better spatial resolution and a lower threshold for stimulation than surface electrode stimulation. However, many issues should be considered because of the specific structure of ON. First, the location for implantation should be selected carefully. There is a rough topographic map around the ON head in humans, but the fibers show a variable degree of scatter with increasing distance from the ON head.69 Therefore, the implantation site of the ON prosthesis should be selected as close as possible to the globe. Second, the parameters to stimulate the ON should be designed with great care. The electric field produced by a large, above-threshold stimulus will activate a larger ON area, which will probably excite fibers arising from different visual field locations and thus degrade the likelihood of a retinotopic stimulus. This requires further study to optimize the stimulus parameters, stimulating electrode array design and image processing strategies to achieve patterned stimulation of the ON by the visual prosthesis.

In addition, the level of mechanical damage resulting from stimulating electrode array implantation and the current intensity level at which axons become injured are crucial issues to be addressed before visual prosthesis based on penetrative ON stimulation becomes a clinically applicable way for vision recovery. It is likely that the axonal injury level will be largely dependent on the size, configuration, and placement of the stimulating electrode array. The mechanical damage and safe current/charge limit of the penetrative ON stimulation will be investigated systematically in future studies with a reduced size of stimulating electrodes and optimally designed electrode array.

In conclusion, EEPs with multipkeaks could be elicited when the ON was stimulated by penetrating electrodes. The faster components might have reflected the inputs from primary afferents, and the slower ones might have been related to the combination of primary inputs through slower fibers, intracortical signaling, and collicular connections. The different components of the EEPs show variable properties, including threshold, implicit time, and time course. The M-channel location of the P1 component showed a spatial correspondence to the ON-stimulating sites. Furthermore, ON stimulation by penetrating electrodes can perform localized stimulation, and the cortical responses can be discriminated when the ON stimulation sites are separated by 150 μm.

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