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Focal damage to macaque photoreceptors produces persistent visual loss

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ABSTRACT

Insertion of light-gated channels into inner retina neurons restores neural light responses, light evoked potentials, visual optomotor responses and visually-guided maze behavior in mice blinded by retinal degeneration. This method of vision restoration bypasses damaged outer retina, providing stimulation directly to retinal ganglion cells in inner retina. The approach is similar to that of electronic visual protheses, but may offer some advantages, such as avoidance of complex surgery and direct targeting of many thousands of neurons. However, the promise of this technique for restoring human vision remains uncertain because rodent animal models, in which it has been largely developed, are not ideal for evaluating visual perception. On the other hand, psychophysical vision studies in macaque can be used to evaluate different approaches to vision restoration in humans. Furthermore, it has not been possible to test vision restoration in macaques, the optimal model for human-like vision, because there has been no macaque model of outer retina degeneration. In this study, we describe development of a macaque model of photoreceptor degeneration that can in future studies be used to test restoration of perception by visual prostheses. Our results show that perceptual deficits caused by focal light damage are restricted to locations at which photoreceptors are damaged, that optical coherence tomography (OCT) can be used to track such lesions, and that adaptive optics retinal imaging, which we recently used for in vivo recording of ganglion cell function, can be used in future studies to examine these lesions.

1. Introduction

The extreme vulnerability of photoreceptors to retinal eye disease has lead to the development of two methods for vision restoration that provide visual information directly to neurons of inner retina, bypassing degenerated photoreceptors. The first method involves optoelectronics, conveying an electrical representation of the visual stimulus to retinal ganglion cells via an array of stimulating electrodes placed above or below the ganglion cell layer, and this approach has recently been approved for therapeutic use in blind humans by the FDA (Humayun et al., 2012; Wilke et al., 2011). A second, optogenetic method, inserts light-gated channels such as channelrhodopsin into bipolar or retinal ganglion cells in order to render these cells light-sensitive and thus able to transduce visual to neural signals in place of the degenerated photoreceptors. The use of light-gated channels has proven effective in rodent animal models, restoring physiological responses to visual neurons, and pupillary and optomotor responses as well as visual guided behavior to mice previously blind due to outer retina degeneration (Bi et al., 2006; Doroudchi et al., 2011; Lagali et al., 2008). Although electronic prostheses have produced partial restoration of vision in blind humans (Wilke et al., 2011) light-gated channels may offer advantages including low cost, avoidance of potentially damaging retina surgery, and the dense spatial sampling of visual images by the many ganglion cells in primate central retina.

However, the potential efficacy of light-gated channels for restoring vision to blind humans is not understood because the rodent animal models in which this technique was developed are not ideal for studying perception. Macaques are an excellent model for evaluating human visual restoration, because, unlike

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rodents, they share the highly specialized fovea that distinguishes primate vision from that of other species (Dacey, 1994). Also, aspects of perception critical to blinded humans such as acuity, contrast sensitivity, form and motion perception can be directly measured psychophysically in macaques (Merigan et al., 1991). In this study, we describe a primate model of focal laser treatment of retina that eliminates photoreceptors over a small region with no alteration in the number of ganglion cells overlying the eliminated photoreceptors. Because macaque vision is similar to human vision, restoration of visual perception can be measured in this model, making testing of monkey vision critical to evaluation of visual prostheses. Psychophysical measures in this study show that vision was eliminated at the location of damaged photoreceptors throughout the followup test period of approximately 7 months, but not disrupted at other nearby locations, including along fiber bundles that would indicate damage to axons of ganglion cells.

The value of macaque monkeys for evaluating vision restoration by light-gated channels is heightened by our recent finding that the function of the output neurons of the retina, retinal ganglion cells, can be monitored in living macaques by adaptive optics calcium imaging (Yin et al., 2012). This advance will permit future studies to measure both loss of RGC function following photoreceptor damage and restoration of function by visual prostheses. The present study demonstrates that structural adaptive optics retinal imaging can be carried out at the site of retinal laser lesions in the same monkey that is used for psychophysical testing.

2. Methods

Subjects. Two adult macaque monkeys were used, each weighing approximately 10 kg, of age between 4 and 5 years at the time lesions were made. Lesions were placed in the left eye (LE) of monkey 1 and the right eye (RE) of monkey 2. Head-posts and scleral eye coils were implanted in both monkeys to permit precise control of fixation locus, so that visual test stimuli could be placed relative to fixation locus with accuracy of approximately 0.1 deg, as previously described (Hayes and Merigan, 2007). This precision is necessary to test central vision because of the high sensitivity and resolution of vision near the fovea. All surgery was performed under isoflurane anesthesia, and all efforts were made to minimize discomfort. The monkeys were pair housed in an AAALAC accredited vivarium, fed ad libitum with a nutritious lab chow, supplemented with fruits and vegetables such as pomegranates and corn on the cob, and were given “browse”, leaf covered tree branches, weekly. Primate enrichment included 2 pieces of manipulata daily, puzzle feeders rotated among all animals, weekly videos and rotating access to a large, free ranging space with swings, perchs, etc. They were cared for by Laboratory Animal Medicine veterinary staff under the supervision of three full-time veterinarians, two with residence training in primatology, as well as 6 veterinary technicians, who monitor the health of the monkeys and check for signs of discomfort at least twice daily. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the University Committee on Animal Resources of the University of Rochester (PHS assurance number: A-3292-01). At the conclusion of the experiments the monkeys were euthanized for histological examination of the retinas by administering an IV overdose of sodium pentobarbital (75 mg/kg), (death verified by thoracotomy) as recommended by the Panel on Euthanasia of the American Veterinary Medical Association.

2.1. Laser lesions

Using a Coherent Novus Omni laser, 12 laser lesions were made, 6 in each of the monkeys within less than ±7 deg of the fovea center: wavelength = 647 nm, duration = 0.02–0.2 s, and intensity = 100–260 mW (Table 1). An additional 3 lesions were made in the retina of monkey 1 to explore morphological changes associated with slightly larger lesions.

2.2. Fluorescein angiography (FA)

The time course of choroidal leakage of fluorescein was tracked for three lesions made at a single time in monkey 1 (lesions 7–9) and the six lesions made at a single time in monkey 2. FA was obtained with a Topcon TRC NW6 fundus camera on days 1, and 4 after placement of lesions in monkey 1 and on days 1, 3 and 5 in monkey 2. For each measure, 0.3 ml sodium fluorescein was injected intravenously and FA images taken for the next five minutes, and fluorescein leakage determined from images taken at approximately 5 min post-injection, when arterial and venous retinal circulation are substantial, and choroidal leakage is prominent.

2.3. Optical coherence tomography (OCT)

OCT images of retinal lesions were obtained with a Zeiss spectral domain OCT (Cirrus) approximately 24 h, 2 or 3 months, and after 6 months in the two monkeys.

2.4. Visual thresholds

The seated monkey faced a 17-inch color monitor, illustrated in Fig. 1, (Nanao, T560i), of illuminance 1.2 cd/m^2, which used only the green gun, at a distance of 57 cm. A small black fixation spot was presented at the center of the monitor, and contrast thresholds were measured for discriminating the orientation of small patches of vertical or horizontal grating displayed on the monitor. The grating targets were Gabor functions (cosinusoidal gratings multiplied by horizontal and vertical Gaussian weighting functions). The

Table 1
Description of the 15 individual laser lesions examined in this study. Lesions 1 – 6 in each monkey were made on one occasion, and then lesions 7 – 9 in monkey 1 were placed 16 months later. Power levels ranged from 100 to 260 uW, and durations of laser exposure from 10 to 200 ms, parameters that are common in clinical treatment for diabetic retinopathy or retinal edema. The diameter of the laser beam was set to 200 and 250 μm, but actual initial lesion size estimated from fundus photos ranged from approximately 250 to 420 um.

Lesion	Lesion parameters			Lesion size (μm)			
	Diameter	Power	Duration	Energy	Fundus	ONL	ONL
	(μm)	(mW)	(ms)	μW/sec	Image	OCT	Histology
Monkey 1							
1	200	260	10	2.6	254	80	57
2	200	200	20	4	329	136	78
3	200	100	100	10	358	112	60
4	200	200	20	4	336	136	95
5	200	100	50	5	291	160	129
6	200	100	50	5	306	176	90
7	250	100	200	20	390		73
8	250	100	100	10	419		279
9	250	100	200	20	375		127
Monkey 2							
1	200	200	20	4	314	136	66
2	200	100	100	10	388	181	138
3	200	100	50	5	343	115	116
4	200	200	50	4	261	146	70
5	200	100	200	20	418	284	230
6	200	260	10	2.6	0	0	0