Published in final edited form as:

Ann Biomed Eng. 2018 September ; 46(9): 1406–1418. doi:10.1007/s10439-018-2059-x.

Eye movements evoked by pulsed infrared radiation of the rat vestibular system

Weitao Jiang¹ and Suhrud M. Rajguru^{1,2,*}

¹Department of Biomedical Engineering

²Department of Otolaryngology

Abstract

Light at infrared wavelengths has been demonstrated to modulate the pattern of neural signals transmitted from the angular motion sensing semicircular canals of the vestibular system to the brain. In the present study, we have characterized physiological eye movements evoked by focused, pulsed infrared radiation (IR) stimuli directed at an individual semicircular canal in a mammalian model. Pulsed IR (1863nm) trains were directed at the posterior semicircular canal in a rat using 200–400µm optical fibers. Evoked bilateral eye movements were measured using a custom-modified video-oculography system. The activation of vestibulo-ocular motor pathways by frequency modulated pulsed IR directed at single posterior semicircular canals evoked significant, characteristic bilateral eye movements. In this case, the resulting eye movements were disconjugate with ipsilateral eye moving upwards with a rotation towards the stimulated ear and the contralateral eye moving downwards. The eye movements were stable through several hours of repeated stimulation and could be maintained with 30+ minutes of continuous, frequency-modulated IR stimulation. Following the measurements, the distance of the fiber from target structures and orientation of the beam relative to vestibular structures were determined using micro-computed tomography. Results highlight the spatial selectivity of optical stimulation. Our results demonstrate a novel strategy for direct optical stimulation of the vestibular pathway in rodents and lays the groundwork for future applications of optical neural stimulation in inner ear research and therapeutic applications.

Keywords

Infrared radiation; infrared neural stimulation; optical stimulation; vestibular system; eye movements

Introduction

Neural stimulation with light is not a novel concept, but has gained momentum over the last decade shaping the field of neuroscience. Optical control of neural activity with cell-specific depolarization or silencing [1–7] shows promise both as a research tool and for development

*Corresponding Author: Suhrud M. Rajguru, PhD, 1251 Memorial Drive, #MEA 210, University of Miami, Miami, FL 33146. s.rajpguru@miami.edu, Phone: 305-284-5434.

Disclosure/conflict of interest: The authors declare no competing financial interests.

of optical neuroprosthetics. Optogenetics and thermogenetics [5, 8] rely on expressing light- or temperature-sensitive channels in the target cell to control its excitation or inhibition. In contrast, infrared (IR, typical wavelengths between 1400-1600 nm and 1840-2100 nm) or near infrared radiation (NIR, 790-850 nm) allow selective stimulation of cells without genetic or pharmacological modification [9]. IR and NIR have been previously shown to consistently elicit both *in vivo* and *in vitro* cell activation. IR wavelengths have been applied *in vitro* to study intracellular responses evoked and contributions of specific organelles to the responses [10-13]. Research studies also suggest that transient cellular responses could be elicited in peripheral and cranial nerves [14-16], vestibular hair cells [3], rat sciatic nerve [9], auditory nerve [14], and quail embryo hearts [17], vestibular nerve [18].

When applied in the inner ear, IR may have potential for significant impact as a scientific tool and possibly as a means of prosthetic stimulation. Photostimulation with IR does not require pharmacological or genetic manipulation of target cells, it elicits no electrical stimulation artifact, and it allows line-of-sight targeting that might result in greater spatial selectivity than possible with electrical stimulation. However, effects of IR stimulation *in vivo* reported to date have been mixed: for example, IR stimulation is reported to evoke action potentials in cochlear neurons [19] and vestibular axons [20], but to decrease action potential activity in some hippocampal neurons [21]. Studies have demonstrated that the vestibular neuroepithelium is highly sensitive to 1862nm pulsed IR stimulation with diverse inhibitory, excitatory and phase-locked afferent responses [20, 22]. This diversity in afferent responses raises the questions of practical usefulness of IR stimuli and whether focused photostimulation of vestibular neuroepithelium via pulsed IR can evoke physiological responses.

The present study is focused on the application of IR stimulation in the rat vestibular system and characterizing the physiological eye movements evoked by direct infrared stimulation of vestibular end organs. The study draws on research by [23-30] to deliver unilateral IR optical stimuli at 1863nm using single optical sources and characterizes eye movements evoked using a video-oculography system. Systematic manipulations of the optical fiber position and IR stimulation parameters were used to evaluate the evoked responses. Post-stimulation micro-computed tomography was used to determine the vestibular end organ stimulated by determination of the orientation of the optical fiber.

Methods

Animal preparation and surgical approach

Care and use of animals were carried out within guidelines of the NIH Guide for the Care and Use of Laboratory Animals and were approved by the University of Miami Institutional Animal Care and Use Committee (IACUC). Long-Evans rats (Charles River Laboratories) of either sex were used in the experiments. Animals were anesthetized by an intraperitoneal injection (i.p., ketamine (44 mg/kg) and xylazine (5 mg/kg). The depth of anesthesia was assessed every 15 minutes with a paw withdraw reflex and anesthesia was maintained by supplemental doses as required. The core body temperature of the animals was maintained between 37-38°C with a heating blanket. A cuffed-based blood pressure and heart rate measuring system was used to continuously monitor the animal's vital signs.

Once the animals were anesthetized, the frontal bony skull was surgically exposed and two screws were anchored into the skull. A custom-designed head post, which was attached to the stereotactic frame, was lowered to the level of skull and fixed to the animal's head with dental acrylic. Once the dental acrylic cured, the head of the animal was secured in the stereotactic frame. The inner ear was accessed via a surgical bullotomy after a C-shaped skin incision was made behind the right ear lobe, the cervicoauricular muscles removed by blunt dissection and the cartilaginous outer ear canal was exposed. The exposed right bulla was opened approximately 1.5×2mm with a motorized drill. The basal turn of the cochlear and the round window were identified. An optical fiber, mounted on a micromanipulator was inserted through the opening of the bulla into the round window (experimental setup is highlighted in Figure 1).

Infrared stimulation

Pulsed IR stimulation was generated by a diode laser (Lockheed Martin Aculight Corp., Bothell, WA). Wavelength, pulse duration, pulse frequency and pulse energy of the laser were modulated. For the present experiments, the wavelength was selected at 1863 nm the pulse duration at 200 μ s, and the baseline pulse frequency at 250 pps. The laser was coupled to polished optical fibers with core diameters of 200 μ m. The pulse energy of the laser was controlled directly by varying the current to the laser diode. The energy per pulse at the tip of the optical fiber ranged between 53 and 109 μ J/pulse at 200 μ s pulse duration as measured in air (between 1.75 and 3.47 mJ/mm²). As discussed previously [31] it is not possible to directly measure the radiation energy delivered to the target structures. The IR energy values reported in the study were measured at the tip of the optical fiber in air and may vary depending upon the distance from the target structure and absorbers (including inner ear fluids) in the optical path. The penetration depth for the lasing wavelength used in the present study in water is approximately 0.8 mm [e.g. 32]. In three experiments, a custom-made 3-channel fiber bundle was used to explore the spatial selectivity of INS. The fiber bundle consisted of three 200 μ m fibers glued along their lengths near the flat polished end placed through the round window.

Pulsed IR were delivered in two modes: continuous pulsed IR trains or frequency modulated (FM) pulsed IR stimuli generated with the diode laser controlled by external function generators (Tektronix, mode CFG250, AFG320) –

$$f = f_0((1 - a) + a\sin(2\pi f_s t))$$

where $f_0 = 250$ Hz is the baseline frequency, $f_s = 0.05$ -1Hz modulation frequency and a is the dynamic range from 0 to 1.

Eye movement recordings and analysis

We used video-based eye tracking system ETL-200 (ISCAN Inc., Woburn, MA) to track and record the evoked eye movements (Figure 1A). The eye-tracking system consists of two IR cameras, two LED IR illuminators and a PC with eye-tracking software installed. The IR camera had the ability to capture videos at 120 frames/s or 240 frames/s (512 pixels 256 pixels). In our system, images (512 pixels 256 pixels) were captured at a sampling rate of

240 Hz. The eye-tracking system recorded position of the center of pupil (P) and center of cornea reflection (CR) (Figure 1B). As discussed by Stahl *et al* [33] in detail, the ISCAN video system outputs signals proportional to the linear positions (P and CR) and need to be converted to angular rotation of eye ball. Following methods outlined [33], we determined R_p , the radius of rotation of the pupil, for rats in 5 animals (Table 1). After the experimental recordings, the position difference (PCR) of pupil center (P) and cornea reflection center (CR) was converted to eye angular rotation in horizontal and vertical direction in MATLAB, Mathworks, MA using the previously calibrated R_p .

Reconstruction of the beam path and target structures

Following measurements of the evoked eye movements during the IR stimulation experiments, animals were euthanized with high dose of Euthasol™ (0.5-2 ml). Using dental acrylic (Lang Dental Manufacturing, Jet Denture repair package), the fiber was secured in its final position. After the dental acrylic cured, the fiber was cut towards the diode end. The inner ears were then fixed in 4% paraformaldehyde overnight and subsequently imaged using a Micro-CT scanning machine (Skyscan 1176 Micro Photonic Inc, Allentown, PA). The tomographic images were imported in Osirix Lite (Pixmeo, Swiss) for 3-D reconstruction, low pass filtered and the fiber orientation relative to the vestibular structures, particularly the posterior canal, was determined.

Results

A total of 10 rats were used for the study. For the present discussion, the animals are divided into two subsets: one where only ipsilateral eye movements were recorded (n=5, Figures 2-6) and another where bilateral eye movement recordings were carried out (n=5, Figures 7,8). The initial data set was obtained with a video-oculography system that had a single camera whereas for the subsequent studies, the system was updated and modified to allow simultaneous, bilateral eye movement recording. The stimulation parameters are detailed for each case below. For the continuous pulsed IR stimulation experiments, the eye movements reported below are the maximum excursion of the eye from the starting position. For the frequency-modulated, pulsed IR stimulation experiments, the eye movements are reported based on the amplitude of the sinusoidal movement (half of peak-to-peak deflection).

IR-evoked ipsilateral eye movements

In 5 rats, we measured ipsilateral eye movements using continuous and frequency-modulated pulsed IR stimulation of the targeted posterior canal. The animals were not physically rotated. Figure 2 shows one example of the ipsilateral (right) eye movement during continuous IR stimulation (1863nm, 200μs and 250Hz) directed towards the right posterior canal. Panel A shows one example of the eye angular position change in both the horizontal and vertical directions in response to IR pulses at 109 μJ/pulse (3.47mJ/mm²). Overall, there was a rotation of the eye towards the contralateral ear, with a vertical, upwards component. Following cessation of IR stimulus, the eye returned to center with a gradual decay that is similar to the classic feature of velocity storage. Velocity storage integrator reflects central processing mechanisms proposed by Raphan et al [34, 35], that prolongs the peripheral vestibular (canal) signal and decays the vestibulo-ocular reflex slower than

expected from semicircular canal dynamics. The time constant is known to vary for different species and may be affected by habituation and/or head position. Panels 2B1 and 2B2 show the trajectory of the eye prior to, during and after IR stimulation. During the stimulation, magnitude of the horizontal eye position change was $31 \pm 1.73^\circ$ over $n=5$ at $109 \mu\text{J/pulse}$ while the magnitude of the vertical position change was $6.3 \pm 1.53^\circ$ over $n=5$ at $109 \mu\text{J/pulse}$ which is significantly different than 0 ($p < 0.05$).

Figure 3 shows the magnitude of eye movements evoked in one animal at different radiant exposures. The top panel (3A1-A3) show the evoked eye movements to three pulse trains at $55 \mu\text{J/pulse}$ (1.75 mJ/mm^2), $77 \mu\text{J/pulse}$ (2.45 mJ/mm^2) and $93 \mu\text{J/pulse}$ (2.96 mJ/mm^2) respectively. The bottom panel (3B1-B3) shows evoked responses from three trials at $109 \mu\text{J/pulse}$ (3.47 mJ/mm^2). In each case, the eye movement followed a characteristic pattern described above – a clockwise rotation (as seen by the observer) of the eye towards the contralateral ear, with an upwards component. During the stimulation, magnitude of the horizontal eye position change varied between 10° at $55 \mu\text{J/pulse}$ to 31° at $109 \mu\text{J/pulse}$ while the magnitude of the vertical position change was 0° at $55 \mu\text{J/pulse}$ to 7° at $109 \mu\text{J/pulse}$. The evoked responses could be elicited multiple times and at different radiant exposures over the duration of the experiment (between 4-8 hours). Overall, the magnitudes of horizontal and vertical eye movements were ($n=5$): H $13.1^\circ \pm 8.1^\circ$, V $2.2^\circ \pm 1.5^\circ$ ($55 \mu\text{J/pulse}$), H $17.4^\circ \pm 8.7^\circ$, V $2.8^\circ \pm 1.4^\circ$ ($77 \mu\text{J/pulse}$), H $21.2^\circ \pm 6.0^\circ$, V $4^\circ \pm 1.7^\circ$ ($93 \mu\text{J/pulse}$), and H $22.8^\circ \pm 5.5^\circ$, V $4.7^\circ \pm 1.1^\circ$ ($109 \mu\text{J/pulse}$).

Figure 4 shows the ipsilateral responses to pulsed IR applied at two modulation frequencies (0.1Hz and 0.2Hz). The baseline frequency was 250Hz. Figure 4A shows the ipsilateral eye movement elicited by frequency modulated IR stimuli at 0.1Hz, $109 \mu\text{J/pulse}$ and dynamic range of 0.9 (modulation between 50-450Hz) over a period of 60s. In this case, the ipsilateral eye instantaneously had the characteristic rotation, a clockwise rotation towards the contralateral ear with a vertical component, followed by sinusoidal rotation. Figure 4B shows the resulting curve-fits for the horizontal and vertical eye position change at 0.1Hz with an R^2 value of 0.94 and 0.86 respectively. The horizontal and vertical peak-to-peak amplitudes across remained consistent across the five cycles, between $3.2\text{-}4.0^\circ$ and $0.8\text{-}2.4^\circ$ respectively.

Figure 4C shows an example of ipsilateral eye movement response to IR stimuli at modulation frequency of 0.2 Hz, $109 \mu\text{J/pulse}$ and dynamic range of 0.9 in the same rat. In this case, there was a 2.5s latency between the IR stimuli and the evoked eye movement. The horizontal and vertical peak-to-peak amplitudes across the frequency-modulated IR cycles were between $0.5\text{-}1.1^\circ$ and $0.3\text{-}0.7^\circ$ respectively. Figure 4D shows the resulting curve-fits of the vertical and horizontal position change. The R^2 value of horizontal fitting was 0.91 and the R^2 value of vertical fitting was 0.84.

Effect of IR parameters on evoked eye movements

Figure 5 shows the effects of different IR parameters (modulation frequency, energy per pulse and dynamic range) on the evoked sinusoidal eye position changes and velocities for 5 animals. Figure 5A shows the effect of modulation frequencies between 0.05-0.5 Hz to the sinusoidal eye movement. The change in horizontal eye position ranged between $2.5^\circ\text{-}6^\circ$ at

0.05 Hz to 0.3° - 1.0° at 0.5 Hz while that of vertical eye position ranged between 1° - 4° at 0.05 Hz to 0.0° - 0.9° at 0.5 Hz. The velocity of horizontal eye movement ranged between $0.6^{\circ}/s$ - $1.6^{\circ}/s$ at 0.05 Hz to $0.2^{\circ}/s$ - $1.2^{\circ}/s$ at 0.5 Hz. The velocity of vertical eye movement ranged between $0.8^{\circ}/s$ - $2.0^{\circ}/s$ at 0.05 Hz to $0.2^{\circ}/s$ - $1.2^{\circ}/s$ at 0.5 Hz. Figure 5B shows the dose-response curve for the effect of pulse energy on the magnitude of eye position change and velocity (modulation frequency = 0.2 Hz dynamic range = 0.9). In the horizontal direction, the magnitude of eye position change varied between 0° - 0.5° at $55 \mu J/pulse$ to 0.5° - 1.7° at $109 \mu J/pulse$, while the velocity of eye movement increased from $0^{\circ}/s$ - $0.6^{\circ}/s$ at $55 \mu J/pulse$ to $0.20^{\circ}/s$ - $1^{\circ}/s$ at $109 \mu J/pulse$. We could not evoke detectable eye movements below $55 \mu J/pulse$ in the rats. In the vertical direction, the magnitude of eye position change varied between 0.1° - 0.3° at $55 \mu J/pulse$ to 0.2° - 0.6° at $109 \mu J/pulse$, while the velocity increased from $0.1^{\circ}/s$ - $0.2^{\circ}/s$ at $55 \mu J/pulse$ to $0^{\circ}/s$ - $1.2^{\circ}/s$ at $109 \mu J/pulse$. Figure 5C shows the effect of dynamic range on the magnitude and velocity (modulation frequency = 0.2 Hz and radiant energy = $109 \mu J/pulse$). In the horizontal direction, the magnitude of eye position change increased from 0.1° - 0.5° at dynamic range of 0.6 to 0.45° - 0.9° at dynamic range of 1, and velocity increased from $0.1^{\circ}/s$ - $0.4^{\circ}/s$ at dynamic range of 0.6 to $0.45^{\circ}/s$ - $1^{\circ}/s$ at dynamic range of 1. In the vertical direction, the magnitude eye position change increased from 0.1° - 0.4° at dynamic range of 0.6 to 0.1° - 0.9° at dynamic range of 1, and the velocity increased from $0.2^{\circ}/s$ - $0.4^{\circ}/s$ at dynamic range of 0.6 to $0.3^{\circ}/s$ - $1^{\circ}/s$ at dynamic range of 1.

Figure 6 shows the magnitude evoked eye velocities over a period of 30 minutes of continuously applied frequency-modulated IR ($55 \mu J/pulse$, $200 \mu s$, 250 Hz, modulation frequency = 0.2 Hz and dynamic range of 1). The data reported as mean $\pm$ S.D. were collected from 5 rats. The left panel shows eye velocities in both vertical and horizontal direction over the 30 minutes of IR stimulation. The eye velocity magnitude was sampled every 5 minutes over this duration. At 5 minutes, the magnitude of horizontal eye movement velocity was $0.6 \pm 0.3^{\circ}/s$, and the magnitude of vertical velocity was $0.3 \pm 0.2^{\circ}/s$. At 25 minutes, the magnitude of horizontal eye movement velocity dropped to $0.3^{\circ} \pm 0.1^{\circ}/s$, and the magnitude of vertical velocity to $0.2^{\circ} \pm 0.1^{\circ}/s$. The right panels A1 and A2 show example sinusoidal eye movements entrained by IR stimulation at 5 minutes and 25 minutes in one animal. At 5 minutes, the magnitude of horizontal eye position change was 0.9° , the magnitude of horizontal eye velocity was $1.1^{\circ}/s$, the magnitude of vertical eye position change was 0.2° , and the magnitude of vertical eye velocity was $0.2^{\circ}/s$. At 25 minutes, the magnitude of horizontal eye position change was 0.4° , the magnitude of horizontal eye velocity was $0.5^{\circ}/s$, the magnitude of vertical eye position change was 0.3° , and the magnitude of vertical eye velocity was $0.4^{\circ}/s$.

Bilaterally measured eye movements

Figure 7 shows the bilateral eye movements evoked during continuous (7A,B) and frequency modulated (7C,D) IR stimuli at 1863 nm. The baseline pulse repetition rate was 250 pps and pulse duration was $200 \mu s$. For the example shown, the evoked responses were elicited multiple times over a duration of 2 hours. Figure 7A,B show the bilateral eye movement during the continuous IR stimuli at $109 \mu J/pulse$. The ipsilateral eye (right, 7A) rotated towards the contralateral ear (clockwise torsion and inward deflection away from the stimulated side), with an upwards component. The total magnitude of horizontal position

change was $\sim 45^\circ$ and the vertical position change was 8° . The contralateral (left, 7B) eye primarily deflected downwards followed by an outward deflection. The magnitude of horizontal position change was 20° and the magnitude of vertical position change was 8° . Figure 7C,D show the bilateral eye movements evoked by 0.1 Hz frequency modulated IR (1863nm and dynamic range=0.9 with radiant energy of 93 $\mu\text{J}/\text{pulse}$). The ipsilateral eye showed a significant clockwise, inward torsional movement accompanied by the sinusoidal pattern that matched the INS modulation frequency. The magnitude of horizontal deflection was 15° and the magnitude of vertical deflection was 7° . In contralateral side, the eye deflected downwards and away from the nose also accompanied by the sinusoidal pattern. The the magnitude of downward deflection was 6° , which followed a lateral deflection of $\sim 25^\circ$. The trajectories of each eye during the low frequency cycle are highlighted in the insets (7C1 and D1) along with the overall stimulation patterns (7C2 and D2).

Beam path and site stimulation

To determine the orientation of the optical fiber and resultant beam path as well as to confirm the spatial selectivity of IR stimulation, the inner ear of each rat was imaged using microcomputer tomography (Micro-CT) following the experimentations. Figure 8 shows reconstructed images (using Osirix and Image J) acquired via the Micro-CT. Figure 8A, B show in two different orientations from an example inner ear with a 200 μm fiber inserted and fixed in place. The vestibular organs and cochlea were imaged and are labeled with the fiber highlighted in red. The fiber was observed pointing towards the base of the posterior canal ampulla. In this case, the distance between fiber and posterior canal was $\sim 300\mu\text{m}$ (one and a half diameter length of the fiber). Figure 8C shows the Micro-CT images from three more animals (R1-R3). For clarity, the posterior canal ampullary region are highlighted along with the orientation of the optical fiber. Figure 8D, E show the imaged inner ear structures from a rat in which a custom-made optical fiber bundle with three 200 μm fibers was inserted. In these experiments ($n=3$), the three fibers were used sequentially to deliver frequency-modulated pulsed IR stimulation. We successfully evoked strong and clear eye movement in only one of the three fibers in each of the experiments. The fiber for which we successfully evoked sinusoidal eye movement is highlighted in red. The inset in each figure shows a closeup of the posterior canal ampullary region along with the highlighted fiber. Although the orientation of the fiber bundle was towards the posterior canal side, only the highlighted fiber could be seen directly pointed towards the basal portion of the posterior canal ampullary region.

Discussion

The present study shows, for the first time, that focused, pulsed optical (infrared wavelength) stimuli delivered selectively to the vestibular neuroepithelium in a mammalian system can evoke physiologically-relevant eye movements. With the current surgical approach, it was possible to deliver optical stimuli to a single vestibular endorgan. In addition, low frequency-modulated IR evoked in-phase eye movement that could be entrained and evoked multiple times during an experiment as well as continuously over a period of 30 minutes or more.

Eye muscle activities, eye movements as well as head and body motions during electrical stimulation of semicircular canal nerve(s) have been characterized previously [23-26, 29, 36-41]. Stimulation of a unilateral posterior canal stimulation, ipsilateral superior oblique and contralateral inferior rectus muscles are expected to elicit the primary contractions, with ipsilateral inferior rectus and/or lateral rectus as well as contralateral inferior oblique expected to elicit weaker responses. The observed eye movements here as well as the time courses are consistent with this expected pattern of eye muscle activity. Following IR stimulation, the bilateral eye movement in each animal in the current study was consistent – the ipsilateral eye always rotated towards the contralateral ear while the contralateral eye primarily deflected downwards (Figs. 2, 7). A smaller upward component in the ipsilateral eye along with a small counterclockwise rotation of the contralateral eye were indeed observed in a majority of the animals (Figure 2). Previous studies have shown that the insertion points and lines of actions of the extraocular muscles are markedly different in lateral-eyed vs front-eyed animals, suggesting differences in resulting eye movements evoked by their contraction. While the primary component of the motion elicited by contraction of the superior oblique is expected to be in-torsion in both the lateral- or front-eyed animals, the secondary action has been shown to be different. For example, in humans the secondary action was observed to be depression and abduction, that in a lateral-eyed animal (rabbit) was elevation and adduction. During the frequency-modulated IR stimulation, the in-phase sinusoidal rotation of the eyes was superimposed on the primary or characteristic eye movement observed from constant rate stimulation. The ipsilateral eye showed a significant inward rotation while continuing to elicit a combination of rotation and adduction during IR cycles. The contralateral eye elicited a cyclic motion in the vertical plane even as the eye rotated off-axis in the counter-clockwise direction. The magnitude of the deflection was much larger than the magnitude of the sinusoidal or cyclic rotation, with evidence during the short-time stimuli used in this study of adaptation. Long-term, chronic experiments are needed to quantify not only any adaptation effects but potential tissue-damaging IR thermal effects.

The potential effects of chronic general anesthesia used in our experiments on the magnitudes of eye movements and the absence of nystagmus must be considered. The anesthetics used in our study are known to significantly suppress the nystagmus and the magnitude of eye movement during stimulation [42-44]. It has been shown that anesthesia influences the reactions to electrical stimuli [45, 46]. These studies showed changes in threshold, a reduction in amplitude of eye movements, and time courses that are similar to observed here in the rodent model. The time constants may also be further affected by habituation and/or static head position used in our study. Future chronic experiments in awake, clinically normal animals as well as animals treated with intratympanic gentamicin to bilaterally ablate vestibular hair cell mechanosensitivity [47] are needed to clarify if IR optical stimulation can evoke eye movement responses of magnitudes similar to typical motion or electrical stimuli.

The site of stimulation was confirmed using micro-CT imaging (Figure 8), as in the previous study [31]. The orientation of the optical fiber confirmed that the beam path primarily targeted the posterior canal ampullary region. The eye movements across animals were consistent, however small variations in the magnitudes of eye movements evoked across

animals were observed (Figure 5). These are likely due to small differences in the orientation of the fibers (Figure 8, A–C). Additionally, when using the custom-made three-fiber bundles, IR directed through only one of the fibers evoked a strong eye movement (Figure 8, D–F). We did not observe eye movements when IR was coupled to either of the other fibers. The three fibers were parallel and within ~200–300µm of each other, suggesting the evoked, physiological eye movements were dependent upon the beam path and not a result of whole-organ temperature changes. Since long-wavelength IR is heavily absorbed by water and the tissue, an elevation of baseline temperature of the endolymph is expected. The accumulated increase in endolymph or neuroepithelium temperature has been measured to be <2.5°C [20, 22]. These results are consistent with our previous study [22] where we compared the changes in temperature and afferent responses evoked from the post-synaptic afferents while irradiating the sensory epithelium directly with IR or while delivering IR with a second optical fiber (see Figure 1, experimental setup in [22]). Individual afferents responded robustly to direct IR of the sensory epithelium (Figure 4A and B, [22]), consistent with the strong, evoked eye movements in the present study. However, identical temperature increases generated by heating the crista ampullaris failed to evoke similar afferent responses in the previous study. For a direct comparison, we measured the temperature change at the site of IR stimulation using wired microthermistors (via the round window approach, n=2). For IR pulse radiant energies between 74.6 to 120.3 µJ per pulse, the temperature increase was 0.2–0.7°C above the baseline (~37°C). This temperature change is not sufficient to generate a warm-caloric like static drift of the eye. In the same animals, raising the temperature of the inner ear using a constant warmed saline perfusion to 41°C did not evoke eye movements. The warm-caloric responses were only observed for round window temperatures >43.5°C. IR stimulation continued to evoke consistent and characteristic eye movements expected from unilateral posterior canal stimulation at 37.2°C, 41°C and 44°C (data now shown). Collectively, these results suggest that direct irradiation of the sensory epithelium is required to elicit the eye movement responses summarized here.

IR is known to evoke a variety of responses in the vestibular afferents: previous studies have shown from different animal models that IR-evokes excitation, inhibition, and phase locking [20, 22]. It has also been demonstrated that trains of infrared (IR) irradiation cause an elevation of cytosolic (and nuclear) $[Ca^{2+}]$ in cultured cardiomyocytes [13], in neurons and glia in the rat brain in vivo [48], and in neural and glial cell lines [49]. We also have evidence that IR-induced $[Ca^{2+}]$ increase in spiral ganglion neurons is caused by the IR photothermal effect, and that much of this $[Ca^{2+}]$ increase represents Ca^{2+} -induced Ca^{2+} -release (CICR) from the endoplasmic reticulum (ER) ([50], *manuscript in review*). Additional evidence suggests that the $[Ca^{2+}]$ increase that initiates CICR results from activation of warm-sensitive TRP channels in the ER. The effects of this IR-induced increase in Ca^{2+} on neural excitability (or inhibition) are yet to be detailed and may limit potential applications of this stimuli in the inner ear. In chinchilla vestibular hair cells, IR pulse trains produced a brief period of increased excitability followed by a longer period of decreased excitability [20]. The decreased excitability was shown to be a result of activation of large-conductance Ca^{2+} -activated K^+ (BK) channels likely activated by this intracellular change in $[Ca^{2+}]$. While, the specific target and mechanism of IR-activated eye movements remain to be identified, it is likely that vestibular hair cells can modulate their neurotransmitter release

in response to IR induced $[Ca^{2+}]$ change. Recently, IR-evoked auditory brainstem responses from the activation of the auditory nerve in Neurog1 knockin mice as well as mice lacking the vesicular glutamate transporter-3 (VGLUT3^{-/-}) have been reported [51]). These mice are known to be congenitally deaf due to loss of glutamate release at the inner hair cell afferent synapse. Results from this study as well as Rabbitt *et al.* [20] suggest that IR optical stimuli targeting primarily hair cells or ganglion neurons can yet find clinical utility under some conditions [52-54].

Conclusions

Despite rapid and substantial progress made towards developing vestibular neuroprostheses to aid patients, significant limitations remain with the contemporary techniques used to probe and control neural function. Optical stimuli do not require physical contact with target tissue and are particularly attractive for applications in the inner ear given their spatial selectivity. We demonstrate the modulation of an important physiological pathway in response to focused, pulsed infrared optical stimulation of individual semicircular canals. Given recent developments, in optical stimulation techniques [7] and available tools, such as waveguides, VCSELs, tapered optical probes, one may be able to build upon our results and develop effective uses of IR and other optical stimuli in the inner ear in the future. It is not difficult to imagine applications of optical neural stimulation to control of neural activity in the inner ear, study the role of vestibular labyrinth in maintaining physiological functions and in pathology and the development of potential therapeutic applications, including bionic vestibular neuroprostheses.

Acknowledgments

Funding: This work was supported by R01DC013798 (SMR).

References

1. Rein ML, Deussing JM. The optogenetic (r)evolution. *Mol Genet Genomics*. 2012; 287(2):95–109. [PubMed: 22183142]
2. Boyden ES, et al. Millisecond-timescale, genetically targeted optical control of neural activity. *Nat Neurosci*. 2005; 8(9):1263–8. [PubMed: 16116447]
3. Rajguru SM, et al. Infrared photostimulation of the crista ampullaris. *The Journal of Physiology*. 2011; 589(6):1283–1294. [PubMed: 21242259]
4. Fenno L, Yizhar O, Deisseroth K. The development and application of optogenetics. *Annu Rev Neurosci*. 2011; 34:389–412. [PubMed: 21692661]
5. Bernstein JG, Garrity PA, Boyden ES. Optogenetics and thermogenetics: technologies for controlling the activity of targeted cells within intact neural circuits. *Current Opinion in Neurobiology*. 2012; 22(1):61–71. [PubMed: 22119320]
6. Deisseroth K, Schnitzer MJ. Engineering approaches to illuminating brain structure and dynamics. *Neuron*. 2013; 80(3):568–77. [PubMed: 24183010]
7. Richter CP, Tan X. Photons and neurons. *Hear Res*. 2014
8. Hernandez VH, et al. Optogenetic stimulation of the auditory pathway. *The Journal of clinical investigation*. 2014; 124(3):1114–1129. [PubMed: 24509078]
9. Wells J, et al. Application of infrared light for in vivo neural stimulation. *Journal of Biomedical Optics*. 2005; 10(6) 064003-064003-12.

10. Katz EJ, et al. Excitation of primary afferent neurons by near-infrared light in vitro. *Neuroreport*. 2010; 21(9):662–6. [PubMed: 20495496]
11. Bec JM, et al. Characteristics of laser stimulation by near infrared pulses of retinal and vestibular primary neurons. *Lasers Surg Med*. 2012; 44(9):736–45. [PubMed: 23018648]
12. Smith NI, et al. A femtosecond laser pacemaker for heart muscle cells. *Opt Express*. 2008; 16(12): 8604–16. [PubMed: 18545573]
13. Dittami GM, et al. Intracellular calcium transients evoked by pulsed infrared radiation in neonatal cardiomyocytes. *J Physiol*. 2011; 589(Pt 6):1295–306. [PubMed: 21242257]
14. Izzo AD, et al. Laser stimulation of the auditory nerve. *Lasers in Surgery and Medicine*. 2006; 38(8):745–753. [PubMed: 16871623]
15. Teudt IU, et al. Optical Stimulation of the Facial Nerve: A New Monitoring Technique? *The Laryngoscope*. 2007; 117(9):1641–1647. [PubMed: 17607145]
16. Fried NM, et al. Noncontact stimulation of the cavernous nerves in the rat prostate using a tunable-wavelength thulium fiber laser. *Journal of Endourology*. 2008; 22(3):409–414. [PubMed: 18257657]
17. Jenkins MW, et al. Optical pacing of the embryonic heart. *Nat Photon*. 2010; 4(9):623–626.
18. Harris DM, et al. Optical nerve stimulation for a vestibular prosthesis. 2009
19. Tian L, et al. Short-wavelength infrared laser activates the auditory neurons: comparing the effect of 980 vs. 810 nm wavelength. *Lasers Med Sci*. 2017; 32(2):357–362. [PubMed: 27987044]
20. Rabbitt RD, et al. Heat pulse excitability of vestibular hair cells and afferent neurons. *J Neurophysiol*. 2016; 116(2):825–43. [PubMed: 27226448]
21. Walsh AJ, et al. Action potential block in neurons by infrared light. *Neurophotonics*. 2016; 3(4): 040501. [PubMed: 27990450]
22. Rajguru SM, et al. Infrared photostimulation of the crista ampullaris. *J Physiol*. 2011; 589(Pt 6): 1283–94. [PubMed: 21242259]
23. Cohen B, Suzuki JI. Eye movements induced by ampullary nerve stimulation. *Am J Physiol*. 1963; 204:347–51. [PubMed: 14022008]
24. Cohen B, Suzuki JI, Bender MB. Eye Movements from Semicircular Canal Nerve Stimulation in the Cat. *Ann Otol Rhinol Laryngol*. 1964; 73:153–69. [PubMed: 14128701]
25. Suzuki JI, Cohen B, Bender MB. Compensatory Eye Movements Induced by Vertical Semicircular Canal Stimulation. *Exp Neurol*. 1964; 9:137–60. [PubMed: 14126123]
26. Suzuki JI, Tokumasu K, Goto K. Eye movements from single utricular nerve stimulation in the cat. *Acta Otolaryngol*. 1969; 68(4):350–62. [PubMed: 5309166]
27. Lewis RF, et al. Spatial and temporal properties of eye movements produced by electrical stimulation of semicircular canal afferents. *J Neurophysiol*. 2012; 108(5):1511–20. [PubMed: 22673321]
28. Merfeld DM, Black FO, Wade S. Clinical use of three-dimensional video measurements of eye movements. *Otolaryngol Head Neck Surg*. 1998; 118(3 Pt 2):S35–8. [PubMed: 9525489]
29. Fridman GY, et al. Vestibulo-ocular reflex responses to a multichannel vestibular prosthesis incorporating a 3D coordinate transformation for correction of misalignment. *J Assoc Res Otolaryngol*. 2010; 11(3):367–81. [PubMed: 20177732]
30. Rahman MA, et al. Restoring the 3D vestibulo-ocular reflex via electrical stimulation: the Johns Hopkins multichannel vestibular prosthesis project. *Conf Proc IEEE Eng Med Biol Soc*. 2011; 2011:3142–5. [PubMed: 22255006]
31. Moreno LE, et al. Infrared neural stimulation: beam path in the guinea pig cochlea. *Hear Res*. 2011; 282(1–2):289–302. [PubMed: 21763410]
32. Hale GM, Querry MR. Optical constants of water in the 200 nm to 200 μ m region. *Appl Opt*. 1973; 12:555–563. [PubMed: 20125343]
33. Stahl JS, van Alphen AM, De Zeeuw CI. A comparison of video and magnetic search coil recordings of mouse eye movements. *J Neurosci Methods*. 2000; 99(1–2):101–10. [PubMed: 10936649]
34. Raphan T, Matsuo V, Cohen B. Velocity storage in the vestibulo-ocular reflex arc (VOR). *Exp Brain Res*. 1979; 35(2):229–48. [PubMed: 108122]

35. Cohen B, et al. Spatial orientation of the angular vestibulo-ocular reflex. *J Vestib Res.* 1999; 9(3): 163–72. [PubMed: 10436469]
36. Curthoys IS. Eye movements produced by utricular and saccular stimulation. *Aviat Space Environ Med.* 1987; 58(9 Pt 2):A192–7. [PubMed: 3675491]
37. Fluor E. Influences of semicircular ducts on extraocular muscles. *Acta Otolaryngol Suppl.* 1959; 149:1–46. [PubMed: 13660800]
38. Fluor E, Mellstrom A. Saccular stimulation and oculomotor reactions. *Laryngoscope.* 1970; 80(11):1713–21. [PubMed: 5481001]
39. Simpson JI, Graf W. Eye-muscle geometry and compensatory eye movements in lateral-eyed and frontal-eyed animals. *Ann N Y Acad Sci.* 1981; 374:20–30. [PubMed: 6978631]
40. Suzuki JI. Vestibular and spinal control of eye movements. *Bibl Ophthalmol.* 1972; 82:109–15. [PubMed: 4568568]
41. Suzuki JI, Cohen B. Head, Eye, Body and Limb Movements from Semicircular Canal Nerves. *Exp Neurol.* 1964; 10:393–405. [PubMed: 14228399]
42. Nair G, et al. Effects of common anesthetics on eye movement and electroretinogram. *Documenta Ophthalmologica.* 2011; 122(3):163–176. [PubMed: 21519880]
43. Mettens P, Godaux E, Chéron G. Effects of ketamine on ocular movements of the cat. *J Vestib Res.* 1991; 1:325–338.
44. Dellepiane M, Mora R, Salami A. Vestibular and optokinetic nystagmus in ketamine-anesthetized rabbits. *International Tinnitus Journal.* 2007; 13(1):15. [PubMed: 17691658]
45. van de Berg R, et al. The modified ampullar approach for vestibular implant surgery: feasibility and its first application in a human with a long-term vestibular loss. *Front Neurol.* 2012; 3:18. [PubMed: 22363317]
46. Poon CC, Irwin MG. Anaesthesia for deep brain stimulation and in patients with implanted neurostimulator devices. *Br J Anaesth.* 2009; 103(2):152–65. [PubMed: 19556271]
47. Dai C, et al. Restoration of 3D vestibular sensation in rhesus monkeys using a multichannel vestibular prosthesis. *Hear Res.* 2011; 281(1–2):74–83. [PubMed: 21888961]
48. Cayce JM, et al. Calcium imaging of infrared-stimulated activity in rodent brain. *Cell Calcium.* 2014; 55(4):183–90. [PubMed: 24674600]
49. Moreau D, et al. Infrared neural stimulation induces intracellular Ca²⁺ release mediated by phospholipase C. *J Biophotonics.* 2017
50. Barrett JN, , et al. Society for Neuroscience Annual Meeting. Washington, D.C: 2017. Pulsed Infrared Releases Ca²⁺ from the Endoplasmic Reticulum of Cultured Spiral Ganglion Neurons.
51. Tan X, et al. Auditory Neural Activity in Congenitally Deaf Mice Induced by Infrared Neural Stimulation. *Sci Rep.* 2018; 8(1):388. [PubMed: 29321651]
52. Kawashima Y, et al. Mechanotransduction in mouse inner ear hair cells requires transmembrane channel-like genes. *J Clin Invest.* 2011; 121(12):4796–809. [PubMed: 22105175]
53. Minor LB. Gentamicin-induced bilateral vestibular hypofunction. *JAMA.* 1998; 279(7):541–4. [PubMed: 9480366]
54. Pan B, et al. TMC1 and TMC2 are components of the mechanotransduction channel in hair cells of the mammalian inner ear. *Neuron.* 2013; 79(3):504–15. [PubMed: 23871232]

Figure 1. The experimental set-up

(A) shows schematic of the animal with the position of two cameras to track eye movements and optical fiber placement. (B) shows micro-CT image of the rat inner ear highlighting important structures: the three semicircular canals (AC, LC, PC) and cochlear oval window. IR targeted region of the PC ampullary region is marked. (C) shows an example image of a rat eye with center of pupil (P) and cornea reflection (CR) markers that are used in off-line analysis to determine horizontal and vertical components of the evoked eye movements. (D) illustrates the stimuli used in each of the experiments. Stim1: continuous IR pulses trains ($f_0=250\text{Hz}$) and Stim2: frequency-modulated IR stimuli ($f_0=250\text{Hz}$, $f_s=0.05\text{-}0.4\text{Hz}$).

Figure 2. Movement of the ipsilateral eye evoked by IR stimulation of the PC

IR was delivered at $\lambda=1863\text{nm}$, $f_0=250\text{Hz}$ and pulse width= $200\mu\text{s}$. (A) shows the horizontal (H, blue curve) and vertical eye (V, black curve) position changes before, during and after IR delivered at 105 mW/mm^2 . Right panel (B1) shows a series of images illustrating ipsilateral eye position changes during IR stimulation. The evoked eye movement response is divided into three phases (I, II and III) and the trajectory of ipsilateral eye movement during these phases are shown in (B2).

Figure 3. Evoked eye movement response to different IR pulse energies

IR was delivered at $\lambda=1863\text{nm}$, $f_0=250\text{Hz}$ and pulse width= $200\mu\text{s}$. (A1-A6) show the horizontal and vertical eye movement responses evoked by $55\mu\text{J/pulse}$, $77\mu\text{J/pulse}$ and $93\mu\text{J/pulse}$ and $109\mu\text{J/pulse}$ respectively.

Figure 4. Ipsilateral eye movements evoked by frequency-modulated IR stimuli

IR was delivered at $\lambda=1863\text{nm}$, $f_0=250\text{Hz}$ and pulse width= $200\mu\text{s}$. (A, B) show the horizontal and vertical eye movements evoked by IR stimuli modulated at $f=0.1\text{Hz}$ and $a=0.9$ (IR shown in green curves, along with H and V in blue and black curves respectively). (B) shows a 0.1Hz curve fit superimposed on the horizontal and vertical sinusoidal eye movements during 40s of the IR stimulation. (C, D) show the horizontal and vertical eye movements evoked by frequency modulated IR stimuli at $f=0.2\text{Hz}$ and $a=0.9$. (D) shows a 0.2Hz curve fit superimposed on the horizontal and vertical sinusoidal eye movements during a 20s period of the IR stimulation.

Figure 5. Influence of different IR parameters

Modulation frequency, pulse energy (or peak power) and dynamic range were altered systematically and the evoked eye movement responses were recorded on the ipsilateral side. The data shown are from 5 rats. (A) shows the position and velocity change of sinusoidal eye movement in horizontal and vertical direction at different IR modulation frequencies (0.05-0.4 Hz). (B) shows the dose-response curves at different pulse energy (55-109 μJ/pulse, corresponding IR peak powers are marked on the x-scale 1.75-3.47 mJ/mm²). (C) shows the effect of dynamic range (0.6 to 1) on the evoked position and velocity change of sinusoidal eye movement in horizontal and vertical directions.

Figure 6. Sinusoidal eye movement evoked by IR

Frequency-modulated IR was delivered for a period of 30 minutes (n=5). (A) shows the mean velocities of sinusoidal eye movement in horizontal and vertical directions measured at multiple time points during the stimulation (2, 5, 10, 15, 20, 25 and 30 mins). (A1 and A2) highlight the evoked responses in one animal at 5 and 25 minutes.

Figure 7. Bilateral eye movements evoked by continuous and frequency modulated IR stimuli Left panels (A, B) show the evoked eye movements bilaterally for IR stimuli delivered at 1863nm, 200 μ s 250Hz and 109 μ J/pulse. Right panels (C, D) show the bilateral eye movements evoked by frequency-modulated IR stimuli at 1863nm, 200 μ s 250Hz, 109 μ J/pulse, $f=0.1$ Hz and $a=0.9$. Insets C1 and D1 show the trajectory of each eye during one period of the IR stimulation. Insets C2 and D2 show the eye positions prior to, during and following IR stimulation – a total of 3 minutes time period.

Figure 8. 3D micro-CT reconstruction

Following each experiment, 3D micro-CT was carried out to identify the optical fiber orientation in relation to vestibular structures, particularly the PC. (A, B) show the reconstructed images in two different orientations from one animal where a single fiber was positioned through the round window and fixed in place post stimulation. The optical fiber is highlighted in red along with the identified cochlea, PC and AC. The insets in each image shows a close up of the posterior canal ampullary region with the optical fiber and endolymphatic space highlighted. (C) shows the magnified images from 3 additional rats (R1, R2 and R3) in which we could successfully evoke eye movements to illustrate the small changes in orientation of the optical fiber but consistent site of stimulation (lower panel). (D, E) the reconstructed images in two different orientations from another animal where a three-fiber bundle was positioned through the round window and fixed in place post stimulation. The fiber highlighted by red circle was the one with which we could successfully report sinusoidal eye movement. The insets show a close-up of the ampullary region with the three-

fiber bundle highlighted. (F) shows the magnified images from 2 additional rats (R4 and R5) in which the three-fiber bundle and vestibular structures were imaged. The fibers highlighted by red circle were the ones that could evoke sinusoidal eye movement.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 1

Calibration for the radius of rat eye (n=5)

Animal #	1	2	3	4	5	Avg.
Radius (mm)	2.0	1.9	2.1	2.3	2.0	2.06