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Evaluation of Effects of Electrical Stimulation in the Retina with Optical Coherence Tomography*

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Abstract

Retinal prostheses provide the capability to blind patients to detect motion and locate large objects. To avoid activating axons of passage, which can create streak-like perceptions, long pulse stimulation can be used to bypass axons and achieve focal retinal activation. Safety is a concern because long pulses require more charge than short pulses to elicit a response from neural tissue. Future implants will require smaller electrodes to improve resolution, but increased charge density may result, which is another safety concern. We developed a method to study the effects of electrical stimulation in the retina in real time using OCT (Optical Coherence Tomography) imaging combined with micropositioning of a stimulating electrode over the retina in an animal model. When using a 250-micron diameter electrode and stimulating for 30 minutes (frequency: 333 Hz), charge density: 1.22 mC/cm^2 , we observed an increase in retinal thickness from $154.3 \mu\text{m} \pm 7.04 \mu\text{m}$ to $179.67 \mu\text{m} \pm 0.47 \mu\text{m}$, a $16.66 \% \pm 5.49\%$ increase compared to baseline. The region of increased thickness extended laterally for $0.56 \text{ mm} \pm 0.009 \text{ mm}$. When stimulating with a charge density of 1.63 mC/cm^2 , we observed an increase in retinal thickness from $160.3 \mu\text{m} \pm 2.05 \mu\text{m}$ to $190 \mu\text{m} \pm 0.81 \mu\text{m}$, a $19.52 \% \pm 1.86\%$ increase compared to baseline. The region of increased thickness expanded laterally for $1.27 \text{ mm} \pm 0.19 \text{ mm}$.

I. Introduction

Retinitis pigmentosa [1] and aged related macular degeneration [2] are two diseases that affect primarily the photoreceptor layer, but leaves the inner retinal cells (bipolar cells and ganglion cells) relatively intact [3], allowing the retina to be electrically stimulated to restore a sense of vision. Retinal prostheses have demonstrated the capability to elicit the sensation of light and to give subjects the ability to detect motion and the location of large objects [4] [5].

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Studies of retinal stimulation safety are needed for higher resolution arrays and new stimulation paradigms. The resolution of a retinal prosthesis is correlated to the number and size of electrodes in its array [6]. To be able to achieve a high-resolution retinal prosthesis, simulations of artificial vision have suggested that 600 to 1000 electrodes, between 50–100 microns diameter will be required in a 5mm diameter on the retina [7] [6]. Small electrodes will focus the stimulus but also require high charge density. Retinal cells (ganglion cells and bipolar cells) can be activated selectively depending on the pulse parameters used for stimulation. Short pulses (1ms) produce elongated phosphenes by activating both ganglion cell axons and bipolar cells [8], while long pulses (25 ms) produce focal phosphenes by activating bipolar cells and avoiding ganglion cell axons [9]. Safety is a concern when using long pulses to stimulate, because long pulses require more charge than short pulses to have the same effect on neural tissue.

Several studies have been reported about the safety of chronic and acute electrical stimulation in the retina. Most of these studies have relied on post-mortem analysis like hematoxylin staining [10], or propidium iodide (PI) fluorescent dye [11]. The effects of electrical stimulation in the retina were evaluated in real time using OCT (Optical Coherence Tomography) imaging in an in-vitro model [12]. These studies have helped to understand the anatomy of the retina at one time point after electrical stimulation [10], [11], [13], and showed the value of real-time imaging to analyze the retina during the stimulation period. However, since this method was in vitro, long-term effects of stimulation could not be investigated. We propose an in vivo experimental model more similar to current and future epiretinal prostheses.

We studied the effects of electrical stimulation in the retina in real time using OCT (Optical Coherence Tomography) images in a rabbit eye, using an in-vivo model and a platinum/Iridium disk electrode (75 and 250 μm diameter). 75 μm corresponds to the electrode size proposed for a high resolution epiretinal prosthesis and 250 μm is similar to electrodes in a commercially available epiretinal prosthesis. Using an in-vivo model allows us to study the retina in its natural condition.

II. METHODS

A. Animal Model

Adult pigmented rabbits (Irish Farms, Norco, CA); approximately 2 months old were used for all experiments. Experiments were performed in the left eye of each animal (n=13). Rabbits were anesthetized with ketamine-xylazine (100 mg/kg and 20 mg/kg) and euthanized at the end of the procedure.

All animals were maintained on a daily 12 h light/day cycle prior experiment. All procedures conformed to the Guide for Care and Use of Laboratory Animals (National Institute of Health). The University of Southern California Institutional Animal Care and Use committee reviewed and approved all procedures.

B. Imaging and stimulation

OCT images were taken as control before stimulation (HRA+OCT Spectralis, HEIDELBERG). After the eye was dilated, an incision was made in the sclera, 3 mm from the limbus and a 25 gauge valved trocar (Alcon) was used to help keep the stimulating electrode in position. The electrode was 90% Pt/10% Ir concentric bipolar (FHC, Bowdoin, ME), with a diameter of 75-micron (Model CBDFG75) or 250-micron (Custom). The stimulating electrode was held by a three axis translational stage (Model 4044 M Parker DAEDAL) mounted on a magnetic based articulating arm. For the 75-micron electrode, the center pole was used for stimulation. A needle electrode was placed on the head for current return. (Figure 1)

The stimulating electrode was placed through the valved trocar and advanced inside the eye until it became visible in the fundus view of the OCT system. The scan line was adjusted to be along the length of the electrode tip to allow simultaneous scanning of the electrode tip and the retina. The micromanipulator was used to advance the electrode towards the retina for better positioning. (Figure 2) Charged-balanced, cathodic first, biphasic current pulses were applied to the retina. Different pulse widths, pulse amplitudes, frequencies, stimulation times, and electrode sizes were used during these experiments (Table 1). OCT images were acquired every 2 minutes during and after stimulation. Retinal thickness was measured to assess retinal damage.

III. RESULTS

Eighteen retinal regions were imaged in thirteen different animals. The electrode was placed on the inferior temporal region, close to the visual streak. (Figure 2).

A. Electrode-retina Distance

OCT images were taken as the electrode was advanced towards the retina. After insertion into the eye, the electrode was advanced from the periphery to a point where the electrode tip was visible in the fundus view of the OCT system. The electrode was placed closed to the epiretinal surface but not touching. The micromanipulator was used to advance the electrode until it was near the retina.

The stimulating electrode was placed inside the left eye $59.25 \mu\text{m} \pm 27.26 \mu\text{m}$ (mean, SD) away from the retina. The distance between the electrode and the retina was measured using the calipers function of the OCT analysis software as shown in figure 2.

B. OCT imaging during stimulation

A fundus image and OCT images were taken before stimulation as control. During the stimulation period, images were acquired every two minutes for a period of 30 minutes. After stimulation, the stimulating electrode was moved away from the retina and images were acquired every 5 minutes for a period of 15 minutes. The effects of the different charge densities applied to the retina are shown on Figure 4. OCT images are shown right before starting stimulating, 20 minutes into the stimulation and 30 minutes after stimulation.

Images shown are for three different charge densities: 0.92 mC/cm², 1.22 mC/cm² and 1.63 mC/cm² applied with a 250 μ m diameter platinum/iridium electrode using 1 ms pulses.

When using the 250 μ m diameter electrode and stimulating for 30 min (frequency: 333 Hz, charge density: 1.63 mC/cm²) we observed an increase in retinal thickness from 160.3 μ m $\pm$ 2.05 μ m (mean, SD) to 190 μ m $\pm$ 0.81 μ m (mean, SD), a 19.52% $\pm$ 1.86% (mean, SD) increase compared to baseline. The region of increased thickness extended laterally for 1.27 mm $\pm$ 0.19 mm (mean, SD), roughly centered about the position of the stimulating electrode. When using a 250 μ m diameter electrode and stimulating for 30 min (frequency: 333 Hz, charge density: 1.22 mC/cm²) we observed an increase in retinal thickness from 154.3 μ m $\pm$ 7.04 μ m (mean, SD) to 179.67 μ m $\pm$ 0.47 μ m (mean, SD), a 16.66 % $\pm$ 5.49% (mean, SD) increase compared to baseline. The region of increased thickness extended laterally for 0.56 mm $\pm$ 0.009 mm (mean, SD).

Experiments using the 75 μ m diameter electrode with any settings listed in Table 2 and using the 250 μ m diameter electrode and charge densities of 0.92 and 1.02 mC/cm² showed no change in retinal thickness.

Figure 3 shows a summary of the OCT measurements of retinal thickness versus current density. Values shown are the averages of all the animals studied per group.

A two-way analysis of variance with Holm-Sidak post-hoc testing for multiple pairwise comparisons showed no significant difference between retinal thicknesses from different groups (1.63, 1.22 and 0.92 mC/cm²) before stimulation, and a significant difference between retinal thicknesses from the same groups after stimulation (Table 2). Similarly, the difference between retinal thicknesses for the 0.92 mC/cm² group pre and post stimulation was not statistically significant (P=1); but there was a significant difference between retinal thicknesses pre and post stimulation for groups 1.22 mC/cm² (P<0.001) and 1.63 mC/cm² (P<0.001).

IV. CONCLUSION

Electrical stimulation at very high charge densities causes swelling of the retina within minutes of stimulation. The stimulus levels at which swelling was noted is significantly higher than parameters used generally for stimulation in humans in terms of rate and charge density, but in limited cases high currents [14] or high rates [15] have been used in humans, on a short term basis in the context of controlled psychophysical testing. Other studies have linked stimulation rate and/or duty cycle to neural damage [16]. Rate may be an important factor for retinal stimulation safety as well.

We developed a new method that allows us to study the effects of electrical stimulation on the retina in an in vivo model during the stimulation period. We used an in vivo model because it allows us to study the retina response in its natural conditions. This technique will also allow study of long-term effects of retinal stimulation, since it is potentially a survival surgery.

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References

1. ur Rehman H. Retinitis Pigmentosa. *N Z Med J*. 2015; 128(1415):54–56.
2. Kerr E. Back to basics : aged-related macular degeneration. *Nurs Resid Care*. 2013; 15(7)
3. Santos A, Humayun MS, De Juan E, Marsh MJ, Klock IB, Milam AH. Preservation of the inner retina in retinitis pigmentosa. *Arch Ophthalmol*. 1997; 115(4)
4. Weiland JD, Member S, Humayun MS. Retinal Prosthesis. 2014; 61(5):1412–1424.
5. Humayun MS, Dorn JD, Da Cruz L, Dagnelie G, Sahel JA, Stanga PE, Cideciyan AV, Duncan JL, Elliott D, Filley E, Ho AC, Santos A, Safran AB, Arditi A, Del Priore LV, Greenberg RJ. Interim results from the international trial of second sight's visual prosthesis. *Ophthalmology*. 2012; 119(4): 779–788. [PubMed: 22244176]
6. Yang, Z. *Neural Computation, Neural Devices, and Neural*. Springer; 2014.
7. Palanker D, Vankov A, Huie P, Baccus S. Design of a high-resolution optoelectronic retinal prosthesis. *J Neural Eng*. 2005; 2(1):S105–20. [PubMed: 15876646]
8. Nanduri, D. Prosthetic vision in blind human patients: Predicting the percepts of epiretinal stimulation. 2011.
9. Weitz AC, Nanduri D, Behrend MR, Gonzalez-Calle A, Greenberg RJ, Humayun MS, Chow RH, Weiland JD. Improving the spatial resolution of epiretinal implants by increasing stimulus pulse duration. *Sci Transl Med*. 2015; 7(318):318ra203–318ra203.
10. Ray A, Lee EJ, Humayun MS, Weiland JD. Continuous electrical stimulation decreases retinal excitability but does not alter retinal morphology. *J Neural Eng*. 2011; 8(4):45003.
11. Butterwick A, Vankov A, Huie P, Freyvert Y, Palanker D. Tissue Damage by Pulsed Electrical Stimulation. 2007; 54(12):2261–2267.
12. Cohen E, Agrawal A, Connors M, Hansen B, Charkhkar H, Pfefer J. Optical coherence tomography imaging of retinal damage in real time under a stimulus electrode.
13. Ray A. Effect of continuous electrical stimulation on retinal structure and function. PhD Propos. Aug.2015 1
14. Humayun MS, Weiland JD, Fujii GY, Greenberg R, Williamson R, Little J, Mech B, Cimmarusti V, Van Boemel G, Dagnelie G, De Juan E. Visual perception in a blind subject with a chronic microelectronic retinal prosthesis. *Vision Res*. 2003; 43(24):2573–2581. [PubMed: 13129543]
15. Horsager A, Greenwald SH, Weiland JD, Humayun MS, Greenberg RJ, McMahon MJ, Boynton GM, Fine I. Predicting visual sensitivity in retinal prosthesis patients. *Investig Ophthalmol Vis Sci*. 2009; 50(4):1483–1491. [PubMed: 19098313]
16. Agnew WF, McCreery DB. Considerations for safety with chronically implanted nerve electrodes. *Epilepsia*. 1990; 31(Suppl 2):S27–S32. [PubMed: 2226363]

Figure 1.

Diagram of the experiment setup. The electrode was placed through the sclera and held in place with a micromanipulator. Return electrode was placed in the rabbit's head. A spectral domain optical coherence tomography (OCT) system was placed in front of the rabbit's left eye and scanned the retina surface while the retina was being stimulated.

Figure 2.

A. Top Image-Left. Fundus Image of the eye. The X scan line was adjusted to be on the tip of the electrode simultaneously while the electrode was advanced toward the retina; which helped for placement of the electrode close to the retina. A. Top Image-Right. OCT image. Electrode tip was placed close to the epiretinal surface. Electrode was advanced towards the retina until the tip of the electrode could be seen on the OCT scan. Distance between the electrode and the retina was measured using the calipers function of the OCT software (shown in red). B. Bottom Image. Fundus image of the eye. Electrode was placed on the inferior temporal region of the eye, close to the visual streak.

Figure 3.

Difference of retinal thickness before and after stimulation for three different groups. 250 micron diameter electrode was used for these three groups. There is a statistically difference between retinal thicknesses pre and post stimulation for 1.63 and 1.22 mC/cm² group and there is no statistically difference for 0.92 mC/cm² group.

Figure 4.

OCT images of the retina during electrical stimulation. Three different current densities are shown in this image. Left 0.92 mC/cm². Middle 1.22 mC/cm². Right 1.63 mC/cm². Three different time points during stimulation are shown per group. Top image of each group shows an OCT scan taken before stimulation was started. Middle image of each group shows an OCT scan taken 20 min during stimulation. Bottom image of each group shows an OCT scan taken 15 minutes after stimulation was stopped. For the middle and right group we can see a swelling of the retina in the scan done 15 minutes after stimulation. Left group doesn't show a difference in retinal thickness in any of the three scans shown. Other parameters evaluated are not shown in this graph because there was no difference in retinal thickness for any of them. Yellow arrow indicates the tip of the stimulating electrode. Blue Arrows indicate the area of retinal thickness increment after stimulation.

Table 1

Parameters used during stimulation. Experimental groups were based on different combinations of charge densities, stimulus frequency, pulse widths, duration of pulse train stimulation and electrode size. –No swelling noted; ++ Swelling noted.

Electrode diameter (µm)	Pulse width (ms)	Amplitude (uA)	Frequency (Hz)	Charge Density (mC/cm ²)	Num of Animals	Duration (min)	Retinal Thickness Increment
75	1	20	100	0.68	1	10	---
		50	100	1.1	1		---
		100	100	2.3	1		---
	25	1	10	0.6	1		---
		2	10	1.1	1		---
		3	10	1.7	1		---
		4	10	2.2	1		---
		450	100	0.92	1		---
250	1		333	0.92	4	30	---
		500	333	1.02	1	20	---
		600	333	1.22	3	30	++
		800	333	1.63	3	30	++

Table 2

Comparison for factors. Retinal thickness between pre and retinal thickness between post.

Group Studied (mC/cm2)	P Value
1.63 vs 1.22 pre/post	0.482/0.037
1.63 vs 0.92 pre/post	0.478/<0.001
0.92 vs 1.22 pre/post	0.824/<0.001