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Ionic direct current modulation for combined inhibition/excitation of the vestibular system

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Abstract

Objective: Prosthetic electrical stimulation delivered to the vestibular nerve could provide therapy for people suffering from bilateral vestibular dysfunction (BVD). Common encoding methods use pulse-frequency modulation (PFM) to stimulate the semicircular canals of the vestibular system. We previously showed that delivery of ionic direct current (iDC) can also modulate the vestibular system. In this study we compare the dynamic range of head velocity encoding from iDC modulation to that of PFM controls.

Methods: Gentamicin-treated wild-type chinchillas were implanted with microcatheter tubes that delivered ionic current to the left ear vestibular canals and stimulated with steps of anodic/cathodic iDC or PFM. Evoked vestibulo-ocular reflex (VOR) eye velocity was used to compare PFM and iDC vestibular modulation.

Results: Cathodic iDC steps effectively elicited eye rotations consistent with an increased firing rate of the implanted semicircular canal afferents. Anodic iDC current steps elicited eye rotations in the opposite direction that, when paired with an adapted cathodic offset, increased the dynamic range of eye rotation velocities in comparison to PFM controls.

Conclusion: Our results suggest that iDC modulation can effectively modulate the vestibular system across a functional range of rotation vectors and velocities, with a potential benefit over a PFM stimulation paradigm.

Significance: In conjunction with a safe DC delivery system, iDC modulation could potentially increase the range of simulated head rotation velocities available to neuroelectric vestibular prostheses.

Index Terms—

Neuroscience; Biomedical Engineering; Neuromodulation; Neural engineering; Neural prosthesis; Neural implants

I. Introduction

THE vestibular system detects head rotation, orientation, and translation to stabilize vision, gait, and posture. In a normal functioning system, the semicircular canals (SSCs) and otolith organs in the inner ear sense head rotation, tilt, and translation via sensory epithelia (vestibular hair cells). In the semicircular canals, vestibular nerve afferents maintain a spontaneous firing rate when the system is at rest (approximately 100 spikes per second in primates [1, 2]) with the direction and amplitude of head rotation encoded by either an increase or decrease from this spontaneous rate [3]. Bilateral loss of this vestibular sensation is a debilitating condition that can dramatically impact independence and quality of life [4]. Although sufferers can adapt with the help of rehabilitation therapies, there remain no comprehensive treatment options available for severe bilateral vestibular loss [5]. Chronically implanted vestibular prostheses modeled on the cochlear implant are currently in development to fill this pressing need. Currently, prostheses designed to replace semicircular canal function in patients with bilateral vestibular disorders use biphasic charge-balanced stimuli to excite neural activity. Because biphasic pulses cannot inhibit activity, to generate bidirectional encoding of motion, implants must artificially elevate the spontaneous rate of the vestibular afferents and then modulate around this increased baseline [6, 7]. This is not ideal as it cannot replicate the full range of inhibition in the normal system: asymmetry of responses to unilateral stimulation of the SSCs has been previously identified as a barrier to the implementation of a high performance vestibular prostheses [6, 8].

The development of a safe delivery method for direct current (DC) neural modulation could allow for neuroprosthetic stimulators that are capable of inhibiting and exciting neural tissue. Anodic DC delivered extracellularly can be used to effectively inhibit neural activity via hyperpolarization of the membrane potential, whereas cathodic DC excites neural activity via depolarization of the membrane potential [9, 10]. DC modulates spike rate in a graded, stochastic fashion which may help emulate neural output in a more physiological manner compared to the phase-locked excitation of a biphasic pulse generator [11, 12]. Despite these potential benefits for DC as a form of neural modulation it has mostly remained an unsuitable option for chronic application. DC modulation inherently violates charge injection criteria and will create toxic reactants and thus cell death at the metal electrode-tissue interfaces [13,14]. Recently, proof-of-concept current delivery strategies have been developed that eliminate or mitigate this otherwise crippling limitation of DC modulation [14–16]. Specifically, in our other work we are developing a ‘safe direct current stimulator’ (SDCS): a current rectifying network of fluidic channels that transforms AC square waves at the electrode-saline interface into ionic DC (iDC) flow across the target tissue [15, 17].

Because iDC could in principle inhibit the vestibular afferent firing rate below the baseline spontaneous rate, it has the potential to deliver a larger range of vestibular function when compared to traditional pulsatile devices. Responses of the vestibular system have been extensively characterized for a more systemic form of direct current application applied transcutaneously, referred to as galvanic stimulation [9, 18, 19]. Based on these studies we hypothesize that modulation of the semicircular canals with cathodic current steps should

elicit a VOR eye response consistent with the excitation of the vestibular afferents and away from the implanted side (“excitatory” response), while anodic current steps should elicit a VOR response consistent with the inhibition of the afferents and toward the implanted side (“inhibitory” response). We further hypothesize that application of a constant current ‘baseline’ should produce a similar adaptation effect to elevated PFM baselines. That is, because cathodic iDC baselines are expected to elevate spontaneous activity, we expect increased inhibitory VOR responses and decreased excitatory VOR responses as we modulate about an increased cathodic baseline, with an opposite effect occurring from anodic baselines (increased excitatory VOR and attenuation of the inhibitory VOR).

While our previous work provided proof-of-concept that supports the notion of effective iDC-facilitated modulation of the vestibular canals [15] it has not yet been established whether an iDC-based approach is capable of creating the functional range of neural modulation necessary for a neural prosthesis. Here we provide evidence for the capability of iDC modulation to create functional responses in an animal model of bilateral vestibulopathy across multiple simultaneously stimulated canals, with benefit over a comparable pulse-based paradigm.

II. Methods

A. Surgical Techniques

Adult wild-type chinchillas ($n = 5$) were used for experiments in this study. All experimental protocols were approved by the Johns Hopkins Animal Care and Use Committee (Protocol #CH15M38). To emulate human bilateral vestibular dysfunction (BVD), animals were placed under anesthesia (Isoflurane, Baxter, Deerfield, IL) and treated bilaterally with 0.5 mL intratympanic injections of 26.7 mg/mL gentamicin. In the chinchilla, intratympanic gentamicin induces loss of vestibular hair cells, particularly type I hair cells, without substantially damaging the vestibular afferents [20]. As animals were prone to losing weight post-treatment, gentamicin injections were performed right ear first with a minimum of one week before the left ear injection to give the animals time to recover.

Surgical techniques followed protocols previously described in detail [7, 8, 15] with modification to allow for iDC modulation of the vestibular canals as detailed here. One month after the second gentamicin injection, animals were placed under isoflurane anesthesia and the dorsal surfaces of both mastoid bullae were exposed and opened using an otologic drill. Plastic microcatheter tubes for the delivery of iDC (20 μ L microloader pipettes; Eppendorf, Hamburg, Germany) were implanted into each left bulla semi-circular canal and the common crus, via small windows exposed with the drill. Microcatheter tubes had a diameter at the end aperture of 200 μ m. Figure 1A shows schematized images of an implanted chinchilla left vestibular organ indicating approximate implanted tube positions relative to the left posterior canal (LP), horizontal canal (LH), superior canal (LS) and common crus (CC). Figure 1B shows the shape and relative size of an example microcatheter tube used in this study and a twisted pair of 75 μ m diameter platinum iridium wires commonly implanted for semicircular canal stimulation in our lab. The plastic microcatheter tubes contained a saline solution suspended in agar, adjusted to match the ionic concentrations of chinchilla perilymph [21] (146.5 mM NaCl; 3.3 mM KCl; 1.3 mM

CaCl₂; 0.9 mM MgCl₂ 0.7 mM NaH₂PO₄; 10 mM HEPES; pH 7.4). Tubes were secured using dental cement. Dental cement plugs protected the internal solutions between experiments. To restrain the head for ocular recordings, a phenolic post was positioned in the midline perpendicular to the skull and fixed in place via dental cement extruded into each bulla. Animals were then allowed to recover for a minimum of 2 weeks following surgery before iDC modulation experiments were performed.

B. Eye Movement Recording

To capture real-time, 3-dimensional video-oculography (3D VOG) we used a modified system described previously in detail [7, 22]. The left eye was topically anesthetized, and the pupil constricted via application of proparacaine (5 mg/mL; Bausch & Lomb, Rochester, NY) and pilocarpine (10 mg/mL; Bausch & Lomb, Rochester, NY) eye drops. An array of three fluorescent yellow squares on a black film was carefully fixed to the cornea using veterinary tissue glue (VetOne, Boise, ID) and the eye illuminated in darkness via four UV-emitting diodes. A USB3 Camera (FL3-U3– 13Y3M-C, Flea3 1.3 MP Mono USB3 Vision; Point Grey, BC, Canada) retrofitted with a 1/4" format, 16.0mm-focal length, f/2.0 C-mount board lens (BL160; Allthings Inc., Australia) was used to acquire 496×400 pixel, 8-bit gray-scale images at 200 Hz using custom software written in LabVIEW (National Instruments, Austin, TX). The output of this system indicates the left eye velocity about each of three axes corresponding to the axes of the semicircular canals: horizontal (H), left anterior/right posterior (LARP), and right anterior/left posterior (RALP).

C. Single Canal Stimulation

As an implantable SDCS device is still in development, we used an acute setup to mimic SDCS iDC delivery over the timeframe of an experiment. Current was delivered using a precision current source (Keithley Model 6221 AC and DC Current Source; Tektronix, Beaverton, OR) via the agar-gel microcatheter tubes with the metal interface (20-gauge steel needles) positioned far from the tissue. The microcatheter tubes served to isolate hazardous byproducts, caused by the electrolysis at the metal-gel interface, from the tissue. We have previously demonstrated the short-term safety of this iDC delivery method into the semicircular canals [15]. A microcatheter tube was also positioned in the common crus to act as a common return for the three semicircular canal tubes. The impedance of this setup ranged from ~300–500 kΩ between animals, but we did not record precise changes in impedances during the experimental phase as long the current source output remained below the 105 V compliance imposed by the device.

Electrical stimulation protocols for AC pulse-frequency modulation (PFM) were similar to those used by our group previously [7, 8, 22], but delivered via the tubes implanted in the canals in the same manner as iDC. We chose to deliver PFM via the ionic tubes to ensure any potential differences in surgical placement of a tube vs lead wire would not confound our comparison of the two stimulation methods. We made this decision based on preliminary data that found delivery of PFM through the ionic tube did not impact current thresholds for stimulation, but repeated removal of the ionic tube and replacement with a lead wire for PFM stimulation compromised the stability of the experimental setup.

Semicircular canals were stimulated with cathodic-first, charge-balanced biphasic pulses with a 200 μ s phase width and a 25 μ s interphase gap. Optimal current amplitudes for pulsatile stimuli were determined individually for each chinchilla by observing VOR responses during 0 to 420 pulses-per-second (PPS) step modulation. The amplitude was increased in 25 μ A steps until a maximal eye velocity was achieved that did not elicit further spread of activation to the other semicircular canals (as determined by change in axis of eye rotation) or to the facial nerve (as determined by facial twitch). To determine the maximal dynamic range of eye responses to pulse rate modulation or iDC from a given baseline, the animal was adapted to a pulse frequency baseline ranging from 0 to 210 PPS or to a constant current anodic iDC 'offset' (with respect to the tube positioned in the common crus) from a range of -40 to $+20$ μ A. The PFM adaptation rate and iDC adaptation amplitude were selected at random for each experiment to eliminate experimental sequence-imposed bias. For PFM we downmodulated from the PFM baseline to 0 PPS and upmodulated from this baseline to 480 PPS using 20 repetitions of 250 ms trapezoid steps with a 750 ms interstep period. For iDC baselines, current was modulated across a ± 40 μ A range around the offset iDC baseline using 20 repetitions of 250 ms current trapezoid steps with a 750 ms interstep period.

PFM waveforms consisted of frequency-modulated biphasic pulses of the same amplitude while iDC waveforms consisted of amplitude-modulated iDC current. Modulation from an adapted baseline of 60 PPS was chosen as the 'control' condition response for PFM because it mimics the physiological spontaneous vestibular afferent firing rate in the chinchilla [23] and previous studies have found that modulation from 60 PPS produced an optimal dynamic range of VOR eye responses [8, 22].

To characterize the frequency response to PFM and DC modulation in our chinchillas, we also examined the peak eye velocity and phase lead/lag characteristics of steady-state VOR responses to sinusoidal modulation. Steady-state responses to sinusoidal modulation were determined via 20 repetitions of 0 to 120 PPS peak-to-peak sinusoidal pulse modulation from a 60 PPS baseline across a 0.5 Hz to 8 Hz frequency range. Steady-state responses to sinusoidal iDC modulation were determined via 20 repetitions of $+10$ to -10 μ A sinusoidal current modulation across a 0.5 Hz to 8 Hz frequency range.

D. Dual Canal Stimulation

In the existing multi-channel vestibular prosthesis design developed by Della Santina et al [7], multiple semi-circular canal afferents are stimulated simultaneously using an interrupt service routine that interleaves pulses to ensure they do not interfere with the timing of pulses on the other channels. As it is not possible to interleave stimulation with an always-on DC modulation strategy, it is necessary to examine whether iDC can deliver functional modulation to multiple canals without breaks in current delivery. Using two Keithley 6221 current sources we delivered either PFM (with timing adjusted to ensure no simultaneous biphasic pulse generation between the two sources) or iDC delivered simultaneously to two semicircular canals (left anterior and left posterior, with respect to the common crus reference). Canals were either downmodulated from 60 to 0 PPS or upmodulated 60 to 420 PPS at a 60 PPS baseline in the PFM control, or from 0 to $+40$ μ A or 0 to -40 μ A at a -10

μA baseline for iDC modulation. The relative contribution of each canal was then varied between 100% and 0% of these values. By varying frequency for PFM or amplitude for iDC in this way, it was possible to direct the axis of the resulting VOR eye rotation across an arbitrary two-dimensional plane that represents the combined contribution to the VOR from these two canals.

E. Data Analysis

Output of the 3D-VOG system was analyzed offline via software developed in LabVIEW [7]. Eye rotation velocity in 3D was calculated from raw positional data interpolated on a 1 kHz time base and then filtered by a low pass filter and a running spline interpolation filter (LabVIEW “cubic spline Fit” module with balance parameter 0.99995). Trials were qualitatively assessed for saccades, blinks, or evidence of marker slippage or lid impact and were manually removed from the data analysis if these errors were detected. Eye velocity for individual trials was determined in MATLAB (MathWorks, Natick, MA) by taking the median peak velocity in the axis of rotation corresponding to the stimulated semicircular canal across 10 step or sinusoidal phase repetitions. Although an analysis of peak amplitude gives information about the differences in dynamic range between baseline and stimulus conditions, it does not address the possibility that the larger eye velocities are paired with greater current spread to other canals. To investigate the extent of current spread to the other semicircular canals we calculated the angle of axis misalignment for each peak velocity. We defined angle of axis misalignment as the difference between the acquired axis of eye rotation and the ‘ideal’ eye rotation vector aligned to the axis of the stimulated semicircular canal (in this case, RALP). A larger misalignment is an indicator of current spread to other canals. The angle of axis misalignment was computed as the angle between the peak eye velocity vector (the resultant vector composed of the three eye movement components) and the desired axis of rotation (the axis of the SCC/s being stimulated). Eye velocity vectors for simultaneous canal modulation were calculated by summing the resultant vector composed of the three eye movement components. Response phase was determined for sinusoidal modulation via a least-squares sinusoidal fit to eye movement velocity traces. We defined ‘phase lead/phase lag’ as the phase difference between peak eye velocity and peak current or pulse frequency amplitude, with negative values corresponding to phase lag. In all graphs we describe eye rotations consistent with an increased firing rate of the vestibular afferent (cathodic iDC steps or an increased PFM) as ‘excitatory’ and eye rotations consistent with a decreased afferent firing rate (anodic iDC steps or a decreased PFM) as ‘inhibitory’. Graphs plot mean peak values across animals ($n=5$) with error bars corresponding to standard error of the mean (SEM). In all figures, stimulus intensity is defined as $100\% * (\text{modulation-baseline}) / (\text{maximum modulation-baseline})$. We defined ‘maximum modulation’ in both DC and PFM as the intensity beyond which the VOR signal rapidly saturated and then attenuated (420 PPS in PFM or $-40 \mu\text{A}$ in DC) likely corresponding to spike saturation of the stimulated neurons as discussed in previous work [22]. We used stimulus intensity rather than absolute modulation to as it allows for a more direct comparison of PFM vs iDC stimuli and conveys more information about how optimum eye velocity magnitudes vary across the range of functionally meaningful stimulus parameters.

F. Statistical Analysis

Analyses were performed using GraphPad Prism v.4 (San Diego, CA, USA) and SigmaPlot v12.5 (Systat Software, San Jose, CA). Results are expressed as the mean $\pm$ standard error of the mean (SEM). All data were analyzed via a Shapiro-Wilk test for normality. Differences in amplitude, angle of axis misalignment and phase lead/lag were examined by one-way or two-way analysis of variance (ANOVA). A Holm-Sidak post hoc test was used to determine significance for multiple pairwise comparisons. Details of individual tests used are also provided in the results. In all figures, statistical significance is expressed as $P < 0.05$.

III. Results

To determine a maximum range of eye rotation velocities, we stimulated the left posterior canal and recorded eye responses to PFM and iDC steps across a range of adapted baselines and stimulus amplitudes. The mean PFM pulse amplitude across the 5 experimental animals was $165 \pm 32.2 \mu\text{A}$. Figures 2A–B show example traces for $175 \mu\text{A}$ amplitude PFM stepped from a 60 PPS adapted baseline to 420 and then to 0 PPS (2A) and iDC steps to -20 and then to $+20 \mu\text{A}$ from a $-20 \mu\text{A}$ baseline (2B). Figures 2C–D show example traces for 2 Hz sinusoidal modulation for PFM from 0 to 120 PPS peak-to-peak modulation centered at a 60 PPS baseline (2C) and for iDC from -10 to $+10 \mu\text{A}$ peak-to-peak at a $0 \mu\text{A}$ baseline (2D). In all traces, the dashed line corresponds to the shape of the modulation waveform, representing change in frequency for PFM and change in amplitude for iDC. The lines indicate left eye rotational velocity about the axes of the left posterior/right anterior (LARP), left anterior/right posterior (RALP) and horizontal (Z) canals. Upmodulation of pulse frequency or a cathodic iDC amplitude produced an ‘excitatory’ eye response vector (i.e. axis of eye rotation) consistent with rotation of the head towards the canal and thus potentially increased activity in the left posterior canal afferents (black arrowheads). Downmodulation of pulse frequency or an anodic iDC amplitude produced an ‘inhibitory’ eye response vector consistent with rotation of the head away from the canal and consistent with the decreased activity in the left posterior canal afferent (grey arrowheads).

We analyzed average peak eye velocities calculated from eye response traces across the range of evocable stimulus intensities and adapted baselines (Figure 3). Figures 3A–C show the excitatory response peak amplitudes evoked by PFM upmodulation (3A), cathodic iDC steps from a cathodic iDC baseline (3B), and cathodic iDC steps from an anodic iDC baseline (3C). Figures 3D–F show inhibitory response peak amplitudes evoked by downmodulated PFM (3D), anodic iDC steps from a cathodic iDC baseline (3E), and anodic iDC steps from an anodic iDC baseline (3F).

Modulation intensity was plotted as percent from maximum evocable response to better compare stimulation protocols. Each line in the plots corresponds to a particular baseline. Darker shades indicated an increased adapted baseline.

For both PFM and iDC modulation, peak eye responses increased as modulation increased towards maximum. Across all baseline conditions, VOR magnitudes increased as stimulus intensity increased (2-way ANOVA; $P < 0.001$; $n = 5$ animals). Maximum magnitudes evocable for each baseline were compared to the 60 PPS PFM baseline (indicated with a

dashed box). All conditions with a maximum amplitude significantly different from the 60 PPS control are marked in the legend with an Asterisk. 120 PPS and 240 PPS PFM baselines had significantly decreased maximum excitatory response amplitudes (1-way ANOVA; $P < 0.0001$ for 120 PPS; $P = 0.011$ for 240 PPS; $n = 5$ animals). Excitatory eye responses associated with the increased cathodic or anodic baseline iDC amplitudes did not significantly differ from those evoked during the 60 PPS PFM control condition (1-way ANOVA; $P > 0.05$; $n = 5$ animals). Inhibitory eye response amplitudes for all cathodic iDC baselines had a significant increase in eye response amplitude, when compared to the 60 PPS PFM control. (1-way ANOVA; $P = 0.037$ for $-5 \mu\text{A}$; $P = 0.004$ for $-10 \mu\text{A}$; $P < 0.001$ for $-20 \mu\text{A}$; $P = 0.002$ for $-30 \mu\text{A}$; $P < 0.001$ for $-40 \mu\text{A}$; $n = 5$ animals) and the 0 PPS PFM condition had significantly reduced peak amplitude (1-way ANOVA; $P = 0.038$; $n = 5$ animals). No other conditions were significantly different from the 60 PPS PFM control (1-way ANOVA; $P > 0.05$; $n = 5$).

Figure 4 shows a box-and-whiskers plot of mean angle of axis misalignment across PFM controls, cathodic iDC baselines and anodic iDC baselines. The 240 PPS amplitudes were only just above the noise floor of ~ 5 degrees per second, and the inhibitory 0 PPS condition was, by definition, noise with no signal. At low signal-to-noise ratios, angle of axis misalignment ceases to be a reliable indicator of current spread. Thus, we removed these datasets from the dataset before analysis. Angle of axis misalignment did not vary significantly between or within stimulus intensity or modulation from baseline (2-way ANOVA; $P > 0.05$; $n = 5$ animals) so these datasets were combined. Angle of misalignment did not significantly vary between any baseline condition (1-way ANOVA; $P = 0.99$). These data suggest that iDC amplitude modulation does not result in a greater spread of current compared to pulse frequency modulation.

Figure 5A shows eye response amplitude as a function of frequency in response to a ± 60 PPS pulse-frequency modulated sinusoid at a 60 PPS baseline or a $\pm 10 \mu\text{A}$ amplitude modulated constant current sinusoid at a 0 or $-10 \mu\text{A}$ baseline, on a log/log scale. Eye response amplitudes decreased as frequency increased (1-way ANOVA; $P < 0.001$; $n = 5$ animals). Figure 5B shows the phase lead/lag of a least-squares sinusoidal fit to eye movement velocity traces as a function of log frequency. The response phase leads the stimulus phase at low frequencies, but this shifted to a phase lag at higher frequencies (1-way ANOVA; $P < 0.001$; $n = 5$ animals). PFM amplitudes were significantly smaller than iDC amplitudes across all frequencies other than at 8 Hz, where there were no significant differences between conditions (2-way ANOVA; Holm-Sidak test for multiple comparisons; P values < 0.001 for 0.5 to 4 Hz; P values > 0.5 at 8 Hz; $n = 5$ animals). There were not significant differences in phase shift between frequencies below 8 Hz conditions, but a significant phase lag for PFM compared to both iDC conditions at 8 Hz (2-way ANOVA; Holm-Sidak test for multiple comparisons; P values > 0.2 for 0.5 to 4 Hz; P values < 0.001 at 8 Hz; $n = 5$ animals). There were no significant differences between the iDC conditions at any frequency for amplitude or phase shift (2-way ANOVA; Holm-Sidak for multiple comparisons; P values > 0.5 for all conditions).

To investigate the extent to which iDC is capable of simultaneously modulating multiple canals without interference we compared eye response magnitude and misalignment across

the range of VOR axes of eye rotation resulting from simultaneous modulation in the left posterior and left anterior canals using either PFM or iDC (Figure 6). One animal did not successfully complete the dual canal protocol due to high impedances in the anterior canal and so was removed from this portion of the study. Shaded quadrants in Figure 6A indicate the combination of ‘excitatory’ (+, corresponding to increased PFM or a cathodic iDC step) or ‘inhibitory’ (-, corresponding to decreased PFM or an anodic iDC step) modulation on each of the two canals necessary to evoke a desired VOR eye response axis located in that quadrant. Figure 6B shows the range of eye response magnitudes achieved via co-modulation of the left anterior and left posterior canals, across the 360° vector space described by Figure 7A. Figure 6C shows the angle of axis misalignment between the experimentally acquired vector angles and the ‘ideal’ vector angles calculated from the relative ratio of the anterior and posterior canal inputs. Eye response magnitudes followed a similar pattern for both PFM control and iDC modulation, with iDC modulation resulting in significantly higher eye response magnitude across the set of stimulated vectors (1-way ANOVA; mean magnitude + SD = 41.35 ± 28.51 for PFM, 93.56 ± 50.77 for iDC; $P < 0.001$; $n = 4$ animals). Angle of axis misalignment varied considerably between trials. Across the set of modulation vectors, PFM had a greater mean angle of axis misalignment, although this P value was only just significant (1-way ANOVA; mean magnitude + SD = 18.07 ± 8.059 for PFM, 13.71 ± 7.614 for iDC; $P = 0.029$; $n = 4$ animals). These data show that iDC modulation in two simultaneous canals is functionally feasible and the simultaneous modulation of two canals did not appear to have any additional effect on the specificity or direction of the resulting response vector compared to the PFM control.

IV. Discussion

This study characterizes the functional range and magnitude of rotational eye velocities in response to iDC modulation in the semicircular canals of the gentamicin-treated chinchilla. We hypothesized that, because iDC could potentially inhibit spontaneous vestibular activity below baseline, a larger magnitude of eye motions associated with inhibition of spontaneous rate should be achievable using iDC modulation vs traditional pulse frequency modulation. iDC and PFM modulation resulted in reflexive eye rotations associated with head movement corresponding to the modulated semicircular canal, with peak eye velocities similar to those reported in previous publications [7, 22]. Upmodulated PFM and cathodic iDC steps effectively elicited excitatory eye rotations associated a head rotation towards the stimulated canal. Anodic iDC current steps elicited inhibitory eye rotations and, when paired with an adapted cathodic offset, resulted in an increased inhibitory magnitude of eye rotation velocities in comparison to the downmodulated PFM responses. This increase in iDC-mediated eye rotational velocities did not appear to come at the cost of greater current spread as evidenced by the lack of significant difference in angle of axis misalignment between iDC and PFM conditions. Importantly, iDC modulation could successfully reproduce a range of eye rotational vectors associated with the modulation of two semicircular canals simultaneously. This suggests that direct current modulation of the semicircular canals can restore a functional range of head rotation detection in the BVD-affected vestibular system with a potential benefit over currently existing modulation strategies.

We originally hypothesized that iDC modulation without a constant current offset would be sufficient to increase the magnitude of inhibitory VOR responses. However, a statistically significant increase in our experiment was evident only once the animal was also adapted to a constant cathodic current offset. It has previously been shown in chinchillas that after gentamicin treatment, the spontaneous firing rate of the vestibular afferent is reduced [20]. It is possible that the cathodic current offsets we provided restored the afferent firing rate to a physiological ‘optimum’ value at or near the 60 PPS firing rate of the normal chinchilla, which then facilitated a greater magnitude of inhibitory motions.

We expected that increasing cathodic iDC baseline would attenuate the excitatory VOR response as can be seen with increasing PFM baselines in the current and previous studies [8]. Surprisingly, higher cathodic iDC baselines did not significantly impact these excitatory responses. This was true even at very high iDC baselines which we assumed would lead to saturation given the large transient responses to identical current amplitudes. Similarly, we could not fully extinguish the inhibitory VOR response with anodic iDC baselines, which we expected would prevent further downmodulation of the afferent. One potential explanation for this is that iDC baseline offsets may not be affecting the afferent firing rates in the same way as transient iDC changes. Rather, there may be a complex relationship between adapted offset and afferent firing rate which is not apparent when this current is modulated transiently. Adaptive changes in the central system due to e.g. tonic vs phasic contribution to VOR pathways [24] might also play a role in this phenomenon. A proper characterization and explanation of this effect is beyond the scope of this study. As our study used visual responses as our assay and did not directly examine neural activity, we cannot make any definitive claims about the effects of iDC on vestibular afferent firing rate. To better understand the mechanisms behind these differences between PFM and iDC modulation it will be necessary to examine the effect of iDC on the vestibular afferents directly via single-unit electrophysiological recordings of the vestibular nerve. Parallel development of computational models of iDC interaction with the vestibular afferents to predict neural responses to tonic/transient iDC modulation could also help to explain our findings.

Given that DC does not have a frequency component and so must modulate amplitude, one potential limitation of iDC neural prostheses is that changes in amplitude might create unintended current spread and thus misalignment of the VOR responses. Previous work in our lab has shown that pulse-amplitude modulation (PAM) of the vestibular nerve leads to greater angles of axis misalignment in the VOR response, likely due to increased current spread to the other semicircular canals [8]. In this study we did not see an increased angle of axis misalignment with increasing iDC intensity. Current amplitudes for iDC modulation were smaller than PFM amplitudes. It is possible then that we did not observe any change in the angle of misalignment because, as spread of current is proportional to current amplitude, iDC amplitudes were too small to spread to the neighboring canals. Our results suggest that changes in current spread resulting from iDC amplitude modulation are not likely to limit implementation of an iDC-mediated vestibular prosthesis.

The amplitude and phase lead/lag characteristics of our VOR responses to sinusoidal modulation using both iDC and PFM modulation strategies showed a decreasing amplitude and increasing phase lag as frequency increased. Previous studies showed high-pass

characteristics of steady state responses that do not appear to be consistent with our findings [6, 7]. Our results are difficult to directly compare with previous studies as frequency characteristics of eye responses have been previously analyzed as gain in relation to rotational or simulated rotational head velocity. This is an appropriate measure in the context of a ratio of evoked eye velocity to encoding of a given head velocity. As we were examining frequency responses in response to electrical modulation alone (rather than encoding of head velocity), gain was not an appropriate measure to compare differences in VOR responses. Thus, the present study did not include a head velocity component but instead described the VOR amplitudes in direct relation to stimulus parameters. Both modulation strategies showed lower amplitudes and greater phase lag as frequency increased, suggesting that the vestibular system responded similarly to iDC and PFM across the range of sinusoidal modulation frequencies used in our study. While our data suggest there may be some intricacies in the relative frequency response functions of iDC vs PFM stimulation strategies (there were differences in how conditions varied at high frequencies, with iDC showing a greater drop in peak amplitude but PFM showing a greater phase lag), head rotation data combined with a full comparison across a larger range of frequencies will be necessary to explore this in further detail.

Chronic iDC safety remains a potential roadblock to the implementation of direct current modulation beyond the current acute studies. Although the SDCS device in development at our lab is designed to eliminate DC-mediated toxicity associated with charge imbalance at the metal-electrode interface [15, 17], the effect of sustained constant current alone on neuron physiology is not well understood. Previous studies have proven the acute viability of separating the metal-electrode interface from the tissue via gel-filled interface [14, 15] and the chinchillas in our study provided stable responses following many repeated acute (1–4 hour) experiments. Chronic in-vivo stimulation experiments including a histological assessment of the stimulated regions will be necessary to determine whether sustained iDC is safe to deliver over extended periods of time. This is especially important given that a constant cathodic offset may be necessary to produce optimal modulation to the vestibular canal.

V. Conclusion

Our results suggest that ionic DC can effectively stimulate and inhibit the BVD-affected vestibular system across a range of functional VOR eye rotational vectors and velocities. In conjunction with a safe DC delivery system, iDC modulation could potentially increase the range of simulated head rotation velocities in neuroelectric prostheses designed to mimic vestibular function. Continued research is necessary to fully characterize the single-unit response of the vestibular afferent to iDC, and to determine the chronic safety of the iDC delivery system.

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VII. References and Footnotes

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Fig 1. Implantation location and size reference of gel-filled microcatheter tubes. (A) approximate implantation position of microcatheter tubes in relation to chinchilla vestibular anatomy. (B) size and shape of a microcatheter tube compared to a twisted pair of 75 μm diameter platinum iridium wires. LP, left posterior canal; LH, left horizontal canal; LS, left superior canal; CC, common crus; V, vestibule. Scale bar: 1 mm.

Fig 2.

Electrically evoked VOR traces in implanted chinchillas. (A-D) representative left eye velocity traces in response to left posterior canal stimulation via stepped pulse frequency modulation (A), stepped iDC amplitude modulation (B), sinusoidal pulse frequency modulation (C) and sinusoidal iDC amplitude modulation. Dashed lines above figures indicate the stimulus waveform. Spike trace in A visualizes the change in pulse rate at a reduced frequency so that individual events are discernable. Solid lines indicate movement in the plane of the horizontal (Z) left anterior/right posterior (LARP) and right anterior/left posterior (RALP) canals. Arrowheads indicate the peak excitatory (black) and inhibitory (grey) eye velocities for a single stimulus waveform.

Fig 3.

Peak eye response amplitudes in response to posterior canal stimulation as a function of stimulus type, intensity, and adapted baseline. (A-C) mean eye response peak amplitudes for a positive pulse frequency change from a PFM baseline (A) and cathodic current steps at cathodic iDC baselines (B) and anodic iDC baselines (C) as a function of stimulus intensity. (D-F) mean eye response peak amplitudes for a negative pulse frequency step from a PFM baseline (D) and anodic current steps at cathodic iDC baselines (E) and anodic iDC baselines (F) as a function of stimulus intensity. Asterisks indicate a statistically significant difference between the maximum intensity peak amplitude and the 60 PPS control maximum intensity amplitude, which is outlined with a dashed box (1-way ANOVA; $P < 0.05$; $n = 5$ animals).

Fig 4.

Box-and-whiskers plot of Angle of axis misalignment as a function of PFM, cathodic iDC and anodic iDC baselines. Misalignment angle did not vary significantly between baselines (1-way ANOVA; $P = 0.99$; $n = 5$ animals).

Fig 5.

Eye response amplitude (A) and phase shift (B) of steady-state eye responses during sinusoidal pulse-frequency or iDC-amplitude modulation. Phase characteristics were calculated using a least-squares sinusoidal fit to eye movement velocity traces.

Fig 6.

Dual canal modulation using DC or PFM stimulation. (A) example plot showing the range of eye rotation vectors theoretically achievable via simultaneous stimulation of the left anterior and left posterior canals. The combined vector space for LARP and RALP has been plotted to an arbitrary 2-dimensional plane for better visualization, with each quadrant representing a space occupied by vectors corresponding to a combination of excitatory (+) and inhibitory (-) responses in one or both canals as indicated. (B) eye response magnitude as a function of stimulation vector in a 60PPS baseline PFM control and for DC modulation with a $-10 \mu\text{A}$ baseline. (C) angle of axis misalignment as a function of stimulation vector in a PFM control and for DC modulation with a $-10 \mu\text{A}$ baseline. Shaded quadrants indicate which areas of the graphs correspond to the areas of the plot in (A).