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Suprachoroidal electrical stimulation: Effects of stimulus pulse parameters on visual cortical responses

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Abstract

Objective: Neural responses to biphasic constant current pulses depend on stimulus pulse parameters such as polarity, duration, amplitude and interphase gap. The objective of this study was to systematically evaluate and optimize stimulus pulse parameters for a suprachoroidal retinal prosthesis.

Approach: Normally sighted cats were acutely implanted with platinum electrode arrays in the suprachoroidal space. Monopolar stimulation comprised of monophasic and biphasic constant current pulses with varying polarity, pulse duration and interphase gap. Multiunit responses to electrical stimulation were recorded in the visual cortex.

Main results: Anodal stimulation elicited cortical responses with shorter latencies and required lower charge per phase than cathodal stimulation. Clinically relevant retinal stimulation required relatively larger charge per phase compared with other neural prostheses. Increasing the interphase gap of biphasic pulses reduced the threshold of activation; however, the benefits of using an interphase gap need to be considered in light of the pulse duration and polarity used and other stimulation constraints. Based on our results, anodal first biphasic pulses between 300 - 1200 μs are recommended for suprachoroidal retinal stimulation.

Significance: These results provide insights into the efficacy of different pulse parameters for suprachoroidal retinal stimulation and have implications for the design of safe and clinically relevant stimulators for retinal prostheses.

1. Introduction

Retinal prostheses are designed to evoke visual percepts by electrically stimulating residual retinal neurons in blind patients or patients with very low vision caused by degenerative retinal disorders (Chowdhury *et al* 2005, Chader *et al* 2009, Cleland 1998, Humayun *et al* 1999, Zrenner *et al* 2011, Jensen *et al* 2009). Electrical stimulation using charge balanced biphasic pulses are considered clinically relevant and safe for use with neural prostheses (Cogan 2008, Lilly 1961, Shepherd *et al* 1983) and are widely used in retinal prostheses (Humayun *et al* 2009, 2003, Rizzo *et al* 2003, Suaning *et al* 1998). These stimulation pulses can be either constant current, where the current (and injected charge per phase) is held constant, or constant voltage, where voltage is held constant (injected charge depends on the impedance of electrode tissue interface) (Cogan 2008). Since neural response is dependent on charge, constant current pulses are generally preferred over constant voltage pulses (Brummer *et al* 1983, Cogan 2008, Shepherd *et al* 2013). Neural responses to electrical stimulation with biphasic constant current pulses depend on several stimulation parameters (Ranck 1975) including pulse polarity (Jensen and Rizzo 2006, Liang *et al* 2011), pulse duration (for biphasic waveforms, pulse duration refers to the duration of the leading phase; 'D', Figure 1) (Crozier 1937) and interphase gap ('G', Figure 1) (Shepherd and Javel 1999).

The most common pulse configuration used in retinal prostheses is a biphasic charge balanced pulse with a cathodal leading phase (Table 1). This is due to the fact that the cathodal phase depolarizes neurons near the stimulating electrode and initiates an action potential while the anodal phase hyperpolarizes proximal neurons (Ranck 1975). Therefore, a cathodal phase (or a biphasic pulse with a cathodal leading phase) requires lower charge to initiate an action potential when the stimulating electrode is positioned close to the neural tissue (Abramian *et al* 2011, Liang *et al* 2011, Shepherd and Javel 1999). However, some *in vitro* studies using subretinal prostheses (Jensen and Rizzo 2006, 2009, Stett *et al* 2007) reported that anodal stimulation required less charge to elicit a response than cathodal stimulation. Furthermore, neural responses also depended on the location (Jensen and Rizzo 2006, Jensen *et al* 2005a) and distance of the stimulating array from the target retinal neurons

(Abramian *et al* 2011, Jensen *et al* 2005b). It is clear that there is a complex relationship between retinal response and location of the stimulating electrode (epiretinal or subretinal).

Pulse duration plays an important role in the excitability of neural tissue (Geddes and Bourland 1985, Grill and Mortimer 1996, Jensen *et al* 2005b). A shorter pulse duration is preferred for two reasons; first, to allow spatially localized stimulation (Grill and Mortimer 1996, Jensen *et al* 2005b) and second, because shorter pulses require less charge per phase to activate neural tissue (Sekirnjak *et al* 2006). However, it is evident from Table 1 that wide ranges of pulse durations have been used with retinal prostheses (0.25-4 ms for epiretinal prostheses; 0.1-4 ms for subretinal prostheses; and 0.25 ms for suprachoroidal prostheses). Furthermore, there is an upper limit to the pulse duration beyond which a further increase in the pulse duration does not result in a change in the charge threshold (Dawson and Radtke 1977). It is unclear how the neural response varies with these large pulse durations and where the upper limit of charge lies for suprachoroidal retinal stimulation.

Studies in cochlear implants have shown that the pulse duration and the charge required to activate neural populations may be reduced by adding an interphase gap (Shepherd and Javel 1999). The first phase of the biphasic current pulse depolarizes the neuron while the second phase hyperpolarizes it. The period immediately following depolarisation of the neuron has been defined as the critical period (Bromm and Frankenhaeuser 1968, Shepherd and Javel 1999). The critical period was estimated to be approximately100 μs for myelinated nerve fibres (Bromm and Frankenhaeuser 1968, van den Honert and Mortimer 1979a, 1979b) . Inserting an interphase gap in this critical period provides sufficient time for the propagation of action potentials generated by local depolarization prior to hyperpolarization by the second phase (Ranck 1975). If the hyperpolarization is large enough it can significantly increase threshold and block the propagation of action potentials (Ranck 1975, van den Honert and Mortimer 1979a, 1979b). The pulse durations and interphase gaps used in retinal prostheses are much wider than those described by Shepherd and Javel (1999) and are longer than the critical period. It is presently unclear how the neural response varies with combinations of long interphase gaps and long pulse durations.

Table 1. Stimulation parameters used in retinal prostheses.

Implant position and study	**Return Configuration**	**Stimulation Configuration**	**Polarity [P]**	**Pulse Duration (D) ms/ ph**	**Interphase Gap (G) ms**	**Suggested Parameters D (G) [P]**
Epiretinal Human [a]	Monopolar/ Bipolar	Biphasic	Cathodal / Anodal	0.1-16	0.1-2	0.25-4ms
Epiretinal *in vivo* [b]	Monopolar/ Bipolar	Biphasic	Cathodal	0.2-1	0.2-0.25	-
Epiretinal *in vitro*[c]	Monopolar/ Bipolar	Biphasic/ Monophasic	Cathodal / Anodal	0.2-50	0.4-2	0.1-0.5 (<0.460)ms [Cathodal first biphasic]
Subretinal *in vivo*[d]	Monopolar	Biphasic	Cathodal / Anodal	0.05-1	0.05-0.25	-
Subretinal *in vitro*[e]	Monopolar/ Bipolar	Biphasic/ Monophasic	Cathodal / Anodal	0.1-50	-	-
Suprachoroidal Human[f]	Monopolar	Biphasic	Cathodal	0.05-5	0-4	0.5-1 (1)ms
Suprachoroidal *in vivo*[g]	Monopolar/ Bipolar	Biphasic/ Monophasic	Cathodal / Anodal	0.1-1	0.01	> 0.25ms [Cathodal first biphasic]

[a](De Balthasar *et al* 2008, Hornig *et al* 2005, Horsager *et al* 2011, 2010, Humayun *et al* 2003, Klauke *et al* 2011, Rizzo *et al* 2003)

[b](Eckhorn *et al* 2006, Eger *et al* 2005, Elfar *et al* 2009, Hesse *et al* 2000, Walter and Heimann 2000)

[c](Grumet *et al* 2000, Humayun 1994, Jensen *et al* 2003, 2005a, Sekirnjak *et al* 2006, Weitz *et al* 2011)

[d](Eckhorn *et al* 2006, Rizzo *et al* 2004, Schanze *et al* 2006, Yamauchi *et al* 2005)

[e](Gerhardt *et al* 2011, Jensen and Rizzo 2006, Tsai *et al* 2009)

[f](Fujikado *et al* 2011, 2007, Sakaguchi *et al* 2009)

[g](Liang *et al* 2011, Nakauchi *et al* 2005, Nishida *et al* 2009, Sakaguchi *et al* 2004, Shivdasani *et al* 2010, Wong *et al* 2009, Yamauchi *et al* 2005)

A further consideration for neural prostheses is stimulation safety. For long-term neural stimulation, a biphasic charge balanced pulse is considered non-damaging (Rose and Robblee 1990, Shepherd *et al* 1990, Shepherd and Javel 1999). However, charge balance alone does not ensure non-damaging stimulation. Charge per phase, charge density, electrode size, pulse duration, stimulus rate and the number of pulses in a pulse train are all factors that may lead to tissue damage (McCreery *et al* 1990, Shannon 1992, Agnew and McCreery 1990, Vankov *et al* 2005, Butterwick *et al* 2007, Shepherd *et al*

2013). Understanding the implications of stimulation on neural safety is critical in establishing clinically relevant stimulation parameters for retinal prostheses.

From the studies discussed above it is evident that pulse polarity, pulse duration and interphase gap have an effect on neural response. Furthermore, there exists a complex relationship between the effect of pulse parameters on neural response and position of the stimulating electrode array. It is therefore necessary to evaluate pulse parameters in the position intended for the stimulating electrode array. In the present study, we used multiunit responses in the visual cortex to suprachoroidal electrical stimulation to investigate the effects of varying pulse polarity and pulse duration for both monophasic and biphasic pulses, and interphase gap for a biphasic pulse.

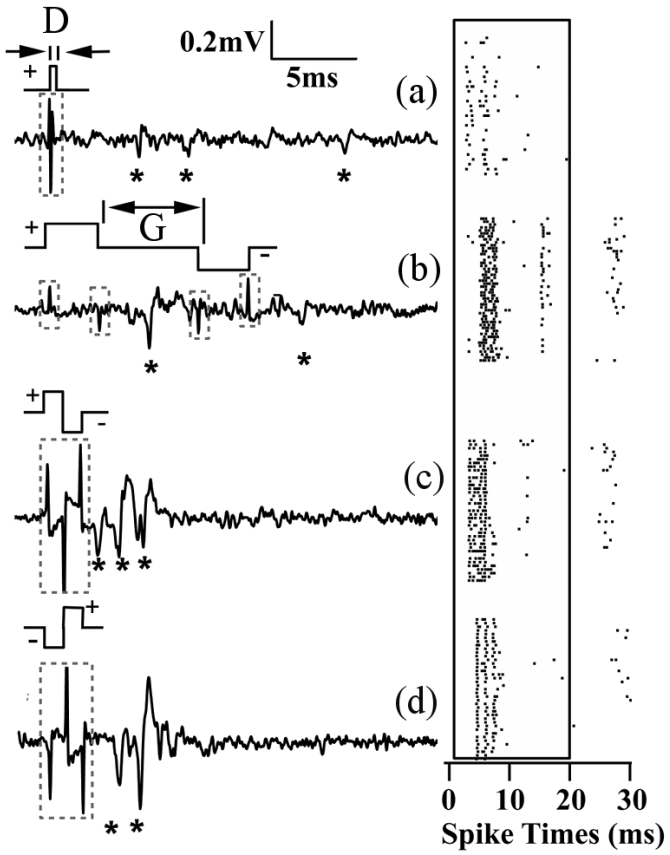

Figure 1: *The left panels show examples of recorded multiunit activity from four different recording sites. Inset shows the stimulus pulse shape, where 'D' is the pulse duration per phase and 'G' is the interphase gap. Stimulus artefacts were removed from the analyses (dashed rectangle) and the stimulus locked multi-unit activity [*] recorded from the visual cortex. Waveform traces are for a*

single trial of 1×current pulse presented at 1 Hz with a current amplitude (I), required to elicit maximum spiking response; (a) I=1 mA, D=0.1 ms; (b) I=0.08 mA, D=3 ms, G=6 ms; (c)(d) I=0.2 mA, D=1 ms, G=0. Waveform traces show (a) monophasic anodal pulse; (b) biphasic anodal first pulse with a long interphase gap; (c) biphasic anodal first pulse with no interphase gap; and (d) biphasic cathodal first pulse with no interphase gap (d). Right panels show the corresponding dot raster representation of the spike times (at current I) for 30 presentations of the stimulus. The rectangular box indicates the post stimulus interval (3-20 ms) in which spikes were further analysed.

2. Methods and Materials

2.1. Experimental Preparation

The study was approved by the Royal Victorian Eye and Ear Hospital animal ethics committee. Subjects were normally sighted adult cats (n = 10) weighing 3.0-5.2 kg. Cats were used in this study as the diameter of the cat eye is similar to that of humans (Bertschinger *et al* 2008, Prince *et al* 1960), and the cat visual system has been well studied (Bertschinger *et al* 2008, Eckhorn *et al* 2006). Animals were anaesthetized with an initial dose of ketamine (intramuscular, 20 mg kg^{-1}) and xylazil (subcutaneous, 2 mg kg^{-1}). Anaesthesia was then maintained with a continuous intravenous infusion of sodium pentobarbitone (0.3 - 0.7 mg kg^{-1} h^{-1}). A continuous intravenous infusion of Hartmann's solution (compound sodium lactate, 2.5 mg kg^{-1} h^{-1}) was administered throughout the experiment to replace body fluid and mineral salts. Animals were prophylactically treated with dexamethasone (intramuscular, 0.1 mg kg^{-1}) to reduce inflammation from surgery, and clavulox (subcutaneous 10 mg kg^{-1}) to reduce bacterial infections. Respiration rate, end-tidal CO_2, blood pressure and body temperature were monitored and maintained within normal levels (Fallon *et al* 2009).

2.2. Stimulating Electrode Array

The stimulating electrode array was comprised of 84 platinum (Pt) electrodes (400 μm diameter; geometric surface area of 0.126 mm^2) arranged in a 7 row x 12 column configuration (Cicione *et al* 2012). The back of each array was coated with silicone (Permatex, CT; Type 65AR flowable) to eliminate sharp edges. The silicone coating on the back of the array was tapered toward the edges (150μm) with the thickest part of the array along the midline (400 μm) (Villalobos *et al* 2012) which allowed the electrode array to conform to the shape of the eye.

2.3. Surgery

Details of the surgery are described in detail elsewhere (Villalobos *et al* 2012). Briefly, under anaesthesia, the choroid was exposed by a lateral canthotomy followed by an incision through the sclera and a tissue pocket was made in the suprachoroidal space. The stimulating electrode array was

inserted 15–17 mm within the tissue pocket beneath the area centralis and fixed to the sclera with sutures (8/0 Vicryl sutures, Johnson & Johnson, Australia). A platinum ball electrode (1.5 mm diameter) was implanted into the vitreous chamber as a monopolar return. The animal was fitted to a stereotaxic frame (David Kopf Instruments, Tujunga, CA) in a dark, electrically shielded Faraday room. After initial visual assessment of the eye, a craniotomy was performed to expose the contralateral visual cortex.

2.4. Electrical Stimulation

Current pulses were delivered to single electrodes on the suprachoroidal array from an optically isolated constant current stimulator via a cross point switch matrix (PXI 2532, National Instruments, Austin, TX) (John *et al* 2011). Monopolar stimulation was performed against the platinum ball electrode placed in the vitreous chamber. The current amplitude, pulse width, interphase gap and electrode shorting used for charge recovery at the end of the stimulation were controlled in software. For biphasic current pulses, electrodes were shorted at the end of each pulse and for monophasic pulses, electrodes were shorted 200 ms from the start of each pulse (capacitive coupling was not used). Charge densities used for stimulation varied up to a maximum of 300 μC/cm^2. Pulse parameters (Figure 1) tested included: (i) pulse polarity (P: cathodal vs. anodal); (ii) pulse duration/ phase (D: 100-3000 μs) for both monophasic and biphasic current pulses and (iii) interphase gap for biphasic pulses. Interphase gaps were presented as a function of the pulse duration per phase (G: 0, 0.5, 1, 1.5 or 2 x pulse duration per phase) and a monophasic pulse was used to represent an infinitely long interphase gap. Current amplitude was varied in a randomized matrix and stimulus pulses were presented at 1 Hz for 10 repetitions of each stimulus combination.

2.5. Cortical Recording

Electrically evoked responses to cathodal first biphasic charge-balanced current pulses (500 μs duration, 25 μs interphase gap) were recorded (100 kHz sampling rate) from the surface of the visual cortex using a large surface area platinum ball electrode (1.5 mm diameter). The recording electrode was connected to a bio amplifier ISO-80 (World Precision Instruments, Inc. Sarasota, FL) and a

National Instruments data acquisition card NI-USB 6251 (National Instruments, Austin, TX). The recording electrode was systematically moved over the surface of the exposed cortex in 2 mm steps. Electrically evoked cortical potentials were recorded at each location in order to identify a single location on the surface of the visual cortex having the lowest threshold response. The dura mater was removed at that location, and a multi-channel recording electrode was implanted. Multiunit recordings were made using either 4 x 8 linear recording arrays (NeuroNexus Technologies, Ann Arbor, MI) or 6 x 10 or 7 x 7 planar silicone arrays (Blackrock Microsystems, Salt Lake City, UT). Linear recording array sampled an area approximately 1.4 mm (depth) × 1.2 mm (width) at a depth of 1-2.4 mm below the surface of the cortex. Planar silicone arrays sampled an area of 2.4 x 4 mm (6 x 10) or 2.8 x 2.8 mm (7x 7) of the cortex at a depth of approximately 1 mm below the surface of the cortex. Recording electrodes were typically inserted contralateral to the stimulated eye, close to the posterior lateral gyrus in the visual cortex, corresponding to the macular region of the retina (Tusa *et al* 1978). Cortical responses to electrical stimuli were recorded at a sampling rate of 30 kHz using the Cerebus data acquisition system (Blackrock microsystems, Salt Lake City, UT).

2.6. Spike Analysis

Multiunit responses were analysed offline using IgorPro (Wavemetrics, Lake Oswego, OE). Stimulus artefacts were removed by replacing sample points on the stimulus artefact with values computed from neighbouring sample points (Heffer and Fallon 2008). Electrophysiological recordings were band-pass filtered using a third order Butterworth filter (300–5000 Hz). Multiunit spikes were detected when 4 x root mean square value of the incoming signal was crossed. The total number of spikes occurring in the analysis window (3-20 ms, Figure 1) was plotted on a rate-level function (Figure 2a, 2b) for each recording site. A sigmoidal function was fitted to the data and the current required to elicit 50% saturating response was defined as I-50 (Figure 2c, 2d) (Fallon *et al* 2009, Cicione *et al* 2012). The I-50 obtained at each pulse duration was plotted on the strength duration curve (SDC, Figure 2c, 2d) (Rushton 1932). The canonical form of the SDC was proposed by Lapicque (1907) and describes the relation between the current ‘I’ and the pulse duration ‘D’. On this curve two critical points are identified; the rheobase ‘r’ and the chronaxie ‘C’ (Rushton 1932). The

rheobase was defined as the current required to activating a neuron using an infinitely long-duration pulse. The chronaxie is the duration of a pulse whose current is twice the rheobase and is considered to be the excitability constant of the given neuron (Geddes and Bourland 1985, Rushton 1932). The rheobase and chronaxie were obtained by fitting the SDC from each cortical channel with a power function, $Y = (a * x^{pow}) + r$ (Figure 2c, 2d), where 'r' was the rheobase (Sekirnjak *et al* 2006). The effect of pulse polarity on cortical response was evaluated from the first spike latencies at I-50 and the I-50 values. The effect of pulse duration on cortical response was evaluated using first spike latencies, the SDC and the charge duration curve (CDC) (Geddes 2004). The effect of pulse polarity and duration was evaluated on rheobase and chronaxie values. Finally, the effect of interphase gap for biphasic pulses was evaluated using change in I-50 (dB) at each interphase gap relative to the I-50 using no interphase gap.

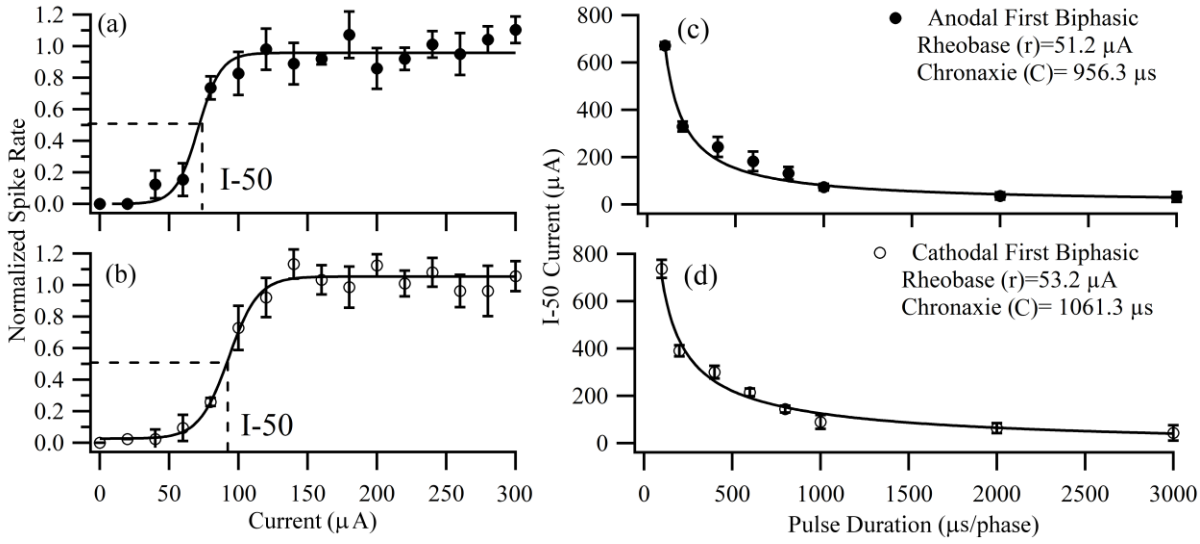

Figure 2: *(a) and (b) show rate-level functions of a single recording site using biphasic pulses with D=1000μs and G=0 (anodal first biphasic - a, cathodal first biphasic - b). The symbols show the spike count 3–20 ms post-stimulus onset at each current level normalised to the plateau value of the fitted sigmoidal function. The threshold (I-50) was defined as the current required for achieving a 50% saturating response. (c) and (d) show the corresponding strength duration curves using the I-50 current for anodal first biphasic stimulation (c) and cathodal first biphasic stimulation (d). The curves were fitted with a power function:* $Y = (a * x^{pow}) + r$ *(the parameter 'r' was defined as the*

rheobase). Each data point indicates the mean of 10 repetitions and the error bars indicate standard error of the mean.

2.7. Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics 21.0, (IBM, Inc., Armonk, NY). Two way repeated measures ANOVAs were performed to determine within subject effects (alpha was set to 0.05).

3. Results

Fifteen retinas in 10 animals were electrically stimulated via electrodes implanted in the suprachoroidal space. Electrically evoked multiunit activity was obtained from 180 responding cortical recording sites across all experiments.

3.1. Response properties

Figure 1 shows stereotypical electrophysiological recordings from four different sites in the visual cortex to different stimulus configurations. An early burst of action potentials was seen at latencies of 3–20 ms post stimulation and represented the majority of neural activity (rectangle drawn on the dot raster plot, Fig. 1). In some instances, a secondary burst at latencies 20-50 ms post stimulation onset and in rare instances a third burst of spiking activity at latencies greater than 50 ms were observed. Previous studies in the visual cortex have shown that the early cortical response (< 20 ms) is the consequence of direct electrical stimulation of the retinal ganglion cells (RGCs) (Eckhorn *et al* 2006, Wong *et al* 2009, Elfar *et al* 2009). The longer-latency activity is thought to be due to indirect activation of the RGC through neurons in the inner retinal layer (Jensen *et al* 2005b) or inter-cortical interactions (Schanze *et al* 2002). In patients with retinal dystrophies who would be candidates for a visual prosthesis, the retina will have undergone some degree of reorganization. Both the functional state and connectivity of retinal neurons will vary significantly from a normal retina (Fletcher *et al* 2011, O'Brien *et al* 2012, Marc *et al* 2003). Therefore, we focussed on the early response as this is most likely due to direct activation of RGCs by electrical stimulation and is less likely to be affected by remodelling of the degenerate retina.

3.2. Effect of Pulse Polarity

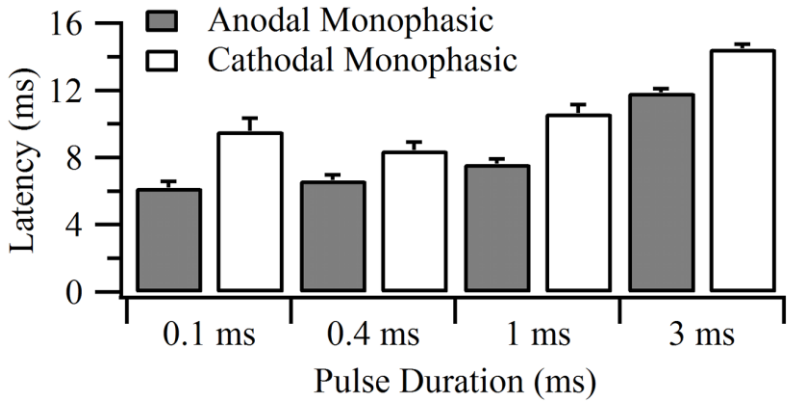

Figure 3: *Cortical response first spike latencies to cathodal and anodal monophasic pulses at I-50. Error bars indicate standard error of the mean (n= 96).*

We measured the first spike latency at I-50 in response to monophasic stimulation (Figure 3). A two-way repeated measures ANOVA was conducted on the latencies (n=96) for monophasic pulses with polarity and pulse duration as factors. There was a statistically significant effect of pulse polarity, pulse duration and an interaction between the two (all p's < 0.05). The first spike latencies at I-50 for anodal pulses were 2-4 ms shorter than cathodal pulses. Post-hoc analysis indicated there was a significant effect of polarity at all pulse durations apart from 0.4 ms (p = 0.19). First spike latencies increased with pulse duration for both anodal and cathodal stimulation.

3.3. Effect of Pulse Duration

The rheobase and chronaxie values for both anodal and cathodal pulses were determined from the SDCs for each cortical site. Figure 4a shows the mean chronaxie and Figure 4b shows the mean rheobase values for both pulse shapes (monophasic and biphasic) and both polarities (anodal and cathodal). Two-way repeated measures ANOVAs were conducted separately on chronaxie and rheobase with polarity and pulse shape as factors (n=98). Despite the apparent increase in both chronaxie and rheobase with biphasic stimulation in Figure 4, there was no significant effect of pulse shape or polarity in either case (all p's > 0.05).

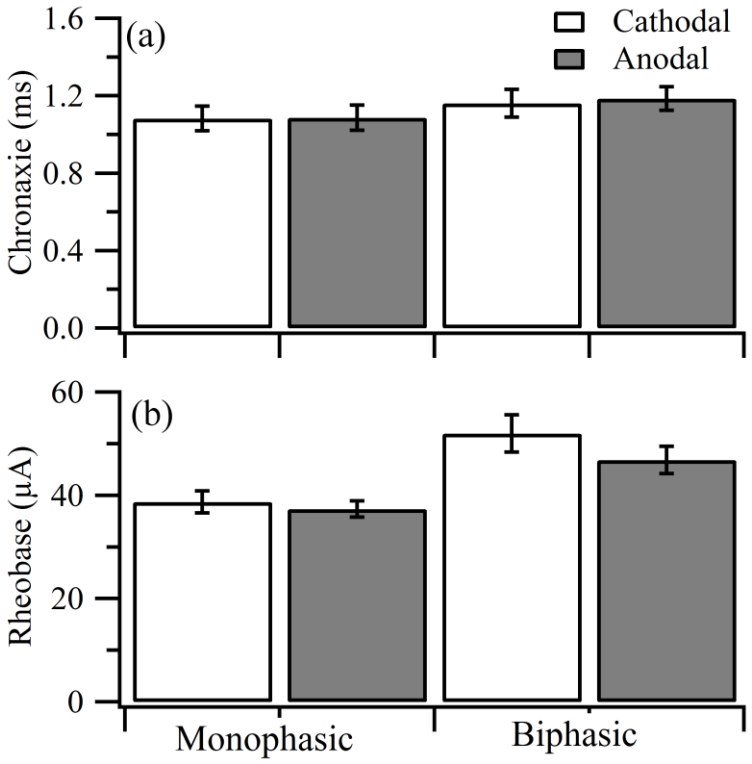

Figure 4: *Chronaxie and Rheobase: (a) Shows the mean chronaxie values and (b) shows the mean rheobase values for both monophasic and biphasic pulses. Error bars show the standard error of the mean (n= 98).*

Figure 5 shows the mean SDCs (Figure 5a and c) and corresponding mean CDCs (Figure 5b and d) for monophasic (Figure 5a and b) and biphasic pulses (Figure 5c and d) and both polarities. As expected, for both monophasic and biphasic pulses the current required to elicit a 50% of maximum cortical response was inversely related to the pulse duration, while the charge per phase required for activation was lowest for the shorter pulse duration. Two-way repeated measures ANOVAs were conducted separately on the I-50 for monophasic and biphasic pulses with polarity and pulse duration as factors. There was a statistically significant effect of pulse polarity, pulse duration and a significant interaction between pulse polarity and pulse duration, for both monophasic (p's < 0.05, $n=140$) and biphasic (p's < 0.05, $n=140$) stimulation. Post-hoc analysis for monophasic stimulation revealed that there was a statistically significant effect of polarity at pulse durations less than 1000 μs ($p < 0.01$). For these pulse durations, I-50 for anodal stimulation was ~3dB lower than cathodal pulses. Post-hoc analysis for biphasic stimulation revealed that there was a statistically significant effect of polarity at

all pulse durations apart from 400 µs (p = 0.8), with I-50 for anodal stimulation ~1dB lower than cathodal pulses. On the mean SDCs and mean CDC's in Figure 5 for biphasic pulses, we identified four regions [R1-R4] of pulse duration [D] as a function of chronaxie [C]. R1= [D ≤ 0.25 x C], was characterized by a steep reduction in the SDC and a corresponding steep increase in the CDC. R2= [0.25 x C < D ≤ 0.5 x C], was characterized by continuing reduction in the current and an increase in the charge nearing the maximum charge. R3= [0.5 x C < D ≤ C], was characterized by a slowly reducing current with no change in the charge. R4= [D > C], was characterized by the asymptotic current and charge.

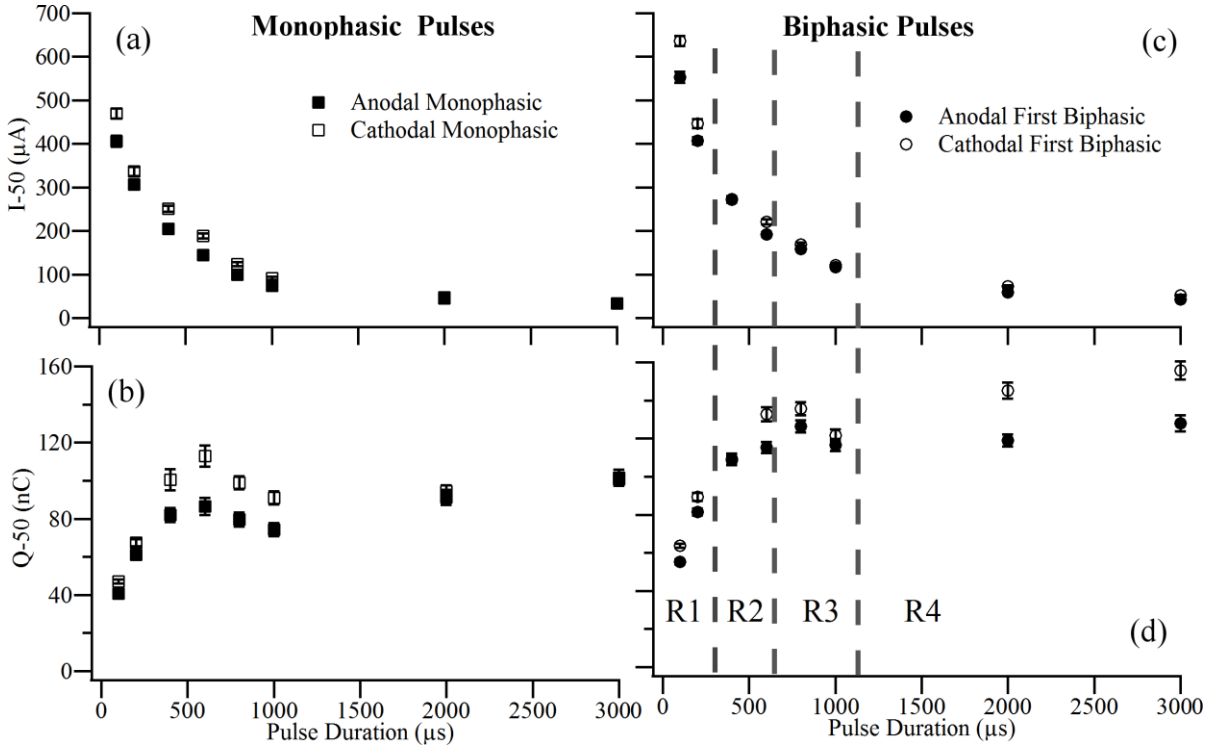

Figure 5: *Effect of pulse duration: I-50 or Q-50 versus pulse duration. (a) Shows the mean I-50 for monophasic pulses; (b) shows the corresponding mean charge level (Q-50) for monophasic pulses; (c) shows the mean I-50 for biphasic pulses; and (d) shows the corresponding mean Q-50 for biphasic pulses. The x-axis shows the pulse duration. The dashed lines indicate the four regions of activation based on the chronaxie-c. R1= [D ≤ 0.25 x C], R2= [0.25 x C < D ≤ 0.5 x C], R3= [0.5 x C < D ≤ C], and R4= [D > C]. Error bars show the standard error of the mean (n= 140).*

3.4. Effect of Interphase Gap

We evaluated the effect of interphase gap for biphasic pulses by analysing the change in I-50 from no interphase gap to a maximum interphase gap of two times the pulse duration in each region defined on the SDC/CDC (with both pulse polarities). A monophasic pulse was used to represent a pulse with an infinitely long interphase gap. Figure 6 summarizes the effect of interphase gap on cortical response with change in I-50 represented in dB. In each region of pulse duration there was variability in the change in I-50 with increasing interphase gap between the anodal and cathodal pulses. However, both anodal and cathodal pulses showed a similar trend of a reduction in I-50 with increasing interphase gap. As expected, the largest drop in I-50 (2-5 dB) was seen with monophasic pulses (infinitely long interphase gap) irrespective of the region of stimulation [R1-R4].

Two-way repeated measures ANOVAs were conducted separately in each region R1-R4 to examine the effect of pulse polarity and interphase gap on the change in I-50. In all regions, there was no significant interaction between interphase gap and pulse polarity (all p's > 0.05). In R1 (n=120), R3 (n=192) and R4 (n=172) both main effects of interphase gap (all p's < 0.001) and pulse polarity were significant (all p's < 0.001). However, in R2 (n=173) the main effect of interphase gap was not significant ($p = 0.19$) but the main effect of polarity was significant ($p < 0.001$). With the exception of R3 at 1.5 x D, cathodal first biphasic pulses showed a larger reduction in I-50 than anodal first biphasic pulses. In both R1 and R2, cathodal first biphasic pulses showed a larger decrease in I-50 (~2 dB) than anodal first biphasic pulses (< 1dB). The largest reduction in I-50 was observed in R4 where, cathodal first biphasic pulses had a ~4 dB drop in I-50 and anodal first biphasic pulses had a ~3 dB drop in I-50.

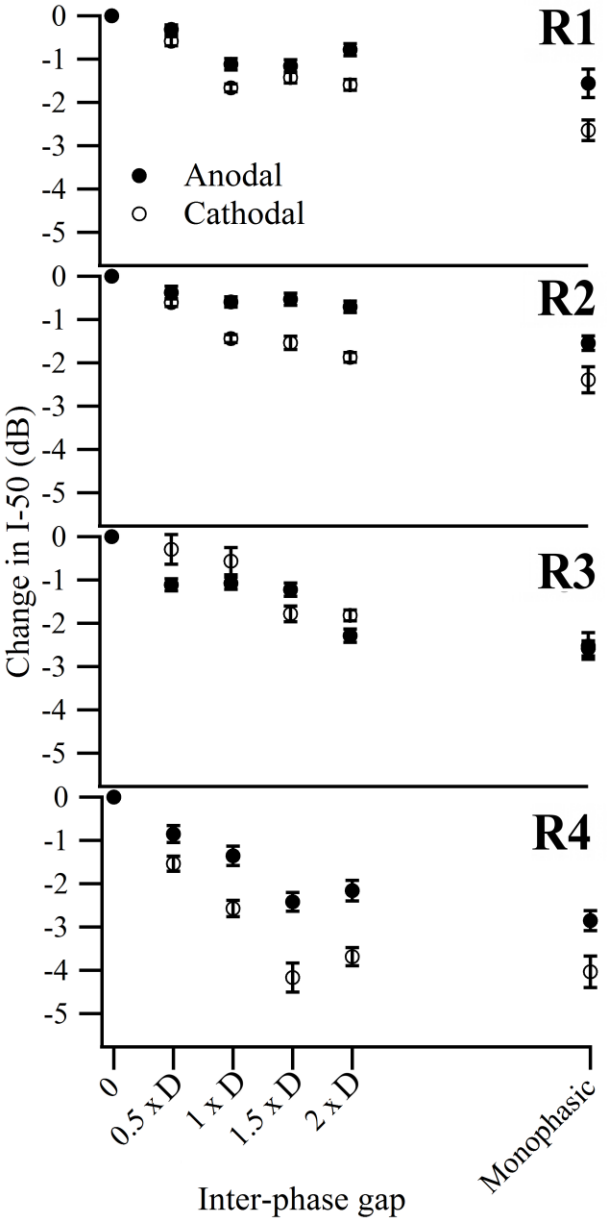

Figure 6: *Effect of inter phase gap: Change in I-50 shown in decibels in four regions: R1= [D ≤ 0.25 x C], R2= [0.25 x C < D ≤ 0.5 x C], R3= [0.5 x C < D ≤ C], and R4= [D > C]. X-axis shows the interphase gap as a function of the pulse duration. Error bars show the standard error of the mean [R1 (n=120), R2 (n=173), R3 (n=192) and R4 (n=172)].*

4. Discussion

The goal of this study was to optimize stimulation pulse parameters for suprachoroidal retinal prostheses by conducting a systematic evaluation of the effects of pulse polarity, pulse duration and interphase gap on cortical response properties using monopolar stimulation. The main findings of this study were: (1) anodal stimulation requires significantly less current than cathodal stimulation to evoke robust cortical activity; (2) relatively large charge per phase is required for clinically relevant retinal stimulation; and (3) adding an interphase gap between the two phases of a biphasic pulse reduces the current required to elicit robust cortical responses compared to no gap.

Cortical response properties: The types of cells that are activated by suprachoroidal stimulation are not known. However, Potts and Inoue (1969), Jensen *et al* (2003) and Rizzo *et al* (2004) among others have suggested that the electrically evoked response represents the activation of cells in the inner nuclear layer (bipolar cells) or RGC's and not the photoreceptors. Furthermore, Kanda *et al* (2004), Liang *et al* (2011) and Fujikado *et al* (2011) suggested that the cortical response to suprachoroidal stimulation was a result of activation of either bipolar cells, RGCs or both. The early response latencies observed (< 20 ms) are consistent with those seen by Eckhorn *et al* (2006) and Elfar *et al* (2009) in the visual cortex of cats using epiretinal stimulation. These latencies appear much earlier than those observed with light stimulation of the photoreceptors (>20ms) (Dawson and Radtke 1977, Potts and Inoue 1969). While Eckhorn *et al* (2006) concluded that the visual cortex responses in their study were a direct consequence of stimulating the RGC's, it is not possible to rule out that other excitable cells within the retina such as bipolar cells, amacrine cells, etc. are also involved in the generation of cortical responses.

Anodal stimulation requires significantly less current than cathodal stimulation: The present results show that anodal pulses resulted in lower I-50 than cathodal pulses for both monophasic and biphasic pulse shapes. This finding is supported by a feasibility study by Kanda *et al* (2004), where anodal monophasic pulses had up to 80% lower evoked potential thresholds in normal rats, and up to 8% lower evoked potential thresholds in blind rats compared to cathodal monophasic pulses. In the

present study, we found 15 % lower thresholds to anodal biphasic stimulation compared to cathodal biphasic stimulation in normally sighted cats (30 % lower thresholds with anodal monophasic stimulation compared to cathodal monophasic stimulation), a reduction much less than that reported by Kanda *et al* (2004). There are a number of differences between the studies that might account for this difference, including: the size of the stimulating electrode (a ball electrode 200-300 µm in diameter in the Kanda *et al* (2004) study as opposed to 400 µm disc electrodes in the present study); the species; the recording location (superior colliculus in Kanda *et al* (2004) as opposed to visual cortex in the present study) and the relative position of the stimulating array in the suprachoroidal space. Another study (Liang *et al* 2011) using suprachoroidal stimulation in normally sighted rabbits showed that cathodal first biphasic pulses resulted in lower cortical evoked potential thresholds than anodal first biphasic pulses, a finding opposite to our observation. A number of technical differences between the present study and Liang *et al* (2011) may have contributed to the observed differences including: the nature of the recordings (multiunit activity vs. evoked responses from the cortical surface); variances in retina between species; areas of sampling in the cortex; and relative position of the stimulating array to the retinal layers.

It is not surprising that the anodal-leading pulses results in shorter latencies than cathodal-leading pulses. Similar results have been previously observed in stimulation of retinal tissue (Jensen *et al* 2005a) as well as other neural sites (Shepherd and Javel 1999). A likely explanation is the variation in the site of action potential initiation (Ranck 1975, Shepherd and Javel 1999, van den Honert and Mortimer 1979b), with the cathodal phase depolarizing cells in close proximity to the active electrode while the anodal phase depolarizes distal neurones (Ranck 1975). A second explanation is offered by Jensen and Rizzo (2006), which takes into consideration the working of the retina and its complex neural composition, and is based on the difference in threshold and latency of responses from the ON and OFF RGC's. Jensen and Rizzo (2006) reported that, in response to subretinal electrical stimulation, OFF RGC's exhibit shorter response latencies and lower thresholds to anodal stimulation than cathodal stimulation. However, ON RGC's exhibit shorter latencies to cathodal stimulation than anodal stimulation but there was no difference in thresholds to anodal and cathodal stimulation. In the

present study, the shorter cortical response latencies and lower I-50s from anodal stimulation could have resulted from preferential stimulation of the OFF cells by the anodal phase. However, the results from the present study cannot be directly compared to those reported by Jensen and Rizzo (2006) for two reasons. Firstly, it is not known whether the responses recorded in the retina by Jensen and Rizzo (2006) would indeed generate a cortical response. Secondly, Jensen and Rizzo (2006) recorded directly from excised rabbit retinal ganglion cells *in vitro* while in the present study we used an *in vivo* cat model and recorded in the cortex.

It is not known which of the two mechanisms discussed above are contributing to the differences in latencies and thresholds between the two polarities. Our study was not designed to determine the type of cells that are activated with suprachoroidal stimulation. Further investigation using long-term blind animal models may provide further insights into the effect of polarity on neural responses as the retina is known to undergo significant reorganisation following loss of the photoreceptors (Marc *et al* 2003). The present study provides baseline information about suprachoroidal electrical stimulation of the normal retina. Based on our results we conclude that pulse polarity plays an important role in neural recruitment and that anodal pulses require less charge to activate neural tissue than cathodal pulses using suprachoroidal stimulation. Data from human psychophysics studies with a suprachoroidal retinal prosthesis agrees with the animal data from this study in that anodal biphasic stimulation results in lower perceptual thresholds than cathodal biphasic stimulation (Blamey *et al* 2013).

Relatively large charge per phase is required for clinically relevant retinal stimulation: The SDCs in this study followed the well-known shape described by Lapicque (1907) and were fit with a power function. The CDCs followed a rising exponential curve with an initial linear rise in the short pulse duration region s (R1 and R2 [0.1-0.6 ms]) followed by a constant charge region at longer pulse durations (R3 and R4 [>0.6 ms]). This indicates that as the pulse duration nears chronaxie, the charge required tends to become constant. Dawson and Radtke (1977) observed a similar result with electrically evoked responses in the cat visual cortex to epiretinal stimulation. Interestingly, response latencies in the present study increased suddenly at pulse durations >1 ms. Greenberg (1998) observed that as the pulse duration was increased > 0.5 ms there was a sudden ~2 ms shift in the latency of the

response and proposed that it resulted from a change in site of activation from RGCs to bipolar cells. Jensen *et al* (2005b) also observed a similar trend with axonal responses; however, indirect activation was only observed at pulse durations > 50 ms. Jensen *et al* (2005b) concluded that the position of the electrode as well as its geometry played a role in the selectivity of retinal neurons. These results indicate that pulse duration may play a role in preferential retinal stimulation with suprachoroidal stimulation. Further investigation is required to establish the role of pulse duration in preferential retinal stimulation using suprachoroidal prostheses.

Shorter pulse durations are generally preferable for neural prostheses as they require lower charge per phase (Grill and Mortimer 1996, Jensen *et al* 2005b) and this was also observed in the present study. Neural implants such as cochlear implants (Clark 2003, Seligman and Shepherd 2004), cortical visual prostheses (Hambrecht 1995), deep brain implants (Kuncel and Grill 2004) and functional electrical stimulation and micturition (Grill *et al* 1999, Nannini and Horch 1991, Schulman *et al* 2007) typically use pulse durations < 200 μs and operate at charge levels below 50 nC/phase. However, most retinal prostheses require relatively long pulses with durations typically between 0.4-1 ms (Table 1) and require relatively larger charge per phase. In the present study, the threshold charge required varied between 40-130 nC/phase and can be expected to increase in the degenerated retinas. Although using a pulse in the R1 (<0.2 ms) region would require less charge per phase (potentially improving battery life for a wearable prosthesis), short duration pulses require more current and consequently require stimulators that can operate at higher voltage compliances. The optimum pulse duration is therefore a trade-off between the current and charge required to elicit a robust response. Based on the present results, pulse durations between 300-1200 μs would be desirable for use with suprachoroidal stimulation, provided long-term safety studies demonstrate that these pulse widths operate within appropriate safety limits.

Adding an interphase gap between the two phases of a biphasic pulse reduces the threshold of activation of retinal neurons:

Weitz *et al* (2011) examined the effect of increasing the interphase gap with epiretinal stimulation using calcium imaging in tiger salamander retina. They found that increasing the interphase gap to a value equal to the pulse duration lead to approximately 21% (~2 dB drop) decrease in threshold for pulses with durations < 460 µs. Interphase gaps < 0.5 times the pulse duration were less effective, with a threshold reduction of about 12% (~1 dB). With longer duration pulses, there was a drop of 28% (~3d B) in threshold. The results from the present study for cathodal first biphasic pulses in R2 (400- 600 µs) are comparable to those of Weitz *et al* (2011). We found the effect of interphase gap depended highly on pulse duration and pulse polarity. Interphase gaps were generally more effective for cathodal first biphasic pulses than anodal first biphasic pulses. This was evident in R1, R2 and R4, however, this has not been previously reported and it is currently unclear how polarity and interphase gap would interact to reduce activation current. Interphase gaps less than the pulse duration were largely ineffective, while an interphase gap of approximately two times the pulse duration gave the same reduction in threshold as an infinitely long interphase gap (i.e. a monophasic pulse). With suprachoroidal stimulation, this can be achieved at durations > 2000 µs, and with interphase gaps > 3000 µs; however, these durations are greater than the pulse duration range (300-1200 µs) recommended in the present study. Furthermore, the implications of these long duration pulses on neural stimulation safety and tissue damage would need to be investigated.

Table 2. Comparison of the change in I-50/Q-50 between two stimulus pulse configurations.

Stimulus configuration [D (G) D]		Configuration I 600 (0) 600	Configuration II 400 (400) 400	dB Change (*re* configuration I)
Stimulus Type				
Anodal First	I-50 (µA)	220.12	329.18	↑ 3.52
Biphasic	Q-50 (nC)	132.07	131.67	↓ 0.02
Cathodal First	I-50 (µA)	240.44	359.94	↑ 3.51
Biphasic	Q-50 (nC)	144.26	143.97	↓ 0.02

Optimizing combinations of pulse duration and interphase gap: Interphase gaps longer than the pulse duration were required to observe a significant reduction in I-50. However, this reduction in I-50 may not be sufficient to warrant the introduction of an interphase gap, particularly when longer pulse durations are used. For example, consider the I-50 and C-50 levels for two biphasic pulse

configurations used in the present study; both having a total pulse duration of 1200 μs (Table 2). For both polarities, configuration II (using a pulse duration of 400 μs and an interphase gap of 400 μs) required approximately 25% (~3 dB) more current and < 1% less charge than configuration I (using a pulse duration of 600 μs and no interphase gap). Greater reductions in current can be observed by increasing the pulse duration than by increasing the interphase gap when pulse duration is greater than 100 μs (critical period for myelinated fibres). For suprachoroidal stimulation, using longer pulse durations without an interphase gap may require less current and charge to establish a visual percept than using shorter pulses with an interphase gap. Similar studies using blind animal models are expected to provide further insight into optimizing pulse parameters for retinal stimulation.

Safety considerations for non-damaging electrical stimulation: Charge balance, charge density and charge per phase are three parameters that are considered critical in establishing non-damaging stimulation parameters (Shepherd *et al* 2013). A minimum requirement for any neural prosthesis involving electrical stimulation is charge-balance, achieved by using biphasic pulses (Lilly 1961). Monophasic pulses without capacitive coupling or shorting allow charge to accumulate at the electrode-tissue interface and therefore should not be used in a clinical setting. It is also important to remain within the charge density limit for the electrode material, as operating above the charge density limit will result in the evolution of hydrogen and oxygen which is harmful for the tissue and electrode (Rose and Robblee 1990). In the present study the maximum charge density was limited to 300 $\mu C/cm^2$ which is the theoretical safe charge density limit for platinum (Cogan 2008). However, one study using a short period of stimulation (1 hour) of platinum electrodes in the suprachoroidal space suggested that a charge density of up to 970 $\mu C/cm^2$ might be safe (Nakauchi *et al* 2007). Nakauchi *et al* (2007) also reported that the threshold for safe stimulation current decreased with increasing pulse duration and the threshold for safe stimulation charge increased with increasing pulse duration. These results are further supported by *in vivo* and *in vitro* studies using chicken embryos and chicken retinas respectively exposed to 2 hour stimulation (Butterwick *et al* 2007, Vankov *et al* 2005). Tissue damage may also arise from over stimulation, as described by McCreery *et al* (1990) based on (7 hours) in the cat cerebral cortex using 400 μs stimulation. Shannon (1992) described an empirical

model based on data from McCreery *et al* (1990) given by [$Log(Qd) = k - \log(Q)$]. Where, Qd is the charge density and Q is the charge per phase and the constant k identifies a theoretical boundary describing non-damaging stimulation (Shannon 1992). It was found that with a value of k=2 the theoretical boundary falls in an area where damage was observed based on histological and ultrastructural changes to cortical neurons following acute stimulation. Based on the model proposed by Shannon (1992) with k=1.7 (most conservative theoretical boundary), over 90% of I-50 activation levels in this study were within the safe limit. While the Shannon model, as well as acute studies by Nakauchi *et al* (2007) and Butterwick *et al* (2007), can be used as a guide in designing safe stimulation parameters, long term chronic stimulation safety studies using the intended electrode array are ultimately required in order to establish safe thresholds to prevent tissue damage using suprachoroidal retinal stimulation.

5. **Conclusion**

In the present study, we evaluated a range of pulse parameters used in retinal prostheses and provided empirical results that can assist in the development and selection of optimal stimulus parameters for retinal prostheses. We found that for both monophasic and biphasic pulses anodal stimulation required significantly less current and charge than cathodal stimulation. Suprachoroidal stimulation required relatively larger charge per phase for clinically relevant retinal stimulation compared to other neural prostheses. Adding an interphase gap between the two phases of a biphasic pulse reduces the threshold of activation of retinal neurons. However, short interphase gaps make little difference to the efficiency of the stimulus and longer interphase gaps (≥ pulse duration) are required. Considering the extant literature and results of the present study, anodal first biphasic pulses between 300- 1200 μs are recommended for suprachoroidal stimulation. The benefit of using an interphase gap varies based on the pulse duration and polarity used and needs to be carefully considered in light of the results. Regardless, long-term safety studies are recommended with pulse durations > 400 μs and large interphase gaps. Furthermore, these studies need to be repeated in animal models of pathologies such as retinitis pigmentosa, in order to address the effectiveness of suprachoroidal stimulation in diseased conditions.

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