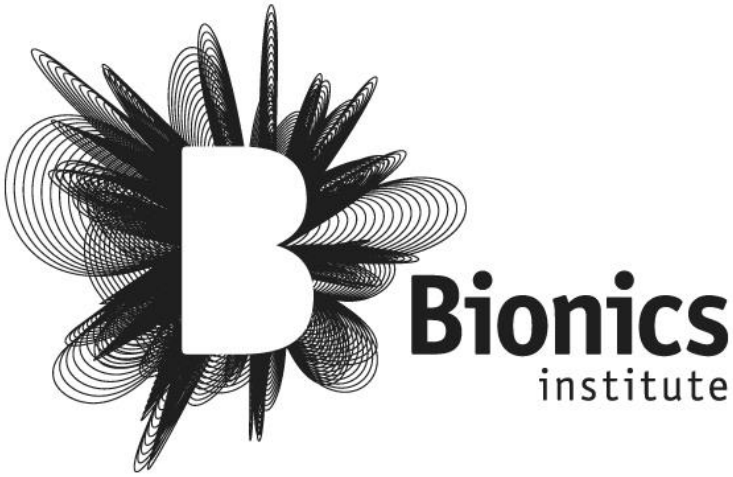

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Spatiotemporal interactions in the visual cortex following paired electrical stimulation of the retina

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ABSTRACT

Purpose

Retinal prostheses employ spatiotemporal patterns of electrical stimulation across multiple electrodes to provide visual percepts to blind patients. It is generally assumed that percepts produced by individual electrodes are independent of one another, which may not be the case. In this study, we aimed to quantify interactions between pairs of electrical stimuli delivered to the retina.

Methods

Normally sighted cats were implanted with a suprachoroidal electrode array. The retina was stimulated with a paired-pulse paradigm that consisted of a conditioning stimulus followed by a test stimulus, whilst recording multi-unit activity in the visual cortex. Conditioning current, and spatial and temporal separation between the conditioning and test stimuli were varied. Cortical interactions were quantified by changes in multi-unit activity elicited by stimulation with the paired-pulse paradigm, compared to stimulation of the test stimulus alone (control).

Results

Interactions varied as a function of conditioning current and temporal separation between the two stimulating pulses. Cortical activity increased compared to the control condition at an inter-stimulus delay of 1.025ms and was significantly suppressed for delays between 20 and 90ms, returning to near control levels for longer delays. The level of interactions increased when the conditioning current was increased. Interactions were found to be similar for electrode separations up to 3mm.

Conclusions

Interactions between sequential stimulation of pairs of electrodes in a suprachoroidal retinal prosthesis occur for delays up to 100ms and electrode separations of several millimetres. Knowledge of these spatiotemporal interactions is essential for developing effective patterns of stimulation for retinal prostheses.

INTRODUCTION

Retinal prostheses are designed for patients suffering from degenerative retinal disorders such as retinitis pigmentosa, and provide sensations of light, termed phosphenes, by electrical stimulation of surviving retinal neurons. In order to provide functional vision to the blind to perform tasks such as navigate a room, or reading, the activation of potentially hundreds of electrodes may be required[1, 2]. Therefore, retinal prostheses will need to use complex spatial and temporal patterns of electrical stimulation. By stimulating appropriate electrodes in a defined spatiotemporal sequence, one can take advantage of the visuotopic organisation of the retina[3] to convey the desired pattern to higher visual centres. Clinical trials have shown that retinal prostheses can provide useful visual cues with patients able to perform tasks in a controlled clinical setting such as identifying orientation of lines, reading letters and identifying simple objects[4-7]. However, testing in humans has shown that electrode interactions can significantly influence the resultant percept (Wilke RG, et al. *IOVS* 2011; 52 ARVO E-Abstract 458)[8, 9]. Therefore, a thorough understanding of the interactions that occur between repetitive electrical stimuli applied to a single retinal electrode, as well as the spatiotemporal interactions that occur between retinal electrodes is integral to the development of efficacious stimulation strategies.

The timing between successive electrical stimuli on a single retinal electrode has been shown to affect retinal ganglion cell (RGC) activity. Studies performing *in vitro* electrical stimulation of the retina suggest that direct stimulation of RGCs may reliably be able to evoke stimulus phase-locked spike activity at stimulating rates of several hundred Hz[10-12], similar to that seen when using visual stimuli[13, 14]. Other studies however report conflicting results and have shown that the number of spikes evoked in RGCs decrease with increasing stimulation rate beyond 50

Hz[15]. With indirect activation of RGCs via depolarization of the neurons in the inner retina, the number of spikes evoked is greatly reduced when stimulation rates in excess of 10Hz are used[10]. The desensitization of RGC spiking activity to fast rates of electrical stimulation is also evident when the retina is activated with only two electrical pulses[16] as opposed to a train of stimulating pulses[10, 15]. Upon presentation of two pulses of equal strength, a slight reduction occurs in the spikes evoked by the second pulse compared to those evoked after the first pulse[16]. The reduction in spiking activity following the second pulse becomes greater when the two pulses are closer in time, such that almost no spikes are evoked by the second pulse with temporal separations less than 25ms (equivalent to 40Hz). As larger numbers of stimulus pulses are presented to the retina in the form of a pulse train, further reductions in spike numbers occur after each subsequent pulse for rates above 4 Hz[16, 17]. These findings may possibly play an important role in the fading of phosphenes observed by patients upon continuous electrical stimulation of the retina[7, 18], whereby phosphenes can disappear into background levels of illumination. However, phosphene fading is complex with great inter-subject variability[18]. Initially upon beginning stimulation, the appearance of the phosphene changes notably. Aside from losing brightness, the phosphene can be marked by changes to its size, shape and even its colour, as well as sometimes reappearing as a bright flash when stimulation ceases[18]. These varying sensations may influence a person's ability to interpret a scene presented by a retinal prosthesis.

Retinal prostheses used for clinical trials employ one of two stimulation strategies to present visual patterns to the retina. The electrodes that comprise the pattern may be simultaneously stimulated[19, 20]. Alternatively, the same group of electrodes can be electrically stimulated in a sequential fashion (Blamey P, et al. *IOVS* 2013; 54: ARVO E-Abstract 1044)[20, 21]. Although implementation of a simultaneous strategy is straightforward, activation of neural prostheses in this manner can result in an uncontrollable percept due to electric field interactions between

electrodes. The use of simultaneous stimulation in a cochlear implant (CI) has demonstrated the existence of interactions between each of the activated electrodes due to overlapping electric fields. It is thought that neurons located between stimulated electrodes receive a summation of stimuli from each of the active electrodes, resulting in unexpected variations to the threshold[22], loudness[23] and pitch[24] of the resultant auditory percept. Therefore, sequential activation of electrodes on an array has been employed in CIs to minimize electrode interactions[25], as it is expected that interactions arising from electric field summation should be eliminated when stimulation of neighbouring electrodes are separated in time. Sequential stimulation has been shown to produce much weaker interactions compared to simultaneous stimulation[22, 24], and the degree of these interactions are found to be a function of both spatial and temporal separation between electrodes. In the animal model, changes to the threshold and spatial profile of recordings from the auditory cortex reflect the spatiotemporal interactions that have been demonstrated to occur with CI recipients[26].

Psychophysical testing of retinal prostheses have shown that patients are able to discern simple lines with simultaneous electrode activation[19, 20]. However, patients experience difficulties discerning multifaceted patterns such as letters, possibly due to interactions between the many, concurrently active electrodes[20]. Multifaceted pattern recognition has been successful in patients with retinal prostheses using sequential electrical stimulation[20]. Furthermore, simultaneous stimulation of a group of retinal electrodes elicits a brighter percept compared to the sequential stimulation of the same group of electrodes[9]. However, stimulation of pairs of retinal electrodes results in more complex changes in perceived brightness, with both increases and decreases in brightness possible[8]. Others have reported that the ability to detect individual phosphenes from adjacent electrodes was compromised if stimulation of the two electrodes occurred closely in both space and time (Wilke RG, et al. *IOVS* 2011; 52 ARVO E-Abstract 458). The extent to which these interactions occur vary widely between the different electrode

placements (epiretinal[8] versus subretinal (Wilke RG, et al. *IOVS* 2011; 52 ARVO E-Abstract 458)) and vastly different electrode array designs. It is difficult to predict the spatial and temporal range over which these interactions may occur based on studies by others. This is especially true of the spatial range in which electrode interactions can occur as each electrode placement and design vary in the area of the retina that is stimulated. Therefore, spatiotemporal interactions need to be evaluated based on electrode array design.

In the present study, we explored spatiotemporal interactions arising between electrical stimuli presented to electrodes on a clinical grade suprachoroidal prosthesis that is currently in trial in three human patients (www.clinicaltrials.gov; trial #NCT01603576). We employed a paired-pulse paradigm[16] to study interactions at the level of the visual cortex in an animal model. This allowed us to systematically investigate in detail how a range of stimulating parameters (current level, spatial shift and temporal shift) influences spatiotemporal interactions, which is not necessarily possible in the limited time frame available with patients during clinical trial.

METHODS

Anaesthesia and Surgery

All experiments were performed with approval from the Royal Victorian Eye and Ear Hospital Animal Ethics Committee and conformed with the ARVO statement for use of animals in ophthalmic research. Anaesthesia prior to surgery was induced in eight normally sighted adult cats with ketamine (intramuscular, i.m., 20mg kg^{-1}) and xylazil (subcutaneous, s.c., 2mg kg^{-1}). Anaesthesia was sustained with an infusion of sodium pentobarbitone (60 mg kg^{-1} hr^{-1}) and vital signs (respiration rate, end tidal CO_2 and temperature) were monitored throughout the experiment. Sodium lactate (Hartmann's solution 2ml kg^{-1} hr^{-1}) was infused intravenously and injections of dexamethosone (i.m. 0.1mg kg^{-1}) to minimise brain swelling, and clavulox (s.c. 10mg kg^{-1}) as an antibiotic were given regularly over the three to four day experiment period.

Surgical implantation involved inserting the suprachoroidal array approximately 15 to 17mm into a pocket created between the sclera and choroid[27], such that the tip of the electrode array was located beneath or near area centralis. Suturing of the array onto the sclera facilitated wound closure and ensured stable positioning of the array throughout the experiment. Following suprachoroidal surgery, the animal was placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) and a craniotomy overlying the visual cortex was performed contralateral to the implanted eye. To determine the optimal placement for a planar 60-channel recording array (Blackrock Microsytems, Foxborough, MA), an evoked potentials mapping procedure was used as previously described[28]. Briefly, a platinum ball electrode was used to measure thresholds of evoked potentials on the surface of the contralateral cortex at various points in response to electrical stimulation of a group of retinal electrodes. The point on the cortex with the lowest evoked potential threshold was chosen for implantation of the recording array, which

corresponded well to the retinotopic positioning of the suprachoroidal array in the visual space[28-32]. Recording electrodes were typically inserted contralateral to the stimulated eye, in the lateral gyrus in the visual cortex, corresponding to the macular region of the retina[33]. Recording areas included both cortical areas 17 and 18.

Suprachoroidal Electrode Array

All experiments utilised a clinical grade suprachoroidal retinal prosthesis, manufactured in house. The prosthesis (Figure 1A) was made of a silicon elastomer substrate that was contoured to conform to the eye. It consisted of an electrode array with 21 platinum disk electrodes (600µm diameter) plus an additional two return electrodes (2mm diameter) placed on the proximal end of the array to complete the current return path for electrical stimulation. Electrodes on the array were arranged in one of two ways. In the first design (4/8 eyes), electrodes were organised into seven rows by three columns. For the second design, which has recently been implanted into three patients as part of a clinical trial (www.clinicaltrials.gov; trial #NCT01603576) (4/8 eyes), electrodes were organised in a pattern such that the 21 stimulating electrodes were surrounded by an additional return made up of the electrodes on the outer edge shorted together. In both designs, electrodes were spaced 1mm, centre-to-centre equating to a separation of approximately 4° visual angle in the cat[34].

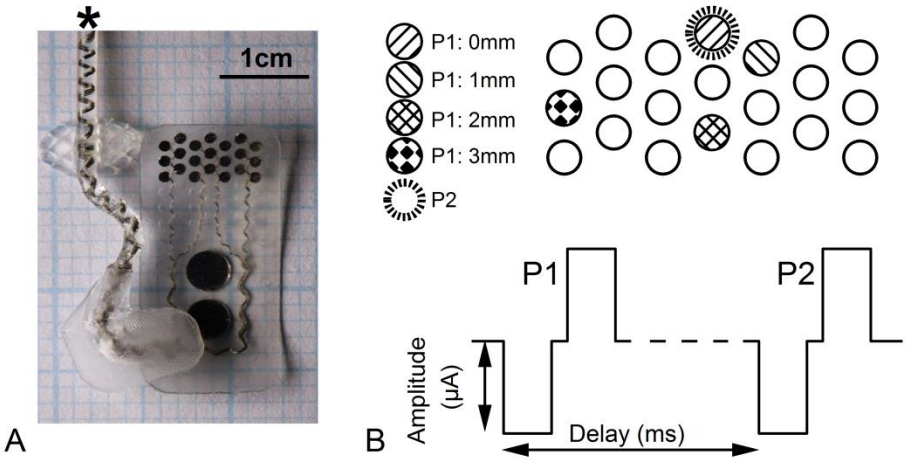

Figure 1. *(A) Photograph of one of the clinical grade suprachoroidal retinal prostheses used during the experiments (design 1). The leadwire (*) allows access to individual electrodes. (B) The paired-pulse protocol. The electrode for the test stimulus (P2) was kept constant while surrounding electrodes of varying separations were chosen to apply the conditioning stimulus (P1). The conditioning stimulus always preceded the test stimulus.*

Electrical Stimulation Protocol

The focus of this study was to investigate how repeated stimulation of the retina affects neural responses in the visual cortex. In particular, we wished to characterize the effect of varying delay, intensity and spatial location of electrical stimuli on multi-unit spiking activity (MUA) in the visual cortex. An electrode on the retinal array was chosen to apply a fixed- amplitude current pulse (test pulse; P2) whilst a conditioning current pulse (P1) was applied to either the same or surrounding electrode as the test stimulus (Figure 1B). Stimuli used were biphasic, cathodic leading current pulses (500μs per phase, 25μs interphase gap). The conditioning current varied from zero to a maximum stimulating level of 1.5mA to ensure that charge densities were below 300μC cm^{-2} [35]. The delay or stimulus onset asynchrony (SOA, i.e. onset of the conditioning stimulus to onset of the test stimulus) was either fixed to 1.025ms (the fastest possible

stimulation rate between electrical stimuli for the given stimulating pulse parameters) in order to assess the combined effect of the two stimuli, or varied in non-linear increments between 20 and 500ms in order to distinguish the response elicited by one stimulus from the other. SOAs between 1.025 and 20ms were not chosen for the paradigm as MUA arising from retinal stimulation usually occurs within a 3-20ms window post-stimulus[28, 36]. Thus, the use of SOAs between 1.025 and 20ms would not allow us to distinguish if the spikes recorded following the paired-pulse protocol were due to the conditioning stimulus, or the test stimulus, or the combination of the two stimuli. Each SOA and conditioning current combination was presented at random with a repetition rate of 1Hz; all possible combinations were repeated 10 times. The situation where the amplitude of the conditioning stimulus was 0μA (i.e. only test stimulus present) was considered as the control. Most of the electrodes used for stimulation were located on either the tip or one of the lateral edges of the stimulating array. While this was the case, the arrays themselves were specifically designed and shaped to conform to the curvature of the eye in the suprachoroidal space, and relied on the active pressure in the eye to keep all electrodes flush against the choroid[37]. Therefore, we did not expect any differences in response properties between different stimulating electrodes according to the location of the electrode on the suprachoroidal array.

In order to choose a suitable current for the test stimulus in the paired-pulse protocol, each retinal electrode was electrically stimulated using single biphasic current pulses (0-1.5mA, Δ50μA, repetition rate of 1Hz) and a spike rate versus stimulating current curve (Figure 2A) was obtained for each recording site. From these curves, we obtained the current required to evoke 50% of the maximum neural activity, chosen to be the midpoint of the fitted sigmoid curve (P50)[28]. The mean P50 current across all recording sites for the given stimulating electrode was taken to be the amplitude of the test stimulus in the paired-pulse protocol.

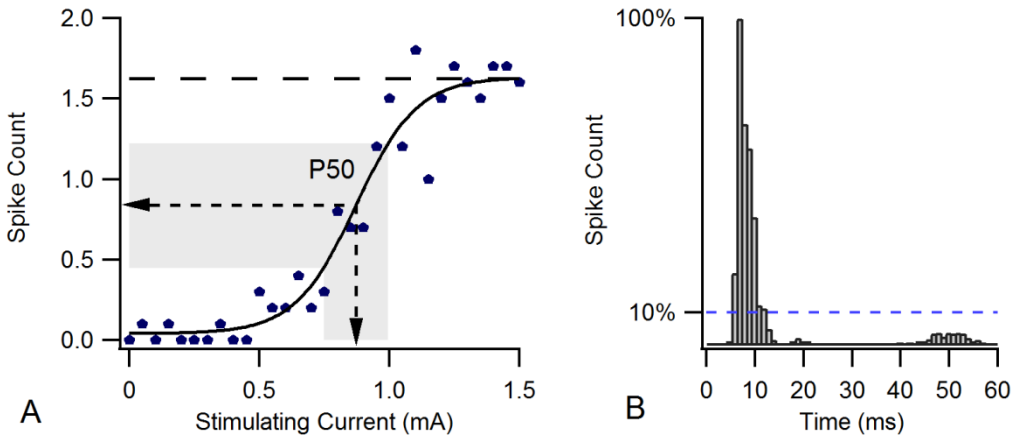

Figure 2. *Example of electrically evoked multi-unit (MUA) in the visual cortex inclusion criteria. (A) Spike rate vs. stimulating current curve. Symbols denote average spike counts for each current level, in the window 3-20ms post-stimulus onset. The test stimulating current, the P50 value, was derived from the fitted sigmoid curve. The shaded area denotes the 25-75% current range. (B) Post-stimulus time histogram of spiking activity for electrical stimulation of a single retinal electrode across all current levels (0-1.5mA). Although there is a second phase of spiking centred at 50ms, this response was classified as a single phase response as per the criterion.*

Data Analysis

MUA was recorded continuously at a sampling frequency of 30 kHz with the Cerebus data acquisition system (Blackrock Microsystems, Salt Lake City, UT). Spiking events collected at the time of the experiment with the Cerebus GUI software were used to determine the test electrode stimulating current. Additional offline processing with custom scripts written in Igor Pro (Wavemetrics, Lake Oswego, OE) were used to identify spikes for subsequent data analysis. Briefly, the methods described by Heffer and Fallon[38], were used to remove signal artefacts due to electrical stimulation, after which the signal was band-passed filtered (Butterworth filter, frequency: 0.3-5 kHz, order: 3). An estimate of the root mean square (RMS) activity was

computed over a 60s moving window and spikes were detected if the signal exceeded four times the RMS value for the given window.

To quantify the degree of stimulus interactions, spike counts on each individual recording site following the test stimulus in the paired-pulse protocol were compared to those obtained in the control condition, i.e. 0μA conditioning stimulus. For each conditioning current – SOA combination, spikes were counted in the time window 3-20ms immediately following the test stimulus. Spike counts were then normalised to the average spike rate obtained for the control conditions across all SOAs, giving a spike ratio that defined the degree of interaction between the conditioning and test stimuli. A spike ratio of one indicated no interactions, with conditioning and test stimuli completely independent of each other. A spike ratio greater than one indicated facilitation or summation caused by presence of both the conditioning and test stimulus, while a spike ratio less than one indicated suppression of neural activity following the test stimulus due to the presence of the conditioning stimulus. The results of such analyses are presented with the conditioning current normalized with respect to the P50 current level obtained for a particular recording site, i.e. $Conditioning\ Current\ (dB) = 20\log\frac{Conditiong\ Current\ (\mu A)}{P50\ Current\ (\mu A)}$. Normalization in this manner better reflects the strength of the conditioning stimulus with respect to its dynamic range. This was previously shown to be approximately 10dB with monopolar stimulation of a single retinal electrode[28], equivalent to ±5dB from the P50 current level, therefore, conditioning currents were restricted to the range of ±5dB.

Cortical Site Inclusion Criteria

A given cortical recording site was included for analysis provided the following criteria were met.

Test stimulating current was within 25-75% of the activation current

The current of the test stimulus was obtained from the average P50 level across cortical recording sites. Therefore, one would expect to see a wide range in the spike counts obtained for the control condition (i.e. no conditioning stimulus) according to where the test stimulating current falls along an individual cortical site's response curve. A recording site was only included in the analyses if the chosen test stimulating current was within the current range that evoked between 25-75% of the maximum activity on that recording site (Figure 2A). It was important to apply this criterion as test pulse stimulating currents much below the P50 current were not large enough to produce a robust spiking response under the control condition, which may confound our results through the inability to observe a drop in spiking activity. Similarly, test pulse currents much larger than the P50 current resulted in cortical sites spiking near to its maximum capacity under the control condition, in which case, we may not observe an increase in spiking activity. In cases where we may still observe small levels of spiking, particularly for test stimulating currents that fall towards the lower end of this range, an additional minimum spike rate criterion for the control condition of 0.5 spikes/pulse needed to be met for a cortical site to be included for analysis.

P1 electrode exhibits a single phase spiking response

Electrical stimulation of the retina can exhibit a multi-phased spiking response in the visual cortex[28, 36]. It is believed that the first phase of spiking is due to the direct and indirect electrical stimulation of RGCs[17]. Later spiking phases may be due to the late stage spiking events shown to occur *in vitro*[17], possibly from other retinal circuits or cortical feedback mechanisms[36]. In this study, analysis was restricted to the first phase occurring 3-20ms after the stimulus presentation. However, in cases where the conditioning stimulus evoked a multi-phase response, it was not possible to distinguish spikes evoked following presentation of the test stimulus with those evoked as part of the multi-phase response to the conditioner. These recording sites were removed from analysis to ensure that any interactions seen were due to

what is presumed to be direct electrical stimulation of RGCs. To ensure unbiased classification of a multi-phased response, a cortical site was classified as multi-phased if the peak amplitude of the second phase of spiking exceeded 10% of the maximum spiking amplitude of the first phase of spiking (Figure 2B).

Statistical Analyses

All statistical analyses were performed in Igor Pro, whereby one-sample t-tests were used to compare computed spike ratios with expected population means, performed at a significance level of 0.05. The conditioning and test pulses were considered to be independent of one another if spike ratios were within 10% of 1, i.e. interactions were considered to be absent for spike ratios between 0.9 and 1.1. Therefore, interactions were deemed facilitatory in nature if average spike ratios exceed a value of 1.1 and suppressive for average spike ratios below 0.9. Furthermore, spike ratios less than 0.5 were defined as heavily suppressed and between 0.5 and 0.9, moderately suppressed. Given that several hypotheses were tested for each classification, Bonferroni adjustment of the p-value to control for family wise error was too conservative a method, therefore, post-hoc adjustments of p-values were performed with the Benjamini and Hochberg correction method[39].

RESULTS

Across the eight animals implanted, 132 cortical recording sites from the stimulation of 16/23 retinal electrodes passed all cortical site inclusion criteria and were analysed when the paired-pulse protocol was applied to the same retinal electrode, i.e. 0mm separation. In addition, analysis of interactions was performed from 55 cortical sites, obtained from 15/21 electrode pairs for a separation of 1mm (centre-to-centre distance, rounded to the nearest mm) and 58 cortical sites, obtained from eight out of 18 electrode pairs for a separation of 2mm. A breakdown of the number of cortical sites that passed each of the inclusion criteria is provided in Table 1.

Table 1: *Details of the number of cortical sites available for analysis*

Electrode Spacing	# Cortical Sites[1]	Passed Test Current Criterion[2]	Passed Minimum Spike Rate Criterion[3]	Passed Single Phase Criterion
0mm	480	194	178	132
1mm	239	100	62	55
2mm	170	89	63	58

[1]Denotes cortical recording sites in which both stimulation of the test and conditioning electrode alone elicited a spike response
[2]Test stimulating current was within 25-75% of the activation current
[3]Minimum spike rate of 0.5 spikes/pulse

Cortical responses to paired-pulse stimulation

Electrical stimulation of the retina with the paired-pulse protocol was found to influence MUA in the visual cortex compared to when the retina was electrically stimulated with the test stimulus

alone (Figure 3). Both SOA and conditioning current were found to influence spikes elicited by the test stimulus in the paired-pulse condition.

Figure 3A demonstrates the effect of SOA on MUA recorded after the test stimulus when the conditioning current was equal to the P50 level for each recording site. Despite a robust pattern of spikes obtained for the control condition when the retina was stimulated with only the test stimulus (Figure 3A, left column), this spike pattern varied with the introduction of the conditioning stimulus, and was dependent on the delay between the two stimuli (Figure 3A, right column). At the shortest SOA (1.025ms), an increase in the number of spikes was observed compared to the control condition, indicating facilitation. However, the next SOA shown in the figure (30ms) resulted in a strong suppression of MUA following the test stimulus where almost no spikes were recorded. As the SOA was increased, the suppression following the test stimulus was found to reduce, with near-normal activity compared to the control condition returned at an SOA of 500ms.

The amplitude of the conditioning stimulus was found to influence the strength of interactions (Figure 3B). As expected, the number of spikes recorded immediately after the conditioning stimulus increased as the amplitude of the conditioning current was increased. However, as the conditioning current was increased, the number of spikes recorded following the test stimulus kept decreasing compared to the control condition when the conditioning stimulus was absent. In fact, at the largest conditioning current (Figure 3B, bottom row), almost all spikes following the test stimulus were eliminated. Thus, larger currents applied to the conditioning stimulus resulted in greater facilitation (SOA = 1.025ms only) or suppression of neural activities following the test stimulus. Interestingly, even conditioning currents corresponding to the lower end of the dynamic range for each recording site (-5dB) and eliciting only a weak response, resulted in suppression of activity elicited by the test stimulus.

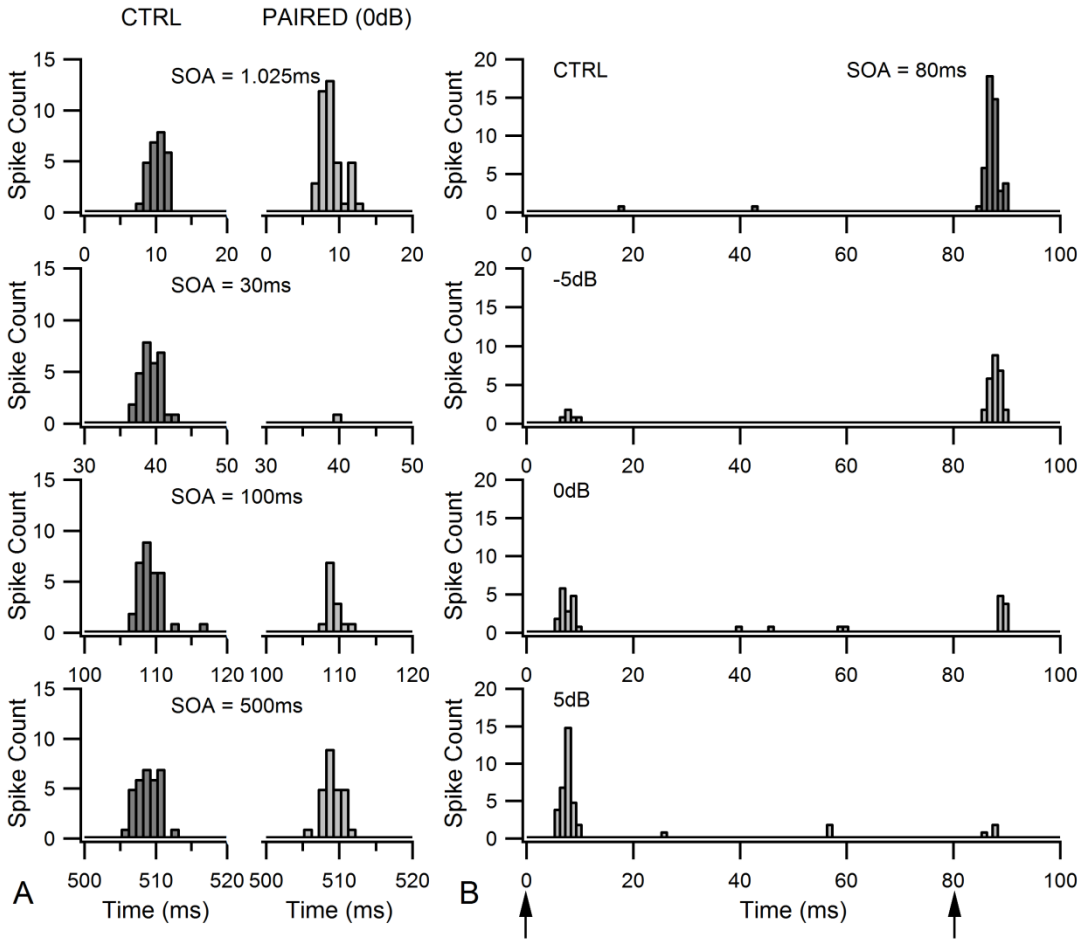

Figure 3. *Post-stimulus time histogram demonstrating the effect of the paired-pulse protocol on MUA. In each example, the conditioning and test stimuli were applied to the same retinal electrode. (A) Fixed conditioning current, varying SOA. Control level of spike activity recorded after the test stimulus with the conditioning current equal to 0μA (left). Spiking activity observed after the test stimulus for SOAs between 1.025 and 500ms with conditioning current fixed to 0dB (right). (B) Varying conditioning current and fixed SOA of 80ms, whereby the arrows on the horizontal axis indicating the timing of the conditioning and test stimulus respectively. Each row represents an increasing conditioning current amplitude. Spiking activity recorded up to 20ms*

post-stimulus is attributed to the conditioning stimulus and between 80 and 100ms post-stimulus, the test stimulus.

Facilitatory neural interactions

Facilitatory interactions occurred between paired pulses separated by 0 (Figure 4A), 1 (Figure 4B) and 2mm (Figure 4C) in the retina, whereby stimulation with the conditioning stimulus resulted in an increase in MUA that followed stimulation of the test stimulus, compared to stimulation with the test stimulus alone. These facilitatory interactions were apparent only for a SOA of 1.025ms, whereby stimulation of the test stimulus immediately followed cessation of the conditioning stimulus. Facilitatory interactions measured in the cortex steadily increased with increasing conditioning current with an approximate two-fold increase in spike ratios for larger conditioning currents. Conditioning currents below the P50 current level (i.e. less than 0dB) were also able to elicit facilitatory interactions (spike ratio > 1.1). One- sample t-tests using the Benjamini and Hochberg correction method showed that the minimum conditioning current required to elicit significant facilitatory interactions was found to be -3dB for 0 and 1mm electrode separations and -1dB for the 2mm electrode separation.

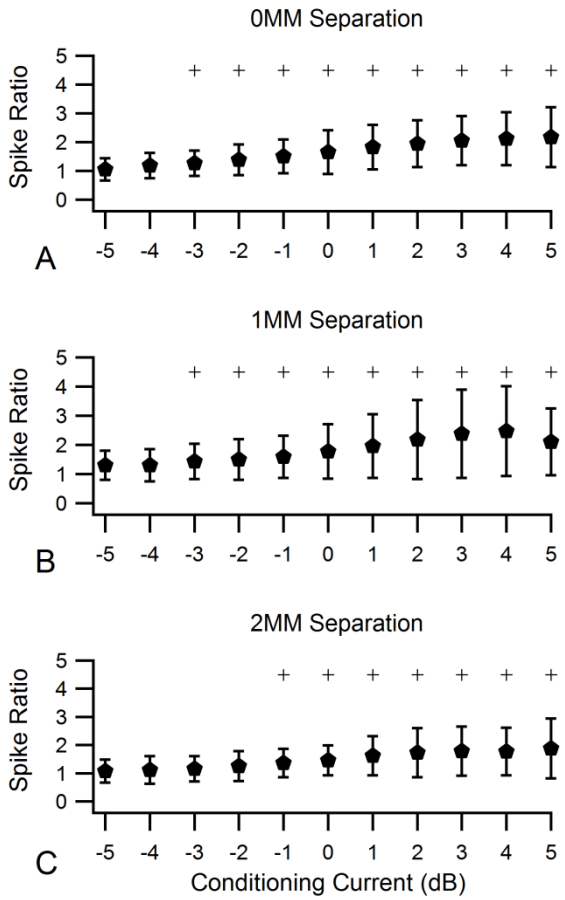

Figure 4. *Spike ratios (mean ± SE) its standard deviation check that others are correct versus conditioning current obtained when SOA between conditioning and test pulse was 1.025ms. Data for each current bin were obtained from (A) 97-130 out of 132, (B) 37-54 out of 55 (C) 40-57 out of 58 cortical recording sites. Number of cortical sites for each conditioning current are indicated in Figure 5. The + sign indicate those conditioning currents that yielded spike ratios which exhibited facilitation (i.e. spike ratios significantly above 1.1).*

Suppressive neural interactions

SOAs ranging from 20 to 500ms exhibited suppressive neural interactions (Figure 5).These interactions were dependent on both conditioning current (abscissa) and SOA (ordinate) and

were very similar for each of the electrode separations tested. Suppressive interactions were strongest for SOAs between 20 to approximately 100ms. In this region, cortical spikes following the test stimulus could be supressed by as much as 90% compared to the control, particularly for large conditioning currents and small SOA combinations. For conditioning currents below the P50 current level (i.e. below 0dB), a 40-50% suppression following the test stimulus was observed. SOAs beyond 100ms marked the beginning of the recovery period for spike activity, with notably higher spike ratios than those obtained with SOAs less than 100ms, although a drop in spike rate by 10-20% was still apparent, even for long SOAs.

One-sample t-tests using the Benjamini and Hochberg correction method were used to compare spike ratio data for each conditioning current and SOA combination to expected population means in order to test for suppression. As there was a varying degree of suppression found, these interactions were further classified as heavily suppressed (spike ratio < 0.5, black dashed line), moderately suppressed ($0.5 \leq$ spike ratio < 0.9, grey dashed line) or absent ($0.9 \leq$ spike ratio ≤ 1.1). Interactions were found to mostly show heavy suppression for SOAs up to 90ms (0mm electrode separation, Figure 5A) and 80ms (1 and 2mm electrode separation, Figure 5B and 5C respectively) and moderate suppression beyond this critical SOA. For SOAs of 400 and 500ms, the two stimuli in the paired-pulse paradigm were found to be independent of one another only if the current of the conditioning stimulus was below the P50 level.

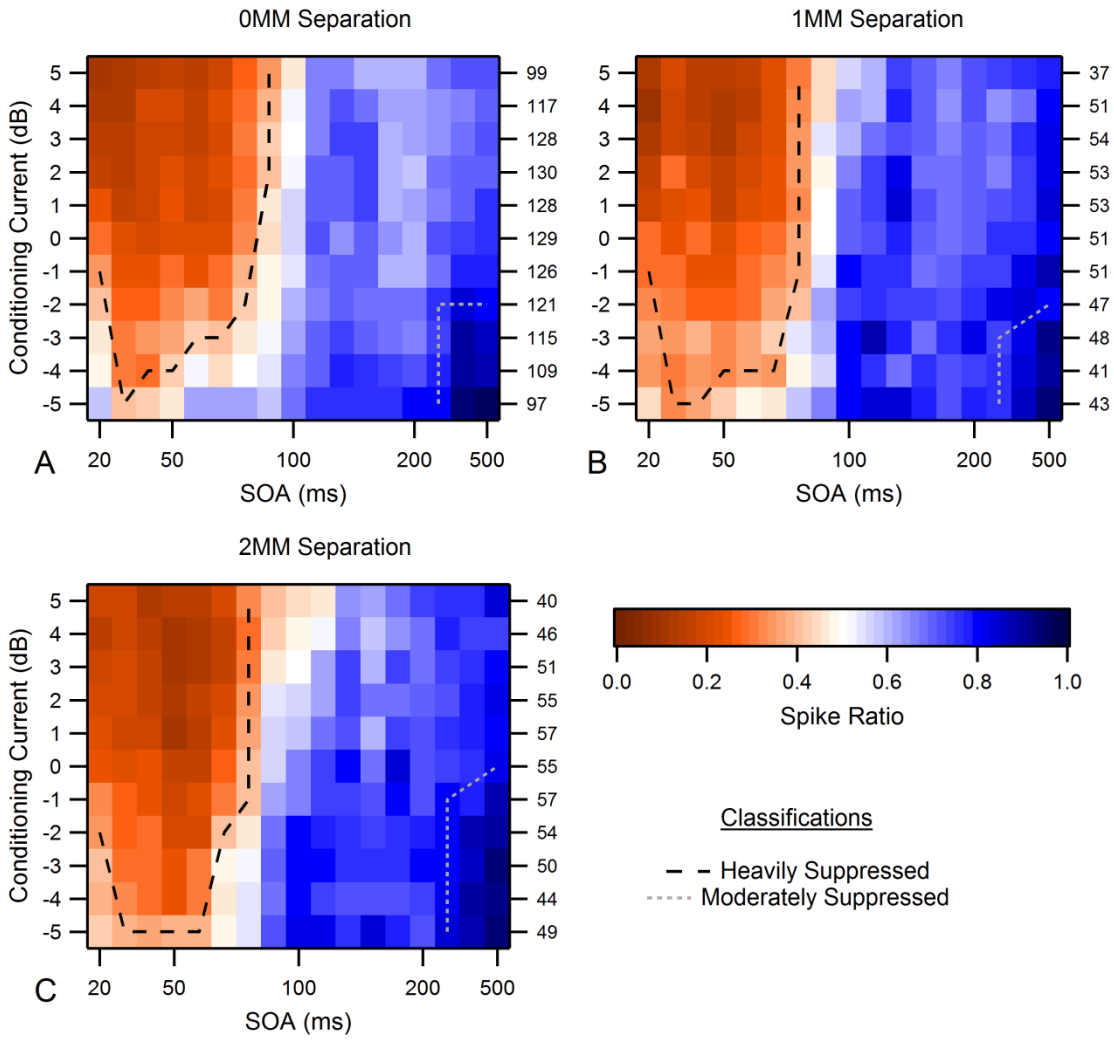

Figure 5. *Colour scale image representing the mean spike ratios obtained for SOAs 20-500ms versus conditioning current. The black dashed line outlines the conditioning current-SOA combinations that yield heavily suppressed interactions and the grey dashed line, moderately suppressed interactions based on statistical analyses of spike ratios to population means. To the right of the moderately suppressed line are the conditioning current-SOA combinations where pulse pairs were found to be independent of one another. Number of cortical sites for each conditioning current are indicated on the right axes. A total of 97-130 (0mm), 37-54 (1mm) and 40-57 (2mm) cortical recording sites were included for analysis for each current bin.*

Differences in interactions with increasing electrode separation

It is evident from Figure 4 and Figure 5 that the conditioning current and SOA combinations that resulted in facilitation, heavy suppression and moderate suppression are comparable for the three electrode spacings that were investigated in this study. Despite these similarities, we may expect that the degree of facilitation and suppression is greater between pulse pairs presented on the same retinal electrode as opposed to two different electrodes. To confirm if this hypothesis was true, differences in spike ratios obtained with single- and paired-electrode stimulation were compared. Where data was available for both, single-electrode and paired-electrode stimulation, spike ratios obtained with single-electrode stimulation were subtracted from those with paired-electrode stimulation to determine differences in the magnitude of interactions (Figure 6). A difference of zero indicated that interactions were not affected by the location of the conditioning stimulus relative to the test stimulus. A two-tailed t-test with Benjamini and Hochberg *post* hoc correction of p-values was used to determine the significance of these differences.

Spike ratio differences between paired-electrode (1mm separation) and single-electrode stimulation are shown in Figures 6A and 6B. For an SOA of 1.025ms (Figure 6A), the spike ratio difference did not deviate significantly from zero. Spike ratios exhibited a trend of positive differences for SOAs between 20 and 500ms (Figure 6B). This suggests that the suppressive interactions between 1mm paired-electrodes tended to be weaker than those obtained with single-electrode stimulation; however, these differences were not significant. Therefore, there was little difference in neural interactions resulting between two stimuli separated by 0 or 1mm in the retina.

Figures 6C and 6D present the analyses of the difference in mean spike ratio for 2mm separated paired-electrode and single-electrode stimulation. Negative differences for facilitatory

interactions (Figure 6C) were obtained with conditioning currents of 4-5dB, which is indicative of weaker facilitation occurring when the conditioning and test stimuli were applied between the 2mm spaced electrode pairs. Generally, suppression of MUA occurring at SOAs longer than 50-60ms was greater when the conditioning and test stimuli were applied to the same site in the retina (Figure 6D). Unexpectedly, for SOAs between 20 and 60ms, suppression was stronger when the two stimuli were separated in the retina by 2mm compared to that seen with single-electrode stimulation. Statistical analyses revealed however, that very few neural interactions between the 2mm separated paired-electrode stimulation were different to those obtained with single-electrode stimulation.

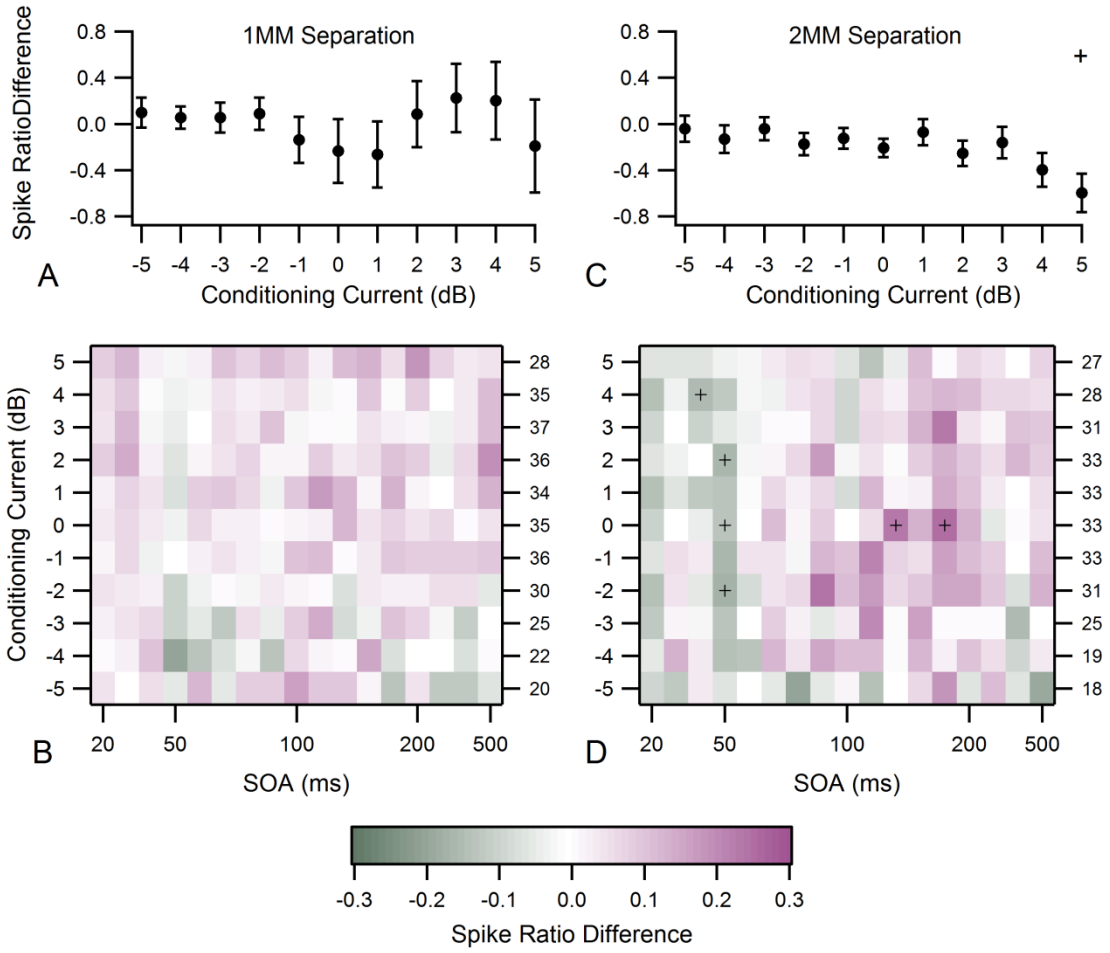

Figure 6. *Differences in mean spike ratios obtained when conditioning and test stimuli were presented on the same electrode compared to 1mm and 2mm electrode pairs. (A) and (C) Differences in facilitatory interactions obtained at an SOA of 1.025ms. (B) and (D) Differences in suppressive interactions obtained with SOAs between 20 and 500ms. Number of cortical sites for each conditioning current are indicated on the right axes (these numbers also apply to panels A and C). Data for the 1mm electrode pair separation were obtained from 20-37 out of 40 cortical recording sites and the 2mm electrode pair 18-33 out of 34 cortical recording sites. The + sign indicates mean differences in spike ratio which were found to be significantly different from zero.*

Interactions between paired-electrode stimulation separated by 3mm in the retina

In some instances, there was the opportunity to apply the paired-pulse paradigm to two retinal electrodes spaced 3mm apart. The results of such stimulation are shown in Figure 7 (six cortical sites obtained from three electrode pairs). It is difficult to make inference from such few cortical sites; however, the results appear to show that facilitation may still be possible between two stimuli separated by 3mm (Figure 7A). Conditioning currents of 0dB and greater resulted in the doubling of spikes evoked by the test stimulus when presented as part of a pair. However, statistical analyses to qualify these interactions revealed no significant facilitation.

In addition, as was seen previously with the 1mm and 2mm paired-electrode stimulation, spikes evoked after electrical stimulation with the test stimulus can be more than halved if the stimulation occurs within 80ms of the conditioning stimulus (Figure 7B). What is notable however is that spiking activity for SOAs greater than 80ms typically range between 80-100% of the number of spikes evoked by electrical stimulation with the test stimulus only. Classifications of neural interactions revealed that for SOAs of 80ms or shorter, interactions between the two stimuli primarily exhibited moderate suppression. When an SOA of 90ms or more separated the two stimuli, the conditioning and test stimuli were found to be independent of one another, exhibiting no interactions.

Following from these results, the differences in spike ratios used to quantify interactions between the 3mm separated paired-electrodes and single-electrode stimulation indicated that stronger facilitation and suppression occurred when the pulse-pairs were applied to the same retinal site (Figure 7C and 7D). In particular for the suppressive interactions, the magnitude of the difference in spike ratios between 0mm and 3mm electrode pairings (Figure 7D) were greater than those obtained with the 1mm (Figure 6B) and 2mm (Figure 6D) electrode pairs.

However, statistical analyses did not show a significant difference in spike ratios between single-electrode and 3mm paired stimulation, which may be a consequence of the small number of cortical recording sites available for analysis (average statistical power = 0.36).

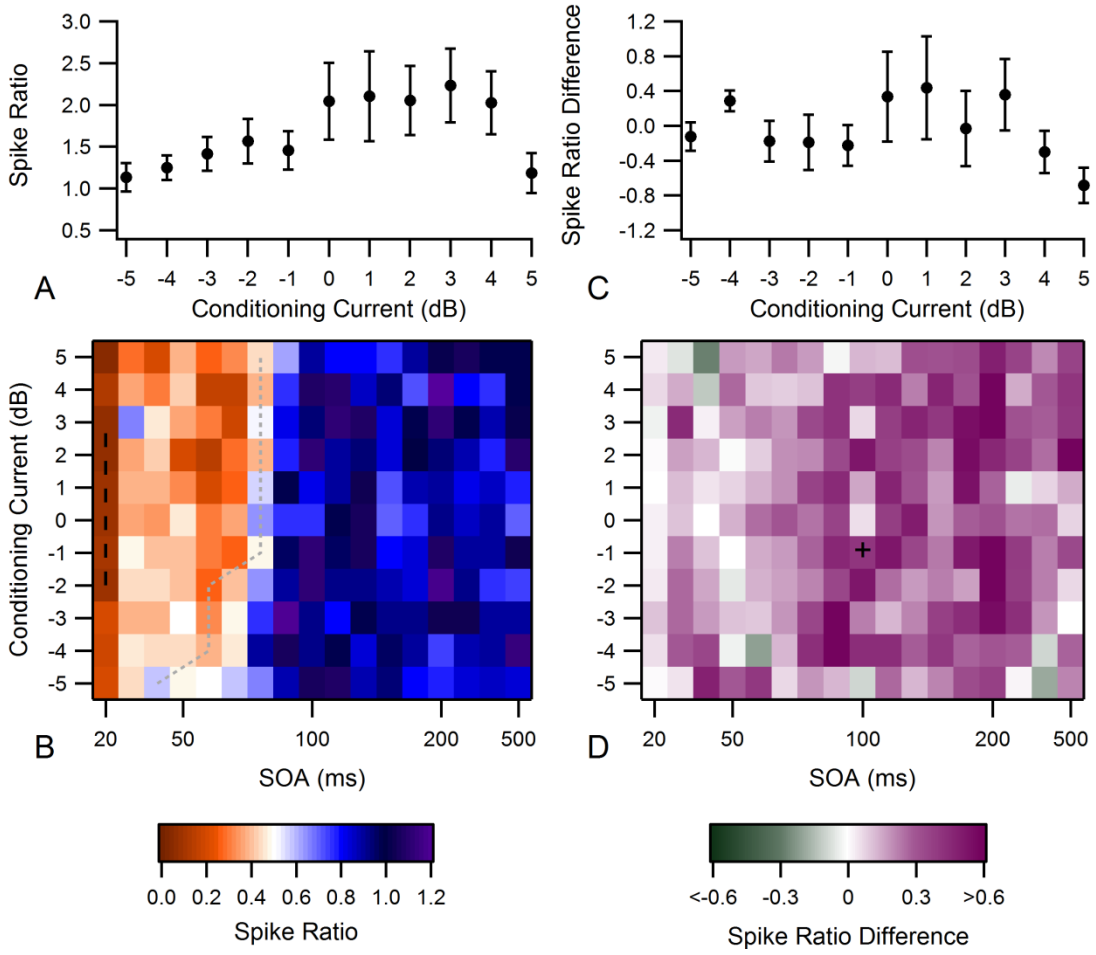

Figure 7. *Interactions obtained between conditioning and test stimuli with a 3mm centre-to-centre electrode spacing (A) Facilitatory interactions. (B) Suppressive interactions. The black dashed line outlines the conditioning current-SOA combinations that yield heavily suppressed interactions and the grey dashed line, moderately suppressed interactions based on statistical analyses of spike ratios to population means. To the right of the moderately suppressed line are the conditioning current-SOA combinations where pulse pairs were found to be independent of one another. (C) Mean difference in facilitatory spike ratios between 0mm and 3mm electrode*

pairs. (D) Mean difference in suppressive spike ratios between 0mm and 3mm electrode pairs. The + sign indicates mean differences in spike ratio which were found to be significantly different from zero.

DISCUSSION

The aim of this study was to evaluate, by recording from the visual cortex, interactions arising from electrical stimulation of the retina with a pair of current pulses presented on a suprachoroidal electrode array. Pairs of stimuli separated in the retina by 0-3mm were found to interact. The resultant interactions showed both facilitation and suppression, dependent on the current of the conditioning stimulus and the delay between the onsets of the conditioning and test stimuli, but not on the spatial distance separating the two stimuli. Interactions were found to begin to abate with wider electrode separations and long delays.

Comparison to other electrophysiological data

Stimulation of the paired-pulse paradigm at the fastest rate possible for the given biphasic pulse parameters resulted in facilitation of MUA. Similar facilitatory responses have been reported with paired-pulse electrical stimulation of auditory nerve fibres[40, 41], whereby the presence of the conditioning stimulus was found to reduce threshold of spiking activity elicited by the test stimulus. The facilitation is a consequence of the charge storing properties of neural membranes[40]; electrical stimuli depolarise the neuron[42], altering the charge on its membrane, which takes time to dissipate[40]. As a result of the altered membrane potential, the neuron is more susceptible to subsequent stimuli presented in quick succession after the first, summing the resultant charge from each pulse, and so, increasing the likelihood of a spiking event. High rates of stimulation, in which we can take advantage of the summation of electrical charge on the neural membrane, could potentially be used as a strategy to reduce thresholds or elicit brighter phosphenes. Whilst phosphene thresholds have been reported to increase[43] and perceptual brightness decrease[44] with increasing stimulation rate in normally sighted individuals following extraocular stimulation, in patients with retinitis pigmentosa, perceptual thresholds

have been shown to decrease when electrical stimulation rates are increased[45, 46] and the phosphenes evoked in such situations have been described as brighter[45, 47, 48]. However, these perceptual observations may not necessarily be solely due to the facilitation described here, but may also reflect the ability of RGCs to follow fast rates of electrical stimulation[47], as has been demonstrated *in vitro*.

In contrast, the majority of SOAs resulted in suppressive interactions occurring between pulse pairs. In particular, SOAs between 20 and 90ms, equivalent to a stimulating pulse rate of approximately between 10-50Hz, resulted in the greatest amount of spike suppression with a reduction in MUA by 50% or greater. The similarities in our results to those obtained *in vitro*[15-17] suggest that the mechanisms behind the heavily suppressed response may be due to the abolishment of the responses from RGCs that occurs with indirect stimulation at fast rates of electrical stimulation (termed as RGC desensitization). There are several mechanisms that have been presented in the literature behind RGC desensitization including the role of the neural network[16] as well as amacrine cell inhibition[17]. It must be noted however that RGC desensitization is known to persist in the absence of amacrine cell inhibition as shown in a study by Freeman and Fried (2011). The same study suggested that desensitization is likely to occur upstream of the spike generator further supporting the role of the neural network including photoreceptors, bipolar cells and horizontal cells in contributing towards RGC desensitization. Furthermore, only a single region of suppression was evident in our study. In an early study examining RGC activity with paired-pulse stimulation, several regions of suppression were evident[49]. In the study by Crapper and Noell, a single electrical stimulus elicited distinct phases of excitation and inhibition, leading to several distinct bursts of spiking activity in the RGCs. To paired-pulse stimulation, the resultant activity measured in the RGCs represented the algebraic sum of the spiking activity from each of the individual pulses in the pair, leading to several regions of suppression[49]. Cortical MUA recorded in response to retinal stimulation also elicits

several phases of spiking. Incorporating these latter phases of spiking into our analyses may possibly reveal additional periods of suppression in our results, as was observed by[49]. However, as the origin of the late phase of spiking is unknown (i.e. possible retinal or cortical mechanisms), our results were conducted on the analysis of cortical recording sites that exhibited only a single phase of spiking.

SOAs in excess of 90ms between the two stimulating pulses also resulted in a reduction in spiking activity to the test stimulus, with spike rates dropping by as much as 10 to 50% compared to when an electrode was stimulated alone. It has been reported that suppressive interactions between pairs of stimulating pulses separated by as much as 400ms can occur, which were only abolished when the pulses were separated by an interval of 650ms[16], a delay outside the range used in this study. Excitatory current produced by inner retina cells to electrical stimuli decrease at pulse repetition rates as low as 2Hz[12], presumably resulting in a reduction in the subsequent excitation of RGCs. These findings could explain why even with SOAs as long as 500ms, spike counts in the cortex after the test stimulus did not match the number of spikes evoked in the control condition.

Although prior studies delving into temporal interactions between electrical stimuli have been conducted in the retina[15-17], evidence of inhibition is also apparent in the electrically evoked potential (EEP), recorded from the visual cortex, whereby the amplitude of the EEP is found to decrease with increasing stimulation frequency[50-52]. Temporal transfer properties are known to differ between retinal and cortical neurons[53], as well as between lateral geniculate nucleus (LGN) neurons and cortical neurons[54]. Therefore, neural processes occurring in either the LGN or visual cortex may also have contributed to the inhibition observed in our study, as well as the known retinal desensitization that occurs with repeated electrical stimuli.

Interactions between pulses exist over several millimetres in the retina

Our results are in accordance with studies performed in CIs[26] and retinal prosthesis simulations[55] which have shown that interactions between stimulus pulses become weaker as a function of the spatial separation between the two stimulating electrodes. The reduced interactions are due to the reduction in the overlap of activated neural populations from each of the electrodes. Electrical stimulation of two suprachoroidal electrodes with the paired-pulse paradigm, even separated by up to 2mm in the retina resulted in comparable cortical interactions to stimulation on a single electrode. Utilising methods developed from an earlier study[28], we estimate that the retinal spread of activation from the 600µm diameter electrode used in this study spans a diameter of 2.9mm. Furthermore, a mathematical modelling study assessing the effects of electrode spacing on crosstalk[55], found that an array placed at a distance of 400µm from the surface of the retina required a centre-to-centre spacing of at least 2.5mm to eliminate crosstalk which is similar to limit of retinal spread of activation found in our study. This suggests that due to spread of current in the retina, a similar population of retinal cells may have been excited from the electrical stimulation of each of the electrodes in the 1mm and 2mm separated pairs. One way in which these spatiotemporal interactions may potentially be reduced is with the appropriate choice of electrode return configuration, that is known to influence the electric field emanating from a stimulating electrode[28, 56-58]. Electrode return configurations that produce focused currents in the retina are expected to exhibit weaker spatiotemporal interactions as current spread between electrodes is minimised. Indeed, with CIs, bipolar and tripolar stimulation have been shown to be effective in reducing electrode interactions between pairs of cochlear electrodes that have been activated simultaneously and with a temporal delay compared to monopolar stimulation[26, 59]. Similarly, simulations in the retina

have demonstrated that hexagonal stimulation can reduce 'cross-talk' between retinal electrodes[55].

For the 3mm electrode spacing, where the distance between the conditioning and test stimuli exceeded the electric receptive field of a cortical site, electrical stimulation with the pulse pairs were found to be more independent of one another. Our current spread data suggests that stimulation of two electrodes spaced 3mm or greater apart should result in two independent phosphenes whose shape and brightness should not be influenced by the stimulation of the other electrode, thus providing an alternative way to reduce spatiotemporal interactions. Preliminary testing with the suprachoroidal electrode array used in this study in human subjects have shown that simultaneous stimulation of two electrodes separated by 3mm can appear as two, non-overlapping phosphenes[60] and support the findings of this study. However, the absence of a statistically significant interaction was apparent only if the SOA between the stimulation of each electrode was at least 90ms. Suppressive interactions still occurred following paired-pulse stimulation with SOAs smaller than 90ms, albeit this suppression tended to be less than the 0, 1 and 2mm electrode separations. Therefore spreading electrodes further apart to abate interactions may not necessarily hold true when several electrodes are stimulated sequentially to form a pattern. Unlike the 1 and 2mm separated electrode pairs, the suppression of neural activity between the 3mm separated electrode pairs is not thought to have arisen from current spread between neighbouring electrodes. Rather, the interactions are likely to arise from the wide-field amacrine cells that span several millimetres in the retina[61, 62], causing inhibition through the release of the neurotransmitter GABA to post synaptic cells[63]. Electrical stimulation of the retina has been shown to excite amacrine cells[12, 64], releasing inhibitory currents that last approximately 100ms[12]. This is of a similar range to the SOAs that resulted in moderate suppression in the 3mm separated electrode pair. We must also consider that in addition to current spread, amacrine cell activation may also have played a role in the suppression of

neural activity in the 1 and 2mm separated electrode pairs; also possibly explaining the stronger suppressive interactions observed with the 2mm electrode pair compared to single-electrode stimulation occurring for SOAs between 20 and 50ms. However, the present work was performed in a normal feline retina, and it is unknown how neurite sprouting and rewiring of retinal cells that occur in conditions such as retinitis pigmentosa[65, 66] will affect such spatiotemporal interactions.

Comparison to human psychophysics data

Recently, Horsager et al.,[8] reported a facilitatory interaction in humans between electrode pairs upon epiretinal stimulation. This resulted in a percept that was brighter than expected based on the brightness of the phosphene evoked by the stimulation of each electrode alone. The facilitatory effect described by Horsager et al.,[8] was strongest for temporally overlapping stimuli and declined as the delay between the stimulation of each electrode increased. In our study, facilitation was only observed with an SOA of 1.025ms, while for the next SOA employed, 20ms, significant suppression was observed. The longest SOA used in the Horsager et al.,[8] study was 9ms. Therefore, we can assume that facilitatory interactions between two suprachoroidal electrodes will occur with SOAs longer than 1.025ms, and that interactions may transition from facilitation to suppression at an SOA less than 20ms. A similar such trend has been observed in humans with paired-pulse paradigms in the form of twin- or two-flash experiments that explore interactions between successive light flash visual stimuli[67-71].

In contrast to the results obtained in this study where both facilitatory and suppressive interactions were seen, only suppressive interactions were observed between electrode pairs in a study with subretinal stimulation in humans (Wilke RG, et al. *IOVS* 2011; 52 ARVO E-Abstract 458). The suppressive interactions reduced the ability to detect the phosphene evoked by the stimulation of the second electrode in the pair. Irrespective of the spatial separation between the

two electrodes, in the study by Wilke, et al., interactions were observed when a delay of 155ms or greater was applied between the stimulation of each electrode. In our study, neural suppression was reduced when an SOA of 100ms (0mm electrode separation) or 90ms (1mm, 2mm and 3mm electrode separation) was applied between the stimulation of the conditioning and test electrode, similar to the range of 155ms reported with subretinal stimulation**.** Therefore, to avoid suppression, it would seem preferable to use stimulating frequencies below approximately 10Hz where cortical suppression was found to be minimal. In practice however, stimulating frequencies greater than 10Hz are utilised so that the patient does not perceive flickering of the phosphene. Testing in patients also show that it is difficult to achieve persistent phosphenes[18], even with stimulating frequencies lower than the 10Hz required to overcome suppression of MUA. Phosphenes initially appear bright and fade to nothing long before electrical stimulation with the pulse train ceases[18]. Similar phenomena have been observed with transcorneal electrical stimulation of normally-sighted and blind individuals, although these phosphenes still maintained their visibility[72], therefore, phosphene fading with electrical stimulation of the retina may be unavoidable. It must be noted that our study was performed using a paired-pulse paradigm while retinal prostheses mostly use pulse trains for stimulation. In such cases, interactions will likely not only occur between the first two pulses in the train but will extend over subsequent pulses as well. For example, while facilitation may occur between the first two pulses, the first pulse may have a suppressive effect on the third or subsequent pulses.

The spatial dynamics of interactions occurring between two suprachoroidal stimuli vary compared to those reported previously in human subjects with epiretinal[8] and subretinal (Wilke RG, et al. *IOVS* 2011; 52 ARVO E-Abstract 458) stimulation, but this may not be surprising due to species differences and the fact that the human studies were conducted in blind individuals, or the different electrode designs. Interactions between epiretinally placed electrodes can occur between two electrodes separated by up to 2.4mm[8], whilst for subretinally placed electrodes,

interactions between electrodes can span a distance of approximately 0.8mm (Wilke RG, et al. *IOVS* 2011; 52 ARVO E-Abstract 458). Based on the results from our study, interactions are likely to exist between two suprachoroidal electrodes spaced anywhere between 2 to 3mm apart. Aside from differences in the models used to investigate electrode interactions, the differences in the size of the stimulating electrodes may also account for the different results among these studies due to differences in the area of the retina that is stimulated. We also need to consider the placement of the retinal prosthesis on the spatial aspects of interactions. Suprachoroidally placed electrodes may activate larger areas of the retina due to a more widely dispersed electric field resulting from an increased distance from the retina, compared to epiretinal and subretinal electrodes[73, 74]. Therefore, for comparably sized electrodes, spatial interactions are expected to occur over a wider distance in the retina with suprachoroidal stimulation compared to epiretinal and subretinal stimulation.

Summary

Interactions between sequential stimulation of a pair of suprachoroidal retinal electrodes span several millimetres in the retina. These interactions were dependent on conditioning current and the spatial and temporal shifts between paired-pulse stimulation. Interactions between stimuli were comparable for 0, 1 and 2mm spaced electrode pairs: successive stimulation with a pair of pulses separated by 1.025ms resulted in an increase in MUA evoked by the test stimulus, a significant reduction in MUA evoked by the test stimulus when separated by less than approximately 80-90ms and slight changes to MUA when paired stimuli were separated by greater than 100ms. Interactions between stimuli were abolished when presented on pairs of electrodes spaced 3mm apart only when combined with temporal shifts of 90ms or greater. Knowledge of these spatiotemporal interactions is essential for developing effective patterns of spatiotemporal stimulation for retinal prostheses.

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REFERENCES

1. Cha K, Horch KW, Normann RA. Mobility performance with a pixelized vision system. *Vision Res* 1992;32:1367-1372.
2. Cha K, Horch KW, Normann RA, Boman DK. Reading speed with a pixelized vision system. *J Opt Soc Am A* 1992;9:673-677.
3. Purves D. Central Visual Pathways. In: Purves D, Augustine GJ, Fitzpatrick D, et al. (eds), *Neuroscience.* Sunderland (MA): Sinauer Associates; 2001.
4. da Cruz L, Coley BF, Dorn J, et al. The Argus II epiretinal prosthesis system allows letter and word reading and long-term function in patients with profound vision loss. *Br J Ophthalmol* 2013;97:632-636.
5. Humayun MS, Dorn JD, da Cruz L, et al. Interim results from the international trial of Second Sight's visual prosthesis. *Ophthalmology* 2012;119:779-788.
6. Stingl K, Bartz-Schmidt KU, Besch D, et al. Artificial vision with wirelessly powered subretinal electronic implant alpha-IMS. *Proc Biol Sci* 2013;280:20130077.
7. Zrenner E, Bartz-Schmidt KU, Benav H, et al. Subretinal electronic chips allow blind patients to read letters and combine them to words. *Proc Biol Sci* 2011;278:1489-1497.
8. Horsager A, Boynton GM, Greenberg RJ, Fine I. Temporal interactions during paired-electrode stimulation in two retinal prosthesis subjects. *Invest Ophthalmol Vis Sci* 2011;52:549-557.
9. Horsager A, Greenberg RJ, Fine I. Spatiotemporal interactions in retinal prosthesis subjects. *Invest Ophthalmol Vis Sci* 2010;51:1223-1233.
10. Ahuja AK, Behrend MR, Kuroda M, Humayun MS, Weiland JD. An in vitro model of a retinal prosthesis. *IEEE Trans Biomed Eng* 2008;55:1744-1753.
11. Cai C, Ren Q, Desai NJ, Rizzo JF, 3rd, Fried SI. Response variability to high rates of electric stimulation in retinal ganglion cells. *J Neurophysiol* 2011;106:153-162.
12. Fried SI, Hsueh HA, Werblin FS. A method for generating precise temporal patterns of retinal spiking using prosthetic stimulation. *J Neurophysiol* 2006;95:970-978.
13. Berry MJ, Warland DK, Meister M. The structure and precision of retinal spike trains. *Proc Natl Acad Sci U S A* 1997;94:5411-5416.
14. O'Brien BJ, Isayama T, Richardson R, Berson DM. Intrinsic physiological properties of cat retinal ganglion cells. *J Physiol* 2002;538:787-802.
15. Sekirnjak C, Hottowy P, Sher A, Dabrowski W, Litke AM, Chichilnisky EJ. Electrical stimulation of mammalian retinal ganglion cells with multielectrode arrays. *J Neurophysiol* 2006;95:3311-3327.
16. Jensen RJ, Rizzo JF, 3rd. Responses of ganglion cells to repetitive electrical stimulation of the retina. *J Neural Eng* 2007;4:S1-6.
17. Freeman DK, Fried SI. Multiple components of ganglion cell desensitization in response to prosthetic stimulation. *J Neural Eng* 2011;8:016008.
18. Perez Fornos A, Sommerhalder J, da Cruz L, et al. Temporal properties of visual perception on electrical stimulation of the retina. *Invest Ophthalmol Vis Sci* 2012;53:2720-2731.
19. Caspi A, Dorn JD, McClure KH, Humayun MS, Greenberg RJ, McMahon MJ. Feasibility study of a retinal prosthesis: spatial vision with a 16-electrode implant. *Arch Ophthalmol* 2009;127:398-401.
20. Wilke R, Gabel VP, Sachs H, et al. Spatial resolution and perception of patterns mediated by a subretinal 16-electrode array in patients blinded by hereditary retinal dystrophies. *Invest Ophthalmol Vis Sci* 2011;52:5995-6003.
21. Klauke S, Goertz M, Rein S, et al. Stimulation with a wireless intraocular epiretinal implant elicits visual percepts in blind humans. *Invest Ophthalmol Vis Sci* 2011;52:449-455.
22. de Balthasar C, Boex C, Cosendai G, Valentini G, Sigrist A, Pelizzone M. Channel interactions with high-rate biphasic electrical stimulation in cochlear implant subjects. *Hear Res* 2003;182:77-87.

23. Shannon RV. Multichannel electrical stimulation of the auditory nerve in man. II. Channel interaction. *Hear Res* 1983;12:1-16.
24. Townshend B, White RL. Reduction of electrical interaction in auditory prostheses. *IEEE Trans Biomed Eng* 1987;34:891-897.
25. Seligman PM, Shepherd RK. Cochlear implants. In: Horch KW, Dhillon GS (eds), *Neuroprosthetics: Theory and Practice.* Singapore: World Scientific; 2004:878-904.
26. Bierer JA, Middlebrooks JC. Cortical responses to cochlear implant stimulation: channel interactions. *J Assoc Res Otolaryngol* 2004;5:32-48.
27. Villalobos J, Allen PJ, McCombe MF, et al. Development of a surgical approach for a wide-view suprachoroidal retinal prosthesis: evaluation of implantation trauma. *Graefes Arch Clin Exp Ophthalmol* 2012;250:399-407.
28. Cicione R, Shivdasani MN, Fallon JB, et al. Visual cortex responses to suprachoroidal electrical stimulation of the retina: effects of electrode return configuration. *J Neural Eng* 2012;9:036009.
29. John SE, Shivdasani MN, Williams CE, et al. Suprachoroidal electrical stimulation: effects of stimulus pulse parameters on visual cortical responses. *J Neural Eng* 2013;10:056011.
30. Matteucci PB, Chen SC, Tsai D, et al. Current steering in retinal stimulation via a quasimonopolar stimulation paradigm. *Invest Ophthalmol Vis Sci* 2013;54:4307-4320.
31. Shivdasani MN, Fallon JB, Luu CD, et al. Visual cortex responses to single- and simultaneous multiple-electrode stimulation of the retina: implications for retinal prostheses. *Invest Ophthalmol Vis Sci* 2012;53:6291-6300.
32. Shivdasani MN, Luu CD, Cicione R, et al. Evaluation of stimulus parameters and electrode geometry for an effective suprachoroidal retinal prosthesis. *J Neural Eng* 2010;7:036008.
33. Tusa RJ, Palmer LA, Rosenquist AC. The retinotopic organization of area 17 (striate cortex) in the cat. *J Comp Neurol* 1978;177:213-235.
34. Hubel DH, Wiesel TN. Receptive fields of single neurones in the cat's striate cortex. *J Physiol* 1959;148:574-591.
35. Brummer SB, Turner MJ. Electrochemical considerations for safe electrical stimulation of the nervous system with platinum electrodes. *IEEE Trans Biomed Eng* 1977;24:59-63.
36. Elfar SD, Cottaris NP, Iezzi R, Abrams GW. A cortical (V1) neurophysiological recording model for assessing the efficacy of retinal visual prostheses. *J Neurosci Methods* 2009;180:195-207.
37. Villalobos J, Nayagam DA, Allen PJ, et al. A wide-field suprachoroidal retinal prosthesis is stable and well tolerated following chronic implantation. *Invest Ophthalmol Vis Sci* 2013;54:3751-3762.
38. Heffer LF, Fallon JB. A novel stimulus artifact removal technique for high-rate electrical stimulation. *J Neurosci Methods* 2008;170:277-284.
39. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B (Methodological)* 1995;289-300.
40. Cartee LA, van den Honert C, Finley CC, Miller RL. Evaluation of a model of the cochlear neural membrane. I. Physiological measurement of membrane characteristics in response to intrameatal electrical stimulation. *Hear Res* 2000;146:143-152.
41. Dynes SBC. Discharge characteristics of auditory nerve fibres for pulsatile electrical stimuli. *Department of Physics*: Massachusetts Institute of Technology; 1996.
42. Durand DM. Electrical Stimulation of Excitable Tissue. In: Bronzino JD (ed), *The Biomedical Engineering Handbook*: Springer; 2000.
43. Motokawa K, Iwama K. Resonance in electrical stimulation of the eye. *Tohoku J Exp Med* 1950;53:201-206.
44. Fujikado T, Morimoto T, Kanda H, et al. Evaluation of phosphenes elicited by extraocular stimulation in normals and by suprachoroidal-transretinal stimulation in patients with retinitis pigmentosa. *Graefes Arch Clin Exp Ophthalmol* 2007;245:1411-1419.

45. Horsager A, Greenwald SH, Weiland JD, et al. Predicting visual sensitivity in retinal prosthesis patients. *Invest Ophthalmol Vis Sci* 2009;50:1483-1491.
46. Shivdasani MN, Sinclair NC, Dimitrov PN, et al. Factors Affecting Perceptual Thresholds in a Suprachoroidal Retinal Prosthesis. *Invest Ophthalmol Vis Sci* 2014.
47. Nanduri D, Fine I, Horsager A, et al. Frequency and amplitude modulation have different effects on the percepts elicited by retinal stimulation. *Invest Ophthalmol Vis Sci* 2012;53:205-214.
48. Greenwald SH, Horsager A, Humayun MS, Greenberg RJ, McMahon MJ, Fine I. Brightness as a function of current amplitude in human retinal electrical stimulation. *Invest Ophthalmol Vis Sci* 2009;50:5017-5025.
49. Crapper DR, Noell WK. RETINAL EXCITATION AND INHIBITION FROM DIRECT ELECTRICAL STIMULATION. *J Neurophysiol* 1963;26:924-947.
50. Li L, Cao P, Sun M, et al. Intraorbital optic nerve stimulation with penetrating electrodes: in vivo electrophysiology study in rabbits. *Graefes Arch Clin Exp Ophthalmol* 2009;247:349-361.
51. Mandel Y, Goetz G, Lavinsky D, et al. Cortical responses elicited by photovoltaic subretinal prostheses exhibit similarities to visually evoked potentials. *Nat Commun* 2013;4:1980.
52. Nadig MN. Development of a silicon retinal implant: cortical evoked potentials following focal stimulation of the rabbit retina with light and electricity. *Clin Neurophysiol* 1999;110:1545-1553.
53. Van De Grind WA, Grüsser OJ, Lunkenheimer HU. Temporal Transfer Properties of the Afferent Visual System Psychophysical,Neurophysiological and Theoretical Investigations. In: Jung R (ed), *Central Processing of Visual Information A: Integrative Functions and Comparative Data*: Springer Berlin Heidelberg; 1973:431-573.
54. Hawken MJ, Shapley RM, Grosof DH. Temporal-frequency selectivity in monkey visual cortex. *Vis Neurosci* 1996;13:477-492.
55. Wilke RG, Moghadam GK, Lovell NH, Suaning GJ, Dokos S. Electric crosstalk impairs spatial resolution of multi-electrode arrays in retinal implants. *J Neural Eng* 2011;8:046016.
56. Jolly CN, Spelman FA, Clopton BM. Quadrupolar stimulation for Cochlear prostheses: modeling and experimental data. *IEEE Trans Biomed Eng* 1996;43:857-865.
57. Kral A, Hartmann R, Mortazavi D, Klinke R. Spatial resolution of cochlear implants: the electrical field and excitation of auditory afferents. *Hear Res* 1998;121:11-28.
58. van den Honert C, Stypulkowski PH. Single fiber mapping of spatial excitation patterns in the electrically stimulated auditory nerve. *Hear Res* 1987;29:195-206.
59. Bierer JA. Threshold and channel interaction in cochlear implant users: evaluation of the tripolar electrode configuration. *J Acoust Soc Am* 2007;121:1642-1653.
60. Sinclair N, Perera T, Shivdasani MN, Blamey PJ, Dimitrov P, Varsamidis M. Shape, location and overlap of phosphenes elicited using a suprachoroidal electrode array. *3rd International Conference on Medical Bionics.* Phillip Island, Victoria; 2013.
61. Kolb H. Amacrine cells of the mammalian retina: neurocircuitry and functional roles. *Eye (Lond)* 1997;11 (Pt 6):904-923.
62. Kolb H, Nelson R, Mariani A. Amacrine cells, bipolar cells and ganglion cells of the cat retina: a Golgi study. *Vision Res* 1981;21:1081-1114.
63. Eggers ED, Lukasiewicz PD. Multiple pathways of inhibition shape bipolar cell responses in the retina. *Vis Neurosci* 2011;28:95-108.
64. Margalit E, Babai N, Luo J, Thoreson WB. Inner and outer retinal mechanisms engaged by epiretinal stimulation in normal and rd mice. *Vis Neurosci* 2011;28:145-154.
65. Fariss RN, Li ZY, Milam AH. Abnormalities in rod photoreceptors, amacrine cells, and horizontal cells in human retinas with retinitis pigmentosa. *Am J Ophthalmol* 2000;129:215-223.
66. Marc RE, Jones BW, Watt CB, Strettoi E. Neural remodeling in retinal degeneration. *Prog Retin Eye Res* 2003;22:607-655.

67. Herrick RM. Increment thresholds for two identical flashes. *J Opt Soc Am* 1972;62:104-110.
68. Ikeda M. Temporal summation of positive and negative flashes in the visual system. *JOSA* 1965;55:1527-1533.
69. Meijer JG, van der Wildt GJ, van den Brink G. Twin-flash response as a function of flash diameter. *Vision Res* 1978;18:1111-1116.
70. Rashbass C. The visibility of transient changes of luminance. *J Physiol* 1970;210:165-186.
71. Schuckman H, Orbach J. Detection thresholds as a function of interval separation between two successive targets. *Science* 1965;150:1623-1625.
72. Xie J, Wang GJ, Yow L, et al. Preservation of retinotopic map in retinal degeneration. *Exp Eye Res* 2012;98:88-96.
73. Fujikado T, Kamei M, Sakaguchi H, et al. Testing of semichronically implanted retinal prosthesis by suprachoroidal-transretinal stimulation in patients with retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 2011;52:4726-4733.
74. Kanda H, Morimoto T, Fujikado T, Tano Y, Fukuda Y, Sawai H. Electrophysiological studies of the feasibility of suprachoroidal-transretinal stimulation for artificial vision in normal and RCS rats. *Invest Ophthalmol Vis Sci* 2004;45:560-566.