Methods and Perceptual Thresholds for Short-Term Electrical Stimulation of Human Retina with Microelectrode Arrays

Perceptual Efficacy of Electrical Stimulation of Human Retina with a Microelectrode Array during Short-Term Surgical Trials

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I. FURTHER DETAILS OF METHODS USED IN BOTH STUDIES

Selection of Volunteers

Visually impaired volunteers contacted us and we discussed their medical history, the goals of our Project, the requirements for their possible participation in the research, and the fact that our testing would not restore vision. Those who seemed suitable were invited to Boston (with a companion, at our expense) to be examined by our team members to verify acuity (Snellen, when possible) and the diagnosis of retinitis pigmentosa, and seek evidence of glaucoma, diabetic retinopathy or media opacities that might complicate testing. The normally sighted volunteer was referred by a physician.

Methods for Preparing Electrode Arrays

The electrode arrays were rinsed in running, deionized water for 24 hours immediately after manufacture, then mounted on a printed circuit (PC) board and electrically tested. Arrays with no short or open circuits were selected for activation (i.e., achieved by growth of a surface iridium oxide layer) in 0.3 M Na2HPO2 by cyclic voltammetry,1 and then rinsed in water for 20 minutes. A thin suture, gold weight and post were glued with silicone adhesive to the upper (non-electrode) side of the distal end of the polyimide strip where the stimulating electrode array was located (Fig. 1, Methods paper). Arrays were washed sequentially for five minutes in acetone, stirred deionized water, and lastly stirred isopropol alcohol, which then evaporated. The assembly was sterilized with ethylene oxide prior to surgery.

Impedance Measurements of Electrodes

Impedance measurements were performed to monitor electrode faults, i.e., open or short circuits. Short circuits were tested with dry arrays using switches on the stimulator box to examine each electrode individually with respect to all other electrodes and ground connections on the array. Local returns on the array (i.e., the annuli that surrounded each electrode) were connected through a PC board to the stimulator via two microfabricated leads built in common to all other local returns. The resistance between these two leads was measured to check that the local return path was not open-circuited.

This test allowed us to measure the resistance per square of the gold traces on the array, given the mask geometry.

Open-circuited electrodes were sought by immersing arrays in saline and measuring impedance to a large immersed return electrode by driving with balanced, square-wave current pulses and monitoring voltages between the electrode and return. For stimulus parameters similar to or lower than those used to obtain our human thresholds, we found the voltage response of our oxidized 400 µm diameter electrodes could be modeled well by a 1.1 kOhm resistor in series with a 0.3 µF capacitor. Impedance tests to detect open circuits were qualitative--any electrode with maximum and minimum voltage responses within 30% of our electrical model was judged to be acceptable. Only arrays without faults were selected for human experiments. At surgery, electrode integrity was verified again. During every stimulation, the impedance of each electrode was monitored qualitatively by observing voltage with an oscilloscope. Immediately after surgery each array was tested for electrical faults.

Surgical Methods

Surgery was performed between 1998 and 2000. Our techniques to non-traumatically contact the retina with an electrode array and electrically stimulate the retina were developed with rabbits.2 Botulinum toxin (type A, ≤ 80 IU; Allergan Co.) was injected into the retrobulbar space and extraocular recti muscles (under electromyographic control) at least 5 days before surgery to achieve akinesia, which reduced the potential for inadvertent retinal damage from unavoidable eye movements in awake volunteers. Topical anesthesia was provided by 0.5% tetracaine and 4% cocaine drops.

An anesthesiologist was present to administer remifentanyl which was hardly required after intraocular insertion of electrode arrays.

The MIS® system, which was sutured at two of three ports, included a fiber optic light source and a rubber gasket sleeve to maintain a water tight seal, especially when instruments were inserted into the eye. We used a hand-held electrode to: 1) have a quasi-standard by which we could compare our results to those of Humayun et al.; 2) judge whether the perceptual results justified insertion of an electrode array, which increased surgical risk; and 3) survey the retina for areas of relatively greater sensitivity.
Choice and Variation of Stimulus Parameters

Several stimulus durations were required to generate the strength-duration curves in the last two experiments. Variations in stimulus duration necessitated variations in stimulus frequency (see below). Both asymmetric and symmetric waveforms were used across the experiments. The asymmetric waveform (Fig. 2, Methods paper) used in the first two experiments was chosen because our rabbit experiments showed relatively lower thresholds with this waveform. A symmetrical waveform was used thereafter because it permitted easier comparison of our results to Humayun et al., and use of longer pulses while maintaining reasonable repetition frequencies.

Stimulation Electronics

The stimulator system shown (Fig. 1, Methods paper) contained ten current sources, each connected to a demultiplexer with ten outputs. Each current source and demultiplexer comprised one column, and the demultiplexer outputs connected to the electrodes via the switches. The ten current sources were driven simultaneously. A digital circuit controlled the demultiplexer, which switched all ten current sources simultaneously from one row to the next. After sweeping through a column, the controller waited until the desired period (i.e., the inverse of repetition frequency) had expired, then it began to sweep through the column again. Multiple current sources and sequential stimulation were used so that each current source would drive only one electrode at a time, thereby ensuring that the current through each electrode was known.

The total time for one biphasic pair (Fig. 2, Methods paper) was twice the pulse width plus the delay between the negative and positive pulses of the pair (usually set to the minimum, 10 &mu;sec), plus 30 &mu;sec delay between pulse pairs (for demultiplexer switching). Take, for example, a 250 &mu;sec pulse width—each biphasic pulse pair would take 540 &mu;sec, and a column of ten electrodes would take 5.4 msec, which could be driven with a repetition frequency at or below 185 Hz. Sequential stimulation continued until the total time of stimulation (i.e., the "pulse train duration," typically 1.5 seconds, occasionally up to 4.0 seconds) had been reached.

Some variation in stimulus frequency resulted from variations in the duration of charge-balanced pulses. For instance, frequencies varied in experiment 2 because we used a range of durations (200 &mu;sec - 2.5 msec) in our attempts to induce vision. In experiment 3, we generally used 30 Hz; however, 6 Hz stimulation occasionally was required when we used 8 msec pulses while driving the large electrodes. One sweep through one such column required 10 repetitions of 16 msec pulse pairs (since 4 large electrodes are located in rows 1, 4, 7 and 10), or 160 msec. Therefore, 6 Hz was the highest possible frequency.

Any potentially negative perceptual consequence of sequential stimulation was not relevant for single electrode trials or those using electrodes from only one row. Further, adjacent electrodes were stimulated with a relative frequency (i.e., the reciprocal of the delay between stimulation of one electrode and stimulation of the next) generally far above the assumed flicker fusion frequency. For example, with a 4 msec pulse width (8 msec biphasic pulse pair), the second electrode in a column was stimulated 8 msec after the first, which is a relative frequency of 125 Hz. In the worst case, the 16 msec pulse width, which was used infrequently, allowed a relative frequency of 30 Hz.

Pre-operative Training of Volunteers

Volunteers were trained pre-operatively by tactile stimulation to describe percepts, which facilitated their ability to efficiently describe: 1) whether percepts were single or multiple; 2) if single, to estimate size by comparison to a pea, dime, quarter, ping-pong ball, baseball or grapefruit, as if viewed at arm's length; and 3) the shape (i.e., line, round, etc.) of the percepts. During surgery, commentary on brightness, color and flicker was encouraged but not rigorously sought to improve efficiency in obtaining data on the more fundamental visual features described above.

Measurement of Perceptual Thresholds

Thresholds were measured by beginning with sub-threshold charge. Successive trials used twice as much current (for a given stimulus duration) until a percept was reported. Then, the charge was reduced by half the difference between the last two trials. If no response was obtained, an upward step (using half the difference in current from the most recent to the higher level of current that had yielded a percept) was made. The "staircase" search was continued until we found the lowest level of current that yielded a percept ≥ 50% of the time, assuming all trials of greater charge yielded a percept. A slightly less rigorous but more time efficient threshold check was used in advance of trials in which multiple electrodes were to be driven to determine if there was reasonable uniformity of thresholds across the array. Non-uniformity, which might occur if the array had become tilted or elevated, was especially important for attempts to produce patterned percepts following stimulation of multiple electrodes. Such "coarse" thresholds were obtained from the two end electrodes of a row or column, and the higher value was then used for both end electrodes for subsequent testing.

II. "METHODS AND PERCEPTUAL THRESHOLDS FOR SHORT-TERM ELECTRICAL STIMULATION OF HUMAN RETINA WITH MICROELECTRODE ARRAYS"

(See http://www.iovs.org/cgi/content/abstract/44/12/5355)

Control Tests

Negative control trials were randomly inserted into the testing protocol. Typically, negative control trials were performed by withholding electrical stimulation after an audible tone was given. This type of trial, about which the volunteers were unaware, comprised 6.5% of all trials across experiments 2-6. We occasionally used a second type of control test to determine if percepts could be created in the absence of electrical stimulation by moving a needle electrode within an illuminated eye. Consistent with our suspicion, three of three volunteers reported false positive percepts (that faded when the intraocular light was dimmed) in this situation. Based partly on this finding, we never performed psychophysical testing with ocular illumination.

Use of Ultra-Thin Electrode Arrays

Ten micron thick polyimide arrays were flexible enough to conform to the retinal curvature without creating obvious damage. Their flexibility allowed them to rest uniformly close to the underlying neurons. Rather than use tacks to stabilize the array, we developed a non-traumatic method of holding the array in position with a small gold weight (attached to the array) and a viscoelastic. Despite our efforts, the array occasionally elevated or turned, but it could fairly easily be re-positioned.

Data Analysis

To permit comparison across patients despite use of different stimulus durations (for reasons stated above), we standardized threshold charge density values to those obtained with 400 &mu;m
electrodes at 4 msec duration in experiments 5 and 6. To permit an approximate comparison to patients 3 and 4 in whom thresholds were obtained at 8 and 2 msec, respectively, we multiplied the 2 msec value of subject 4 by the average ratio of 4 to 2 msec thresholds (1.05) obtained from the threshold charge density versus duration plots of the last two patients (Fig. 7, Methods paper). The 8 msec threshold of patient 3 was multiplied by the average ratio of 4 to 8 msec thresholds (0.55) obtained by interpolation of the same plots.

**Charge Injection Density**

The only convincing method to establish safe limits for electrical stimulation of human retina would be microscopic examination of electrodes and histological evaluation of tissue following chronic stimulation of mammalian retina through a set of electrodes with size, composition, current waveforms, pulse durations and frequencies typical of those proposed for use with human prostheses. Despite several relevant publications,6,7,8,9 only one study approaches these requirements.9 Weiland et al. used 4 x 4 arrays of 460 μm diameter platinum disc electrodes for epiretinal stimulation of 6 dogs for up to 60 days. No retinal damage was evident at lower stimulation levels (0.05 and 0.10 mC/cm²/phase delivered through 8 electrodes simultaneously). The 0.10 mC/cm²/phase level approximates the single-electrode threshold for our normally sighted patient, but falls substantially short of the single-electrode thresholds of our blind patients. As such, the electrical safety limit for human retinal stimulation is an incompletely resolved issue.

A range of studies, from consideration of purely physical effects in saline solutions to histopathological observations, has been made to assess allowable limits. For the former, stimulation levels are judged by whether they avoid potential differences between electrodes and fluid sufficient to cause electrolysis of water (i.e., evolution of H₂ or O₂ gases), which is typically accompanied by pH changes and often electrode damage. In these studies, electrodes usually are driven by charge-balanced biphasic pulses, in which the charge in the first phase is quickly canceled by charge of equal magnitude but opposite polarity in the second phase of stimulation to prevent a continuous charge buildup on the electrodes, which would damage the electrode and potentially the tissue.

Electrodes made of iridium oxide have become favored because of their multi-layer porous structure that increases the effective surface area and the voltage-dependent transition between the Ir⁺³ and Ir⁺⁴ oxidation states in the oxide layer.1,3,10-13 Both features greatly increase capacitance per unit area, thereby reducing the voltage change per unit charge passed per unit area. The result is a dramatic increase in charge capacity, i.e., the charge that can be passed per unit electrode area in a single phase of stimulation without causing electrolysis. One often cited paper13 reports that hydrogen evolves when the potential of an iridium oxide electrode reaches ~0.6 V on a sustained basis, and oxygen evolves when +0.8 V is reached, both measured with respect to a saturated calomel electrode in bicarbonate buffered saline. Beebe and Rose recommended that electrode potentials be kept within this narrow “water window,” even though gas evolution does not occur during rapid cycling of a small electrode through potential excursions that pass somewhere outside the window.

Iridium oxide electrode capacitance and the resulting charge capacity per unit area vary significantly with the thickness of the underlying iridium metal and the method of oxide growth and deposition. In one popular method, anodic formation of activated iridium oxide film (AIROF), multiple oxide layers are formed by repeatedly cycling the electrode area in a single phase of stimulation without causing electrolysis. One often cited paper13 reports that hydrogen evolves when the potential of an iridium oxide electrode reaches ~0.6 V on a sustained basis, and oxygen evolves when +0.8 V is reached, both measured with respect to a saturated calomel electrode in bicarbonate buffered saline. Beebe and Rose recommended that electrode potentials be kept within this narrow “water window,” even though gas evolution does not occur during rapid cycling of a small electrode through potential excursions that pass somewhere outside the window.

III. **“Perceptual Efficacy of Electrical Stimulation of Human Retina with a Microelectrode Array during Short-Term Surgical Trials”**

(See http://www.iovs.org/cgi/content/abstract/44/12/5362)

**Additional Details of Perceptual Results**

The first two volunteers reported only vague percepts. The first reported “indistinct flashes” and “fireworks” following bipolar stimulation with 10 electrodes at 30 μA. No improvement occurred with 50 electrodes up to 80 μA. With monopolar stimulation (50 electrodes, 6 – 80 μA), only 1 of 11 trials yielded a percept, which occurred with 80 μA and was vague. The experiment was discontinued when the maximum current output had been reached and a drop of blood that appeared behind the crystalline lens reduced visibility.

Charge capacity is usually quantified in terms of charge per phase per unit electrode area, but use of charge per unit area can be misleading. Even flat disc electrodes pass charge in a highly non-uniform manner, with the bulk of the charge passing through the periphery of the disc.14-18 Our unpublished experiments have shown that, as a result, capacitance grows more slowly than area as electrode size is increased, and thus, total charge capacity grows more slowly than area.

There is not a consensus on whether tissue damage arises from electrochemical reactions or from processes associated with passage of stimulus current through tissue, such as neuronal hyperactivity.20 This question has been studied by chronic stimulation of tissue through purely capacitive electrodes in which no electrochemical reactions occur. In one study,14 tantalum pentoxide capacitive electrodes and conventional platinum electrodes of 1 mm diameter were implanted in the subdural space over cat parietal cortex and pulsed at 50 Hz for seven hours with charge-balanced pulses at a very modest charge density of 0.08 – 0.10 mC/cm²/phase. Damaged neurons were found beneath both types of electrodes, but little damage was noted beneath unstimulated electrodes. A subsequent study by the same group14 revealed that local anesthesia with procaine or lidocaine protected the peroneal nerves from electrically induced damage. Collectively, these two studies strongly suggest that damage may result from neural hyperactivity following stimulation under circumstances where electrochemical damage is implausible (i.e., low charge densities or capacitive electrodes).

A consensus is also lacking on the relative roles of charge per unit area versus charge per phase in determining biologically safe stimulation levels.8,19 The only systematic attempt to quantitatively relate these variables revealed that the product of charge per phase and charge per unit area per phase is a better predictor of biological damage than either quantity alone.20 From this Shannon estimated, perhaps conservatively, that 32 μC/cm²/phase is a safe upper limit. Using the same data and method of reasoning, we calculated a less conservative limit of 79 μC/cm²/phase (see dotted lines in Fig. 7, Methods paper).

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With the second volunteer thresholds (using a single electrode and monopolar stimulation) could not be obtained with 1 msec pulses up to 3 mA, 2.5 msec pulses up to 1.5 mA, or 200 µsec pulses up to 12 mA. In 3 of 12 trials he reported “something real dim” or a “circle” (3 mA, 1 msec pulses). Given the poor responses, the electrode array was not introduced since this would have increased the surgical risk.

Regarding hypothesis 1, responses from a 100 µm electrode were not smaller than those obtained with a 400 µm electrode, although a stimulus-matched (i.e., identical charge density and duration) comparison was not made. Regarding hypothesis 3, bipolar stimulation (1860 µm spacing) produced percepts that were similar to those produced by monopolar stimulation.

Regarding hypothesis 5, percepts did not differ substantially between the normal subject and blind volunteers (hypothesis 1) when a single 100 vs. 400 µm diameter electrode was used. The normal volunteer underwent 22 trials to determine “accuracy.” The challenge of generating spatially detailed vision is exemplified by the following excerpt. Stimulation of a row of four large electrodes (250 µA per electrode, previously determined to be at or slightly above threshold for both end electrodes) initially yielded three circles. To understand why four percepts were not seen, we drove only the two end electrodes (250 µA each), which produced only one percept in each of four trials, which suggested sub-threshold current at one electrode. Increasing the stimulus to 350 µA for both end electrodes produced two circles, which supported our suspicion of earlier sub-threshold stimulation. Letter recognition was attempted in 3 of the 22 trials, none of which were successful. (She merely described one or more “circles” or “dots.”)

Regarding hypothesis 6, the third volunteer inconsistently reported size, and the fourth had little training because of pre-arranged surgery for cancer. Hence, trials that assessed perceptual size were useful only for the fifth and sixth volunteers. With respect to the first method used to study size, we also examined the potential confounding effect of stimulus duration. Using 400 µm electrodes and a range of currents, ≤ 1 msec pulses yielded a pea 10 of 14 times (71%), whereas > 1 msec pulses yielded a pea 36 of 52 times (59%), which is not obviously different.

References
9. Weiland JD et al. Chronic electrical stimulation of the canine retina. 2nd Joint Meeting of BMES and IEEE EMBS. Houston, TX, 2002.