Retinal electrostimulation in rats: Activation thresholds from superior colliculus and visual cortex recordings

Author:
Barriga-Rivera, A; Guo, T; Morley, JW; Lovell, NH; Suaning, GJ

Publication details:
Proceedings of the Annual International Conference of the IEEE Engineering in Medicine and Biology Society, EMBS
v. 2017
Medium: Print
pp. 1166 - 1169
9781509028092 (ISBN)
1557-170X (ISSN); 1558-4615 (ISSN)

Event details:
2017 39th Annual International Conference of the IEEE Engineering in Medicine and Biology Society [EMBC]
Seogwipo, South Korea
2017-07-11 - 2017-07-15

Publication Date:
2017-09-13

Publisher DOI:
https://doi.org/10.1109/EMBC.2017.8037037

License:
https://creativecommons.org/licenses/by-nc-nd/4.0/
Link to license to see what you are allowed to do with this resource.

Downloaded from http://hdl.handle.net/1959.4/unsworks_50787 in https://unsworks.unsw.edu.au on 2024-08-04
Retinal Electrostimulation in Rats: Activation Thresholds from Superior Colliculus and Visual Cortex Recordings

Alejandro Barriga-Rivera, Tianruo Guo, John W. Morley, Nigel H. Lovell, Fellow, IEEE, Gregg J. Suaning, Senior Member, IEEE,

Abstract - Retinal neuromodulation is an emerging therapeutic approach to restore functional vision to those suffering retinal photoreceptor degeneration. The retina encodes visual information and transmits it to the brain. Replicating this retinal code through electrical stimulation is essential to improving the performance of visual prostheses. In doing so, the first step relies on precise neural recordings from visual centers that allow studying the response of these neurons to electrical stimulation of the retina. This paper demonstrates the feasibility of a rat model to conduct highly reliable electrophysiological studies in the field of retinal neuromodulation. A disc electrode, implanted in the retrobulbar space was used to stimulate the retina of Long-Evans rats. Buzsaki multi-electro arrays were inserted in the superior colliculus (SC) to record electrical activity propagated from the retinal ganglion cells (RGCs). Activation thresholds calculated from local field potentials (visual cortex) and from neural spikes (SC) were contrasted. Both values were comparable to those in humans and in other animal models, and were slightly higher when estimated from SC recordings. However, differences were not statistically significant.

I. INTRODUCTION

Electrical stimulation of the retina can restore aspects of functional vision to those blinded by retinitis pigmentosa and other conditions of the retina [1]. In physiological conditions, visual stimuli are transduced by the photoreceptors and the resulting information encoded by the retinal network. This information is sent to visual centers in the brain as a series of action potentials through the optic nerve. In the diseased retina, photoreceptors are degenerated thus impeding normal function of the retina [2]. However, the retinal ganglion cells (RGCs), the sole neurons that connect the eye with the brain, typically remain viable and therefore, electrical stimulation of those can elicit visual perception. The axons of these cells collectively form the optic nerve and project their synaptic terminals to the dorsal surface of the superior colliculus (SC) and the lateral geniculate nucleus (LGN) [3].

Neural recordings at these locations provide an excellent tool to investigate the electrical activation of RGCs under different stimulation conditions. In this vein, normally sighted cats have been traditionally used to answer questions related to field manipulation in retinal neuromodulation [4-6]. However, a murine model can be an alternative to larger animals with the possibility of using strains with retinal degeneration such as the Royal College of Surgeons or Tg P23H-1 rats [7]. The aim of this study is to investigate the activation thresholds in the visual cortex (VC) and the SC from a retrobulbar prosthesis in a rat model. This represents an initial assessment when investigating more complex stimulation paradigms.

II. METHODS

A. Ethics Statement

This study was approved by the Animal Care & Ethics Committee of the University of New South Wales, Australia. Experiments were conducted in compliance with the Australian code for care and use of animals for scientific purposes and the ARVO statement for the Use of Animals in Ophthalmic and Vision Research.

B. Animal Preparation and Surgery

Three male Long-Evans rats of between 12 and 15 weeks of age were included in this study. To ensure the wellbeing of the animals during induction of anesthesia, they were sedated within an anesthetic chamber filled with increasing levels of isoflurane in oxygen. To induce a surgical anesthetic plane, an intraperitoneal injection of ketamine/xylazine (80/10 mg·kg⁻¹) was administered afterwards. Anesthesia was then maintained by a combination of inhaled isoflurane in oxygen (0-1%), delivered via anesthetic mask (Stoelting, Illinois, USA), and intravenous infusion of ketamine (10-50 mg·kg⁻¹·h⁻¹) and xylazine (4-15 mg·kg⁻¹·h⁻¹). These drugs were prepared in equal amounts of saline and Hartman’s solution and delivered at constant flows of between 0.5 and 1.5 mL·h⁻¹. An injection of the anti-inflammatory agent dexamethasone was administered via intramuscular injection (0.06 mg·kg⁻¹). The antibiotic combination amoxicillin/clavulanic acid was also administered intramuscularly (20/5 mg·kg⁻¹) as a prophylaxis to reduce the risk of infections. A subcutaneous injection of atropine (0.05 mg·kg⁻¹) was given to decrease fluid sections from airways. Corena hydration was maintained using hydroxypropyl methylcellulose routinely. Respiratory rate, electrocardiogram, rectal temperature and oxygen saturation were continuously monitored, and body temperature was maintained using a thermal blanket placed underneath the animal.

Immediately prior to placing the animal within a stereotaxic holder (Stoelting, Illinois, USA), lignocaine was sprayed inside the ears. With the animal secured, the conjunctiva was dissected from the limbus to clear approximately 90 degrees. A pocket was made by blunt dissecting tenons and conjunctiva from the superior surface of the eye. The superior rectus muscle was resected to avoid unwanted contractions from electrode activation. Then, a platinum disc electrode, 2 mm radius, was implanted between...
A thin sponge (~0.5 x 1 x 2 mm) was positioned behind the electrode to maintain intimate contact between the sclera and the stimulating electrode. The incision was sutured to secure the electrode in place. Then, a 1 mm diameter platinum braided wire, 4 mm long, acting as a distant return electrode, was placed on the surface of the sclera.

Following implantation of the stimulating electrode, a midline incision was made in the skin to access the skull. After retracting the periosteum to the edges of the skull, the bone was gently scraped to expose a dry and clean surface. Next, three stainless steel screw electrodes were placed, one over the midline, 3 mm rostral to the bregma suture, and two over both visual cortices (area 17, 7 mm caudal to the bregma suture and 3 mm lateral to the midline). To access the SC, a small craniotomy (2 mm x 1 mm approximately) was made contralateral to the implanted eye using Paxinos coordinates [8]. After reflecting the dura, a 32-channel multi-electrode array (MEA) (Neuronexus Buzsaki32, Michigan, USA) was inserted using a micromanipulator for subsequent recording of neural activity from the dorsal surface of the SC.

C. Stimulation Paradigms

Prior to electrically stimulating the retina, viability of the eye and the correct location of the recording electrodes were assessed using a strobe light. Each flash was repeated 50 times.

A custom retinal stimulator capable of up to 14 channels of concomitant stimulation [9] was used to stimulate the retina. The neurostimulator was connected to a personal computer. Neural recordings and stimulus delivery were synchronized by a trigger signal [10]. Stimulation waveforms were biphasic, cathodic-first constant-current pulses with a phase time of 400 µs and an interphase delay of 10 µs. Six channels, each implemented as push/pull current source, were connected in parallel to the retinal electrode to deliver waveforms of amplitudes between 0 and 3.6 mA in steps of 300 µA. Stimuli were randomized and each level was delivered 25 times with an inter-stimulus time of 1 s.

D. Local Field Potentials (VC)

The screw electrodes were connected to an 1800 AM-System microelectrode amplifier (AM-System, Washington, USA). Biopotentials were acquired at 24.4 kHz using a RZ2-8 BioAmp Processor (Tucker Davis Technology, Florida, USA) as described in a previous publication [10]. To reduce noise, neural responses were obtained after calculating the ensemble average of all repeats of each stimulus delivered. Resulting waveforms were normalized relative to the maximum voltage recorded during stimulation.

E. Extracellular Activity (SC)

Using a PZ5 digitizer (Tucker Davis Technology, Florida, USA) the MEA was interfaced to the RZ2-8 processor previously described (Tucker Davis Technology, Florida, USA) [4, 5]. Signals were also acquired at 24.4 kHz. Stimulation artifacts were removed by linearly interpolating the traces 1 ms before and after the stimulus delivery. Next, the recordings were band-pass filtered between 300 Hz and 5 kHz using a zero-phase Butterworth filter. As in previous publications, neural spikes were detected using a threshold established as 3.8 times the value of the root mean square level of the recording acquired 100 ms prior to the stimulus delivery [5, 11]. The number of spikes in the first 2 ms after the onset of the stimulus was accounted as the response of the RGCs. Responding channels were only considered when they reached a spike rate of at least twice that in the absence of the stimulus.

F. Data Analysis

A sigmoidal curve was fitted to the value of the peak of the local field potentials recorded in the first 20 ms following stimulus delivery against the stimulation current density. The F50 activation threshold, defined as the mid-point of the sigmoidal regression, was then calculated [12]. Similarly, the overall spike rates of all responding channels were fitted to a sigmoidal curve to estimate the P50 threshold. The thresholds obtained from cortical and subcortical recordings were compared using one-way ANOVA analysis. Significance was defined at the 95% confidence level. Confidence intervals were given by the standard error of the mean (SEM).

III. RESULTS

First, visual evoked potentials (VEPs) were obtained and contrasted with those reported in the literature [13]. An example of the tracing is shown in Fig. 1.

After verifying the viability of the operated eye, the retina was electrically stimulated as defined in the methodology. A first peak, with a latency of approximately 10-20 ms occurs via the direct activation of the RGCs [14]. A second peak occurring between 35 and 70 ms after the stimulus is caused by the activation of the retinal network. The second peak was of lower amplitude and larger duration compared to the first peak. The overall activation threshold obtained in the visual cortex was 7.51 ± 1.07 µC·cm⁻². It should be noted that the P50 threshold was estimated by fitting the first peak of the evoked response to a sigmoidal profile. However, as illustrated in Fig. 2, activation of the visual cortex occurred at lower stimulating
currents, suggesting a different activation threshold $(4.62 \pm 0.42 \mu\text{C}\cdot\text{cm}^{-2})$. For example, fitting the first value above twice the baseline level, as shown in Fig. 2, would result in a significantly lower activation threshold.

Subcortical activity in the SC arises from RGCs directly. To isolate the direct contribution from RGCs, the first burst of spikes was considered only, that is, those with latency less than 2 ms. This is clearly illustrated in Fig. 3, where for high amplitude electrical stimulation, several bursts of spikes occurred as a consequence of both the activation of the retinal network and the activation of SC neurons. The $P_{50}$, calculated as previously described, represents the activation threshold of the RGCs (see Fig. 4). The overall number of responding channels was $8 \pm 4$ and the resulting threshold was $9.79 \pm 0.28 \mu\text{C}\cdot\text{cm}^{-2}$.

Differences in the activation thresholds were not statistically significant at the 95% confidence level ($p = 0.1$). However, the value obtained from the SC had a substantially lower dispersion across the subjects, indicating that the second method can be more accurate and reliable when estimating the activation threshold of the RGCs.

IV. Discussion

In previous publications, cortical thresholds were obtained in a sheep model [12]. Resulting activation thresholds with relatively large electrodes were comparable to those found in this study. Thresholds in the rat were also in the same order of magnitude as those found in humans [15].

A larger sample size is required to determine whether differences between thresholds obtained from SC were significantly lower than those observed from the VC. However, recordings from the SC show more clearly direct activation of the RGCs, whereas cortical recordings represent highly processed information from thalamic inputs [3]. By considering the first spike elicited, it is possible to determine the probability of a stimulus to activate the target cells. Nevertheless, it is difficult to distinguish between responses...
due to activation of the retinal network and the subsequent activation of SC neurons.

In this study spikes were not sorted. With recording electrodes of 15 μm in diameter, such as those used in this study, it is possible to isolate responses from single units [16]. With the help of synaptic blockade, this would allow identifying different functional RGC types and therefore to explore whether activation produced by more complex stimulation strategies propagates to higher visual centers, as in the case of high frequency stimulation [17]. This combined with the possibility of using animals with degeneration of the retina makes this model a powerful tool when answering some of the key questions of visual neuromodulation such as, for example, the effects of concomitant electrical stimulation [6].

V. CONCLUSIONS

Activation thresholds in the rat are comparable with those in other animal models and similar to those found in humans. Neural recordings from the SC allow for accurate monitoring of the electrical activity of the RGCs. The use of high-density MEAs can help in identifying single unit responses of RGCs and therefore to study the performance of complex stimulation paradigms in retinal prosthesis.

ACKNOWLEDGMENT

The authors would like to acknowledge the assistance of Veronica Tatarinoff and Naomi Craig for the animal care and preparation, and their technical support during the experiment.

REFERENCES