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Research Paper

Electrical stimulation of different retinal components and the effect of asymmetric pulses

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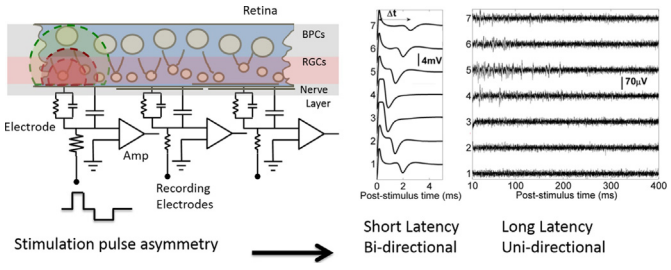
HIGHLIGHTS

- MEA recording of the chick retina offers a straight forward mapping of axonal activation in direct and indirect responses.
- Recording from developing chick retina with a multi electrode array (MEA), combined with calcium imaging, can reveal detail on axonal activation such as orientation and conduction velocity.
- Asymmetric electrical stimulations allow control over proportion of direct versus indirect activation and can thus contribute to increased resolution in prosthetic devices.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: High resolution electrical stimulation of neural tissue is a fundamental challenge in applications such as deep brain stimulation and artificial vision. In artificial vision, achieving and validating local selective epi-retinal stimulation of different layers in the retina is particularly challenging owing to plurality of retinal cell types and delocalized wiring.
Results: Strong selectivity and non-localized responses to epi-retinal stimulation, over a wide range of realistic stimulation parameters, was achieved and validated using asymmetric pulses.
New method: The reported method consists of multi electrode array (MEA) stimulation and recording from a developing chick retina combined with calcium imaging. Data show direct and indirect neuronal activation in the chick retina model. In particular, axonal activation, orientation and conduction velocity are derived, and the non-local nature of the responses to direct axonal stimulation is demonstrated.
Comparison with existing methods: Some of the previous research with mammalian retinas demonstrated local responses around the stimulating electrode, revealing little as to axonal activation. Recent studies showed activation along the nerve fibers and studied the effect of pulse duration to improve stimulation localization (Twyford and Fried, 2016; Weitz et al., 2015). The chick retina offers a straight forward mapping of axonal activation. Here we demonstrate that the chick retina, combined with MEA recording and stimulation along with calcium imaging is a powerful tool to study retinal activation and in particular the effect of asymmetry on axonal activation.

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Conclusions: MEA recording and stimulation from the chick retina is exceptionally powerful in distinguishing between direct and indirect responses. This method facilitates comparison between different stimulation strategies. We show that asymmetric electrical stimulations allow control over the intensity of direct activation.

1. Introduction

Artificial retina is one of the most demanding neuro-stimulation applications requiring very high resolution to achieve visual repair. The technology has been proposed for retina degeneration diseases, such as Retinitis Pigmentosa (RP) and age related macular degeneration (AMD). AMD is the leading cause of vision loss threatening to affect a substantial part of the aging population in the future decades. In retinal degeneration diseases, the inner retinal layers often remain functional (Margolis et al., 2008), and despite occurrence of rewiring of retinal neurons during retinal degeneration (Marc et al., 2007), the retinotopic map in the visual cortex is generally preserved (Xie et al., 2012). An artificial approach to bypass the adverse effects of photoreceptor degeneration is therefore possible through localized electrical stimulation of inner retinal neurons. Micro electrodes for retinal stimulation were extensively explored over the past several decades for artificial retina applications. Several general schemes including epi- and sub- retinal approaches have been proposed, all sharing the common principle of a multi-electrode array (MEA) for electrical stimulation of the retina (Weiland et al., 2011; Zrenner et al., 2011).

The development of a functional retinal prosthesis that can mimic visual perception is hindered by a multitude of challenges, ranging from biocompatibility and electrode miniaturization to resemble physiological signaling. A major challenge is the complex signal processing executed by the retina. Questions such as how to stimulate specific cell populations, as well as how to limit the spatial and temporal resolution of the response, remain open (Freeman et al., 2011b).

Three activation scenarios are possible in epi-retinal stimulation(Margalit et al., 2011). The first involves activation of remaining photoreceptors or bipolar cells (BPC) and subsequent retinal ganglion cell (RGC) activation (indirect activation). A second possibility involves the direct activation of a single, or several, RGCs, positioned close to the stimulating electrode. This activation process is typified by a single unit response at very close proximity to the stimulating electrode. A third process involves activation of axons crossing the stimulating electrode. In this case, both forward (orthodromic) and backward (antidromic) axonal propagation is expected. The first two scenarios yield spatially localized groups of activated RGCs (the second one in time as well). The third scenario is highly non-localized as the information transmitted to the optic nerve is equivalent to the one that would have originated from an entire ganglion cell region (numerous ganglion cells with axons crossing the stimulating electrode are evoked). Although the activation thresholds among different cell layers, cell types and cell compartments are different (Sekirnjak et al., 2008), it is likely that, under realistic conditions, all three activation scenarios coexist. Indeed, patients with epi-retinal implants often report longitudinal phosphenes as a result of stimulation (Nanduri et al., 2012). Clearly, choosing a specific activation scenario to achieve a desired visual perception is a major challenge in epi-retinal stimulation.

Despite extensive investigations in the field of electrical retinal stimulation over the last two decades, there is still a lack of *ex-vivo* quantitative and functional characterization of axonal activation, and very little is known about how to elicit direct versus in-direct activation. Since retinal stimulation involving direct electrical activation of retinal ganglion cell axons can lead to highly non-localized retina-brain mapping, it can significantly hamper the spatial resolution of epi-retinal implants. Therefore, properly identifying axonal activation and controlling it is important to fully capture the effect of electrical stimulation. Rodent models are commonly used in *ex-vivo* studies of retinal physiology and disease mechanisms (Homma et al., 2009; Ye et al., 2008). Some previous research with mammalian retinas demonstrated local distribution of responses around the stimulating electrode (Sekirnjak et al., 2006), revealing little as to axonal activation. Recent studies showed activation along the nerve fibers (Petoe and Shivdasani, 2016; Twyford and Fried, 2016). The chick retina offers (as we will show below) a straightforward mapping of axonal activation. In this study, we therefore chose the chick retina to study the effect of asymmetry on axonal activation. We used charged balanced current stimulation to guarantee stimulation safety while modifying systematically the asymmetry between the phases (Weitz et al., 2015). By applying asymmetric electrical stimulations, we could directly control, for the first time, the ratio between direct versus indirect dominated activation.

2. Methods

2.1. Retinal model, preparation and handling

We used embryonic chick retinas as a model system. The cell composition of the chick retina[13], as well as fibre diameter and conduction velocity (Rager, 1976) during different developmental stages, are well known. We chose to use retinas from embryonic day 14, when most of the BPC and RGC are developed, but the photoreceptors are not fully developed. Indeed, in this developmental stage, the retina does not show response to optical stimulation, but shows intensive spontaneous neural activity (Wong et al., 1998), as do degenerated retinas (Menzler and Zeck, 2011). Moreover, recordings from RGC axons are easily achieved with a conventional MEA setup. All animal procedures were conducted under the institutional animal care standards. After chick sacrifice and eye enucleation, retinas were dissected under a binocular microscope under physiological conditions. Isolated retinas were transferred using a physiological solution-filled pipette to the experimental chamber. The physiological medium used contains 124 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 1.2 mM HEPES and 10 mM glucose in deionized water (DW). Tension-relieving incisions were made in the retina to allow its flattening, and the retina was placed, nerve fibres layer (NFL) facing down (as in epi-retinal implant), on the MEA. Excess physiological solution between the retina and the electrodes was carefully sucked out using a fine pipette. Better coupling between the tissue and the electrodes was achieved by placing a small piece of polyester membrane filter (5 μm pores, PET5013100, Sterlitech, Kent, WA, USA) on the retina, followed by a slice anchor holder. The time between chick sacrifice and retina positioning was less than 5 min. Next, the experimental chamber was filled with physiological medium, and during the whole experiment the medium was continually perfused (1–5 ml/minute), oxygenated by 95% O_2 and 5% CO_2, and kept at 34 °C, using a heated cannula (PH01, MultiChannel Systems,