Vision Research 137 (2017) 40–49

Contents lists available at ScienceDirect

Vision Research

journal homepage: www.elsevier.com/locate/visres

Cellular origin of intrinsic optical signals in the rabbit retina

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ARTICLE INFO

Article history:
Received 2 September 2016
Received in revised form 26 April 2017
Accepted 30 April 2017

Number of reviewers = 2

Keywords:
Imaging
Bipolar cells
Photoreceptors
Ganglion cells
Electroretinogram

ABSTRACT

Optical imaging of retinal intrinsic signals is a relatively new method that provides spatiotemporal patterns of retinal activity through activity-dependent changes in light reflectance of the retina. The exact physiological mechanisms at the origin of retinal intrinsic signals are poorly understood and there are significant inter-species differences in their characteristics and cellular origins. In this study, we re-examined this issue through pharmacological dissection of retinal intrinsic signals in the rabbit with simultaneous ERG recordings. Retinal intrinsic signals faithfully reflected retinal activity as their amplitude was strongly associated with stimulation intensity ($r^2 = 0.85$). Further, a strong linear relation was found using linear regression ($r^2 = 0.98$) between retinal intrinsic signal amplitude and the ERG b wave, which suggests common cellular origins. Intravitreal injections of pharmacological agents were performed to isolate the activity of the retina's major cell types. Retinal intrinsic signals were abolished when the photoreceptors' activity was isolated with aspartate, indicative that they are not at the origin of this signal. A small but significant decrease in intrinsic response (20%) was observed when ganglion and amacrine cells' activity was inhibited by TTX injections. The remaining intrinsic responses were abolished in a dose-dependent manner through the inhibition of ON-bipolar cells by APB. Our results indicate that, in rabbits, retinal intrinsic signals reflect stimulation intensity and originate from the inner retina with a major contribution of bipolar cells and a minor one from ganglion or amacrine cells.

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1. Introduction

In recent years, the development of imaging techniques (Chen et al., 2005; Costa et al., 2006; Kiernan, Mieler, & Hariprasad, 2010; Sharp & Manivannan, 1997; Yannuzzi et al., 2004) has provided clinicians and researchers the means to reveal the structure of the retina at a very high spatial resolution. While these techniques have the potential to detect anatomical changes associated with retinal diseases, they do not provide functional assessments of retinal activity. Therefore, medical investigation of retinal integrity often requires the use of complementary functional techniques. As such, the electroretinogram (ERG) is the most frequently employed method to study retinal function, both in clinical settings and research laboratories (Heckenlively John. R AGB, 2006). However, ERG recordings typically reflect the activity of relatively large volumes of retinal cells and are thus characterized by a coarse spatial resolution. Hence, both the clinical and research communities could potentially benefit from the development of higher resolution retinal functional assessment techniques.

Optical imaging of intrinsic signals is a technique that measures activity-dependent changes in intrinsic optical properties of a tissue (Grinvald, Lieke, Frostig, Gilbert, & Wiesel, 1986; Grinvald et al., 2004) and it has been successfully used to reveal anatomo-functional maps of the cortex (Grinvald et al., 1986; Zepeda, Arias, & Sengpiel, 2004). Pioneering experiments have demonstrated that intrinsic signals can also be captured in the retina and generated hope that this non-invasive technique may be used as an anatomo-functional diagnosis tool in the near future (Hanazono, Tsunoda, Kazato, Tsubota, & Tanifuji, 2008; Hanazono et al., 2007; Inomata et al., 2008; Schallek, McLellan, Viswanathan, & Ts'o, 2012; Schallek & Ts'o, 2011; Schallek et al., 2009; Ts'o et al., 2009; Tsunoda, Oguchi, Hanazono, & Tanifuji, 2004). Indeed, optical imaging of retinal intrinsic signals (RIS) may enable the detection of functional disorders before the emergence of symptomatic or anatomical changes in the retina.

Several groups have studied the origins and characteristics of intrinsic signals in the retina in both *in vivo* (Hanazono et al., 2007, 2008; Inomata et al., 2008; Mihashi et al., 2011; Schallek, McLellan, Viswanathan, & Ts'o, 2012; Schallek & Ts'o, 2011;

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http://dx.doi.org/10.1016/j.visres.2017.04.015
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Schallek et al., 2009; Ts'o et al., 2009; Tsunoda et al., 2004) and *in vitro* experiments (Li et al., 2010). Two collective conclusions can be drawn from these studies. First, there is high spatial correspondence between the localization of intrinsic signals and stimulation loci, suggesting that they reflect the activity of retinal neurons. Second, RIS are constituted of distinct temporal components that are cell-type and topographically specific. For example, in non-human primates (Ivo Vanzetta & Grinvald, 2014; Tsunoda et al., 2009), RIS result from the combination of both fast and slow kinetics components. Further, the RIS time course, shape and origin vary according to the portion of the retina that is stimulated. Studies have suggested that the photoreceptors are responsible for the fast component and that the slow component arises from the inner retina, mostly from ganglion cells (Li et al., 2010; Tsunoda et al., 2009).

In the cat, by examining RIS in response to stimuli of different spatial frequencies, it was demonstrated that RIS reflect not only the activity of the photoreceptors but also the activity of the inner part of the retina (Hirohara et al., 2013). However, in the same animal species, Schallek et al. (2009) investigated the cellular origin of RIS through the use of pharmacological agents and, contrary to what was reported in other species (Hanazono et al., 2007; Tsunoda et al., 2009; Zhang et al., 2012), concluded that photoreceptors were the sole cells responsible for RIS.

Notwithstanding the numerous studies on RIS, there is yet no consensus regarding the anatomical origins of these signals. In this study, we re-examined this issue through the pharmacological dissection of RIS and simultaneous ERG recordings in the rabbit. This animal model was chosen for both phylogeny and practical reasons. Practically, the size of the rabbit eye is comparable to the human eye, thus facilitating experimental manipulations and enabling the use of equipment calibrated for human eye imaging. In addition, data on RIS from the lagomorph order would supplement the available data from primates and carnivores and further highlight the differences and similitudes in RIS among mammals.

Our results indicate that retinal intrinsic signals are activity-dependent signals. Concomitant ERG recordings showed that RIS amplitude tightly co-varied with the b-wave amplitude. Through sequential intravitreal injections of cell-type specific inhibitors, we observed a dominant impact of the activity of bipolar cells and a minimal influence of ganglion cells and/ or amacrine cells on the generation of RIS in the rabbit.

2. Material and methods

Experiments were performed on Dutch Belt (n = 34, 1–2.5 kg, Covance, NJ, United States) and Polish Dwarf hutch rabbits (n = 7, 1–2.5 kg, Aubin, QC, Canada). Data from both breeds were pooled together since the two samples were not statistically different. Seventeen animals were used to study the effects of stimulus intensity and 22 animals were used for pharmacological experiments (see below). The animals were treated according to the guidelines of the Canadian Council on Animal Care and in accordance with the Code of Ethics of the World Medical Association. The experimental protocol was accepted by the Animal Ethics Committee of the Université de Montréal. All efforts were made to minimize any discomfort of the animals.

Animals were pre-medicated by the subcutaneous injection of glycopyrolate (0.1 mg/kg) to reduce tracheal secretions, and Atravet (0.5 mg/kg), a muscle relaxant. Thirty minutes later, animals were anesthetized by an intramuscular injection of a mix of ketamine (30 mg/kg) and xylazine (5 mg/kg). After tracheotomy, animals were transferred into a stereotaxic apparatus, immobilized by a gallamine perfusion (10 mg/kg/h for a solution of 2%) and artificially ventilated by a respiratory pump. During the experiments, anesthesia was maintained under isoflurane (1%) in a mixture of O_2 (30%) and N_2O (70%). Pupils were dilated with eye drops of 1% tropicamide and 2.5% phenylephrine hydrochloride. Corneas were protected against dryness by contact lenses and periodic

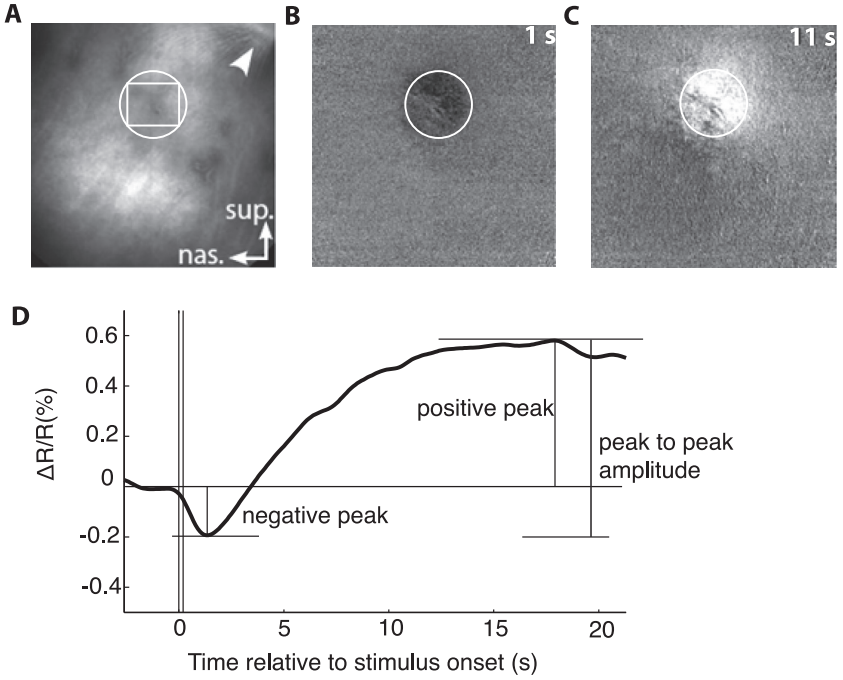

Fig. 1. Retinal intrinsic responses in rabbits. (A) Image of the fundus, under near infrared illumination. The arrowhead points to the retinal blind spot. The circle represents the retinal surface stimulated by a 200 ms flash of green light at an intensity of 16 cd.m^{-2} and the square indicates the region of interest for quantification. In this control experiment, the area stimulated covered 14 degrees. (B)-(C) Differential images obtained at different times following stimulation. (D) Retinal intrinsic responses are biphasic, with an initial drop in light reflectance (negative peak, see B), followed by an increase in light reflectance (positive peak, see C). The two vertical lines indicate the time and duration of stimulation.

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