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Rodent electroretinography: Methods for extraction and interpretation of rod and cone responses

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Abstract

The flash electroretinogram (ERG) represents a serial ensemble of neural responses that can be used to objectively evaluate retinal function on a layer-by-layer basis. In this review, the seminal concepts of Granit are developed within the modern context to demonstrate how the ERG waveform can be decomposed to isolate the activity of individual neural populations and their circuitry. The contribution of rods and cones to the ERG waveform can be precisely defined with simple methods that yield the veridical cone response, which allows identification of rod-isolated components. This knowledge will afford an enhanced capacity to understand retinal development and ageing as well as to interpret the effects of insult, genetic manipulation and disease processes on photoreceptor and neuron-specific components.

This review integrates conclusions drawn from a large body of past work and presents new data that enables the provision of detailed methodology for ERG assessment in rodents. Emphasis is placed on protocols that allow efficient acquisition of useful information for the major ERG components with minimal complexity. In particular, specific guidelines for the isolation of rod and cone contributions from the full-field ERG in rodents are provided. This is complemented with detailed and novel methodology for determining parameters that describe individual neuronal generators of rod and cone responses. The effect of stimulus energy on the kinetics of ERG response recovery and photopigment bleaching and regeneration are also discussed. The guidelines presented here are applicable to a wide range of investigations of retinal disease in rodent models

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1. Introduction

Electroretinography (ERG) provides a non-invasive measure of retinal function following light stimulation. However, the ERG waveform reflects an ensemble response from many neuronal and glial populations as a consequence of the serial processing within the retina. This review details how signal collection protocols can be tailored to identify the specific physiological process under study e.g. the photoreceptors. This is a bit like wishing to hear only the violins of a symphony orchestra, obviously a difficult task in some compositions, but not impossible if the piece is well conducted to emphasise the violin section. So it is with the ERG waveform where the nature of the neuronal input differs depending on how the stimulus is presented. Given the frequent selection of rodents as models of human disease, it is imperative that the ERG of these species is well understood. This article reviews approaches for collection of rat electroretinograms and discusses how methods can be applied to identify specific retinal cell populations for targeted investigation and assessment of disease processes. It complements and extends a similar review on mouse electrophysiology (Peachey and Ball, 2003) by incorporating new work that defines particular attributes of the rat response to enable appropriate signal collection and component analysis.

At the outset it must be recognised that the International Society for Clinical Electrophysiology of Vision (ISCEV) provides recommendations for retinal evaluation using the flash ERG (Marmor et al., 2004). The ISCEV recommendation incorporates a protocol that includes extraction of the rod response to a weak flash, presumably to define the maximal rod-driven waveform, the mixed (rod–cone) response to a strong flash in the dark-adapted eye, the definition of oscillatory potentials under light or dark adaptation, a response to a strong flash in the light-adapted eye representing a cone signal, and the response to 30 Hz flicker stimulation to identify a cone-driven post-receptoral standing potential. Although these protocols have been well designed to identify human disease and disorders of the ERG their direct application to rodents and other species is limited. Moreover, they provide little scope for the detailed understanding of retinal physiology, which is often the desired application of such methods. In particular, the ISCEV logic fails to define the underlying processes mediating the waveform, as needed to formally test scientific hypotheses. This review therefore extends the ISCEV guidelines to demonstrate how specific retinal elements contribute to the ERG waveform and details how these can be identified.

Contemporary understanding of retinal neural circuitry allows procedures that identify rod pathway contributions to the ERG to be devised. Detailed methods are given for the application of single flash ERG recordings made at relatively high energies that expose rod-specific photoreceptoral activation and the deactivation of photoproducts. Photoreceptor activation is quantified by considering the *a*-wave in terms of the gain and sensitivity characteristics of the phototransduction cascade and these analyses are scrutinised and extended to the rat. The advantages and limitations of using a twin-flash protocol for defining rod and cone contributions are discussed and methods for optimising this technique are provided. Component extraction is described to identify rod-specific post-receptoral responses at brighter light levels and mathematical descriptors of second-order neuron light- and time-dependent responses are given. This review also considers the