Vision Research 50 (2010) 2176–2187

Contents lists available at ScienceDirect

Vision Research

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

ERG changes in albino and pigmented mice after optic nerve transection

Luis Alarcón-Martínez [a,1], Marcelino Avilés-Trigueros [a,1], Caridad Galindo-Romero [a], Javier Valiente-Soriano [a], Marta Agudo-Barriuso [c], Pedro de la Villa [b], Maria P. Villegas-Pérez [a], Manuel Vidal-Sanz [a,*]

[a] Departamento de Oftalmología, Facultad de Medicina, Universidad de Murcia, 30100 Murcia, Spain
[b] Departamento de Fisiologia, Facultad de Medicina, Universidad de Alcalá, 28871 Alcalá de Henares, Spain
[c] Unidad de Investigación, Hospital Universitario Virgen de la Arrixaca, Servicio Murciano de Salud, Fundación para la Formación e Investigación Sanitarias de la Región de Murcia, 30120 Murcia, Spain

ARTICLE INFO

Article history:
Received 2 June 2010
Received in revised form 12 August 2010

Keywords:
Electroretinogram (ERG)
Scotopic threshold response (STR)
Optic nerve transection (ONT)
Axotomy
Retinal ganglion cells (RGCs)
Adult albino and pigmented mice
Neuronal degeneration
Retina

ABSTRACT

Optic nerve transection (ONT) triggers retinal ganglion cell (RGC) death. By using this paradigm, we have analyzed for the first time in adult albino and pigmented mice, the effects of ONT in the scotopic threshold response (STR) components (negative and positive) of the full-field electroretinogram. Two weeks after ONT, when in pigmented mice approximately 18% of the RGC population survive, the STR-implicit time decreased and the p and nSTR waves diminished approximately to 40% or 55%, in albino or pigmented, respectively, with respect to the values recorded from the non-operated contralateral eyes. These changes were maintained up to 12 weeks post-ONT, demonstrating that the ERG–STR is a useful parameter to monitor RGC functionality in adult mice.

1. Introduction

In response to a flash of light the electrical response than can be recorded at the cornea is known as the electroretinogram (ERG) (Dowling, 1987; Frishman, 2006). The ERG is formed by different individual components that can be associated to their cells of origin. The initial negative wave recorded after a bright full-field stimulus (the so named *a*-wave) is accepted to be generated by photoreceptor phototransduction, while the prominent positive wave that follows the *a*-wave (so named *b*-wave) is mainly generated by depolarization of ON-bipolar cells and Müller cells (Dowling, 1987; Frishman, 2006). In addition to the *a*- and *b*-waves, other main components of the ERG that appear superimposed on the *b*-wave are the oscillatory potentials which are thought to arise from feedback circuitries as well as from amacrine cells (Wachtmeister, 1998).

When very dim light stimuli are presented in scotopic conditions a small cornea-negative potential, called negative scotopic threshold response (nSTR) (Sieving, Frishman, & Steinberg, 1986), dominates the ERG. The nSTR has been recorded in humans (Frishman, Reddy, & Robson, 1996; Korth, Nguyen, Horn, & Martus, 1994; Sieving & Nino, 1988), cats (Sieving et al., 1986), monkeys (Frishman, Shen, et al., 1996), rats (Alarcón-Martínez et al., 2009; Bui & Fortune, 2004) and mice (Saszik, Frishman, & Robson, 2002). Intraretinal recordings (Frishman & Steinberg, 1989a, 1989b; Sieving et al., 1986) and studies with pharmacological substances (Naarendorp & Sieving, 1991; Robson & Frishman, 1995; Saszik et al., 2002) have indicated that this potential corresponds to inner retina neurons; amacrine and/or ganglion cells. Moreover, there is a cornea-positive component forming the STR (pSTR), and this is abolished with pharmacological agents inhibiting the response of the inner retina (Naarendorp & Sieving, 1991; Saszik et al., 2002).

Intraorbital optic nerve transection (ONT) is a classic model to study injury-induced RGC regenerative and degenerative responses (Aguayo, Vidal-Sanz, Villegas-Pérez, & Bray, 1987; Avilés-Trigueros, Sauvé, Lund, & Vidal-Sanz, 2000; Vidal-Sanz, Bray, & Aguayo, 1991; Vidal-Sanz, Bray, Villegas-Perez, Thanos, & Aguayo, 1987; Vidal-Sanz, Villegas-Perez, Bray, & Aguayo, 1993; Vidal-Sanz et al., 2000; Whiteley, Sauvé, Avilés-Trigueros, Vidal-Sanz, & Lund, 1998), including cell loss (Nadal-Nicolás et al., 2009; Parrilla-Reverter, Agudo, Sobrado-Calvo, et al., 2009; Peinado-Ramón, Salvador, Villegas-Pérez, & Vidal-Sanz, 1996; Villegas-Pérez, Vidal-Sanz, Bray, & Aguayo, 1988; Villegas-Pérez, Vidal-Sanz, Rasminsky, Bray, &

* Corresponding author. Address: Departamento de Oftalmología, Facultad de Medicina, Universidad de Murcia, Campus de Espinardo, E-30100 Espinardo, Murcia, Spain. Fax: +34 968 363962.
E-mail address: ofmmv01@um.es (M. Vidal-Sanz).
[1] These authors contributed equally to this work.

0042-6989/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.visres.2010.08.014

Aguayo, 1993). In adult rats, ONT induces within 2 weeks the loss of approximately 80% of the RGC population, while the remaining RGCs undergo a slower degeneration rate (Villegas-Pérez et al., 1993). ONT also results in a number of alterations in their functional (Casson, Chidlow, Wood, Vidal-Sanz, & Osborne, 2004; McKerracher, Vidal-Sanz, & Aguayo, 1990; McKerracher, Vidal-Sanz, Essagian, & Aguayo, 1990; Parrilla-Reverter, Agudo, Nadal-Nicolás, et al., 2009; Schlamp, Johnson, Li, Morrison, & Nickells, 2001) and metabolic (Chidlow, Casson, Sobrado-Calvo, Vidal-Sanz, & Osborne, 2005; Lindqvist, Peinado-Ramónn, Vidal-Sanz, & Hallböök, 2004; Lindqvist, Vidal-Sanz, & Hallböök, 2002; Lindqvist et al., 2010) properties, as well as in the regulation of a substantial number of genes (Agudo et al., 2008, 2009). This experimental model appears suitable to study the ERG components generated by the RGC population in adult mammals because, it is widely accepted that ONT results in selective loss of RGCs but not of other non-RGC retinal neurons (Carter, Vidal-Sanz, & Aguayo, 1987; Villegas-Pérez et al., 1993) or in the impairment of the outer retinal neurons functionality (Alarcón-Martínez et al., 2009; Bui & Fortune, 2004).

There is a number of studies in rats analyzing the electroretinographic responses after ONT (Alarcón-Martínez et al., 2009; Bui & Fortune, 2004; Mojumder, Sherry, & Frishman, 2008), however such information is lacking for mice. Transgenic and knock-out mice have become an important tool to study a number of relevant questions in the adult mammalian visual system. Moreover, albino or pigmented mice are usually the animal of choice for many experimental models involving RGC injury, including ocular hypertension induced by laser photocoagulation of the limbar tissues (Cuenca et al., 2010; Fu & Sretavan, 2010; Grozdanic et al., 2003; Holcombe, Lengefeld, Gole, & Barnett, 2008; Morrison, Johnson, & Cepurna, 2008; Salinas-Navarrov, Alarcón-Martínez, et al., 2009; Salinas-Navarro et al., 2010), thus it is important to ascertain the origin of the STR waves in this species. In the present work we have studied the ERG response after ONT at different time intervals for albino and pigmented mice to provide further evidence for the relationship between the generation of the STR components of the full-field electroretinogram and the anatomical integrity of the RGC population. Using a classic model of axotomy-induced RGC injury, we have investigated in adult albino and pigmented mice the different components of the full-field flash ERG at various time intervals after ONT as well as RGC survival. Retrogradely transported neuronal tracers were applied to both superior colliculi (SCi) to identify RGCs, and we report that optic nerve (ON) injury results by 2 weeks in the loss of approximately 80% of the RGC population. Moreover, ONT resulted in major permanent reductions of the early components of the ERG, mainly the positive scotopic threshold response, and alterations of the STR-implicit time. Overall, our data provide new and original information for the electrophysiology of the adult albino and pigmented mice after ONT and document that ERG STR could be a useful parameter to monitor RGC functionality in adult mice (short accounts of this work have been published in abstract format; Alarcón-Martínez et al., 2010; Galindo-Romero et al., 2010).

2. Materials and methods

2.1. Animals

Female adult albino (BALB/c; 30–35 g) and pigmented (C57BL/6; 30–35 g) mice of the same age were treated according to institutional guidelines, European Union regulations for the use of animals in research, the ARVO statement for the use of animals in ophthalmic and vision research, and were comparable to those published by the Institute for Laboratory Animal Research (Guide for the Care and Use of Laboratory Animals). Mice were anaesthetized with an intraperitoneal (i.p.) injection of a mixture of ketamine (70 mg/kg Ketolar®, Pfizer, Alcobendas, Madrid, Spain) and xylazine (10 mg/kg Rompur®, Bayer, Kiel, Germany) in 0.1 ml saline. Mice were kept in a 12-h light/dark cycle. For electrophysiological studies, three groups of albino mice; group I ($n = 9$), II ($n = 6$) or III ($n = 5$), were processed at 2, 4 or 12 weeks after ONT, respectively, and three groups of pigmented mice; group IV ($n = 7$), V ($n = 6$) or VI ($n = 9$) were processed at 2, 4 or 12 weeks

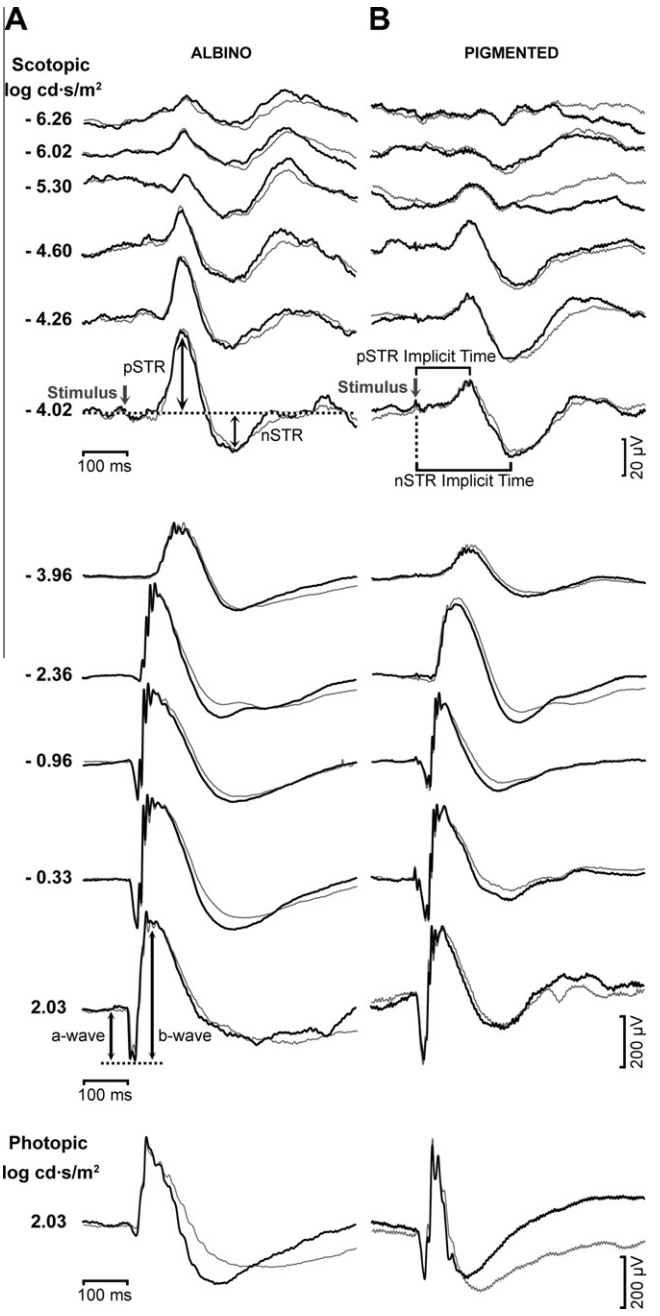

Fig. 1. Scotopic and photopic electroretinographic recordings in albino and pigmented mice. Examples of the ERG traces recorded in an albino (A) or pigmented (B) control mouse in response to flash stimuli of increasing intensity (indicated in log cd s/m^2 units at the left of the recording traces) for the right eye (thin trace) and for the left eye (bold trace). The scotopic threshold responses (STR) were elicited by weak light stimuli (−6.26 to −4.02 log cd s/m^2). Responses mediated by rods and by rods and cones (mixed response) were elicited by light intensities from −3.96 to 2.03 log cd s/m^2. Photopic response was recorded after a light adaptation of 5 min and it was elicited by the highest light stimulus (2.03 log cd s/m^2). No significant differences in the ERG amplitudes between left and right eyes were observed. Examples for the measurement of wave amplitudes and implicit times are shown.