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Light-induced retinal degeneration causes a transient downregulation of melanopsin in the rat retina

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ABSTRACT

In this work we study the effects of an acute light-induced retinal degeneration on the population of melanopsin positive retinal ganglion cells (m^+RGCs) and the expression of the melanopsin protein in the retina. The m^+RGCs may be more resistant than other RGCs to lesion, but the effects of an acute light exposure in this population are unknown. Albino rats were exposed to white light (3000 lux) continuously for 48 h and processed 0, 3, 7 or 30 days after light exposure (ALE). Whole-mounted retinas were immunodetected with antibodies against melanopsin, Brn3a, and rhodopsin to study the populations of m^+RGC, Brn3a$^+$RGC and rods (which are the most abundant photoreceptors in the rat retina). Three days ALE there was substantial rod loss in an arciform area of the superior retina and with time this loss expanded in the form of rings all throughout the retina. Light exposure did not affect the number of Brn3a$^+$RGCs but diminished the numbers of m^+RGCs. Immediately ALE there was a significant decrease in the mean number of immunodetected m^+RGCs that was more marked in the superior retina. Later, the number of m^+RGCs increased progressively and reached normal values one month ALE. Western blot analysis showed that melanopsin expression down-regulates shortly ALE and recovers thereafter, in accordance with the anatomical data. This study demonstrates that there is a transient downregulation of melanopsin expression in the RGCs during the first month ALE. Further studies would be needed to clarify the long-term effect of light exposure on the m^+RGC population.

Intrinsically photosensitive retinal ganglion cells (ipRGCs) represent a special subtype of RGC that expresses the photopigment melanopsin and thus responds directly to light (Pickard and Sollars, 2012; Provencio et al., 2000). These neurons have changed the traditional conception that classical photoreceptors (cones and rods) were the only cells able to sense light in the retina. We will refer to the ipRGCs as melanopsin positive RGCs (m^+RGCs), as most works on these cells rely on melanopsin immunodetection.

m^+RGCs have important roles related to non–image forming visual functions (Berson, 2003; Chen et al., 2011; Hattar et al., 2003; LeGates et al., 2014; Sand et al., 2012; Panda et al., 2003; Schmidt et al., 2011; Semo et al., 2014; Vugler et al., 2015) such as the regulation of circadian rhythms, part of the pupillary light reflex, melatonin secretion, regulation of sleep, or masking behaviour. Moreover, recent studies have suggested that these neurons could also have a role in image-forming visual function (Estevez et al., 2012; Schmidt et al., 2011) and that they may also influence vision through the transmission of luminance signals to the outer retina (Prigge et al., 2016).

Our group has demonstrated that the transcription factor Brn3a is expressed by most RGCs (~96%) except those that express melanopsin (~2.6% of the total RGC population; Galindo-Romero et al., 2013; García-Ayuso et al., 2015) and half of their ipsilateral projection (~1.3% of the total RGC population; Nadal-Nicolas et al., 2012; 2014). While melanopsin is expressed by photosensitive RGCs that mainly carry non-image forming visual information, Brn3a is expressed by the population of RGCs that convey image-forming visual information but do not respond to light

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(henceforward the general RGC population or Brn3a$^+$RGCs). Thus, double immunodetection of melanopsin and Brn3a is an effective tool to study these two functionally different types of RGCs in parallel but independently (Agudo-Barriuso et al., 2016; Vidal-Sanz et al., 2015a). This approach has allowed us to document the total number and spatial distribution of m$^+$RGCs in the rat retina in relation to the general RGC population (Galindo-Romero et al., 2013; Nadal-Nicolas et al., 2012; 2014, 2015a,b), their co-expression of Brn3a (Galindo-Romero et al., 2013; Nadal-Nicolas et al., 2012) and that this co-expression changes as photoreceptors die in a model of retinal dystrophy (García-Ayuso et al., 2015).

m$^+$RGCs may be more resistant to different injuries than the general RGC population (Cui et al., 2015; DeParis et al., 2012; Moura et al., 2013; Nadal-Nicolás et al., 2015b; Pérez de Sevilla Müller et al., 2014). However, the higher resilience of m$^+$RGCs to some degenerations is not clear and they may die at the same rates as the rest of the RGCs in some diseases such as experimental glaucoma (Rovere et al., 2016; Valiente-Soriano et al., 2015a,b) or retinitis pigmentosa (Esquiva et al., 2013; García-Ayuso et al., 2015).

Exposure to light is often used to induce retinal degeneration (Noell et al., 1966). Our method of phototoxicity has been well characterized in rats (García-Ayuso et al., 2011; Marco-Gomariz et al., 2006) and mice (Montalbán-Soler et al., 2012). We have described in detail how phototoxicity affects first photoreceptors

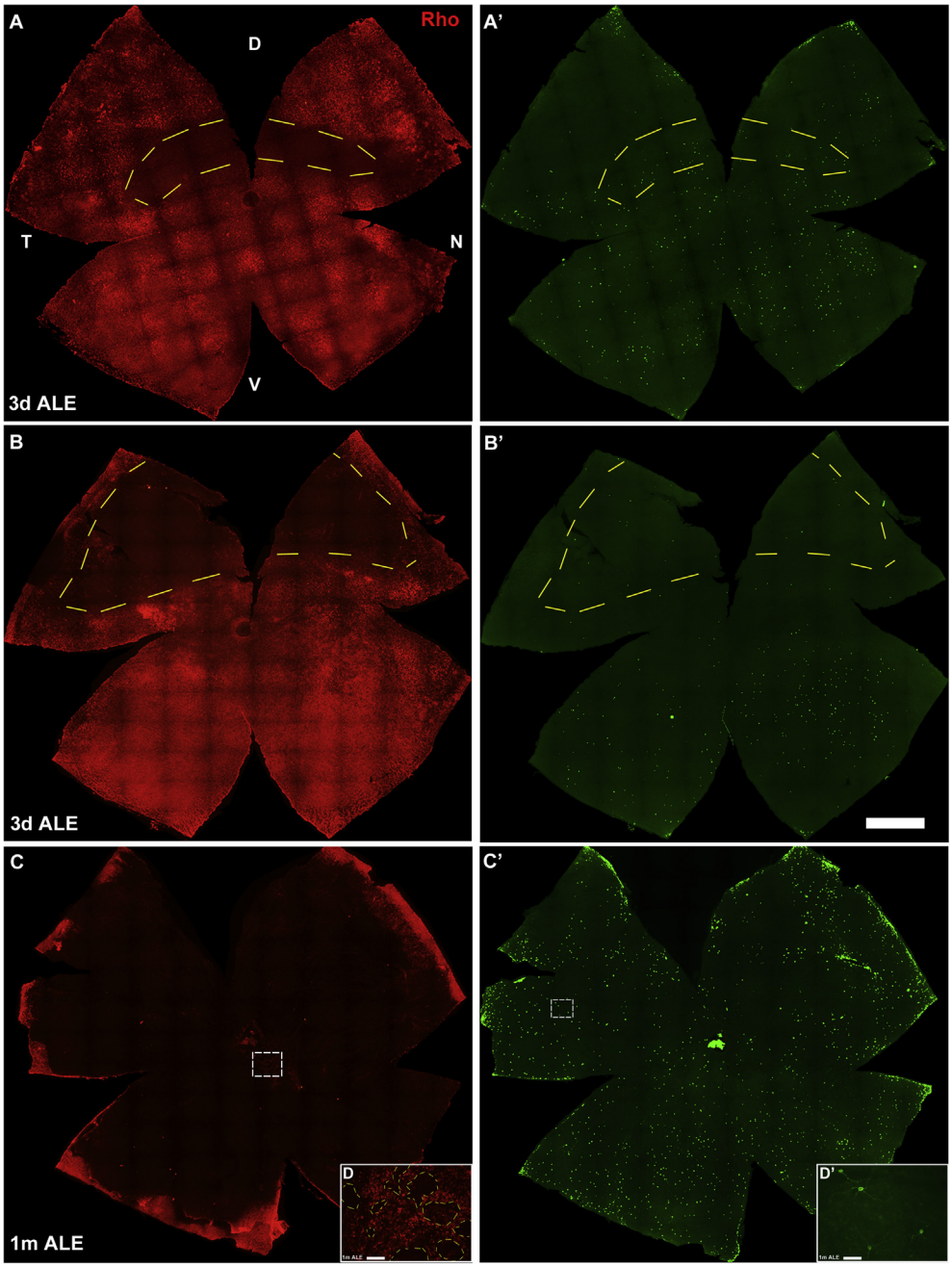

Fig. 1. Light exposure causes a progressive rod loss and a transitory melanopsin downregulation. Retinal whole-mounts showing rhodopsin (A–C) and melanopsin (A′-C′) immunodetection in three representative retinas from animals processed 3 days (A, A′, B, B′), and 1 month (C, C′) ALE. The arciform area of rod degeneration is clearly observed at 3 days ALE. D and D′, magnifications showing the areas highlighted in C and C′, respectively. In D, rings delineated by rods are observed at 1 month ALE (C). In D′, specific melanopsin immunostaining. Scale bars: 1 mm in (A-C, A′-C′) and 100 μm in (D, D′).