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Research Report

# Perinatal development of melanopsin expression in the mouse retina

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ABSTRACT

The melanopsin-expressing retinal ganglion cells are specialized in measuring irradiance for several functions, including daily photoentrainment and regulation of pupil size. In the present study, these cells were analyzed in mice during their perinatal period, from embryonic day (E) 15 to postnatal day (P) 1. Melanopsin expression was detected at E15 in cells that did not co-express the transcription factor Brn3a. Under light/dark (LD) cycles, the number of melanopsin-expressing cells did not change between E16 and E19, while a very significant increase was observed during the short interval around birth, between E19 (the day before birth) and P0 (the day of birth). As these samples were collected after lights on, to determine whether such increase in melanopsin expression was driven by light, we also analyzed samples collected 0–4 hours after birth (during the night period), which revealed that the cell number increase was already present and, therefore, was not induced by the early post-birth light exposure. To clarify the role of ambient light conditions during this period, P1 retinas from pups under constant light or darkness conditions were also analyzed and compared to those of mice under LD cycles. No variation in the number of immunostained cells was detected among the groups studied, indicating that ambient conditions did not provoke the increase in melanopsin expression detected. Rather, it might have been induced by either a maternal or a developmental signal and is likely related to the first connections between the retina and the suprachiasmatic nucleus reported by other authors.



## 1. Introduction

Photoreception in mammals extends beyond the classical retinal photoreceptors (rods and cones) to include a small subpopulation of intrinsically photosensitive retinal ganglion cells (ipRGCs) (Hattar et al., 2002; Peirson and Foster, 2006). These ipRGCs express the photopigment melanopsin (Provencio et al., 2002), and are specialized in measuring

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*Abbreviations:* DD, constant darkness; E, embryonic day; ipRGCs, intrinsically photosensitive retinal ganglion cells; LD, light/dark; LL, constant light; P, postnatal day; RGCs, retinal ganglion cells; RHT, retinohypothalamic tract; SCN, suprachiasmatic nucleus; ZT, zeitgeber time

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ambient levels of light (irradiance) for a wide variety of functions, including synchronization of circadian clocks to light–dark cycles, regulation of pupil size, sleep propensity and pineal melatonin production (Nayak et al., 2007). The ipRGCs have also proved to be directly involved in important aspects of image-forming vision. In fact, Brown et al. (2010) showed that melanopsin-based photoreception constitutes a significant source of sensory input to the thalamo-cortical visual system, providing irradiance information, even in the absence of rods and cones. More recently, Brown et al. (2011) have demonstrated that both cone and melanopsin photoreceptors drive contrast and irradiance responses in the mouse hypothalamus.

Melanopsin immunoreactivity has been shown at postnatal day (P) 1 (Fahrenkrug et al., 2004; Sekaran et al., 2005). The melanopsin-expressing cells are responsive to light stimulation since P0 (Sekaran et al., 2005; Tu et al., 2005) and, depending on the intensity of the stimulus, light was able to induce expression of the immediate early gene *c-fos* at P0–P1 in the suprachiasmatic nucleus (SCN) (Lupi et al., 2006) and the retina (Matejů et al., 2010). Moreover, it has been demonstrated that the retina maintains a rather constant number of melanopsin expressing retinal ganglion cells (RGCs) from P1 on (González-Menéndez et al., 2010a). In addition to this, a daily rhythm (González-Menéndez et al., 2009; Hannibal et al., 2007) and changes in melanopsin expression (González-Menéndez et al., 2010b; Hannibal et al., 2005) in response to both constant darkness or light were also detected in neonatal rodents, suggesting that melanopsin-expressing cells can adapt their responsiveness to the external illumination conditions by regulating their melanopsin content even in the absence of functional rod/cone photoreceptors. Taken together, all these data support the general idea that the non-image-forming system, in which melanopsin cells are mainly involved, is functional at the very early postnatal stage. McNeill et al. (2011), nevertheless, reported that some ipRGCs follow a delayed time-course development and extends beyond that of other RGCs.

Melanopsin transcripts could be detected in the mouse retina as early as embryonic day (E) 10.5 (Tarttelin et al., 2003) and the SCN begins to function as a circadian pacemaker during late fetal development (Reppert et al., 1985; Reppert et al., 1988). However, little is known to date about the embryonic development of the ipRGCs. The goal of the present study was to analyze the prenatal and perinatal periods of such melanopsin-expressing cells in mice, which will undoubtedly contribute to the understanding of their physiology, particularly at this very early stage.

## 2. Results

Melanopsin-expressing cells could be observed in mice as early as E15 in the whole retinal area (Fig. 1). During prenatal development, immunostaining was relatively weak in the somata and even lesser in the dendrites or axons, which were only occasionally observed. Although in adults melanopsin-expressing cells can be classified attending to the different location of their somata and dendritic processes, the different melanopsin-cell subpopulations could not be identified in the stages analyzed in the present study, because the sparse and weakly immunostained dendrites observed did not yet stratify in the two characteristic plexuses seen in adult retinas.

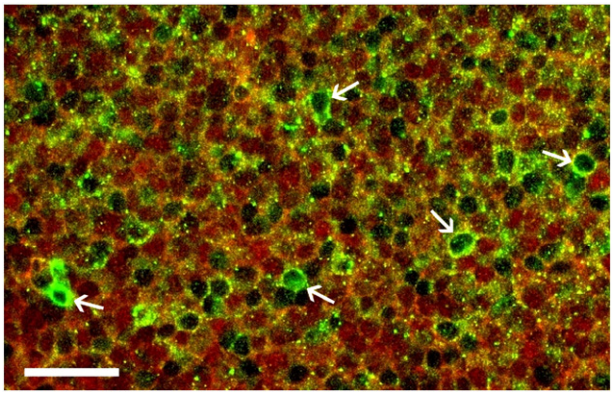

**Fig. 1 – Representative micrograph showing double immunostaining for melanopsin (green) and Brn3a (red) proteins at E15 in a flat-mounted retina. Melanopsin cell somata (some of which were pointed with arrows) are located in the ganglion cell layer, in which the lens was focused for taking the picture. Immunolabeled neurites are rather scarce. Brn3a can be observed in most nuclei; however, no colocalization of both proteins was detected in these prenatal retinas. Scale bar: 50 µm.**

Brn3a nuclear marker was detected in the nuclei of most ganglion cells of the E15 retinas. However, when double immunostaining for Brn3a and melanopsin was analyzed no co-localization of both proteins was detected at this prenatal stage (Fig. 1).

The total retinal area was estimated and a significant increase was observed between E16 and E19 (E16: 3.36±0.33 $mm^2$; E19: 5.13±0.25 $mm^2$; ANOVA test: $p<0.01$), which is consistent with an increase in size of the eye during fetal development. However, no significant increase was detected between E19 and P0 (P0: 5.38±0.58 $mm^2$). The counts in whole-mounted retinas determined that the total number of melanopsin-expressing cells did not change between E16 and E19 (ANOVA test, $p>0.05$), while a very significant increase of immunopositive cells was detected during the perinatal period (E19: 924±63 immunopositive cells/ retina; P0: 1580±51 immunopositive cells/ retina; ANOVA test, $p<0.01$; Fig. 2). Obviously, the global density of melanopsin-immunopositive cells also increased within the same period (E19: 180.68±15.52 cells/$mm^2$; P0: 294.52±8.26 cells/$mm^2$; Student's $t$ test, $p<0.01$). Moreover, the melanopsin-immunostained dendrites and axons also became more visible (Fig. 3). No significant differences were detected between P0 and P1 (ANOVA test, $p>0.05$).

To determine whether the early post birth light exposure of the LD cycle had an effect on this increase, we also analyzed "early" P0 retinas, which were collected from 0 to 4 hours after delivery. These pups were born during the dark phase of the LD cycle and their retinas were collected during the night period. Contrary to those of the P0 group, which were collected at zeitgeber time (ZT) 3, these "early" P0 retinas have not been subjected to the first light exposure of the LD