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Brain Research 1060 (2005) 40 – 46
BRAIN RESEARCH
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Research Report

Ganglion cell densities in normal and dark-reared turtle retinas

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Accepted 8 August 2005
Available online 6 October 2005

Abstract

In dark-reared, neonatal turtle retinas, ganglion cell receptive fields and dendritic trees grow faster than normal. As a result, their areas may become, on average, up to twice as large as in control retinas. This raises the question of whether the coverage factor of dark-reared ganglion cells is larger than normal. Alternatively, dark rearing may lead to smaller-than-normal cell densities by accelerating apoptosis. To test these alternatives, we investigated the effect of light deprivation on densities and soma sizes of turtle retinal ganglion cells. For this purpose, we marked these cells using retrograde labeling of fixed turtle retinas with DiI (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate). Control turtles were maintained in a regular 12-h light/dark cycle from hatching until 4 weeks of age, whereas dark-reared turtles were maintained in total darkness for the same period. Ganglion cells in the control and dark-reared retinas were found to be similar in density and soma sizes. These results show that the mean coverage factor of turtle dark-reared ganglion cells is larger than normal.

Theme: Sensory systems
Topic: Retina and photoreceptors

Keywords: Ganglion cell; Dark rearing; Retina; Turtle

1. Introduction

Visual deprivation during development dramatically alters the normal refinement of connections and function in the visual system. The alterations occur both in the retina [34,37,38] and in the rest of the brain [5,13,18,26]. In the former, visual deprivation interferes with the normal refinement of synaptic circuits that occur after exposure to light [39,37,41]. This synaptic refinement is reflected in changes of retinal ganglion cell connectivity and synaptic activity [37,38]. In addition, amplitudes of ganglion cell light responses in cat, ferret, and mouse increase after eye opening [39,37,41].

In normal turtle retinas, ganglion cell receptive field areas expand significantly as soon as the animal hatches. They stabilize to their mature size at about 4 weeks post-hatching, when the spontaneous bursting stops [34]. In contrast, in the dark-rearing animal, spontaneous bursting is stronger than normal from hatching and continues past 4 weeks for many months [24]. Moreover, receptive field areas grow faster than normal since hatching [34], becoming twice that in control animals by 4 weeks [34]. Exposure to light does not cause receptive fields to shrink after that [24]. There is a correlation between the exuberant expansion of receptive fields and that of dendritic trees in dark-reared turtle retinas [35].

The exuberant receptive field growth with dark rearing raises a question about the coverage factors of ganglion cells. Coverage factors, i.e., receptive field areas times cell densities, are normally relatively small (typically between 1 and 7) for different kinds of ganglion cells [6,7,28,43]. Coverage factor appears to be so important that, across species [28], it is invariant with development [15] and eccentricity despite wide variation of density [6,7,28,43].

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doi:10.1016/j.brainres.2005.08.007

Invariances like this and developmental studies led to the suggestion that specific competitive mechanisms control the final size of ganglion cell dendritic trees [44]. Developmental studies suggest that this competition exists and is cell-class specific [44,22]. For instance, during a critical postnatal period of mammalian development, removing patches of ganglion cells induces dendrites of neighbor neurons of the same type to grow into the vacated territory [8,17,29]. Therefore, it is widely assumed that homotypic cells interact with one another to constrain the lateral extent of their dendritic fields [1,29,31,43].

In the present study, we investigated the cell number (and soma size) of ganglion cells in control and dark-reared turtle retinas using the lipophilic tracer DiI to test whether the mean coverage factor has been affected.

2. Materials and methods

2.1. Animals

We used ten 4-week-old turtles (*Trachemys scripta elegans*) of either sex for both control (five turtles) and dark-rearing (five turtles) experiments. We obtained retinas from 4-week-old animals because a previous study showed that normally reared ganglion cells stop bursting spontaneously and their receptive field reaches adult size by that time [34]. Before hatching, eggs were incubated in a 30 °C dark oven. This oven mimicked natural conditions, in which turtles bury eggs, leaving them in darkness before hatching. The oven temperature was between the extremes in which development is reasonably satisfactory [46]. Control turtles were fed and housed under cyclic 12-h light/12-h dark conditions from hatching. In turn, we fed and housed dark-reared turtles under completely dark conditions from hatching. Trained personnel conducted all procedures of daily monitoring and routine maintenance of dark-reared turtles under infrared illumination. (For this purpose, they used a pair of infrared sensitive goggles — B.E. Meyers and Co. Inc., Redmond, WA). All procedures were in conformance with the *Guide for Care and Use of Laboratory Animals* (National Institutes of Health). The University of Southern California Institutional Animal Care and Use Committee reviewed and approved all procedures.

2.2. Tissue fixation and DiI labeling

Turtles were decapitated, pithed, and the eyes were removed. Afterwards, we fixed the eyes by immersion in 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4, for 1 week. Following fixation, we made an incision in the optic nerve stump and implanted crystals of the lipophilic tracer DiI (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate) (Molecular Probes, Eugene, OR) within the nerve cut. After 8 weeks, we removed whole retinas from the eyes and cover-slipped with 10% glycerol in 0.1 M PB. We observed retinal images with a fluorescence microscope (Leica Microsystems; Leica DM LB2, Bannockburn, IL) at an excitation wavelength of 560 to 590 nm (rhodamine filter). We then imported images into Adobe Photoshop version 7.0 (Adobe Systems, Mountain View, CA). For presentation, we carried out all manipulations equally on all images (brightness and contrast only).

2.3. Hematoxylin staining

The anterior segments of the eyeballs were removed, and the eyecups were fixed in 4% paraformaldehyde in 0.1 M

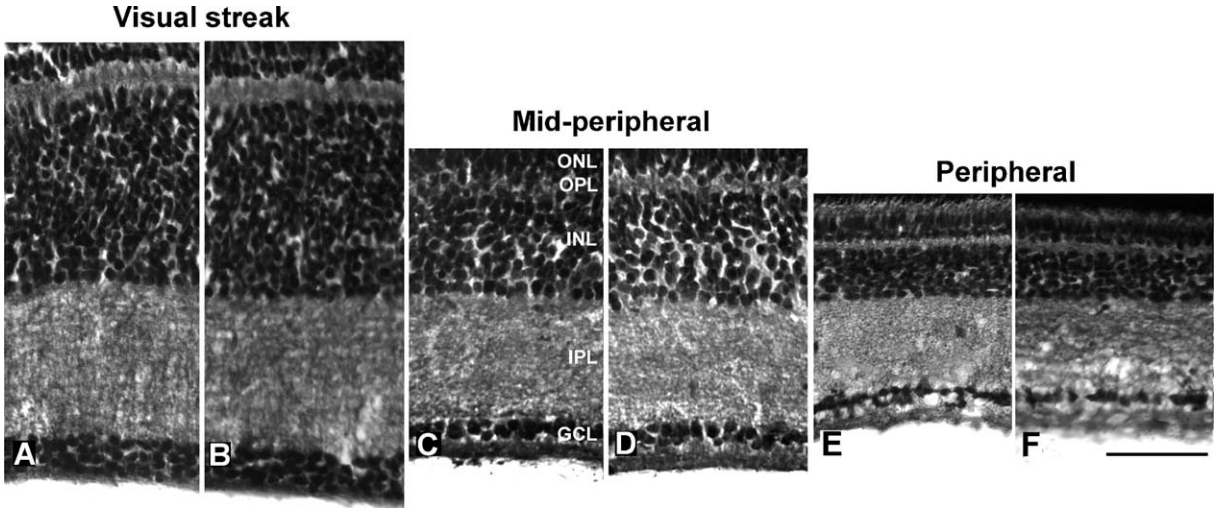

Fig. 1. Light micrographs taken from 15-μm-thick vertical cryostat sections processed by hematoxylin staining. Hematoxylin staining in visual streak (A, B), mid-periphery (C, D), and periphery (E, F) of control (A, C, E) and dark-reared retinas (B, D, F) is seen. Dark rearing induces no detectable changes in the thicknesses of retinal layers. GCL, ganglion cell layer; INL, inner nuclear layer; IPL, inner plexiform layer; ONL, outer nuclear layer; OPL, outer plexiform layer. Scale bar, 50 μm.