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Research article

The retino–retinal projection: Tracing retinal ganglion cells projecting to the contralateral retina

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HIGHLIGHTS

- A direct retino–retinal projection between the eyes via the optic chiasm, as evidenced by retino–retinal ganglion cells, persists into adulthood in rodents.
- Optic nerve stump labeling yields greater numbers of retino–retinal ganglion cells.
- Retino–retinal ganglion cells can have collateral axon projections to the brain.

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ABSTRACT

We investigated the presence of a direct retino–retinal (R–R) projection between the two eyes via the optic chiasm of retinal ganglion cells (RGCs) in adult Long-Evans rats. We also explored the presence of collateral projections originating from these cells to the brain. In the first group of animals, right optic nerves (ONs) were orbitally transected approximately 2 mm behind the globe followed by application of fluorochrome (2% Fluorogold [FG]) to the optic nerve stump to retrogradely label the R–R projection RGCs (R–RGCs) on the contralateral side. Animals were then sacrificed after 3, 5, 7, or 21 days. Contralateral retinas were fixed, whole-mounted, and imaged for R–RGCs. In a second group of animals, RGCs were retrogradely labeled with 15% rhodamine-β-isothiocynate (RITC) at the superior colliculi, where approximately 96% of rat RGCs synapse. Seven days later, the right ONs were transected and 2% FG applied to the proximal and distal ON stumps. Animals were then sacrificed after 5 days. Contralateral retinas were examined for co-labeled (RITC/FG) RGCs. Control rats underwent the same procedures excluding fluorescent tracer application. In the first group of animals, the number of R–RGCs in the contralateral eye ranged from 3 to 25 and did not depend on survival time. The second group of animals revealed evidence of co-labeled contralateral RGCs. Results suggest that a greater number of R–RGCs persist into adulthood than previously reported [M. Müller, H. Holländer, 1988]. Furthermore, the presence of co-labeled RGCs in the contralateral eye indicates that in adult rodents some R–R projections have a collateral projection to the brain, whereas previous reports had only found collateral projections in newborns.

1. Introduction

The eye transmits visual information from the retina to the brain via the optic nerve (ON). In mammals, ONs are composed of retinal ganglion cell (RGC) axons and support cells, such as glia, including astrocytes, microglia and oligodendrocytes, and connective tissue. In humans, RGC axons from each eye partially cross at the chiasm before reaching the first subcortical relay. Beyond the chiasm, the axons that form the optic tracts are channeled to relay neurons in the lateral geniculate nucleus (LGN) and midbrain before final connections are made in the cortex. The pathway, however, varies between mammalian species. For instance, in adult rats, over 96% of RGCs project to the contralateral superior colliculus (SC) the dorsal division of the LGN [1,2]. From adulthood, the number of RGCs and the topographic nature of the projection persist unchanged throughout the rat's life. The refinement of the crossed retinal

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projections to the SC is thought to involve selective elimination of RGCs with inappropriate connections [3–5].

Intriguingly, there is evidence of axons that project directly from one retina to the other via the optic chiasm in the so-called retino–retinal (R–R) projection pathway. The R–R projection has been reported in both lower and higher vertebrates, including, *anurans* [6–10], chick [11,12], quokka [13], cat [14], rabbit [15], and rat [15–17]. The majority of these studies, however, observed the projection only during pre- and postnatal stages of development.

Although there have been reports on the presence of a R–R projection persisting into adulthood in rats and rabbits, these reports are few [15]. Previous research with vitreous humor dye injection techniques in adult rats and rabbits revealed a very small number of R–RGCs [15]. This technique depends on diffusion of the dye through the vitreous to the RGC and may not be as reliable a technique for identifying R–RGCs as applying a tracer directly to the transected ON, such that the tracer is transported by the axon itself. We are not aware of any published studies employing this technique in adult rodents. Researchers have also reported evidence for an axon collateral to the SC in newborn rabbits [15] however this topic remains uninvestigated in adult vertebrates. We sought to establish further evidence for the presence of a R–R projection persisting in the adult rat visual system, and to determine whether any R–RGCs also have a collateral projection to the SC. Here we report the first observations of R–RGCs with a collateral projection in adult rodents.

2. Material and methods

Adult male Long Evans rats [weight, 225–250 g; aged 8–12 weeks, Charles River Laboratories (Saint-Constant, QC, Canada)] were housed in a 12 h light–dark cycle environment at the Carleton Animal Care Facility at Dalhousie University with food and water *ad libitum*. All protocols and procedures complied with the Association for Research in Vision and Ophthalmology Statement on the Use of Animals in Ophthalmology and Vision Research. Ethics approval was granted by the Dalhousie University Committee on Laboratory Animals.

In preliminary experiments, a time-course study of retrograde labeling of RGCs from the SC was performed with the tracer fluorochrome (2% Fluorogold [FG]; Fluorochrome Inc. Denver, CO) to establish the starting time point for R–R projection labeling. This rationale was based on the fact that the distance between the SC to RGCs (~19.9 mm) is comparable to that of the R–R projection (~19 mm) [18]. Animals underwent bilateral retrograde labeling with FG placed at the SC and sacrificed at 1, 2, 3, and 7 days post-application ($n = 5$ for each time point). Retinas were whole-mounted and RGCs quantified (see below for detailed methodology). While RGC densities differed significantly across the 4 survival times ($p < 0.01$, analysis of variance), there was no difference between the 3-day and 7-day post-transection RGC densities [mean (SD), 1996 (41) and 2096 (52) cells/mm^2, respectively; $p = 0.02$] (Tukey's post-hoc comparison). Therefore, 3 days post-labeling was chosen as the time-point to quantify R–RGCs in the contralateral retina.

A total of 33 animals were used for subsequent experiments. Twenty rats (Group 1) were used for time-course labeling of R–RGCs, 10 rats (Group 2) underwent co-labeling to identify the presence of a collateral projection to the SC, and 3 were used as negative controls (see below).

Group 1 animals were anaesthetized for surgery with a 1 ml/kg intraperitoneal injection of 55.5 mg/ml ketamine hydrochloride (Vetalar; Vetrepharm, Belleville, ON, Canada), 5.5 mg/ml xylazine (Rompun; Bayer Inc. Toronto, ON, Canada) and 1 mg/ml acepromazine (Atravet, Ayerst Veterinary Laboratories, Guelph, ON, Canada). Animals were stereotaxically secured and parasagittal incisions close to the superior orbital rim made. With a limbal suture, the right eye was pulled downward for easier visibility and access to the ON. The ON was exposed by partially resecting the lacrimal gland and spreading the superior extraocular muscles apart. Following a longitudinal incision of the meningeal sheath, the nerve was transected completely approximately 2 mm from the globe. To ensure complete labeling of RGCs, resorbable gelfoam soaked in FG was applied to the cross-sectional surface of the distal and proximal orbitally transected ON stumps. Rats were sacrificed after 3, 5, 7, and 21 days ($n = 5$, for each time-point) with a barbituate overdose of 240 mg/ml pentobarbital (Euthanyl, CDMV, Saint-Hyacinthe, QC, Canada). Control rats ($n = 3$) underwent the same procedures, excluding tracer application but with application of saline soaked gelfoam at the orbitally transected ON distal stumps to determine whether any FG positive cells were falsely detected as R–RGCs by autofluorescence alone.

Group 2 animals underwent co-labeling of RGCs to examine the presence of a collateral projection to the brain. RGCs were retrogradely labeled from the SC with 15% rhodamine-β-isothiocynate (RITC; Sigma Aldrich, Oakville, ON, Canada) following the same technique as previously detailed with FG [19]. Gelfoam soaked

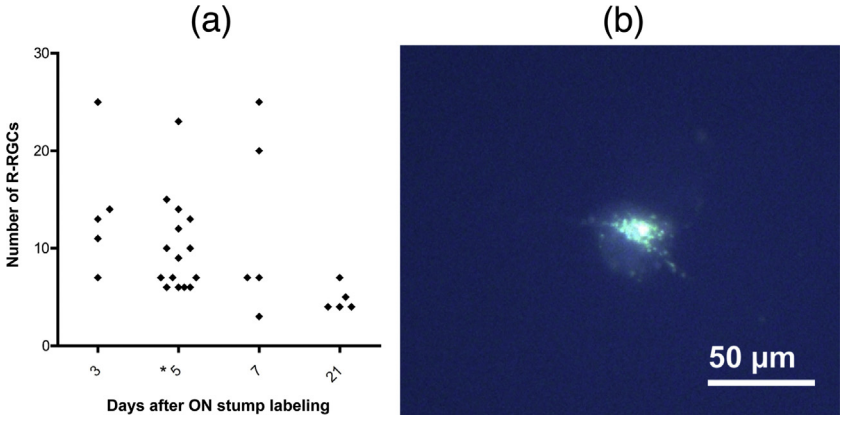

Fig. 1. Retino–retinal projecting retinal ganglion cells (R–RGCs) identified by fluorescence detection in the left retinas of Group 1 animals after fluorochrome application to the right transected optic nerve stump. (a) Quantification of R–RGCs at 3, 5, 7 and 21 days after optic nerve stump labeling ($n = 5$, for each time point). *Note, the additional 10 counts are from Group 2 double-labeling experiments. Data are jittered along the x-axis for better visibility. (b) A single R–RGC.