Differential visual system organization and susceptibility to experimental models of optic neuropathies in three commonly used mouse strains

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Abstract

Mouse disease models have proven indispensable in glaucoma research, yet the complexity of the vast number of models and mouse strains has also led to confusing findings. In this study, we evaluated baseline intraocular pressure, retinal histology, and retinofugal projections in three mouse strains commonly used in glaucoma research, *i.e.* C57Bl/6, C57Bl/6-Tyr^{c}, and CD-1 mice. We found that the mouse strains under study do not only display moderate variations in their intraocular pressure, retinal architecture, and retinal ganglion cell density, also the retinofugal projections to the dorsal lateral geniculate nucleus and the superior colliculus revealed striking differences, potentially underlying diverging optokinetic tracking responses and visual acuity. Next, we reviewed the success rate of three models of (glaucomatous) optic neuropathies (intravitreal N-methyl-D-aspartic acid injection, optic nerve crush, and laser photocoagulation-induced ocular hypertension), looking for differences in disease susceptibility between these mouse strains. Different genetic backgrounds and albinism led to differential susceptibility to experimentally induced retinal ganglion cell death among these three mouse strains. Overall, CD-1 mice appeared to have the highest sensitivity to retinal ganglion cell damage, while the C57Bl/6 background was more resistant in the three models used.

Key words

mouse, retina, visual system, glaucoma, disease models, mouse strains, genetic background

Highlights

- C57Bl/6, C57Bl/6-Tyr^{c}, and CD-1 mice were studied
- Intraocular pressure, retinal histology, and RGC density varied in these strains
- Different retinofugal projections to the dLGN and SC were observed
- Optokinetic tracking responses and visual acuity were strikingly different
- The three strains were differentially susceptible to experimental RGC death

Abbreviations

CTB	cholera toxin subunit β
dLGN	dorsal lateral geniculate nucleus
dpi	days post injury/injection
IOP	intraocular pressure
L-Dopa	L-3,4-dihydroxyphenylalanine
LP	laser photocoagulation
NMDA	N-methyl-D-aspartic acid
ONC	optic nerve crush
PBS	phosphate-buffered saline
PFA	paraformaldehyde
rd1	*retinal degeneration 1* mutation
RGC	retinal ganglion cell
ROI	region of interest
(SD-)OCT	(spectral domain-) optical coherence tomography
(s)SC	(superficial layers of the) superior colliculus
TBS	tris-buffered saline
Tyr	Tyrosinase gene
VGluT2	vesicular glutamate transporter 2

1. Introduction

Glaucoma is a heterogeneous group of disorders that have in common the progressive death of retinal ganglion cells (RGCs) and degeneration of the optic nerve. Worldwide, over 60 million people are believed to be at risk to become irreversible blind, due to this neurodegenerative disease (Quigley and Broman, 2006; Tham et al., 2014). Although elevated intraocular pressure (IOP) is considered the major risk factor –and sole target for clinical treatment– glaucoma etiology is still not completely understood and thought to involve a dynamic interplay of genetic predisposition and age-related and environmental stressors (Calkins, 2012; Calkins and Horner, 2012; Leske et al., 2007; Nickells, 2007). This complexity and multifactorial nature of glaucoma is challenging scientists and clinicians to understand the underlying mechanisms leading to neurodegeneration and to find novel therapeutic approaches to fight this blinding disease. Mouse disease models have proven indispensable in this quest, yet the complexity of the vast number of models and mouse strains has also led to confusing findings.

Previous papers reported that CD1- mice are more susceptible to ocular hypertension-induced glaucoma as compared to C57Bl/6 mice (Cone et al., 2010; Cone et al., 2012; Nguyen et al., 2013). This greater susceptibility could derive from structural differences in the eye/visual system of either mouse strain or from a differential response to elevated IOP. The assess whether albinism alone is the main factor predisposing CD-1 mice to more severe glaucomatous neurodegeneration, we tested CD-1, albino C57Bl/6 and pigmented C57Bl/6 mice in a model for ocular hypertension-induced glaucoma and two other optic neuropathy models (*i.e.* intravitreal N-methyl-D-aspartic acid (NMDA) injection and optic nerve crush (ONC)).

Whether or not related to albinism, many morphological and functional characteristic of the eye have been shown to vary among mouse strains, including IOP (Cone et al., 2012; John et

al., 1997; Savinova et al., 2001), aqueous humor outflow resistance (Boussommier-Calleja and Overby, 2013), scleral biomechanics (Nguyen et al., 2013), RGC and cone density (Salinas-Navarro et al., 2009b; Whitney et al., 2011; Williams et al., 1996), congenital retinal degeneration (Chang et al., 2013; Clapcote et al., 2005; Mattapallil et al., 2012; Serfilippi et al., 2004; Wong and Brown, 2006), susceptibility to photoreceptor death (Matsumoto et al., 2014), visual projection patterns (Drager and Olsen, 1980; Rebsam et al., 2012; Rice et al., 1995), and performance in vision-guided behavior tasks (Balkema and Drager, 1991; Wong and Brown, 2006). We therefore first investigated the baseline phenotype of the retina and retinofugal projection in the three wild type mouse strains mentioned above, looking for traits that might relate to the differential glaucoma susceptibility.

2. Methodology

2.1. Experimental animals

Three mouse strains/stocks[1] were used in this study: C57Bl/6, C57Bl/6-Tyr^c, and CD-1 mice. The CD-1 and C57Bl/6-Tyr^c mouse strains both carry a homozygous Cys103Ser mutation (designated *Tyr^c*) in the tyrosinase (*Tyr*) gene, resulting in oculocutaneous albinism (Beermann et al., 2004; Lavado and Montoliu, 2006). Although C57Bl/6-Tyr^c and CD-1 are both albino mice, they have a very distinct genetic background: while C57Bl/6-Tyr^c inbred mice share the same genetic background as the C57Bl/6 strain, CD-1 mice are an outbred strain, implying high genetic heterogeneity (Chia et al., 2005).

All studies were conducted in compliance with the European Communities Council Directive of 22 September 2010 (2010/63/EU) and the Belgian legislation (KB of 29 May 2013), and

[1] Outbred colonies are usually referred to as ‘stocks’, whereas inbred ones are referred to as ‘strains’ or ‘lines’. However, to facilitate reading we will refer to all of them as ‘strains’.

were approved by the KU Leuven institutional ethical committee. Adult (2-4 months) C57Bl/6, C57Bl/6-Tyr^{c}, and CD-1 mice were obtained from the university breeding colony. Animals were kept under a 12/12 light-dark cycle and had *ad libitum* access to food and water.

2.2. Genotyping

Mice were genotyped for the *retinal degeneration 1 (rd1)* mutation of the *Pde6b* gene. Briefly, DNA was extracted from tail biopsies, and genotyping PCR was performed with the Fast Hotstart Genotyping PCR kit (Kapa Biosystems), in 30 cycles at the following temperatures: denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and polymerization at 72°C for 30 seconds. The amplified DNA products were analyzed by electrophoresis on a 2% agarose gel. The following primer sequences were used, producing 237 bp and 562 bp bands for wild type and *rd1* genotypes, respectively:
5' ACCTGCATGTGAACCCAGTATTCTATC 3' (*Pde6b1*);
5' CTACAGCCCCTCTCCAAGGTTTATAG 3' (*Pde6b2*);
5' AAGCTAGCTGCAGTAACGCCATTT 3' (*Pde6b3*).

2.3. Intra-ocular pressure measurement

The IOP was measured in awake animals with a calibrated rebound tonometer (Tono-Lab, iCare) (Haddadin et al., 2009; Haddadin et al., 2012). IOP was measured before LP (day 0) and on day 1 till 7 following LP. In short, the mouse was hold with one hand by loosely grabbing the fur in the neck and care was taken to avoid stress and pressure on the neck region. Next, 10 independent IOP measurements were taken per eye, from which the highest two and lowest two values are excluded in order to reduce variability. An average IOP for each eye was calculated from the remaining six values. IOP measurements were always

performed in the morning, to avoid potential variability due to diurnal IOP variations. The untreated, contralateral eye was used as a control.

2.4. Spectral domain optical coherence tomography

Thickness of the retinal layers was evaluated using a spectral domain optical coherence tomography (SD-OCT) system (Envisu R2210, Bioptigen) (Buys et al., 2013). Upon general anesthesia (i.p. 75 mg/kg body weight ketamine, Anesketin, Eurovet; i.p. 1 mg/kg medetomidine, Domitor, Pfizer), pupils were dilated by topical application of 0.5% tropicamide (0.5% Tropicol, Thea). SD-OCT was performed using 100 consecutive B-scan lines composed of 1000 A-scans, in a 1.4x1.4mm field. After the procedure, anesthesia was reversed by means of atipamezol (i.p. 1 mg/kg, Antisedan, Pfizer) and antibiotic ointment was applied to the eye (tobramycin 3 mg/g, Tobrex, Alcon). Total retinal thickness and thickness of different retinal layers were analyzed using InVivoVue Diver 2.2 software (Bioptigen).

2.5. Optokinetic tracking response

The optokinetic tracking response was measured under photopic conditions in a virtual-reality chamber (OptoMotry, Cerebral Mechanics), as described by Prusky *et al.* (Douglas et al., 2005; Prusky et al., 2004). Briefly, a virtual cylinder comprised of a vertical sine wave grating was projected on four computer screens facing into a box. The animal was placed on a platform in the center of the arena and a video camera, situated above the animal, provided real-time video feedback. Visual acuity was measured using a staircase procedure, in which different spatial frequencies (100% contrast, 12° per second speed) varied randomly and separate for each eye. The experimenter judged whether the mouse displayed optokinetic

tracking, and the maximum spatial frequency at which optokinetic tracking seen, was determined.

2.6. Surgical procedures

All surgical procedures were performed under general anesthesia with ketamine and domitor (see above). After the procedure, anesthesia was reversed by means of atipamezol (see above), and antibiotic ointment (see above) was applied to avoid corneal desiccation and infection of the eye. Untreated eyes from a separate cohort of untreated animals served as controls.

2.6.1. Intravitreal injections

Intravitreal injections were performed as described (Lebrun-Julien et al., 2009), to deliver either anterograde tracer or NMDA. Briefly, a total volume of 2 µl (0.5 µl/second) was injected into the superior quadrant of the right eye using a glass capillary with a 50-70 µm outer diameter, connected to a Hamilton syringe. In addition to general anesthesia, eye drops with topical anesthesia (oxybuprocaine 0.4%, Unicaïne, Thea) were given. For anterograde tracing of the dorsal lateral geniculate nucleus (dLGN) and the superior colliculus (SC), Alexa Fluor-488-conjugated cholera toxin β subunit (CTB) (0.5% in phosphate-buffered saline (PBS) containing 0.5% DMSO; Life Technologies) was injected in the right eye, and mice were sacrificed at 7 dpi. In order to induce retinal excitotoxicity, NMDA (7.5 mM, in PBS; Sigma-Aldrich) was injected, and animals were sacrificed at 4 days post injection (dpi).

2.6.2. Optic nerve crush

Intraorbital ONC was performed as described (Parrilla-Reverter et al., 2009). Briefly, an incision was made in the skin overlying the superior orbital rim, the supero-external orbital contents were dissected, and the superior and external rectus muscles were transected. The exposed optic nerve was then crushed 1 mm from the globe with a watchmaker's forceps for

10 seconds. Funduscopy was performed before and after the procedure to assess retinal perfusion. Animals were sacrificed at 7 days post injury (dpi).

2.6.3. Laser photocoagulation (LP)

Tropicamide eye drops (1% Mydriacyl, Alcon) were administered to ensure pupil dilatation. Next, monocular hypertension in the left eye was induced via LP of the episcleral and perilimbal vessels, as described (Salinas-Navarro et al., 2009a; Valiente-Soriano et al., 2015). Briefly, a 532 nm diode laser (Vitra, Quantel Medical) was used to deliver a number of laser burns in one single session. The number of spots, power, and duration of the laser pulse were adjusted according to the mouse strain. For C57Bl/6 mice, the vessels were photocauterized with 140 spots, with a laser power and duration of 100 mW and 0.05 seconds, respectively. For C57Bl/6-Tyr^{c} mice, the vessels were photocauterized with 100 spots, with a laser power and duration of 200 mW and 0.5 seconds, respectively. For CD-1 mice, the vessels were photocauterized with 70 spots, with a laser power and duration of 300 mW and 0.5 seconds, respectively. Animals were sacrificed at 14 dpi.

2.6.4. Retrograde labeling from the superior colliculus

To identify the population of RGCs with functional retrograde axonal transport in the ocular hypertension model, hydroxystilbamidine methanesulfonate (OHSt) (Life Technologies) was applied to both SC (Galindo-Romero et al., 2013; Salinas-Navarro et al., 2009b). In brief, after exposing the midbrain, a small pledge of gelatin sponge (Espongostan, Ferrosan) soaked in saline containing 10% OHSt and 10% DMSO, was applied over the entire surface of both SC following previously described methods (Salinas-Navarro et al., 2009). Retinas were dissected 4 days post LP, and subsequently immunostained for Brn3a (see below). Mosaic z-stack images of the entire retina were taken with a confocal microscope (FV1000, Olympus), equipped with FluoViewer 4.0 software (Olympus).

2.7. Hematoxylin and eosin staining

Mice were deeply anaesthetized (i.p. 30 mg/kg sodium pentobarbital, Nembutal, Ceva), perfused transcardially with 1% phosphate-buffered paraformaldehyde (PFA) and eyes were dissected. Eyes were fixed overnight in 1% PFA, processed for paraffin-embedding and transverse sections (10 µm) of the whole eye were made. Sections were first deparaffinized and rehydrated, followed by a histological staining with hematoxylin (Sigma) and 1% eosin (Prosan). Next, sections were dehydrated and mounted using DPX neutral mountant (Prosan). Images were taken with a light microscope (Zeiss Axio Imager Z.1), equipped with an AxioCam MRm camera and ZEN software (Zeiss).

2.8. Immunohistochemistry

In order to quantify retinal ganglion cell density, a Brna3 immunostaining was performed on retinal flatmounts (Galindo-Romero et al., 2011; Nadal-Nicolas et al., 2009). Mice were deeply anaesthetized with sodium pentobarbital (see above) and sacrificed by cervical dislocation. Eyes were dissected and fixed for 1 hour in 4% PFA. Next, retinas were flatmounted and again fixed for 1 hour in 4% PFA. Flatmounted retinas were frozen for 15 minutes at -80°C, before applying the primary antibody goat anti-Brn3a (Santa Cruz, C-20, sc-31984) (1:750), which was diluted in PBS containing 2% Triton X-100 and 2% rabbit pre-immune serum. The next day, Alexa Fluor-488-conjugated rabbit anti-goat IgG antibody (Life Technologies) (1:500) was applied for 2 hours. Retinal flatmounts were rinsed with PBS with 0.5% Triton X-100 in between steps, and mounted using mowiol mounting medium (10% mowiol 4-88 (Sigma-Aldrich), 40% glycerol, 0.1% 1,4-diazabicyclo-[2,2,2]-octane in 0.2 M Tris-HCl [pH 8.5]). Mosaic z-stack images of the entire retina were taken with a multiphoton microscope (BX61WI, Olympus), equipped with a MaiTai HP DeepSee laser (690-1020 nm, Spectra Physics) and FluoViewer 4.0 software (Olympus).

In order to delineate dLGN boundaries and SC stratification, an immunostaining for vesicular glutamate transporter 2 (VGluT2), a discrete marker for presynaptic glutamatergic terminals, was performed (Dekeyster et al., 2015a; Fujiyama et al., 2003). Mice were deeply anesthetized with sodium pentobarbital (see above) and perfused transcardially with 4% PFA. Brains were dissected and post-fixed overnight in 4% PFA. Vibratome sections (50 µm) of the dLGN and SC brain regions, between Bregma -1.70 mm and -4.84 mm, were mounted on gelatin-coated microscopy slides and dried overnight at 37°C. After rehydration, sections were incubated in PaxD permeabilization buffer (PBS with 5 % bovine serum albumin, 0.3% Triton X-100, and 0.3% sodium deoxycholate) and blocking solution (0.01 M Tris-buffered saline (TBS) with 0.5% blocking reagent (Perkin-Elmer) and 20% normal donkey serum (Life technologies)). Next, sections were incubated overnight with rabbit anti-VGluT2 (Life Technologies, 42-7800) (1:300), diluted in PBS containing 0.3% Triton X-100 and 10% normal donkey serum. The next day, Alexa Fluor-647-conjugated donkey anti-rabbit IgG antibody (Life Technologies) (1:200) was applied for 2 hours. Sections were rinsed with PaxD in between steps, and cover slipped using mowiol mounting medium (see above). 4',6-diamidino-2-phenylindole (1 µg/ml in PBS, Applichem) was used as a fluorescent nuclear counterstaining. Images were taken with an inverted confocal microscope (FV1000, Olympus) and were processed with FluoViewer 4.0 (Olympus) and Photoshop CS5 (Adobe) software.

2.9. Morphometric analyses and quantification of RGC density

Total retinal thickness, as well as thickness of the different retinal layers, was measured on 6 retinal sections per eye, using Fiji software (Schindelin et al., 2012). For each section, measurements were performed at two locations in the peripheral retina and two locations in the central retina.

RGC density was evaluated on entire retinal flatmounts after immunostaining for Brn3a. RGC density (number of RGCs/mm²) was semi-automatically computed using Fiji software and an in-house made macro (Geeraerts et al., 2015). Briefly, the mosaic picture of a full Brn3a-stained retinal flatmount was subjected to a series of operations, consisting of noise removal via Fiji's 'Remove Outlier' plugin, enhancement of the $Brn3a^+$ signal of RGCs via the determinant of the Hessian, and counting of the resulting local maxima as RGCs. Next, the retinal flatmount was outlined, and its total area, total number of RGCs and average RGC density were calculated.

The number of retrogradely labeled RGCs was manually counted after tracing with OHSt using the "cell counter" plugin of Fiji software. $OHSt^+$ RGCs were quantified in twelve 250x250 µm frames per retina, by two independent, blinded observers.

RGC axon terminals, labeled via CTB tracing, were quantified using Fiji software. For the dLGN, four regions of interest (ROI) were outlined: three ROIs in the contralateral dLGN (in respect to CTB-injected eye) and one ROI in the ipsilateral dLGN. A schematic overview of these ROIs, further referred to as 'contralateral', 'contra patch', 'contra in ipsi region', and 'ipsilateral', is depicted in Figure 2c. The ROIs 'contralateral', 'contra in ipsi region', and 'ipsilateral' were analyzed on three 100 µm-spaced coronal sections per animal within the rostrocaudal region between Bregma -2.10 mm and -2.60 mm. The ROI 'contra patch' was analyzed on all sections from Bregma -2.40 mm till -2.80 mm showing this feature. Immunofluorescence intensity (per µm²) was analyzed in randomly placed frames (50 µm diameter) in these ROIs and, after background subtraction, a mean value was calculated per mouse. For the SC, CTB tracing was analyzed qualitatively on sections between Bregma -3.16 mm and -4.84 mm. The boundary between the visually driven superficial layers of the SC, including the *stratum zonale*, *stratum griseum superficiale*, and *stratum opticum*, and the

underlying deep SC, was outlined based on a VGluT2 immunostaining. A scheme of the theoretical contralateral and ipsilateral RGC projection zones in the SC is depicted in Figure 3b.

2.10. Statistics

Normal distribution was verified using a Kolmogorov–Smirnov test and parallel equal variance between groups was tested. Outliers were identified and excluded, based on a Grubbs' test. Statistical tests are mentioned in the figure captions. Briefly, all baseline characteristics were either analyzed via a paired Student's *t*-test, a one-way ANOVA with *post hoc* Turkey's multiple comparisons test, or a two-way ANOVA with *post hoc* Dunnett's multiple comparisons test. Differences in experimentally induced RGC death were analyzed among the different strains using a two-way ANOVA with *post hoc* Turkey's multiple comparisons test. IOP profiles after LP were analyzed using a repeated measures two-way ANOVA with *post hoc* Dunnett's multiple comparisons test. A probability level (α-level was set to 0.05) of <0.05 was accepted as statistically significant (*$p<0.05$, **$p<0.01$, ***$p<0.005$). All data are presented as mean ± SEM, unless indicated otherwise. For all statistical analyses GraphPad Prism 6 (GraphPad Software) was used.

3. Results

3.1. Evaluation of baseline characteristics of three commonly used mouse strains in glaucoma research

3.1.1. Ocular phenotype

As elevated IOP has been identified as one of the major risk factors for glaucoma, we first evaluated baseline IOP in the three mouse strains under study. A significant difference in IOP was seen between C57Bl/6 and CD-1 mice, which had an IOP of 14.2 ± 0.1 mmHg and 13.2 ± 0.1 mmHg, respectively ($p<0.0001$). C57Bl/6-Tyr^{c} mice displayed an even higher IOP,

318 which –with a value of 16,6 ± 0.2 mmHg–t was 2.4 mmHg and 3.4 mmHg higher than the
319 IOP of C57Bl/6 and CD-1 mice, respectively ($p<0.001$).
320 Next, we evaluated retinal histology via *in vivo* OCT imaging (Figure 1a-d) and on
321 hematoxylin and eosin-stained retinal cross-sections (Figure 1e-h). No significant differences
322 in the thickness of any individual retinal layer, and no signs of neurodegeneration were seen
323 (Figure 1i). In addition, RGC density was quantified on retinal flatmounts, revealing a small
324 yet significant, biological variation. Despite their elevated IOP, C57Bl/6-Tyr^c mice showed a
325 similar RGC density as C57Bl/6 mice, which share an identical genetic background. CD-1
326 mice, on the other hand, exhibited significantly higher RGC densities than C57Bl/6 and
327 C57Bl/6-Tyr^c mice (+28.5% and +22.4%, respectively) ($p<0.0001$) (Figure 1j). A similar
328 accordance was seen for the total RGC counts in these strains (data not shown).
329 Overall, the histological architecture of the three strains in this study is highly similar. Small,
330 biological variations do exists, yet seem to be unrelated to developmental or
331 neurodegenerative differences.
332 Importantly, OCT imaging and histological analysis sporadically revealed severe retinal
333 dystrophy in CD-1 mice: the outer plexiform layer, outer nuclear layer, and photoreceptor
334 layer were completely absent in a subset of these animals (Figure 1d, h). A large-scale
335 genotyping experiment (N=106) confirmed that 21% of the mice in our CD-1 colony were
336 homozygous for the *rd1* mutation of the *Pde6b* gene, leading to photoreceptor degeneration.
337 These mice were excluded from the study.
338
339 3.1.2. Retinofugal projections
340 Retinofugal projections to the two major subcortical relay stations, the dLGN and SC, were
341 studied after anterograde tracing with fluorescently labeled CTB (Figure 2c). Analysis of the
342 CTB signal (fluorescence intensity/μm^2) on coronal brain sections, unveiled prominent

differences in the patterns of RGC synapses in the dLGN and SC of C57Bl/6, C57Bl/6- Tyr^{c}, and CD-1 mice.

In the dLGN, ipsilateral projections from the CTB-injected eye (ROI 'ipsilateral') appeared more diffuse in C57Bl/6- Tyr^{c} and CD-1 mice, as compared to C57Bl/6 mice ([y] $p<0.05$) (Figure 2a, b). In addition, when looking at the contralateral dLGN, the area with ipsilateral termini was not devoid of CTB signal (ROI 'contra in ipsi region') in C57Bl/6-Tyr^{c} and CD-1 mice: a black hole in the CTB^{+} contralateral dLGN signal is seen in C57Bl/6 mice, but not in the albino mice ([z] $p<0.001$ *versus* C57Bl/6-Tyr^{c}, $p<0.01$ *versus* CD-1) (Figure 2a (arrow), b). Moreover, in C57Bl/6 animals, the density of RGC synapses did not differ between the contralateral zone of the contralateral dLGN (ROI 'contralateral') and the ipsilateral zone of the ipsilateral dLGN (ROI 'ipsilateral'). However, in C57Bl/6-Tyr^{c} and CD-1 mice, ipsilateral projections showed lower fluorescence intensity/µm² values *versus* contralateral projections (ROI 'ipsilateral' *versus* ROI 'contralateral') ([x] $p<0.05$ for C57Bl/6-Tyr^{c}, $p<0.001$ for CD-1) (Figure 2a, b).

Finally, a cluster of contralaterally projecting axon terminals that appeared separated from the other contralateral projections (ROI 'contra patch') was found in the contralateral, caudal dLGN, adjacent to the optic tract (Figure 2d). Interestingly, this phenomenon was only sporadically observed in CD-1 mice, in which it spanned a distance of less than 200 µm, while it was seen in all C57Bl/6-Tyr^{c} mice in this study, spanning a distance of approximately 500 µm along the rostrocaudal axis. Quantification of the CTB signal in this ROI 'contra patch' in the C57Bl/6-Tyr^{c} strain, showed that RGC axon termini were more dense as compared to the adjacent contralateral zone (ROI 'contra') ($p<0.05$) (Figure 2e).

In summary, these results confirm a less strict segregation of ipsilateral and contralateral projections to the dLGN, and reduced – or at least more diffuse –, ipsilateral projections in C57Bl/6-Tyr^{c} and CD-1 albino animals, compared to the C57Bl/6 strain. Moreover, a spatial

segregation of densely packed contralateral RGC terminals was described in the caudal dLGN of C57Bl/6-Tyr^{c}, and to a lesser extent CD-1, mice.

In the SC, in all three mouse strains, contralateral RGCs terminated in the superficial layers of the SC (sSC), including the stratum zonale, stratum griseum superficiale, and stratum opticum, and no apparent changes were noted in this dominant projection zone. In contrast, differential segregation of the ipsilateral RGC axons to the lower stratum opticum was seen (Figure 3a, b). In the rostral sSC, these ipsilateral projections were packed in a series of delineated patches along the lateromedial axis (Figure 3a, cross-section 1). Similar to the dLGN, ipsilateral projections to the SC were less dense and more diffuse in C57Bl/6-Tyr^{c} and CD-1 mice, compared to C57Bl/6 animals, and seemingly interconnected (Figure 3a, cross-section 1). More caudal along the rostrocaudal axis, the ipsilateral RGC axons bundled in a tube running parallel to the brain midline (Figure 3a, cross-sections 2 and 3). In CD-1 mice, this rostrocaudal tube appeared somewhat stretched along the lateromedial axis, rather than being round, as was seen in the C57Bl/6 mice (Figure 3a, cross-section 3 (arrow)). Of note, these disparities of the retinocollicular projections were most pronounced when comparing C57Bl/6 with CD-1. The organization of the retinocollicular ipsilateral projections in C57Bl/6-Tyr^{c} mice appeared to be somewhat in between the C57Bl/6 and CD-1 phenotypes, with rostral patches being more delineated as compared to CD-1 mice, yet less dense as compared to C57Bl/6. Furthermore, the rostrocaudal tube appeared less delineated and slightly larger as compared to C57Bl/6 strain, but not as stretched as seen in the CD-1 mice. Strikingly, all C57Bl/6 and C57Bl/6- Tyr^{c} mice displayed a small, densely labeled patch of ipsilaterally projecting RGC axons terminating in the medial stratum zonale (Figure 3a, cross-section 1 (arrowhead)), which was only visible over a rostrocaudal distance of less than 200 µm. This was never observed in CD-1 animals and might therefore be linked to the C57Bl/6 genetic background. Of note, the strain-related differences in the visual projection to

393 the SC shown in this study relate to the ipsilateral projection zones, which make up only 1 to
394 3% of the SC. Anterograde/retrograde axonal tracing to/from the SC therefore remains a
395 legitimate ways of assessing axonal transport differences in these strains (see below).
396 Taken together, as with the retinogeniculate projections, ipsilateral projections to the SC were
397 more diffuse in C57Bl/6-Tyr^{c} and CD-1 albino animals, compared to the C57Bl/6 strain.
398 Moreover, their segregation pattern seemed to be affected as well, with interconnected
399 patches of ipsilateral termini in the stratum opticum, and a deformation of the rostrocaudal
400 tube of ipsilateral axons. Finally, in the C57Bl/6 genetic background, a densely labeled patch
401 of ipsilateral RGC termini was seen in the medial stratum zonale. Importantly, while these
402 strain differences seem to be confined to the 'subordinate' – in comparison to the dominant,
403 contralateral projections to the sSC – ipsilateral projection areas in the SC, behavioral
404 analyses suggest that they may have major implications on SC function (see below).
405
406 3.1.3. Optokinetic tracking response
407 In addition to the morphological study of the retinofugal projections to the dLGN and SC, the
408 retinocollicular circuit was also evaluated by means of a functional test, *i.e.* the optokinetic
409 tracking response. Visual acuity, measured as the highest spatial frequency (cycles/degree)
410 eliciting an optokinetic response, was found to be divergent among the C57Bl/6, C57Bl/6-
411 Tyr^{c}, and CD-1 mouse strains ($p<0.0001$ for ANOVA). C57Bl/6 mice displayed normal
412 visual acuity (0.40 ± 0.004 c/d) (Figure 3c), conform to what has been reported for this strain
413 (Prusky et al., 2004), yet the optokinetic tracking response in C57Bl/6-Tyr^{c} and CD-1 mice
414 was severely affected. Strikingly, normal tracking reflexes were almost completely absent in
415 these albino mice, rather they displayed counter-directive head movements. As this behavior
416 clearly consisted of reflexive movements in response to the moving gratings, we decided to
417 catalog them as true responses and to determine visual acuity based on these optokinetic

418 ‘anti-tracking’ responses. Still, in comparison to C57Bl/6 mice, reduced and more variable
419 visual acuities of 0.32 ± 0.01 c/d and 0.28 ± 0.01 c/d were found for C57Bl/6-Tyr^{c} ($p<0.001$)
420 and CD-1 mice ($p<0.001$), respectively (Figure 3c).
421 To summarize, assessment of the optokinetic tracking response revealed that vision is
422 preserved in all three mouse strains. Nevertheless, visual acuity is lower in albino mice as
423 compared to the C57Bl/6 mice, and their optokinetic tracking behavior is severely disturbed.
424 Of note, the latter might potentially relate to the differential segregation of ipsilateral
425 projections to the SC seen in these albino strains.
426
427 **3.2. Evaluation of the three most commonly used glaucoma models in C57Bl/6, C57Bl/6-**
428 **Tyr^{c}, and CD-1 mice**
429 In a second part of this study, we evaluated differences in the success and applicability of
430 three commonly used glaucoma models in C57Bl/6, C57Bl/6-Tyr^{c}, and CD-1 mice. In
431 addition, we also evaluated the susceptibility of each of the three mouse strains to these
432 disease models. Importantly, given the complexity of the disease, there is still no ideal animal
433 model that mimics all aspects of glaucoma pathogenesis. Nonetheless, a variety of different
434 models does exist, each with their strengths and limitations, which allow to investigate at
435 least defined processes contributing to glaucomatous damage.
436 The first optic neuropathy model tested, consisted of an intravitreal injection of the glutamate
437 analogue NMDA. The importance of this excitotoxic model is often somewhat underscored,
438 due to the controversy about excitotoxicity as a contributing factor to glaucoma.
439 Nevertheless, it is a valuable model to investigate neuroprotective strategies to treat
440 secondary RGC loss and excitotoxic insults in the central nervous system (CNS) (Almasieh et
441 al., 2012; Casson, 2006; Kalia et al., 2008; Tilleux and Hermans, 2007). Intravitreal injection
442 of NMDA had a nearly 100% success rate in inducing RGC death, although a moderate

443 degree of variability existed. Intriguingly, intravitreal injection of NMDA in the three mouse
444 strains resulted in very divergent degrees of RGC degeneration ($p<0.001$ for ANOVA).
445 Evaluation of RGC survival at 4 dpi, proved C57Bl/6 mice to be the most resistant to
446 NMDA-induced RGC death, while CD-1 mice were the most sensitive (Figure 4a).
447 Next, we evaluated the effect of ONC on RGC survival in C57Bl/6, C57Bl/6-Tyr^c, and CD-1
448 mice. Although the acuteness of this model does not correspond to human glaucoma patients,
449 at least part of the glaucomatous damage in patients with ocular hypertension is believed to
450 result from IOP-induced mechanical forces damaging the optic nerve axons. As such, the
451 ONC model is of particular benefit when studying the contribution of axonal damage to
452 glaucoma pathogenesis (Johnson and Tomarev, 2010; McKinnon et al., 2009). Evaluation of
453 RGC survival at 7 dpi revealed that C57Bl/6, C57Bl/6-Tyr^c, and CD-1 mice responded
454 identically to ONC, resulting in highly reproducible, low-variability results among the three
455 strains under study (Figure 4b).
456 Finally, RGC degeneration following LP-induced monocular hypertension was investigated.
457 Given that elevated IOP remains the most important modifiable risk factor for glaucoma,
458 animal models of OHT are highly valued in the elucidation of the pathology of RGC
459 degeneration in humans (Vidal-Sanz et al., 2012). Monocular hypertension models have been
460 extensively described in CD-1 mice (Cuenca et al., 2010; Dekeyster et al., 2015a; Fu and
461 Sretavan, 2010; Salinas-Navarro et al., 2009a). Due to pigmentation of the sclera, ciliary
462 body, and iris, as well as strain-related differences in the size of the eye, however, the laser
463 settings used for CD-1 mice are insufficient to induce monocular hypertension in C57Bl/6
464 and C57Bl/6-Tyr^c mice. Therefore, laser settings were optimized to yield an optimal set of
465 parameters for each of the three mouse strains (own observations, (Valiente-Soriano et al.,
466 2015)). These settings resulted in an IOP profile that mimics the well-established response in
467 CD-1 mice, with a success rate of nearly 100% in all three strains. More in detail, a steep rise

in IOP was seen at 1 day post LP, resulting in an average IOP of 41.9 ± 1.6 mmHg. In general, this IOP elevation was sustained for 5 days (4 days for C57Bl/6-Tyr^{c} mice) ($p<0.001$), after which the IOP gradually decreased to return to its baseline value by day 6 (Figure 4c).

At 14 dpi, a sectorial pattern of RGC death, in addition to diffuse loss of RGCs, was observed in all three mouse strains under study. However, notwithstanding the overlap in IOP profiles, the extent of RGC degeneration following LP-induced temporary monocular hypertension did vary. While no significant difference in average RGC death was observed between C57Bl/6 and C57Bl/6-Tyr^{c} mice (26,0 ± 3.8% *versus* 41.0 ± 10.0%), RGC death was significantly higher in CD-1 mice (86.9 ± 2.1%) as compared to the C57Bl/6 background ($p<0.0001$) (Figure 4d). A correlation analysis for each strain, plotting individual IOP exposure (X, *i.e.* integral ΔIOP) and RGC loss (Y) for each animal, revealed that the observed intra-strain variation in RGC loss is unrelated to IOP for CD-1 (Pearson R = -0.3429; $p>0.05$) and C57Bl/6-Tyr^{c} (Pearson R = -0.4957; $p>0.05$), but not for C57Bl/6 mice (Pearson R = -0.8524; $p<0.01$). Overall, as no correlation between RGC survival and IOP exposure was found for two out of the three strains, these results confirm that the inter-strain differences in RGC death that were observed in this study reflect the effect of 'strain-related' factor(s) rather than IOP differences.

Intriguingly, the effect of the genetic background on RGC survival within the ocular hypertension model, was also apparent when axonal transport integrity and visual function were evaluated. At 4 dpi, all RGCs were still alive, yet the earliest signs of dysfunctional axonal transport became apparent, similar to what has been reported previously (Salinas-Navarro et al., 2009a; Valiente-Soriano et al., 2015; Vidal-Sanz et al., 2012) (Figure 5). Indeed, the number of retrogradely labeled RGCs was immediately lower in CD-1 mice as compared to mice with a C57Bl/6 background ($p<0.001$ *versus* C57Bl/6, $p<0.05$ *versus*

C57Bl/6-Tyr^{c}) (Figure 5b). Also the rate of visual acuity deterioration was faster in CD-1 mice ($p<0.001$ for ANOVA) (Figure 5d), indicative for a higher susceptibility of the RGCs and their axons to ocular hypertension-induced neurodegeneration.

Overall, genetic background appeared to have a clear impact on RGC degeneration, although this was very much depending on the kind of glaucoma disease models used. While RGC degeneration resulting from ONC proved to be equal in the three mouse strains studied, NMDA- and ocular hypertension-induced RGC death were dramatically modulated by the genetic background. Notably, in both models, CD-1 mice appeared to display the highest sensitivity, while the C57Bl/6 background was the most resistant to RGC damage.

4. Discussion

In this study, we set out to discover whether differential susceptibility to glaucomatous RGC degeneration in CD-1, albino C57Bl/6 and pigmented C57Bl/6 mice, is related to structural differences in the eye/visual system of either mouse strain, to a differential response to elevated IOP, and/or to other not-yet-known factors. A central question in this investigation was whether albinism alone is the main factor predisposing mice to more severe glaucomatous neurodegeneration, or whether other factors inherent to the genetic background are at play as well.

4.1. Differential ocular morphology and visual system organization in pigmented *versus* albino mice

No fewer than 30.000 genes are believed being expressed in the mammalian nervous system, and it is to be suspected that a small yet important subset of alleles of this extraordinarily large number of genes has well-defined effects on the structure and function of the mammalian CNS (Williams et al., 1996). In the eye/retina, many morphological and

functional characteristic have been shown to vary among mouse strains: IOP and aqueous humor outflow resistance, scleral biomechanics, RGC and cone density, congenital retinal degeneration, visual projection patterns, performance in vision-guided behavior tasks, *etc.* (see introduction). In a first step of this investigation, we set out by defining to what extent albinism (*i.e.* the *Tyr*^{c} mutation) contributes to these baseline characteristics – that might ultimately influence the response to glaucomatous injury. Unraveling this research question, we took advantage of the genetic lineage of C57Bl/6 and C57Bl/6-Tyr^{c} mice, which share the exact same genetic background except for one mutation in the *Tyr*^{c} gene. Besides, CD-1 mice were included as a third strain, sharing the *Tyr*^{c} mutation with the C57Bl/6-Tyr^{c} mice yet further belonging to a very distinct genetic background. As such, the combinatorial study of these three mouse strains allowed us to dissect the relative importance of albinism *versus* genetic background.

Given the crucial role of L-3,4-dihydroxyphenylalanine (L-Dopa) during the development of the iridocorneal angle and retinofugal projections (Bhansali et al., 2014; Cronin et al., 2003; Eisenhofer et al., 2003; Gimenez et al., 2004; Jeffery et al., 1997; Jeffery et al., 1994; Libby et al., 2003; Roffler-Tarlov et al., 2013; Savinova et al., 2001), we hypothesized that L-Dopa depletion in albino mice with the *Tyr*^{c} mutation – tyrosinase is a rate-limiting in L-Dopa biosynthesis – would result in ocular hypertension and misguidance of RGC axons at the optic chiasm. Indeed, significant differences in baseline IOP were observed amongst the three mouse strains included in this study. However, although a higher IOP was seen in C57Bl/6-Tyr^{c} mice, confounding genetic factors appear to counteract IOP elevation in CD-1 mice. Second, and in agreement with the hypothesis, missegregation of retinogeniculate projections came to light in C57Bl/6-Tyr^{c} and CD-1 mice: a reduced density and rather diffuse appearance of ipsilateral projection zones was seen in the dLGN, and to a lesser extent also in the SC. Moreover, these disturbed retinotopic visual field representations

appeared to affect visual functioning, as C57Bl/6-Tyr^{c} and CD-1 mice not only had reduced visual acuity, but also displayed so-called optokinetic 'anti-tracking' responses. Of note, this striking 'misbehavior' of albino mice in the optomotor test has never been described before, and suggests that the full implications of axonal misrouting in the albino visual system are still being uncovered. The present study furthermore discovered a densely labeled, well-delineated patch of ipsilaterally projecting RGCs in the medial stratum zonale of the SC, in C57Bl/6 and C57Bl/6-Tyr^{c} mice, but not in CD-1 animals. By consequence, this feature seems to be related to the C57Bl/6 genetic background, not to tyrosinase deficiency.

Also related to the genetic background rather than directly originating from the *Tyr^{c}* mutation, is the variance in RGC numbers in different mouse strains (Williams et al., 1996). Significant differences, both in the total number and density of the RGC population, were seen between the C57Bl/6 and the CD-1 genetic background in this study, yet not among C57Bl/6 and C57Bl/6-Tyr^{c} mice. In contrast, the gross organization of the retina, *i.e.* the thickness of its different layers, was similar in C57Bl/6, C57Bl/6-Tyr^{c}, and CD-1 mice. Our data confirm previous reports (Salinas-Navarro et al., 2009b; Williams et al., 1996) and once more point out that CD-1 mice carrying a homozygous *rd1* mutation of the *Pde6b* gene – which does not occur in the C57Bl/6 background – are doubtfully suited for research into ocular pathologies (Hart et al., 2005; Serfilippi et al., 2004). It is thus advisable for any retina study using this albino strain, to determine the presence of the mutation and to monitor potential interference with experimental outcomes.

Altogether, in the visual system, albino mutations in the mouse *Tyr* gene appear to result in a set of diverging phenotypes that are the result of differential modifier genes in the genetic background. While the function of tyrosinase in melanin synthesis results in a phenotype, *i.e.* oculocutaneous albinism, that remains unaltered regardless of the genetic background, L-

567 Dopa depletion in these mice leads to a set of developmental abnormalities that are modified
568 by complex, multifactorial influences of the genetic background.
569
570 **4.2. Why are mouse strains differentially susceptible to RGC degeneration induced by**
571 **different optic neuropathy models?**
572 In a second aspect of this study, we continued to investigate whether the greater susceptibility
573 to RGC degeneration that has been reported in CD-1 mice (Cone et al., 2010; Cone et al.,
574 2012; Nguyen et al., 2013), could derive from the aforementioned structural differences in the
575 eye/visual system, from a differential response to elevated IOP, or from other disease-
576 modifying genes encoded in the genetic background.
577 Altogether, susceptibility to RGC degeneration is a complex trait modulated by several
578 distinct genomic loci (Dietz et al., 2008; Li et al., 2007; Templeton et al., 2009). The present
579 results can be interpreted in the context of a study by Libby *et al.*, who dissected the
580 multifactorial complexity of genetic susceptibility factors into two broad classes of genes that
581 can affect the outcome of glaucomatous damage. The first class modulates the intrinsic
582 susceptibility of RGCs and comprises genes that directly affect RGC survival, including pro-
583 and anti-apoptotic genes, genes encoding free radical scavengers and heat shock proteins, *etc.*
584 The second class of genes modulates extrinsic susceptibility of RGCs, e.g. genes predicted to
585 influence micro- and macroglia reactivity, ECM composition and dynamics, density and
586 regulation of retinal and optic nerve vasculature, the immune responses in the eye (Li et al.,
587 2007; Libby et al., 2005).
588 For a start, the experimental outcomes in the NMDA and ONC models of optic neuropathy
589 are excellent illustrations of this concept of genes modulating the intrinsic *versus* extrinsic
590 susceptibility of RGCs to apoptosis. In the NMDA model, higher susceptibility to excitotoxic
591 neurodegeneration in C57Bl/6-Tyr^{c} and CD-1 mice can be directly related to the lack of

592 tyrosinase activity and the resulting shortage in L-Dopa – and therefrom synthetized
593 dopamine – in these mice. In this optic neuropathy model, the *Tyr* gene modulates extrinsic
594 susceptibility of RGCs via its profound effect on retinal dopamine levels and melanin
595 synthesis, which have been shown to confer neuroprotection by regulating physiological
596 glutamate signaling and restricting glutamate-induced excitotoxicity (Kitaoka et al., 2003;
597 Palumbo et al., 2000; Safa and Osborne, 2000), by inhibiting NMDA receptor activity
598 (Castro et al., 1999; Kashii et al., 1994; Kitaoka et al., 2003; Vaarmann et al., 2013), and by
599 scavenging free radicals (Bilgihan et al., 1995; Corsaro et al., 1995; Porebska-Budny et al.,
600 1992; Scalia et al., 1990; Valverde et al., 1996). Together these tyrosinase-dependent actions
601 explain the high rate of RGC death in C57Bl/6-Tyr^{c} and CD-1 mice *versus* pigmented
602 C57Bl/6 mice. However, with CD-1 mice being far more sensitive than C57Bl/6-Tyr^{c} mice,
603 an additional interplay of disease-modifying genes encoded in their distinct genetic
604 backgrounds seems to be at play as well. In contrast, given that axonal lesion is expected to
605 directly activate apoptosis, the subset of disease-modifying genes can likely be reduced to
606 intrinsic susceptibility genes in the ONC model (Li et al., 2007; Libby et al., 2005). As these
607 genes appear to be well-conserved among different genetic lineages (Reed et al., 2003), the
608 lower complexity of the of the ONC models – in terms of number and interplay of
609 contributing signaling pathways – might thus explain why genetic background seems to be
610 sidelined.
611 On the other hand, comparative studies revealed overlapping gene expression profiles in
612 murine models of ONC and ocular hypertension-induced glaucoma, indicating that a
613 glaucomatous insult with many of the same molecular changes is evoked in both models
614 (Panagis et al., 2011; Schlamp et al., 2001; Steele et al., 2006). This can also be deduced from
615 the fact that many neuroprotective strategies have been shown to be effective after ONC as
616 well as ocular hypertension-induced glaucoma. Of note, these therapeutic strategies, e.g. dual

leucine zipper kinase inhibition, neurotrophin supplementation, TNF-α inhibition (Dekeyster et al., 2015b; Domenici et al., 2014; Fernandes et al., 2014; Roh et al., 2012; Tezel et al., 2004; Welsbie et al., 2013), all act at somal degeneration pathways. Overall, we propose that the neurodegeneration evoked by ONC mainly appeals to the intrinsic susceptibility of RGCs, while ocular hypertension-induced RGC death – characterized by a complex interplay of risk factors that go beyond the acute effects of the elevated IOP-related mechanical injury – is influenced by a complexity of genetic susceptibility factors, both intrinsic and extrinsic. This might explain why RGC survival rates were found to diverge in the latter model. Integration of the data from the ONC and ocular hypertension-induced glaucoma models in this study, comparing the C57Bl/6 *versus* CD-1 genetic background, indeed revealed a differential strain-dependent effect on RGC survival after ONC and LP – *i.e.* the same RGC death after ONC, but worse after LP for CD-1. First of all, these findings thus support the concept that diverging RGC survival is due to factors other than the somal response to any kind of axonal injury (*i.e.* intrinsic susceptibility), and is rather related to how and when the injury is delivered (*i.e.* to differential involvement of extrinsic susceptibility factors). Second, strain-related differences in RGC survival appear to be more centrally associated with ocular hypertension-induced glaucomatous damage than broad optic neuropathy models such as ONC.

Finally, what factors underlie the generally higher susceptibility of CD-1 mice to glaucomatous RGC death (Cone et al., 2010; Cone et al., 2012; Li et al., 2007)? Current knowledge of risk factors at play in CD-1 mice, is limited to altered scleral biomechanical behavior and increased axial length (Cone et al., 2010; Nguyen et al., 2013). Nevertheless, based on the present findings – relating independent outcomes of the RGC population, visual projection and visual behavior –, we can suggest several other contributing factors that might deserve more in-depth investigation in follow-up studies. First, it is to be noted that baseline

IOP appears to be irrelevant in the LP model used, as CD-1 mice – with the lowest IOP of all three strains – would theoretically be at low risk for developing glaucoma. Second, (secondary) RGC loss due to excitotoxicity is an integral component of ocular hypertension-induced glaucoma (Almasieh et al., 2012; Casson, 2006). The increased susceptibility to ocular hypertension-induced RGC death in CD-1 mice might therefore be (partially) related to their decreased ability to cope with excitotoxic stress – note the similarities in the RGC survival graphs for the NMDA and LP models (Figure 4a and d). In addition, given the higher density of RGCs in the retina of CD-1 mice, glutamate release might be manifold and result in an exponential cascade of paracrine pro-apoptotic signaling. Third, in line with e.g. (Buckingham et al., 2008; Fahy et al., 2015; Martin et al., 2006; Vidal-Sanz et al., 2012), our data suggest that RGC axonal transport deficits precede RGC loss in the LP model, and that early changes in visual function may be caused by axonal dysfunction rather than by cell loss. Furthermore, this loss in axonal transport integrity and visual acuity appeared to be larger – already at day 1 post injury – and to progress faster in CD-1 mice, eventually resulting in a higher RGC death. It remains entirely speculative whether the aberant segregation of retinofugal axons does confer to this increased susceptibility to axonal injury. Differential axonal transport rates in albino *versus* pigmented animals (Lund, 1975), abrogated retrograde (neurotrophic) support due to mistargeting to neurons in the dLGN and SC – *cfr.* developmental refinement of mistargeted retinal projections by BDNF (Ernst et al., 2000; Schmidt, 2004) –, as well as differential susceptibility to ocular hypertension-induced damage depending on the anatomical position of the axon in the optic nerve head/visual projection (Giolli and Creel, 1973; Osborne et al., 2001), are only a few possible explanations for our observations. Moreover, our behavioral analyses suggest that visual acuity and SC functionality is already severely disturbed at baseline in CD-1 mice, potentially leading to a state that is more vulnerable to additional losses of axonal projections.

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References

Almasieh, M., Wilson, A.M., Morquette, B., Cueva Vargas, J.L., Di Polo, A., 2012. The molecular basis of retinal ganglion cell death in glaucoma. Progress in Retinal and Eye Research 31, 152-181.

Balkema, G.W., Drager, U.C., 1991. Impaired visual thresholds in hypopigmented animals. Visual neuroscience 6, 577-585.

Beermann, F., Orlow, S.J., Lamoreux, M.L., 2004. The Tyr (albino) locus of the laboratory mouse. Mammalian genome : official journal of the International Mammalian Genome Society 15, 749-758.

Bhansali, P., Rayport, I., Rebsam, A., Mason, C., 2014. Delayed neurogenesis leads to altered specification of ventrotemporal retinal ganglion cells in albino mice. Neural development 9, 11.

Bilgihan, A., Bilgihan, M.K., Akata, R.F., Aricioglu, A., Hasanreisoglu, B., 1995. Antioxidative role of ocular melanin pigment in the model of lens induced uveitis. Free Radic Biol Med 19, 883-885.

Boussommier-Calleja, A., Overby, D.R., 2013. The influence of genetic background on conventional outflow facility in mice. Invest Ophthalmol Vis Sci 54, 8251-8258.

Buckingham, B.P., Inman, D.M., Lambert, W., Oglesby, E., Calkins, D.J., Steele, M.R., Vetter, M.L., Marsh-Armstrong, N., Horner, P.J., 2008. Progressive ganglion cell degeneration precedes neuronal loss in a mouse model of glaucoma. J Neurosci 28, 2735-2744.

Buys, E.S., Ko, Y.C., Alt, C., Hayton, S.R., Jones, A., Tainsh, L.T., Ren, R., Giani, A., Clerte, M., Abernathy, E., Tainsh, R.E., Oh, D.J., Malhotra, R., Arora, P., de Waard, N., Yu, B., Turcotte, R., Nathan, D., Scherrer-Crosbie, M., Loomis, S.J., Kang, J.H., Lin, C.P., Gong, H., Rhee, D.J., Brouckaert, P., Wiggs, J.L., Gregory, M.S., Pasquale, L.R., Bloch, K.D., Ksander, B.R., 2013. Soluble guanylate cyclase alpha1-deficient mice: a novel murine model for primary open angle glaucoma. PLoS One 8, e60156.

Calkins, D.J., 2012. Critical pathogenic events underlying progression of neurodegeneration inÂ glaucoma. Progress in Retinal and Eye Research 31, 702-719.

Calkins, D.J., Horner, P.J., 2012. The cell and molecular biology of glaucoma: axonopathy and the brain. Invest Ophthalmol Vis Sci 53, 2482-2484.

Casson, R.J., 2006. Possible role of excitotoxicity in the pathogenesis of glaucoma. Clin Experiment Ophthalmol 34, 54-63.

Castro, N.G., de Mello, M.C., de Mello, F.G., Aracava, Y., 1999. Direct inhibition of the N-methyl-D-aspartate receptor channel by dopamine and (+)-SKF38393. Br J Pharmacol 126, 1847-1855.

Chang, B., Hurd, R., Wang, J., Nishina, P., 2013. Survey of common eye diseases in laboratory mouse strains. Invest Ophthalmol Vis Sci 54, 4974-4981.

Chia, R., Achilli, F., Festing, M.F., Fisher, E.M., 2005. The origins and uses of mouse outbred stocks. Nat Genet 37, 1181-1186.
Clapcote, S.J., Lazar, N.L., Bechard, A.R., Wood, G.A., Roder, J.C., 2005. NIH Swiss and Black Swiss mice have retinal degeneration and performance deficits in cognitive tests. Comparative medicine 55, 310-316.
Cone, F.E., Gelman, S.E., Son, J.L., Pease, M.E., Quigley, H.A., 2010. Differential susceptibility to experimental glaucoma among 3 mouse strains using bead and viscoelastic injection. Exp Eye Res 91, 415-424.
Cone, F.E., Steinhart, M.R., Oglesby, E.N., Kalesnykas, G., Pease, M.E., Quigley, H.A., 2012. The effects of anesthesia, mouse strain and age on intraocular pressure and an improved murine model of experimental glaucoma. Exp Eye Res 99, 27-35.
Corsaro, C., Scalia, M., Blanco, A.R., Aiello, I., Sichel, G., 1995. Melanins in Physiological Conditions Protect Against Lipoperoxidation. A Study on Albino and Pigmented Xenopus. Pigment Cell Research 8, 279-282.
Cronin, C.A., Ryan, A.B., Talley, E.M., Scrable, H., 2003. Tyrosinase expression during neuroblast divisions affects later pathfinding by retinal ganglion cells. J Neurosci 23, 11692-11697.
Cuenca, N., Pinilla, I., Fernandez-Sanchez, L., Salinas-Navarro, M., Alarcon-Martinez, L., Aviles-Trigueros, M., de la Villa, P., Miralles de Imperial, J., Villegas-Perez, M.P., Vidal-Sanz, M., 2010. Changes in the inner and outer retinal layers after acute increase of the intraocular pressure in adult albino Swiss mice. Exp Eye Res 91, 273-285.
Dekeyster, E., Aerts, J., Valiente-Soriano, F.J., De Groef, L., Vreysen, S., Salinas-Navarro, M., Vidal-Sanz, M., Arckens, L., Moons, L., 2015a. Ocular Hypertension Results in Retinotopic Alterations in the Visual Cortex of Adult Mice. Curr Eye Res 23, 1-15.
Dekeyster, E., Geeraerts, E., Buyens, T., Van den Haute, C., Baekelandt, V., De Groef, L., Salinas-Navarro, M., Moons, L., 2015b. Tackling Glaucoma from within the Brain: An Unfortunate Interplay of BDNF and TrkB. PLoS One 10, e0142067.
Dietz, J.A., Li, Y., Chung, L.M., Yandell, B.S., Schlamp, C.L., Nickells, R.W., 2008. Rgcs1, a dominant QTL that affects retinal ganglion cell death after optic nerve crush in mice. BMC Neurosci 9, 1471-2202.
Domenici, L., Origlia, N., Falsini, B., Cerri, E., Barloscio, D., Fabiani, C., Sansò, M., Giovannini, L., 2014. Rescue of Retinal Function by BDNF in a Mouse Model of Glaucoma. PLoS One 9, e115579.
Douglas, R.M., Alam, N.M., Silver, B.D., McGill, T.J., Tschetter, W.W., Prusky, G.T., 2005. Independent visual threshold measurements in the two eyes of freely moving rats and mice using a virtual-reality optokinetic system. Visual neuroscience 22, 677-684.
Drager, U.C., Olsen, J.F., 1980. Origins of crossed and uncrossed retinal projections in pigmented and albino mice. J Comp Neurol 191, 383-412.
Eisenhofer, G., Tian, H., Holmes, C., Matsunaga, J., Roffler-Tarlov, S., Hearing, V.J., 2003. Tyrosinase: a developmentally specific major determinant of peripheral dopamine. The FASEB Journal 17, 1248-1255.
Ernst, A.F., Gallo, G., Letourneau, P.C., McLoon, S.C., 2000. Stabilization of growing retinal axons by the combined signaling of nitric oxide and brain-derived neurotrophic factor. J Neurosci 20, 1458-1469.
Fahy, E.T., Chrysostomou, V., Crowston, J.G., 2015. Impaired Axonal Transport and Glaucoma. Curr Eye Res, 1-11.
Fernandes, K.A., Harder, J.M., John, S.W., Shrager, P., Libby, R.T., 2014. DLK-dependent signaling is important for somal but not axonal degeneration of retinal ganglion cells following axonal injury. Neurobiology of Disease 69, 108-116.
Fu, C.T., Sretavan, D., 2010. Laser-induced ocular hypertension in albino CD-1 mice. Invest Ophthalmol Vis Sci 51, 980-990.
Fujiyama, F., Hioki, H., Tomioka, R., Taki, K., Tamamaki, N., Nomura, S., Okamoto, K., Kaneko, T., 2003. Changes of immunocytochemical localization of vesicular glutamate transporters in the rat visual system after the retinofugal denervation. J Comp Neurol 465, 234-249.
Galindo-Romero, C., Aviles-Trigueros, M., Jimenez-Lopez, M., Valiente-Soriano, F.J., Salinas-Navarro, M., Nadal-Nicolas, F., Villegas-Perez, M.P., Vidal-Sanz, M., Agudo-Barriuso, M., 2011.

763 Axotomy-induced retinal ganglion cell death in adult mice: quantitative and topographic time course
764 analyses. Exp Eye Res 92, 377-387.
765 Galindo-Romero, C., Valiente-Soriano, F.J., Jiménez-López, M., García-Ayuso, D., Villegas-Pérez,
766 M.P., Vidal-Sanz, M., Agudo-Barriuso, M., 2013. Effect of Brain-Derived Neurotrophic Factor on
767 Mouse Axotomized Retinal Ganglion Cells and Phagocytic Microglia. Investigative Ophthalmology
768 & Visual Science 54, 974-985.
769 Geeraerts, E., Dekeyster, E., Gaublomme, D., Salinas-Navarro, M., De Groef, L., Moons, L., 2015. A
770 freely available semi-automated method for quantifying retinal ganglion cells in entire retinal
771 flatmounts. submitted to Experimental Eye Research.
772 Gimenez, E., Lavado, A., Giraldo, P., Cozar, P., Jeffery, G., Montoliu, L., 2004. A transgenic mouse
773 model with inducible Tyrosinase gene expression using the tetracycline (Tet-on) system allows
774 regulated rescue of abnormal chiasmatic projections found in albinism. Pigment cell research /
775 sponsored by the European Society for Pigment Cell Research and the International Pigment Cell
776 Society 17, 363-370.
777 Giolli, R.A., Creel, D.J., 1973. The primary optic projections in pigmented and albino guinea pigs: an
778 experimental degeneration study. Brain Res 55, 25-39.
779 Haddadin, R.I., Oh, D.-J., Kang, M.H., Filippopoulos, T., Gupta, M., Hart, L., Sage, E.H., Rhee, D.J.,
780 2009. SPARC-null Mice Exhibit Lower Intraocular Pressures. Investigative Ophthalmology & Visual
781 Science 50, 3771-3777.
782 Haddadin, R.I., Oh, D.-J., Kang, M.H., Villarreal, G., Kang, J.-H., Jin, R., Gong, H., Rhee, D.J., 2012.
783 Thrombospondin-1 (TSP1)–Null and TSP2-Null Mice Exhibit Lower Intraocular Pressures.
784 Investigative Ophthalmology & Visual Science 53, 6708-6717.
785 Hart, A.W., McKie, L., Morgan, J.E., Gautier, P., West, K., Jackson, I.J., Cross, S.H., 2005.
786 Genotype-phenotype correlation of mouse pde6b mutations. Invest Ophthalmol Vis Sci 46, 3443-
787 3450.
788 Jeffery, G., Brem, G., Montoliu, L., 1997. Correction of retinal abnormalities found in albinism by
789 introduction of a functional tyrosinase gene in transgenic mice and rabbits. Brain research.
790 Developmental brain research 99, 95-102.
791 Jeffery, G., Schutz, G., Montoliu, L., 1994. Correction of abnormal retinal pathways found with
792 albinism by introduction of a functional tyrosinase gene in transgenic mice. Dev Biol 166, 460-464.
793 John, S.W., Hagaman, J.R., MacTaggart, T.E., Peng, L., Smithes, O., 1997. Intraocular pressure in
794 inbred mouse strains. Invest Ophthalmol Vis Sci 38, 249-253.
795 Johnson, T.V., Tomarev, S.I., 2010. Rodent models of glaucoma. Brain Research Bulletin 81, 349-
796 358.
797 Kalia, L.V., Kalia, S.K., Salter, M.W., 2008. NMDA receptors in clinical neurology: excitatory times
798 ahead. Lancet Neurol 7, 742-755.
799 Kashii, S., Takahashi, M., Mandai, M., Shimizu, H., Honda, Y., Sasa, M., Ujihara, H., Tamura, Y.,
800 Yokota, T., Akaike, A., 1994. Protective action of dopamine against glutamate neurotoxicity in the
801 retina. Invest Ophthalmol Vis Sci 35, 685-695.
802 Kitaoka, Y., Kumai, T., Isenoumi, K., Kitaoka, Y., Motoki, M., Kobayashi, S., Ueno, S., 2003.
803 Neuroprotective effect of nitric oxide against NMDA-induced neurotoxicity in the rat retina is
804 associated with tyrosine hydroxylase expression. Brain Research 977, 46-54.
805 Lavado, A., Montoliu, L., 2006. New animal models to study the role of tyrosinase in normal retinal
806 development. Front Biosci 11, 743-752.
807 Lebrun-Julien, F., Duplan, L., Pernet, V., Osswald, I., Sapieha, P., Bourgeois, P., Dickson, K., Bowie,
808 D., Barker, P.A., Di Polo, A., 2009. Excitotoxic death of retinal neurons in vivo occurs via a non-cell-
809 autonomous mechanism. J Neurosci 29, 5536-5545.
810 Leske, M.C., Heijl, A., Hyman, L., Bengtsson, B., Dong, L., Yang, Z., 2007. Predictors of long-term
811 progression in the early manifest glaucoma trial. Ophthalmology 114, 1965-1972.
812 Li, Y., Semaan, S.J., Schlamp, C.L., Nickells, R.W., 2007. Dominant inheritance of retinal ganglion
813 cell resistance to optic nerve crush in mice. BMC Neuroscience 8, 19-19.
814 Libby, R.T., Gould, D.B., Anderson, M.G., John, S.W., 2005. Complex genetics of glaucoma
815 susceptibility. Annu Rev Genomics Hum Genet 6, 15-44.

Libby, R.T., Smith, R.S., Savinova, O.V., Zabaleta, A., Martin, J.E., Gonzalez, F.J., John, S.W., 2003. Modification of ocular defects in mouse developmental glaucoma models by tyrosinase. Science 299, 1578-1581.
Lund, R.D., 1975. Variations in the laterality of the central projections of retinal ganglion cells. Exp Eye Res 21, 193-203.
Martin, K.R., Quigley, H.A., Valenta, D., Kielczewski, J., Pease, M.E., 2006. Optic nerve dynein motor protein distribution changes with intraocular pressure elevation in a rat model of glaucoma. Exp Eye Res 83, 255-262.
Matsumoto, H., Kataoka, K., Tsoka, P., Connor, K.M., Miller, J.W., Vavvas, D.G., 2014. Strain difference in photoreceptor cell death after retinal detachment in mice. Invest Ophthalmol Vis Sci 55, 4165-4174.
Mattapallil, M.J., Wawrousek, E.F., Chan, C.C., Zhao, H., Roychoudhury, J., Ferguson, T.A., Caspi, R.R., 2012. The Rd8 mutation of the Crb1 gene is present in vendor lines of C57BL/6N mice and embryonic stem cells, and confounds ocular induced mutant phenotypes. Invest Ophthalmol Vis Sci 53, 2921-2927.
McKinnon, S.J., Schlamp, C.L., Nickells, R.W., 2009. Mouse models of retinal ganglion cell death and glaucoma. Exp Eye Res 88, 816-824.
Nadal-Nicolas, F.M., Jimenez-Lopez, M., Sobrado-Calvo, P., Nieto-Lopez, L., Canovas-Martinez, I., Salinas-Navarro, M., Vidal-Sanz, M., Agudo, M., 2009. Brn3a as a marker of retinal ganglion cells: qualitative and quantitative time course studies in naive and optic nerve-injured retinas. Invest Ophthalmol Vis Sci 50, 3860-3868.
Nguyen, C., Cone, F.E., Nguyen, T.D., Coudrillier, B., Pease, M.E., Steinhart, M.R., Oglesby, E.N., Jefferys, J.L., Quigley, H.A., 2013. Studies of scleral biomechanical behavior related to susceptibility for retinal ganglion cell loss in experimental mouse glaucoma. Invest Ophthalmol Vis Sci 54, 1767-1780.
Nickells, R.W., 2007. From ocular hypertension to ganglion cell death: a theoretical sequence of events leading to glaucoma. Can J Ophthalmol 42, 278-287.
Osborne, N.N., Melena, J., Chidlow, G., Wood, J.P.M., 2001. A hypothesis to explain ganglion cell death caused by vascular insults at the optic nerve head: possible implication for the treatment of glaucoma. British Journal of Ophthalmology 85, 1252-1259.
Palumbo, A., Poli, A., Di Cosmo, A., d'Ischia, M., 2000. N-Methyl-D-aspartate receptor stimulation activates tyrosinase and promotes melanin synthesis in the ink gland of the cuttlefish Sepia officinalis through the nitric Oxide/cGMP signal transduction pathway. A novel possible role for glutamate as physiologic activator of melanogenesis. J Biol Chem 275, 16885-16890.
Panagis, L., Zhao, X., Ge, Y., Ren, L., Mittag, T.W., Danias, J., 2011. Retinal Gene Expression Changes Related to IOP Exposure and Axonal Loss in DBA/2J Mice. Investigative Ophthalmology & Visual Science 52, 7807-7816.
Parrilla-Reverter, G., Agudo, M., Nadal-Nicolas, F., Alarcon-Martinez, L., Jimenez-Lopez, M., Salinas-Navarro, M., Sobrado-Calvo, P., Bernal-Garro, J.M., Villegas-Perez, M.P., Vidal-Sanz, M., 2009. Time-course of the retinal nerve fibre layer degeneration after complete intra-orbital optic nerve transection or crush: a comparative study. Vision Res 49, 2808-2825.
Porebska-Budny, M., Sakina, N.L., Stepien, K.B., Dontsov, A.E., Wilczok, T., 1992. Antioxidative activity of synthetic melanins. Cardiolipin liposome model. Biochim Biophys Acta 5, 11-16.
Prusky, G.T., Alam, N.M., Beekman, S., Douglas, R.M., 2004. Rapid quantification of adult and developing mouse spatial vision using a virtual optomotor system. Invest Ophthalmol Vis Sci 45, 4611-4616.
Quigley, H.A., Broman, A.T., 2006. The number of people with glaucoma worldwide in 2010 and 2020. Br J Ophthalmol 90, 262-267.
Rebsam, A., Bhansali, P., Mason, C.A., 2012. Eye-specific projections of retinogeniculate axons are altered in albino mice. J Neurosci 32, 4821-4826.
Reed, J.C., Doctor, K., Rojas, A., Zapata, J.M., Stehlik, C., Fiorentino, L., Damiano, J., Roth, W., Matsuzawa, S.-i., Newman, R., Takayama, S., Marusawa, H., Xu, F., Salvesen, G., Riken Ger Group Laboratory for Genome Exploration Research Group, R.G.S.C.R.Y.I.S.-c.T.-k.Y.K.J., Gsl Members Genome Science Laboratory, R.H.W.S.J., Godzik, A., 2003. Comparative Analysis of Apoptosis and Inflammation Genes of Mice and Humans. Genome Research 13, 1376-1388.

Rice, D.S., Williams, R.W., Goldowitz, D., 1995. Genetic control of retinal projections in inbred strains of albino mice. J Comp Neurol 354, 459-469.
Roffler-Tarlov, S., Liu, J.H., Naumova, E.N., Bernal-Ayala, M.M., Mason, C.A., 2013. L-Dopa and the Albino Riddle: Content of L-Dopa in the Developing Retina of Pigmented and Albino Mice. PLoS One 8, e57184.
Roh, M., Zhang, Y., Murakami, Y., Thanos, A., Lee, S.C., Vavvas, D.G., Benowitz, L.I., Miller, J.W., 2012. Etanercept, a Widely Used Inhibitor of Tumor Necrosis Factor-α (TNF- α), Prevents Retinal Ganglion Cell Loss in a Rat Model of Glaucoma. PLoS One 7, e40065.
Safa, R., Osborne, N.N., 2000. Retinas from albino rats are more susceptible to ischaemic damage than age-matched pigmented animals. Brain Res 862, 36-42.
Salinas-Navarro, M., Alarcon-Martinez, L., Valiente-Soriano, F.J., Ortin-Martinez, A., Jimenez-Lopez, M., Aviles-Trigueros, M., Villegas-Perez, M.P., de la Villa, P., Vidal-Sanz, M., 2009a. Functional and morphological effects of laser-induced ocular hypertension in retinas of adult albino Swiss mice. Mol Vis 15, 2578-2598.
Salinas-Navarro, M., Jimenez-Lopez, M., Valiente-Soriano, F.J., Alarcon-Martinez, L., Aviles-Trigueros, M., Mayor, S., Holmes, T., Lund, R.D., Villegas-Perez, M.P., Vidal-Sanz, M., 2009b. Retinal ganglion cell population in adult albino and pigmented mice: a computerized analysis of the entire population and its spatial distribution. Vision Res 49, 637-647.
Savinova, O.V., Sugiyama, F., Martin, J.E., Tomarev, S.I., Paigen, B.J., Smith, R.S., John, S.W., 2001. Intraocular pressure in genetically distinct mice: an update and strain survey. BMC genetics 2, 12.
Scalia, M., Geremia, E., Corsaro, C., Santoro, C., Baratta, D., Sichel, G., 1990. Lipid Peroxidation in Pigmented and Unpigmented Liver Tissues: Protective Role of Melanin. Pigment Cell Research 3, 115-119.
Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P., Cardona, A., 2012. Fiji: an open-source platform for biological-image analysis. Nat Meth 9, 676-682.
Schlamp, C.L., Johnson, E.C., Li, Y., Morrison, J.C., Nickells, R.W., 2001. Changes in Thy1 gene expression associated with damaged retinal ganglion cells. Mol Vis 7, 192-201.
Schmidt, J.T., 2004. Activity-driven sharpening of the retinotectal projection: the search for retrograde synaptic signaling pathways. J Neurobiol 59, 114-133.
Serfilippi, L.M., Pallman, D.R., Gruebbel, M.M., Kern, T.J., Spainhour, C.B., 2004. Assessment of retinal degeneration in outbred albino mice. Comparative medicine 54, 69-76.
Steele, M.R., Inman, D.M., Calkins, D.J., Horner, P.J., Vetter, M.L., 2006. Microarray analysis of retinal gene expression in the DBA/2J model of glaucoma. Invest Ophthalmol Vis Sci 47, 977-985.
Templeton, J.P., Nassr, M., Vazquez-Chona, F., Freeman-Anderson, N.E., Orr, W.E., Williams, R.W., Geisert, E.E., 2009. Differential response of C57BL/6J mouse and DBA/2J mouse to optic nerve crush. BMC Neuroscience 10, 90-90.
Tezel, G., Yang, X., Yang, J., Wax, M.B., 2004. Role of tumor necrosis factor receptor-1 in the death of retinal ganglion cells following optic nerve crush injury in mice. Brain Res 996, 202-212.
Tham, Y.-C., Li, X., Wong, T.Y., Quigley, H.A., Aung, T., Cheng, C.-Y., 2014. Global Prevalence of Glaucoma and Projections of Glaucoma Burden through 2040. Ophthalmology 121, 2081-2090.
Tilleux, S., Hermans, E., 2007. Neuroinflammation and regulation of glial glutamate uptake in neurological disorders. Journal of Neuroscience Research 85, 2059-2070.
Vaarmann, A., Kovac, S., Holmstrom, K.M., Gandhi, S., Abramov, A.Y., 2013. Dopamine protects neurons against glutamate-induced excitotoxicity. Cell Death Dis 4, e455.
Valiente-Soriano, F.J., Salinas-Navarro, M., Jiménez-López, M., Alarcón-Martínez, L., Ortín-Martínez, A., Bernal-Garro, J.M., Avilés-Trigueros, M., Agudo-Barriuso, M., Villegas-Pérez, M.P., Vidal-Sanz, M., 2015. Effects of Ocular Hypertension in the Visual System of Pigmented Mice. PLoS One 10, e0121134.
Valverde, P., Manning, P., McNeil, C.J., Thody, A.J., 1996. Activation of tyrosinase reduces the cytotoxic effects of the superoxide anion in B16 mouse melanoma cells. Pigment cell research / sponsored by the European Society for Pigment Cell Research and the International Pigment Cell Society 9, 77-84.

Vidal-Sanz, M., Salinas-Navarro, M., Nadal-Nicolas, F.M., Alarcon-Martinez, L., Valiente-Soriano, F.J., Miralles de Imperial, J., Aviles-Trigueros, M., Agudo-Barriuso, M., Villegas-Perez, M.P., 2012. Understanding glaucomatous damage: Anatomical and functional data from ocular hypertensive rodent retinas. Prog Retin Eye Res 31, 1-27.
Welsbie, D.S., Yang, Z., Ge, Y., Mitchell, K.L., Zhou, X., Martin, S.E., Berlinicke, C.A., Hackler, L., Jr., Fuller, J., Fu, J., Cao, L.H., Han, B., Auld, D., Xue, T., Hirai, S., Germain, L., Simard-Bisson, C., Blouin, R., Nguyen, J.V., Davis, C.H., Enke, R.A., Boye, S.L., Merbs, S.L., Marsh-Armstrong, N., Hauswirth, W.W., DiAntonio, A., Nickells, R.W., Inglese, J., Hanes, J., Yau, K.W., Quigley, H.A., Zack, D.J., 2013. Functional genomic screening identifies dual leucine zipper kinase as a key mediator of retinal ganglion cell death. Proc Natl Acad Sci U S A 110, 4045-4050.
Whitney, I.E., Raven, M.A., Lu, L., Williams, R.W., Reese, B.E., 2011. A QTL on chromosome 10 modulates cone photoreceptor number in the mouse retina. Invest Ophthalmol Vis Sci 52, 3228-3236.
Williams, R.W., Strom, R.C., Rice, D.S., Goldowitz, D., 1996. Genetic and environmental control of variation in retinal ganglion cell number in mice. J Neurosci 16, 7193-7205.
Wong, A.A., Brown, R.E., 2006. Visual detection, pattern discrimination and visual acuity in 14 strains of mice. Genes, brain, and behavior 5, 389-403.

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

LDG and ED designed the study, performed the experiments and prepared the manuscript; EG, EL, and MSN performed the experiments; LDG, ED, IS, and LM contributed to the analysis and interpretation of the data; LM designed the study and prepared the manuscript. All authors read and approved the final manuscript.

Figure captions

Figure 1.

Retinal histology and RGC density in C57Bl/6, C57Bl/6-Tyr^c, and CD-1 mice. (a-d) *In vivo* OCT scans of C57Bl/6, C57Bl/6-Tyr^c, and CD-1 mice. Representative image of N=10 per strain; scale bar: 50 µm. (e-h) Hematoxylin and eosin-stained transverse sections of the retina of C57Bl/6, C57Bl/6-Tyr^c, and CD-1 mice. Representative image of N=4 per strain; scale bar: 20 µm. (d, h) In our colony, 21% of the CD-1 mice are carrying a homozygous *rd1/rd1* mutation, resulting in a total loss of the outer retinal layers due to photoreceptor degeneration. (i) A detailed morphometric analysis on retinal sections reveals, upon exclusion of CD-1 mice carrying the *rd1* mutation, no significant differences in the thickness of any retinal layer (N=4, two-way ANOVA). (j) Evaluation of RGC density on retinal flatmounts points out a higher number of RGCs per mm² in CD-1 mice (N=10, one-way ANOVA). Data are presented as mean ± SEM. NFL: nerve fiber layer, GCL: ganglion cell layer; IPL: inner plexiform layer, INL: inner nuclear layer; OPL: outer plexiform layer, ONL: outer nuclear layer; PRL: photoreceptor layer; total: thickness of the entire retina, measured from the NFL till the ONL.

Figure 2.

Anterograde tracing of retinofugal projections to the dLGN in C57Bl/6, C57Bl/6- Tyr^c, and CD-1 mice, within the rostrocaudal region between Bregma -2.10 mm and -2.60 mm. (a) The dLGN is outlined based on a VGluT2 immunostaining. In C57Bl/6-Tyr^c and CD-1 mice, the ipsilateral retinogeniculate projection is more diffuse, as compared to the C57Bl/6 mice, and the core of the ipsilateral zone within the contralateral dLGN (arrow) is not well delineated. (b) In C57Bl/6 mice, there is no difference in the density of ipsilateral *versus* contralateral RGC axon termini. In contrast, in C57Bl/6-Tyr^c and CD-1 mice, ipsilateral termini are less dense compared to contralateral projections (N=3-4, two-way ANOVA) [x]. When comparing ipsilateral projections among the three strains, both albino strains show lower values as compared to the pigmented C57Bl/6 strain (N=3-4, one-way ANOVA) [y]. In addition, C57Bl/6-Tyr^c and CD-1 mice have more contralateral projecting RGCs terminating within the ipsilateral core (N=3-4, one-way ANOVA) [z]. (c) Transverse section of the dLGN, with a schematic representation of the theoretical RGC projection zones, along with the ROIs (cfr. methodology section) in which CTB labeling is analyzed. (d) Separated from the bulk of contralateral axon terminals, a patch of contralateral termini (arrowhead) is detected in all four C57Bl/6-Tyr^c and in two out of four CD-1 mice. This phenotype is never observed in C57Bl/6 wild types. (e) In C57Bl/6-Tyr^c mice, the density of these RGC axon terminals in the ROI ‘contra patch’ is higher compared to the density within the neighboring contralateral zone (N=4, Student’s *t*-test). Data are presented as mean ± SEM.

Figure 3.

Anterograde tracing of retinofugal projections to the SC in C57Bl/6, C57Bl/6- Tyr^c, and CD-1 mice, within the rostrocaudal region between Bregma -3.16 mm and -4.84 mm. (a) The boundary (dotted line) between the visually driven layers of the sSC, including the stratum zonale (SZ), stratum griseum superficiale (SGS), and stratum opticum (SO), and the underlying deep SC, is outlined based on a VGluT2 immunostaining. Ipsilateral retinocollicular projections mainly terminate in the lower sSC. Cross-section 1: clearly distinct ipsilateral patches in the SO are visible in the rostral sSC of C57Bl/6 and C57Bl/6-Tyr^c mice, but this zone is more continuous in CD-1 animals. In addition, in C57Bl/6 and C7Bl/6-Tyr^c mice, a densely labeled small patch of ipsilateral projections is observed in the medial SZ (arrowhead). Cross-sections 2 and 3: the rostrocaudal tube-like ipsilateral projection zone (arrows) is densely labeled with CTB signal in C57Bl/6 mice, while in both albino strains, the CTB signal appears more diffuse. In CD-1 mice particularly, the shape of this tube is rather stretched along the lateromedial axis as compared to the round shape in C57Bl/6 and C57Bl/6-Tyr^c mice. Scale bars: 200 μm. (b) Left: top view on both SCi, dotted lines mark the rostrocaudal position of the coronal sections. Right: coronal sections of the SCi, illustrating theoretical projections from the CTB-injected eye. (c) Visual acuity in albino mice is significantly reduced in comparison to the C57Bl/6 strain. Moreover, CD-1 mice display an even worse visual acuity than C57Bl/6-Tyr^c mice (N=10, one-way ANOVA). Data are presented as mean ± SEM.

Figure 4.

RGC survival in C57Bl/6, C57Bl/6-Tyr^{c}, and CD-1 mice after induction of glaucoma by means of intravitreal injection of NMDA, ONC, and LP of the episcleral and perilimbal vessels. (a) At 4 dpi of NMDA, C57Bl/6, C57Bl/6-Tyr^{c} and CD-1 mice display significantly different RGC survival rates (N=5-9, two-way ANOVA). (b) Survival of Brn3a^{+} RGCs at 7 days post ONC is identical in C57Bl/6, C57Bl/6-Tyr^{c}, and CD-1 mice (N=7-11, one-way ANOVA). (c) Adjustment of the laser parameters for each strain results in overlapping IOP profiles for C57Bl/6, C57Bl/6-Tyr^{c}, and CD-1 mice after LP of the episcleral and perilimbal vessels (N=10-11, repeated measures two-way ANOVA). (d) Average survival of Brn3a^{+} RGCs at 14 days post LP is similar in C57Bl/6 and C57Bl/6-Tyr^{c}, yet lower in CD-1 mice (N=10-11, one-way ANOVA). Data are presented as mean ± SEM.

Figure 5.

Loss of axonal transport and diminished visual function, precede ganglion cell loss in an ocular hypertension-induced glaucoma model. (a) At 14 dpi, C57Bl/6 and C57Bl/6-Tyr^{c} *versus* CD-1 mice display significantly different RGC survival rates (N=9-10, two-way ANOVA). The number of Brn3a^{+} RGCs is expressed relative (%) to the number of Brn3a^{+} RGCs in naive retinas. (b) A similar trend, *i.e.* higher disease severity in CD-1 mice compared to the C57Bl/6 background, is seen when axonal transport integrity is evaluated by means of OHSt retrograde labeling at 4 dpi (N=7-9, two-way ANOVA). The number of OHSt^{+} RGCs is expressed relative (%) to the number of OHSt^{+} RGCs in naive retinas. (c) At 4 dpi, no RGC death is observable, while the first signs of axonal transport deficits are emerging. As a result, all RGCs retain Brn3a labeling yet only a subset can still be visualized via retrograde labeling with OHSt. Arrow: example of Brn3a^{+} OHSt^{-} RGC. Scale bar, 50 µm. (d) Visual acuity starts to decrease as soon as 1 dpi, and this deterioration in visual function appears to progress faster in CD-1 mice as compared to C57Bl/6-Tyr^{c} mice (N=8-10, two-way ANOVA). Data are presented as mean ± SEM.