In search for the “idyllic” animal model to evaluate ocular pathologies and translate new therapies to improve human health

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The retina is a highly specialized neural tissue that encodes information of vision, a vital sensory modality for most species. Although the retina also contributes to other non-image-forming signals (e.g. entrainment of circadian clocks), vision chiefly enables animals to interpret and navigate their environment (despite the diverse variety of species and lifestyles; Baden et al., 2020) and also to interact with other animals. Thus, features such as high visual resolution and color discrimination are important for interactive behaviors like prey capture and also for social animals, including primates, that establish a dominance hierarchy.

Among vertebrates, the laminar structure of the retina is notably conserved. The photoreceptor layer (rods and cones) initiates the process of converting light energy into nerve impulses by absorbing photons and transferring the signals to interneurons. These signals ultimately converge onto retinal ganglion cells (RGCs), the only afferent neurons in the retina. RGCs are responsible for communicating the pre-processed information to the brain through their axons that form the optic nerve (ON). Mature RGCs, like the rest of the central nervous system, lack regenerative ability. Because all visual information is transmitted by RGCs, their dysfunction or degeneration can lead to significant visual impairment and permanent vision loss. Currently, there is no effective treatment to prevent RGC loss due to injury or degeneration.

RGC death occurs after damage to the optic nerve resulting from traumatic injury, glaucoma, other optic neuropathies, and may occur in other neurodegenerative disorders such as Parkinson’s disease and Alzheimer’s disease (La Morgia et al., 2017). Thus, to elucidate the underlying events of different neuro-ocular disorders, scientists model human diseases in an extensive variety of biological systems. Although eye development, anatomy, and function are empirically similar among vertebrates, some animal models are more amenable to genetic manipulation, while others are more suitable to be successfully translated to therapeutic strategies in humans. However, it is difficult to find a perfect animal model that shares all these advantages and fully phenocopies human disease. Thus, the need to study human pathologies in experimental animals that are most similar to us is widely recognized. Non-human primates are ideal (Picaud et al., 2019), but their use has practical limitations, which include high cost, time, and more important ethical considerations. For these reasons, small mammals became a popular choice in biomedical research.

Experiments conducted on rodents have contributed to scientific discoveries for decades and their use is still growing. They reproduce quickly, are highly adaptable, and easy to handle. Among them, mouse and rat are most commonly used. In fact, due to the availability of tools for manipulation of the mouse genome, scientists have the ability to precisely add, mutate, or remove genes developing more accurate models of human disease and pathological processes (Nguyen and Xu, 2008). Recent advances in genome editing have revolutionized the field of biomedical research and further reinforce why mice have become the principal animal model selected for the study. However, as nocturnal mammals, mouse and rat retinas have anatomical differences from primates. Thus, although nocturnal rodents also possess cone photoreceptors for color vision (Nadal-Nicolás et al., 2020), the retina is rod-dominated and therefore more sensitive and better adapted to scotopic conditions. Rods also greatly outnumber cones in the primate retina and are present at high density throughout the retina, but their density declines in the central retina which contains a higher density of cones. This specialized region is a unique feature of primate vision, called the macula, and is characterized histologically by the presence of a ganglion cell layer with multiple RGC-rows in addition to being a highly cone-rich region. Its importance lies in contributing to high-resolution central vision. At its center is the fovea centralis, a depression that pushes aside most of the retinal layers, except the narrow and elongated cones for maximizing visual acuity.

In our pursuit to establish a more representative model for RGC degenerative diseases, we have explored the threeline-ground squirrel (TLGS) retina (Xiao et al., 2021). Unlike the above-mentioned rodents, the TLGS is diurnal with a cone-dominated retina (Li, 2020). These cones have peak density at the equatorial retina termed the visual streak. Comparative analysis in central retinas demonstrated remarkable parallelism between the visual streak in TLGS and the perifoveal area of the macula in primates. Both possess a ganglion cell layer that contains many RGC-rows with RGCs predominantly placed towards the inner layers, while other neurons are settled at the outer row (e.g. ChAT+ amacrine cells; Figure 1A). The total RGC population in TLGS identified using Brn3a, a specific RGC marker (Nadal-Nicolás et al., 2009, 2015), is approximately half that estimated for primate retina (~60,000 and 1.1–1.3 million RGCs in TLGS and primates respectively; Curcio and Allen, 1990; Figure 1B). However, if the difference in retinal area is taken into consideration, the thickness of the retina also share a similar average density. Although the TLGS retina lacks a fovea, the high density of RGCs at the visual streak more closely resembles the macula in primates (~10,000 and ~15,000 RGCs/mm², TLGS, and primate respectively; Curcio and Allen, 1990; Xiao et al., 2021). Thus, it is fair to speculate that, the visual streak in TLGS provides a better representation of the central visual field than other rodents because (i) the higher cone density provides high-resolution image formation and, (ii) the higher RGC density extends large projections to the higher visual centers in the brain. Both features are anatomically shared by the macula in primates and contribute to making them the thickest portion of the retina as observed in OCT scans (optical coherence tomography; Figure 1C).

Although a thicker retina would be detrimental for visual sensitivity due to higher light scattering and image blurring, the visual streak and macula of primates could potentially compensate by implementing mechanisms to better guide and focus the incident light. Regardless, the visual streak is not expected to reach the acuity level provided by the thinner fovea in primates (Figure 1C).

Although our vision naturally declines gradually with age, macular damage (brought on by acute trauma or chronic degenerative disease) usually causes severe vision loss because it compromises central vision. Although most macular degenerations are related to progressive cone loss, whether RGCs in the central retina are also more vulnerable in humans remains uncertain. Thus, the visual streak, a macula-like structure in TLGS, offers an excellent surrogate to explore RGC vulnerability to degeneration. Obviously, the resulting loss in vision will depend on the severity of damage to the RGCs. Therefore, to characterize RGC degeneration in the central retina, we performed a single traumatic injury at the ON in TLGS. The ON crush is characterized by progressive RGC loss that consistently leads to more than 80% RGC loss at 14 days in different species (Galindo-Romero et al., 2011; Nadal-Nicolás et al., 2009, 2015; Xiao et al., 2021; Figure 1D and E). However, we noticed more severe RGC death in the visual streak than in the peripheral retina (Xiao et al., 2021; Figure 1E), which would be the closer proximity of central RGCs to the injury site than those in the periphery. However, the degenerative kinetics of RGCs suggest that all RGCs should evenly degenerate after significant time.
Figure 1 | Retinal ganglion cell (RGC) anatomy in different mammalian species and degenerative response to axonal damage in thirteen-lined ground squirrel (TLGS).

(A) Cross-section of ganglion cell layer (GCL) in the central retina of mouse, rat, TLGS, and monkey retinas. (B) Topographical distribution, total Brn3a RGC number (shown in the upper right), and highest density (in red) of RGCs in mouse, rat, and TLGS retinas, and illustration of macaque RGC distribution based on a previous report (Curcio and Allen, 1990). (C) Images of OCT b-scans from mouse, rat, TLGS, and monkey retinas. Note similarities of RGC thickness between the visual streak in TLGS and the macular area in the macaque retina. (D) Number and topography of surviving RGCs at 14 days after optic nerve injury (arrowheads depict apoptotic RGC nuclei, cleaved-Casp3+). (E) RGCs in the peripheral retina showed a higher survival rate than those in the central retina after the same insult (**P \leq 0.01; images shown in 1A are similar to those published previously in Xiao et al., 2021). Brn3a: Brain-specific homeobox/POU domain protein 3A (RGCs); Casp3: cleaved caspase 3; CHAT: choline acetyltransferase (CHAT amacrine cells); DAPI: 4′,6-diamidino-2-phenylindole (nuclei); ONC: optic nerve crush; ONH: optic nerve head; pNFH: phosphorylated neurofilament heavy (RGC axons); RBPMs: RNA binding protein with multiple splicing (RGCs).

had elapsed, but a higher RGC proportion remained alive at the periphery by 6 or 15 months after ON axotomy in rats (Nadal-Nicolas et al., 2015). The higher metabolic rate and oxidative stress in the central retina suggest another explanation. Photoreceptors in the central retina receive more light and all this visual information is transmitted to the RGCs which may induce comparable stress on to the RGCs enhancing their vulnerability to additional pathological events (Nadal-Nicolas et al., 2015; Xiao et al., 2021).

To date, there is limited evidence indicating that RGCs are more susceptible in the macula of patients (Hood, 2015). Inferences based on experimental evidence from animal studies are difficult to translate to patients as pathological studies can only be performed postmortem. Fortunately, the eye’s transparent structure provides an open window to assess the retina, and imaging advances have exploited this feature to non-invasively evaluate the retina. Thus, the progression of RGC loss can be determined by scanning the thickness of the retinal nerve fiber layer (RNFL), constituted almost exclusively by RGC axons. Our results, at 14 days after the ON crush revealed that ~80% of the RNFL thickness remained present in the retina while only ~15% of RGC survived, corroborating previous studies (Rovere et al., 2015; Xiao et al., 2021). These findings suggest that intraretinal axons are preserved longer than RGC cell bodies even though the axons lack nutrient supply from the soma. Despite axons constituting most of the cell volume, apoptotic cell body clearance appears to be prioritized. Thus, the axonal disintegration may be delayed, driven by genetically different molecular pathways (Freeman, 2014), or even prevented by astrocytes.

We hypothesized that cell-cell interactions contributed to the delay in axonal disintegration and explored the interlocking astrocytes/axon processes in the central retinas of different mammals. The visual streak of TLGS possesses elongated astrocytes in a pattern that precisely mirrors the vitreal surface and lateral sides of the axon bundles (Figure 2A and 2B). The number of astrocytes decreases as the RNFL thins, but they remain parallel to the axons, and only a few dispersed astrocytes with typical stellate shape laid in the extreme periphery (Figure 2A).

Interestingly, astrocytes ensheathe the nerve fibers in the central retina of TLGS (Figure 2B), a pattern previously described for primates (Ogden, 1978). By contrast, rats and mice only possess star-shaped astrocytes that form punctual contacts with the axons (Figure 2B). Astrocyte/axons colocalization analysis in central retinas provides evidence of a much tighter interlock in TLGS and macaque retinas (Figure 2B). Astrocytes could contribute to the maintenance and the persistence of RGC-axons post-injury by supplying neurotrophic factors or maintaining mechanical integrity especially after astrocyte activation (enlarged somas and thicker processes) in response to pathological events. However, this enhanced RGC survival in the periphery was also observed in rats that have significantly fewer astrocyte/axon interlocks. Thus, whether a more resistant subtype of RGCs resides towards the periphery has yet to be discovered. In perspective, RGC degeneration after axonal damage occurs in a neuroinflammatory environment that involves the participation of resident microglia, infiltrating monocytes, macroglia, and their interactions. Astrocytes also contact retinal blood vessels closely following their pattern. Interestingly, in glaucomatous conditions, the GFAP reactivity of astrocytes increases around the blood vessels (Wang et al., 2002), which may indicate altered cellular properties. Thus, it is fair to speculate that under pathological conditions astrocytes undergo morpho-functional changes that may impact vascular properties and therefore influence the integrity of the blood/brain barrier or even drive neuronal death.

Therefore, it remains relatively unknown whether RGCs in the macula in humans are more susceptible to degeneration. The mismatch between RGC loss and RNFL thinning is clinically relevant and could be taken into consideration during the diagnosis of RGC loss by OCT. In this perspective, we emphasize the advancement of using the ground squirrel to model retinal degenerative diseases and to expose key anatomical differences between commonly used rodents and non-human primates. Obviously, the TLGS is not the perfect animal model, having its limitations like higher cost, lower availability, housing requirements, difficulty breeding, and lack of genetic tools (Xiao et al., 2021). However, in addition to those abovementioned similarities between TLGS and primates, the size of the eye of TLGS provides another practical advantage in comparison to other rodents. Their large vitreous body (4 µL in mouse, 20 µL in the rat, 280 µL in TLGS, 540 µL in marmoset monkey, 2–3.2 mL in large primate, and 4 mL in human), and the existence of pars plana, a peripheral extension from the neuroretina, provides easy access to the vitreous chamber to perform a variety of surgical interventions without damaging the retina (Figure 2C).

Thus, rodents still provide many insights and valuable knowledge of neuronal anatomy and function, and the TLGS retina, with its...
simplified model of the macula, can pave the way for (i) studying neurodegenerative events that compromise central (macular) vision, (ii) development of biomarkers, and (iii) evaluating strategies to target RGC neuroprotection, axon regeneration, and synaptic circuit reinnervation. Importantly, the inherent ability of TLGS to hibernate offers a novel way to develop revolutionary neuronal therapies for clinical translation and to improve human health and welfare.

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