The age factor in optic nerve regeneration: intrinsic and extrinsic barriers hinder successful functional recovery in killifish

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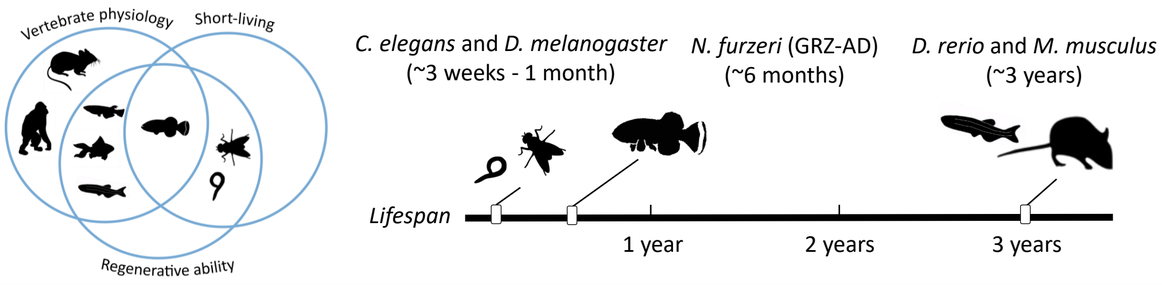
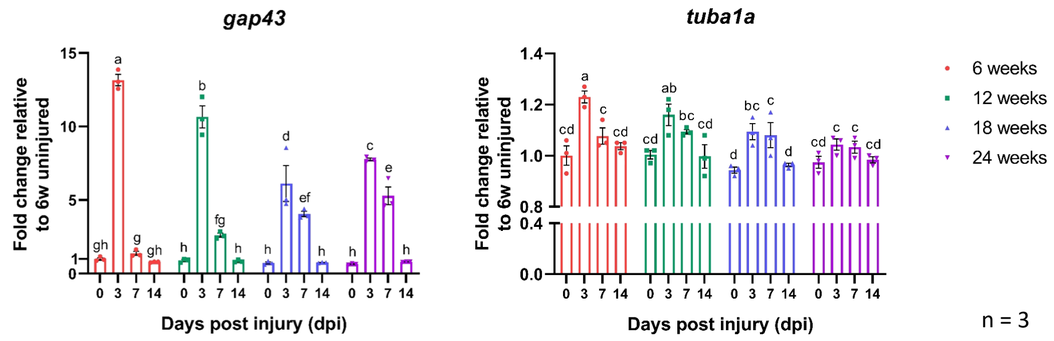
Purpose

Worldwide, people are getting older. This prolonged lifespan, however, is not necessarily accompanied by an improved health span but results in an increased prevalence of CNS insults and age-related neurodegenerative diseases. Accordingly, novel scientific challenges have emerged, and many research efforts currently focus on triggering repair in the damaged or diseased brain. Yet, stimulating neuroregeneration remains ambitious, especially in an aged environment. Over the last years, the African turquoise killifish (*Nothobranchius furzeri*) has surfaced as a very promising gerontology model, with the unique trait of being a vertebrate while having an invertebrate-like lifespan and displaying many of the aging hallmarks described for human aging. Moreover, as a teleost, this fish has a remarkable regenerative potential in its adult CNS, making it ideally suited to unravel the impact of aging on CNS repair.

The African turquoise killifish as regeneration-competent aging model



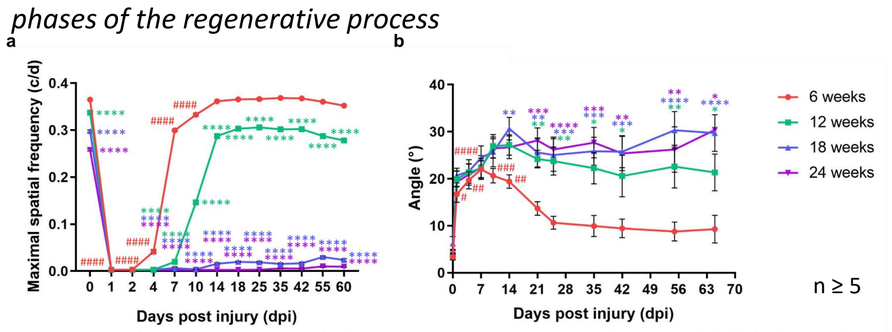
Regeneration of the optic nerve is intrinsically impaired in aged killifish



n = 3

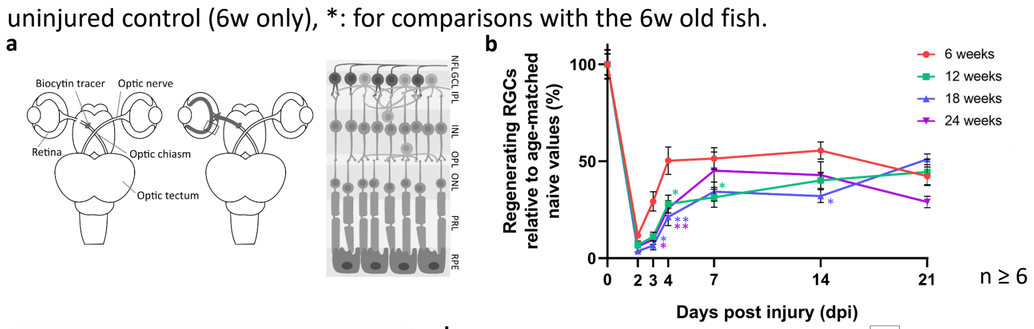
The intrinsic growth potential of RGCs is diminished in older killifish. mRNA expression studies of the growth-associated genes *gap-43* and *tuba1a* reveal a significant reduction in injury response levels at 3 dpi in 18w and 24w old killifish for *gap-43*, and in all older age groups for *tuba1a* when compared to young adult killifish. In addition to a declined expression following injury, the RGC growth response also seems delayed as *gap-43* expression levels are still elevated at 7 dpi in 12w, 18w and 24w old fish.

Depending on the age, optic nerve regeneration is impaired in different phases of the regenerative process

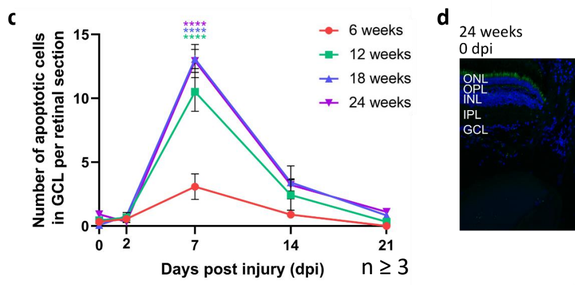


n ≥ 5

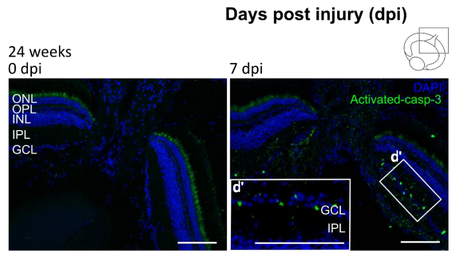
Recovery of primary vision following ONC is impaired in aged killifish. Strikingly, and in contrast to what has been observed in zebrafish, primary vision, evaluated via the optokinetic response test **(a)** and the dorsal light reflex test **(b)**, is not restored in older age groups when subjected to ONC. #: for comparisons with the uninjured control (6w only), *: for comparisons with the 6w old fish.

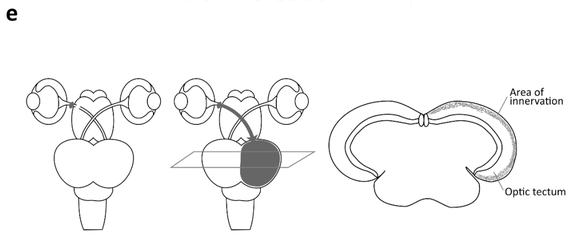


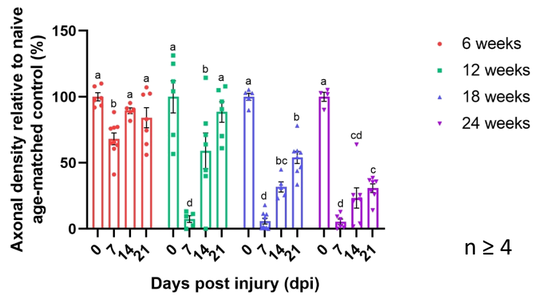
n ≥ 6



n ≥ 3

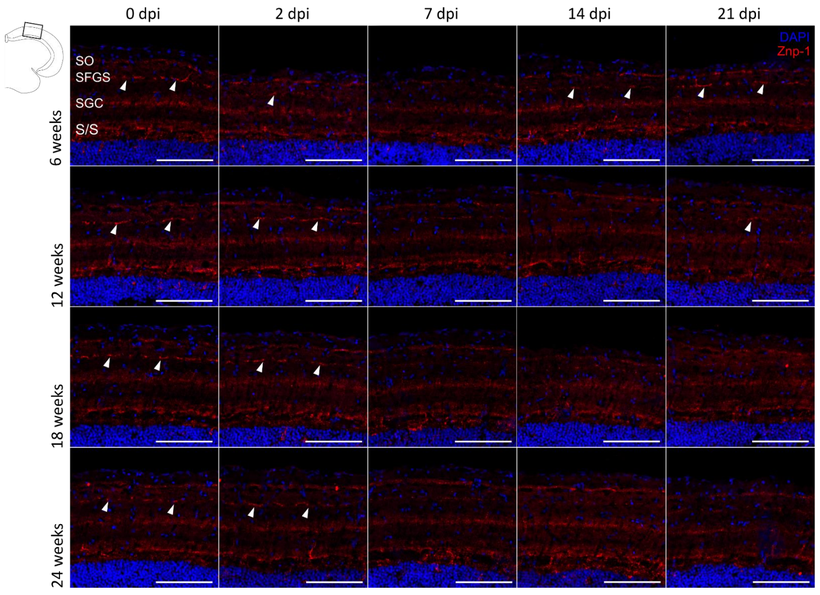






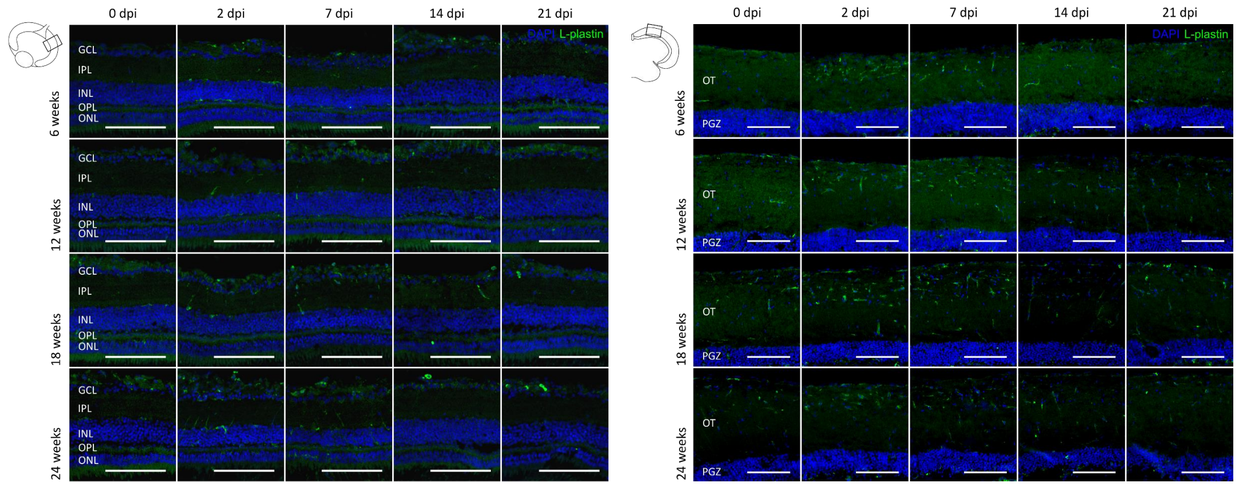
n ≥ 4

Axonal regrowth initiation and elongation are affected in aged killifish following ONC. Quantification of the number of biocytin-positive RGCs after retrograde tracing **(a)** reveals an age-related reduction in the number of regenerating RGCs in the older age groups, already visible at 3 dpi, suggesting that axonal outgrowth is impaired in the early phases of regeneration **(b)**. Contrary to zebrafish, ONC seems to induce apoptosis of RGCs in all age groups, but more pronounced in older killifish, studied via immunostainings for activated caspase-3 **(c)** and cell countings in the ganglion cell layer (data not shown). Apoptotic cells were mainly found in the central retina, where the older, more vulnerable cells are located **(d)** (magnification in d'). Scale bar = 100 µm. Anterograde biocytin tracing **(e)** shows that, while in young killifish the majority of RGC axons have reinnervated the optic tectum at 14 dpi, tectal reinnervation is strongly diminished in aged killifish. Indeed, while tectal reinnervation seems to be delayed in 12w old fish, axonal density levels within the SFGS and the SO layers of the tectum, quantified by an in-house FIJI script, do never approach naive values in 18w and 24 weeks old fish at 21 dpi **(f)**. *: for comparisons with the 6w old fish. Means with a different letter are significantly different.

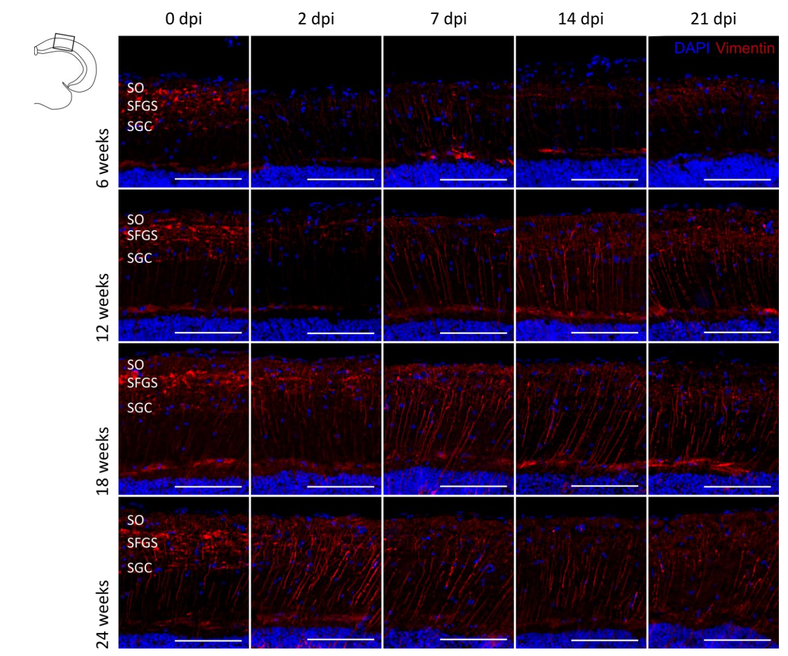


Synaptic repair following ONC is impeded in older killifish. Immunostainings for synaptotagmin, using the Znp-1 antibody on coronal brain sections, illustrate a downregulation of the pre-synaptic signal in the SFGS layer of the optic tectum in all age groups following ONC when compared to uninjured fish. In contrast to young adult killifish, the synaptotagmin signal does not reapproach baseline levels at any time point after ONC. While in middle-aged fish the synaptic vesicles might reappear at 21 dpi, no signal can be detected in the SFGS of 18w and 24w old fish. White arrows indicate the presence of pre-synaptic vesicles in the SFGS. Scale bar = 100 µm.

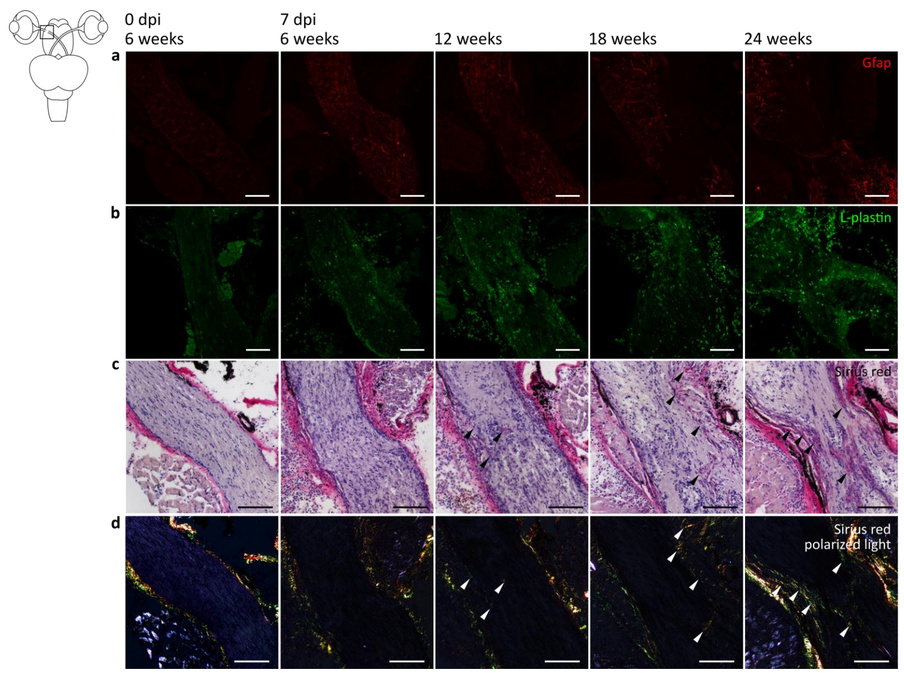
Optic nerve regeneration is hindered by extrinsic factors



Inflammaging sets the stage for a dysregulated immune response following ONC in aged killifish. Immunolabeling of L-plastin in the retina and optic tectum of the young adult, middle-aged, old and very old killifish subjected to ONC reveals a transient rise in immune cell number in young adults, while the immune response in killifish of older age seems to be more extensive and prolonged. Scale bar = 100 µm.



Radial glia respond differently to ONC in aged killifish. Immunostaining for vimentin in the optic tectum of young adult, middle-aged, old and very old killifish subjected to ONC shows a more pronounced and prolonged upregulation of vimentin in the radial fibers *post* ONC in the older age groups compared to 6w old fish. In the SGC, SFGS and SO layers of the optic tectum, vimentin labeling disappears in all age groups following ONC. While labeling increases again in young adults, it does not return in older killifish. Scale bar = 100 µm.



Glial scar formation in older killifish following ONC. Gfap- **(a)** and L-plastin- **(b)** labeling of frontal sections of the visual system of 6w, 12w, 18w and 24w old killifish subjected to ONC reveal signs of a gliotic and inflammatory response at the crush site in older fish. Interestingly, Sirius red staining **(c, d)** shows a clear presence of both immature (green) and mature (red) collagen fibers in 18w and 24w old fish, pointing towards the development of a collagen-rich glial scar in aged fish. Scale bar = 100 µm.

Abbreviations: CNS, central nervous system; dpi, days *post* injury; ONC, optic nerve crush; RGC, retinal ganglion cell; SFGS, *stratum fibrosum et griseum superficiale*; SGC, *stratum griseum centrale*; SO, *stratum opticum*; w, weeks.

Conclusion

Altogether, our current results point towards an age-dependent decline in optic nerve regeneration in the African turquoise killifish. Interestingly, while the repair process following nerve injury highly resembles that of zebrafish at young age, the killifish's ability to regenerate is more mammalian-like at old age. These findings urge further investigations into the cellular and molecular mechanisms underlying this impairment, thereby contributing to the search for effective neuroregenerative therapies .