Tissue stretching in the fast-growing killifish central nervous system, an confounding factor to study neurodegeneration

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By now, the fast-ageing killifish has been accepted as an strong gerontology model to study age-related processes and neurodegeneration. Indeed, it is the first vertebrate model organism that shows physiological neuron loss at old age in its central nervous system (CNS), including its brain and retina. However, the fact that the killifish CNS is an ever-growing organ complicates studying neurodegenerative events in aged fish. Recent studies showed that the method of tissue sampling, either using sections or whole-organs, has a large effect on the observed cell densities in the fast-expanding CNS. Here, we elaborated on how these two sampling methods affect neuronal counts in the aged retina and how this tissue grows throughout life. Layer specific analysis of retinal cryosections revealed an age-dependent reduction in cellular density which could not be observed on whole-mount retinas, this as a result of an extremely fast retinal expansion with age. Using lineage tracing experiments, we showed that the young adult killifish retina mainly grows by cell addition. However, the neurogenic potency of the retina declines with increasing age while the tissue keeps on growing. Further histological analyses revealed tissue stretching, including cell size increase, as the main driver of retinal growth at old age. Indeed, both cell size and inter-neuronal distance augment with ageing, thereby decreasing neuronal density. All in all, our findings urge the 'ageing science' community to consider cell quantification bias and employ tissue-wide counting methods to reliably quantify neuronal numbers in this unique gerontology model.