

# In vivo imaging of axonal transport of mitochondria in the diseased and aged mammalian CNS

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The lack of intravital imaging of axonal transport of mitochondria in the mammalian CNS precludes characterization of the dynamics of axonal transport of mitochondria in the diseased and aged mammalian CNS. Glaucoma, the most common neurodegenerative eye disease, is characterized by axon degeneration and the death of retinal ganglion cells (RGCs) and by an age-related increase in incidence.

RGC death is hypothesized to result from disturbances in axonal transport and in mitochondrial function. Here we report minimally invasive intravital multiphoton imaging of anesthetized mouse RGCs through the sclera that provides sequential time-lapse images of mitochondria transported in a single axon with submicrometer resolution. Unlike findings from explants, we show that the axonal transport of mitochondria is highly dynamic in the mammalian CNS in vivo under physiological conditions.

Furthermore, in the early stage of glaucoma modeled in adult (4-mo-old) mice, the number of transported mitochondria decreases before RGC death, although transport does not shorten. However, with increasing age up to 23-25 mo, mitochondrial transport (duration, distance, and duty cycle) shortens. In axons, mitochondria-free regions increase and lengths of transported mitochondria decrease with aging, although totally organized transport patterns are preserved in old (23- to 25-mo-old) mice. Moreover, axonal transport of mitochondria is more vulnerable to glaucomatous insults in old mice than in adult mice.

These mitochondrial changes with aging may underlie the age-related increase in glaucoma incidence. Our method is useful for characterizing the dynamics of axonal transport of mitochondria and may be applied to

other submicrometer structures in the diseased and aged mammalian CNS in vivo.

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