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Imaging of neuronal mitochondria *in situ*

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Neuronal mitochondria are receiving a rapidly increasing level of attention. This is to a significant part due to the ability to visualize neuronal mitochondria in novel ways, especially *in vivo*. Such an approach allows studying neuronal mitochondria in an intact tissue context, during different developmental states and in various genetic backgrounds and disease conditions. Hence, *in vivo* imaging of mitochondria in the nervous system can reveal aspects of the 'mitochondrial life cycle' in neurons that hitherto have remained obscure or could only be inferred indirectly. In this survey of the current literature, we review the new insights that have emerged from studies using mitochondrial imaging in intact neural preparations ranging from worms to mice.

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Introduction

The brain consumes energy well out of proportion to its weight [1]. Neurons appear to have especially high energy requirements to sustain ion gradients and synaptic vesicle recycling. At the heart of neuronal energy metabolism reside mitochondria, organelles that are at the focus of rapidly growing interest. While mitochondria are ubiquitous in almost all eukaryotic cells, there appears to be something special about neuronal mitochondria: First, while being essential for bioenergetics, mitochondria are also endowed with a range of additional functions, which can regulate genuine 'neuronal' physiology. This includes calcium buffering, which directly impacts synaptic transmission and plasticity. Second, mitochondria appear to be signaling hubs during neuronal development [2–6]. Third, mitochondria are the clavigers of intrinsic apoptotic signaling — a role that is particularly critical in neural tissues given the post-mitotic nature of most mature neurons and that might also impact more subtle regressive phenomena than overt cell death [7]. Finally, many of these aforementioned roles link neuronal mitochondria to disease, a nexus strengthened by a growing body of genetic evidence. It is remarkable, indeed, in how many neurological conditions mitochondrial dysfunction has been implicated. Obviously it is not clear in all cases, whether this is a true causal link or rather an epiphenomenon affecting a particularly vulnerable organelle. Still, the fact that neurons are particularly vulnerable to dysfunction of mitochondria, and — in converse — that amongst mitochondria those in neurons might be particularly vulnerable seems indisputable. For example, mutations in the mitochondrial genome manifest preferentially as pathology of excitable tissues and especially of neurons [8]. Similarly, many mutations that affect mitochondrial dynamics and turn-over seem to impact neurons first [9]. Hence, neuronal mitochondria rightly have come under scrutiny not only as one of many organelles in a special cell type, but as special organelles in their own right. Imaging studies of neuronal mitochondria offer a unique glimpse on how this remarkable organelle lives out its life in its native habitat [10].

The mitochondrial life cycle

More than for almost any other cellular structure, the ability to visualize neuronal mitochondria in *in vivo* contexts — originally in intact cells, but increasingly *in situ* in intact tissues or even in living multicellular organisms — has influenced our interpretation of this organelle's function. From a practical point of view, the propensity of many membrane-permeant vital dyes to partition into mitochondria, following an electrical driving force, and the fact that mitochondria are just within the resolving power of diffraction-limited light microscopy, has allowed capturing the fascinating dynamics of these organelles. From this emerged the view that the mitochondria within a cell form a constantly intermixing network of organelles, and that the original ultrastructural interpretation of mitochondria as well-isolated and stable entities might have been somewhat misleading. The first imaging studies that characterized neuronal mitochondria taking advantage of intrinsic contrast [11] or mitochondria-specific dyes and time-lapse *in vivo* imaging of isolated neurons [12–14] confirmed the impression that also in neurons many mitochondria are highly dynamic. Still, this 'network view' of a neuron's mitochondrial population remained slightly at odds with the extended geometry that characterizes neuronal cells and the compartmentalization of mitochondria with distinct

morphologies at different densities to somatic, dendritic, axonal and synaptic sites [15]. Thus, while it is clear that mutations affecting mitochondrial fusion and fission often manifest as neurological diseases with an axonopathic phenotype [9], where the disrupted mitochondrial dynamics take place physiologically remains unclear. Similar unresolved issues also affect our current interpretation of other important steps of a mitochondria's life inside a neuron: For example, one peculiarity of neurons is electrical activity, which comprises ion fluxes to maintain excitability, action potential firing and synaptic transmission. This activity results in variable energy demands across a neuron's compartments. It is undisputed that the resulting local needs for energy provision and ion buffering impact mitochondrial trafficking, dynamics and shape. However, in which way exactly this influence of activity unfolds and how strongly it affects the homeostatic distribution and dynamics of neuronal mitochondria is an area of ongoing confusion and study.

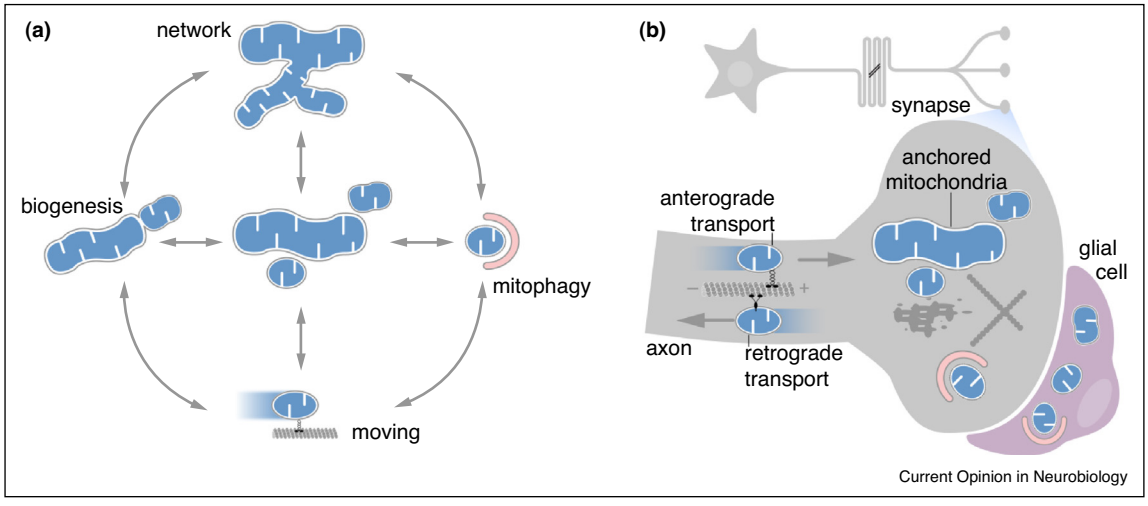
Similarly, the process of degrading mitochondria at the end of their life time — a process that involves autophagic digestion, i.e. 'mitophagy' — has been clearly implicated by genetics in neurodegeneration [16]. Still, even basic facts about mitochondrial degradation *in vivo* remain a matter of debate. For example, it is unresolved where in a mature neuron *in vivo* mitophagy actually takes place [17–19]. Related arguments can be put forward about the neuronal topography of mitochondrial biogenesis, as well as the interactions of neuronal mitochondria with other organelles, such as the axoplasmic reticulum.

Together the outlined processes (Figure 1): *Biogenesis — Transport — Fusion/Fission — Organelle contact — Mitophagy* represent seminal steps of what can be called the 'mitochondrial life cycle'. Thus a central complex of open and timely questions regarding neuronal mitochondria is how this cycle maps onto a neuron's extended geometry. For this quest, new approaches to visualize the morphology, dynamics and functional state of neuronal mitochondria in intact tissue preparations derived from invertebrate and vertebrate model organisms have become available. Compared to the classical dissociated neuronal cell culture preparations, in which mitochondria have long been studied, these approaches have two seminal advantages albeit at the expense of accessibility and reductionist appeal: First, neuronal geometry, compartmentalization and tissue context — and hence the corresponding aspects of mitochondrial distribution and specialization — are preserved; second, at least some of these preparations can be used in the mature state and can be followed longitudinally over time. This allows investigations into mitochondrial homeostasis under conditions of aging or late-onset neurological conditions.

In vivo imaging of mitochondrial dynamics

Although mitochondria have been identifiable and well-characterized transport cargoes during early studies of axonal transport in invertebrate neurons using intrinsic contrast, the use of fluorescent protein specifically targeted to neuronal mitochondria, first in *Drosophila*, and subsequently in mice, *Caenorhabditis elegans* and zebrafish has transformed our ability to track mitochondria in more

Figure 1



(a) Schematic representation of the mitochondrial life cycle with various steps of unclear localization in mature neurons. **(b)** Illustration of the synaptic compartment as an example of a site of remote mitochondrial accumulation. Although there is broad consensus that a substantial fraction of microtubule-dependent transport delivers mitochondria to peripheral locations, the exact size of the synaptically-delivered fraction is unclear. Similarly, while the origin of retrogradely transported mitochondria can clearly be synaptic, how the retrogradely transported pool exactly relates to the local resident pool is unresolved. Similarly elusive remain the relative importance of local autophagic digestion versus export; the role and extent of inter-organellar interactions involving mitochondria in distal locations; or the role of the non-microtubular cytoskeleton in placing and retaining peripheral mitochondria.