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Emerging roles of mitochondria in synaptic transmission and neurodegeneration

Annie Lee^{1,2,#}, Yusuke Hirabayashi^{1,2,#}, Seok-Kyu Kwon^{1,2,4,#}, Tommy L. Lewis Jr^{1,2,#}, and Franck Polleux^{1,2,3,*}

¹Department of Neuroscience, Columbia University, New York, NY 10032, USA

²Mortimer B. Zuckerman Mind Brain Behavior Institute

³Kavli Institute for Brain Science at Columbia University

Abstract

Mitochondria play numerous critical physiological functions in neurons including ATP production, Ca²⁺ regulation, lipid synthesis, ROS signaling, and the ability to trigger apoptosis. Recently developed technologies, including *in vivo* 2-photon imaging in awake behaving mice revealed that unlike in the peripheral nervous system (PNS), mitochondrial transport decreases strikingly along the axons of adult neurons of the central nervous system (CNS). Furthermore, the improvements of genetically-encoded biosensors have enabled precise monitoring of the spatial and temporal impact of mitochondria on Ca²⁺, ATP and ROS homeostasis in a compartment-specific manner. Here, we discuss recent findings that begin to unravel novel physiological and pathophysiological properties of neuronal mitochondria at synapses. We also suggest new directions in the exploration of mitochondrial function in synaptic transmission, plasticity and neurodegeneration.

Introduction

Neurons communicate with each other through specialized contacts termed synapses. Synaptic transmission between individual neurons affects neuronal development, maintenance, and brain function. The ability of mitochondria to control both cytosolic Ca²⁺ dynamics and ATP availability, both of which are crucial for proper synaptic function, makes this organelle particularly important during development and in the adult brain. Therefore, interrogating mitochondrial function in neurons is both critical for understanding normal synaptic transmission as well as in neurodegenerative diseases where synaptic function and maintenance are disrupted. We focus here on recent studies deciphering novel mechanisms

*Address correspondence to (Lead contact): Franck Polleux, Columbia University, Department of Neuroscience, Mortimer B. Zuckerman Mind Brain Behavior Institute, Jerome L. Greene Science Center, 3227 Broadway, L-5-050, MC 9853, New York, NY 10027, fp2304@cumc.columbia.edu, Tel: 212-853-0407.

#These authors contributed equally to this work

⁴Present address: Center for Functional Connectomics, Brain Science Institute, Korea Institute of Science and Technology, Seoul, South Korea

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in maintaining mitochondrial structure, morphology and distribution in the developing and adult CNS as well as their implications for the pathophysiological mechanisms underlying various neurodegenerative conditions.

Cell biology of mitochondria in CNS neurons

Neurons are truly unique cells whose function is largely dictated by their extreme level of compartmentalization at the cellular and molecular levels. This high degree of compartmentalization is also evident for intracellular organelles such as mitochondria which differ in their localization, morphology and function in neurons of the central nervous system (CNS). The term “mitochondrial dynamics” encompasses a set of five distinct mitochondrial cell biological events: biogenesis, fission and fusion, trafficking, and mitophagy, a specialized form of macro-autophagy (Cagalinec et al., 2016; Mishra and Chan, 2016; Pagliuso et al., 2017). Mitochondria display distinct morphologies and distributions in the two main compartments characterizing neurons: the axon and dendrites (Fig. 1A-F). For example, in pyramidal neurons, the main excitatory neuronal subtype in the cortex, dendritic mitochondria display long and tubular shapes, forming a complex network filling ~70-80% of the dendritic arbor. In contrast, axonal mitochondria display a remarkably standard size and are small and punctate (~1µm length), occupying <10% of axonal volume (Chicurel and Harris, 1992; Harris and Stevens, 1989; Kasthuri et al., 2015) (Fig. 1A-F). In non-neuronal cell types, mitochondrial morphology is controlled mostly by fusion and fission mechanisms. Therefore, one would hypothesize that the striking differences in mitochondrial morphology between the axon and dendrites is the result of a high level of fission in the axon and high degree of fusion in dendrites. Although some studies have suggested that this is the case (Berman et al., 2009; Chang and Reynolds, 2006), the molecular mechanisms underlying compartment-specific differences in mitochondrial fission/fusion remains to be identified (Fig. 1G-H). It also seems clear that these different morphologies will be accompanied by alterations in the functional abilities and output of these mitochondria, which likely include an increased or decreased ability to produce ATP or buffer calcium. How these differences in mitochondrial function affect neuronal development, function or health remain completely open.

The best studied process underlying mitochondrial dynamics in neurons is their trafficking along microtubules by the microtubule-based motors kinesin and dynein (Hirokawa et al., 2010; Loss and Stephenson, 2017). In axons, microtubules display a unique orientation with their polymerizing plus-end oriented away from the cell body (Baas et al., 1988). Using a number of different methods, many studies have revealed that there are at least two pools of mitochondria: a motile and an immobile (or stationary) pool (Lewis et al., 2016; Loss and Stephenson, 2017; Obashi and Okabe, 2013). The current consensus in developing neurons is that each pool comprises around 50% of the mitochondria (Fig. 2A). However, recent work from multiple labs using models from flies to mice, both *in vitro* and *in vivo*, revealed that mitochondrial trafficking decreases significantly with neuronal maturation (Faits et al., 2016; Lewis et al., 2016; Smit-Rigter et al., 2016; Vagnoni et al., 2016). For instance, in axons of adult layer 2/3 cortical pyramidal neurons, the ratio of stationary to motile mitochondria is approximately of 9:1 both *in vitro* and *in vivo* (Fig. 2B-D). At least two sites of mitochondrial capture have been identified in developing axons: ‘*en passant*’ presynaptic

synapses and branch points (Courchet et al., 2013; Spillane et al., 2013). The axon of cortical pyramidal neurons form thousands of ‘*en passant*’ synapses which are synapses made along the entire length of the axon as opposed to the axon ‘terminal’ of specialized neuronal subtypes, such as motor neurons forming only synapses at the end of their axon i.e. the neuromuscular junction. In these cortical axons, like many other central nervous system neuron subtypes, >90% of mitochondria appear immobile for long periods of time (~45% of axonal mitochondria are captured at presynaptic sites while 45% are associated with sites of currently unknown relevance (Courchet et al., 2013; Obashi and Okabe, 2013)). These observations raise a number of important questions currently being addressed by the field: What are the molecular mechanisms underlying the long-term “capture” of mitochondria? What are the specific locations where mitochondria are captured along axons? Is mitochondrial function altered upon capture at different sites? The first of these questions has already been partially answered by several studies suggesting that the first step in arresting mitochondria trafficking along the axon depends on Ca^{2+} -dependent release from microtubules through the combined functions of Miro/Milton and/or syntaphilin (Kang et al., 2008; Macaskill et al., 2009; Wang and Schwarz, 2009) which are key adaptors/modifiers of the microtubule motors, kinesins and dyneins. However, we propose that other currently unknown mechanisms also likely exist to mediate a more permanent ‘anchoring’ of mitochondria at presynaptic boutons or at other locations along the axon.

Synaptic functions of mitochondria

The ability of mitochondria to generate ATP and uptake Ca^{2+} , both of which affect presynaptic release properties, has been extensively studied. Neurotransmitter-containing synaptic vesicle (SV) exocytosis is triggered by Ca^{2+} entry through voltage-gated Ca^{2+} channels (VGCC) upon action potential invasion of the presynaptic bouton, where synapses occur in axons. This Ca^{2+} -dependent exocytosis of neurotransmitter vesicles is coupled to endocytosis in order to maintain SV pool size under physiological conditions. Therefore, regulating presynaptic Ca^{2+} homeostasis and controlling SV pool size are critical for proper synaptic transmission.

Presynaptic cytoplasmic Ca^{2+} can be cleared by multiple, potentially complementary mechanisms: (1) through plasma membrane Ca^{2+} -ATPase (PMCA) to the extracellular space, (2) through smooth endoplasmic reticulum Ca^{2+} -ATPase (SERCA) to the endoplasmic reticulum, or (3) through mitochondrial Ca^{2+} uniporter (MCU) to the mitochondrial matrix (Billups and Forsythe, 2002; Castonguay and Robitaille, 2001; David and Barrett, 2003; Jensen et al., 2007; Scullin and Partridge, 2010). The relative contribution of each of these mechanisms is unclear and might actually be synapse-specific (Kwon et al., 2016a). Mitochondrial contribution to presynaptic Ca^{2+} buffering has been suggested from studies including large synaptic terminals such as *Drosophila* neuromuscular junction (NMJ), mammalian NMJ, and the calyx of Held (Kwon et al., 2016a). Moreover, recent studies using genetically encoded sensors probing Ca^{2+} dynamics and SV release demonstrated critical roles for mitochondria at individual presynaptic release sites of CNS axons making thousands of small (~1micron length) *en passant* boutons (Gazit et al., 2016; Kwon et al., 2016b; Vaccaro et al., 2017). As mentioned above, approximately 50% of presynaptic *en passant* boutons are associated with mitochondria, and our work as well as

others, recently showed that mitochondria-free boutons of cortical or hippocampal neurons accumulate more Ca^{2+} upon repetitive (5-20 action potentials (AP) at 10Hz) stimulation, thereby triggering progressively more SV release during train of AP (Kwon et al., 2016b; Vaccaro et al., 2017). Furthermore, reduced MCU-dependent presynaptic mitochondrial Ca^{2+} uptake caused increased cytoplasmic presynaptic Ca^{2+} accumulation and impaired short-term synaptic plasticity and asynchronous release (Kwon et al., 2016b). Variation in levels of neuronal activity level can scale (up or down) neuronal excitability in order to bring neural network activity within a certain range, and mitochondrial occupancy at individual presynaptic boutons can play an important role in this form of homeostatic rescaling (Vaccaro et al., 2017). Paradoxically, with the notion that presynaptic mitochondrial function is primarily to generate ATP (Rangaraju et al., 2014), these recent results suggest that the presence of mitochondria corresponds to boutons releasing at lower probability compared to boutons devoid of mitochondria along the same axon (Kwon et al., 2016b; Vaccaro et al., 2017).

Historically, ATP has been proposed to play critical roles presynaptically, such as SV endocytosis and neurotransmitter re-uptake in presynaptic vesicles. Therefore, mitochondrial ATP generation has been suggested to maintain SV pool size. Moreover, a recently developed ATP sensor directly showed activity-driven ATP generation at presynaptic sites (Rangaraju et al., 2014). However, in these studies, ATP is monitored during presynaptic neurotransmitter release evoked by high, non-physiological regimes of action potential stimulations (600AP i.e. 60sec stimulation at 10Hz). Moreover, even in these extreme conditions, (never encountered by cortical or hippocampal pyramidal neurons *in vivo*), blocking either glycolysis or the mitochondrial ATP synthase results in only modest changes in presynaptic ATP concentrations. Even more intriguing, as mentioned above, in axons of cortical pyramidal neurons, only 50% of presynaptic release sites are associated with a mitochondria (Lewis et al., 2016). In fact, a recent study compared changes in ATP concentration (using a genetically-encoded ATP sensor) in presynaptic boutons with or without mitochondria and found no difference, even during strong stimulation of neurotransmitter release (600AP) (Pathak et al., 2015). Collectively, these results suggest that presynaptic mitochondria in adult mammalian CNS axons are likely not the major source of ATP and that glycolysis or other means of generating ATP provide a sizeable proportion of the ATP required for synaptic activity. Interestingly, glycolysis-related proteins are enriched at presynaptic sites upon synaptic stimulation, suggesting that glycolysis may supply enough ATP to sustain physiological levels of activity in axons (Ashrafi et al., 2017; Jang et al., 2016).

In dendrites of cortical pyramidal neurons, long and tubular mitochondria occupy a significant volume of the dendritic arbor (~70%), but are restricted to the dendritic shaft and largely excluded from spines (Kasthuri et al., 2015; Wu et al., 2017) (Fig. 1). Postsynaptic/dendritic Ca^{2+} dynamics also plays critical roles including synaptic integration and gene expression regulation. In dendrites, extracellular and ER-stored Ca^{2+} are the main sources of dendritic Ca^{2+} . However, in this context, the role of mitochondria in postsynaptic function is largely unknown despite the large volume they occupy. Recent 3D-serial electron microscopy (3D-SEM) reconstruction data revealed that dendritic ER and mitochondria make many contacts along the dendrite (Wu et al., 2017). Furthermore, our lab recently

identified that a significant fraction of synaptically-induced Ca^{2+} released from the ER is directly transferred to mitochondria at these ER-mitochondria contact sites. This was uncovered through the identification of PDZD8, a novel ER-mitochondria tethering protein in multiple mammalian cell types (Hirabayashi et al., 2017) which represents a functional paralog of the yeast protein Mmm1, one of the four proteins composing the ERMES ER-mitochondria tethering complex in this cell type. Interestingly, in dendrites of PDZD8-deficient cortical pyramidal neurons, a significantly higher fraction of synaptically-evoked Ca^{2+} release from the ER leaks to the cytosol, therefore increasing local dendritic Ca^{2+} concentration. These results suggest that the distribution and extent of ER-mitochondria contacts in neuronal dendrites might control the spatial and temporal aspects of dendritic Ca^{2+} dynamics and thereby might underlie dendritic branch-specific properties of synaptic integration and/or plasticity.

In addition, 3D-serial EM reconstructions also visualized the structure of axonal ER and mitochondria, where they also form large contacts (Wu et al., 2017). Our own reconstructions from serial EM of layer 4/5 mouse cortex demonstrate that presynaptic mitochondria form extended contacts with the axonal ER (Fig. 3A and C-D). Interestingly, in dendrites, ER-released Ca^{2+} can be conveyed to mitochondria through their contact sites, as mentioned above; however this has not been observed yet at presynaptic boutons of CNS axons. Surprisingly, a novel genetically-encoded ER Ca^{2+} sensor demonstrates that axonal ER can effectively uptake Ca^{2+} without any detectable release of Ca^{2+} at presynaptic boutons of cortical axons *in vitro* (de Juan-Sanz et al., 2017). Therefore, understanding how mitochondria-ER contacts regulate presynaptic function will be pivotal for the field.

Disruption of mitochondrial dynamics and neurodegeneration

Several studies have highlighted the frequent role of mitochondria in the pathogenesis of a wide range of neurodegenerative diseases (ND). Due to the limitation of space, we focus this portion of our review on prototypical examples of ND as listed in Table 1. Below, we explore some recent reports demonstrating disruptions in mitochondrial homeostasis, motility, and dynamics observed upon neurodegeneration.

Mitochondria quality control through fission, fusion, and mitophagy

In non-neuronal cells, mitochondrial homeostasis is tightly regulated by a dynamic balance of fission, fusion and mitochondrial autophagy (mitophagy). Changes in mitochondrial morphology have been implicated in Alzheimer's Disease (AD) through observations of altered mitochondrial fission and fusion protein levels in AD patient brains. Corroborating some of the postmortem analysis of human patient samples, primary neuronal culture *in vitro* showed shorter mitochondria upon exposure to Amyloid- β Derived Diffusible Ligand (ADDL), which appears critical for synapse elimination during early stages of the disease progression (Wang et al., 2009). More recent studies using 3D-SEM reconstructions showed that various AD mouse models display a “mitochondrial-on-a-string” phenotype in neurites from CA1 where reduced mitochondrial matrix volume is observed together with continuous but constricted outer mitochondrial membrane (OMM). Although the authors do not distinguish the specific cell type they are evaluating, they hypothesized that the MOAS

phenotype that is observed in AD mouse models occur due to potentially disrupted function of Drp1, ultimately resulting in an incomplete fission pattern (Zhang et al., 2016). In line with morphological changes, increased mitochondrial stress response, including increased mitophagy genes, have recently been reported across-species from *C. elegans* to humans, in response to A β accumulation (Sorrentino et al., 2017). Currently, the exact molecular mechanisms underlying these changes in mitochondrial morphology and mitophagy dynamics are unclear. Whether these changes play a causal role in the neuropathology or represent an attempt for neurons to cope with a form of cellular stress induced by A β is still unclear, but disruption of mitochondrial homeostasis is becoming a common phenotype in various models of AD (Schon and Przedborski, 2011; Wang et al., 2009; Zhang et al., 2016).

Multiple lines of evidence suggesting disrupted mitochondrial homeostasis in Parkinson's Disease (PD) have emerged from studying mutations of genes linked to familial forms of PD including Parkin, a E3 ubiquitin ligase, and PINK1 kinase. These two genes are involved in mitophagy, implicating their mutations in the disruption of mitochondrial integrity (Matheoud et al., 2016; Schon and Przedborski, 2011). However, how these two proteins directly influence mitochondrial morphology and function is still under debate, as Parkin has many downstream effectors, and because many of the functions of Pink1/Parkin have been studied under non-physiological conditions (Schon and Przedborski, 2011). Another interesting autosomal recessive mutation in a familial form of PD affects the gene encoding DJ1, which localizes to mitochondria. However, one of the biggest caveats in studying these genes is that PINK1, Parkin, and DJ1 knockout mice do not display any dramatic phenotypes in neurons, bring into question the importance of mitochondrial quality control in the disease pathogenesis (McWilliams and Muqit, 2017). However, a recent study emphasized how DJ1 mutations in human iPSCs differentiated into dopaminergic neurons (DA) influences mitochondrial function. Compared to mouse iPSCs where the same mutations show little phenotypic effects, human iPSCs showed elevated mitochondrial oxidative stress. How the elevated mitochondrial oxidative stress can directly influence their morphology is unclear, but this is one of first papers highlighting the unique susceptibility PD mutations might have on human, but not mouse, DA neurons (Burbulla et al., 2017). Lastly, PD associated mutation of VPS35, a membrane protein found on the recycling retromer complex, has been proposed to regulate mitochondrial morphology through disruption of Drp1 recycling (Wang et al., 2016b). Further study is needed to test if these changes in mitochondrial structure and function have a causal role in Parkinson's disease progression.

Structural evidence of altered and aggregated mitochondria has been observed in motor neurons of ALS patients (Smith et al., 2017). Interestingly, many of the genes mutated in the familial forms of ALS, such as optineurin, p62, and TBK1, regulate mitophagy. Several familial ALS mutations also disrupt ATP generation, ROS production, and Ca²⁺ buffering in part by altered interaction with the ER. The loss of ER-mitochondria communication is an emerging mechanism for disrupted Ca²⁺ due to mutations of SOD1, TDP43, and FUS-related ALS, where reduced Ca²⁺ uptake in mitochondria from the ER leads to increased cytosolic Ca²⁺ (Smith et al., 2017). However, the molecular players involved in this remain unclear, especially because novel ER-mitochondrial proteins are still being discovered (Hirabayashi et al., 2017). A recent study also suggested that mutation on TDP43 results in

increased localization of the protein to mitochondria, and that this ALS-associated mutation allows TDP43 to bind to mtDNA to disrupt its transcription, ultimately leading to mitochondrial fragmentation and dysfunction (Wang et al., 2016a). Despite the different consequences of these mutations, disrupted mitochondrial homeostasis is emerging as a common feature of ALS.

Mitochondrial trafficking and distribution in neurodegenerative diseases

Mitochondrial trafficking allows the specific distribution of mitochondria in subcellular compartments, permitting them to carry out localized functions. This is particularly important in neurons due to their polarized morphology. Disrupted mitochondrial trafficking was hypothesized in AD when patient samples showed axonal swelling filled with mitochondria as well as other vesicles, vacuoles, and multi-vesicular bodies (MVB). Subsequent studies using mouse models overexpressing familial AD linked mutations also suggested disrupted mitochondrial trafficking in relatively immature cortical axons *in vitro* (Schon and Przedborski, 2011; Vossel et al., 2010). However, recent data demonstrated that mitochondrial trafficking in mature, adult axons of cortical pyramidal neurons both *in vitro* and *in vivo* is dramatically lower than previously reported (Lewis et al., 2016; Smit-Rigter et al., 2016). Therefore, these previous results obtained *in vitro* will need to be re-evaluated using either mature cortical neuron cultures (>21DIV) or ideally using *in vivo* 2-photon imaging approaches in adult or aging AD mouse models.

Although the direct consequence of mutations linked with PD on mitochondrial trafficking is still unclear, many studies suggest disrupted mitochondrial motility in axons. PINK1 has been shown to interact with Miro directly on depolarized mitochondria which ultimately leads to Parkin dependent degradation of Miro, potentially as a mechanism to stall and isolate damaged mitochondria (Wang et al., 2011). Other PD-associated genes such as Parkin, α -synuclein, and leucine-rich repeat kinase 2 (LRRK2) have been linked to alteration of microtubule (MT) dynamics, suggesting that MT-dependent trafficking of several axonal cargoes such mitochondria, could be disrupted by these mutations (Schon and Przedborski, 2011). Interestingly, recent studies suggest that some mutations associated with neurodegeneration influence the expression level of MT adaptors. Mutations of LRRK2, for example, delay the degradation of Miro from the outer mitochondria membrane under stress. This not only alters mitochondrial motility, but also slows down the recruitment of the mitophagic players for proper degradation of damaged mitochondria (Hsieh et al., 2016). The ultimate effect of LRRK2 mutations on mitochondrial morphology/function in mammalian CNS neurons is still unclear, but the authors propose that the function of LRRK2 to degrade Miro1 is Pink1/Parkin-independent (Hsieh et al., 2016).

Changes in mitochondrial dynamics have also been observed in animal mouse models and patients samples from Huntington Disease (HD) patients. Although the exact function of Huntingtin (Htt) is unclear in this context as it is a large pleiotropic adaptor protein, studies on HD-associated mutations of Htt suggested altered mitochondrial dynamics, especially since Htt can interact with mitochondria and regulators of mitochondrial trafficking (Lin and Beal, 2006; Saudou and Humbert, 2016).

Emerging Views and Concluding Remarks

It is important to note that many studies have a neuro-centric view in determining how mitochondria disruption influences neurons in the brain during neurodegeneration. However, the field is beginning to recognize the role of other cell types in neurodegenerative diseases. In the case of AD, a recent gene variant associated with late-onset AD, TREM2, has been identified to function as an immune receptor on microglia. Mutations of this gene lead to lowered mitochondrial mass and decreased metabolites from the TCA and glycolysis pathways in microglia, and are thought to result in global microglial dysfunction correlating with the progression of AD (Ulland et al., 2017). The role of immune response in PD has also been raised. Interestingly, a novel function of Parkin and PINK1 include inhibiting the dynamics of mitochondrial-derived vesicles (MDVs). These MDVs can be exported to present antigens on the cell surface to initiate autoimmune responses that ultimately lead to neuronal cell death (Matheoud et al., 2016). Mutations of Parkin and PINK1 are hypothesized to increase MDV dependent antigen presentation, eliciting a neuro-inflammatory response. Although the above studies highlight a common mitochondrial dysfunction in many ND, it is often unclear whether mitochondria have a causal role in disease progression or if their dysfunction is merely a consequence due to other forms of cellular toxicities and various forms of stress responses.

Despite the tremendous efforts to treat these diseases, many of the available treatments show mild results at best, suggesting a need for novel approaches. Because mitochondrial dysfunction and or remodeling has been reported in multiple forms of ND, enhancing mitochondrial function or maintaining their structural integrity has been proposed to delay the onset of ND (Schon and Przedborski, 2011). Before diving into this endeavor, it will be critical to understand and dissect the key mechanisms behind the observed mitochondrial dysfunction in hopes of identifying new potential therapeutic approaches for neurodegenerative diseases.

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Abbreviations

CNS	central nervous system
PNS	peripheral nervous system
ER	endoplasmic reticulum
SV	synaptic vesicle
AP	action potential
SERCA	sarcoendoplasmic reticulum calcium transport ATPase
NCX	sodium-calcium exchanger
VGCC	voltage gated calcium channel
PMCA	plasma membrane calcium ATPase
ND	neurodegenerative disease
DA	dopaminergic neurons
MT	microtubule
AD	Alzheimer's Disease
PD	Parkinson's Disease

HD	Huntington's Disease
ALS	Amyotrophic Lateral Sclerosis

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Highlights

- Neuronal mitochondria show compartment-specific morphology in dendrites and axons
- Genetically-encoded tools allow for monitoring of many mitochondrial functions in neurons
- Dendritic ER and mitochondria are functionally connected
- Mitochondrial dysfunction play critical roles in multiple neurodegenerative diseases

Figure 1. Mitochondrial morphology is compartmentalized in CNS neurons

(A) Single cortical neuron cultured for 21 days *in vitro* (DIV) following *ex utero* electroporation at E15.5 with plasmids encoding tdTomato (magenta) and mitochondrial targeted YFP (mt-YFP, green). (B) Isolated mt-YFP fluorescence from panel A. Mitochondria are long and tubular in dendrites (arrow) occupying a large fraction of the dendritic arbor, whereas in the highly branched axon (arrowhead), mitochondria are uniformly small (~ 1 micron length) and occupy a small fraction of the axonal volume.. (C-F) Higher magnification images of a dendrite segment (C-D) and an axon segment (E-F) from the cortical pyramidal neuron shown in A and B. (G) The increased occupancy and length of mitochondria in the dendrites suggests that the balance of fission and fusion is strongly weighted towards fusion in the dendritic compartment of cortical neurons. (H) The much smaller size of mitochondria in the axon suggests the opposite is true in the axon where fission must be more prominent than fusion. Future work will be required to determine the cellular and molecular mechanisms that regulate this compartmentalization of mitochondria morphology in neurons. See text for details.

Figure 2. Mitochondrial trafficking decreases with axonal development as mitochondria become captured at specific points along the axon

(A) Kymograph of mitochondrial movement (visualized via time lapse microscopy of YFP-labeled mitochondria at 0.1 frame per second (fps) in the axon of a 7DIV cortical neuron demonstrating a profile of ~50% motile and ~50% stationary pools of mitochondria. (B) Kymograph of mitochondrial movement in the axon of a 21DIV cortical neuron showing ~95% captured mitochondria and an ~5% motile pool. (C) Kymograph of mitochondrial movement in the distal axon of a layer 2/3 cortical neuron in a (P)ostnatal day 32 awake behaving mouse. (D) Kymograph of mitochondrial movement in the distal axon of a layer 2/3 cortical neuron in a P45 awake behaving mouse. C and D illustrate that ~90% of mitochondria in mature axons *in vivo* are captured at specific points along the axon. (E) Schematic representation of mitochondrial localization along mature cortical axons *in vitro* and *in vivo*. Future work will be necessary to identify the different locations of mitochondrial capture as well as the molecular mechanisms that regulate these long term capture events.

Figure 3. Contacts between the ER and mitochondria in the dendrites and axons

(A, B) Serial electron microscopy (EM) images of axon and dendritic segment of pyramidal neurons in layer 4/5 of the primary somatosensory area of mouse neocortex (publicly available data- Kasthuri et al., 2015; <https://neurodata.io/data/kasthuri15>). Individual mitochondria and the ER network located in segments of the axon (A) and dendrite (B) are highlighted in green and magenta, respectively. Arrowheads indicate ER-mitochondria contact sites. (C-F) 3D reconstructions of the serial EM images shown in A (C, D) and B (E, F). The ER is made of a thin, tubular structure along the axon shaft that systematically bulges specifically at the presynaptic sites (C, D), while having a complex network-like structure in dendrites (E, F). There are extensive contacts between the ER and mitochondria both in the axon and dendrites (D, F). (C) Synaptic vesicles; blue, Mitochondria; green, ER; Magenta, (D) ER-mitochondria contact site; green, ER; Magenta, (E) Mitochondria; green, ER; Magenta, (F) Mitochondria; green, ER-mitochondria contact site; Magenta. Scale bar; 300 nm. (G) A diagram showing ER and mitochondrial-dependent regulation of Ca^{2+} dynamics in the axons and dendrites (modified from ** Hirabayashi et al. 2017). Synaptic stimulation induces Ca^{2+} entry through VGCC (Voltage-gated Ca^{2+} channel) at the presynaptic sites. Ca^{2+} is partially cleared by MCU-dependent mitochondrial Ca^{2+} uptake. In dendrites, Ca^{2+} entry through ligand-gated ion channels and VGCC can induce Ca^{2+} release from RyR (Ryanodine Receptor). In addition, IP3 (Inositol 1,4,5-trisphosphate) generated downstream of a metabotropic Glutamate receptors (mGluR) activation induces Ca^{2+} release from the ER through IP3R (IP3 receptor). Mitochondria buffers a significant fraction of Ca^{2+} released from the ER, which at the expense of the fraction of Ca^{2+} ending in the cytoplasm.

Table 1

Disease	Genetic Cause	Description of Gene	Potential Consequences of Mutations on Mitochondrial Function	References
Alzheimer's Disease (AD)	APP	Trans-membrane protein that give rise to A β peptides Oligomeric form of A β are thought to have a causal role in AD and aggregate to form senile plaques	Altered levels of mitochondrial fission and fusion proteins that ultimately leads to abnormal mitochondrial distribution Disrupted mitochondrial trafficking Reduced mitochondrial membrane potential and ATP levels Increase of mitochondrial on a string (MOAS) phenotype Increased apoptotic response and free radical production	Reviewed by (Schon & Przedborski, 2011) (Wang et al., 2009) (Lin & Beal, 2006) (Zhang et al., 2016)
	PS1 & 2	Component of γ secretase that cleaves APP to produce A β peptides	Altered mitochondrial motility and mitochondrial activity that ultimately leads to changes in brain energetics	(Zhang et al., 2016)
	TREM2	Surface receptor required for microglial response	Lowered mitochondrial mass and activated caspase in microglia thought to disrupt microglial function to degrade aggregates or proteinopathy	(Ulland et al., 2017)
Parkinson's Disease (PD)	α -Synuclein	Function unclear; thought to be involved in membrane remodeling at nerve terminals Major component of Lewy bodies	Disrupted mitochondrial trafficking Fragmented mitochondria	Reviewed by (Schon & Przedborski, 2011)
	Parkin	Cytosolic E3 ubiquitin ligase localized to mitochondria Novel function shown to inhibit formation of mitochondrial derived vesicles (MDV) involved in mitochondrial antigen presentation (MitoAP)	Altered mitochondrial morphology and mitophagy Increased oxidative stress Increased MDV formation and activated MitoAP thought to elicit cytotoxic response	Reviewed by (Schon & Przedborski, 2011) (Matheoud et al., 2016)
	PINK1	Kinase localized to mitochondria Novel function to inhibit formation of MDV involved in MitoAP	Altered mitochondrial morphology and mitophagy Increased MDV and activated MitoAP thought to elicit cytotoxic response	Reviewed by (Schon & Przedborski, 2011) (Matheoud et al., 2016)
	DJ-1	Hypothesized to be involved in regulating oxidative stress & protecting against cell death	Increased mitochondrial oxidative stress Decreased oxygen consumption rate by mitochondria	(Burbulla et al., 2017)
	LRRK2	Kinase with unclear function Recently proposed to remove Miro1 in stalled mitochondria	Delayed mitophagy in part by stabilizing Miro1 on damaged mitochondria and prolonging active transport Altered mitochondrial morphology is controversial	(Hsieh et al., 2016)
	VPS35	Membrane protein part of recycling retromer complex	Fragmented mitochondria Reduce levels of ATP and membrane potential Impaired bioenergetics	(Wang et al., 2016)
Amyotrophic Lateral Sclerosis (ALS)	SOD1	Cu/Zn superoxide dismutase; antioxidative function	Disrupted mitochondrial trafficking and energy metabolism Reduced calcium-loading capacity in mitochondria Increased cytochrome c release and apoptosis Clogged protein importation machinery and reduced ETC activity Altered ROS production (but note, it's thought that there is toxic forms of	Reviewed by (Schon & Przedborski, 2011) (Lin & Beal, 2006) (Smith et al., 2017)

Disease	Genetic Cause	Description of Gene	Potential Consequences of Mutations on Mitochondrial Function	References
			mutants that drive the phenotype, and NOT the reduction of its dismutase activity)	(Taylor, Brown, and Cleveland, 2016)
	ALS2	Guanine nucleotide exchange factor (GEF)	Decreased mitophagy causing recessive juvenile form of ALS	(Smith et al., 2017)
	FUS	RNA-binding protein	Disrupted mitochondrial function including decreased ATP generation, loss of mitochondrial calcium uptake, increased ROS production Disrupted mitochondrial trafficking, mitochondrial morphology, and mitophagy Disrupted ER-mitochondrial contacts	(Smith et al., 2017)
	VAPB	Function unclear but implicated in vesicle trafficking and calcium homeostasis	Impaired mitochondrial calcium uptake Disrupted mitochondrial trafficking Disrupted ER-mitochondria contacts	(Smith et al., 2017)
	TARDBP	RNA-binding protein	Reduced mitochondrial length and mal-distribution of mitochondria in cell body and reduced mitochondrial levels in distal motor axon terminals Altered complex I disassembly Impaired ER-mito communication that leads to calcium mishandling	Reviewed by (Schon & Przedborski, 2011) (Smith et al., 2017) (Wang et al., 2016)
	OPTN	LC3 adaptor involved in mitophagy	Disrupted recruitment to the mitochondria and therefore disrupted mitophagy	Reviewed by (Schon & Przedborski, 2011) (Smith et al., 2017)
	SQSTM1	LC3 adaptor also involved in mitophagy	Disrupted interaction with LC3 and therefore disrupted mitophagy Reduced mitochondrial membrane potential	(Smith et al., 2017)
	TBK1	Kinase that can phosphorylate OPTN and SQSTM1 to promote LC3 binding	Impaired clearance of LC3 labeled autophagic cargoes including mitochondria	(Smith et al., 2017)
	VCP	Type II AAA+ ATPase Involved in dislodging damaged ubiquitinated proteins from complexes for proteasomal dependent degradation, essential for mitophagy	Disrupted mitochondrial trafficking Increased mitochondrial uncoupling and reduced ATP production Reduced mitophagy	Reviewed by (Schon & Przedborski, 2011) (Smith et al., 2017)
	SIGMA-1	Localizes to ER-mitochondria contacts and	Disrupted mitochondrial trafficking	(Smith et al., 2017)
	Receptor ($\sigma 1R$)	modulates IP3 receptor-dependent Ca^{2+} release from ER	Disrupted ER-mitochondrial contacts and dysregulated calcium homeostasis	
	C9orf72	Unclear function hypothesized to be guanine nucleotide exchange factor	Altered mitochondrial morphology and membrane potential Increased oxidative stress (increased mtROS production)	(Smith et al., 2017) (Lopez-Gonzales et al., 2016)
	CHCHD10	Mitochondrial protein localized between inner mitochondrial membrane and outer mitochondrial membrane	Disrupted of mitochondrial cristae morphology Reduced oxidative phosphorylation	(Smith et al., 2017)

Disease	Genetic Cause	Description of Gene	Potential Consequences of Mutations on Mitochondrial Function	References
Huntington's Disease (HD)	Huntingtin	Very large cytoplasmic protein playing pleiotropic functions in neurons Regulates trafficking of mitochondria and other cargoes such as synaptic vesicles (can bind with Huntingtin-associated protein, which interact with mitochondria and both kinesin and dynein/dynactin)	Impaired mitochondria trafficking Altered mitochondrial fission/fusion protein expression Fragmented mitochondria and altered mitochondrial dynamics Disrupted mitochondrial respiration	Reviewed by (Schon & Przedborski, 2011) and (Saudou and Humbert, 2016) (Lin & Beal, 2006)