Published in final edited form as:

Glia. 2018 June ; 66(6): 1213–1234. doi:10.1002/glia.23252.

Regulation of mitochondrial dynamics in astrocytes: mechanisms, consequences, and unknowns

Joshua G. Jackson^{1,2} and Michael B. Robinson^{1,2,3}

¹Children's Hospital of Philadelphia Research Institute, University of Pennsylvania, Philadelphia, PA 19104

²Department of Pediatrics, University of Pennsylvania, Philadelphia, PA 19104

³Department of Pharmacology University of Pennsylvania, Philadelphia, PA 19104

Abstract

Astrocytes are the major glial cell in the central nervous system. These polarized cells possess numerous processes that ensheath the vasculature and contact synapses. Astrocytes play important roles in synaptic signaling, neurotransmitter synthesis and recycling, control of nutrient uptake, and local blood flow. Many of these processes depend on local metabolism and/or energy utilization. While astrocytes respond to increases in neuronal activity and metabolic demand by upregulating glycolysis and glycogenolysis, astrocytes also possess significant capacity for oxidative (mitochondrial) metabolism.

Mitochondria mediate energy supply and metabolism, cellular survival, ionic homeostasis, and proliferation. These organelles are dynamic structures undergoing extensive fission and fusion, directed movement along cytoskeletal tracts, and degradation. While many of the mechanisms underlying the dynamics of these organelles and their physiologic roles have been characterized in neurons and other cells, the roles that mitochondrial dynamics play in glial physiology is less well understood. Recent work from several laboratories has demonstrated that mitochondria are present within the fine processes of astrocytes, that their movement is regulated, and that they contribute to local Ca^{2+} signaling within the astrocyte. They likely play a role in local ATP production and metabolism, particularly that of glutamate. Here we will review these and other findings describing the mechanism by which mitochondrial dynamics are regulated in astrocytes, how mitochondrial dynamics might influence astrocyte and brain metabolism, and draw parallels to mitochondrial dynamics in neurons. Additionally, we present new analyses of the size, distribution, and dynamics of mitochondria in astrocytes *in vivo* using 2-photon microscopy.

Keywords

mitochondria; mobility; astrocytes

Introduction

Mitochondria are essential organelles that play multifaceted roles in eukaryotic cells. They provide energy, coordinate local metabolism, regulate Ca^{2+} signals, and integrate survival and death cues (reviewed in Jacobson and Duchen, 2004; Rizzuto et al., 2012). Mitochondria are dynamic organelles; they move, divide from and fuse with one another, and are removed if damaged. In neurons, mitochondria are transported, often over long distance, to sites of elevated $[\text{Ca}^{2+}]$ and ATP utilization to match energetic demand with supply and provide local Ca^{2+} buffering capacity (for reviews see Hollenbeck and Saxton, 2005; Cai and Sheng, 2009; MacAskill and Kittler, 2010; Schwarz, 2013). Mitochondria may be part of large reticular networks or exist as discrete structures of varying size or shape. Even here, constant remodeling of shape and content takes place facilitating the distribution of mitochondria and homogenization of their content. In addition, mechanisms exist to remove damaged or otherwise dysfunctional mitochondria from the mitochondrial network (for reviews see Chen and Chan, 2009; Youle and Narendra, 2011; Maday et al., 2014; Mishra and Chan, 2014). The totality of these behaviors establishes the steady state distribution of mitochondria (and energy/ Ca^{2+} buffering) within the cell. Much of what we know regarding the mechanisms underlying these behaviors were gleaned from studies conducted in primary neuronal cultures. By contrast less is known regarding the roles and regulation of mitochondrial dynamics in glia, particularly astrocytes.

Astrocytes, thought to be the predominant type of glial cell in the brain, are involved in a wide range of CNS functions, including control of blood flow, glucose metabolism, glutamate clearance, ionic homeostasis (particularly K^+), synaptic development, and neuronal plasticity (for reviews see Araque et al., 1999; Attwell et al., 2010; Clarke and Barres, 2013; Dienel, 2013; Haydon and Nedergaard, 2014; Weber and Barros, 2015). The wide range of functions ascribed to astrocytes is facilitated anatomically by processes that contact synapses and blood vessels. While primary cultures of astrocytes are largely flat and retain few cellular processes (Shao and McCarthy, 1994; Derouiche and Frotscher, 2001), astrocytes in the CNS, particularly the so called protoplasmic astrocytes, possess numerous, highly branched processes, lamellipodia, and veil-like structures. The smallest of these processes, also known as the peripheral astrocytic processes (PAPs), may be 20–200 nm in diameter and possess very little cytoplasm (Kosaka and Hama, 1986; Ogata and Kosaka, 2002; for review see Benjamin Kacerovsky and Murai, 2015). This gives astrocytes a very large surface area to volume ratio. In the cortex, these PAPs often are intimately associated with synapses (Kosaka and Hama, 1986; Ventura and Harris, 1999; Witcher et al., 2010). In fact, a single astrocyte in the rodent brain may contact ~100K synapses and may contact in excess of 2 million synapses in the human brain (Oberheim et al., 2009).

Based in part on their small size, it was assumed that the fine astrocyte processes were too small to accommodate mitochondria (whose diameter may exceed 1 μm in other cell types). However, new studies, and re-examination of older studies, have revealed the presence of mitochondria within the fine astrocytic processes both *in situ* and *in vivo* (Mugnaini, 1964; Aoki et al., 1987; Lovatt et al., 2007; Oberheim et al., 2009; Mathiisen et al., 2010; Genda et al., 2011; Jackson et al., 2014b; Derouiche et al., 2015; Stephen et al., 2015; Agarwal et al., 2017; for review, see Stephen et al., 2014; Benjamin Kacerovsky and Murai, 2015; Robinson

and Jackson, 2016) and demonstrated that astrocytes exhibit a high rate of oxidative metabolism (Hertz et al., 2007). To better understand how mitochondrial dynamics and distribution were regulated in adult animals *in vivo*, we generated DNA constructs to drive expression of fluorescent proteins (mitochondrially-targeted enhanced green fluorescent protein (mitoEGFP) and a membrane-targeted tandem-dimer tomato (Lck-TdTomato) fluorescent protein) under control of a minimal glial fibrillary acid protein promoter (GFA_{ABC1D}; Lee et al., 2008). These were packaged into adeno-associated virus 2 (AAV2) particles that express the coat-protein from AAV5 (AAV2/5) which displays a tropism for astrocytes (Duale et al., 2005). A similar strategy, combining viral tropism with an astrocyte-selective promoter, has been used previously to confine expression of transgenes to astrocytes *in vivo* (Ortinski et al., 2010; Shigetomi et al., 2013). Viral particles were injected at a total dose of 3×10^9 genome copies per animal into the superficial layers of somatosensory cortex of adult mice of either sex. The distribution and trafficking of mitochondria in astrocyte processes *in vivo* were examined two weeks post-injection. As has been observed in organotypic cultures (Benediktsson et al., 2005) or *in vivo* (Shigetomi et al., 2013; Benjamin Kacerovsky and Murai, 2015), membrane-targeted TdTomato fluorescence revealed the complex morphology of the astrocyte with many fine processes and branchlets (Fig 1A, A'). EGFP-labelled mitochondria were readily visualized throughout the processes of the cortical astrocytes using confocal microscopy of fixed brain tissue (Fig 1B, B' or overlay Fig 1C, C') and *in vivo* using two-photon microscopy (Fig 1D, E), including distal processes and vascular endfeet (see also Mathiisen et al., 2010; Motori et al., 2013; Benjamin Kacerovsky and Murai, 2015). Interestingly, this approach reveals dense labeling of mitochondria in the astrocyte processes along vessel walls. Mitochondria within the astrocyte processes *in vivo* possessed a similar, but slightly smaller distribution of mitochondrial lengths to that previously observed in hippocampal organotypic cultures (Fig 1F. median length=1.4 vs 1.8 μ m, respectively; Jackson et al., 2014b). These contribute to the many converging lines of evidence that mitochondria are present within astrocytic processes *in situ* and *in vivo*.

Recently, we and others have examined aspects of mitochondrial dynamics within astrocyte processes in both acute slices, organotypic cultures, and *in vivo* (Motori et al., 2013; Jackson et al., 2014b; Jackson and Robinson, 2015; Stephen et al., 2015; O'Donnell et al., 2016). Here we review these studies and try to link them to the broader context of what is known about mitochondrial dynamics. In particular, we will compare the dynamics of mitochondria within astrocytes with the more extensively characterized neuronal population. We try to point out what is yet unknown about the dynamics of mitochondria within astrocytes and to highlight cellular functions in astrocytes that might be impacted by changes in mitochondrial distribution and dynamics.

Mitochondrial Trafficking/Transport

Mitochondria within neurons maintained in culture are actively transported in axons and dendrites (Morris and Hollenbeck, 1995; Saxton and Hollenbeck, 2012). Experiments have consistently shown that 20–40% of mitochondria are mobile within neuronal processes (axons and dendrites; Overly et al., 1996; Chen et al., 2007; for reviews see Cai and Sheng, 2009; MacAskill and Kittler, 2010; Saxton and Hollenbeck, 2012; Schwarz, 2013). The

percentage of mobile mitochondria decreases with age or time in culture and varies between brain regions (Lewis et al., 2016; Smit-Rigter et al., 2016). Recently, several groups have examined factors regulating the distribution and transport of mitochondria within the processes of astrocytes *in situ* and *in vivo* (Genda et al., 2011; Bauer et al., 2012; Jackson et al., 2014b; Jackson and Robinson, 2015; Stephen et al., 2015; O'Donnell et al., 2016). While many of the features of mitochondrial movement are similar in astrocytes and neurons, there are differences.

Direct comparisons of mitochondrial movement in organotypic hippocampal cultures shows that a greater fraction of mitochondria is mobile in neuronal dendrites than in astrocytic processes (defined at displacement > 2 $\mu\text{m}/15\text{min}$). We found that ~40% of mitochondria in dendrites were mobile over this 15-minute imaging window vs ~15–20% in astrocyte processes (Jackson et al., 2014b). Using a similar preparation, Stephen et al observed greater, but qualitatively similar results (~54% mobile in neurons vs ~31% in astrocytes; Stephen et al., 2015). In order to determine if similar mobility is observed *in vivo*, we used two-photon imaging to monitor the movement of mitochondria in transduced cortical astrocytes (AAV2/5-GFA_{ABC1D}-mitoEGFP) in adult mice (Fig 1D, E). Mice were anesthetized (α -chloralose) and the EGFPmito fluorescence visualized through an acute cranial window. As we previously observed in organotypic cultures (Jackson et al., 2014b), the percentage of mobile mitochondria *in vivo* (displacement > 0.67 $\mu\text{m}/5\text{min}$) was low ($9.7 \pm 2.5\%$; n=4 mice). This observation is consistent with the apparent stability of mitochondrially-dependent $[\text{Ca}^{2+}]$ microdomains in astrocyte processes *in vivo* that was recently reported (Agarwal et al., 2017). Whether this reflects a difference in the preparation (organotypic cultures vs *in vivo*) or a difference in the developmental age of the astrocytes (neonatal vs adult) is an open question. However, others have observed that the mitochondrial movement decreases in neurons *in vivo* (Lewis et al., 2016; Smit-Rigter et al., 2016). Using 2-photon *in vivo* imaging of mTurquoise2-labeled mitochondria, Smit-Rigter and colleagues report that ~1% of mitochondria are mobile in layer 2/3 pyramidal neuron axons (V1) in either adult or neonatal mice. Similarly, Lewis et al report <5% of mitochondria are mobile in layer 2/3 pyramidal axon collaterals *in vivo*. While they found that the percentage of mobile mitochondria decreased coincident with axon maturation *in vitro*, the percentage of mobile mitochondria did not increase from P10-adulthood *in vivo*.

The movement of mitochondria in neurons is characterized by brief pauses, accompanied by changes in speed and direction (Chang et al., 2006; Macaskill et al., 2009b). We found that the movement of mitochondria in astrocytes is similarly characterized by brief pauses, as well as changes in speed and direction (Jackson et al., 2014b). However, the features of this movement differ between astrocytes and neurons. Stephen et. al. found that in astrocytic processes, mitochondria exhibited a more oscillatory pattern of motion compared to the directed motion observed in neurons. Differences within the rates at which mitochondria move are apparent between astrocytes and neurons as well. We found that the instantaneous velocity in astrocytes (0.15 $\mu\text{m}/\text{s}$ anterograde and 0.2 $\mu\text{m}/\text{s}$ retrograde) was significantly slower than in neurons (0.55 $\mu\text{m}/\text{s}$ anterograde and 0.65 $\mu\text{m}/\text{s}$ retrograde; Jackson et al., 2014b). Stephen et. al. (2015) observed a similar difference in the rates of mitochondrial movement. What might explain these differences? In other cells, differences in the rates of movement, destination, and distance traveled have been ascribed to differences in motor

apparatus, adaptor proteins, and cytoskeletal elements. In neurons, for example, long range movements is often attributed to kinesin and dynein motors moving along microtubules, while short range movements depend on myosin-directed movement along the actin microfilaments (Nangaku et al., 1994; Morris and Hollenbeck, 1995; Tanaka et al., 1998; Pilling et al., 2006; Tanaka et al., 2011; reviewed in MacAskill and Kittler, 2010; Schwarz, 2013). In axons of neurons in culture, mitochondria move along both microtubules and actin microfilaments albeit with different velocities (Morris and Hollenbeck, 1995). Even within the kinesin superfamily, different motor proteins move at velocities that range from 0.02 to 1.5 $\mu\text{m}/\text{second}$ and vary with respect to processivity (see Martin et al., 1999). Additionally, different adaptor proteins (TRAK1 and TRAK2) associate with different machineries to transport mitochondria to axons or dendrites, with TRAK1 associating with kinesin-1 and dynactin and TRAK2 associating with dynein-dynactin to mediate axonal or dendritic targeting, respectively (van Spronsen et al., 2013). The rates of mitochondrial movement in astrocytes ($\sim 0.15 \mu\text{m}/\text{sec}$), match the rates of mitochondrial movement along actin microfilaments (Morris and Hollenbeck, 1995). Based on these differences, we would speculate that the transport of mitochondria in astrocytes may depend on different motor or adaptor proteins (Jackson et al., 2014b; Stephen et al., 2014; Stephen et al., 2015) than are utilized by neurons (please see subsection on motor and adaptor proteins).

Recently, mitochondria have been observed in the primary process of oligodendrocytes and their myelin sheaths (Rinholm et al., 2016). There are fascinating differences between these mitochondria and those found in astrocytes and neurons. Mitochondria within the oligodendrocyte primary process or myelin sheath are generally smaller (lengths of ~ 1.2 and $0.8 \mu\text{m}$, respectively) than in astrocytes or neurons ($\sim 1.4\text{--}3 \mu\text{m}$). This is particularly interesting as mitochondrial oxidative capacity is thought to vary inversely with size (Bertoni-Freddari et al., 2003; Bertoni-Freddari et al., 2005; Tondera et al., 2009). Supporting the notion of reduced oxidative capacity, Rinholm and colleagues report that putative mitochondria present in the myelin sheath are relatively devoid of cristae, another factor that correlates with respiratory potential (Palade, 1953; Mannella et al., 2001; Rinholm et al., 2016; reviewed in Zick et al., 2009; Mannella et al., 2013). Oligodendrocyte resident mitochondria are significantly less mobile than in either astrocytes or neurons ($\sim 12\%$ and $\sim 8\%$ in primary processes or sheaths, respectively) and move slower ($< 0.1 \mu\text{m}/\text{s}$; Rinholm et al., 2016). There are very striking differences in the regulation of mitochondrial movement between oligodendrocytes and either astrocytes or neurons. Whereas mitochondrial movement is arrested in both neurons and astrocytes by glutamate and Ca^{2+} , the movement of mitochondria in oligodendrocyte processes is enhanced (by $\sim 76\%$) by application of glutamate. Conversely, removal of Ca^{2+} abolished mitochondrial movement in oligodendrocytes. The authors suggest that these differences may serve an important function in oligodendrocytes; activity dependent dispersal of mitochondria may preserve lactate production for export to axons (Rinholm et al., 2016). The differences in mitochondrial mobility and its regulation further strengthen the notion that different cells may utilize different strategies to position mitochondria.

Role of activity in regulating mitochondrial movement

Given the energetic demands of continuously moving mitochondria, one might ask “what is the purpose of moving mitochondria?”. The coordinated movement of mitochondria along cellular processes probably serves several roles. First, the movement of mitochondria facilitates the distribution of mitochondria into cellular processes. While mitochondrial biogenesis (which involves nuclear transcription as well as mtDNA replication and fission) can occur locally in neuronal processes (Amiri and Hollenbeck, 2008), most mitochondria are thought to be generated in the cell body and subsequently transported in an anterograde direction into the processes (Davis and Clayton, 1996; for review, see Schwarz, 2013). Second, the retrograde movement of mitochondria out of cellular processes probably facilitates the degradation of depolarized, damaged, or aged mitochondria (Frederick and Shaw, 2007). Third, mitochondrial transport facilitates fusion and fission processes that allow the exchange of proteins and genetic material between discrete mitochondria (Misko et al., 2010). Fourth, mitochondrial transport allows for the relatively rapid redistribution of mitochondria, at least in culture, to sites of elevated activity (for reviews, see MacAskill et al., 2010; Schwarz, 2013) such as active synapses or nodes of Ranvier in a neuron or the perisynaptic astrocytic processes where they can provide local energy and regulate Ca^{2+} signaling. Finally, relatively recent evidence suggests a role for mitochondrial trafficking in regulating the transfer of mitochondria between adjacent cells, termed horizontal transfer (Ahmad et al., 2014; Berridge et al., 2016b).

Arguably one of the most important features of mitochondrial movement, particularly in neurons, is its directed distribution and the consequent accumulation of mitochondria at sites of elevated metabolic load and $[\text{Ca}^{2+}]$. In neurons, mitochondria actively accumulate in areas of high-energy demand and Ca^{2+} flux including synaptic terminals, nodes of Ranvier and growth cones (Li et al., 2004; Miskeld et al., 2007; Ohno et al., 2011; Bertholet et al., 2013). Blocking mitochondrial redistribution to these areas, either by manipulating mitochondrial transport, decreasing mitochondrial anchoring, or blocking fission (necessary for mitochondrial entry to spines) inhibits synapse formation, axonal/dendritic branching, and maintenance of these structures (Li et al., 2004; Guo et al., 2005; Verstreken et al., 2005; Ishihara et al., 2009; Courchet et al., 2013; Lopez-Domenech et al., 2016). In fact, blocking mitochondrial trafficking (Miro1 knock-out) leads to progressive neurodegeneration in peripheral nerves (Nguyen et al., 2014). Thus, it seems clear that mitochondrial distribution in neurons is important to neuronal maturation and the maintenance of neuronal structures. While it is unknown whether mitochondrial distribution controls the maturation and elaboration of astrocyte or oligodendrocyte processes, it is an intriguing possibility.

The accumulation of mitochondria at sites of elevated activity presumes a signal arresting their movement and retaining them. In cultured neurons, mitochondrial movement is inversely dependent upon neuronal activity. Decreasing neuronal activity (with tetrodotoxin or omega-conotoxin GVIA) increases mitochondrial movement, while conversely increasing neuronal activity (KCl depolarization or activation of NMDA receptors) decreases movement (Rintoul et al., 2003; Li et al., 2004; Mironov, 2006). In addition to increases in local $[\text{Ca}^{2+}]$, increases in [ADP] subsequent to increased ATP consumption have also been implicated in the arrest of mitochondria at synapses (Mironov, 2006, 2007).

Much of astrocytic activity is tuned to neuronal activity, and in particular excitatory (glutamatergic) activity (Araque et al., 1999; Halassa et al., 2007; Araque and Navarrete, 2010). Using transfected organotypic cultures of rat hippocampus, we found that the percentage of mobile mitochondria in astrocytes was low (~15%). Treatment of the slices with tetrodotoxin, a Na^+ channel antagonist that blocks action potential generation, increased the percentage of mobile mitochondria to ~45% (Jackson et al., 2014b). Similarly, simultaneous blockade of AMPA, NMDAR, and GABAR receptors (DNQX, APV, bicuculline, respectively) increased the percentage of mobile mitochondria. Recently, work from Joseph Kittler's lab, confirmed the observation that inhibiting neuronal activity increases mitochondrial mobility in astrocytes (Stephen et al., 2015). As a greater proportion of mitochondria were mobile in their preparations, they were also able to demonstrate that treatment with glutamate or 4-AP (increasing neuronal activity) decreased the percentage of mobile mitochondria (Stephen et al., 2015). Both the Robinson and Kittler laboratories have implicated glutamate in the mechanism of mitochondrial arrest (Jackson et al., 2014b; Stephen et al., 2015), however we differ in some of the mechanistic details of this arrest.

Perhaps one of the most important roles that astrocytes play is in the clearance of glutamate following excitatory neurotransmission. Glutamate, the principal excitatory neurotransmitter in the forebrain, is almost entirely cleared into astrocytes via GLAST and GLT1 (EAAT1 and EAAT2, respectively; Rothstein et al., 1994; Tanaka et al., 1997; for review see Danbolt, 2001). Glutamate uptake is steeply Na^+ dependent. The glutamate transporters catalyze the movement of glutamate (and aspartate) against its concentration gradient along with 3 Na^+ ions and a H^+ , and the counter-transport of a K^+ ion (Zerangue and Kavanaugh, 1996). This large increase in Na^+ influx activates the Na^+/K^+ -ATPase (utilizing energy) and causes local reversal of the $\text{Na}^+/\text{Ca}^{2+}$ -exchanger (NCX), leading to increase in $[\text{Ca}^{2+}]$ (Pellerin and Magistretti, 1996; Kirischuk et al., 1997; Pellerin and Magistretti, 1997; Rojas et al., 2013). We, and subsequently others, have observed co-compartmentalization of both GLT1 and GLAST with mitochondrial proteins and the Na^+/K^+ -ATPase in astrocyte processes (Genda et al., 2011; Bauer et al., 2012; Jackson et al., 2014a; Shan et al., 2014). Based on this, and the large depolarization that accompanies glutamate (Na^+) influx, we hypothesized that mitochondrial movement might be regulated by glutamate uptake. We found that inhibition of glutamate uptake (with TFB-TBOA) or the Na^+/K^+ -ATPase (with ouabain) increased the percentage of mobile mitochondria in astrocyte processes. Conversely, application of D-aspartate decreased the percentage of mobile mitochondria in the presence of TTX (Jackson et al., 2014b). Further, two structurally dissimilar inhibitors of reversed-mode $\text{Na}^+/\text{Ca}^{2+}$ -exchange both increased the percentage of mobile mitochondria. These results demonstrate that activation of glutamate transport is necessary and sufficient to stop mitochondria moving and suggest that increases in $[\text{Ca}^{2+}]$ might underlie their arrest.

By contrast, Stephen et al (2015) implicate activation of NMDARs on astrocytes in the arrest of mitochondrial movement. In hippocampal slice cultures, they found that glutamate decreased the percentage of mobile mitochondria. This decrease was not blocked or only partially blocked by inhibiting AMPA receptors (with 2,3-Dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide; NBQX) or mGluRs (with α -Methyl-4-carboxyphenylglycine; MCPG), respectively. However, the decrease in mobility was blocked by the NMDAR antagonist D-APV or by removing extracellular Ca^{2+} . They also found that

MK801 was able to block glutamate-induced mitochondrial shortening in slices or in pure astrocyte cultures (Stephen et al., 2015). While both groups broadly agree that glutamate is decreasing mitochondrial movement, and that this proceeds through increases in $[Ca^{2+}]$, we differ in mechanisms by which glutamate mediates this $[Ca^{2+}]$ increase. These differences could reflect differences in culture preparation. Alternatively, perhaps transporter mediated-depolarization facilitates opening of NMDA receptors on astrocytes. We would suggest that a possible resolution to this would involve knocking out NMDARs specifically in astrocytes.

The Ca^{2+} -dependent arrest of mitochondria is thought to facilitate the activity-dependent positioning of mitochondria and underlie the observed steady-state accumulation of mitochondria at synapses and other active regions (Macaskill et al., 2009b; Wang and Schwarz, 2009). Like in neurons, mitochondria in astrocytes are not uniformly distributed. Previously, we observed that mitochondria co-localize with clusters of glutamate transporters in the processes of transfected astrocytes; a phenomenon that occurred more than would be predicted by chance (Genda et al., 2011). This accumulation depends on neuronal activity. We examined the spatial relationship of individual mitochondria with glutamate transporter puncta and synapses in the presence and absence (+TTX) of neuronal activity. We found that ~80% of mitochondria were within 1 μm of an endogenous glutamate transporter puncta (and VGLUT1 positive pre-synaptic terminal). In the absence of neuronal activity (+TTX) the distance between mitochondria and glutamate transporters (and synapses) increased (Jackson et al., 2014b). In co-cultures of hippocampal neurons and astrocytes, glutamate application or electrical field stimulation decreased the mean distance between astrocytic mitochondria and VGLUT+ puncta, as did application of 4-AP in hippocampal slices (Stephen et al., 2015). Thus, multiple lines of evidence from multiple labs suggest that neuronal activity increases the apposition of astrocytic mitochondria with synapses.

Role of motor proteins and adaptors

The movement and distribution of mitochondria to active cellular compartments, whether into the synapse in neurons or perisynaptic processes of astrocytes, is likely determined by their engagement with the cellular transport machinery (reviewed in MacAskill and Kittler, 2010; Saxton and Hollenbeck, 2012; Schwarz, 2013). While, neurons can utilize both the actin and microtubule cytoskeletons for the directed transport of mitochondria, long-range transport proceeds mainly along the microtubule cytoskeleton (reviewed in Hollenbeck and Saxton, 2005; MacAskill et al., 2009a; Schwarz, 2013; Maday et al., 2014; see Fig 2 for model). Microtubules are polymers comprised of α and β -tubulin dimers joined end-to-end to form a polarized structure with β (+) and α (–) ends (Watanabe et al., 2005). In axons, microtubules display uniform polarity with the β (+) end oriented distally toward the axon terminal while they display a mixed orientation in dendrites (Baas et al., 1988). Most plus-end mediated transport is mediated by kinesin superfamily motors, while minus-end mediated transport is via cytoplasmic dynein (Pilling et al., 2006; Hirokawa et al., 2009). Thus, in axons, most anterograde movement is via kinesin motors while retrograde movement is via dynein. In dendrites, because of mixed microtubule polarity, both kinesin and dynein motors can operate in either the retrograde or anterograde direction. The orientation of the microtubule cytoskeleton in astrocytes is not known. However,

mitochondria move at similar rates and in similar proportions in both the anterograde and retrograde directions (Jackson et al., 2014b; Stephen et al., 2015), perhaps implying a mixed orientation, as in dendrites.

What motor proteins are responsible for the movement of mitochondria in astrocytes? The kinesin family of motors is relatively large, including at least 45 members (Hirokawa et al., 2009). Several kinesin family members have been implicated in the transport of mitochondria. In neurons, most plus-end mediated movement is mediated by Kif5 (kinesin-1) superfamily members (MacAskill et al., 2010). Of the three Kif5 superfamily members, Kif5a and Kif5c are principally neuronal, while Kif5b is ubiquitously expressed (Nangaku et al., 1994; Tanaka et al., 1998; Kanai et al., 2000; Zhang et al., 2014). Deletion of Kif5b leads to the perinuclear accumulation of mitochondria (Tanaka et al., 1998) in cultured extraembryonic cells, whilst expression of a KIF5B mutant lacking the motor domain blocks transit of mitochondria into neurites in Neuro2 cells (Tanaka et al., 2011). In addition to Kif5, other kinesin family members have been associated with mitochondrial transport. Kif1B (kinesin-3) localizes to mitochondria in neurons and purified protein can transport mitochondria along microtubules *in vitro*. Kinesin-like protein 6 (Klp6) mutants inhibit anterograde transport of mitochondria in differentiated neuro 2a. By contrast, only one dynein type motor, cytoplasmic dynein, has been implicated as the primary retrograde mitochondrial motor (Pilling et al., 2006). It is, as yet, unknown which motor protein(s) mediates mitochondrial movement in astrocytes.

The movement of mitochondria along the microtubule cytoskeleton depends on several adaptor proteins that link mitochondria to the motor proteins (Glater et al., 2006; for review see MacAskill and Kittler, 2010; Sheng and Cai, 2012; Schwarz, 2013). Amongst these are the mitochondrial Rho proteins (Miro; also known as RhoTs) and the trafficking kinesin binding proteins (TRAKs, known as Milton in *drosophila*). Mutations in Milton lead to loss of mitochondria from photoreceptor terminals and blindness in *drosophila* (Stowers et al., 2002). The Milton/TRAK proteins bind kinesin motors (KIF5) and Miro protein (Glater et al., 2006; Smith et al., 2006; Macaskill et al., 2009b). The Miro proteins (Miro1 and Miro2) possess an n-terminal outer mitochondrial membrane anchoring motif, two EF-hand Ca^{2+} binding domains, and 2 Rho-GTPase domains (Fransson et al., 2003; Fransson et al., 2006). In neurons and other cells the Ca^{2+} -dependent arrest and accumulation of mitochondria in response to elevated $[\text{Ca}^{2+}]$ is thought to be largely dependent on the Miro proteins (Saotome et al., 2008; Macaskill et al., 2009b; Wang and Schwarz, 2009). Mutations in the Ca^{2+} binding domain of Miro proteins block the Ca^{2+} -dependent arrest of mitochondrial movement. In neurons, both sets of proteins are important for correct trafficking of mitochondria to processes (Glater et al., 2006; MacAskill et al., 2009a; Brickley and Stephenson, 2011; van Spronsen et al., 2013).

Similar roles for the TRAK and Miro proteins in astrocytes have recently started to be appreciated. In primary cultures of astrocytes, the TRAK proteins are not expressed. Upon co-culture with neurons, astrocytes increase expression of TRAK2, allowing transit of mitochondria into astrocyte process (Ugbode et al., 2014). Both Miro1 and Miro2 are expressed in astrocytes and localize to mitochondria within the processes of astrocytes (Zhang et al., 2014; Jackson and Robinson, 2015; Stephen et al., 2015; reviewed in Stephen

et al., 2014). As in neurons, the Miro proteins appear to mediate the Ca^{2+} sensitive arrest of mitochondria within the processes of astrocytes in response to neuronal activity and glutamate (Jackson et al., 2014b; Jackson and Robinson, 2015; Stephen et al., 2015). Exogenous expression of wild-type Miro proteins results in an increase in the percentage of mobile mitochondria (Jackson and Robinson, 2015; Stephen et al., 2015), suggesting that Miro availability might limit mobility in astrocytes. Expression of Ca^{2+} -insensitive Miro1 or Miro2 mutants increase the percentage of mobile mitochondria in astrocyte processes and blocks the arrest of mitochondria in response to increased Ca^{2+} signaling (Jackson and Robinson, 2015; Stephen et al., 2015).

In addition to regulating mitochondrial movement, Miro proteins may play additional roles in regulating mitochondrial function and signaling. Mitochondria closely approach endoplasmic reticulum (ER) Ca^{2+} release channels and form physical structures with the ER in multiple cell types (Rizzuto et al., 1993; Rizzuto et al., 1994; Rizzuto et al., 1998; Csordas et al., 1999; Csordas et al., 2006). Close apposition of the mitochondria and ER facilitates Ca^{2+} transfer between these organelles (Csordas et al., 2006). Miro proteins may be involved in the formation of tethers linking the ER with mitochondria. Gem1, a Miro homologue found in yeast, was identified as a regulator of the endoplasmic reticulum-mitochondrial encounter structure (ERMES; Kornmann et al., 2011). This complex, which includes Fis1 and Mfn2, forms a physical link between the ER and mitochondria and may facilitate lipid exchange between the organelles. The existence of mitochondrial/ER tethers has not been explored in astrocytes, however there is evidence that the ER exists within the astrocyte processes (Spacek, 1982; Benjamin Kacerovsky and Murai, 2015).

Like in neurons, the majority of mitochondria in astrocytes are, in fact, immobile (Morris and Hollenbeck, 1993, 1995; Miller and Sheetz, 2004; Kang et al., 2008; Jackson et al., 2014b; Stephen et al., 2015). We found that up to 90% of mitochondria were immobile over a 15 minute viewing window. Even in the absence of neuronal activity, (TTX) the percentage of mobile mitochondria was not 100% (Jackson et al., 2014b; Stephen et al., 2015). This suggests either that other processes regulate mitochondrial mobility in astrocytes or that the mitochondria might be physically tethered to domains within astrocytes. Such physical tethering/anchoring proteins have been identified in neurons. One of these, syntaphilin, a microtubule binding protein, has been suggested to mediate the permanent capture/immobilization of mitochondria within axons (Kang et al., 2008). However, syntaphilin mRNA is absent from astrocytes (Zhang et al., 2014). The existence of different proteins regulating this immobile pool of mitochondria in astrocytes is an interesting area for future exploration.

In addition to the microtubule cytoskeleton, the actin cytoskeleton and myosin motors contribute to the distribution of cellular cargo. In contrast to microtubule-based transport, the myosin family of proteins are the only known actin-based motor (reviewed in Hartman and Spudich, 2012). While the motor/head domain of the myosin proteins is largely conserved, many of these proteins possess variable tail domains that enable specificity with respect to the binding of different cargos or adaptor proteins (reviewed in Boldogh and Pon, 2006; Akhmanova and Hammer, 2010).

While most long-range mitochondrial movement in neurons depends on microtubule-based motors, there is evidence, sometimes conflicting, of mitochondrial interactions with the actin cytoskeleton and myosin motors (Bradley and Satir, 1979; Morris and Hollenbeck, 1993; Ligon and Steward, 2000; Saxton and Hollenbeck, 2012). In neurons, it has been hypothesized that mitochondrial movement might proceed along actin filaments where microtubules might be sparse, such as at active growth cones (Bradley and Satir, 1979). In cultures of hippocampal neurons, Ligon and Steward found that mitochondrial movement was arrested by treatment with cytochalasin D, which aggregates actin filaments, although latrunculin (an actin polymerizing agent) had no effect (Ligon and Steward, 2000). In cultures of dorsal root ganglion neurons, mitochondria accumulate near to nerve growth factor (NGF) beads. This accumulation is blocked by Latrunculin B without blocking overt mitochondrial transport (Chada and Hollenbeck, 2004); suggesting a role for the actin cytoskeleton in anchoring/retaining mitochondria.

Recently, several myosin motors have been identified that associate with mitochondria and influence mitochondrial dynamics in other cell types. Myo 19 is anchored to the outer-mitochondrial membrane through its c-terminus. Its overexpression resulted in a nearly 2-fold increase in mitochondrial mobility in A549 cells (an epithelial cell line), while a dominant-negative tail domain mutant decreased movement (Quintero et al., 2009). Myo19 also facilitates the localization of mitochondria to filopodial extensions in U2OS cells (Shneyer et al., 2016). RNAi-mediated knockdown of Myo19 decreased filopodial size and number. Moreover, mitochondria were absent from the remaining filopodia, suggesting that myo19 might regulate filopodial extension and mitochondrial transit to those filopodia. By contrast, RNAi-mediated knockdown of myosin IV and V increased both the percentage of mobile mitochondria and their velocity in cultured drosophila neurons (Pathak et al., 2010), suggesting that myosin motors might oppose mitochondrial movement in neurons. In astrocytes, we found that application of cytochalasin D or vinblastine decreased the percentage of mobile mitochondria, suggesting that both the actin and microtubule cytoskeletons, respectively, contribute to the movement of mitochondria in astrocytic processes (Jackson et al., 2014b). Further, while only suggestive, the rates of mitochondrial movement in astrocytes ($\sim 0.15 - 0.2 \mu\text{m}/\text{sec}$), match the rates of mitochondrial movement along actin microfilaments (Morris and Hollenbeck, 1995). The identity of the motor proteins, whether microtubule or actin resident, that mediate the movement of mitochondria in astrocyte processes, and their relative contributions to that movement remains unknown.

In addition to actin and microtubule networks, intermediate filament (IF) proteins constitute part of the cytoskeleton. Unlike the actin or microtubule cytoskeletons, there are no known motors that operate along intermediate filaments. Astrocytes express three types of intermediate filament proteins (vimentin, nestin, and glial fibrillary acid protein (GFAP); Pekny and Pekna, 2004). Vimentin and GFAP, in particular, increase dramatically during reactive astrogliosis (Eng et al., 1987; Chen et al., 1993; Janeczko, 1993). Intermediate filaments associate with mitochondria in a number of cell types, including muscle and neurons (Mose-Larsen et al., 1982; Summerhayes et al., 1983; Linden et al., 1989; Leterrier et al., 1994; Tang et al., 2008). In fact, vimentin directly bind to mitochondria (Nekrasova et al., 2011). Further, the association of vimentin with mitochondria regulates its movement and function. Disruption of vimentin IFs increases mitochondrial movement and alters the

distribution of mitochondria in cell lines and fibroblasts (Nekrasova et al., 2011). shRNA mediated knockdown of vimentin decreases mitochondrial membrane potential (Tang et al., 2008; Chernoiivanenko et al., 2015). It is unclear whether changes in vimentin or GFAP expression in astrocytes might alter mitochondrial dynamics or function in astrocytes, however Motori et al (2013) found that pro-inflammatory stimuli in astrocyte cultures (LPS + IFN γ) or *in vivo* (cortical stab wound) elicited transient changes in mitochondrial dynamics favoring fission over fusion (discussed in the next section).

Fission/Fusion

While we often conceptualize mitochondria as discrete organelles, the reality is more complicated. Mitochondria in most cells exist as a complex continuum between a reticular network of connected mitochondria and discrete organelles. The shape and interconnectedness of this network varies between cells, within cells, and is dynamic. The mitochondrial network is characterized by the near constant division and condensation of individual mitochondria. These opposing activities, termed fission and fusion, determine the size and shape of the mitochondrial network in eukaryotic cells and, along with movement, determine its distribution. These structural rearrangements also facilitate the exchange of mitochondrial components, including mtDNA, lipids, and proteins (Parone et al., 2008; Ban-Ishihara et al., 2013; Mishra and Chan, 2014; Ishihara et al., 2015; Bertholet et al., 2016), and contribute to homogenization of the mitochondrial pool.

Mitochondria are membrane encapsulated organelles that possess both inner and outer membranes. Mitochondrial fusion, in which two or more mitochondria condense into one larger mitochondrion, involves the merger of both the inner and outer mitochondrial membranes of the parent mitochondria. Mitochondrial fusion is controlled by the mitofusin proteins (Mfn1, and Mfn2) and optic atrophy 1 (OPA1; Santel and Fuller, 2001; for review see Chen and Chan, 2009; Liesa et al., 2009). Fission, by contrast, describes the division of a single mitochondria into two organelles. Mitochondrial fission is controlled by dynamin-like protein 1, (DRP1) and fission 1 protein (Fis1; Chang and Blackstone, 2010). The balance between mitochondrial fission and fusion events determines the shape, connectivity, distribution, and density of mitochondria within neuronal, and presumably, astrocytic processes. This balance between fission/fusion is controlled both at the level of expression of these proteins and by post-translational modification (e.g. phosphorylation, ubiquitination, etc; Cribbs and Strack, 2007; Ziviani et al., 2010; for review see Flippo and Strack, 2017).

Mitochondrial fission and fusion are critical for proper development of the nervous system. Neural cell-specific ablation of the mitochondrial fission factor Drp1 is embryonic lethal in mice (Ishihara et al., 2009). Primary cultures of forebrain neurons derived from Drp1 knockout mice possess fewer neurites and display defective synapse formation (Ishihara et al., 2009). Mutations in the genes encoding the fusion-related proteins MFN2 and OPA1 are associated with neurodegenerative diseases. Mutations in MFN2 lead to Charcot-Marie-Tooth subtype 2A (Zuchner et al., 2004), while mutations in OPA1 lead to autosomal optic atrophy (Alexander et al., 2000; Delettre et al., 2000; Delettre et al., 2002). Mitochondrial fragmentation and perturbations in mitochondrial fission and fusion have been observed in other neurodegenerative disorders, including amyotrophic lateral sclerosis (ALS),

Huntington's and Parkinson's disease, however the precise role that these events play in the etiology of these disorders is yet unclear (Calkins et al., 2011; Shirendeb et al., 2011; DuBoff et al., 2012; Shirendeb et al., 2012; for reviews see Reddy et al., 2011; Anne Stetler et al., 2013; Hroudova et al., 2014; Burte et al., 2015; Flippo and Strack, 2017).

In neuronal somata, mitochondria often resemble a reticular network, while mitochondria, particularly within the axons, exist as discrete organelles. The distribution of mitochondria within astrocytes appears to mirror that of neurons, with cell body resident mitochondria resembling the reticular network seen in neuronal soma (Li et al., 2004; Popov et al., 2005; Jackson et al., 2014b), whilst mitochondria in processes are discrete, vermiform structures ranging from 0.2–6 μm in length (Genda et al., 2011; Motori et al., 2013; Jackson et al., 2014b; Derouiche et al., 2015; Stephen et al., 2015; O'Donnell et al., 2016). Recently, we, and others, demonstrated mitochondria distributed throughout the astrocytic arborization, even into the small distal processes where the volume of the mitochondria occupies almost the entire process (Genda et al., 2011; Motori et al., 2013; Jackson et al., 2014b; Stephen et al., 2015; see also Fig 1). Indeed, the existence of a population of diminutive mitochondria (0.2 μm in diameter) in the very distal tips of the peripheral astrocyte processes was described in astrocyte cultures (Derouiche et al., 2015). The small size and discrete nature of these mitochondria would suggest a prominent role for Drp1-mediated mitochondrial fission in astrocyte processes. There is a precedent for this. In neurons, activity drives mitochondrial fission and subsequent recruitment of very small mitochondria ($<1 \mu\text{m}$) to spines and filopodia (Li et al., 2004). Drp1 inhibition reduces the number of mitochondria that are present in dendrites (Li et al., 2004) and synaptic terminals (Verstreken et al., 2005). Thus, Drp1 mediated mitochondrial fission facilitates the entry of mitochondria into the distal processes of neurons, perhaps it serves a similar role in astrocytes.

Using viral expression of a mitochondrially-targeted photoconvertible fluorescent protein, mito-Dendra, in organotypic hippocampal cultures, Stephen et al (2015) showed evidence for mitochondrial fusion on a constitutive basis in the astrocyte processes. While, no direct comparison of the fission/fusion dynamics between astrocytes and neurons has been conducted, the slower movement and greater percentage of immobile mitochondria in astrocyte processes (relative to neurons), as well as the often-tortuous geometry of these processes, might suggest that mitochondrial fusion (and exchange of mitochondrial content) might occur less frequently than in neurons. Limited fusion-mediated exchange of mitochondrial content would be expected to manifest as heterogeneity of individual mitochondria within astrocyte processes. Supporting this notion, α -ketoglutarate dehydrogenase is heterogeneously distributed in mitochondria within individual astrocytes in primary culture (Waagepetersen et al., 2006).

As with trafficking, the dynamics and morphology of mitochondria in astrocytes appears to be regulated by neuronal activity (glutamate). Bath application of glutamate decreased mitochondrial fusion in astrocytes in organotypic cultures. Whether this was a primary effect on fusion or secondary to decreases in mitochondrial transport is unclear (Stephen et al., 2015). In addition to decreasing fusion, increasing neuronal activity (4-AP) or glutamate decreased the length of mitochondria from $\sim 3 \mu\text{m}$ to $\sim 1 \mu\text{m}$ (Stephen et al., 2015). This is

similar to what was observed in primary cultures of hippocampal neurons in response to glutamate (Rintoul et al., 2003).

Astrocytes respond to a wide range of noxious stimuli or damage by a set of changes in morphology and expression patterns that are collectively termed “reactivity”. In the case of prolonged or severe injury this reactivity can be accompanied by the proliferation of astrocytes (gliosis; reviewed in Sofroniew, 2009). These changes vary according to the type of stimulus and can be beneficial or harmful (Bush et al., 1999; Menet et al., 2003; Okada et al., 2006; Zamanian et al., 2012). Changes in metabolic pathways have been observed in the response of astrocytes to inflammation and tissue injury (Hamby et al., 2012; Zamanian et al., 2012). In addition, changes in mitochondrial morphology and mitochondrial dynamics have been observed post injury. In organotypic hippocampal cultures oxygen-glucose deprivation (OGD) leads to decreased mitochondrial length and increased mitochondria number (due to either increased fission or decreased fusion) beginning several hours following the insult. This is followed by the loss of ~50% of mitochondria from the astrocyte processes (O'Donnell et al., 2016). Similarly, cortical stab wound or inflammatory stimuli (LPS + IFN γ) result in large scale remodeling of mitochondrial networks in astrocytes *in vivo* and *in situ*, respectively, favoring fission over fusion. These effects are mediated by phosphorylated Drp1 and result in decreases in respiratory capacity (Motori et al., 2013). Along a similar line, blocking reactive astrocytosis, by disruption of astrocytic STAT3, decreases mitochondrial function and increases mitochondrial oxidative stress (Sarafian et al., 2010). Conversely, others have found that increases in inflammation stimulate mitochondrial oxidative metabolism and mitogenesis in astrocytes (Jiang and Cadenas, 2014). It is clear, however, that the large scale structural and phenotypic remodeling of astrocytes to injury and inflammation is associated with similar changes in the mitochondrial network.

Remodeling of the mitochondrial network plays a role in the response of astrocytes to injury and inflammation. Following pilocarpine induced status epilepticus (SE), Ko et al (2017) observed apoptosis of astrocytes in the molecular layer of the dentate gyrus and clasmatodendrotic astrocytes in area CA1 (characterized by round edematous cell bodies, short processes, loss of distal processes, nuclear dissolution, and LAMP-1⁺ lysosomes) of rat hippocampus. This astrocytic damage was accompanied by regionally-distinct mitochondrial remodeling, with mitochondrial length decreasing in dentate gyrus but increasing in area CA1 (Ko et al., 2016). Pharmacologic inhibition of mitochondrial fission (Mdivi-1) attenuates, while enhancing fission (WY14643) increases, the cell death seen in the dentate gyrus post-SE. Increasing mitochondrial fission increased astrocytic autophagy in area CA1. In related work, SE-evoked increases in astrocytic death and reactive astrocytosis were attenuated by inhibitors of cyclin-dependent kinase 5 (CDK5) acting upstream of Drp1-s616 phosphorylation (Hyun et al., 2017).

Similarly, changes in the fission/fusion dynamics and the astrocytic mitochondrial network are observed in several neurodegenerative diseases. Drp1, the human homologue of DLP1, is decreased in astrocytes of Parkinson's disease patients (Hoekstra et al., 2015). SiRNA-mediated knockdown of Drp1 leads to a hyperfused mitochondrial morphology, altered Ca²⁺ signaling and decreases glutamate (aspartate) uptake (by ~30%). This loss of glutamate

uptake is associated with an impaired ability of astrocytes to protect dopaminergic neurons in culture against the addition of excess glutamate (Hoekstra et al., 2015).

The mechanisms regulating mitochondrial transport, mitogenesis, and fission/fusion do not operate in isolation. There are considerable interactions between the fission/fusion apparatus and various transport-mediating proteins. Dynein and dynactin recruit the fission related protein Drp1 to the outer mitochondrial membrane (Varadi et al., 2004). The fusion protein Mfn2 physically interacts with Miro proteins (Misko et al., 2010) and knock-out of MFN2 or expression of disease-causing variants of Mfn2 decrease axonal mitochondrial transport (Baloh et al., 2007; Misko et al., 2010) in neurons. In addition, mutations disrupt axonal mitochondrial positioning and promote axon degeneration (Misko et al., 2012). Conversely, proteins that are conventionally associated with mitochondrial transport can influence mitochondrial fusion/fission and shape. Expression of Miro1/2 isoforms harboring null mutations in their GTPase or Ca^{2+} -binding domains results in increases in mitochondrial length (Fransson et al., 2006; Saotome et al., 2008). Similarly, expression of Ca^{2+} -insensitive mutants of Miro1 or Miro2 modestly increase the length of mitochondria in astrocytes (Jackson et al., 2014b; Stephen et al., 2015). Myosin V increases length of axonal mitochondria (Pathak et al., 2010). The coordination of mitochondrial trafficking and fission/fusion dynamics likely contributes to appropriate distribution of mitochondria within neuronal and astrocytic processes.

Quality control/mitophagy

Depolarized or damaged mitochondria are degraded through a specialized, cargo specific form of macroautophagy termed mitophagy (Lemasters, 2005; reviewed in Youle and Narendra, 2011; Wong and Holzbaur, 2015). Mitophagy is often preceded by the arrest of mitochondrial movement and mitochondrial fission (Twig et al., 2008), possibly to isolate individual dysfunctional mitochondria from the larger network and to make mitochondrial fragments small enough to be engulfed by the autophagosome apparatus.

PTEN-induced putative kinase protein (Pink1) and the cytosolic ubiquitin E3 ligase, Parkin (PARK2) play important roles in the initialization of this pathway. Mitochondrial damage or collapse of the mitochondrial matrix potential (ψ_m) results in the accumulation of Pink1 (due to loss of inner-mitochondrial membrane localized protease presenilin-associated rhomboid-like protein (PARL) -mediated Pink1 degradation) and the subsequent recruitment of parkin from the cytosol to the outer mitochondrial membrane (Jin et al., 2010; Deas et al., 2011; Greene et al., 2012; Meissner et al., 2015; Reviewed in Wong and Holzbaur, 2015). Mutations in the genes encoding parkin and Pink1 have been linked to forms of familial Parkinson's disease (Kitada et al., 1998; Valente et al., 2004b; Valente et al., 2004a). Parkin catalyzes the ubiquitination of various substrates on the outer mitochondrial membrane, including Drp1 (Wang et al., 2011), VDAC (Geisler et al., 2010), Miro (Weihs et al., 2009; Liu et al., 2012), and Mfn1/2 (Poole et al., 2010; Ziviani et al., 2010). This is followed by autophagosome formation and the engulfment and degradation of damaged mitochondria (reviewed in Narendra et al., 2012; Wong and Holzbaur, 2015).

In neurons, autophagosomes are continuously formed in the distal tips of neurites and are transported retrogradely to the cell body (Maday and Holzbaur, 2012; Maday et al., 2012; Ashrafi et al., 2014). A small percentage (~10%) of these autophagosomes contain mitochondrial fragments (Maday et al., 2012), suggesting a constitutive, but low level turnover of mitochondria in neuronal processes. Similarly, basal autophagosome formation (and mitophagy) appears to be low in astrocyte processes (Motori et al., 2013; O'Donnell et al., 2016). In astrocyte processes *in vivo*, Motori et al observed that ~1% of GFP+ mitochondria co-localized with LC3BII (a marker of autophagosomes). Cortical stab wound resulted in an increase in autophagosome formation, increased mitochondrial fragmentation, and resulted in a 3-fold increase in the percentage mitochondria that co-localize with the autophagosome marker (Motori et al., 2013). Using organotypic hippocampal cultures, we found that oxygen-glucose deprivation (OGD) increased the colocalization of mitochondria with the autophagosome marker LC3B and caused a delayed loss of mitochondria (~50%) from astrocytic processes between 8–24 hrs post injury (O'Donnell et al., 2016). These results are consistent with observation in neurons demonstrating (mito)autophagosome generation in distal axons in response to damaged mitochondria (Ashrafi et al., 2014). It remains to be seen whether autophagosome maturation is local or whether these organelles are transported to the soma (retrograde) for degradation in astrocytes.

In neurons and other cells, removal of damaged or dysfunctional mitochondria is thought to protect the cell from oxidative damage (Loeb et al., 2005). In astrocytes, blocking of mitophagy is associated with an inability to re-establish the mitochondrial network and increased cell death. Blocking autophagosome induction (either by knockout of ATG7 or expression of a dominant-negative ATG4B mutant), altered mitochondrial fission/fusion balance, increased ROS formation, and increased inflammation-mediated astrocyte cell death (Motori et al., 2013). Genetic ablation of Pink1 decreases mitochondrial function (decreased mitochondrial mass, ψ m, ATP production, and increased ROS generation) and attenuates astrocyte proliferation in primary cultures of astrocytes (Choi et al., 2013). Given the role of astrocytes in coordinating neuronal metabolism, survival, and synaptic maintenance, it will be interesting to determine the extent to which mitochondrial quality control in astrocytes contributes to neuronal death and disease progression in neurodegenerative diseases.

Transcellular/ intercellular transfer of mitochondria

Until recently, the conventional view of mitochondrial inheritance was that mitochondria are transmitted during cell division, with newly generated mitochondria split between the mother and daughter cells. However, recent studies have demonstrated the existence of other mechanisms that allow the transfer of mitochondria between cells in the absence of cell division. This process, termed horizontal transfer, can be broadly placed into two categories, those favoring degradation of mitochondria and those that allow incorporation of mitochondrial DNA and proteins into the existing mitochondrial network of the acceptor cell (for reviews see Las and Shirihai, 2014; Berridge et al., 2016b; Torralba et al., 2016).

The first report of horizontal mitochondrial transfer involved the rescue of mitochondria-depleted cells by transfer of healthy mitochondria from human stem cells (Spees et al.,

2006). A549 cells were exposed to ethidium bromide to mutate and deplete mtDNA, rendering them incapable of aerobic respiration and growth unless grown in a permissive medium containing uridine and pyruvate. Co-culture with non-haematopoietic progenitor cells from human bone marrow (hMSCs) resulted in a rescue of the A549 cells. Genetic analysis demonstrated that rescued cells contained mtDNA from donor cells that correlated with increase in mitochondrial activity (decreased production of extracellular lactate, decreased levels of reactive oxygen species, increased [ATP], and increased oxygen consumption). Since this initial report, multiple groups have demonstrated that the horizontal transfer of mitochondria can occur in a number of cell types both in culture and *in vivo* (Islam et al., 2012; Pasquier et al., 2013; Ahmad et al., 2014; Tan et al., 2015; Hayakawa et al., 2016; reviewed in Berridge et al., 2016b; Torralba et al., 2016). A common feature involved in the horizontal transfer of mitochondria is cell stress and loss of mitochondrial function in the acceptor cell (Torralba et al., 2016).

Several mechanisms have been proposed to underlie the transfer of mitochondria between cells, including extracellular vesicles (exosomes and microvesicles), membrane evulsions (during transcellular mitophagy) and tunneling nanotubes (see Torralba et al., 2016). Extracellular vesicles have been suggested to facilitate the transfer of proteins (cytosolic and membrane-bound), lipids, and RNA, between cells (reviewed in Budnik et al., 2016). Exosomes (30–100 nm microvesicles) containing mtDNA have been isolated from astrocyte or glioblastoma-conditioned medium (Guescini et al., 2010). In addition, several groups have isolated larger microvesicles (0.3–8 μ m) that contain intact mitochondria (Falchi et al., 2013; Hayakawa et al., 2016). In addition, mitochondria can be transferred along bridging structures termed tunneling nanotubes (Rustom et al., 2004). These are 50–100 nm long structures containing actin and microtubules that are assembled between cells in response to cell stress. Interestingly, transfer of mitochondria along tunneling nanotubes requires Miro1. Knockdown of Miro1 decreases the transfer of mitochondria from mesenchymal stem cells to injured (rotenone-treated) epithelial cells, while overexpression increases mitochondrial transfer and facilitates functional rescue (Ahmad et al., 2014; reviewed in Las and Shirihi, 2014).

Recently, Hayakawa et al proposed that mitochondria derived from astrocytes might serve to rescue neurons whose mitochondria are damaged, such as occurs following stroke. As others have observed (Falchi et al., 2013), they described microvesicles (300–1100 nm diameter) containing functional mitochondria and displaying surface expression of β 1-integrin in medium from primary cultures of astrocytes. In primary cultures of cortical neurons, they observed loss of [ATP] and neuronal viability following oxygen-glucose deprivation. This was rescued by addition of astrocyte-conditioned medium containing mitochondrial-laden microvesicles, but was not mimicked by addition of ATP-containing liposomes or microvesicle-depleted medium. Using confocal microscopy, they demonstrated that “treatment” of OGD injured neurons with astrocyte results in an accumulation of astrocytic mitochondria (labelled with mitoTracker Red CMXRos) in neuronal soma and axons in culture. Some of these transferred mitochondria colocalize with neuronally born mitochondria (labelled with CellLight Mitochondria-GFP), suggesting fusion and incorporation of astrocyte-derived mitochondria into the pre-existing neuronal mitochondrial network (Hayakawa et al., 2016; although see Berridge et al., 2016a for caveats). While the

mechanisms involved in mitochondrial release and subsequent neuronal internalization are not yet clear, this involves cyclic ADPribose-mediated Ca^{2+} increases in astrocytes and integrin signaling (Hayakawa et al., 2016). This mechanism has been proposed to provide healthy mitochondria to neurons following stroke aid in their functional recovery.

Several lines of evidence suggest that astrocytes might serve as acceptors for mitochondria destined for degradation. Davis et al recently identified a new mechanism that might mediate the degradation of axonal mitochondria from retinal ganglion cells in astrocytes, which they termed transmitophagy. Using serial block-face scanning electron microscopy, they identified axonal protrusions filled with mitochondria that appose astrocytic processes. These protrusions are pinched off from the axons to form membrane enclosed evulsions that are surrounded by axonal cytoplasm. These axonally-derived evulsions containing mitochondria appear to be degraded via fusion with the lysosome in astrocytes (Davis et al., 2014).

How widespread is this? Davis and colleagues suggest that the degradation of retinal ganglion cell mitochondria in the optic nerve head astrocytes might exceed that seen within the retinal ganglion cell soma (Davis et al., 2014). In addition, they report the existence of morphologically similar structures (mitochondrial filled evulsions) within superficial layers of cortex, although with a significantly decreased frequency. The astrocyte-mediated phagocytosis of membranous evulsions though is reminiscent of astrocyte-mediated phagocytosis and removal of synapses identified in the retinogeniculate system (Chung et al., 2013). As noted by Davis et al, it is possible that the molecular machinery for both activities is conserved.

Role of mitochondria in astrocytic processes

Like neurons, astrocyte processes are decorated with receptors and transporters for numerous neurotransmitters, allowing astrocytes to sense and regulate multiple aspects of brain function. Unlike neurons, astrocytes are not electrically active (i.e. their activation does not lead to the generation of action potentials). Instead, the activation of astrocytic neurotransmitter receptors and transporters is frequently coupled, either directly, or indirectly, to increases in $[\text{Ca}^{2+}]_i$. There are multiple sources for these Ca^{2+} increases, including IP_3 receptor-mediated Ca^{2+} release from the endoplasmic reticulum, release from mitochondria, and influx across the plasma through a plethora of ion channels and exchangers. The attribution of Ca^{2+} signal to source has, however, proven contentious (for recent discussion and reviews see Rusakov et al., 2014; Volterra et al., 2014; Bazargani and Attwell, 2016). Increases in intracellular $[\text{Ca}^{2+}]$ are implicated in numerous astrocyte functions, including regulation of local blood flow (Petzold et al., 2008; Schummers et al., 2008; Petzold and Murthy, 2011; Otsu et al., 2015, reviewed in MacVicar and Newman, 2015), release of transmitter (Nedergaard, 1994; Parpura et al., 1994; Marchaland et al., 2008; Navarrete et al., 2013), and regulation of synaptic plasticity (Di Castro et al., 2011; Min and Nevean, 2012).

Calcium signals in astrocytes are highly varied, ranging from small, local, uncoordinated elevations in $[\text{Ca}^{2+}]$ (Nett et al., 2002; Shigetomi et al., 2010a; Di Castro et al., 2011) to

large, somatic $[Ca^{2+}]$ increases that are coordinated between many cells, such as occur in response to adrenergic signaling *in vivo* (Ding et al., 2013). Numerous groups have observed spatially restricted $[Ca^{2+}]_i$ increases within the processes of astrocytes (Grosche et al., 1999; Perea and Araque, 2005; Shigetomi et al., 2010b; Di Castro et al., 2011; Jackson and Robinson, 2015; Srinivasan et al., 2015). Using hippocampal organotypic cultures expressing membrane tethered Ca^{2+} indicators, we observed that these Ca^{2+} signaling microdomains frequently co-localized with mitochondria (Jackson and Robinson, 2015; O'Donnell et al., 2016).

Mitochondria play important roles in regulating intracellular $[Ca^{2+}]$. In addition to providing ATP necessary to fuel the various Ca^{2+} pumps that maintain low levels of intracellular $[Ca^{2+}]$, mitochondria can also actively import Ca^{2+} . Electron transport generates a proton electrochemical gradient ($-\psi_m$) across the inner mitochondrial membrane. The membrane potential associated with this gradient (~ 180 – 200 mV) facilitates the import of Ca^{2+} into the mitochondria against its concentration gradient via a Ca^{2+} uniporter (MCU) protein in the inner mitochondrial membrane (Kirichok et al., 2004). Mitochondria can release accumulated Ca^{2+} via the mitochondrial Na^+/Ca^{2+} exchanger (NCXL) (Werth and Thayer, 1994) or via transient opening of the mitochondrial permeability transition pore (mPTP; Bernardi and Petronilli, 1996). Together, these activities allow mitochondria to serve as both sink and source of Ca^{2+} (see Fig 3 for model).

Several lines of evidence suggest a role for mitochondria controlling Ca^{2+} signaling within astrocytes. Dissipating the mitochondrial membrane potential with carbonyl cyanide p-trifluoromethoxy-phenyl-hydrazine (FCCP; a proton uncoupler) slows the rate of decay of $[Ca^{2+}]$ transients both in primary cultures of astrocytes and within astrocyte process, where it also increases the amplitude of spontaneous Ca^{2+} transients and increases the distance over which these transients propagate (Boitier et al., 1999; Jackson and Robinson, 2015). Both we, and Joseph Kittler's group have used Ca^{2+} -insensitive Mito mutant proteins to block the Ca^{2+} sensitive positioning of mitochondria within astrocyte processes. Both groups see an increase in Ca^{2+} signaling characterized by increases in the frequency, amplitude, and half-life of spontaneous events. Further, we have found that the ablation (KillerRed-mito) of individual mitochondria leads to similar increases in Ca^{2+} signaling within astrocyte processes. Additionally, loss of mitochondria from astrocyte processes following oxygen-glucose deprivation is associated with large increases in Ca^{2+} signals (O'Donnell et al., 2016). Together, these observations suggest a role for mitochondrial Ca^{2+} uptake in the clearance of physiological $[Ca^{2+}]$ signals in astrocytes. This may contribute to the restriction of these signals into microdomains within the astrocyte processes.

Similarly, evidence suggests that under certain circumstances, mitochondria may also serve as sources of Ca^{2+} signaling within the astrocyte. Mitochondria within astrocytes co-localize with domains of elevated $[Ca^{2+}]$ within the processes (Jackson and Robinson, 2015; O'Donnell et al., 2016), suggesting that mitochondria might contribute to Ca^{2+} release. In cultures of astrocytes, inhibition of the NCLX decreased exocytotic glutamate release, wound closure, and proliferation (Parnis et al., 2013). Recently, a role for mitochondria has been suggested in generating Ca^{2+} signals within the processes of astrocytes (Agarwal et al., 2017) *in vivo*. Using two-photon *in vivo* imaging of awake mice, Agarwal et al determine

that a significant proportion (up to 85%) of spontaneous microdomain $[Ca^{2+}]_i$ increases correlated with mitochondrial localization. Further, they found that ~35% of these microdomain elevations could be blocked by inhibition of the mitochondrial permeability pore opening (mPTP; using cyclosporine A + rotenone). In addition, they found the application of picrotoxin (to increase neuronal activity) increased efflux of Ca^{2+} from the mitochondria (increased microdomain number and frequency) and that this was blocked by mPTP inhibition. Together, these results strongly suggest a role for mitochondrial Ca^{2+} efflux in the generation of Ca^{2+} signals in astrocyte processes and adds to the diversity of Ca^{2+} signals that are present in astrocytes.

In addition to contributing to the termination and compartmentalization of Ca^{2+} signals within astrocyte processes, the positioning of mitochondria near sources of elevated $[Ca^{2+}]$ may also be important in matching ATP supply with demand. Simultaneous measurement of [ATP] and $[Ca^{2+}]$ in HeLa cells revealed that mitochondrial $[Ca^{2+}]$ increases are matched by increases in ATP production (Jouaville et al., 1999). Work from the Foskett lab has shown that loss of ER-to-mitochondrial Ca^{2+} transfer in unstimulated DT40B lymphocytes results in cell starvation even in the presence of nutrients (Cardenas et al., 2010), highlighting the importance of matrix Ca^{2+} for cell survival. Several mechanisms have been proposed to explain the Ca^{2+} -dependent increases in respiration. Increases in intra-mitochondrial $[Ca^{2+}]$ increase ATP production through activation of the mitochondrial dehydrogenases (FAD-glycerol phosphate dehydrogenase, pyruvate dehydrogenase, NAD-isocitrate dehydrogenase and oxoglutarate dehydrogenase (Denton et al., 1972; Denton, 2009). Increases in matrix $[Ca^{2+}]$ also increase the velocity of ATP production via the ATP-synthase at a given mitochondrial membrane potential (Territo et al., 2000; Territo et al., 2001a; Territo et al., 2001b; reviewed in Balaban, 2009) in cardiac mitochondria. More recently, it was found that nanomolar ($S_{0.5}$ ~300 nM) elevations in extramitochondrial $[Ca^{2+}]$ increase activation of the aspartate-glutamate carrier-1 (AGC1/Aralar) (Palmieri et al., 2001). This results in an increased transfer of reducing equivalents (NADH) into the mitochondria and has been proposed to increase glutamate-dependent respiration by increasing substrate supply (Safer et al., 1971; Palmieri et al., 2001; Pardo et al., 2006; Contreras et al., 2007; Gellerich et al., 2012; reviewed in Gellerich et al., 2013; Llorente-Folch et al., 2015). Interestingly, glutamate-dependent increases in intracellular $[Na^+]$ and $[Ca^{2+}]$ stimulate the glucose transporter GLUT1 in astrocytes, a mechanism that is mimicked by D-aspartate application and inhibited by TBOA (Loaiza et al., 2003; Porras et al., 2008). Regardless of the site of Ca^{2+} activation (intra- or extra-mitochondrial) active positioning of mitochondria near to sites of elevated $[Ca^{2+}]$ would favor the local production of ATP.

As well as providing local ATP and Ca^{2+} buffering, the movement of mitochondria to sites of high activity and glutamate uptake within the astrocyte may be related to the metabolism of glutamate itself (see Fig 3 for model). Most glutamate in the forebrain is cleared into astrocytes via the glial glutamate transporters GLT1 and GLAST (Rothstein et al., 1994; Danbolt, 2001). It is generally assumed that most of this glutamate is converted to glutamine via the astrocyte selective enzyme glutamine synthetase (Martinez-Hernandez et al., 1977; Norenberg and Martinez-Hernandez, 1979). This glutamine is, in turn, recycled to neurons to regenerate glutamate; the glutamate-glutamine cycle. However, a relatively large percentage (~30%) of the glutamate that is taken up by astrocytes is converted to α -

ketoglutarate and subsequently metabolized via the TCA cycle on an ongoing basis (Yu et al., 1982; McKenna et al., 1996; for review see McKenna, 2013). In fact, Yu et al found that in primary cultures of astrocytes, ~50% more glutamate was oxidized vs converted to glutamine. In addition, the percentage of glutamate that is oxidized increases as a function of glutamate concentration (McKenna et al., 1996). Thus, the positioning of mitochondria within astrocytes may allow selective fueling of glutamate uptake by catabolism of glutamate itself (for more extensive reviews see (Dienel, 2013; McKenna, 2013; Olsen and Sonnewald, 2015; Robinson and Jackson, 2016).

From the perspective of the astrocyte, the release of glutamine (derived from α -ketoglutarate) can be regarded as a loss of a TCA cycle intermediate. By some estimates, ~60% of TCA cycle intermediates are lost in astrocytes (Hassel et al., 1994; Hassel and Sonnewald, 1995; for review see Hassel, 2000). Anaplerosis is necessary to replace these intermediates. Pyruvate (derived from glucose metabolism) can be carboxylated via the astrocyte- selective, mitochondrial enzyme, pyruvate decarboxylase, to form the TCA cycle intermediate oxaloacetate (Yu et al., 1983; Shank et al., 1985). Thus, one consequence of perisynaptic mitochondrial positioning in astrocytes may be to facilitate the replenishment of TCA cycle intermediates.

Conclusion

Until recently, the existence of mitochondria and mitochondrial metabolism within astrocyte processes was highly contested. It is increasingly clear that, as in neurons, the dynamics of mitochondria in astrocytes is highly regulated and influences multiple aspects of mitochondrial, and presumably, astrocytic functions. Given that astrocyte signaling and metabolism is highly integrated with that of neurons, one possible avenue of investigation should be to determine whether mitochondrial dynamics in astrocytes influences neuronal signaling and function. In addition to the physiologic influence of mitochondria, dysfunction of mitochondria has been implicated in the pathology of both neuro-developmental and neurodegenerative diseases (Chen and Chan, 2009; Liesa et al., 2009). Relatively unexplored is whether mitochondrial dynamics in astrocytes contributes to these conditions. Understanding the differences underlying the mechanisms regulating mitochondrial dynamics between neurons and glia may yield new targets for pharmacologic manipulation of these disorders.

Acknowledgments

This work was supported by grants (RO1 NS077773 and R56 NS077773) to M.B.R. from the National Institute of Neurological Disorders and Stroke. J.G.J was partially supported by a Foerderer Foundation grant. The Institutional Intellectual and Developmental Disabilities Research Center U54 HD086984 Neuroimaging and Neurocircuitry Core also provided valuable support for these studies. We would also like to thank members of the Robinson and Coulter laboratories for their advice and suggestions during the conduct of this research. We would like to thank Dr. Douglas Coulter for use of equipment and Dr. Hajime Takano for help with the two-photon microscopy. We would like to thank Elizabeth Krizman and Drs. Zila Martinez-Lozando and Donald Joseph for critical reading of the manuscript.

References

- Agarwal A, Wu PH, Hughes EG, Fukaya M, Tischfield MA, Langseth AJ, Wirtz D, Bergles DE. Transient Opening of the Mitochondrial Permeability Transition Pore Induces Microdomain Calcium Transients in Astrocyte Processes. *Neuron*. 2017; 93:587–605. e587. [PubMed: 28132831]
- Ahmad T, Mukherjee S, Pattnaik B, Kumar M, Singh S, Kumar M, Rehman R, Tiwari BK, Jha KA, Barhanpurkar AP, Wani MR, Roy SS, Mabalirajan U, Ghosh B, Agrawal A. Miro1 regulates intercellular mitochondrial transport & enhances mesenchymal stem cell rescue efficacy. *The EMBO journal*. 2014; 33:994–1010. [PubMed: 24431222]
- Akhmanova A, Hammer JA 3rd. Linking molecular motors to membrane cargo. *Curr Opin Cell Biol*. 2010; 22:479–487. [PubMed: 20466533]
- Alexander C, Votruba M, Pesch UE, Thiselton DL, Mayer S, Moore A, Rodriguez M, Kellner U, Leo-Kottler B, Auburger G, Bhattacharya SS, Wissinger B. OPA1, encoding a dynamin-related GTPase, is mutated in autosomal dominant optic atrophy linked to chromosome 3q28. *Nat Genet*. 2000; 26:211–215. [PubMed: 11017080]
- Amiri M, Hollenbeck PJ. Mitochondrial biogenesis in the axons of vertebrate peripheral neurons. *Dev Neurobiol*. 2008; 68:1348–1361. [PubMed: 18666204]
- Anne Stetler R, Leak RK, Gao Y, Chen J. The dynamics of the mitochondrial organelle as a potential therapeutic target. *J Cereb Blood Flow Metab*. 2013; 33:22–32. [PubMed: 23093069]
- Aoki C, Milner TA, Sheu KF, Blass JP, Pickel VM. Regional distribution of astrocytes with intense immunoreactivity for glutamate dehydrogenase in rat brain: implications for neuron-glia interactions in glutamate transmission. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 1987; 7:2214–2231. [PubMed: 3302125]
- Araque A, Navarrete M. Glial cells in neuronal network function. *Philos Trans R Soc Lond B Biol Sci*. 2010; 365:2375–2381. [PubMed: 20603358]
- Araque A, Parpura V, Sanzgiri RP, Haydon PG. Tripartite synapses: glia, the unacknowledged partner. *Trends Neurosci*. 1999; 22:208–215. [PubMed: 10322493]
- Ashrafi G, Schlehe JS, LaVoie MJ, Schwarz TL. Mitophagy of damaged mitochondria occurs locally in distal neuronal axons and requires PINK1 and Parkin. *The Journal of cell biology*. 2014; 206:655–670. [PubMed: 25154397]
- Attwell D, Buchan AM, Charpak S, Lauritzen M, Macvicar BA, Newman EA. Glial and neuronal control of brain blood flow. *Nature*. 2010; 468:232–243. [PubMed: 21068832]
- Baas PW, Deitch JS, Black MM, Banker GA. Polarity orientation of microtubules in hippocampal neurons: uniformity in the axon and nonuniformity in the dendrite. *Proceedings of the National Academy of Sciences of the United States of America*. 1988; 85:8335–8339. [PubMed: 3054884]
- Balaban RS. The role of Ca(2+) signaling in the coordination of mitochondrial ATP production with cardiac work. *Biochimica et biophysica acta*. 2009; 1787:1334–1341. [PubMed: 19481532]
- Baloh RH, Schmidt RE, Pestronk A, Milbrandt J. Altered axonal mitochondrial transport in the pathogenesis of Charcot-Marie-Tooth disease from mitofusin 2 mutations. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2007; 27:422–430. [PubMed: 17215403]
- Ban-Ishihara R, Ishihara T, Sasaki N, Mihara K, Ishihara N. Dynamics of nucleoid structure regulated by mitochondrial fission contributes to cristae reformation and release of cytochrome c. *Proceedings of the National Academy of Sciences of the United States of America*. 2013; 110:11863–11868. [PubMed: 23821750]
- Bauer DE, Jackson JG, Genda EN, Montoya MM, Yudkoff M, Robinson MB. The glutamate transporter, GLAST, participates in a macromolecular complex that supports glutamate metabolism. *Neurochemistry international*. 2012; 61:566–574. [PubMed: 22306776]
- Bazargani N, Attwell D. Astrocyte calcium signaling: the third wave. *Nature neuroscience*. 2016; 19:182–189. [PubMed: 26814587]
- Benediktsson AM, Schachtele SJ, Green SH, Dailey ME. Ballistic labeling and dynamic imaging of astrocytes in organotypic hippocampal slice cultures. *J Neurosci Methods*. 2005; 141:41–53. [PubMed: 15585287]

- Benjamin Kacerovsky J, Murai KK. Stargazing: Monitoring subcellular dynamics of brain astrocytes. *Neuroscience*. 2015
- Bernardi P, Petronilli V. The permeability transition pore as a mitochondrial calcium release channel: a critical appraisal. *Journal of bioenergetics and biomembranes*. 1996; 28:131–138. [PubMed: 9132411]
- Berridge MV, Schneider RT, McConnell MJ. Mitochondrial Transfer from Astrocytes to Neurons following Ischemic Insult: Guilt by Association? *Cell Metab*. 2016a; 24:376–378. [PubMed: 27626198]
- Berridge MV, McConnell MJ, Grasso C, Bajzikova M, Kovarova J, Neuzil J. Horizontal transfer of mitochondria between mammalian cells: beyond co-culture approaches. *Curr Opin Genet Dev*. 2016b; 38:75–82. [PubMed: 27219870]
- Bertholet AM, Millet AM, Guillermin O, Daloyau M, Davezac N, Miquel MC, Belenguer P. OPA1 loss of function affects in vitro neuronal maturation. *Brain*. 2013; 136:1518–1533. [PubMed: 23543485]
- Bertholet AM, Delerue T, Millet AM, Moulis MF, David C, Daloyau M, Arnaune-Pelloquin L, Davezac N, Mils V, Miquel MC, Rojo M, Belenguer P. Mitochondrial fusion/fission dynamics in neurodegeneration and neuronal plasticity. *Neurobiol Dis*. 2016; 90:3–19. [PubMed: 26494254]
- Bertoni-Freddari C, Fattoretti P, Paoloni R, Caselli U, Giorgetti B, Solazzi M. Inverse correlation between mitochondrial size and metabolic competence: a quantitative cytochemical study of cytochrome oxidase activity. *Naturwissenschaften*. 2003; 90:68–71. [PubMed: 12590300]
- Bertoni-Freddari C, Fattoretti P, Giorgetti B, Spazzafumo L, Solazzi M, Biliotti M. Age-related decline in metabolic competence of small and medium-sized synaptic mitochondria. *Naturwissenschaften*. 2005; 92:82–85. [PubMed: 15619093]
- Boitier E, Rea R, Duchon MR. Mitochondria exert a negative feedback on the propagation of intracellular Ca²⁺ waves in rat cortical astrocytes. *The Journal of cell biology*. 1999; 145:795–808. [PubMed: 10330407]
- Boldogh IR, Pon LA. Interactions of mitochondria with the actin cytoskeleton. *Biochimica et biophysica acta*. 2006; 1763:450–462. [PubMed: 16624426]
- Bradley TJ, Satir P. Evidence of microfilament-associated mitochondrial movement. *J Supramol Struct*. 1979; 12:165–175. [PubMed: 397369]
- Brickley K, Stephenson FA. Trafficking kinesin protein (TRAK)-mediated transport of mitochondria in axons of hippocampal neurons. *The Journal of biological chemistry*. 2011; 286:18079–18092. [PubMed: 21454691]
- Budnik V, Ruiz-Canada C, Wendler F. Extracellular vesicles round off communication in the nervous system. *Nat Rev Neurosci*. 2016; 17:160–172. [PubMed: 26891626]
- Burte F, Carelli V, Chinnery PF, Yu-Wai-Man P. Disturbed mitochondrial dynamics and neurodegenerative disorders. *Nat Rev Neurol*. 2015; 11:11–24. [PubMed: 25486875]
- Bush TG, Puvanachandra N, Horner CH, Polito A, Ostensfeld T, Svendsen CN, Mucke L, Johnson MH, Sofroniew MV. Leukocyte infiltration, neuronal degeneration, and neurite outgrowth after ablation of scar-forming, reactive astrocytes in adult transgenic mice. *Neuron*. 1999; 23:297–308. [PubMed: 10399936]
- Cai Q, Sheng ZH. Mitochondrial transport and docking in axons. *Exp Neurol*. 2009; 218:257–267. [PubMed: 19341731]
- Calkins MJ, Manczak M, Mao P, Shirendeb U, Reddy PH. Impaired mitochondrial biogenesis, defective axonal transport of mitochondria, abnormal mitochondrial dynamics and synaptic degeneration in a mouse model of Alzheimer's disease. *Human molecular genetics*. 2011; 20:4515–4529. [PubMed: 21873260]
- Cardenas C, Miller RA, Smith I, Bui T, Molgo J, Muller M, Vais H, Cheung KH, Yang J, Parker I, Thompson CB, Birnbaum MJ, Hallows KR, Foscett JK. Essential regulation of cell bioenergetics by constitutive InsP3 receptor Ca²⁺ transfer to mitochondria. *Cell*. 2010; 142:270–283. [PubMed: 20655468]
- Chada SR, Hollenbeck PJ. Nerve growth factor signaling regulates motility and docking of axonal mitochondria. *Curr Biol*. 2004; 14:1272–1276. [PubMed: 15268858]

- Chang CR, Blackstone C. Dynamic regulation of mitochondrial fission through modification of the dynamin-related protein Drp1. *Ann N Y Acad Sci.* 2010; 1201:34–39. [PubMed: 20649536]
- Chang DT, Honick AS, Reynolds IJ. Mitochondrial trafficking to synapses in cultured primary cortical neurons. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* 2006; 26:7035–7045. [PubMed: 16807333]
- Chen H, Chan DC. Mitochondrial dynamics--fusion, fission, movement, and mitophagy--in neurodegenerative diseases. *Human molecular genetics.* 2009; 18:R169–176. [PubMed: 19808793]
- Chen H, Chopp M, Schultz L, Bodzin G, Garcia JH. Sequential neuronal and astrocytic changes after transient middle cerebral artery occlusion in the rat. *J Neurol Sci.* 1993; 118:109–106. [PubMed: 8229058]
- Chen S, Owens GC, Crossin KL, Edelman DB. Serotonin stimulates mitochondrial transport in hippocampal neurons. *Molecular and cellular neurosciences.* 2007; 36:472–483. [PubMed: 17904380]
- Chernoivanenko IS, Matveeva EA, Gelfand VI, Goldman RD, Minin AA. Mitochondrial membrane potential is regulated by vimentin intermediate filaments. *FASEB J.* 2015; 29:820–827. [PubMed: 25404709]
- Choi I, Kim J, Jeong HK, Kim B, Jou I, Park SM, Chen L, Kang UJ, Zhuang X, Joe EH. PINK1 deficiency attenuates astrocyte proliferation through mitochondrial dysfunction, reduced AKT and increased p38 MAPK activation, and downregulation of EGFR. *Glia.* 2013; 61:800–812. [PubMed: 23440919]
- Chung WS, Clarke LE, Wang GX, Stafford BK, Sher A, Chakraborty C, Joung J, Foo LC, Thompson A, Chen C, Smith SJ, Barres BA. Astrocytes mediate synapse elimination through MEGF10 and MERTK pathways. *Nature.* 2013; 504:394–400. [PubMed: 24270812]
- Clarke LE, Barres BA. Emerging roles of astrocytes in neural circuit development. *Nat Rev Neurosci.* 2013; 14:311–321. [PubMed: 23595014]
- Contreras L, Gomez-Puertas P, Iijima M, Kobayashi K, Saheki T, Satrustegui J. Ca²⁺ Activation kinetics of the two aspartate-glutamate mitochondrial carriers, aralar and citrin: role in the heart malate-aspartate NADH shuttle. *The Journal of biological chemistry.* 2007; 282:7098–7106. [PubMed: 17213189]
- Courchet J, Lewis TL Jr, Lee S, Courchet V, Liou DY, Aizawa S, Polleux F. Terminal axon branching is regulated by the LKB1-NUAK1 kinase pathway via presynaptic mitochondrial capture. *Cell.* 2013; 153:1510–1525. [PubMed: 23791179]
- Cribbs JT, Strack S. Reversible phosphorylation of Drp1 by cyclic AMP-dependent protein kinase and calcineurin regulates mitochondrial fission and cell death. *EMBO Rep.* 2007; 8:939–944. [PubMed: 17721437]
- Csordas G, Thomas AP, Hajnoczky G. Quasi-synaptic calcium signal transmission between endoplasmic reticulum and mitochondria. *The EMBO journal.* 1999; 18:96–108. [PubMed: 9878054]
- Csordas G, Renken C, Varnai P, Walter L, Weaver D, Buttke KF, Balla T, Mannella CA, Hajnoczky G. Structural and functional features and significance of the physical linkage between ER and mitochondria. *The Journal of cell biology.* 2006; 174:915–921. [PubMed: 16982799]
- Danbolt NC. Glutamate uptake. *Prog Neurobiol.* 2001; 65:1–105. [PubMed: 11369436]
- Davis AF, Clayton DA. In situ localization of mitochondrial DNA replication in intact mammalian cells. *The Journal of cell biology.* 1996; 135:883–893. [PubMed: 8922374]
- Davis CH, Kim KY, Bushong EA, Mills EA, Boassa D, Shih T, Kinebuchi M, Phan S, Zhou Y, Bihlmeyer NA, Nguyen JV, Jin Y, Ellisman MH, Marsh-Armstrong N. Transcellular degradation of axonal mitochondria. *Proceedings of the National Academy of Sciences of the United States of America.* 2014; 111:9633–9638. [PubMed: 24979790]
- Deas E, Wood NW, Plun-Favreau H. Mitophagy and Parkinson's disease: the PINK1-parkin link. *Biochimica et biophysica acta.* 2011; 1813:623–633. [PubMed: 20736035]
- Delettre C, Lenaers G, Pelloquin L, Belenguer P, Hamel CP. OPA1 (Kjer type) dominant optic atrophy: a novel mitochondrial disease. *Mol Genet Metab.* 2002; 75:97–107. [PubMed: 11855928]
- Delettre C, Lenaers G, Griffoin JM, Gigarel N, Lorenzo C, Belenguer P, Pelloquin L, Grosgeorge J, Turc-Carel C, Perret E, Astarie-Dequeker C, Lasquelléc L, Arnaud B, Ducommun B, Kaplan J,

- Hamel CP. Nuclear gene OPA1, encoding a mitochondrial dynamin-related protein, is mutated in dominant optic atrophy. *Nat Genet.* 2000; 26:207–210. [PubMed: 11017079]
- Denton RM. Regulation of mitochondrial dehydrogenases by calcium ions. *Biochimica et biophysica acta.* 2009; 1787:1309–1316. [PubMed: 19413950]
- Denton RM, Randle PJ, Martin BR. Stimulation by calcium ions of pyruvate dehydrogenase phosphate phosphatase. *The Biochemical journal.* 1972; 128:161–163. [PubMed: 4343661]
- Derouiche A, Frotscher M. Peripheral astrocyte processes: monitoring by selective immunostaining for the actin-binding ERM proteins. *Glia.* 2001; 36:330–341. [PubMed: 11746770]
- Derouiche A, Haseleu J, Korf HW. Fine Astrocyte Processes Contain Very Small Mitochondria: Glial Oxidative Capability May Fuel Transmitter Metabolism. *Neurochemical research.* 2015; 40:2402–2413. [PubMed: 25894677]
- Di Castro MA, Chuquet J, Liaudet N, Bhaukaurally K, Santello M, Bouvier D, Tiret P, Volterra A. Local Ca²⁺ detection and modulation of synaptic release by astrocytes. *Nature neuroscience.* 2011; 14:1276–1284. [PubMed: 21909085]
- Dienel GA. Astrocytic energetics during excitatory neurotransmission: What are contributions of glutamate oxidation and glycolysis? *Neurochemistry international.* 2013; 63:244–258. [PubMed: 23838211]
- Ding F, O'Donnell J, Thrane AS, Zeppenfeld D, Kang H, Xie L, Wang F, Nedergaard M. alpha1-Adrenergic receptors mediate coordinated Ca²⁺ signaling of cortical astrocytes in awake, behaving mice. *Cell Calcium.* 2013; 54:387–394. [PubMed: 24138901]
- Duale H, Kasparov S, Paton JF, Teschemacher AG. Differences in transductional tropism of adenoviral and lentiviral vectors in the rat brainstem. *Exp Physiol.* 2005; 90:71–78. [PubMed: 15542614]
- DuBoff B, Gotz J, Feany MB. Tau promotes neurodegeneration via DRP1 mislocalization in vivo. *Neuron.* 2012; 75:618–632. [PubMed: 22920254]
- Eng LF, Reier PJ, Houle JD. Astrocyte activation and fibrous gliosis: glial fibrillary acidic protein immunostaining of astrocytes following intraspinal cord grafting of fetal CNS tissue. *Progress in brain research.* 1987; 71:439–455. [PubMed: 3588961]
- Falchi AM, Sogos V, Saba F, Piras M, Congiu T, Piludu M. Astrocytes shed large membrane vesicles that contain mitochondria, lipid droplets and ATP. *Histochem Cell Biol.* 2013; 139:221–231. [PubMed: 23108569]
- Filippo KH, Strack S. Mitochondrial dynamics in neuronal injury, development and plasticity. *Journal of cell science.* 2017; 130:671–681. [PubMed: 28154157]
- Fransson A, Ruusala A, Aspenstrom P. Atypical Rho GTPases have roles in mitochondrial homeostasis and apoptosis. *The Journal of biological chemistry.* 2003; 278:6495–6502. [PubMed: 12482879]
- Fransson S, Ruusala A, Aspenstrom P. The atypical Rho GTPases Miro-1 and Miro-2 have essential roles in mitochondrial trafficking. *Biochemical and biophysical research communications.* 2006; 344:500–510. [PubMed: 16630562]
- Frederick RL, Shaw JM. Moving mitochondria: establishing distribution of an essential organelle. *Traffic.* 2007; 8:1668–1675. [PubMed: 17944806]
- Geisler S, Holmstrom KM, Skujat D, Fiesel FC, Rothfuss OC, Kahle PJ, Springer W. PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. *Nat Cell Biol.* 2010; 12:119–131. [PubMed: 20098416]
- Gellerich FN, Gizatullina Z, Gainutdinov T, Muth K, Seppet E, Orynbayeva Z, Vielhaber S. The control of brain mitochondrial energization by cytosolic calcium: the mitochondrial gas pedal. *IUBMB Life.* 2013; 65:180–190. [PubMed: 23401251]
- Gellerich FN, Gizatullina Z, Trumbekaite S, Korzeniewski B, Gaynutdinov T, Seppet E, Vielhaber S, Heinze HJ, Striggow F. Cytosolic Ca²⁺ regulates the energization of isolated brain mitochondria by formation of pyruvate through the malate-aspartate shuttle. *The Biochemical journal.* 2012; 443:747–755. [PubMed: 22295911]
- Genda EN, Jackson JG, Sheldon AL, Locke SF, Greco TM, O'Donnell JC, Spruce LA, Xiao R, Guo W, Putt M, Seeholzer S, Ischiropoulos H, Robinson MB. Co-compartmentalization of the astroglial glutamate transporter, GLT-1, with glycolytic enzymes and mitochondria. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* 2011; 31:18275–18288. [PubMed: 22171032]

- Glater EE, Megeath LJ, Stowers RS, Schwarz TL. Axonal transport of mitochondria requires mltin to recruit kinesin heavy chain and is light chain independent. *The Journal of cell biology*. 2006; 173:545–557. [PubMed: 16717129]
- Greene AW, Grenier K, Aguilera MA, Muise S, Farazifard R, Haque ME, McBride HM, Park DS, Fon EA. Mitochondrial processing peptidase regulates PINK1 processing, import and Parkin recruitment. *EMBO Rep*. 2012; 13:378–385. [PubMed: 22354088]
- Grosche J, Matyash V, Moller T, Verkhratsky A, Reichenbach A, Kettenmann H. Microdomains for neuron-glia interaction: parallel fiber signaling to Bergmann glial cells. *Nature neuroscience*. 1999; 2:139–143. [PubMed: 10195197]
- Guescini M, Genedani S, Stocchi V, Agnati LF. Astrocytes and Glioblastoma cells release exosomes carrying mtDNA. *J Neural Transm (Vienna)*. 2010; 117:1–4. [PubMed: 19680595]
- Guo X, Macleod GT, Wellington A, Hu F, Panchumarthi S, Schoenfield M, Marin L, Charlton MP, Atwood HL, Zinsmaier KE. The GTPase dMiro is required for axonal transport of mitochondria to *Drosophila* synapses. *Neuron*. 2005; 47:379–393. [PubMed: 16055062]
- Halassa MM, Fellin T, Haydon PG. The tripartite synapse: roles for gliotransmission in health and disease. *Trends Mol Med*. 2007; 13:54–63. [PubMed: 17207662]
- Hamby ME, Coppola G, Ao Y, Geschwind DH, Khakh BS, Sofroniew MV. Inflammatory mediators alter the astrocyte transcriptome and calcium signaling elicited by multiple G-protein-coupled receptors. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2012; 32:14489–14510. [PubMed: 23077035]
- Hartman MA, Spudich JA. The myosin superfamily at a glance. *Journal of cell science*. 2012; 125:1627–1632. [PubMed: 22566666]
- Hassel B. Carboxylation and anaplerosis in neurons and glia. *Mol Neurobiol*. 2000; 22:21–40. [PubMed: 11414279]
- Hassel B, Sonnewald U. Glial formation of pyruvate and lactate from TCA cycle intermediates: implications for the inactivation of transmitter amino acids? *Journal of neurochemistry*. 1995; 65:2227–2234. [PubMed: 7595511]
- Hassel B, Sonnewald U, Unsgard G, Fonnum F. NMR spectroscopy of cultured astrocytes: effects of glutamine and the gliotoxin fluorocitrate. *Journal of neurochemistry*. 1994; 62:2187–2194. [PubMed: 8189227]
- Hayakawa K, Esposito E, Wang X, Terasaki Y, Liu Y, Xing C, Ji X, Lo EH. Transfer of mitochondria from astrocytes to neurons after stroke. *Nature*. 2016; 535:551–555. [PubMed: 27466127]
- Haydon PG, Nedergaard M. How Do Astrocytes Participate in Neural Plasticity? *Cold Spring Harbor perspectives in biology*. 2014;7.
- Hertz L, Peng L, Dienel GA. Energy metabolism in astrocytes: high rate of oxidative metabolism and spatiotemporal dependence on glycolysis/glycogenolysis. *J Cereb Blood Flow Metab*. 2007; 27:219–249. [PubMed: 16835632]
- Hirokawa N, Noda Y, Tanaka Y, Niwa S. Kinesin superfamily motor proteins and intracellular transport. *Nat Rev Mol Cell Biol*. 2009; 10:682–696. [PubMed: 19773780]
- Hoekstra JG, Cook TJ, Stewart T, Mattison H, Dreisbach MT, Hoffer ZS, Zhang J. Astrocytic dynamin-like protein 1 regulates neuronal protection against excitotoxicity in Parkinson disease. *Am J Pathol*. 2015; 185:536–549. [PubMed: 25482923]
- Hollenbeck PJ, Saxton WM. The axonal transport of mitochondria. *Journal of cell science*. 2005; 118:5411–5419. [PubMed: 16306220]
- Hroudova J, Singh N, Fisar Z. Mitochondrial dysfunctions in neurodegenerative diseases: relevance to Alzheimer's disease. *Biomed Res Int*. 2014; 2014:175062. [PubMed: 24900954]
- Hyun HW, Min SJ, Kim JE. CDK5 inhibitors prevent astroglial apoptosis and reactive astrogliosis by regulating PKA and DRP1 phosphorylations in the rat hippocampus. *Neurosci Res*. 2017
- Ishihara N, Nomura M, Jofuku A, Kato H, Suzuki SO, Masuda K, Otera H, Nakanishi Y, Nonaka I, Goto Y, Taguchi N, Morinaga H, Maeda M, Takayanagi R, Yokota S, Mihara K. Mitochondrial fission factor Drp1 is essential for embryonic development and synapse formation in mice. *Nat Cell Biol*. 2009; 11:958–966. [PubMed: 19578372]
- Ishihara T, Ban-Ishihara R, Maeda M, Matsunaga Y, Ichimura A, Kyogoku S, Aoki H, Katada S, Nakada K, Nomura M, Mizushima N, Mihara K, Ishihara N. Dynamics of mitochondrial DNA

nucleoids regulated by mitochondrial fission is essential for maintenance of homogeneously active mitochondria during neonatal heart development. *Mol Cell Biol.* 2015; 35:211–223. [PubMed: 25348719]

Islam MN, Das SR, Emin MT, Wei M, Sun L, Westphalen K, Rowlands DJ, Quadri SK, Bhattacharya S, Bhattacharya J. Mitochondrial transfer from bone-marrow-derived stromal cells to pulmonary alveoli protects against acute lung injury. *Nat Med.* 2012; 18:759–765. [PubMed: 22504485]

Jackson JG, Robinson MB. Reciprocal Regulation of Mitochondrial Dynamics and Calcium Signaling in Astrocyte Processes. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* 2015; 35:15199–15213. [PubMed: 26558789]

Jackson JG, O'Donnell JC, Krizman E, Robinson MB. Displacing hexokinase from mitochondrial voltage-dependent anion channel impairs GLT-1-mediated glutamate uptake but does not disrupt interactions between GLT-1 and mitochondrial proteins. *Journal of neuroscience research.* 2014a

Jackson JG, O'Donnell JC, Takano H, Coulter DA, Robinson MB. Neuronal activity and glutamate uptake decrease mitochondrial mobility in astrocytes and position mitochondria near glutamate transporters. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* 2014b; 34:1613–1624. [PubMed: 24478345]

Jacobson J, Duchen MR. Interplay between mitochondria and cellular calcium signalling. *Mol Cell Biochem.* 2004; 256–257:209–218.

Janezko K. Co-expression of GFAP and vimentin in astrocytes proliferating in response to injury in the mouse cerebral hemisphere. A combined autoradiographic and double immunocytochemical study. *Int J Dev Neurosci.* 1993; 11:139–147. [PubMed: 8328297]

Jiang T, Cadenas E. Astrocytic metabolic and inflammatory changes as a function of age. *Aging Cell.* 2014; 13:1059–1067. [PubMed: 25233945]

Jin SM, Lazarou M, Wang C, Kane LA, Narendra DP, Youle RJ. Mitochondrial membrane potential regulates PINK1 import and proteolytic destabilization by PARL. *The Journal of cell biology.* 2010; 191:933–942. [PubMed: 21115803]

Jouaville LS, Pinton P, Bastianutto C, Rutter GA, Rizzuto R. Regulation of mitochondrial ATP synthesis by calcium: evidence for a long-term metabolic priming. *Proceedings of the National Academy of Sciences of the United States of America.* 1999; 96:13807–13812. [PubMed: 10570154]

Kanai Y, Okada Y, Tanaka Y, Harada A, Terada S, Hirokawa N. KIF5C, a novel neuronal kinesin enriched in motor neurons. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* 2000; 20:6374–6384. [PubMed: 10964943]

Kang JS, Tian JH, Pan PY, Zald P, Li C, Deng C, Sheng ZH. Docking of axonal mitochondria by syntrophin controls their mobility and affects short-term facilitation. *Cell.* 2008; 132:137–148. [PubMed: 18191227]

Kirichok Y, Kravinsky G, Clapham DE. The mitochondrial calcium uniporter is a highly selective ion channel. *Nature.* 2004; 427:360–364. [PubMed: 14737170]

Kirischuk S, Kettenmann H, Verkhratsky A. Na⁺/Ca²⁺ exchanger modulates kainate-triggered Ca²⁺ signaling in Bergmann glial cells in situ. *FASEB J.* 1997; 11:566–572. [PubMed: 9212080]

Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, Yokochi M, Mizuno Y, Shimizu N. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature.* 1998; 392:605–608. [PubMed: 9560156]

Ko AR, Hyun HW, Min SJ, Kim JE. The Differential DRP1 Phosphorylation and Mitochondrial Dynamics in the Regional Specific Astroglial Death Induced by Status Epilepticus. *Front Cell Neurosci.* 2016; 10:124. [PubMed: 27242436]

Kornmann B, Osman C, Walter P. The conserved GTPase Gem1 regulates endoplasmic reticulum-mitochondria connections. *Proceedings of the National Academy of Sciences of the United States of America.* 2011; 108:14151–14156. [PubMed: 21825164]

Kosaka T, Hama K. Three-dimensional structure of astrocytes in the rat dentate gyrus. *J Comp Neurol.* 1986; 249:242–260. [PubMed: 3525618]

Las G, Shrihail OS. Miro1: new wheels for transferring mitochondria. *The EMBO journal.* 2014; 33:939–941. [PubMed: 24711517]

- Lee Y, Messing A, Su M, Brenner M. GFAP promoter elements required for region-specific and astrocyte-specific expression. *Glia*. 2008; 56:481–493. [PubMed: 18240313]
- Lemasters JJ. Selective mitochondrial autophagy, or mitophagy, as a targeted defense against oxidative stress, mitochondrial dysfunction, and aging. *Rejuvenation Res*. 2005; 8:3–5. [PubMed: 15798367]
- Leterrier JF, Rusakov DA, Nelson BD, Linden M. Interactions between brain mitochondria and cytoskeleton: evidence for specialized outer membrane domains involved in the association of cytoskeleton-associated proteins to mitochondria in situ and in vitro. *Microsc Res Tech*. 1994; 27:233–261. [PubMed: 8204913]
- Lewis TL, Turi GF, Kwon SK, Losonczy A, Polleux F. Progressive Decrease of Mitochondrial Motility during Maturation of Cortical Axons In Vitro and In Vivo. *Current Biology*. 2016; 26:2602–2608. [PubMed: 27641765]
- Li Z, Okamoto K, Hayashi Y, Sheng M. The importance of dendritic mitochondria in the morphogenesis and plasticity of spines and synapses. *Cell*. 2004; 119:873–887. [PubMed: 15607982]
- Liesa M, Palacin M, Zorzano A. Mitochondrial dynamics in mammalian health and disease. *Physiol Rev*. 2009; 89:799–845. [PubMed: 19584314]
- Ligon LA, Steward O. Role of microtubules and actin filaments in the movement of mitochondria in the axons and dendrites of cultured hippocampal neurons. *J Comp Neurol*. 2000; 427:351–361. [PubMed: 11054698]
- Linden M, Nelson BD, Leterrier JF. The specific binding of the microtubule-associated protein 2 (MAP2) to the outer membrane of rat brain mitochondria. *The Biochemical journal*. 1989; 261:167–173. [PubMed: 2775202]
- Liu S, Sawada T, Lee S, Yu W, Silverio G, Alapatt P, Millan I, Shen A, Saxton W, Kanao T, Takahashi R, Hattori N, Imai Y, Lu B. Parkinson's disease-associated kinase PINK1 regulates Miro protein level and axonal transport of mitochondria. *PLoS genetics*. 2012; 8:e1002537. [PubMed: 22396657]
- Llorente-Folch I, Rueda CB, Pardo B, Szabadkai G, Duchen MR, Satrustegui J. The regulation of neuronal mitochondrial metabolism by calcium. *J Physiol*. 2015
- Loaiza A, Porras OH, Barros LF. Glutamate triggers rapid glucose transport stimulation in astrocytes as evidenced by real-time confocal microscopy. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2003; 23:7337–7342. [PubMed: 12917367]
- Loeb LA, Wallace DC, Martin GM. The mitochondrial theory of aging and its relationship to reactive oxygen species damage and somatic mtDNA mutations. *Proceedings of the National Academy of Sciences of the United States of America*. 2005; 102:18769–18770. [PubMed: 16365283]
- Lopez-Domenech G, Higgs NF, Vaccaro V, Ros H, Arancibia-Carcamo IL, MacAskill AF, Kittler JT. Loss of Dendritic Complexity Precedes Neurodegeneration in a Mouse Model with Disrupted Mitochondrial Distribution in Mature Dendrites. *Cell Rep*. 2016; 17:317–327. [PubMed: 27705781]
- Lovatt D, Sonnewald U, Waagepetersen HS, Schousboe A, He W, Lin JH, Han X, Takano T, Wang S, Sim FJ, Goldman SA, Nedergaard M. The transcriptome and metabolic gene signature of protoplasmic astrocytes in the adult murine cortex. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2007; 27:12255–12266. [PubMed: 17989291]
- MacAskill AF, Kittler JT. Control of mitochondrial transport and localization in neurons. *Trends Cell Biol*. 2010; 20:102–112. [PubMed: 20006503]
- MacAskill AF, Atkin TA, Kittler JT. Mitochondrial trafficking and the provision of energy and calcium buffering at excitatory synapses. *Eur J Neurosci*. 2010; 32:231–240. [PubMed: 20946113]
- MacAskill AF, Brickley K, Stephenson FA, Kittler JT. GTPase dependent recruitment of Grif-1 by Miro1 regulates mitochondrial trafficking in hippocampal neurons. *Molecular and cellular neurosciences*. 2009a; 40:301–312. [PubMed: 19103291]
- Macaskill AF, Rinholm JE, Twelvetrees AE, Arancibia-Carcamo IL, Muir J, Fransson A, Aspenstrom P, Attwell D, Kittler JT. Miro1 is a calcium sensor for glutamate receptor-dependent localization of mitochondria at synapses. *Neuron*. 2009b; 61:541–555. [PubMed: 19249275]

- MacVicar BA, Newman EA. Astrocyte regulation of blood flow in the brain. Cold Spring Harbor perspectives in biology. 2015;7.
- Maday S, Holzbaur EL. Autophagosome assembly and cargo capture in the distal axon. Autophagy. 2012; 8:858–860. [PubMed: 22617438]
- Maday S, Wallace KE, Holzbaur EL. Autophagosomes initiate distally and mature during transport toward the cell soma in primary neurons. The Journal of cell biology. 2012; 196:407–417. [PubMed: 22331844]
- Maday S, Twelvetrees AE, Moughamian AJ, Holzbaur EL. Axonal transport: cargo-specific mechanisms of motility and regulation. Neuron. 2014; 84:292–309. [PubMed: 25374356]
- Mannella CA, Lederer WJ, Jafri MS. The connection between inner membrane topology and mitochondrial function. J Mol Cell Cardiol. 2013; 62:51–57. [PubMed: 23672826]
- Mannella CA, Pfeiffer DR, Bradshaw PC, Moraru II, Slepchenko B, Loew LM, Hsieh CE, Buttle K, Marko M. Topology of the mitochondrial inner membrane: dynamics and bioenergetic implications. IUBMB Life. 2001; 52:93–100. [PubMed: 11798041]
- Marchaland J, Cali C, Voglmaier SM, Li H, Regazzi R, Edwards RH, Bezzi P. Fast subplasma membrane Ca²⁺ transients control exo-endocytosis of synaptic-like microvesicles in astrocytes. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2008; 28:9122–9132. [PubMed: 18784293]
- Martin MA, Hurd DD, Saxton WM. Kinesins in the nervous system. Cell Mol Life Sci. 1999; 56:200–216. [PubMed: 11212348]
- Martinez-Hernandez A, Bell KP, Norenberg MD. Glutamine synthetase: glial localization in brain. Science. 1977; 195:1356–1358. [PubMed: 14400]
- Mathiisen TM, Lehre KP, Danbolt NC, Ottersen OP. The perivascular astroglial sheath provides a complete covering of the brain microvessels: an electron microscopic 3D reconstruction. Glia. 2010; 58:1094–1103. [PubMed: 20468051]
- McKenna MC. Glutamate pays its own way in astrocytes. Frontiers in endocrinology. 2013; 4:191. [PubMed: 24379804]
- McKenna MC, Sonnewald U, Huang X, Stevenson J, Zielke HR. Exogenous glutamate concentration regulates the metabolic fate of glutamate in astrocytes. Journal of neurochemistry. 1996; 66:386–393. [PubMed: 8522979]
- Meissner C, Lorenz H, Hehn B, Lemberg MK. Intramembrane protease PARL defines a negative regulator of PINK1- and PARK2/Parkin-dependent mitophagy. Autophagy. 2015; 11:1484–1498. [PubMed: 26101826]
- Menet V, Prieto M, Privat A, Gimenez y, Ribotta M. Axonal plasticity and functional recovery after spinal cord injury in mice deficient in both glial fibrillary acidic protein and vimentin genes. Proceedings of the National Academy of Sciences of the United States of America. 2003; 100:8999–9004. [PubMed: 12861073]
- Miller KE, Sheetz MP. Axonal mitochondrial transport and potential are correlated. Journal of cell science. 2004; 117:2791–2804. [PubMed: 15150321]
- Min R, Nevian T. Astrocyte signaling controls spike timing-dependent depression at neocortical synapses. Nature neuroscience. 2012; 15:746–753. [PubMed: 22446881]
- Mironov SL. Spontaneous and evoked neuronal activities regulate movements of single neuronal mitochondria. Synapse. 2006; 59:403–411. [PubMed: 16485263]
- Mironov SL. ADP regulates movements of mitochondria in neurons. Biophys J. 2007; 92:2944–2952. [PubMed: 17277190]
- Misgeld T, Kerschensteiner M, Bareyre FM, Burgess RW, Lichtman JW. Imaging axonal transport of mitochondria in vivo. Nat Methods. 2007; 4:559–561. [PubMed: 17558414]
- Mishra P, Chan DC. Mitochondrial dynamics and inheritance during cell division, development and disease. Nat Rev Mol Cell Biol. 2014; 15:634–646. [PubMed: 25237825]
- Misko A, Jiang S, Wegorzewska I, Milbrandt J, Baloh RH. Mitofusin 2 is necessary for transport of axonal mitochondria and interacts with the Miro/Milton complex. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2010; 30:4232–4240. [PubMed: 20335458]

- Misko AL, Sasaki Y, Tuck E, Milbrandt J, Baloh RH. Mitofusin2 mutations disrupt axonal mitochondrial positioning and promote axon degeneration. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2012; 32:4145–4155. [PubMed: 22442078]
- Morris RL, Hollenbeck PJ. The regulation of bidirectional mitochondrial transport is coordinated with axonal outgrowth. *Journal of cell science*. 1993; 104(Pt 3):917–927. [PubMed: 8314882]
- Morris RL, Hollenbeck PJ. Axonal transport of mitochondria along microtubules and F-actin in living vertebrate neurons. *The Journal of cell biology*. 1995; 131:1315–1326. [PubMed: 8522592]
- Mose-Larsen P, Bravo R, Fey SJ, Small JV, Celis JE. Putative association of mitochondria with a subpopulation of intermediate-sized filaments in cultured human skin fibroblasts. *Cell*. 1982; 31:681–692. [PubMed: 6186387]
- Motori E, Puyal J, Toni N, Ghanem A, Angeloni C, Malaguti M, Cantelli-Forti G, Berninger B, Conzelmann KK, Gotz M, Winklhofer KF, Hrelia S, Bergami M. Inflammation-induced alteration of astrocyte mitochondrial dynamics requires autophagy for mitochondrial network maintenance. *Cell Metab*. 2013; 18:844–859. [PubMed: 24315370]
- Mugnaini E. Helical Filaments in Astrocytic Mitochondria of the Corpus Striatum in the Rat. *The Journal of cell biology*. 1964; 23:173–182. [PubMed: 14228509]
- Nangaku M, Sato-Yoshitake R, Okada Y, Noda Y, Takemura R, Yamazaki H, Hirokawa N. KIF1B, a novel microtubule plus end-directed monomeric motor protein for transport of mitochondria. *Cell*. 1994; 79:1209–1220. [PubMed: 7528108]
- Narendra D, Walker JE, Youle R. Mitochondrial quality control mediated by PINK1 and Parkin: links to parkinsonism. *Cold Spring Harbor perspectives in biology*. 2012:4.
- Navarrete M, Perea G, Maglio L, Pastor J, Garcia de Sola R, Araque A. Astrocyte calcium signal and gliotransmission in human brain tissue. *Cereb Cortex*. 2013; 23:1240–1246. [PubMed: 22581850]
- Nedergaard M. Direct signaling from astrocytes to neurons in cultures of mammalian brain cells. *Science*. 1994; 263:1768–1771. [PubMed: 8134839]
- Nekrasova OE, Amargo EV, Smith WO, Chen J, Kreitzer GE, Green KJ. Desmosomal cadherins utilize distinct kinesins for assembly into desmosomes. *The Journal of cell biology*. 2011; 195:1185–1203. [PubMed: 22184201]
- Nett WJ, Oloff SH, McCarthy KD. Hippocampal astrocytes in situ exhibit calcium oscillations that occur independent of neuronal activity. *Journal of neurophysiology*. 2002; 87:528–537. [PubMed: 11784768]
- Nguyen TT, Oh SS, Weaver D, Lewandowska A, Maxfield D, Schuler MH, Smith NK, Macfarlane J, Saunders G, Palmer CA, Debattisti V, Koshiba T, Pulst S, Feldman EL, Hajnoczky G, Shaw JM. Loss of Miro1-directed mitochondrial movement results in a novel murine model for neuron disease. *Proceedings of the National Academy of Sciences of the United States of America*. 2014; 111:E3631–3640. [PubMed: 25136135]
- Norenberg MD, Martinez-Hernandez A. Fine structural localization of glutamine synthetase in astrocytes of rat brain. *Brain research*. 1979; 161:303–310. [PubMed: 31966]
- O'Donnell JC, Jackson JG, Robinson MB. Transient Oxygen/Glucose Deprivation Causes a Delayed Loss of Mitochondria and Increases Spontaneous Calcium Signaling in Astrocytic Processes. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2016; 36:7109–7127. [PubMed: 27383588]
- Oberheim NA, Takano T, Han X, He W, Lin JH, Wang F, Xu Q, Wyatt JD, Pilcher W, Ojemann JG, Ransom BR, Goldman SA, Nedergaard M. Uniquely hominid features of adult human astrocytes. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2009; 29:3276–3287. [PubMed: 19279265]
- Ogata K, Kosaka T. Structural and quantitative analysis of astrocytes in the mouse hippocampus. *Neuroscience*. 2002; 113:221–233. [PubMed: 12123700]
- Ohno N, Kidd GJ, Mahad D, Kiryu-Seo S, Avishai A, Komuro H, Trapp BD. Myelination and axonal electrical activity modulate the distribution and motility of mitochondria at CNS nodes of Ranvier. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2011; 31:7249–7258. [PubMed: 21593309]

- Okada S, Nakamura M, Katoh H, Miyao T, Shimazaki T, Ishii K, Yamane J, Yoshimura A, Iwamoto Y, Toyama Y, Okano H. Conditional ablation of Stat3 or Socs3 discloses a dual role for reactive astrocytes after spinal cord injury. *Nat Med.* 2006; 12:829–834. [PubMed: 16783372]
- Olsen GM, Sonnewald U. Glutamate: Where does it come from and where does it go? *Neurochemistry international.* 2015; 88:47–52. [PubMed: 25447768]
- Ortinski PI, Dong J, Mungenast A, Yue C, Takano H, Watson DJ, Haydon PG, Coulter DA. Selective induction of astrocytic gliosis generates deficits in neuronal inhibition. *Nature neuroscience.* 2010; 13:584–591. [PubMed: 20418874]
- Otsu Y, Couchman K, Lyons DG, Collot M, Agarwal A, Mallet JM, Pfrieger FW, Bergles DE, Charkpak S. Calcium dynamics in astrocyte processes during neurovascular coupling. *Nature neuroscience.* 2015; 18:210–218. [PubMed: 25531572]
- Overly CC, Rieff HI, Hollenbeck PJ. Organelle motility and metabolism in axons vs dendrites of cultured hippocampal neurons. *Journal of cell science.* 1996; 109(Pt 5):971–980. [PubMed: 8743944]
- Palade GE. An electron microscope study of the mitochondrial structure. *J Histochem Cytochem.* 1953; 1:188–211. [PubMed: 13069686]
- Palmieri L, Pardo B, Lasorsa FM, del Arco A, Kobayashi K, Iijima M, Runswick MJ, Walker JE, Saheki T, Satrustegui J, Palmieri F. Citrin and aralar1 are Ca(2+)-stimulated aspartate/glutamate transporters in mitochondria. *The EMBO journal.* 2001; 20:5060–5069. [PubMed: 11566871]
- Pardo B, Contreras L, Serrano A, Ramos M, Kobayashi K, Iijima M, Saheki T, Satrustegui J. Essential role of aralar in the transduction of small Ca²⁺ signals to neuronal mitochondria. *The Journal of biological chemistry.* 2006; 281:1039–1047. [PubMed: 16269409]
- Parnis J, Montana V, Delgado-Martinez I, Matyash V, Parpura V, Kettenmann H, Sekler I, Nolte C. Mitochondrial exchanger NCLX plays a major role in the intracellular Ca²⁺ signaling, gliotransmission, and proliferation of astrocytes. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* 2013; 33:7206–7219. [PubMed: 23616530]
- Parone PA, Da Cruz S, Tondera D, Mattenberger Y, James DI, Maechler P, Barja F, Martinou JC. Preventing mitochondrial fission impairs mitochondrial function and leads to loss of mitochondrial DNA. *PloS one.* 2008; 3:e3257. [PubMed: 18806874]
- Parpura V, Basarsky TA, Liu F, Jęftinija K, Jęftinija S, Haydon PG. Glutamate-mediated astrocyte-neuron signalling. *Nature.* 1994; 369:744–747. [PubMed: 7911978]
- Pasquier J, Guerrouahen BS, Al Thawadi H, Ghiabi P, Maleki M, Abu-Kaoud N, Jacob A, Mirshahi M, Galas L, Raffi S, Le Foll F, Raffi A. Preferential transfer of mitochondria from endothelial to cancer cells through tunneling nanotubes modulates chemoresistance. *J Transl Med.* 2013; 11:94. [PubMed: 23574623]
- Pathak D, Sepp KJ, Hollenbeck PJ. Evidence that myosin activity opposes microtubule-based axonal transport of mitochondria. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* 2010; 30:8984–8992. [PubMed: 20592219]
- Pekny M, Pekna M. Astrocyte intermediate filaments in CNS pathologies and regeneration. *J Pathol.* 2004; 204:428–437. [PubMed: 15495269]
- Pellerin L, Magistretti PJ. Excitatory amino acids stimulate aerobic glycolysis in astrocytes via an activation of the Na⁺/K⁺ ATPase. *Dev Neurosci.* 1996; 18:336–342. [PubMed: 8940604]
- Pellerin L, Magistretti PJ. Glutamate uptake stimulates Na⁺,K⁺-ATPase activity in astrocytes via activation of a distinct subunit highly sensitive to ouabain. *Journal of neurochemistry.* 1997; 69:2132–2137. [PubMed: 9349559]
- Perea G, Araque A. Properties of synaptically evoked astrocyte calcium signal reveal synaptic information processing by astrocytes. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* 2005; 25:2192–2203. [PubMed: 15745945]
- Petzold GC, Murthy VN. Role of astrocytes in neurovascular coupling. *Neuron.* 2011; 71:782–797. [PubMed: 21903073]
- Petzold GC, Albeanu DF, Sato TF, Murthy VN. Coupling of neural activity to blood flow in olfactory glomeruli is mediated by astrocytic pathways. *Neuron.* 2008; 58:897–910. [PubMed: 18579080]

- Pilling AD, Horiuchi D, Lively CM, Saxton WM. Kinesin-1 and Dynein are the primary motors for fast transport of mitochondria in *Drosophila* motor axons. *Mol Biol Cell*. 2006; 17:2057–2068. [PubMed: 16467387]
- Poole AC, Thomas RE, Yu S, Vincow ES, Pallanck L. The mitochondrial fusion-promoting factor mitofusin is a substrate of the PINK1/parkin pathway. *PloS one*. 2010; 5:e10054. [PubMed: 20383334]
- Popov V, Medvedev NI, Davies HA, Stewart MG. Mitochondria form a filamentous reticular network in hippocampal dendrites but are present as discrete bodies in axons: a three-dimensional ultrastructural study. *J Comp Neurol*. 2005; 492:50–65. [PubMed: 16175555]
- Porras OH, Ruminot I, Loaiza A, Barros LF. Na(+)-Ca(2+) cosignaling in the stimulation of the glucose transporter GLUT1 in cultured astrocytes. *Glia*. 2008; 56:59–68. [PubMed: 17924581]
- Quintero OA, DiVito MM, Adikes RC, Kortan MB, Case LB, Lier AJ, Panaretos NS, Slater SQ, Rengarajan M, Feliu M, Cheney RE. Human Myo19 is a novel myosin that associates with mitochondria. *Curr Biol*. 2009; 19:2008–2013. [PubMed: 19932026]
- Reddy PH, Reddy TP, Manczak M, Calkins MJ, Shirendeb U, Mao P. Dynamin-related protein 1 and mitochondrial fragmentation in neurodegenerative diseases. *Brain Res Rev*. 2011; 67:103–118. [PubMed: 21145355]
- Rinholm JE, Vervaeke K, Tadross MR, Tkachuk AN, Kopek BG, Brown TA, Bergersen LH, Clayton DA. Movement and structure of mitochondria in oligodendrocytes and their myelin sheaths. *Glia*. 2016; 64:810–825. [PubMed: 26775288]
- Rintoul GL, Filiano AJ, Brocard JB, Kress GJ, Reynolds IJ. Glutamate decreases mitochondrial size and movement in primary forebrain neurons. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2003; 23:7881–7888. [PubMed: 12944518]
- Rizzuto R, Brini M, Murgia M, Pozzan T. Microdomains with high Ca²⁺ close to IP₃-sensitive channels that are sensed by neighboring mitochondria. *Science*. 1993; 262:744–747. [PubMed: 8235595]
- Rizzuto R, De Stefani D, Raffaello A, Mammucari C. Mitochondria as sensors and regulators of calcium signalling. *Nat Rev Mol Cell Biol*. 2012; 13:566–578. [PubMed: 22850819]
- Rizzuto R, Bastianutto C, Brini M, Murgia M, Pozzan T. Mitochondrial Ca²⁺ homeostasis in intact cells. *The Journal of cell biology*. 1994; 126:1183–1194. [PubMed: 8063855]
- Rizzuto R, Pinton P, Carrington W, Fay FS, Fogarty KE, Lifshitz LM, Tuft RA, Pozzan T. Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca²⁺ responses. *Science*. 1998; 280:1763–1766. [PubMed: 9624056]
- Robinson MB, Jackson JG. Astroglial glutamate transporters coordinate excitatory signaling and brain energetics. *Neurochemistry international*. 2016; 98:56–71. [PubMed: 27013346]
- Rojas H, Colina C, Ramos M, Benaim G, Jaffe E, Caputo C, Di Polo R. Sodium-calcium exchanger modulates the L-glutamate Ca(i) (2+) signalling in type-1 cerebellar astrocytes. *Adv Exp Med Biol*. 2013; 961:267–274. [PubMed: 23224886]
- Rothstein JD, Martin L, Levey AI, Dykes-Hoberg M, Jin L, Wu D, Nash N, Kuncel RW. Localization of neuronal and glial glutamate transporters. *Neuron*. 1994; 13:713–725. [PubMed: 7917301]
- Rusakov DA, Bard L, Stewart MG, Henneberger C. Diversity of astroglial functions alludes to subcellular specialisation. *Trends Neurosci*. 2014; 37:228–242. [PubMed: 24631033]
- Rustom A, Saffrich R, Markovic I, Walther P, Gerdes HH. Nanotubular highways for intercellular organelle transport. *Science*. 2004; 303:1007–1010. [PubMed: 14963329]
- Safer B, Smith CM, Williamson JR. Control of the transport of reducing equivalents across the mitochondrial membrane in perfused rat heart. *J Mol Cell Cardiol*. 1971; 2:111–124. [PubMed: 4329775]
- Santel A, Fuller MT. Control of mitochondrial morphology by a human mitofusin. *Journal of cell science*. 2001; 114:867–874. [PubMed: 11181170]
- Saotome M, Safiulina D, Szabadkai G, Das S, Fransson A, Aspenstrom P, Rizzuto R, Hajnoczky G. Bidirectional Ca²⁺-dependent control of mitochondrial dynamics by the Miro GTPase. *Proceedings of the National Academy of Sciences of the United States of America*. 2008; 105:20728–20733. [PubMed: 19098100]

- Sarafian TA, Montes C, Imura T, Qi J, Coppola G, Geschwind DH, Sofroniew MV. Disruption of astrocyte STAT3 signaling decreases mitochondrial function and increases oxidative stress in vitro. *PloS one*. 2010; 5:e9532. [PubMed: 20224768]
- Saxton WM, Hollenbeck PJ. The axonal transport of mitochondria. *Journal of cell science*. 2012; 125:2095–2104. [PubMed: 22619228]
- Schummers J, Yu H, Sur M. Tuned responses of astrocytes and their influence on hemodynamic signals in the visual cortex. *Science*. 2008; 320:1638–1643. [PubMed: 18566287]
- Schwarz TL. Mitochondrial trafficking in neurons. *Cold Spring Harbor perspectives in biology*. 2013; 5:a011304. [PubMed: 23732472]
- Shan D, Mount D, Moore S, Haroutunian V, Meador-Woodruff JH, McCullumsmith RE. Abnormal partitioning of hexokinase 1 suggests disruption of a glutamate transport protein complex in schizophrenia. *Schizophr Res*. 2014; 154:1–13. [PubMed: 24560881]
- Shank RP, Bennett GS, Freytag SO, Campbell GL. Pyruvate carboxylase: an astrocyte-specific enzyme implicated in the replenishment of amino acid neurotransmitter pools. *Brain research*. 1985; 329:364–367. [PubMed: 3884090]
- Shao Y, McCarthy KD. Plasticity of astrocytes. *Glia*. 1994; 11:147–155. [PubMed: 7927644]
- Sheng ZH, Cai Q. Mitochondrial transport in neurons: impact on synaptic homeostasis and neurodegeneration. *Nat Rev Neurosci*. 2012; 13:77–93. [PubMed: 22218207]
- Shigetomi E, Kracun S, Khakh BS. Monitoring astrocyte calcium microdomains with improved membrane targeted GCaMP reporters. *Neuron glia biology*. 2010a; 6:183–191. [PubMed: 21205365]
- Shigetomi E, Kracun S, Sofroniew MV, Khakh BS. A genetically targeted optical sensor to monitor calcium signals in astrocyte processes. *Nature neuroscience*. 2010b; 13:759–766. [PubMed: 20495558]
- Shigetomi E, Bushong EA, Hausteiner MD, Tong X, Jackson-Weaver O, Kracun S, Xu J, Sofroniew MV, Ellisman MH, Khakh BS. Imaging calcium microdomains within entire astrocyte territories and endfeet with GCaMPs expressed using adeno-associated viruses. *The Journal of general physiology*. 2013; 141:633–647. [PubMed: 23589582]
- Shirendeb U, Reddy AP, Manczak M, Calkins MJ, Mao P, Tagle DA, Reddy PH. Abnormal mitochondrial dynamics, mitochondrial loss and mutant huntingtin oligomers in Huntington's disease: implications for selective neuronal damage. *Human molecular genetics*. 2011; 20:1438–1455. [PubMed: 21257639]
- Shirendeb UP, Calkins MJ, Manczak M, Anekonda V, Dufour B, McBride JL, Mao P, Reddy PH. Mutant huntingtin's interaction with mitochondrial protein Drp1 impairs mitochondrial biogenesis and causes defective axonal transport and synaptic degeneration in Huntington's disease. *Human molecular genetics*. 2012; 21:406–420. [PubMed: 21997870]
- Shneyer BI, Usaj M, Henn A. Myo19 is an outer mitochondrial membrane motor and effector of starvation-induced filopodia. *Journal of cell science*. 2016; 129:543–556. [PubMed: 26659663]
- Smit-Rigter L, Rajendran R, Silva CA, Spierenburg L, Groeneweg F, Ruimschotel EM, van Versendaal D, van der Togt C, Eysel UT, Heimel JA, Lohmann C, Levelt CN. Mitochondrial Dynamics in Visual Cortex Are Limited In Vivo and Not Affected by Axonal Structural Plasticity. *Curr Biol*. 2016; 26:2609–2616. [PubMed: 27641766]
- Smith MJ, Pozo K, Brickley K, Stephenson FA. Mapping the GRIF-1 binding domain of the kinesin, KIF5C, substantiates a role for GRIF-1 as an adaptor protein in the anterograde trafficking of cargoes. *The Journal of biological chemistry*. 2006; 281:27216–27228. [PubMed: 16835241]
- Sofroniew MV. Molecular dissection of reactive astrogliosis and glial scar formation. *Trends Neurosci*. 2009; 32:638–647. [PubMed: 19782411]
- Spacek J. Ribosome-associated membrane contacts between astrocytes in the anoxic brain. *Acta Neuropathol*. 1982; 57:270–274. [PubMed: 7136505]
- Spees JL, Olson SD, Whitney MJ, Prockop DJ. Mitochondrial transfer between cells can rescue aerobic respiration. *Proceedings of the National Academy of Sciences of the United States of America*. 2006; 103:1283–1288. [PubMed: 16432190]

- Srinivasan R, Huang BS, Venugopal S, Johnston AD, Chai H, Zeng H, Golshani P, Khakh BS. Ca signaling in astrocytes from Ip3r2 mice in brain slices and during startle responses in vivo. *Nature neuroscience*. 2015; 18:708–717. [PubMed: 25894291]
- Stephen TL, Gupta-Agarwal S, Kittler JT. Mitochondrial dynamics in astrocytes. *Biochemical Society transactions*. 2014; 42:1302–1310. [PubMed: 25233407]
- Stephen TL, Higgs NF, Sheehan DF, Al Awabdh S, Lopez-Domenech G, Arancibia-Carcamo IL, Kittler JT. Miro1 Regulates Activity-Driven Positioning of Mitochondria within Astrocytic Processes Apposed to Synapses to Regulate Intracellular Calcium Signaling. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2015; 35:15996–16011. [PubMed: 26631479]
- Stowers RS, Megeath LJ, Gorska-Andrzejak J, Meinertzhagen IA, Schwarz TL. Axonal transport of mitochondria to synapses depends on Milton, a novel Drosophila protein. *Neuron*. 2002; 36:1063–1077. [PubMed: 12495622]
- Summerhayes IC, Wong D, Chen LB. Effect of microtubules and intermediate filaments on mitochondrial distribution. *Journal of cell science*. 1983; 61:87–105. [PubMed: 6350334]
- Tan AS, et al. Mitochondrial genome acquisition restores respiratory function and tumorigenic potential of cancer cells without mitochondrial DNA. *Cell Metab*. 2015; 21:81–94. [PubMed: 25565207]
- Tanaka K, Sugiura Y, Ichishita R, Mihara K, Oka T. KLP6: a newly identified kinesin that regulates the morphology and transport of mitochondria in neuronal cells. *Journal of cell science*. 2011; 124:2457–2465. [PubMed: 21693574]
- Tanaka K, Watase K, Manabe T, Yamada K, Watanabe M, Takahashi K, Iwama H, Nishikawa T, Ichihara N, Kikuchi T, Okuyama S, Kawashima N, Hori S, Takimoto M, Wada K. Epilepsy and exacerbation of brain injury in mice lacking the glutamate transporter GLT-1. *Science*. 1997; 276:1699–1702. [PubMed: 9180080]
- Tanaka Y, Kanai Y, Okada Y, Nonaka S, Takeda S, Harada A, Hirokawa N. Targeted disruption of mouse conventional kinesin heavy chain, kif5B, results in abnormal perinuclear clustering of mitochondria. *Cell*. 1998; 93:1147–1158. [PubMed: 9657148]
- Tang HL, Lung HL, Wu KC, Le AH, Tang HM, Fung MC. Vimentin supports mitochondrial morphology and organization. *The Biochemical journal*. 2008; 410:141–146. [PubMed: 17983357]
- Territo PR, French SA, Balaban RS. Simulation of cardiac work transitions, in vitro: effects of simultaneous Ca²⁺ and ATPase additions on isolated porcine heart mitochondria. *Cell Calcium*. 2001a; 30:19–27. [PubMed: 11396984]
- Territo PR, Mootha VK, French SA, Balaban RS. Ca(2+) activation of heart mitochondrial oxidative phosphorylation: role of the F(0)/F(1)-ATPase. *Am J Physiol Cell Physiol*. 2000; 278:C423–435. [PubMed: 10666039]
- Territo PR, French SA, Dunleavy MC, Evans FJ, Balaban RS. Calcium activation of heart mitochondrial oxidative phosphorylation: rapid kinetics of mVO₂, NADH, AND light scattering. *The Journal of biological chemistry*. 2001b; 276:2586–2599. [PubMed: 11029457]
- Tondera D, Grandemange S, Jourdain A, Karbowski M, Mattenberger Y, Herzig S, Da Cruz S, Clerc P, Raschke I, Merkwirth C, Ehses S, Krause F, Chan DC, Alexander C, Bauer C, Youle R, Langer T, Martinou JC. SLP-2 is required for stress-induced mitochondrial hyperfusion. *The EMBO journal*. 2009; 28:1589–1600. [PubMed: 19360003]
- Torrallba D, Baixauli F, Sanchez-Madrid F. Mitochondria Know No Boundaries: Mechanisms and Functions of Intercellular Mitochondrial Transfer. *Front Cell Dev Biol*. 2016; 4:107. [PubMed: 27734015]
- Twig G, Elorza A, Molina AJ, Mohamed H, Wikstrom JD, Walzer G, Stiles L, Haigh SE, Katz S, Las G, Alroy J, Wu M, Py BF, Yuan J, Deeney JT, Corkey BE, Shirihai OS. Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. *The EMBO journal*. 2008; 27:433–446. [PubMed: 18200046]
- Ugbode CI, Hirst WD, Rattray M. Neuronal influences are necessary to produce mitochondrial co-localization with glutamate transporters in astrocytes. *Journal of neurochemistry*. 2014; 130:668–677. [PubMed: 24814819]

- Valente EM, Salvi S, Ialongo T, Marongiu R, Elia AE, Caputo V, Romito L, Albanese A, Dallapiccola B, Bentivoglio AR. PINK1 mutations are associated with sporadic early-onset parkinsonism. *Ann Neurol*. 2004a; 56:336–341. [PubMed: 15349860]
- Valente EM, et al. Hereditary early-onset Parkinson's disease caused by mutations in PINK1. *Science*. 2004b; 304:1158–1160. [PubMed: 15087508]
- van Spronsen M, Mikhaylova M, Lipka J, Schlager MA, van den Heuvel DJ, Kuijpers M, Wulf PS, Keijzer N, Demmers J, Kapitein LC, Jaarsma D, Gerritsen HC, Akhmanova A, Hoogenraad CC. TRAK/Milton motor-adaptor proteins steer mitochondrial trafficking to axons and dendrites. *Neuron*. 2013; 77:485–502. [PubMed: 23395375]
- Varadi A, Johnson-Cadwell LI, Cirulli V, Yoon Y, Allan VJ, Rutter GA. Cytoplasmic dynein regulates the subcellular distribution of mitochondria by controlling the recruitment of the fission factor dynamin-related protein-1. *Journal of cell science*. 2004; 117:4389–4400. [PubMed: 15304525]
- Ventura R, Harris KM. Three-dimensional relationships between hippocampal synapses and astrocytes. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 1999; 19:6897–6906. [PubMed: 10436047]
- Verstreken P, Ly CV, Venken KJ, Koh TW, Zhou Y, Bellen HJ. Synaptic mitochondria are critical for mobilization of reserve pool vesicles at *Drosophila* neuromuscular junctions. *Neuron*. 2005; 47:365–378. [PubMed: 16055061]
- Volterra A, Liaudet N, Savtchouk I. Astrocyte Ca(2)(+) signalling: an unexpected complexity. *Nat Rev Neurosci*. 2014; 15:327–335. [PubMed: 24739787]
- Waagepetersen HS, Hansen GH, Fenger K, Lindsay JG, Gibson G, Schousboe A. Cellular mitochondrial heterogeneity in cultured astrocytes as demonstrated by immunogold labeling of alpha-ketoglutarate dehydrogenase. *Glia*. 2006; 53:225–231. [PubMed: 16206171]
- Wang X, Schwarz TL. The mechanism of Ca²⁺-dependent regulation of kinesin-mediated mitochondrial motility. *Cell*. 2009; 136:163–174. [PubMed: 19135897]
- Wang X, Winter D, Ashrafi G, Schlehe J, Wong YL, Selkoe D, Rice S, Steen J, LaVoie MJ, Schwarz TL. PINK1 and Parkin target Miro for phosphorylation and degradation to arrest mitochondrial motility. *Cell*. 2011; 147:893–906. [PubMed: 22078885]
- Watanabe T, Noritake J, Kaibuchi K. Regulation of microtubules in cell migration. *Trends Cell Biol*. 2005; 15:76–83. [PubMed: 15695094]
- Weber B, Barros LF. The Astrocyte: Powerhouse and Recycling Center. *Cold Spring Harbor perspectives in biology*. 2015:7.
- Weihofen A, Thomas KJ, Ostaszewski BL, Cookson MR, Selkoe DJ. Pink1 forms a multiprotein complex with Miro and Milton, linking Pink1 function to mitochondrial trafficking. *Biochemistry*. 2009; 48:2045–2052. [PubMed: 19152501]
- Werth JL, Thayer SA. Mitochondria buffer physiological calcium loads in cultured rat dorsal root ganglion neurons. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 1994; 14:348–356. [PubMed: 8283242]
- Witcher MR, Park YD, Lee MR, Sharma S, Harris KM, Kirov SA. Three-dimensional relationships between perisynaptic astroglia and human hippocampal synapses. *Glia*. 2010; 58:572–587. [PubMed: 19908288]
- Wong YC, Holzbaur EL. Autophagosome dynamics in neurodegeneration at a glance. *Journal of cell science*. 2015; 128:1259–1267. [PubMed: 25829512]
- Youle RJ, Narendra DP. Mechanisms of mitophagy. *Nat Rev Mol Cell Biol*. 2011; 12:9–14. [PubMed: 21179058]
- Yu AC, Schousboe A, Hertz L. Metabolic fate of 14C-labeled glutamate in astrocytes in primary cultures. *Journal of neurochemistry*. 1982; 39:954–960. [PubMed: 6126524]
- Yu AC, Drejer J, Hertz L, Schousboe A. Pyruvate carboxylase activity in primary cultures of astrocytes and neurons. *Journal of neurochemistry*. 1983; 41:1484–1487. [PubMed: 6619879]
- Zamanian JL, Xu L, Foo LC, Nouri N, Zhou L, Giffard RG, Barres BA. Genomic analysis of reactive astrogliosis. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2012; 32:6391–6410. [PubMed: 22553043]
- Zerangue N, Kavanaugh MP. Flux coupling in a neuronal glutamate transporter. *Nature*. 1996; 383:634–637. [PubMed: 8857541]

- Zhang Y, Chen K, Sloan SA, Bennett ML, Scholze AR, O'Keeffe S, Phatnani HP, Guarnieri P, Caneda C, Ruderisch N, Deng S, Liddelow SA, Zhang C, Daneman R, Maniatis T, Barres BA, Wu JQ. An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2014; 34:11929–11947. [PubMed: 25186741]
- Zick M, Rabl R, Reichert AS. Cristae formation-linking ultrastructure and function of mitochondria. *Biochimica et biophysica acta*. 2009; 1793:5–19. [PubMed: 18620004]
- Ziviani E, Tao RN, Whitworth AJ. Drosophila parkin requires PINK1 for mitochondrial translocation and ubiquitinates mitofusin. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107:5018–5023. [PubMed: 20194754]
- Zuchner S, et al. Mutations in the mitochondrial GTPase mitofusin 2 cause Charcot-Marie-Tooth neuropathy type 2A. *Nat Genet*. 2004; 36:449–451. [PubMed: 15064763]

Main Points

- This review highlights what is known about mitochondrial dynamics in astrocytes, their regulation, and how these dynamics influence local signaling and metabolism. New analyses of mitochondrial distribution and movement *in vivo* are presented.

Figure 1. Visualization of mitochondrial distribution *in vivo*

DNA constructs were generated to drive expression of a membrane-targeted TdTomato fluorescent protein (Lck-TdTomato) and a mitochondrially-targeted enhanced green fluorescent protein (mitoEGFP) under control of a minimal GFAP promoter (GFAP_{ABC1D}). These were packaged into AAV2 particles containing the coat-protein from AAV5 (AAV2/5; UPenn viral vector core). Viral particles were injected into the superficial cortical layers of adult mice of either sex. (A–C) Representative images (maximal z-projections) from astrocytes transduced with Lck-TdTomato (A, C; magenta) and mitoEGFP (B, C; green). Images A'–C' are cross-sections from z-projection images above (A–C) that depict a dense mitochondrial network throughout the astrocyte. Individual mitochondria can be seen in the smaller distal processes and are particularly concentrated near vascular end-feet (arrows). (D–E) Representative maximal z-projection images of the vasculature (visualized by injection of dextran conjugated AlexaFluor633 hydrazide into the tail vein; magenta) and

mitochondria (mitoEGFP; green) in an anesthetized (α -chloralose) mouse visualized through an acute cortical window via a two-photon microscope, representative of 4 animals). (F) Distribution of mitochondrial lengths *in vivo* (magenta; new data) compared with mitochondrial lengths in organotypic cultures (green; data from Jackson et al., 2014b).

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 2. Cartoon model depicting mitochondrial trafficking in astrocytes

Neuronal activity increases the concentration of glutamate (and other transmitters) at the synaptic cleft. Glutamate is cleared by a family of Na⁺-dependent glutamate transporters, in particular, GLAST and GLT-1 (EAAT1 and EAAT2), that reside on the perisynaptic astrocyte membranes. Activation of glutamate uptake activates Na⁺/K⁺-ATPase isoforms and leads to a reversal (Na⁺ out, Ca²⁺ in) of the Na⁺/Ca²⁺ exchanger (NCX) in the astrocyte processes, thus consuming ATP and increasing local [Ca²⁺], respectively. In addition, astrocytes possess receptors for numerous neurotransmitters (glutamate, ATP, etc) that couple to increases in [Ca²⁺]_i increases. Increases in Ca²⁺ result in the arrest of mitochondria within astrocyte processes via a Ca²⁺-dependent disengagement of Miro proteins from the motor apparatus. The identity of motor proteins mediating mitochondrial movement in astrocytes is unknown.

Figure 3. Cartoon representation depicting proposed roles of mitochondria in astrocyte processes
Astrocytic mitochondria apposed to synapses coordinate local metabolism and Ca^{2+} signaling in astrocyte processes. Increases in neuronal activity result in increases in local $[\text{Ca}^{2+}]$ in astrocytes. Calcium is removed from the cytosol into the mitochondria via the mitochondrial Ca^{2+} uniporter (MCU). Increases in matrix $[\text{Ca}^{2+}]$ stimulate dehydrogenases in the TCA cycle and the ATP-synthase, while increases in extramitochondrial $[\text{Ca}^{2+}]$ stimulate the glutamate-aspartate carrier, aralar. Ca^{2+} accumulated by the mitochondria may be released via transient opening of the permeability transition pore or by the mitochondrial $\text{Na}^+/\text{Ca}^{2+}$ -exchanger (NCLX). Glutamate can be transferred into the mitochondria via aralar and subsequently converted to α -ketoglutarate (α -KG) for entry into the TCA cycle.