Are mitochondria the key to reduce the age-dependent decline in axon growth after spinal cord injury?

Theresa C. Sutherland, Cédric G. Geoffroy*

Spinal cord injury (SCI) is a debilitating condition resulting in varying degrees of functional impairment and exhibits only limited repair. Currently there is no cure for SCI, and no proven treatment to promote restoration of function. One area that has received extensive attention, with the goal of promoting functional recovery, is promoting axonal regeneration and growth in the injured cord. However, one factor that is likely to impede the translation of promising restorative therapies from the bench to the clinic is the lack of consideration of the aging factor in SCI research and its impact on axon regeneration in particular. In the United States, the average age of occurrence of SCI is 43 years old (National Spinal Cord Injury Statistical Center), with a peak in incidence in young (20–30 years) and in aging (≥ 65 years) adults (GBD 2016 Traumatic Brain Injury and Spinal Cord Injury Collaborators, 2019). Patients are also living longer with the injury, with approximately 80% of all people with a SCI being over 40 (One Degree of Separation, Christopher & Dana Reeve Foundation). This demographic change has largely not been addressed in pre-clinical research. Indeed, the bulk of pre-clinical research is performed in young adult rodents (2–4 months), despite 6 month old mice more appropriately representing the 20–25 year old population in humans, and less than 0.35% of experimental rodents being 12 months or older, mimicking 40 years of age in humans (Fouad et al., 2020). In fact, an age-dependent decline in axon growth has been reported in a variety of model organisms, mediated by both neuron-intrinsic (Geoffroy et al., 2017) and extrinsic mechanisms (Sutherland and Geoffroy, 2020). While there has been significant progress made in understanding and manipulating axon growth after injury, even genetic manipulations promoting growth seem age-sensitive (Geoffroy et al., 2017), suggesting that other factors are needed to enhance axon growth in aging neurons. One of these factors is mitochondria. The mitochondrial theory of aging is one of the main mechanisms proposed to explain the biological process of aging. With age, mitochondrial function is reduced, which has been associated with a wide range of age-related diseases, including neurodegeneration (Haas, 2019). Importantly, mitochondria are essential for axonal growth and cell maintenance. Both normal aging and traumatic injury to the central nervous system (CNS) are highly associated with mitochondrial dysfunction and oxidative stress, this poses a great challenge for an aging SCI population as the two elements can compound one another to worsen injury outcomes. Observations from our laboratory has found detrimental changes in mitochondria in the aging CNS across a range of functional areas that will have a significant effect on neuronal health and ability to promote axonal growth in the event of injury. These observations suggest an important role for mitochondria in the age-dependent decline in axon growth potential that has been previously observed, and also may suggest targeting mitochondria as a promising therapeutic avenue for SCI regardless of age.

While aging has been associated with a decline in mitochondrial activity, successful axon regeneration requires an increase in energy and mitochondrial dynamics. The primary role of the mitochondria is the generation of intracellular energy via the production of ATP through oxidative phosphorylation. Cortical neurons rely on significant ATP production to maintain normal biological functions, and even higher energy production in active axonal growth cones to promote growth, which is essential for regeneration and restoration of any functionality after injury (Vaarmann et al., 2016). The disruption of mitochondrial energy production is a significant detriment to axon growth in both aging and injury. Observations from our laboratory suggest decreases in the expression of significant oxidative phosphorylation complexes in the mitochondrial electron transport chain in aging mice, compared to young adults (**Figure 1B**). This may have a significant impact on the mitochondrial bioenergetics, and subsequently the ability to produce sufficient energy to support axon sprouting and growth.

The body's response to SCI comprises a wide range of injury cascades, mechanisms and disfunctions. Among these is mitochondrial disfunction and oxidative stress. Oxidative stress occurs in cells when there is an imbalance between the production of reactive oxygen species (ROS) and the antioxidant systems required to detoxify these, in which mitochondria play a central role. Oxidative stress and the formation of free radicals, such as reactive oxygen and nitrogen species, and their significant proposed role in the pathophysiology of SCI have long been acknowledged and previously described in detail (Jia et al., 2012). Mitochondrial bioenergetics decreases with age in the brain and spinal cord and is associated with a detrimental increase in ROS (Yonutas et al., 2015). Unbalanced oxidative phosphorylation and antioxidant systems is a feature seen in both aging and injury, and may compound when the two intersect. Increased ROS in the injury environment and oxidative stress has been seen to inhibit axonal transport and promote degeneration of axons (Islam, 2017; Sheng, 2017). Increased ROS, especially superoxide anions, has been linked to mitochondrial DNA damage and down-regulation of the electron transport chain, and therefore subsequent mitochondrial dysfunctions as well as further promoting metabolic oxidative stress in mitochondria (**Figure 1A**).

Further, synaptic mitochondria are involved in buffering calcium (Ca^{2+}) to regulate neurotransmission (Islam, 2017). In previous work from Murchison and Griffith (2017), mitochondria in aged neurons have been observed to have Ca^{2+} buffering deficits as well as reduced membrane potential. Altered Ca^{2+} homeostasis associated with neuronal excitotoxicity and oxidative stress is one significant mechanism in axonal damage and also a barrier to axonal growth (Islam, 2017). Altered membrane permeability in aging mitochondria may be attributed to multiple disfunctions, and impact antioxidant balance and Ca^{2+} buffering. With increased ROS and oxidative stress the mitochondrial permeability transition pore is more active and through prolonged opening of this channel will effect both ROS balance as well as Ca^{2+} homeostasis (**Figure 1D**). Alteration in expression and function of membrane complexes involved in the translocation of proteins to the mitochondria, such as TOM and TIM, and decreases in membrane potential also affect mitochondrial ability to effectively buffer calcium and import essential molecules (**Figure 1C**). Our own observations suggest that there is a decrease in the expression of mitochondrial transmembrane proteins in aging neurons. This may be suggestive of decreased mitochondrial functionality or productivity, and show a diminished ability of mitochondria to shuttle necessary molecules into the mitochondrial matrix.

The very active oxygen metabolism and high lipid content of the CNS leave it particularly vulnerable to oxidative stress and subsequent free-radical mediated damages, such as lipid peroxidation (Jia et al., 2012). The effects of oxidative stress and neuronal excitotoxicity after injury are wide reaching and seen in a range of cells, especially the supporting glia. Increased ROS has been associated with pro-inflammatory microglia and reactive astrocytes involved in the detrimental progression of the SCI lesion (Sutherland and Geoffroy, 2020). The increase in ROS, in both aging and SCI, is coupled with a decrease in antioxidant capacity with aging to create a greater vulnerability to oxidative stress and damage in aged individuals after an injury to the CNS (Sutherland and Geoffroy, 2020). To date there have been several clinical and pre-clinical trials of different anti-oxidant therapies for SCI (Rabchevsky et al., 2020), however, the aging factor has received little attention. Observations from our own research suggest that mitochondria in both aging cortical neurons and astrocytes, in dissociated conditions, exhibit an increased stress and dysfunctional state. This is likely to impair the ability of these mitochondria to efficiently produce the necessary energy and also contribute to increases in oxidative stress. The increased oxidative stress observed in the injury environment is also seen, more subtly in the normal aging CNS, once again leading to an additive effect when age and injury meet.

Axonal trafficking is essential for growth, shuttling necessary supplies to the extending axon, including organelles, cytoskeletal proteins and other necessary components. The cellular morphology of neurons, and especially projecting motor neurons with extremely long axons, presents unique challenges for energy homeostasis and distribution. Mitochondrial energy production is particularly in demand at the growth cones of axons and in synapses, requiring efficient mitochondrial trafficking and axonal transport to these areas (Sheng, 2017). Axonal trafficking has been seen to decline with age (**Figure 1**). In aged peripheral nervous system, the reduced speed of axon growth has been correlated with a diminished rate of axonal transport. There is an age-related decline in mitochondrial transport, both in the peripheral nervous system and the CNS, that may have significant implications for the age-related decline in axon growth potential (Geoffroy et al., 2017). Mitochondrial biogenesis and turnover of proteins is important for the maintenance of bioenergetics, the prevention of detrimental ROS build-up, and mitochondrial fission/fusion. Disfunctions in mitochondrial biogenesis and fission/fusion have been associated with aging. This may be linked to changes in protein expression or potentially to the maturation of the final products within the mitochondrial matrix resulting in decreased functionality as well as decreased turnover of dysfunctional mitochondria (López-Lluch et al., 2008). Therefore, an important strategy to enhance axon growth in aging neurons is to promote mitochondrial trafficking, in addition to promoting biogenesis.

Mitochondria have been investigated as a potential therapeutic target in a range of disorders, especially cancers, and in recent years has begun to be explored in CNS injury. A mitochondrial approach, 'MitoCeuticals', may be promising in treating SCI for an assortment of reasons (Rabchevsky et al., 2020). The secondary injury phase of SCI is complex and multifaceted, with many of the signaling cascades involved intersecting and diverging, and effecting multiple targets. This complexity has led to the proposition that targeting a single specific pathway or cascade may prove a less effective approach to promote recovery of function after an injury, and a broader subcellular approach is necessary (McEwen et al., 2011). There are a variety of ways that mitochondria may be used and manipulated, genetically and pharmacologically, in SCI therapeutics. These include anti-apoptotic strategies, boosting mitochondrial bioenergetics, alternative biofuels, mitochondrial

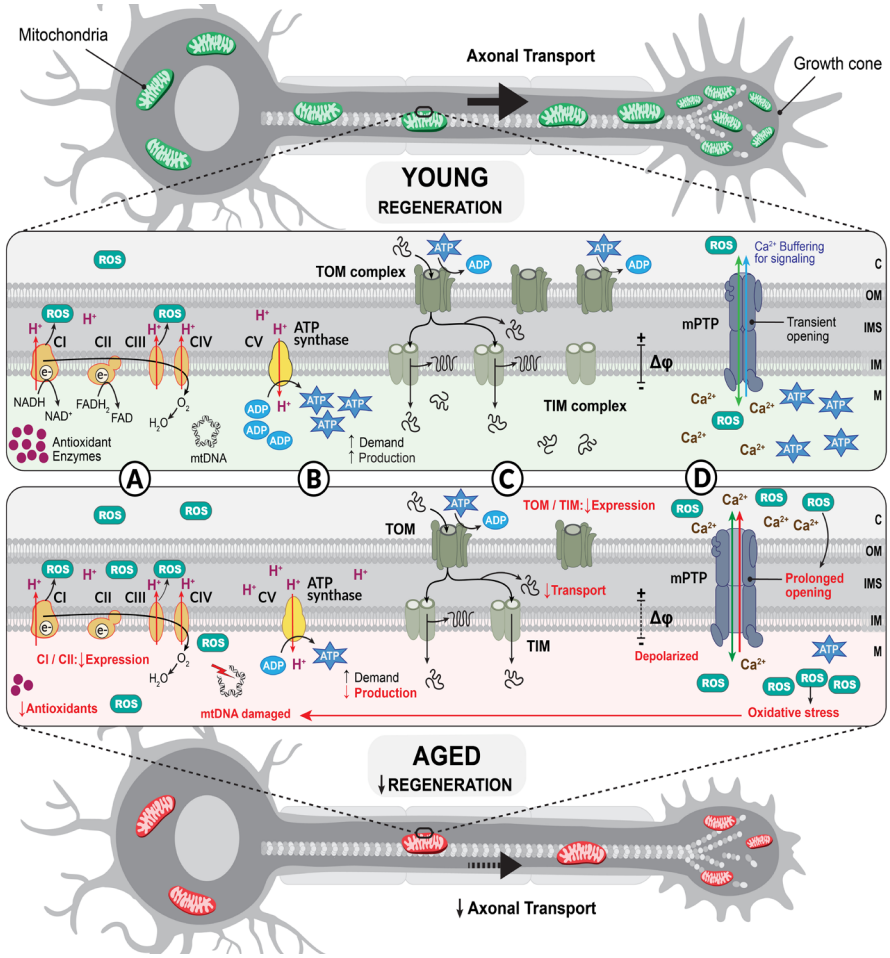

Figure 1 | **Mitochondria in aging neurons show decline from their young counterparts in a range of functional areas.**
(A) Antioxidant homeostasis: aged mitochondria produce excessive reactive oxygen species and show decreased antioxidant capability, leading to oxidative stress. (B) Bioenergetics: aged mitochondria exhibit decreased ability to efficiently and effectively meet the high energy demand of the cell especially in the growing axon. (C) Transmembrane transport: decreased expression and/or function of transmembrane transport channels in aged mitochondria may result in decreased transport of essential molecules into the matrix and impaired protein turn-over. (D) Calcium buffering and the mitochondrial permeability transition pore (mPTP): with increased ROS and an oxidative stress state the mPTP in aged mitochondria is more active and shows prolonged opening, effecting calcium buffing ability and contributing to mitochondrial membrane depolarization. All of these elements are detrimental to neuronal health and may contribute to the age-dependent decline in axon growth potential. ADP: Adenosine diphosphate; ATP: adenosine triphosphate; C: cytoplasm; CI-CV: OXPHOS complex 1–5; Δφ: membrane potential; IM: inner membrane; IMS: inter-membrane space; M: mitochondrial matrix; mPTP: mitochondrial permeability transition pore; OM: outer membrane; ROS: reactive oxygen species; TIM: translocase inner membrane; TOM: translocase outer membrane.

permeability transition pore, Ca^{2+} signaling and transmembrane channels, as well as antioxidant strategies (McEwen et al., 2011; Rabchevsky et al., 2020). 'MitoCeuticals' may be an effective strategy to improve neuroprotection after SCI, as well as potentially promote axon growth. Our pilot experiments indeed demonstrate that pharmacologically enhancing mitochondrial biogenesis increases neurite outgrowth of adult cortical neurons *in vitro*. To our knowledge, enhancing mitochondrial biogenesis to stimulate axon growth *in vivo* in a more chronic injury phase and in older animals has not been attempted to date.

An interesting idea in recent years is the transplantation of mitochondria as a therapeutic agent. There is evidence that transplantation of autologous mitochondria, either directly or systematically, is beneficial in a variety of models such as neurodegeneration and cardiac ischemia. The transplantation of mitochondria in SCI is based on the emerging idea that mitochondria released from cells after CNS injury may transfer between cells to assist in oxidative phosphorylation. It is postulated that transplanted mitochondria supplement endogenous antioxidant systems allowing for increased Ca^{2+} buffering and greater energy production (Rabchevsky et al., 2020). One particularly interesting proposition arising from this may be to transplant mitochondria from young mice into aging mice to promote mitochondrial bioenergetics and improve axon growth. These therapeutic strategies may be promising but have yet to be explored in SCI in an aging paradigm.

It is clear that mitochondria play important roles in normal aging, in both axon regeneration and sprouting, and in the progression of SCI. We contend that alterations in mitochondrial functioning and bioenergetics with age is detrimental to axon growth potential, and will compound the mitochondrial disfunctions occurring after a SCI. Conversely, this also makes them an interesting target for potential therapeutic manipulation to improve SCI outcomes, regardless of the age of the patient. The promotion of mitochondrial function may be a key element to promote axon growth in injured neurons regardless of age or time post injury, a contention that we will be assessing in the context of SCI. However, to effectively harness mitochondria as a therapeutic target in the wider SCI population we must more fully understand what impact both age and injury has on mitochondrial function, and how this will correlate to axon growth.

This work was partially presented at Theresa C. Sutherland, Alexandra H Lopez and Cédric G. Geoffroy: Molecular Alterations in the Aging Cortex Associated with an Age-Dependent Decline in Axon Growth; In: Mission Connect: 2019; Houston TX, US; 2019.

This work was in part supported by Mission Connect and the Craig Neilsen Foundation to CGG.

Theresa C. Sutherland, Cédric G. Geoffroy*
Department of Neuroscience and Experimental Therapeutics, Texas A&M Health Science Center, Bryan, TX, USA
***Correspondence to:** Cédric G. Geoffroy, PhD, geoffroy@tamu.edu.
https://orcid.org/0000-0002-7671-147X (Cédric G. Geoffroy);
https://orcid.org/0000-0002-2919-1504 (Theresa C. Sutherland)
Date of submission: August 3, 2020
Date of decision: August 28, 2020
Date of acceptance: September 26, 2020
Date of web publication: December 12, 2020

https://doi.org/10.4103/1673-5374.301016
How to cite this article: *Sutherland TC, Geoffroy CG (2021) Are mitochondria the key to reduce the age-dependent decline in axon growth after spinal cord injury? Neural Regen Res 16(7):1444-1445.*
Copyright license agreement: *The Copyright License Agreement has been signed by both authors before publication.*
Plagiarism check: *Checked twice by iThenticate.*
Peer review: *Externally peer reviewed.*
Open access statement: *This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.*

References

Fouad K, Bixby JL, Callahan A, Grethe JS, Jakeman LB, Lemmon VP, Magnuson DS, Martone ME, Nielson JL, Schwab JM (2020) FAIR SCI ahead: the evolution of the open data commons for pre-clinical spinal cord injury research. J Neurotrauma 37:831-838.
GBD 2016 Traumatic Brain Injury and Spinal Cord Injury Collaborators (2019) Global, regional, and national burden of traumatic brain injury and spinal cord injury, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol 18:56-87.
Geoffroy CG, Meves JM, Zheng B (2017) The age factor in axonal repair after spinal cord injury: A focus on neuron-intrinsic mechanisms. Neurosci Lett 652:41-49.
Haas RH (2019) Mitochondrial dysfunction in aging and diseases of aging. Biology (Basel) 8:48.
Islam MT (2017) Oxidative stress and mitochondrial dysfunction-linked neurodegenerative disorders. Neurol Res 39:73-82.
Jia Z, Zhu H, Li J, Wang X, Misra H, Li Y (2012) Oxidative stress in spinal cord injury and antioxidant-based intervention. Spinal Cord 50:264-274.
López-Lluch G, Irusta PM, Navas P, de Cabo R (2008) Mitochondrial biogenesis and healthy aging. Exp Gerontol 43:813-819.
McEwen ML, Sullivan PG, Rabchevsky AG, Springer JE (2011) Targeting mitochondrial function for the treatment of acute spinal cord injury. Neurotherapeutics 8:168-179.
Murchison D, Griffith WH (2007) Calcium buffering systems and calcium signaling in aged rat basal forebrain neurons. Aging Cell 6:297-305.
Rabchevsky AG, Michael FM, Patel SP (2020) Mitochondria focused neurotherapeutics for spinal cord injury. Exp Neurol 330:113332.
Sheng ZH (2017) The interplay of axonal energy homeostasis and mitochondrial trafficking and anchoring. Trends Cell Biol 27:403-416.
Sutherland TC, Geoffroy CG (2020) The influence of neuron-extrinsic factors and aging on injury progression and axonal repair in the central nervous system. Front Cell Dev Biol 8:190.
Vaarmann A, Mandel M, Zeb A, Wareski P, Liiv J, Kuum M, Antsov E, Liiv M, Cagalinec M, Choubey V, Kaasik A (2016) Mitochondrial biogenesis is required for axonal growth. Development 143:1981-1992.
Yonutas HM, Pandya JD, Sullivan PG (2015) Changes in mitochondrial bioenergetics in the brain versus spinal cord become more apparent with age. J Bioenerg Biomembr 47:149-154.

C-Editors: Zhao M, Li JY; T-Editor: Jia Y