Potential Mechanisms Driving Mitochondrial Motility Impairments in Developing Iron-Deficient Neurons

Journal of Experimental Neuroscience
Volume 13: 1–3
© The Author(s) 2019
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/1179069519858351

Thomas W Bastian

Department of Pediatrics, University of Minnesota, Minneapolis, MN, USA.

ABSTRACT: Brain development is highly demanding energetically, requiring neurons to have tightly regulated and highly dynamic metabolic machinery to achieve their ultimately complex cellular architecture. Mitochondria are the main source of neuronal adenosine 5′-triphosphate (ATP) and regulate critical neurodevelopmental processes including calcium signaling, iron homeostasis, oxidative stress, and apoptosis. Metabolic perturbations during critical neurodevelopmental windows impair neurological function not only acutely during the period of rapid growth/development, but also in adulthood long after the early-life insult has been rectified. Our laboratory uses iron deficiency (ID), the most common nutrient deficiency, as a model of early-life metabolic disruptions of neuronal metabolism because iron has a central role in mitochondrial function. Recently, we published that ID reduces hippocampal neuronal dendritic mitochondrial motility and size. In this commentary, we delve deeper into speculation about potential cellular mechanisms that drive the effects of neuronal ID on mitochondrial dynamics and quality control pathways. We propose that understanding the basic cellular biology of how mitochondria respond and adapt to ID and other metabolic perturbations during brain development may be a key factor in designing strategies to reduce the risk of later-life psychiatric, cognitive, and neurodegenerative disorders associated with early-life ID.

KEYWORDS: mitochondria, hippocampus, iron, development, dendrite, energy metabolism, neuron

RECEIVED: May 23, 2019. **ACCEPTED:** May 29, 2019.

TYPE: Commentary

FUNDING: The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The writing of this manuscript was supported by National Institutes of Health Grants R01 HD094809 and F32 HD085576.

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

CORRESPONDING AUTHOR: Thomas W Bastian, Department of Pediatrics, University of Minnesota, Minneapolis, MN 55455, USA. Email: bast0068@d.umn.edu

COMMENT ON: Bastian TW, von Hohenberg WC, Georgieff MK, Lanier LM. Chronic energy depletion due to iron deficiency impairs dendritic mitochondrial motility during hippocampal neuron development. *J Neurosci.* 2019 Jan 30;39(5):802-813. doi:10.1523/JNEUROSCI.1504-18.2018. Epub 2018 Dec 6. PubMed PMID: 30523068; PubMed Central PMCID: PMC6382976.

Commentary

The brain accounts for ~60% of a human baby's total oxygen consumption (compared with ~20% in the adult brain),[1] indicating the high metabolic requirements of brain development. Metabolic insults, including dietary macronutrient and micronutrient deficiencies, anemia, gestational diabetes, and hypoxic-ischemia, are relatively common developmental health problems and can have lasting deleterious effects on cognitive function and predispose to adult mental health diseases.[2–5] Neurodevelopmental, psychiatric, and neurodegenerative diseases/conditions (eg, autism spectrum disorder, schizophrenia, major depressive disorder, bipolar disorder, Parkinson disease, Huntington disease, Alzheimer disease) are also characterized by impaired brain energy metabolism.[6,7] Therefore, it is not surprising that there has been a recent resurgence in biomedical research on the mitochondrion and its functional role(s) in a wide array of diseases.[8] Once thought of mainly as "cellular powerhouses," we now have a more comprehensive understanding that mitochondria are not just "energy hubs" but rather are dynamic and multifunctional organelles with unique tissue-specific and age-specific roles. Not surprisingly, mitochondrial dynamics are particularly important in tissues/cells with high energy demands, such as in postmitotic rapidly developing neurons, where adenosine 5′-triphosphate (ATP) is mainly produced through mitochondrial oxidative phosphorylation.[9] In developing neurons, mitochondria are trafficked between the cell soma and distal dendritic and axonal processes, regulating local ATP production, calcium signaling, iron homeostasis, apoptotic signaling, and multiple other metabolic pathways.[10] Together these mitochondrial processes impinge on critical neurodevelopmental processes including axonal/dendritic growth and branching and synapse formation and activity.[6,7,10] Because of these critical and diverse roles for mitochondria in the developing brain, it is important that we further our understanding of how early-life energy metabolism perturbations alter neuronal mitochondrial dynamics.

Iron is a critical regulator of neuronal energy metabolism, providing structural and catalytic components for all 4 complexes of the electron transport chain (ETC) and multiple tricarboxylic acid (TCA) cycle enzymes.[11] The mitochondrion, in turn, is the intracellular site of heme and iron-sulfur cluster biosynthesis. When developing neurons have insufficient iron, they enter a hypometabolic state with impaired ETC complex activity[12] and an overall reduction of mitochondrial oxygen consumption and glycolysis.[13] Iron deficiency (ID) is also the most common nutrient deficiency, affecting 40% to 50% of pregnant women and children, and impairs neurodevelopment.[2] Early-life ID increases the risk for many of the same neuropsychiatric diseases (eg, autism, schizophrenia, depression, impaired cognitive function) in which energy impairment has been implicated as part of the pathophysiology.[2] Thus, developmental neuronal ID provides a physiologically and clinically relevant model of the effects of early-life metabolic insufficiencies on developing neurons.

Despite iron's well-known role in important mitochondrial functions, little is known about how insufficient iron alters

Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (http://www.creativecommons.org/licenses/by-nc/4.0/) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (https://us.sagepub.com/en-us/nam/open-access-at-sage).

mitochondrial dynamics in neurons. Our recent study[14] aimed to comprehensively assess the effect of neuronal ID on mitochondrial motility in actively growing dendrite branches in relation to neuronal energy status and to begin to form hypotheses related to mitochondrial fusion, fission, and mitophagy. To do this, we used our mouse primary embryonic hippocampal neuron culture model of ID. At 11 days in vitro (DIV), we assessed mitochondrial motility in terminal dendrite segments and oxygen consumption rate, ATP levels, and expression of mitochondrial fusion/fission genes in whole cultures. We also measured fusion/fission gene expression and dendritic mitochondrial density 1 week later at 18 DIV. As the first study of neuronal ID on mitochondrial dynamics, our findings highlight several important remaining questions and areas for future investigation related to both basic mitochondrial biology and translation to human health.

The major novel finding of this study was that chronic ID in developing neurons reduces mitochondrial motility in terminal dendrite branches with a concomitant reduction in whole neuron mitochondrial respiration and ATP levels. In diving deeper, we determined that the reduced motility is not caused by reduced mitochondrial speed (in fact iron-deficient mitochondria trended toward an increased speed when moving) but rather by more frequent pausing. An important area of future investigation is determining what drives the increased mitochondrial pausing. Several potential cellular mechanisms are possible:

1. *Transient local ATP depletion.* This mechanism, whereby there is a transient decrease in ATP in the immediate area surrounding the paused mitochondria, was the focus of our speculation in the original paper. Experimentally induced acute, local neuronal energy depletion has been shown to cause mitochondrial pausing.[10,15] Mechanistically, the pausing may be driven by a decreased ATP to ADP ratio as the functional state of microtubule motor proteins depends on this ratio.[15] Our data showing that iron-deficient neurons have impaired mitochondrial respiratory capacity and decreased ATP support this mechanism. However, the effect of neuronal ID on ATP:ADP ratios in the area immediately surrounding motile vs stationary mitochondria will need to be assessed.
2. *Altered neuronal glucose metabolism*. In neurons, high cytosolic glucose induces mitochondrial arrest through glycosylation of milton (mitochondrial motor adaptor protein) by O-GlcNAc transferase, which acts as a cellular nutrient sensor.[16] Early-life ID increases mRNA expression levels of the BBB and neuronal glucose transporters (ie, *Glut1* and *Glut3*) in the developing hippocampus and glucose concentrations in the developing striatum,[17–19] suggesting that iron-deficient dysregulation of neuronal glucose may play a role in mitochondrial motility impairments.
3. *Altered neuronal calcium signaling*. Increased cytosolic calcium levels can also induce pausing of dendritic mitochondria. Calcium binds to the EF-hand motif of Miro (a mitochondrial motor complex adaptor protein) and disrupts its interaction with Kif5 (a kinesin motor protein), causing mitochondria to dissociate from microtubules and/or bind to the anchoring protein Syntaphilin.[10] This is thought to recruit mitochondria to sites of high energy demand and calcium flux such as presynaptic and postsynaptic sites. The question of whether or not the paused mitochondria have been released from the motor protein complex and microtubules is of potential importance. Our data showing that mitochondrial pause duration is similar between ID and controls suggests that pausing mitochondria in iron-deficient neuron dendrites stay on the microtubules at the same frequency, which is not consistent with the current model of a calcium-induced increase in pause frequency. This still may be a fruitful area of future research as ID impairs synaptic function[20,21] and the effect of ID on calcium signaling in developing neurons has not been assessed.
4. *A combination of signals or unknown signal*. Given ID's global metabolic effects, it is certainly possible that alterations in ATP, glucose, and calcium all contribute vital aspects to the disrupted mitochondrial motility. In addition, mitochondrial motor complex-microtubule interactions can be disrupted by other signals (that may or may not also alter ATP, glucose, or calcium) including mitochondrial membrane depolarization, reactive oxygen species (ROS), and interactions with myosin and actin filaments.[10,15,22,23] Most of the work on signals regulating the pool of motile vs stationary mitochondria has been performed in axons or mixed neurite populations, and thus, there are dendrite-specific mechanisms that are still unclear. For example, Syntaphilin serves as a synaptic mitochondrial anchoring protein in axons but an equivalent dendritic anchoring protein has yet to be discovered.[10] In addition, neuronal mitochondrial motility is regulated in a developmental age-specific manner, decreasing as the neuron matures. Whether the mitochondrial motor complex is differentially regulated throughout development has yet to be determined.

The second major finding of our study was that neuronal ID shifts the size distribution of mitochondria toward smaller mitochondria, with a striking increase in a population of small (<0.5 μM), round mitochondria. We also showed a corresponding decrease in messenger RNA (mRNA) expression of genes involved in mitochondrial fusion (*Opa1, Mfn1*, and *Mfn2*) and fission (*Drp1*) at this time point. These data raise several questions:

1. What is the identity/source of the small, round mitochondria population in the ID neuronal dendrites? Do they come from mitochondria with a low membrane potential? Or are they normal, small mitochondria that

are just a product of increased fission/decreased fusion? ID effects on mitochondrial fusion/fission rates have not been directly assessed but our gene expression data suggest that these processes may be altered.
2. What happens to the small mitochondria? Are they targeted for mitophagy? Or, do they end up undergoing fusion with another mitochondrion?
3. Are the membrane and cristae structures of the small mitochondria altered? How metabolically functional are these small mitochondria?

It is becoming increasingly clear that mitochondrial dynamics and quality control pathways play an important role in normal brain development and the pathophysiology of multiple neurological diseases. It is also clear that programming of mitochondrial metabolic rate based on early-life environment can have long-lasting effects on brain function and risk of dysfunction.[2,5] Thus, identifying and alleviating the early-life mitochondrial impairments associated with ID and perturbations of other metabolic substrates critical for neuronal mitochondrial function may be a key factor in reducing the risk of psychiatric and neurodegenerative disorders later in life. Understanding how nutritional/metabolic regulation of mitochondrial dynamics is programmed during brain development is critical to this endeavor.

Acknowledgement

The author would like to thank Michael Georgieff for his helpful suggestions.

Author Contributions

TWB wrote the manuscript.

ORCID iD

Thomas W Bastian https://orcid.org/0000-0001-5486-9471

REFERENCES

1. Kuzawa CW. Adipose tissue in human infancy and childhood: an evolutionary perspective. *Am J Phys Anthropol*. 1998;27:177–209.
2. Georgieff MK, Ramel SE, Cusick SE. Nutritional influences on brain development. *Acta Paediatr*. 2018;107:1310–1321.
3. McKenna MC, Scafidi S, Robertson CL. Metabolic alterations in developing brain after injury: knowns and unknowns. *Neurochem Res*. 2015;40:2527–2543.
4. Hay WW. Care of the infant of the diabetic mother. *Curr Diabet Rep*. 2012;12:4–15.
5. Georgieff MK, Brunette KE, Tran PV. Early life nutrition and neural plasticity. *Dev Psychopathol*. 2015;27:411–423.
6. Manji H, Kato T, Di Prospero NA, et al. Impaired mitochondrial function in psychiatric disorders. *Nat Rev Neurosci*. 2012;13:293–307.
7. Kim Y, Vadodaria KC, Lenkei Z, et al. Mitochondria, metabolism, and redox mechanisms in psychiatric disorders [published online ahead of print February 1, 2019]. *Antioxid Redox Signal*. doi:10.1089/ars.2018.7606.
8. Picard M, Wallace DC, Burelle Y. The rise of mitochondria in medicine. *Mitochondrion*. 2016;30:105–116.
9. Zheng X, Boyer L, Jin M, et al. Metabolic reprogramming during neuronal differentiation from aerobic glycolysis to neuronal oxidative phosphorylation. *eLife*. 2016;5:e13374.
10. Mishra P, Chan DC. Metabolic regulation of mitochondrial dynamics. *J Cell Biol*. 2016;212:379–387.
11. Ward DM, Cloonan SM. Mitochondrial iron in human health and disease. *Ann Rev Physiol*. 2019;81:453–482.
12. De Ungria M, Rao R, Wobken JD, Luciana M, Nelson CA, Georgieff MK. Perinatal iron deficiency decreases cytochrome c oxidase (CytOx) activity in selected regions of neonatal rat brain. *Pediatr Res*. 2000;48:169–176.
13. Bastian TW, von Hohenberg WC, Mickelson DJ, Lanier LM, Georgieff MK. Iron deficiency impairs developing hippocampal neuron gene expression, energy metabolism, and dendrite complexity. *Dev Neurosci*. 2016;38:264–276.
14. Bastian TW, von Hohenberg WC, Georgieff MK, Lanier LM. Chronic energy depletion due to iron deficiency impairs dendritic mitochondrial motility during hippocampal neuron development. *J Neurosci*. 2019;39:802–813.
15. Mironov SL. Complexity of mitochondrial dynamics in neurons and its control by ADP produced during synaptic activity. *Int J Biochem Cell Biol*. 2009;41:2005–2014.
16. Pekkurnaz G, Trinidad JC, Wang X, Kong D, Schwarz TL. Glucose regulates mitochondrial motility via Milton modification by O-GlcNAc transferase. *Cell*. 2014;158:54–68.
17. Bastian TW, Santarriaga S, Nguyen TA, Prohaska JR, Georgieff MK, Anderson GW. Fetal and neonatal iron deficiency but not copper deficiency increases vascular complexity in the developing rat brain. *Nutr Neurosci*. 2015;18:365–375.
18. Carlson ES, Tkac I, Magid R, et al. Iron is essential for neuron development and memory function in mouse hippocampus. *J Nutr*. 2009;139:672–679.
19. Ward KL, Tkac I, Jing Y, et al. Gestational and lactational iron deficiency alters the developing striatal metabolome and associated behaviors in young rats. *J Nutr*. 2007;137:1043–1049.
20. McEchron MD, Paronish MD. Perinatal nutritional iron deficiency reduces hippocampal synaptic transmission but does not impair short- or long-term synaptic plasticity. *Nutr Neurosci*. 2005;8:277–285.
21. Jorgenson LA, Sun M, O'Connor M, Georgieff MK. Fetal iron deficiency disrupts the maturation of synaptic function and efficacy in area CA1 of the developing rat hippocampus. *Hippocampus*. 2005;15:1094–1102.
22. Debattisti V, Gerencser AA, Saotome M, Das S, Hajnóczky G. ROS control mitochondrial motility through p38 and the motor adaptor Miro/Trak. *Cell Rep*. 2017;21:1667–1680.
23. Pathak D, Sepp KJ, Hollenbeck PJ. Evidence that myosin activity opposes microtubule-based axonal transport of mitochondria. *J Neurosci*. 2010;30:8984–8992.