



Stimulation of mitochondrial motility in hippocampal neurons improves neurite distribution and bioenergetic capacity

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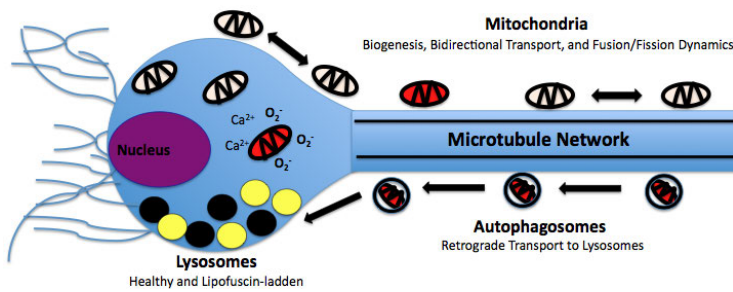
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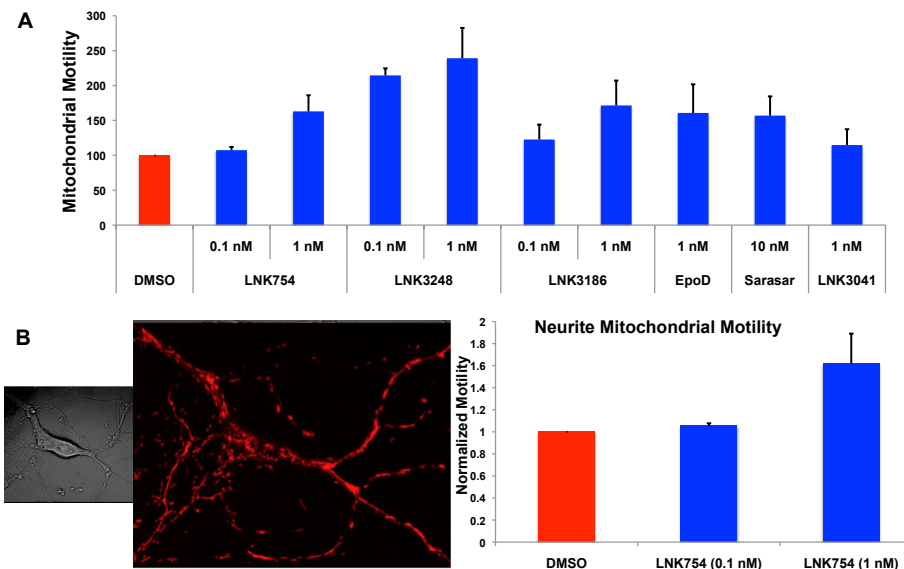
ABSTRACT

Background. Neurons are highly dependent on exchange of mitochondria between soma and neurites. Microtubules mediate anterograde delivery of mitochondria from the soma to peripheral regions in neurites where they replace or complement older and less functional mitochondria. Microtubules are also required for retrograde delivery of damaged mitochondria to lysosomes for recycling through mitophagy. Several conditions that are characterized by disrupted motility are associated with neurodegeneration. For these reasons, therapeutic agents that enhance microtubule stabilization are an attractive option for the treatment of many neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases. We hypothesize that enhancing mitochondrial motility will improve bioenergetic capacity and also lead to redistribution of mitochondria towards the neurites. Here we report on the effects on mitochondrial motility and bioenergetics by compounds that enhance microtubule assembly. Farnesyltransferase inhibitors (FTIs) stabilize microtubules by increasing acetylation while epothilone D stabilizes microtubules by reducing $\alpha\beta$ -tubulin heterodimer dissociation. While their mechanisms of action differ, the effects on mitochondrial motility and respiration were quite similar. Our data suggest that enhancing microtubule stability is a viable strategy for increasing mitochondrial function in neurons and thus could potentially serve as a novel therapeutic strategy for treating neurodegenerative diseases associated with mitochondrial dysfunction.



RESULTS

Microtubule Stabilizing Compounds Increase Mitochondrial Motility



A) Normalized rates of motility after 72 hour treatment with FTIs or epothilone D

B) Hippocampal neuron with mitochondria labeled with TMRE.

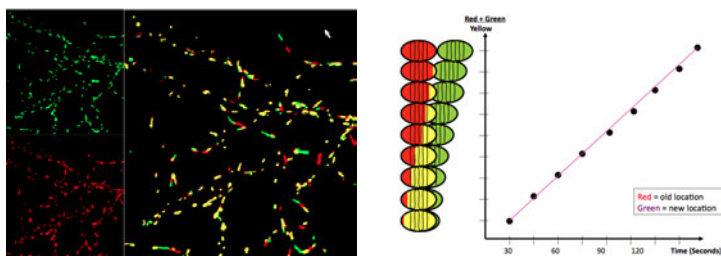
C) Rates of mitochondrial motility exclusively within neurites

All data shown as mean with SEM

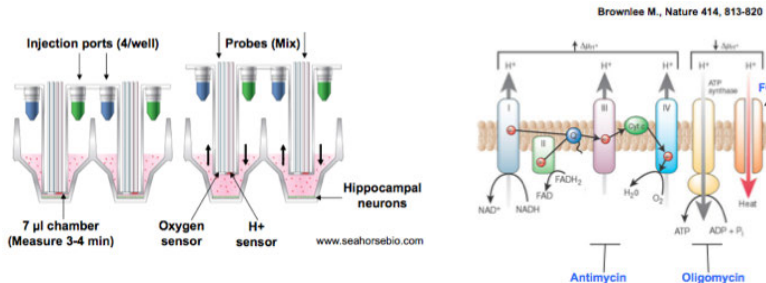
METHODS

Culture of primary hippocampal neurons: Hippocampal tissue was harvested from E14 BL6/C57 mice and cultured on poly-D-lysine/laminin coated MatTek dishes and Seahorse plates. Cells were maintained in neurobasal media with B27 supplement (Gibco) with half media changes every other day. On day 8 *in vitro* (DIV8) cultures were treated with farnesyl transferase inhibitors, epothilone D, or DMSO. After 72 hours treatment, neurons were assessed.

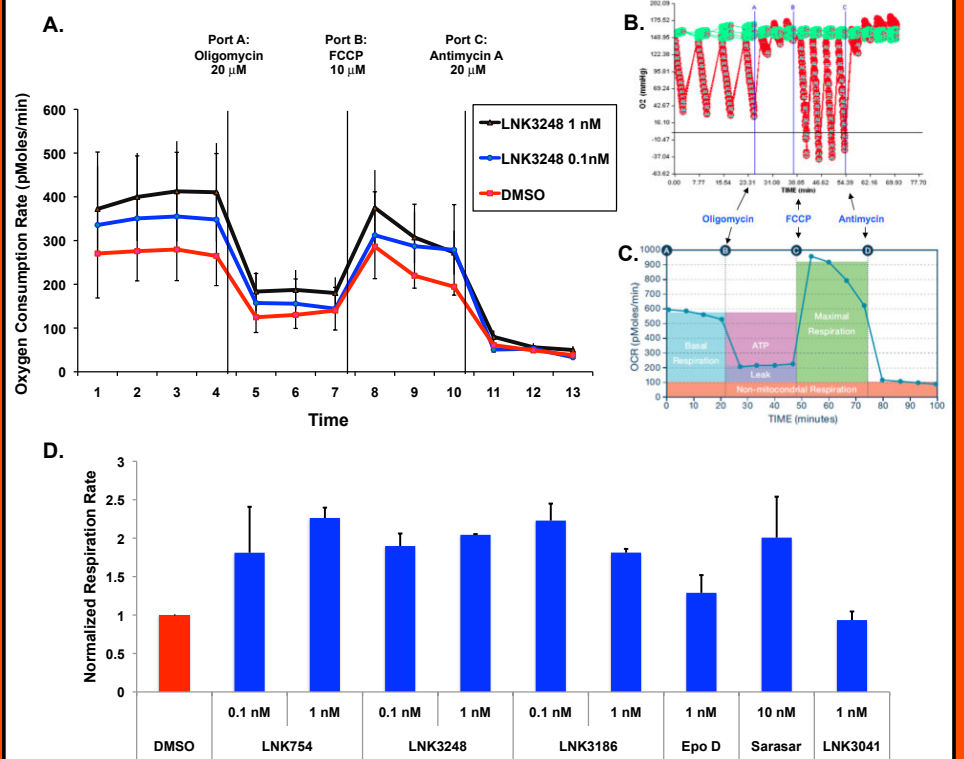
Mitochondrial Motility Assay: Primary hippocampal cultures were plated into glass bottom culture dishes (150,000 cells/dish). The neurons were imaged with a Zeiss LSM710 confocal microscope. Prior to imaging, the neurons were loaded with TMRE (Tetramethylrhodamine ethyl ester perchlorate, 7 nM). Individual neurons were selected and followed over a time series (10 timepoints, 20 sec intervals) with a 100x objective and HeNe 543nm laser. Images were processed with Metamorph analysis software. Individual pixels from each timepoint were compared for the amount of overlap (yellow) and displacement (red + green) with the first timepoint. A quality control assessment was used to exclude rare neurons with drifts in focus. The average pixel intensity for each time series was compared to the maximum and minimum values, variation beyond 5% of the average was deemed grounds for exclusion. Linear regression analysis identified the rate of change, slopes were averaged and compared between treatment groups.



Respirometry of primary hippocampal neurons using Seahorse XF24: Primary mouse hippocampal neurons were plated onto poly-D-lysine/laminin-coated 24-well Seahorse plates (80,000 cells/well, n=5 replicates per treatment). Neurons were treated on day 8 *in vitro* (DIV8) with LINK FTI compounds, Epothilone D (1 nM), or vehicle (DMSO). After 72 hours neuronal respiration was assayed through measurement of the rate of oxygen consumption with the Seahorse XF24. Prior to analysis neurobasal culture media was replaced with Seahorse media (with 1x wash). The three sequential injection ports of the Seahorse cartridge contained: A (first port injected): 50 μ l Oligomycin 20 μ M; B: 55 μ l FCCP; C: 60 μ l Antimycin 20 μ M. Oxygen consumption was measured every 8 minutes for 13 intervals.



Enhancers of Mitochondrial Motility Increase Neuronal Respiration



A) Respiration trial with the Seahorse XF24 measuring Oxygen Consumption Rates (OCR) in hippocampal cultures.

B) Example of oxygen probe measurements during respiration trial

C) Schematic of respiration trial

D) Normalized basal respiration rates after 72 hour treatment with FTIs or Epothilone D

All data are shown as mean with SEM. *, p<0.05. **, p<0.01

CONCLUSIONS

Inhibition of farnesyltransferase and stabilizing tubulin heterodimers both increased mitochondrial movement in cultured primary mouse hippocampal neurons. Treatment with FTIs or Epothilone D also resulted in increased rates of respiration. These data suggest that multiple strategies for microtubule stabilization represent novel therapeutic approaches for treatment of neuropathologies associated with mitochondrial dysfunction, such as Alzheimer's and Parkinson's diseases. Stabilization of the microtubule network within neurons has the potential to benefit not only mitochondrial dynamics but also other key processes essential for survival, such as macroautophagy, endocytosis, and synaptic vesicle transport.