Loss of NRF2 results in decreased neuronal arborization and synaptic density and causes exacerbated age-related cognitive impairment.

Mikah Brandes  
*Portland State University*

Nora Gray  
*Oregon Health and Science University*

Maya Caruso  
*Oregon Health and Science University*

Jonathan Zweig  
*Oregon Health and Science University*

Amala Soumyanath  
*Oregon Health and Science University*

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Presenter Information
Mikah Brandes, Nora Gray, Maya Caruso, Jonathan Zweig, Amala Soumyanath, and Joseph F. Quinn

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Loss of NRF2 results in impaired decreased neuronal arborization and synaptic density and causes exacerbated age-related impairment.

Mikah Branded1,3, Jonathan A. Zweig1, Maya Caruso1, Amala Soumyanath1, Joseph F. Quinn1,2, Nora E. Gray1,
1. Department of Neurology, Oregon Health and Science University, Portland, OR, USA 97239
2. Department of Neurology and Parkinson’s Disease Research Education and Clinical Care Center (PADRECC), VA Portland Healthcare System, Portland, OR, USA 97239
3. BUILD EXITO, Portland State University, Portland, OR, USA 97201

Background

- Reactive oxygen species (ROS) are free radicals or molecules with an unpaired valence electron. They are typically highly reactive and short-lived.
- ROS are critical signaling molecules but in excess can cause damage to cellular macromolecules (i.e., lipids, proteins and DNA) which is known as oxidative stress.
- As the body ages ROS accumulate over time causing cellular damage.
- The cell’s antioxidant response capacity also diminishes with aging leading to even greater oxidative damage.

The brain is particularly susceptible to oxidative stress as it is a high energy consuming organ with a high lipid content.
- Increased oxidative stress is thought to contribute to the synaptic loss and cognitive impairment that occurs during aging.

The endogenous antioxidant response pathway protects cells from oxidative stress by increasing transcription of cytoprotective genes through the binding of the transcription factor NRF2 (nuclear factor erythroid 2-related factor 2) to antioxidant response elements (AREs) in the promoters of antioxidant genes.

Activation of NRF2 has been shown to improve neuronal health in models of aging and neurodegenerative disease although its exact role in maintaining synaptic and cognitive function has not been fully elucidated.

Purpose

The goal of this study was to determine how loss of NRF2 affects both synaptic plasticity in isolated hippocampal neurons and cognitive performance in aged mice.

Methods

Primary Hippocampal Neuron Culture and Analysis

Embryonic hippocampal neurons were isolated as previously described. Briefly, breeding pairs of NRF2KO and C57BL/6J mice were acquired from Jackson Laboratory. All animals were weaned at 10 days of gestation. Hippocampi were dissected, gently minced, and suspended in 10x minimum essential medium. 10 days in vitro, hippocampi were fixed with paraformaldehyde stained with anti-NeuN and Goat anti mouse (1:200). Immunostained neurons were imaged with a Zeiss Axioshot microscope and blinded Shi analysis were performed using the Fiji platform with the plug-in created by Ferreira et al.12 Thirty isolated, non-overlapping cells were analyzed per coverslip. Statistical differences between treatment groups were calculated using Student’s unpaired t-test.

Animals

- NRF2KO mice were generated from homozygous NRF2KO breeding pairs (on a C57BL/6J background). Age C57BL/6 control mice (WT) were obtained through the NIA aged colony.
- Mice were maintained in a temperature-controlled environment with a 12-12 light/dark cycle. Food and water were supplied ad libitum, except during behavioral testing.
- Animals used in behavioral testing were aged to 20 months prior to testing. Following 2 weeks of behavioral testing animals were sacrificed and tissue extracted.

Primary hippocampal neurons were plated on glass coverslips. The neurons were incubated with a Zeiss Axioshot microscope and imaged using the Fiji platform with the plug-in created by Ferreira et al.12 Thirty isolated, non-overlapping cells were analyzed per coverslip. Statistical differences between treatment groups were calculated using Student’s unpaired t-test.

Behavioral Testing

- Odor Discrimination Reversal Learning Test (ODRL): Pretraining was required for learning and executive function.
- Animals are exposed to two cups containing one of two digging materials (dried leaves or snow) with one of two odors (peppermint or vanilla).
- Acquisition phase: Animals are trained to dig to a food reward paired with one odor regardless of the digging material as associated with that odor. The number of trials necessary to reach criteria (8/10 correct in a set of 10 trials) recorded.
- Shift phase: The food reward is now paired with a specific digging material irrespective of odor. The number of trials to learn the new association is recorded.
- Conditioned Fear Response (CFR): Contextual or object mediated by the hippocampus and amygdala.
- Habituation phase: Animals are exposed to the test chamber for five minutes. Baseline amount of time freezing is recorded.
- Conditioning phase: Immediately following habituation 3 sessions are randomly administered over a 3-hour period with no more than one shock per minute.
- Test phase: 24 hours later mice are re-exposed to the test chamber with the shock present. Amount of time freezing is quantified and the difference between time freezing in the test phase and time freezing in the habituation phase is recorded.

Conclusions and Future Directions

- In this study we demonstrate that the antioxidant regulatory transcription factor NRF2 plays an important role in modulating synaptic plasticity and cognitive performance in aged mice.
- Loss of NRF2 resulted in diminished dendritic complexity and a reduction in synaptic gene expression in isolated hippocampal neurons.
- A similar reduction in synaptic gene expression was also observed in the hippocampus and frontal cortex of aged NRF2KO mice.
- Aged NRF2KO mice also displayed significant impairments in contextual memory as well as learning and executive function.
- These results suggest that NRF2 participates in maintaining synaptic plasticity and cognitive function in normal aging and suggest that targeting NRF2 pharmacologically could be an effective cognitive enhancing strategy.
- Because oxidative stress accompanies cognitive impairment in a variety of neurodegenerative conditions as well, the findings of this study suggest that NRF2 activation may be a more broadly relevant therapeutic strategy beyond healthy aging.
- Research in our lab is ongoing to explore the beneficial effects of NRF2 activating plant-derived compounds in mouse models of aging and Alzheimer’s disease.
- Future studies are planned to look at whether activation of NRF2 in young animals could slow or prevent age-related cognitive decline.