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

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

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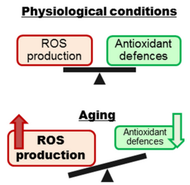
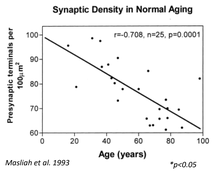
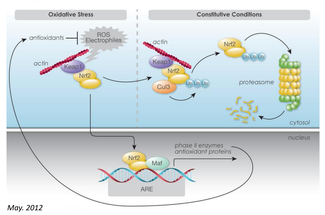
Background

- 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[1].
- 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[2-4].



- Activation of NRF2 has been shown to improve neuronal health in models of aging and neurodegenerative diseases[5-6] 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 [7]. Briefly breeding pairs of NRF2KO and C57BL6 mice were acquired from Jackson Laboratories and embryos were harvested at 18 days of gestation. Hippocampi were dissected, gently minced, and trypsinized to generate suspensions of dispersed neurons, which were then plated onto glass coverslips.
- For Sholl analyses of dendritic complexity, 30,000 hippocampal neurons were plated on coverslips in 60 mm dishes containing glial feeder cells. At 19 days in vitro, coverslips were fixed with paraformaldahyde stained with Anti-MAP2B and Goat anti-mouse IgG1-Cy3.
- Immunostained neurons were imaged with a Zeiss ApoTome2 microscope and blinded Sholl analyses were performed using the Fiji platform with the plug-in created by Ferreira et al. [8-10] Thirty isolated, non-overlapping cells were analyzed per coverslip. Statistical differences between treatment groups were calculated using Student's unpaired t-tests.

Animals

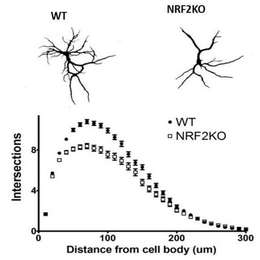
- NRF2KO mice were generated from homozygous NRF2KO breading pairs (on a C57BL6 background). Aged C57BL6 control mice (WT) were obtained through the NIA aged rodent colony.
- Mice were maintained in a climate-controlled environment with a 12-hr light/12-hr dark cycle. Diet and water were supplied ad libitum, except during behavioral testing.
- Animals used in behavioral testing were aged to 20 months prior to testing, following 3 weeks of behavioral testing animals were sacrificed and tissue harvested.

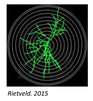
Behavioral Testing

- Odor Discrimination Reversal Learning Test (ODRL):
  - Prefrontal cortex mediated assessment of learning and executive function.
  - Animals are exposed to two cups containing one of two digging materials (dried beans or string) with one of two odors (peppermint or vanilla).
  - Acquisition phase: Animals learn to dig for a food reward paired with one odor regardless of the digging material is associated with that odor. The number of trials necessary to reach criteria (8/10 correct in a set of 10 trials) isrecorded.
  - 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 emory test mediated by the hippocampus and the 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 one second shocks (0.5mA) are randomly administered over a 3 minute period with no more than one shock per minute.
  - Test phase: 24 hours later mice are re-introduced to the test chamber but without the shock stimulus. 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.

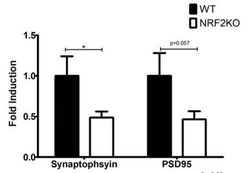
Results: *In Vitro*

NRF2KO neurons have reduced dendritic arborization and decreased synaptic gene expression.





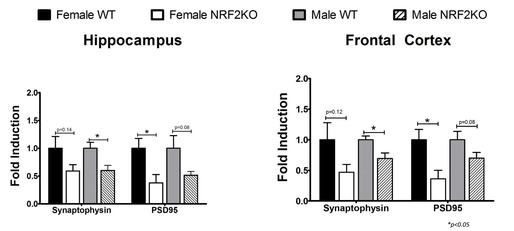
- We found that after three weeks in culture isolated embryonic NRF2KO hippocampal neurons had reduced dendritic arborization relative to cultured WT neurons.
- NRF2KO neurons also displayed reduced expression of the synaptic genes synaptophysin and PSD95 as compared to WT neurons.



Results: *In Vivo*

Aged NRF2KO animals have reduced hippocampal and cortical synaptic gene expression

- Synaptic gene expression was also reduced in the hippocampus (A) and frontal cortex (B) of aged NRF2KO mice as compared to age-matched WT animals.
- Similar reductions were observed in both brain regions for male and female NRF2KO animals.



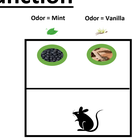
Aged NRF2KO mice have impaired learning, memory and executive function

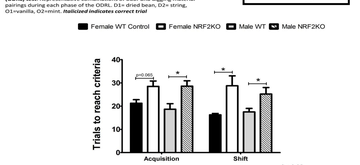
Odor Discrimination Reversal Learning Test (ODRL)

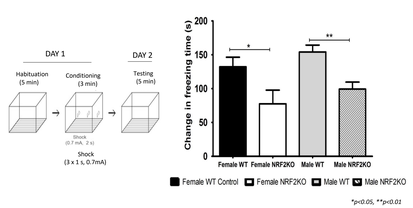
- Aged NRF2KO mice showed deficits in the ODRL test of learning and executive function.
- Male NRF2KO mice took significantly more trials to reach criteria in the acquisition phase of the ODRL than age matched WT. The same trend was evident in female mice.
- Both male and female NRF2KO mice performed significantly worse in the shift phase of the test relative to WT mice.

	Right Position	Left Position
Acquisition Phase	D1+O1	***D2+O2***
	D1+O2	D2+O1
	D2+O1	***D1+O2***
	D2+O2	D1+O1
Shift Phase	***D1+O1***	D2+O2
	D1+O2	D2+O1
	D2+O1	***D1+O2***
	D2+O2	***D1+O1***

Table 1: Example of test pairings for Odor Discrimination Reversal Learning (ODRL) test. Representative combinations of odor and digging material pairings during each phase of the ODRL. D1= dried bean, D2= string, O1=vanilla, O2=mint. ***Italicized indicates correct trial***







Conditioned Fear Response

- NRF2KO mice had significantly impaired performance in the contextual conditioned fear response test.
- Aged male and female NRF2KO mice displayed significantly reduced freezing behavior than aged WT mice.

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.

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