CELL AND SEX SPECIFIC EFFECTS OF MITOCHONDRIAL OXIDATIVE STRESS ON AGE-RELATED COGNITIVE DYSFUNCTION

Sreemathi Logan[1,3], Raghavendra Yelahanka Nagaraja[2], Hannah Yarbrough[3], Anthony Masingale[1,3], Martin-Paul Agbaga[2], William Sonntag[3], Holly Van Remmen[3,4]

[1]Department of Rehabilitation Sciences, College of Allied Health, [2]Department of Cell Biology,
[3]Oklahoma Center for Geroscience and Healthy Brain Aging, Department of Biochemistry and Molecular Biology,
University of Oklahoma Health Sciences Center;
[4]Aging & Metabolism Research Program, Oklahoma Medical Research Foundation

Background

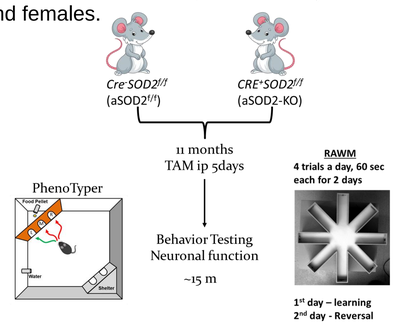
Cognitive decline is a debilitating aspect of aging and neurodegenerative diseases such as Alzheimer's disease that is closely associated with mitochondrial dysfunction, increased oxidative stress. Astrocytes, the major cell-type in the brain, play an integral role in neuroprotection and neurotransmission by maintaining integrity of the synaptic milieu. The age-related decline in cognitive function is correlated with increased astrogliosis.

We hypothesize that the age-related increase in mitochondrial dysfunction in astrocytes impairs cognitive and increases susceptibility to neuro-degeneration.

The purpose of this study was to investigate the effects of increased mitochondrial oxidative stress specifically in astrocytes on neuronal function and cognitive performance.

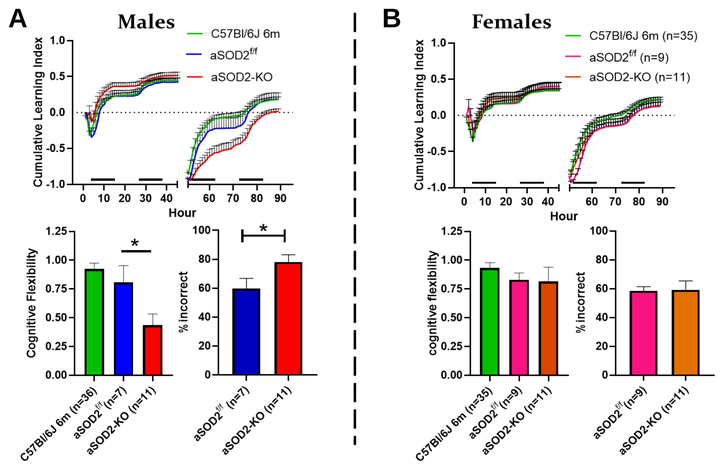
Methods

- We reduced astrocyte levels of mitochondrial superoxide dismutase (SOD2) that quenches mitochondrial generated peroxides, in tamoxifen-inducible astrocyte-specific knockout of SOD2 (GFAP-Cre^{ERT2+}/*Sod2$^{f/f}$*; aSOD2-KO) in both males and females.

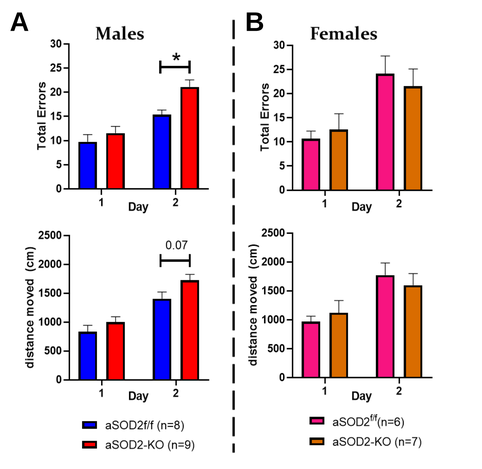


- Learning and memory was assessed using the radial arm water maze (RAWM) and Automated Home-Cage Testing (PhenoTyper)
- Hippocampal neuronal function was assessed using field recordings of long term potentiation and paired pulse facilitation.

Astrocyte SOD2 activity is necessary for hippocampal spatial working memory in males

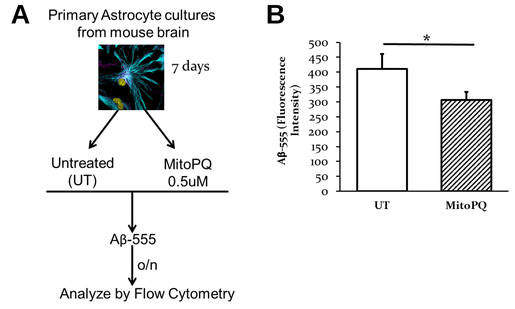


Spatial working memory is impaired in male mice that are deficient in astrocytic SOD2. Mice were tested in the PhenoTyper over a 90 hr day/night period. Mice show an increase in cumulative learning index ((correct – incorrect/total entries) to receive a spatially cued food reward. No differences were seen in male (A; top panel) or female (B; top panel) mice during initial discrimination phase (first 45 hr). Male mice were deficient in cognitive flexibility (hours 51-61 learning index) in the reversal phase (last 45 hr) when the spatial location for food reward is switched to a new location. Bar graphs are plotted as the Mean ± SE and analyzed by one-way ANOVA and post-hoc Holm-Sidak multiple comparison.



Spatial working memory using the radial arm water maze shows impairment in aSOD2-KO male and not in female mice. Mice were tested in the RAWM over a 2 day period (1st day – Learning; 2nd day – Reversal) where they learn to find a hidden platform in one arm of an 8-arm water maze. Data are plotted as Total Errors (incorrect arms entered; top panels) and Pathlength (total distance moved; bottom panels) to find the target arm. Male mice again showed deficiency in reversal learning in increased number of error and pathlength (A). No differences were observed in aSOD2-KO females (B). Data are plotted as Mean ± SE and analyzed by two-tailed students t-test.

SOD2 activity in astrocytes is necessary for Aβ$^{1-42}$ uptake

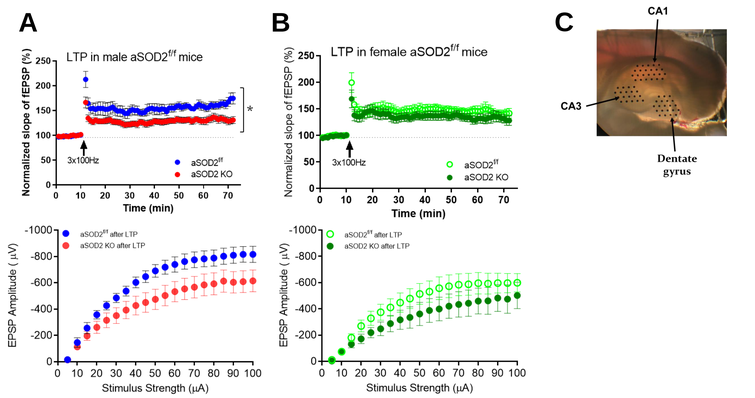


Astrocytic Aβ uptake is deficient in astrocytes with mitochondrial oxidative stress. Primary astrocytes were cultured from post-natal day 1-3 pups. Mature astrocytes in culture were treated with Mitochondrial Paraquat (MitoPQ) to induce mitochondrial stress and then treated with fluorescent labelled Aβ$^{1-42}$ oligomers (Aβ-555) as shown in (A). Cells were harvested and analyzed by flow cytometry to measure internalized Aβ$^{1-42}$. Mitochondrial stress reduced Aβ$^{1-42}$ uptake into astrocytes. Data are plotted as Mean ± SE and analyzed by two-tailed students t-test.

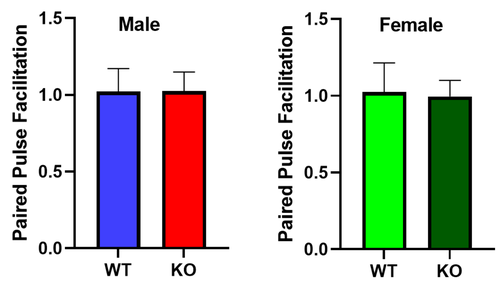
Summary

- No effect on initial learning in either PhenoTyper or RAWM in either sex
- Significant decline in Cognitive flexibility in Reversal (PhenoTyper and RAWM) and increased errors in males but not females.
- RAWM shows significant decline in spatial working memory in males and not in females
- Decreased induction of LTP in males; not in females
- No differences in PPF
- Mitochondrial stress leads to reduced trafficking of amyloid beta (Aβ$^{1-42}$) for clearance.

Astrocyte SOD2 activity is necessary for Hippocampal Long Term Potentiation But not Paired-Pulse Facilitation



Hippocampal long term potentiation was measured using field recordings (C) from the CA1 region of hippocampal slices to a tetanus (100 Hz) stimulus. Slope (A) and Amplitude (B) of EPSPs generated showed impaired LTP in males and not in females.



Paired pulse facilitation is a measure of short term synaptic plasticity and is indicative of presynaptic Ca^{2+} influx into axon terminals. measures No differences in paired pulse facilitation was observed for a range of stimuli (10-100 Hz) in male or female mice.

Conclusions

- Suggests post-synaptic mechanisms are affected rather than presynaptic
- Increased mitochondrial stress in astrocytes may contribute to sensitivity of the aged brain to neurodegeneration.
- Sexual dichotomy on cognitive function may suggest a role for estrogens in neuroprotection.
- **These data highlight the importance of therapeutic interventions targeting mitochondrial health and metabolism in the brain may be an avenue for preserving cognitive health.**

Acknowledgements

We are grateful for the support of: R00AG056662 awarded to Dr. Logan; OCAST and 3R01AG057424-04S1 to Dr. William Sonntag.

Email: Sreemathi-Logan@ouhsc.edu

COLLEGE OF ALLIED HEALTH
DEPARTMENT OF REHABILITATION SCIENCES
The UNIVERSITY *of* OKLAHOMA HEALTH SCIENCES CENTER