NEW IMAGING APPROACH TO EVALUATE THE EFFECTS OF DEVELOPMENTAL ALCOHOL EXPOSURE ON BRAIN GREY AND WHITE MATTER AND INTERVENTION OUTCOMES

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10th International Conference on Adolescents and Adults with FASD
Seattle, April 19, 2026

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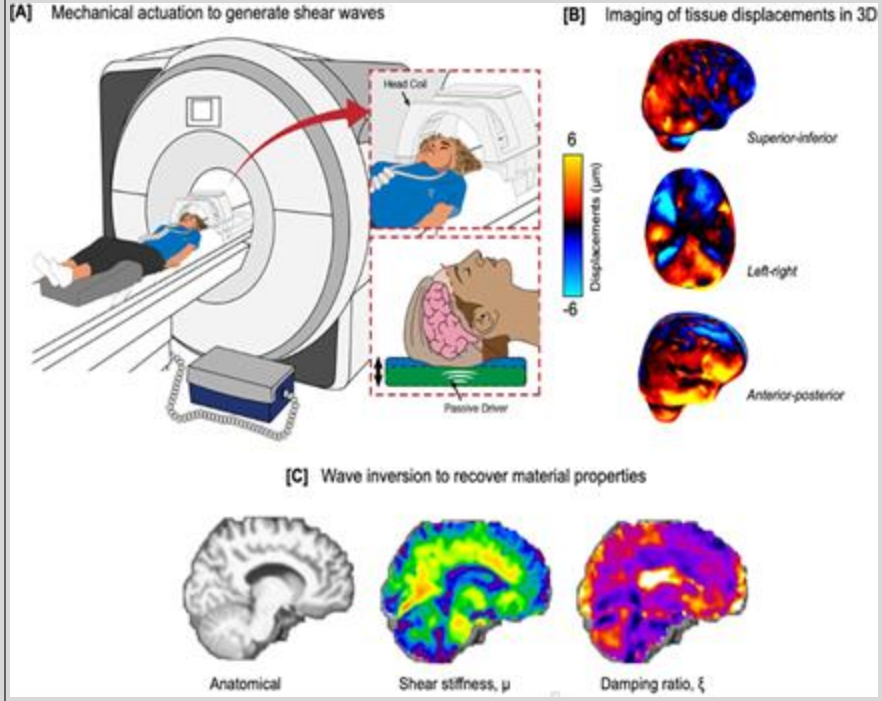
Brain Mechanical Properties are Defined by Organized Structures, Including *Myelinated Fibers* and *Extracellular Matrix*



Hiscox et al. (2021) *NeuroImage*

Magnetic resonance elastography (MRE) is a noninvasive MR imaging technique that produces quantitative maps of viscoelastic mechanical properties in soft biological tissues (including brain)

Shear stiffness (resistance to deformation)

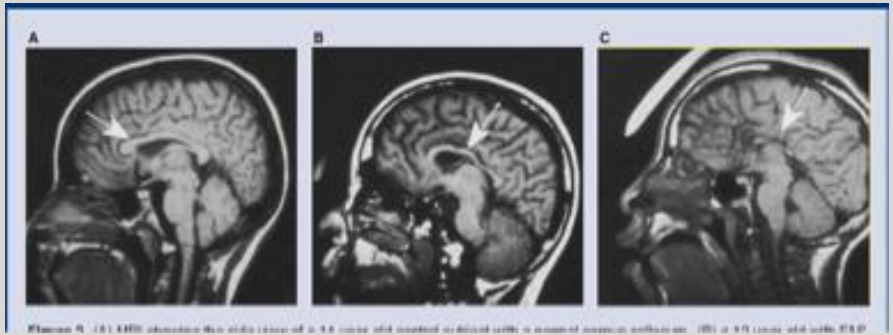
Damping ratio (how quickly the oscillations in the system die)

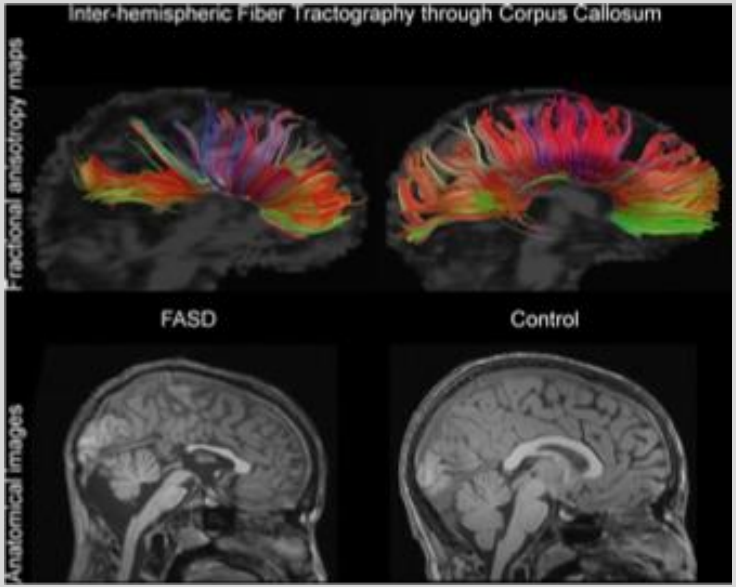
In preclinical animal models, changes to mechanical properties of brain tissue relate to the composition and organization of brain: significant correlations with neuronal density, myelin content and oligodendrocytes, microglia, and astrocytes

Used as a marker of disease, injury, and recovery

Bayly & Garbow (2018) *J Magn Reson*; Hiscox et al (2016) *Physics in Med and Biol*
Sack et al. (2013) *Soft Matter*

Defects of the Corpus Callosum Have Been Proven to be a Reliable Indicator of Prenatal Alcohol Exposure

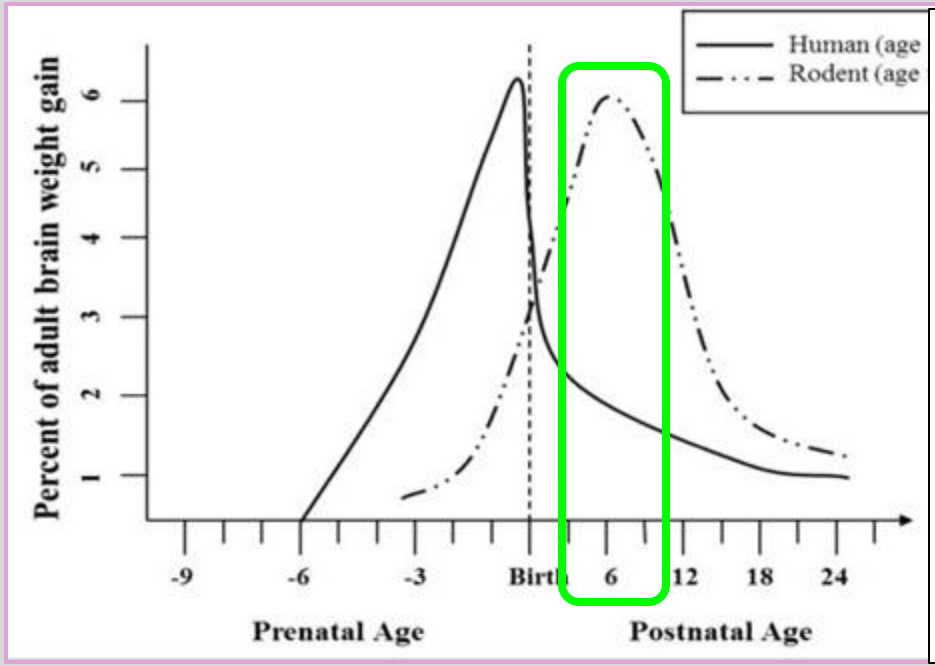


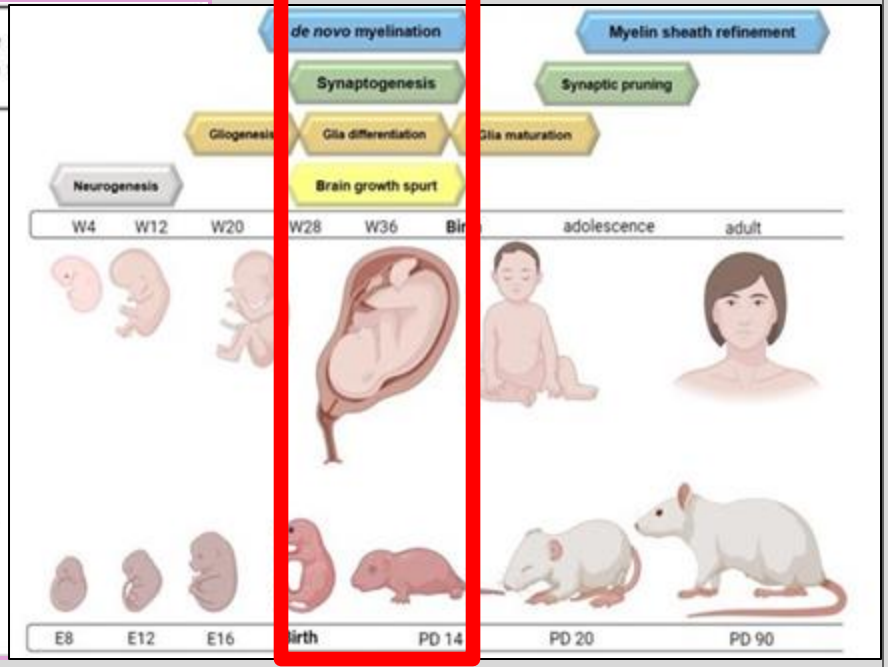


- FASD - umbrella term for FAS, FAE, ARND
- Range of anatomical abnormalities and behavioral deficits; CNS damage
- Completely preventable cause of mental/cognitive disabilities
- Prevalence: FAS - 0.1% live births; FASD – 1-5% live births

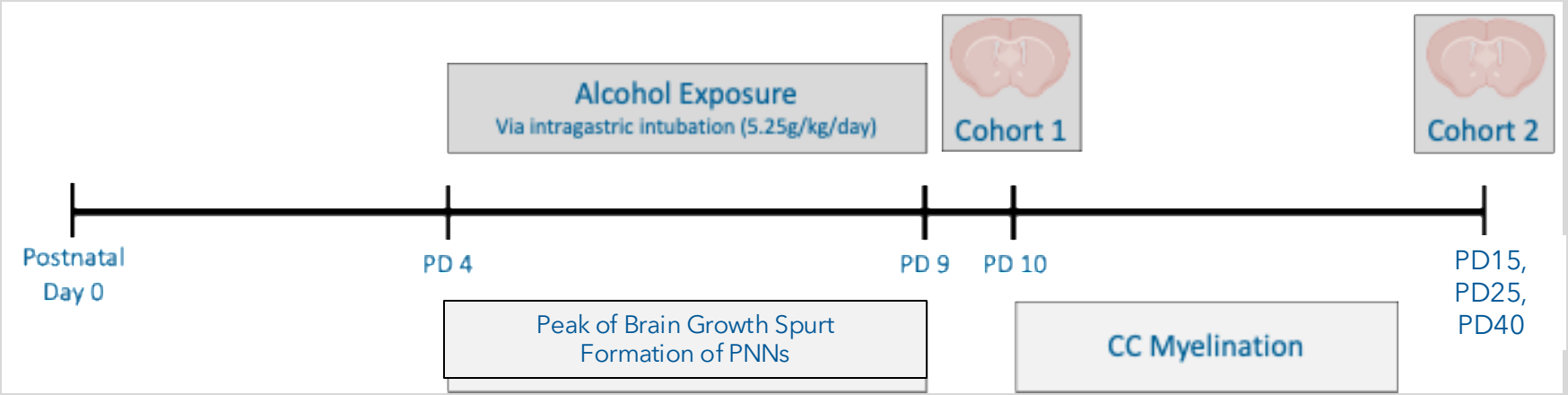
(Left - Mattson, Jernigan, Riley (1994). *Alcohol Health and Research World*; Right - Wozniak et al. (2008, 2019). *The Lancet*)

Preclinical Rat Model: Exposure to Alcohol During the 3rd Trimester

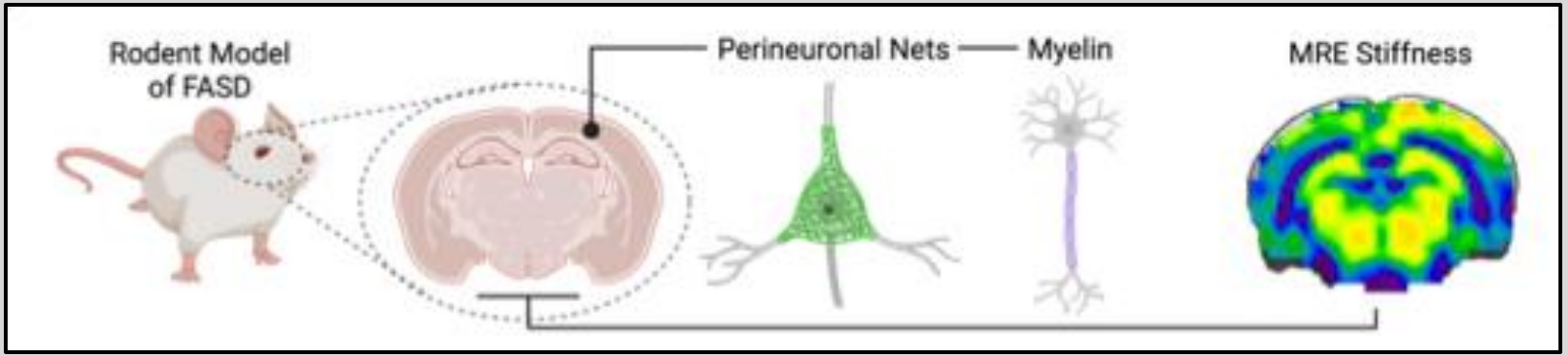




Experimental Timeline

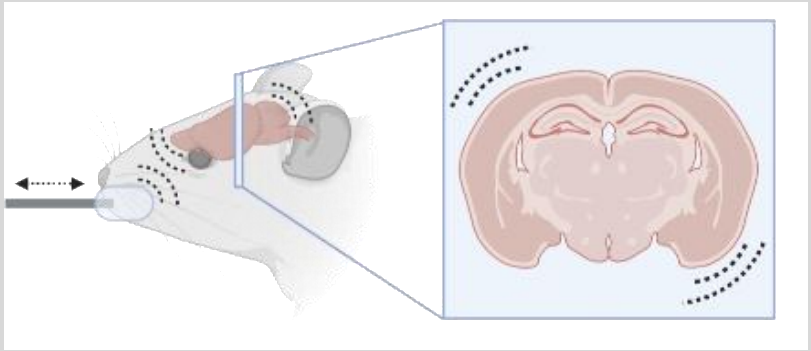


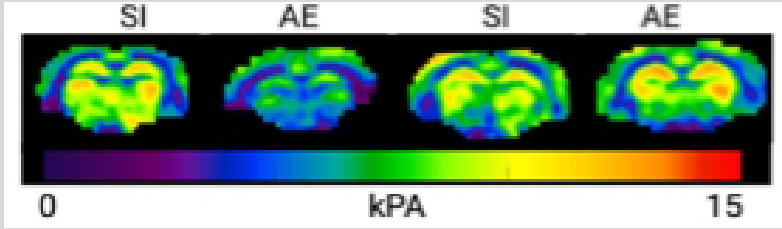
Brain Mechanical Properties are Defined by Organized Structures: Myelinated Fibers and Extracellular Matrix [Perineuronal Nets (PNNs)]



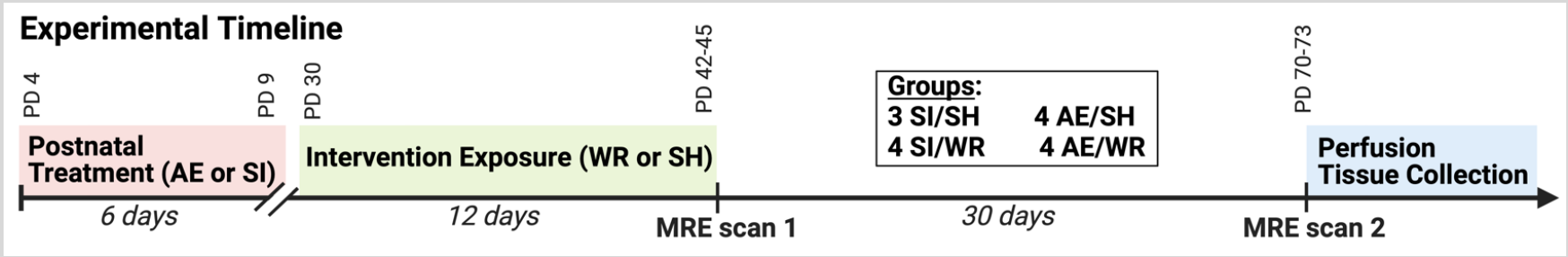
Magnetic Resonance Elastography Setup

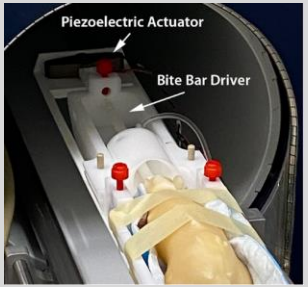
- 9.4T Bruker Biospec scanner
- 1-3% isoflurane in oxygen continuous anesthesia
- 4 channel surface coil
- Vibrations (800Hz) applied using piezoelectric actuator attached to a bite bar driver

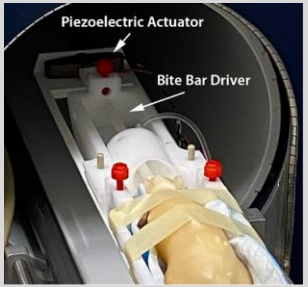




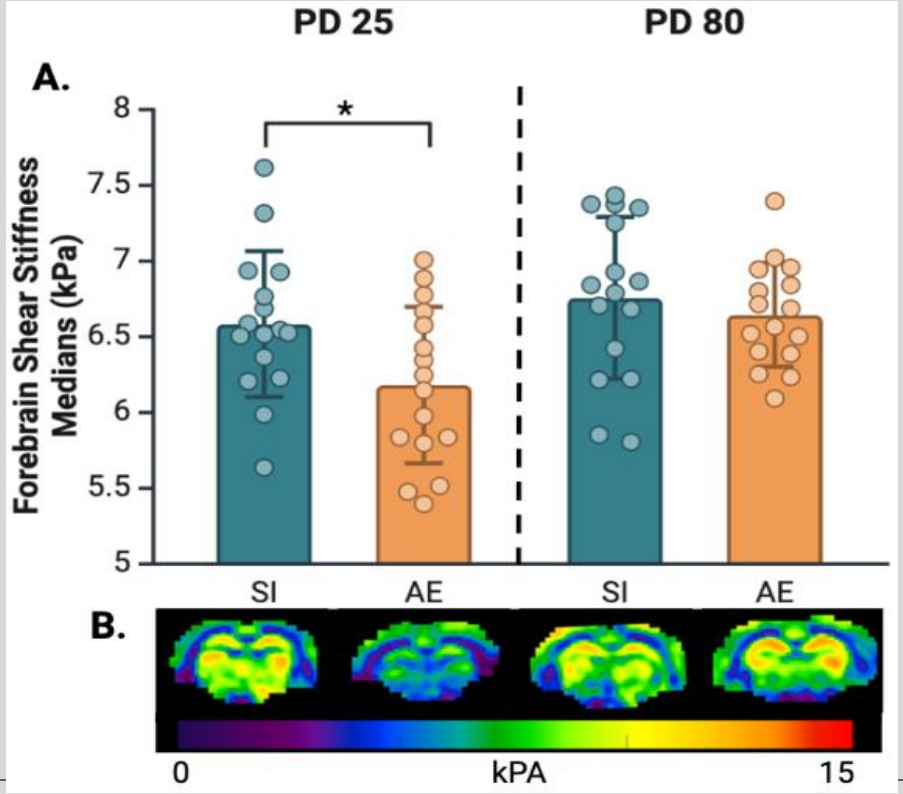
Can Voluntary Exercise (Wheel-Running) Mitigate Changes to Brain Mechanical Properties?



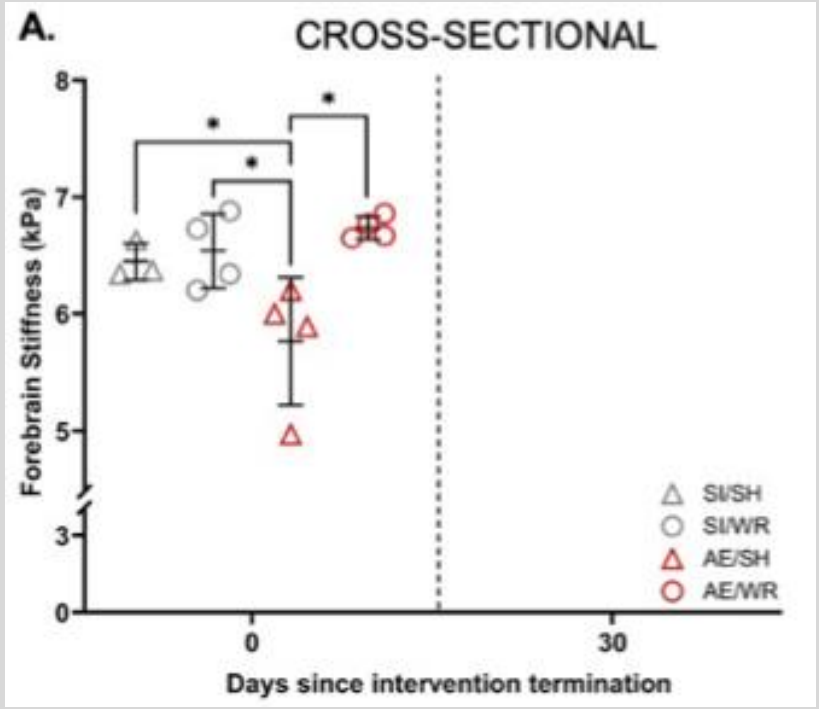


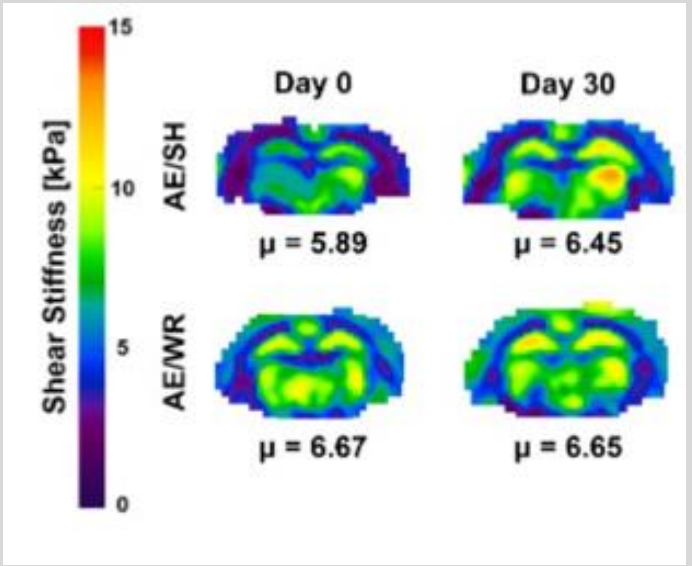


Brain Stiffness Measured by MRE Is Reduced in AE Rats in Adolescence (PD 25) but Not in aAdulthood (PD 80)

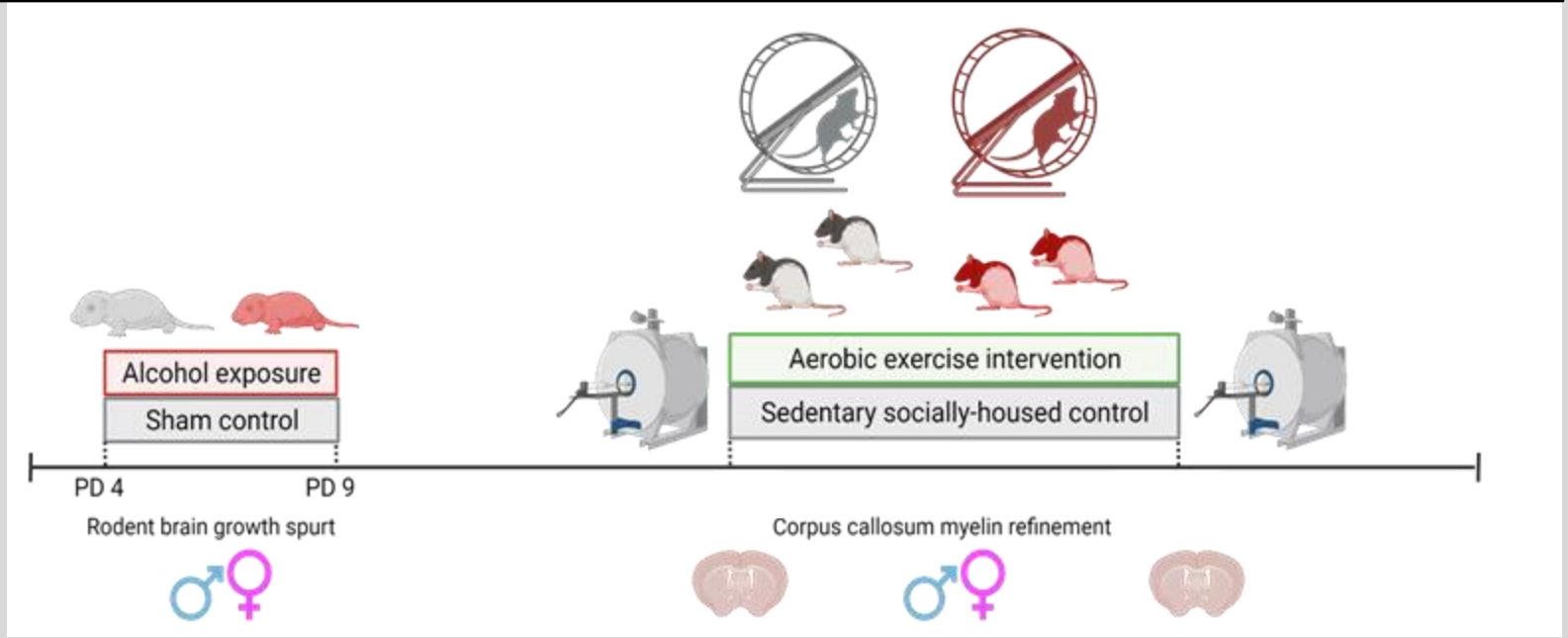


Alcohol Exposure Decreases Brain Shear Stiffness; WR Intervention Mitigates This Effect



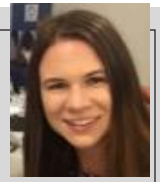


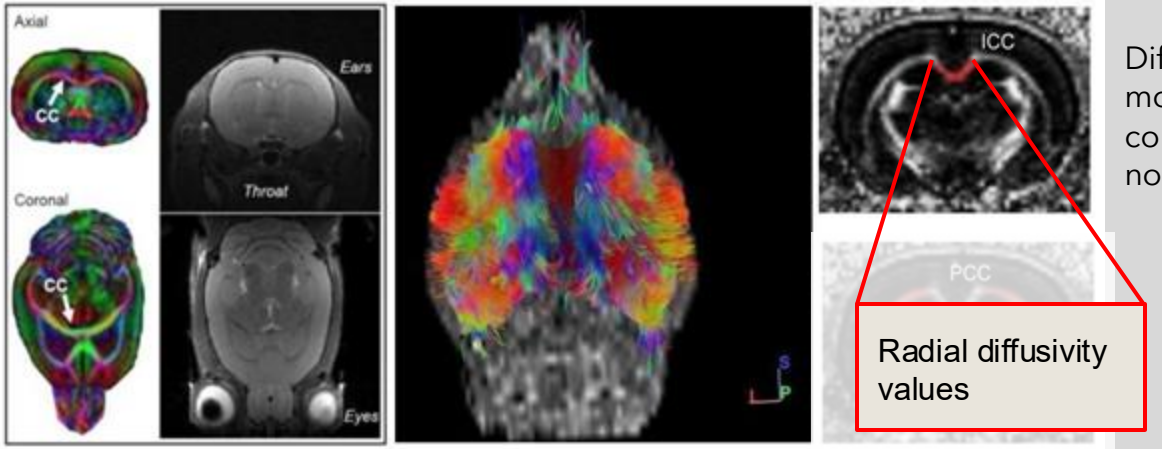
Is Myelination Delayed in a Rat Model of FASD? If So, Can We Stimulate This Process in Adolescence?



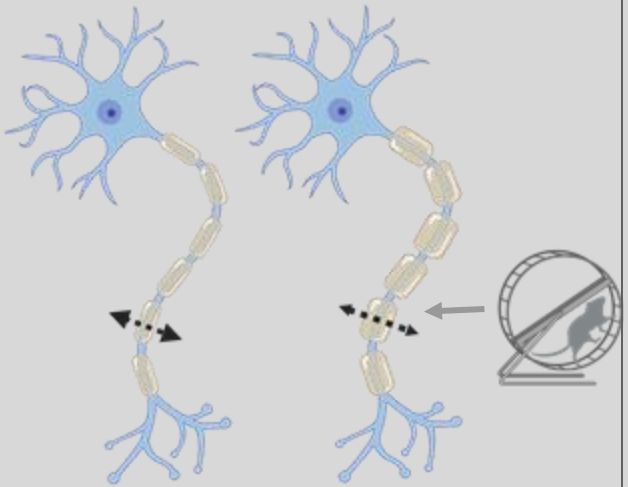
Adapted from Milbocker et al. (2022) *Scientific Reports*.

Investigating the Impact of Exercise Intervention on Corpus Callosum ***STRUCTURE*** in a Rat Model of FASD

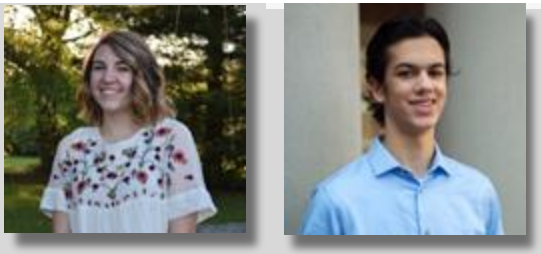




Diffusion Tensor Imaging (DTI): uses the movement of protons in water molecules to collect data describing neural tissue <u>structure</u> noninvasively



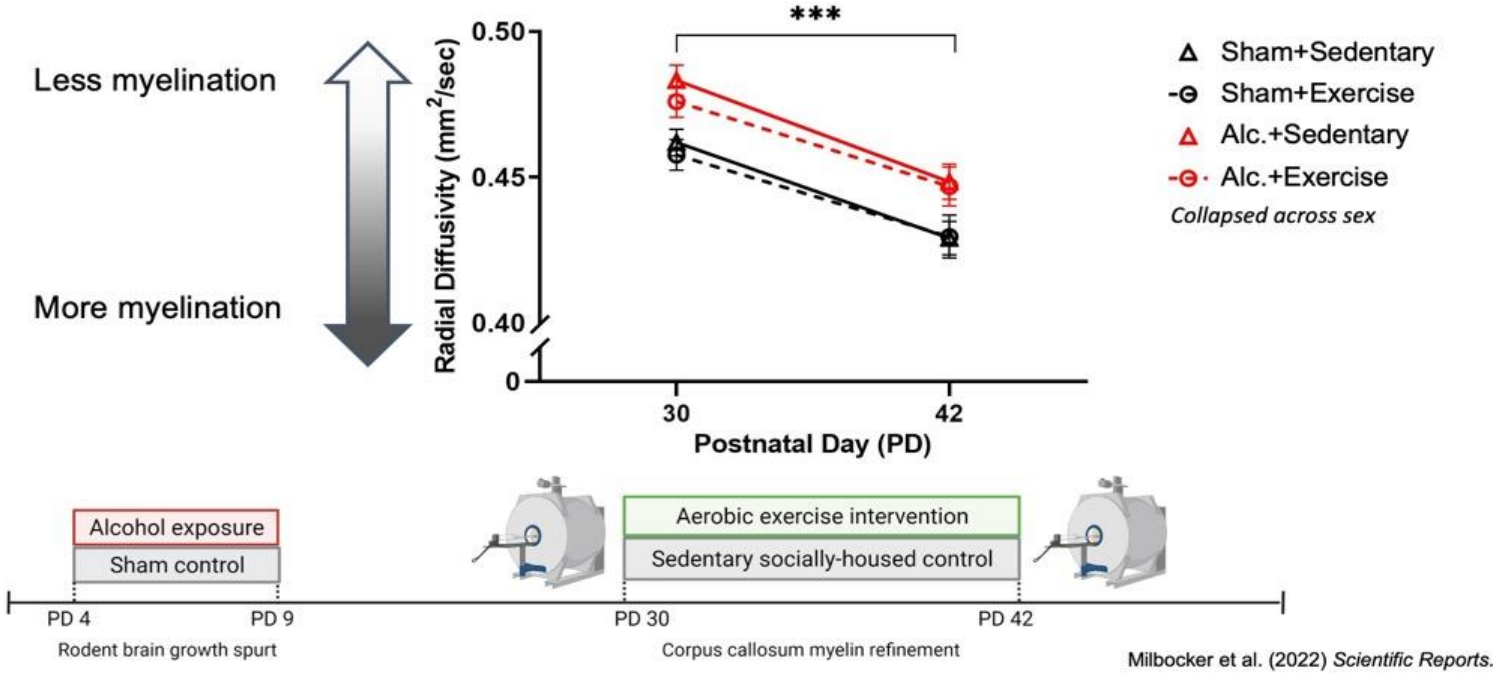
Radial diffusivity (biological interpretation)



Gillian LeBlanc and Eric Brengel

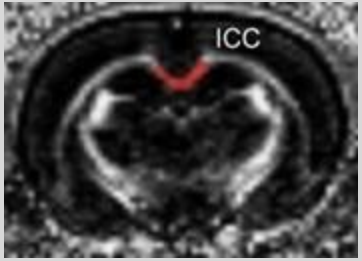
Adapted from Milbocker et al. (2022) *Scientific Reports*.

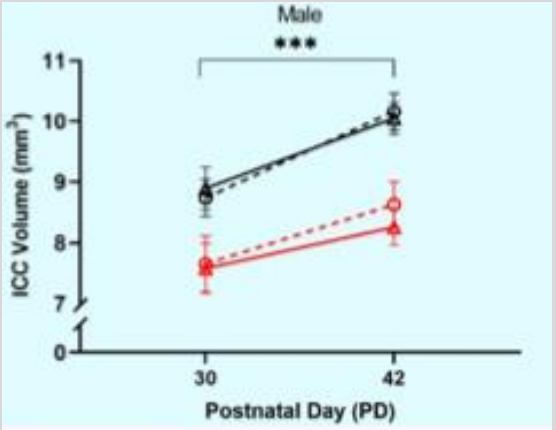
TRAJECTORY OF INTERHEMISPHERIC MYELINATION IS DELAYED IN THE RAT MODEL OF FASD - NO EFFECT OF INTERVENTION

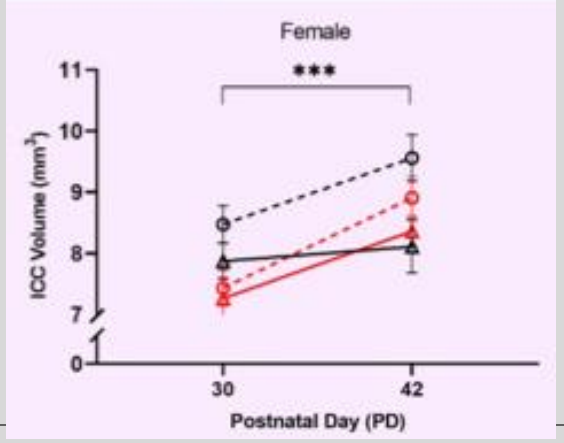


Milbocker et al. (2022) *Scientific Reports.*

Alcohol Exposure During the BGS Delays the Trajectory of Corpus Callosum Growth in Adolescence; Female Rats Benefit From the Exercise Intervention

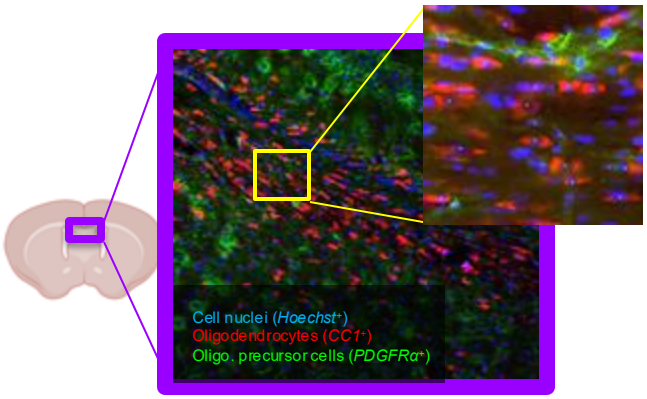


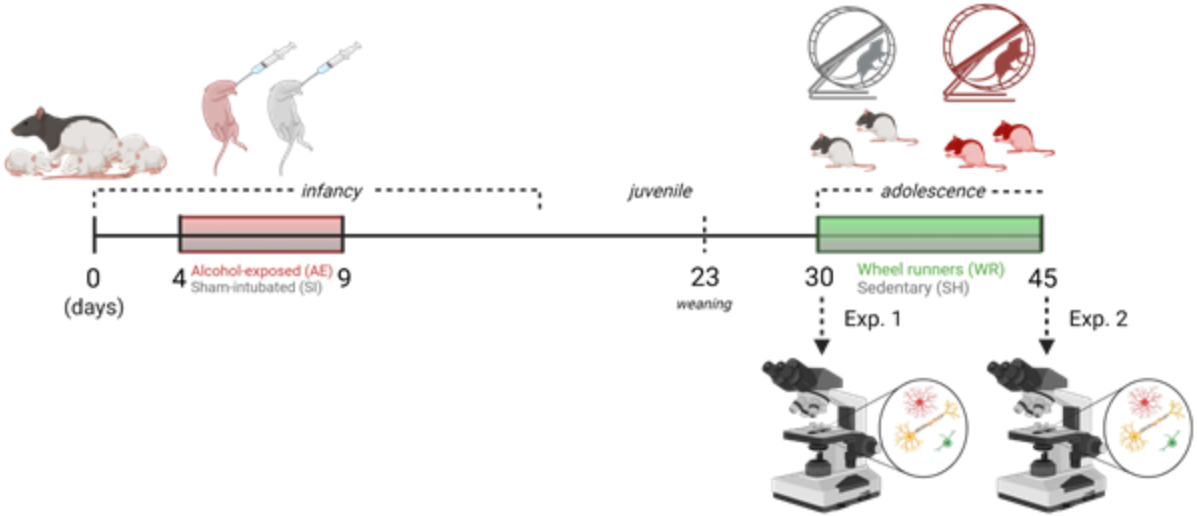




Milbocker et al. (2022) *Scientific Reports*.

Validation of Neuroimaging Results with Immunocytochemistry





OLIGODENDROCYTE PRECURSOR CELL NUMBER UNAFFECTED BY ALCOHOL TERATOGENESIS IN ADOLESCENCE

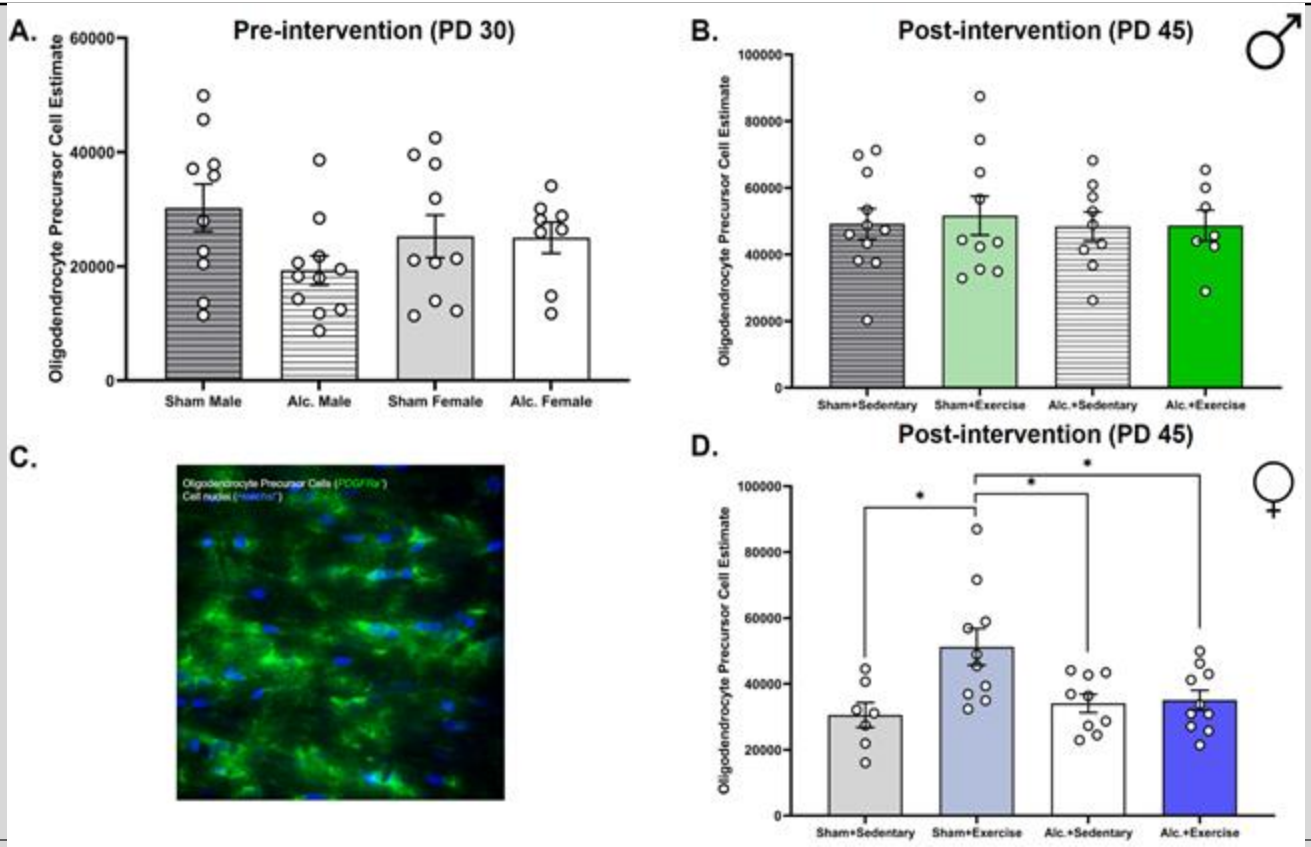
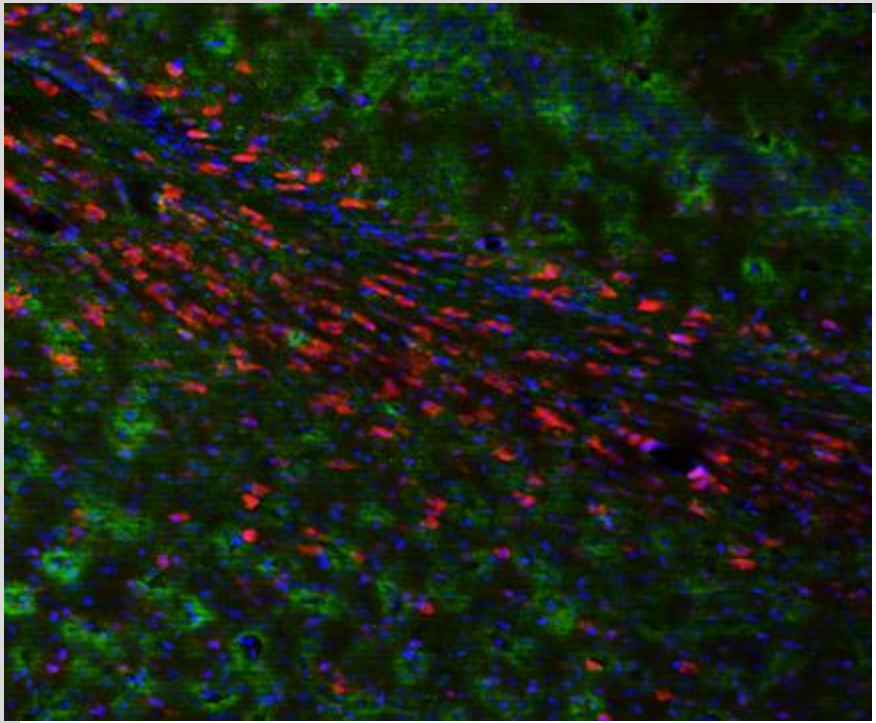


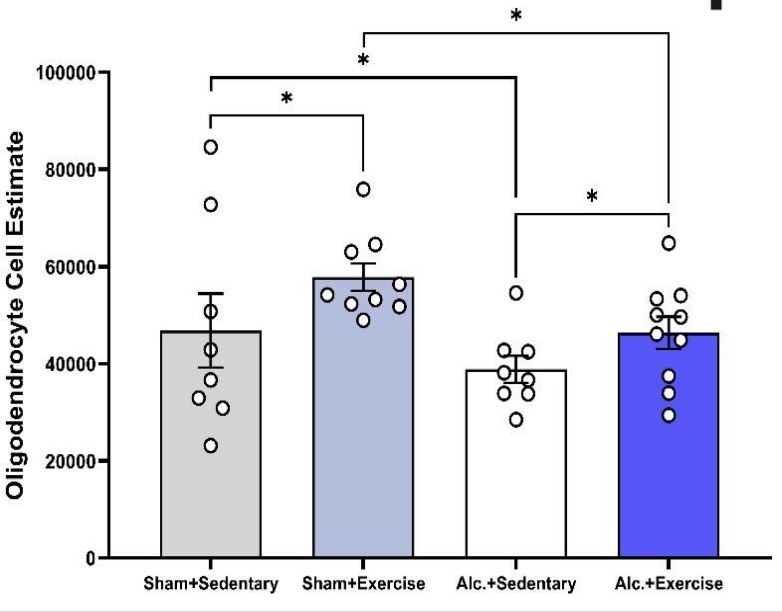
Figure error bars represent ± SEM.
*$p \leq .05$

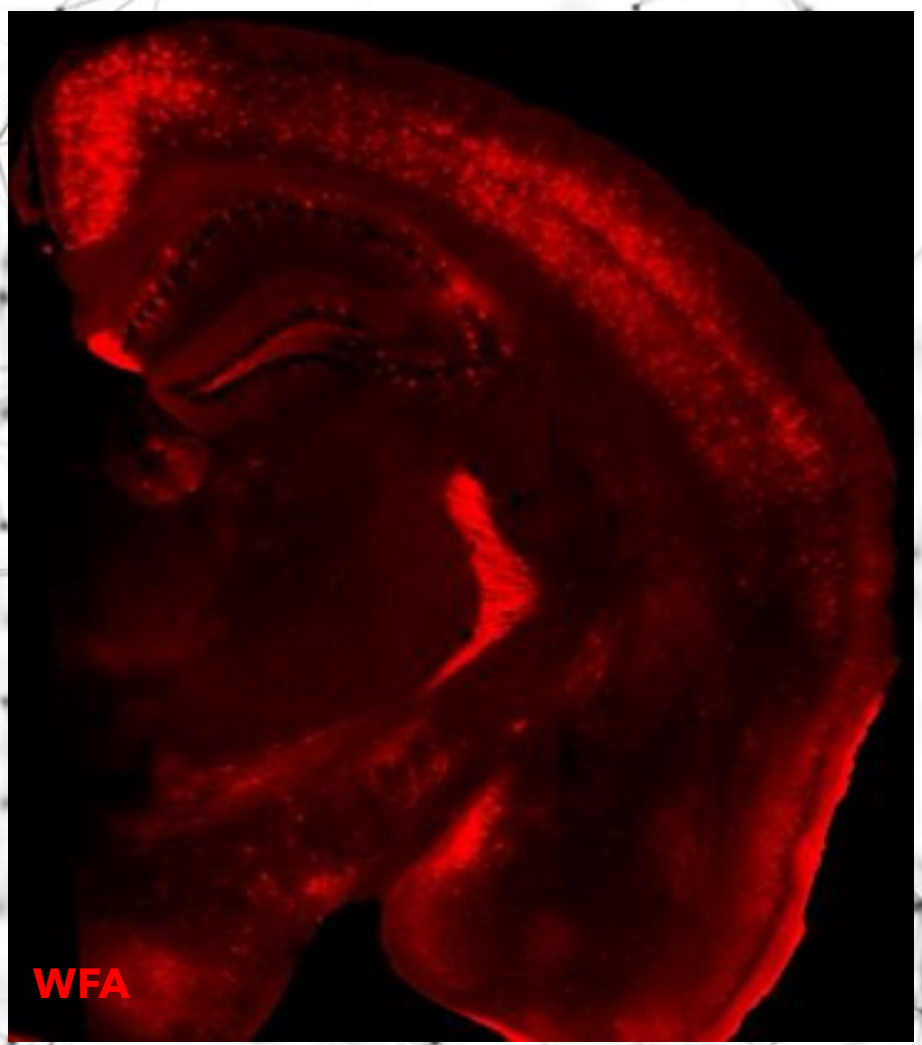
Myelinating Oligodendrocyte Cell Number Increased by Exercise Intervention in ***FEMALE*** Brain



OPC (PDGFR)
OL (CC1)
Nuclei (Hoechst 33342)

Postnatal Day 45 (females) ♀



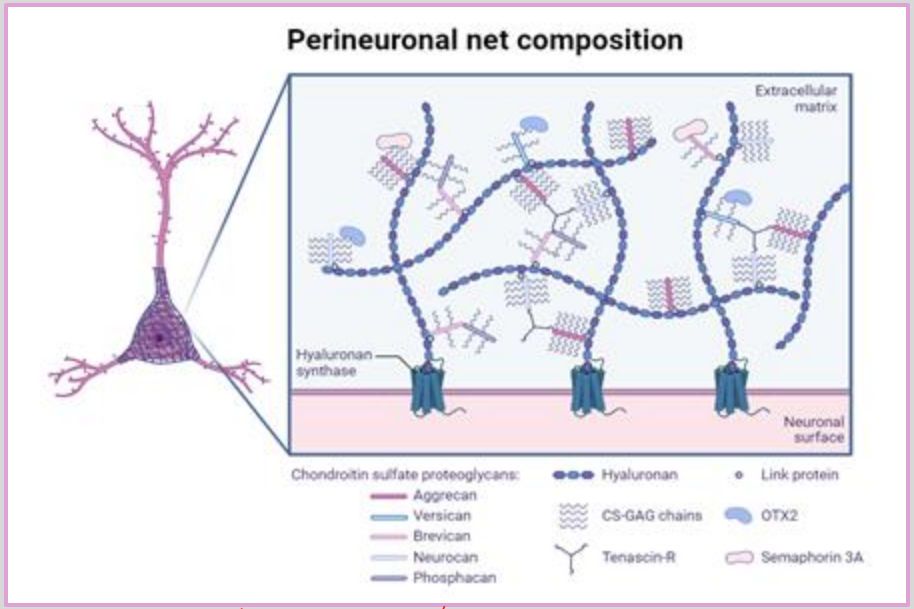


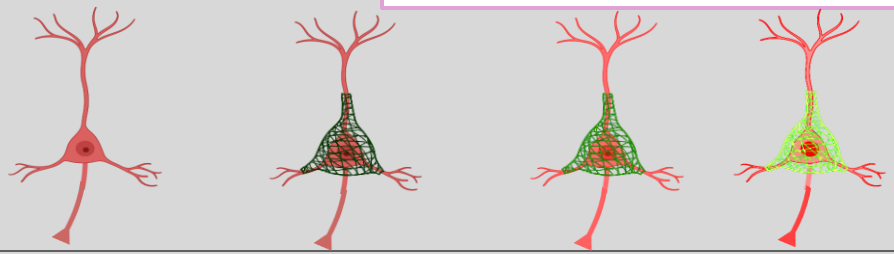
Perineuronal nets (PNNs) are unique, specialized extracellular matrix in the CNS

- First described by C.Golgi in 1882
- First detected in late development
- Unique distribution in different CNS regions (cerebellum - DCN, thalamus – TRN, hippocampus – CA2…)
- Surround primarily PV+ GABAergic interneurons, Glu+ neurons
- Important regulator of CNS plasticity
- PNNs disruption can reactivate plasticity
- Composed of chondroitin sulfate proteoglycans (CSPGs)

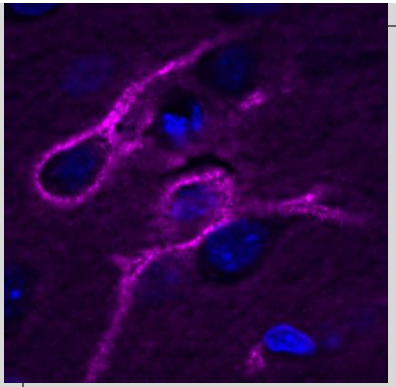
Pizzorusso et al., *Science*, 2002
Rowlands et al., *J. Neurosci.*, 2018

Perineuronal Nets

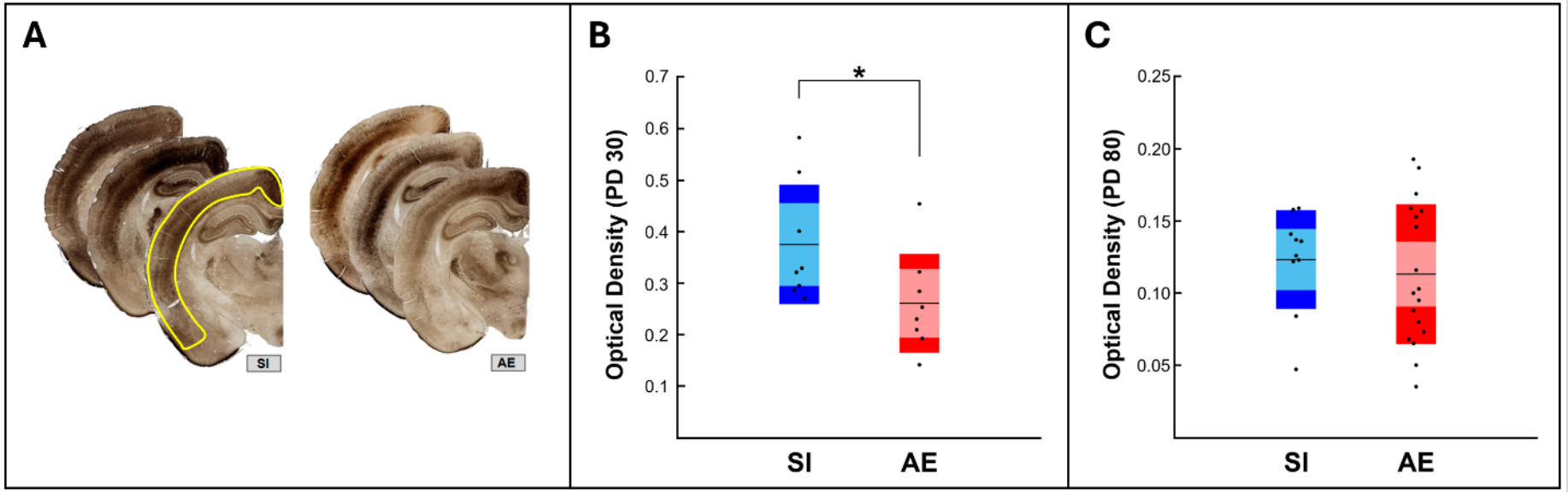




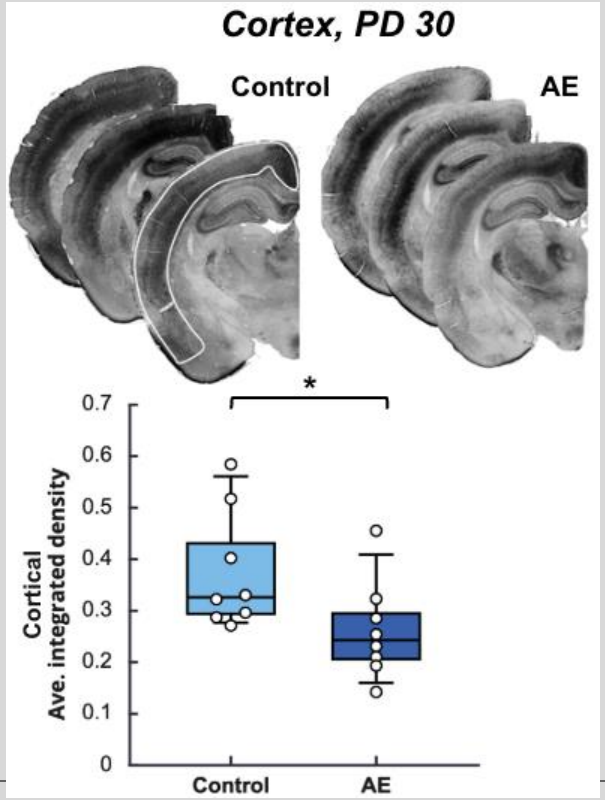
Formation marks the end of sensitive period of brain development (e.g. visual cortex)



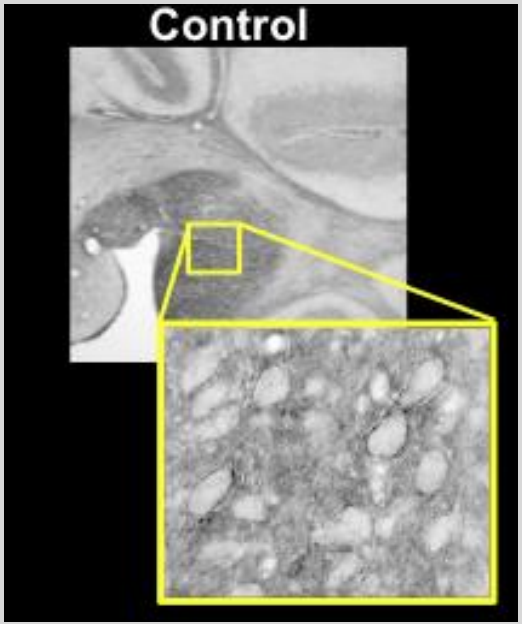
The density of WFA^{+} PNNs is greater
in the cortex of SI rats compared to AE rats
in juvenility (**B**, PD 30)
but not in adulthood (**C**, PD80)

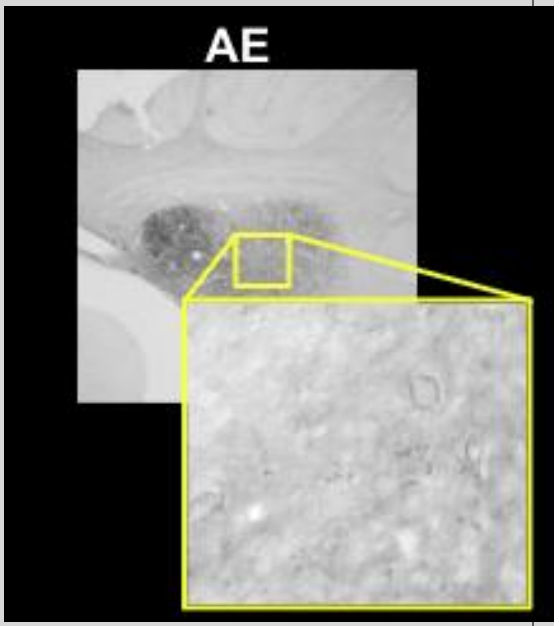


Perineuronal Nets in Cortex and Cerbellum after Alcohol Exposure on PD 4-9



Cerebellum, PD 15

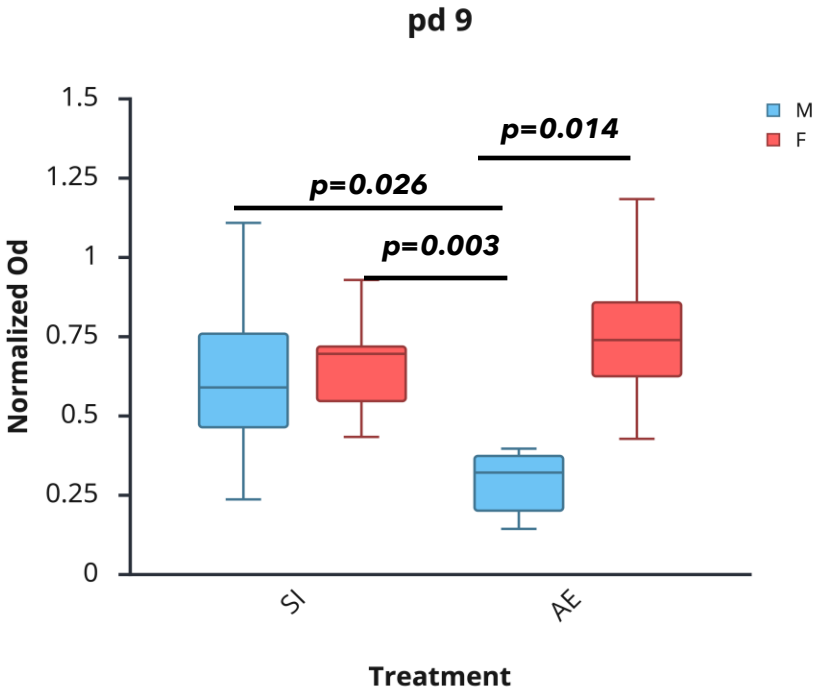




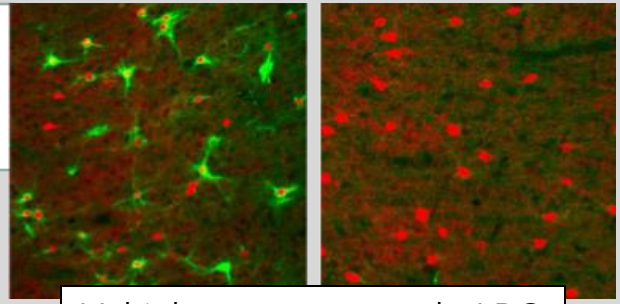
PNNs Expression in the Male Thalamic Reticular Nucleus Is Significantly Reduced after Single Binge Exposure on PD9

PD9 AE

PD9 SI

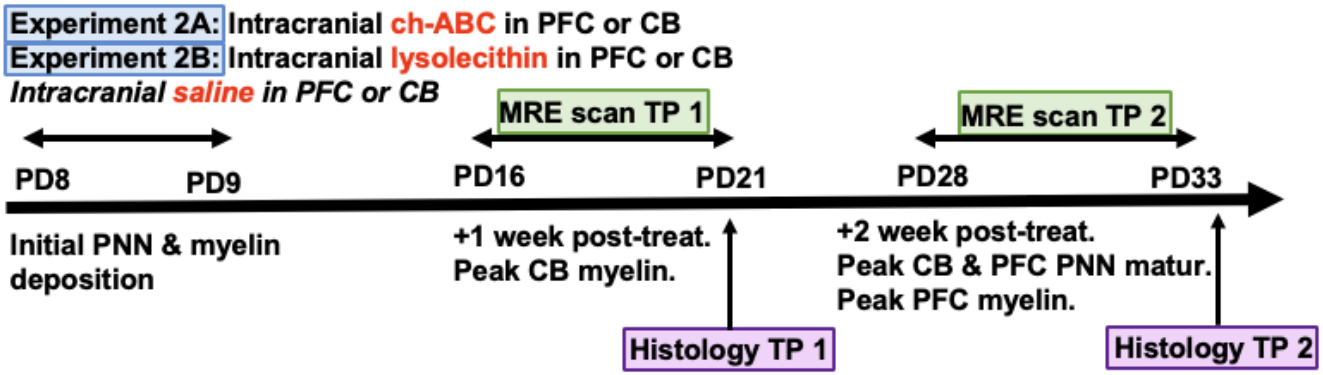


PNNs and Brain Mechanical Properties: What Is Next?



Vehicle ch-ABC

- Proof of causation:
  - Reversible PNNs degradation via chondroitinase-ABC microinjections
  - Reversible focal demyelination via lysolecithin microinjections



Conclusions

- MRE is sensitive to small brain tissue property variations in rodents.
- Exercise has a positive effect on rodent brain mechanical properties.
- Brain stiffness in alcohol exposed rats is a sensitive measure of changes in myelination and extracellular matrix/PNNs integrity – and could be used (potentially) as a diagnostic tool for FASD abnormalities.

MANY THANKS!

Collaborators: Drs. Katrina Milbocker and Curtis Johnson (both - UD); Craig Ferris (Northeastern U)

Klintsova Lab (Past & Present):

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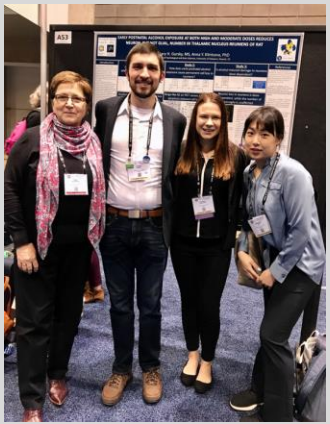
Eric Brengel, MS
Grace Lyons
Sneha Indrakanti



Center for Biomedical & Brain Imaging (UD) :

Dr. Keith Schneider, Director

OLAM Staff

Dr. Gwen Talham

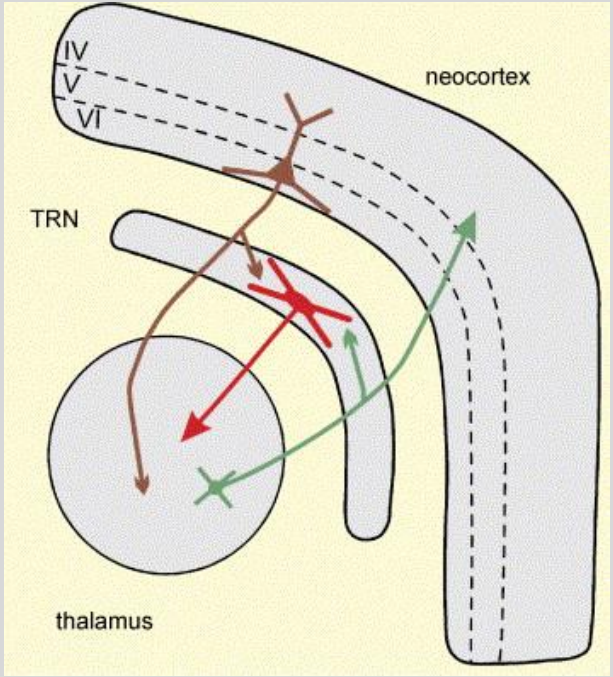
Funding:

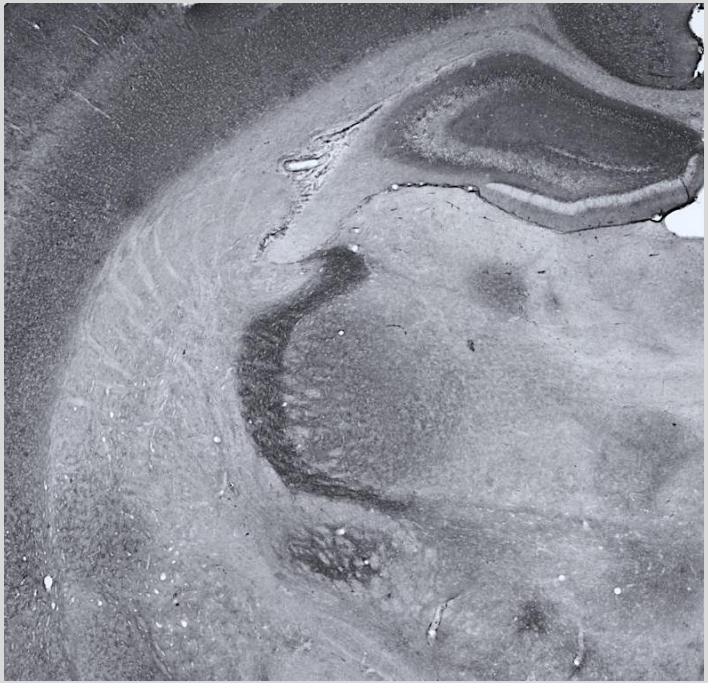
NIH/NIAAA R01 to AYK AA027269

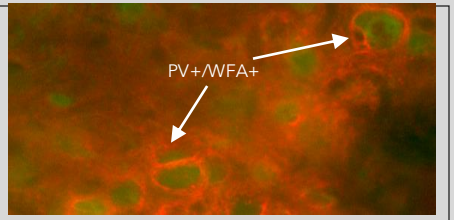
COBRE MRI Pilot Funding 2P20GM103653-06

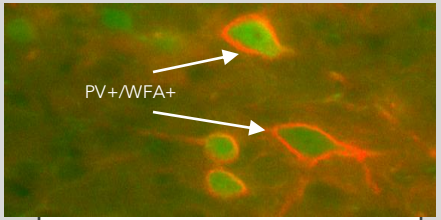
INBRE pilot award to AYK

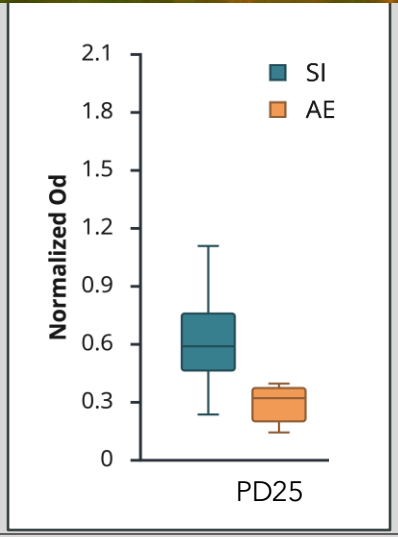
Perineuronal Nets In Thalamic Reticular Nucleus (TRN)



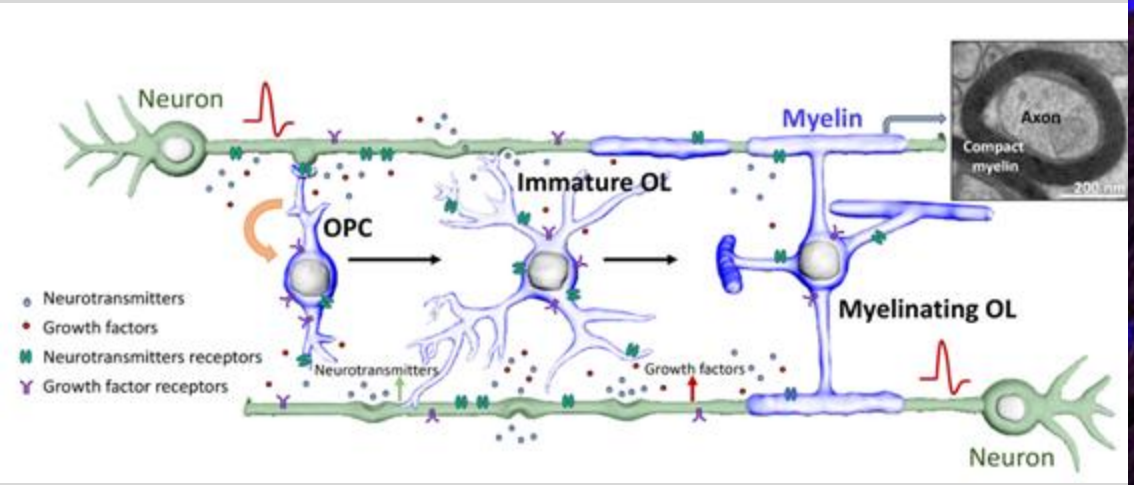


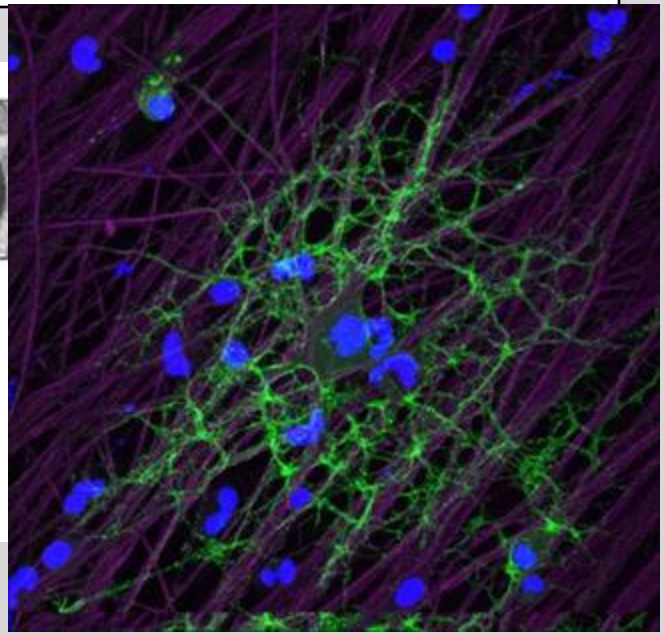






MYELINATION OF CNS AXONAL TRACTS BEGINS DURING THE ***BRAIN GROWTH SPURT*** IN MAMMALS

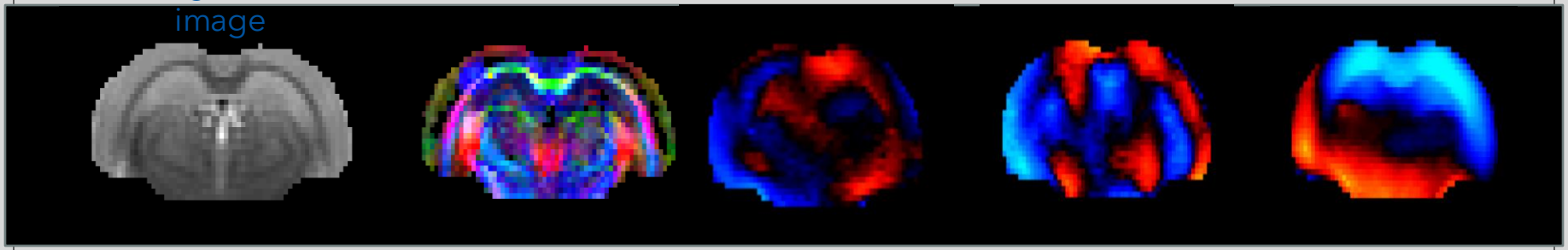


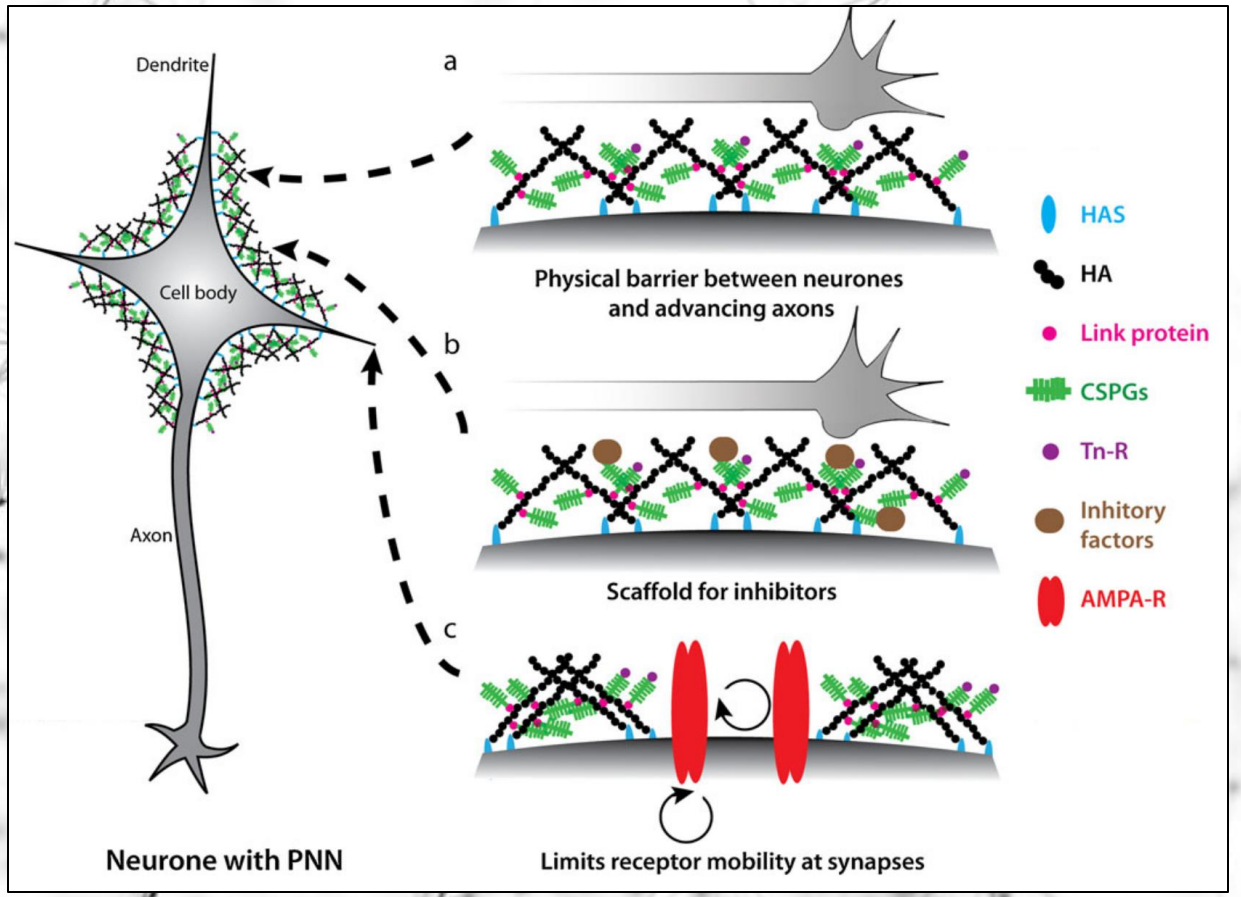


Left: Faria Jr. et al. (2019) *J. Neurochem*. **Right**: Fields & Wake (2008) *NIH*

Imaging sequence

- MRE-EPI sequence: TE/TR = 60/3400ms, slices = 40, slice thickness = 0.5 mm, FOV = 20 x 20 mm^2, matrix = 80 x 80, averages = 24, resolution = 0.25 x 0.25 x 0.5 mm^3, scan time = 32 minutes
- DTI sequence: TE/TR = 30/3200, 30 directions, $5b_0$, averages = 4, scan time = 15 minutes



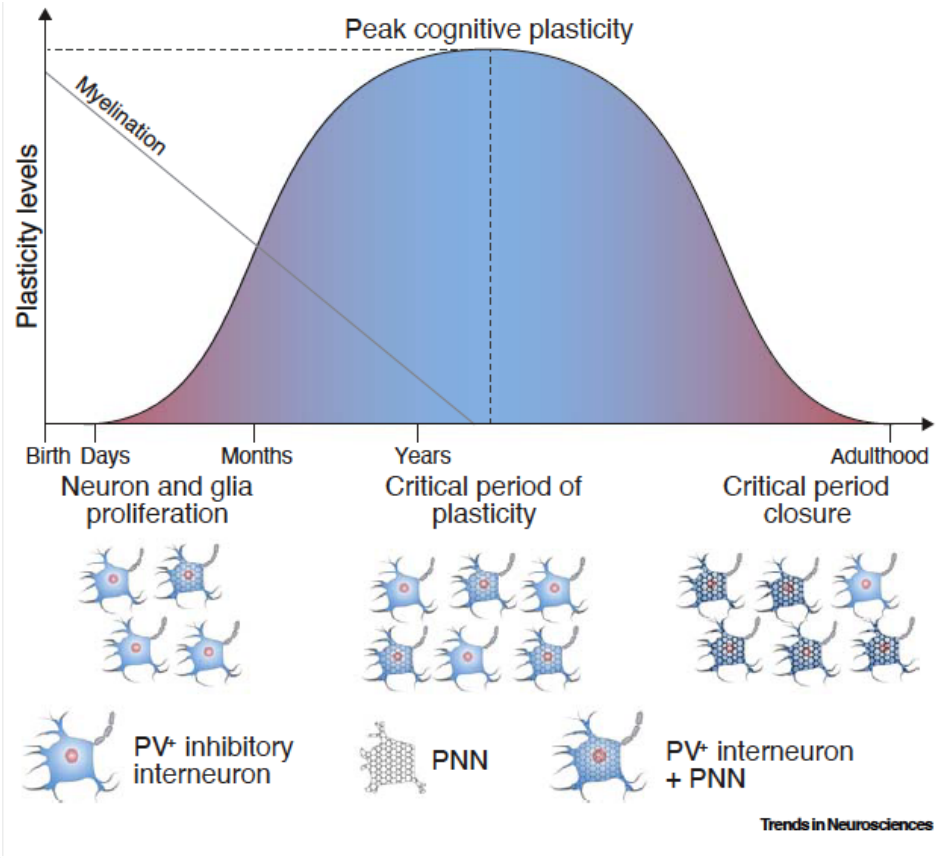


Composition and function of PNNs

- Hyaluronan (HA) and its synthases HAS)
- Chondroitin sulphate proteoglycans (CSPGs)
- Tenascins (Tn-R)
- Hyaluronan and proteoglycan link proteins

Wang, Fawcett (2012) *Cell Tissue Res.*

Panel presenters will discuss the function of PNNs in the context of developmental and adult drug exposure and Alzheimer's disease.



- Dr. Kim Green, UC-Irvine, will present on the regulation of PNNs by microglia under both homeostatic and disease/inflammatory conditions, and relate this to loss of PNNs in the Alzheimer's disease brain
- Dr. Barbara Sorg, Legacy Research Institute, will present on the role of PNNs for acquiring and maintaining drug-associated memories and hippocampal-mPFC communication in rodent models of cocaine use disorder
- Ms. Alexia Zylko, a graduate student at Dr. Amy Lasek Lab @ Virginia Commonwealth University, will present her work on the effects that psilocybin has on perineuronal nets.
- Dr. Anna Klintsova will conclude the panel with demonstration of the effects of developmental alcohol exposure on the expression of PNNs in the cortex, hippocampus, and subcortical structures (thalamic reticular nucleus), and correlation of these changes with brain mechanics.

Schematic of PNNs as part of ECM surrounding PV interneurons in the brain

- Astrocytes (green) and oligodendrocytes (yellow), which supports axonal myelin.
- These features create a tissue matrix that anchors the cells in place.