Doctoral Dissertation Public Defense Announcement

Thiago Arzua

Brain Organoids and Long non-coding RNAs: Neuroprotective pathways in alcohol-induced developmental neurotoxicity

Candidate for Doctor of Philosophy in Physiology with a Concentration in Basic and Translation Sciences

Graduate School of Biomedical Sciences
Medical College of Wisconsin

Wednesday, December 8th, 2021 at 11am (CST)

https://mcw-edu.zoom.us/s/92583489491?pwd=NnBGZTBiY0lRSkVuOGpsQlhtYTNHZ09

Committee in Charge:
Xiaowen Bai, M.D., Ph.D.
Matthew Hodges, Ph.D.
Alison Kriegel, Ph.D.
Amy Wagner, M.D.
Yun Li, Ph.D.
Graduate Studies

Biochemistry of the Cell
Molecular and Cellular Biology
Mechanism of Cellular Signaling
Fundamentals of Neuroscience
Graduate Neuroanatomy
Neuroscience Journal Club
Ethics and Integrity in Science
Special Problems in Physiology
Graduate Human Physiology
Endocrine Regulation and Common Disease
Complement to General Human Physiology
Current Topics in Physiology
Critical Reading in Respiratory Physiology
Physiological Genomics
Boundaries of Science and Medical Practice
Cellular and Molecular Neurobiology
Research Ethics Discussion Series
Developing Soft Skills
Advanced Systems Neuroscience
Seminar in Physiology
Reading and Research
Doctoral Dissertation
Abstract

Prenatal alcohol exposure (PAE) can adversely affect the developing fetus, resulting in deficiencies collectively known as Fetal Alcohol Spectrum Disorders (FASD), with an estimated prevalence of 1-5% of the population in the United States. Socioeconomic and cultural factors, including unplanned pregnancies, and alcohol use disorder, make it difficult to prevent PAE with education alone. A consequence of FASD is alcohol-induced developmental neurotoxicity (AIDN) manifesting as cognitive impairments and behavioral problems throughout life. Despite extensive research FASD does not have a clear mechanism, or a protective therapeutic approach.

This stems in part due to barriers in conducting research on human subjects. Animal models have played a major role in studying FASD and have facilitated our initial understanding of how alcohol affects the developing brain, but their translatability is a contentious issue. Recently, researchers have turned to human-induced pluripotent stem cell (iPSC)-derived 3D brain organoids to investigate the neurotoxic effects of alcohol. Brain organoids can structurally and developmentally recapitulate human fetal brains with higher fidelity than other 2D cell cultures, and a higher translational potential than animal models. The goals of this dissertation are then, to use a human brain organoid model to investigate AIDN, to examine underlying long non-coding RNA (lncRNA) molecular mechanisms, and to further develop neuroprotective approaches.

Based on preliminary data from our laboratory, our overarching hypothesis is that AIDN acts through the lncRNA AK156531/Neuronal PAS domain protein 4 (NPAS4) signaling axis in brain organoids, with honokiol being neuroprotective against the neurotoxicity. Three specific aims were developed to test this hypothesis: 1) to characterize and quantify the toxic effects of alcohol in a novel human brain organoid model, 2) to investigate the role of AK156531/NPAS4 signaling in AIDN, and 3) to determine the neuroprotective effects of honokiol in in AIDN.

We first quantified the toxic effects of alcohol using 2-month-old organoids exposed to a binge-drinking-like episode. The results revealed that alcohol induced apoptosis on organoids in a dose-dependent manner. Alcohol-treated organoids also exhibited disrupted mitochondria structure and function. Microarray showed that alcohol dysregulated 199 out of 17,195 analyzed genes, and this dysregulation was associated with clinically relevant pathways. We then focused on the never previously studied lncRNA AK156531 and its neighbor gene NPAS4 – a neuron-specific transcription factor known to be neuroprotective. Through Rapid Amplification of cDNA Ends, we discovered and characterized the human AK156531 gene, located upstream of NPAS4. Knocking down AK156531 reduced NPAS4 expression, and both genes were downregulated following alcohol exposure. Gain-and-loss of function studies demonstrated that the downregulation of either gene lead to an increase in alcohol-induced neuroapoptosis, while their overexpression protected the brain organoids. Lastly, we investigated honokiol as a potential neuroprotective agent. In different injury models, honokiol offers neuroprotection via its antioxidant activity and effects on the mitochondria. Our results demonstrated that pretreatment with honokiol reduced alcohol-induced apoptosis in brain organoids.

This dissertation shows for the first time that 1) brains organoids offer an unprecedented opportunity by modeling the toxic effects of alcohol at the cellular, subcellular, metabolic, and transcriptomic levels 2) alcohol induces apoptosis via AK156531-mediated regulation of its neighbor gene NPAS4, and 3) honokiol is neuroprotective against AIDN. PAE remains the leading preventable cause for intellectual disabilities, yet no therapeutic approach exists. This dissertation addresses this issue by advancing brain organoids as complex models for AIDN, while examining a novel mechanism and neuroprotective compound. These studies serve as a foundation for further work aimed at improving the prognosis and treatment of FASD patients.
Thiago Arzua

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Education
Doctor of Philosophy – Neuroscience  
Concentration in Basic and Translational Science Program  
Medical College of Wisconsin – Milwaukee, WI  
Dissertation Title: Brain Organoids and Long Non-Coding RNAs: Neuroprotective pathways in alcohol-induced developmental neurotoxicity

Interactive Track Certificate – Neuromatch Academy  
Neuromatch is a worldwide academy to train neuroscientists to learn computational tools. Trained with different datasets of calcium imaging and animal behavior.

Bachelor of Arts – Chemistry  
University of South Florida – Honors College – Tampa, FL  
Thesis Title: The Neurochemical Effects of Choline in Chemotherapy-Induced Cognitive Deficits  
French Minor

Research Experience
Medical College of Wisconsin  
Doctoral Student – Department of Physiology – Mentor: Xiaowen Bai, M.D., Ph.D.  
• Wide-array of cell culture experience especially with human stem cells and cerebral organoids  
• Advanced molecular biology techniques, including CRISPR-Cas9 gene editing, with a focus on lncRNAs  
• Whole-systems physiology studies, involving rodent models and behavioral assays  
• Bioinformatics analysis of transcriptomic data, such as single-cell RNA-sequencing

University of South Florida  
Thesis Candidate – Department of Psychiatry and Neuroscience  
Focus: Behavioral and cognitive paradigms in rodent models of cancer

Byrd Alzheimer’s Institute  
Research Assistant – Molecular Pharmacology and Physiology  
Focus: Cellular and molecular mechanisms behind Alzheimer’s and Angelman Syndrome
Publications


Honors and Awards

Outstanding Scholars in Neuroscience Award Program – National Institutes of Health 2021
Trainee Professional Development Award – Society for Neuroscience 2020
Early Career Policy Ambassador – Society for Neuroscience 2020
Graduate Student Travel Award – Medical College of Wisconsin 2019
Undergraduate Scholar – University of South Florida 2016
Clear Springs Land Undergraduate Research Award – University of South Florida 2014
Martin Travel Awards for Undergraduates – University of South Florida 2013
1st Place Poster Award – Castle Student Research Conference 2013

Invited Talks

Loyola University – Seminar in Molecular/Cellular Neuroscience 11/2021
Brain organoids: Modeling neuroprotective signaling in developmental neurotoxicity

Federal University of Rio de Janeiro – Caxias É Live 10/2021
Black in Neuro – Empoderando Negros na Ciência (Portuguese)

Loyola University – Seminar in Molecular/Cellular Neuroscience 04/2021
Modeling Alcohol-induced Neurotoxicity using hiPSC-derived Cerebral Organoids

Federal University of Santa Catarina – Postgraduate Program in Neuroscience 11/2020
Black in Neuro Week: A Successful Diversity, Equity, and Inclusion Initiative

Loyola University – Neuroscience Society 10/2020
Brain Organoids as Models for Alcohol-induced Neurotoxicity

Congresso Brasileiro Interligas de Neurologia, Neurocirurgia e Neurociências 09/2020
Carreira Acadêmica no Exterior (Portuguese)

Grad Chat with PhD Balance 07/2020
International Students and Being Abroad

Ask Me, I'm a Scientist with Dr. Susanna Harris 03/2020
Why Pregnant Moms Shouldn't Drink

2nd Annual Student Health Sciences Conference 01/2020
Discovery of Purpose, Passion, and Perseverance in the Health Sciences

Underground Science Society 07/2019
Mini Brains: The Exciting World of Lab Grown Mini Organs

Scientific Talks

Regenerative Medicine and Stem Cell Biology Forum 10/2021
Studying IncRNA regulation of neuroprotective signaling in developmental neurotoxicity

Department of Physiology 06/2021
NPAS4-mediated Neuroprotection from Alcohol-Induced Neurotoxicity on Brain Organoids

Developmental Biology Club 11/2020
Modeling Neuroprotection from Alcohol-Induced Neurotoxicity with Brain Organoids

Developmental Biology Club 09/2019
Mini Brains and Alcohol-Induced Developmental Brain Injury

Basic and Translational Science Seminar Series 08/2019
Stem Cells and Disease Modeling

Department of Physiology 07/2019
Long noncoding RNAs and Alcohol-Induced Developmental Brain Injury
Conference Presentations

Society for Neuroscience Annual Meeting 11/2021
Honokiol acts as a neuroprotective in alcohol-induced developmental neurotoxicity in brain organoids

Society for Neuroscience Global Connectome 01/2021
Brain Organoids as Models for Alcohol-induced Developmental Neurotoxicity

Organoids: Modelling Organ Development and Disease in 3D Culture 10/2020
Stem Cell-derived Cerebral Organoids as Models for Studying Alcohol-induced Developmental Neurotoxicity: Analyses at Tissue, Cellular, Subcellular, and Gene Levels

International Society for Stem Cell Research Annual Meeting 06/2020
Modeling Alcohol-Induced Neurotoxicity using Human iPSC-Derived Cerebral Organoids

MCW Graduate Student Poster Session 11/2019
Exposure to ethanol leads to abnormal signaling network of long non-coding RNAs and mRNAs in neonatal mouse brains

Society for Neuroscience Annual Meeting 11/2018
Excitatory and inhibitory imbalance may contribute to the propofol-induced developmental neurotoxicity through NPAS4 signaling

Graduate Student Symposium 03/2018
Anesthetic-induced downregulation of NPAS4 might contribute to developmental neurotoxicity in neonatal mice

MCW Graduate Student Poster Session 10/2016
Light-modulated Keratinocytes and Sensory Neurons: Insights into Mechanotransduction

Undergraduate Research and Arts Colloquium 04/2016
The Neurochemical Effects of Choline in Chemotherapy-Induced Cognitive Deficits

USF Health Day 02/2015
Determining the Neurobiological Effects of Minocycline in Angelman Syndrome Mouse Model

Raymond N. Castle Student Research Conference 04/2013
N=N Bond Formation via Metalloradical Catalysis (MRC): An Efficient Synthesis of Fluoroazo-compounds

Relevant Experience

Black in Neuro 2020 – Present
Co-founder of Black In Neuro and #BlackinNeuroWeek
  ○ Black in Neuro is a unique professional development program highlighting Black contributions to neuroscience, neurology, and related fields.
    ● Helped the team create events, including multiple seminars, journal clubs, and digital content with preeminent Black neuroscientists across the world.

Clubes de Ciência (Science Clubs) Brasil 2020 – Present
Organizer
  ○ Science Clubs is an organization aiming at inspiring and developing scientific engagement in teenagers and young adults by offering free workshops and mentoring sections.
    ● Organizers prepare educational and scientific content for high school students in Brazil with a focus on neuroscience and stem cell biology.
Massive Science Consortium 2019 – 2021
Freelance Writer
- Massive Science is a content and media company delivering bleeding-edge scientific research and expertise, and providing trustworthy, entertaining, and shareable science content.
  - Authored several different articles about various neuroscience topics, including brain-machine interfaces, organoids, space radiation among others.

Society for Neuroscience 2020 – 2021
Early Career Policy Ambassador
- This program is designed to develop skills to advocate for science and to encourage science policy conversations with policymakers at different levels.
  - Engaged with members of Congress about the value of a strong national investment in scientific research, especially advocating for neuroscience research.

Milwaukee Area Science Advocates 2017 – 2019
Social Media Manager
- Milwaukee Area Science Advocates is a local non-profit aimed to increase scientific enthusiasm, understanding, and legislative value in the Milwaukee area.
  - Managed daily online content to build meaningful connections and encourage community members to take action on different social media platforms.

BRASA Impāctus 2016 – 2018
Co-founder and Content Coordinator
- Impāctus is a platform of social impact initiatives that intend to connect Brazilian students abroad with the reality of economically challenged communities in Brazil.
  - Directed and analyzed case competitions in partnership with the biggest companies in Brazil.

Service and Committees

Society for Neuroscience Trainee Advisory Committee 2021 – Present
Provided a trainee’s perspective to the Society as it supports neuroscience trainee development, as well as helped monitor the effectiveness of SfN programs in reaching trainees.

Diversity and Inclusion Action Committee 2019 – 2021
Represented the graduate students in an institution-wide committee dedicated to developing an institutional culture that fosters the recruitment, nurturing, and retention of a diverse population.

Graduate Outreach Program 2016 – 2021
Assisted in planning visits and recruiting students for the graduate program and summer programs.

Graduate School Public Relations 2019 – 2020
Served as liaison between the Graduate Student Association and the general public by maintaining the webpage, email account, and social media accounts.

Mentoring

Summer Program for Undergraduate Research (SPUR) Mentor 2019
Mentee: Lauren Gray

Diversity Summer Health-Related Research Education Program (DSHREP) Mentor 2018 – 2019
Mentees: Adora Wang, Jennifer Landeta