Grace Y. Qian
Undergraduate
Tulane University, New Orleans, Louisiana

Michael Salling, Ph.D.
LSUHSC, Department of Cell Biology and Anatomy

"Structural assessment of adolescent alcohol's impact on a prefrontal corticothalamic circuit using expansion microscopy"

Heavy alcohol use during adolescence is associated with an increased risk of developing alcohol use disorders later in life, as well as deficits in behaviors mediated by the prefrontal cortex (PFC). The specific neurobiological mechanisms for how adolescent alcohol impacts PFC function is not well understood, which may be due to generalizing the effects of alcohol on the PFC and not focusing on specific neural circuits. The major thalamic output nuclei of the PFC is the mediodorsal thalamus (MDT) and this connectivity is specifically associated with behaviors known to be impacted by adolescent alcohol: working memory and response inhibition. In our previous studies using a preclinical mouse model of adolescent binge drinking, we found that the PFC to MDT circuit is uniquely vulnerable to the effects of adolescent alcohol consumption, including a reduction in glutamatergic transmission. In this study, we sought to extend these findings using a mouse model of binge-like alcohol consumption during adolescence to assess if there are structural changes in neurons projecting from the PFC to the MDT that are associated with behavioral deficits. Based on our previous work, we hypothesized that mice given access to alcohol during adolescence will show a reduction in spine density of PFC to MDT neurons that is associated with impaired performance on working memory and on a version of the novel object recognition task (NORT) that is sensitive to PFC and MDT lesions.

Male and female C57BL/6J mice (n=21) were stereotaxically injected with a retrograde viral tracer (AAVrg-CAG-GFP) in the MDT on post-natal day (PND) 27-29. Mice were then given voluntary intermittent access to alcohol (IA EtOH) through the two-bottle choice drinking procedure from PND 32-61, for a total of 15 sessions of alcohol access. On the 13th alcohol access day, blood was collected at 6 hours into the dark cycle to measure blood alcohol levels. The daily alcohol dose consumed increased from 5.01 g/kg on drinking session one to an average of 13.15 g/kg over the last three drinking sessions. Following IA EtOH, we will use a battery of behavioral tasks to assess the impact of adolescent alcohol consumption, including the elevated plus maze (EPM), spontaneous locomotor activity test, standard 2-Object NORT, PFC/MDT-dependent 4-Object NORT, and Y-maze. We will then collect brain tissue from the mice and perform immunohistochemical and structural analyses of GFP-positive neurons from both groups. A subset of the tissue sections will be expanded using the Magnify expansion microscopy protocol, which enables higher resolution imaging by physically expanding tissue while retaining relative protein locations. We have previously applied this protocol to tissue sections with successful expansion and imaging of perineuronal nets stained with Wisteria floribunda agglutinin. We anticipate the Magnify protocol will allow for clearer analysis of spine density, spine classification, and protein localization. Findings from these studies will improve our understanding of the impact of adolescent alcohol consumption on the highly relevant PFC-MDT circuit.