Submitter Name: Kelly Wingfield
Submitted Email: kelwing@bu.edu
PI Name: Camron Bryant, PhD
PI Email: c.bryant@northeastern.edu

Exploring mouse ultrasonic vocalizations as a tool to study negative affective states in mice using a mouse model of neonatal opioid withdrawal syndrome (NOWS)

Kelly K. Wingfield[1,2], Teodora Misic[1], Kaahini Jain[1], Nalia Abney[1], Carly McDermott[1], Kayla T. Richardson[1,4], Mia B. Rubman[5], Jacob A. Beierle[1,2], Sophia Miracle[1,3], Kristyn N. Borrelli[1,2,3], Emily J. Yao[1], Camron D. Bryant[1,6]

[1]Department of Pharmacology, Physiology, and Biophysics, Boston University Chobanian & Avedisian School of Medicine; [2]T32 Biomolecular Pharmacology Training Program, Boston University Chobanian & Avedisian School of Medicine; [3]Graduate Program for Neuroscience, Boston Univeristy; [4]Post-Baccalaureate Research Education Program, Boston University Chobanian & Avedisian School of Medicine; [5]NIH/NIDA Summer Undergraduate Fellowship Program. [6]Center for Drug Discovery, Northeastern University.

Opioid use during pregnancy is a growing public health concern, as gestational opioid exposure often leads to neonatal opioid withdrawal syndrome (**NOWS**) in infants. NOWS refers to the set of symptoms due to spontaneous cessation of opioid exposure, including hyperirritability and excessive crying. There is no standard care approach to treat NOWS due to variability in NOWS symptom onset and severity, which can be attributed to genetic and environmental factors. We use a mouse model for NOWS to assess several phenotypes during spontaneous morphine withdrawal (16 h) on P7 and P14, including ultrasonic vocalizations (**USVs**). We found that neonatal morphine exposure alters the USV profile, as demonstrated by a significant increase in the proportion of “Complex 3” syllables during withdrawal on P14, suggesting that Complex 3 syllables could serve as a potential marker for the aversive state of opioid withdrawal and thus a strategy to model distress severity and alleviation in mice. Brainstem transcriptomics revealed upregulation of genes associated with addiction and withdrawal, including the kappa opioid receptor (KOR; *Oprk1)*. Pre-treatment with the selective KOR antagonist, norBNI reduced Complex 3 emission in female but not male neonates on P16, indicating that KORs are necessary for this phenotype. We are currently examining whether KOR activation with the KOR agonist U50,488H is sufficient to increase Complex 3 syllables in neonates. Given that KOR activation is dysphoric and USVs are indicative of neonatal distress, these results implicate Complex 3 syllables as a potential marker for the aversive internal state associated with morphine withdrawal.