Temple University
Department of Biology

-Final Doctoral Thesis Defense-

TITLE

"Reward and drug induced molecular neuroadaptations - the role of circular RNAs and m6A RNA modifications"

Konrad Robert Dabrowski

TIME AND PLACE

Monday, March 11, 2024
12:00 PM
Bio-life Building, room 234

Light refreshments starting at 11:30 am
Any questions, please contact the Biology Department @ 215-204-8854

Dissertation Committee

Dr. Stephanie Sillivan, Advisory Co-Chair, Center for Substance Abuse Research at Lewis Katz School of Medicine
Dr. Anna Moore, Advisory Co-Chair, Department of Biology, Temple University
Dr. Richard Waring, Examining Committee Chair, Department of Biology, Temple University
Dr. Mathieu Wimmer, External Examiner, Department of Psychology, Temple University

Abstract: The reward system is a network of structures in the brain responsible for the feelings of pleasure, motivation, and decision making. It is comprised of the prefrontal cortex, orbitofrontal cortex (OFC), nucleus accumbens, ventral tegmental area, amygdala and the hippocampus, brain regions that come together to process rewarding stimuli, commonly referred to as rewards, to positively shape behavior. Rewards are well known to induce a range of molecular changes within the reward system that mediate reinforcing effects of rewards—neuroadaptations. These neuroadaptations can not only support adaptive behavior but also can mediate negative symptoms of psychiatric disorders such as anhedonia, withdrawal, or drug tolerance. Hence, aberrant functioning of the reward circuitry is present in patients with psychiatric disorders such as addiction, bipolar disorder, eating disorders, major depressive disorder, and schizophrenia. The molecular mechanisms underlying the function of the reward system are not fully understood and therefore elucidating the reward-induced neuroadaptations could inform future therapeutic approaches for symptoms caused by aberrant reward processing associated with psychiatric disorders. This thesis aims to characterize two types of neuroadaptations, circular RNA (circRNA) transcriptomic changes as well as N6-methyladenosine (m6A) epitranscriptomic adaptations, in the context of appetitive reward and opioids, respectively. First, we focused on describing circRNA related neuroadaptations within the OFC, and their functional implications, in the context of sucrose seeking behavior. We reported the first circRNA profile associated with appetitive reward and identified a regulation of 92 OFC circRNAs by sucrose self-administration. Among these changes we observed a downregulation of circNrxn3, a circRNA originating from neurexin 3 (Nrxn3), a gene involved in synaptogenesis, learning, and memory. Transcriptomic profiling via RNA sequencing and qPCR of the OFC following in vivo knock-down of circNrxn3 revealed differential regulation of genes associated with pathways important for learning and memory and altered splicing of Nrxn3. Furthermore, circNrxn3 knock-down enhanced sucrose self-administration and motivation for sucrose. Using RNA-immunoprecipitation, we reported binding of circNrxn3 to the known Nrxn3 splicing factor SAM68. circNrxn3 is the first reported circRNA capable of regulating reward behavior. In addition, circNrxn3-mediated interactions with SAM68 may impact subsequent downstream processing of RNAs such as the regulation of gene expression and splicing. We then went to characterize m6A epitranscriptomic adaptations induced by a commonly misused drug, the opioid morphine. m6A modifications have not been studied in opioid use disorder, despite being the most common RNA modification. We detected significant regulation of m6A-modifying enzymes in rat primary cortical cultures following morphine treatment, including AlkB Homolog 5 (Alkbh5). The m6a demethylase Alkbh5 functions as an m6A eraser, removing m6A modifications from mRNA. We hypothesized that chronic opioid treatment regulates m6A modifications through modulation of Alkbh5 and profiled m6A modifications in primary cortical cultures following chronic morphine treatment and Alkbh5 knock-down. We observed differential regulation of m6A modifications for 568 transcripts following morphine and 2865 following Alkbh5 knock-down. 103 transcripts were commonly regulated by both morphine and Alkbh5 knock-down, and the two treatments elicited concordant m6A epitranscriptomic profiles, suggesting that a subset of morphine-driven m6A modifications may be mediated through downregulation of Alkbh5 in cortical cultures. Together, this volume expands our understanding of molecular neuroadaptations induced by both appetitive reward and opioids. We have identified potential facilitators that could impact reward seeking, motivation and drug induced molecular adaptations that could inform future studies.