“New” UROP Proposal

Title of Proposal: Investigating the Role of Kdm3 in Alcohol Sensitivity and Tolerance

STATE THE PROBLEM/TOPIC

According to the 2022 National Survey on Drug Use and Health (NSDUH), 29.5 million people aged 12 and older (10.5% in this age group) had alcohol use disorder (AUD) in the past year (SAMHSA). AUD is defined as the uncontrolled and excessive use of alcohol despite adverse social, psychological, and physical consequences. Two of the main risk factors for AUD are decreased sensitivity and the development of tolerance. Sensitivity refers to the naïve response to the effects of alcohol, and tolerance is the lowered effects of alcohol after repeated exposures. Many genetic risk factors and neurobiological mechanisms of AUD, including sensitivity and tolerance, are not well understood. Understanding the mechanisms can help us better diagnose and treat those with AUD.

Many studies have found that alcohol exposure leads to changes in gene expression, associated genetic networks, and neuronal cellular functions (Iancu et al., 2017; Tulisiak et al., 2017). One mechanism involved in alcohol-induced gene modifications is epigenetic changes via chromatin and histone modifiers (Ponomarev, 2013). Histones are proteins that provide structural support for the chromosomes and contribute to the regulation of gene expression. A promising avenue of AUD research investigates epigenetic regulators such as histone demethylases, which remove methyl groups from histones and other proteins (Dimitrova et al., 2015), which promotes transcriptional activation of target genes. Determining how these epigenetic regulators change gene expression allows us to understand the neurobiological mechanisms that are altered after ethanol exposure. Kdm3 is a histone demethylase that acts on H3K9me1/2 (histone 3, lysine 9, mono-and di-methyl) (Shen et al., 2017). Human KDM3C has been shown to be downregulated in the amygdala of people diagnosed with AUD (Ponomarev et al., 2012), and Kdm3a expression is downregulated in the nucleus accumbens of mice predisposed to drink alcohol (Ferguson et al., 2019). However, the specific neurons and neurotransmitter systems that are critical for Kdm3 function have not been explored.

Drosophila melanogaster is a great model to study AUD because of its accessibility to genetic manipulations and its ability to display behaviors that are relevant to human alcohol sensitivity and tolerance. Like humans, when flies are exposed to low doses of alcohol, they exhibit hyperactivity and disinhibition, while high doses eventually lead to sedation, indicating similarities in naive responses (Rodan and Rothenfluh, 2010; Narayanan & Rothenfluh, 2016). Acquired tolerance to ethanol-induced incapacitation is also found in flies. Both initial sensitivity as well as acquired tolerance with repeat alcohol exposure, are easily assayed in Drosophila (Scholz et at., 2000). Drosophila has one Kdm3 ortholog, and Dr. XXX’s lab has discovered that Kdm3 knock-out flies are sensitive to ethanol-induced sedation and develop less tolerance to ethanol upon repeat exposure (Pinzón et al., 2017). To better understand the role of Kdm3 in behavioral alcohol responses, my research will determine which neurotransmitter system is relevant for Kdm3's effects on ethanol sensitivity and tolerance.

This will further our understanding of the biological mechanisms of alcohol-related behaviors and discover possible therapeutic interventions for alcohol use disorder.

RELEVANT BACKGROUND/LITERATURE REVIEW

As mentioned before, the XXX lab has shown that Kdm3 loss of function mutant flies are more sensitive to ethanol and develop less tolerance compared to control flies (Pinzón et al., 2017). It was also found that knockdown in all neurons recapitulates the phenotype of flies that are missing the Kdm3 gene (Pinzón et al., 2017). These findings show that neuronal Kdm3 function plays an important role in normal ethanol-induced behavior.

Neurotransmitters are the messengers between neurons that exert inhibitory, excitatory, or modulatory effects on their downstream targets, which are mostly other neurons. Different neurons and neurotransmitters have distinct functions in the brain and manipulating a gene in one set of neurons can result in the opposite phenotype than manipulating the same gene in a different set of neurons. This highlights our need to understand the neurons that require Kdm3 for normal sensitivity and tolerance responses to alcohol.

Drosophila is a useful organism to study neurotransmitters because neurotransmitters are well conserved between flies and mammals and they also show similar effects on behavior (Chvilicek et al., 2020). For example, GABA is the main inhibitory transmitter and acetylcholine acts as an excitatory transmitter in both flies and humans. Neurotransmitters can help determine how behavior is affected after alcohol exposure. In this research, I will focus on 7 conserved neurotransmitters that are relevant for addiction. These 7 neurotransmitter systems are GABA, glutamate, acetylcholine, dopamine, glycine, serotonin, and octopamine (the fly ortholog of norepinephrine). It is still unclear which neurotransmitter systems are involved in ethanol sensitivity and tolerance, but determining the different neurotransmitter circuits involved will allow narrowing down the specific type of neurons involved in alcohol responses.

A useful tool to study the genetics of behavior in flies is the GAL4/UAS system. Transgenic flies carry the yeast transcriptional activator (GAL4) which is under the control of a specific promoter. Additionally, a gene of interest is under the control of Upstream Activating Sequence (UAS) in a second transgene. The system is activated when GAL4 binds to the UAS which promotes the expression of the gene of interest in spatial and temporally defined ways. This GAL4/UAS system allows us to knock down gene expression using RNA interference (UAS-RNAi) and observe how behavior is affected. The XXX lab found that knocking down Kdm3 in all neurons resulted in the same ethanol phenotype as whole Kdm3 loss of function in Drosophila, suggesting that Kdm3's effects on alcohol behaviors are brain-specific (Pinzón et al. 2017). To further understand how Kdm3 activity in the brain affects ethanol-induced behavior, we will be exploring which neural circuits, specifically which neurotransmitter systems, mediate such phenotypes.

PROPOSAL: SPECIFIC ACTIVITIES AND TIMELINE

To investigate which neural circuits mediate ethanol-induced behavior, I will first establish the control and UASKdm3-RNAi lines to be in a homogeneous genetic background. Then, I will knock down Kdm3 in neurons using pan-neuronal drivers (elav and n-Syb) to reproduce the findings of the lab and ensure that Kdm3 knock-down flies are more sensitive and less tolerant to ethanol. Next, I will cross the control and UAS-Kdm3-RNAi fly lines to different neurotransmitter GAL4 drivers. The neurotransmitter systems I will investigate include GABA, glutamate, acetylcholine, dopamine, glycine, serotonin, and octopamine. To determine the role of Kdm3 in these neurotransmitter systems, I will conduct the Maples assay to measure ethanol sensitivity and tolerance (Maples and Rothenfluh, 2011). In the Maples assay, flies are placed in a vial and exposed to ethanol, and the time to 50% of the flies becoming sedated (ST50) is quantified. The flies will be exposed twice, four hours apart, with increased ST50 in the second exposure reflecting the development of tolerance.

May 13-24

- Collect flies
- Extract genomic DNA
- Perform PCR to verify genotype
- Make working fly stock of control and Kdm3 RNAi lines
- Collect virgins for pan-neuronal GAL4 lines [elavc155-GAL4, nSyb-GAL4] and set up crosses for Maples assay

May 27- June 7

- Collect flies for Maples assay
- Conduct Maples assay for ethanol sensitivity and tolerance
- Analyze data and run statistics to make figures
- Collect following virgins of GABAergic GAL4 lines [vGAT-GAL4, Gad1-GAL4] and set up crosses for Maples assay
- Cross GABAergic GAL4 lines to control and Kdm3 RNAi lines June 10- June 28
- Collect flies from crosses of GABAergic GAL4 to conduct the Maples assay
- Analyze data and run statistics to make figures
- Collect virgins of glutamatergic [vGluTTrojanGAL4] and cholinergic [ChATTrojan-GAL4] GAL4 lines and set up crosses with control and Kdm3 RNAi lines for Maples assay - Present in lab meeting

July 1- July 12

- Collect flies from crosses of glutamatergic [vGluTTrojanGAL4] and cholinergic [ChATTrojan-GAL4] GAL4 for Maples assay
- Collect virgins of serotonergic [Trh2AGAL4] and serotonergic/dopaminergic [Dcd-GAL4] GAL4 lines and set up crosses for Maples assay
- Conduct the Maples assay for ethanol sensitivity and tolerance
- Analyze data and run statistics to make figures
- Cross the following GAL4 lines to control and Kdm3 RNAi lines

July 15-31

- Analyze data from the semester
- Repeat any experiments that need to be redone
- Presentation in lab meeting - Prepare the poster

Additionally, I will be attending weekly lab meetings with the XXX lab. We also share a lab space with the Rodan lab and have weekly joint meetings which I will also be attending. I will be presenting twice about my research during the semester.

RELATIONSHIP OF WORK TO THE EXPERTISE OF THE MENTOR

Dr. XXX is an associate professor in the Psychiatry Department and Molecular Medicine Program at the University of Utah. Dr. XXX received his Ph.D. in genetics from Rockefeller University and completed his postdoc at the University of California, San Francisco, focusing on neurogenetics. With over 30 years of experience working with Drosophila, his research primarily revolves around genetics, molecular mechanisms, and neural circuits related to behavior concerning alcohol or substance abuse. While Dr.
XXX's work predominantly focuses on Drosophila, he collaborates with human consortia to explore gene involvement in both Drosophila and humans for alcohol use disorder (AUD). Dr. XXX has mentored over 70 mentees and continues to create a diverse and equal environment that promotes academic and personal growth.

Dr. XXX is a research associate in the XXX lab at the University of Utah. She obtained her Ph.D. in molecular biology from the University of Paris Diderot in France. Having worked with Dr. XXX for7 years, her current research centers on Kdm3 and ethanol sensitivity and tolerance in Drosophila. Her willingness to share her knowledge and skills makes her an ideal mentor.

RELATIONSHIP OF THE WORK TO YOUR FUTURE GOALS

As a 5th-year senior and pre-medical student, I have always been intrigued by the intricacies of the human body and the sciences associated with it. I've always been interested in behavior and this research allows me to perform my own independent experiments to investigate the molecular and neural mechanisms that underlie behavior. Learning about the molecular mechanisms behind addiction has helped to strengthen my intellectual curiosity and change how I view addiction. This research has also sparked an interest in addiction medicine and psychiatry. I hope to further explore this field and contribute to finding new therapeutic interventions for those who are suffering from alcohol use disorder.

PROPOSAL: REFERENCES (Works Cited)

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