Sex Differences in Ethanol Modulation of Dopamine Release in the Mesolimbic Reward System

Dr. Scott Steffensen – Psychology

Project Purpose

The purpose of this project is to evaluate the effects of ethanol on dopamine release in female rats during diestrus, proestrus, and estrus using microdialysis to better understand how different phases in the menstrual cycle affect dopamine (DA) release due to alcohol intoxication in the female brain.

Project Importance

According to the Alcohol-related Disease Impact (ARDI) report, nearly 88,000 people (approximately 62,000 men and 26,000 women) die from alcohol-related causes annually, making it the third leading preventable cause of death in the United States (2). According to the ARDI report, the costs of AUD to the US in 2006 was $223.5 billion, and almost three-quarters of the total cost was related to binge drinking. As the most abused legal drug in the world, the prevalence of alcohol addiction indicates a need for change and the discovery of the processes that will enable researchers to break the cycle of addiction. The mesolimbic pathway has been implicated in reward processing. Specifically, current dogma holds that DA release in the nucleus accumbens (NAc) is rewarding. Studying the effects of drugs on DA release sheds light on the mechanism of release and the possible discovery of treatment that might aid in the prevention and treatment of addiction.

Additionally, the relative dearth of animal studies conducted in females has led NIH to require researchers to consider sex as a variables in their studies in order to elucidate key sex related differences. It is hoped that this will lead to better treatments for both men and women in conditions such as alcohol use disorder. However, despite these mandates, research has not been conducted on the effects of ethanol throughout the menstrual cycle. In human females, Ryback has determined that chronic alcohol consumption does disrupt the menstruation cycle in humans. This project seeks to determine how the effects of alcohol on DA release in the NAc differ throughout the menstrual cycle thus improving our understanding of the neural substrates of alcohol addiction in women.

Project Overview

Humans have been abusing drugs as early as 8,000 B.C. when mead, an alcoholic drink containing fermented honey and water, was highly popular through Europe and Asia. It is clear that there are properties of certain substances that create an altered consciousness that is pleasurable when introduced into the body. Chemically, this feeling of pleasure is thought to be related to the release of dopamine in the NAc. Dopamine (DA) is the canonical neurotransmitter implicated in motivated behavior and reward learning. Current dogma maintains that DA neuron activation and release in the mesocorticolimbic DA system originating in the midbrain ventral
tegmental area (VTA) and projecting to the nucleus accumbens (NAc) and other limbic structures is literally a scalar index of reward. This system is known to be involved in reward from natural behaviors such as feeding, drinking, and other rewards such as intracranial self-stimulation. It has also been implicated in the habit-forming actions of several addictive drugs [for review see]. The dogma is that any drug or behavior that increases mesolimbic DA neuron activity and release will be reinforcing, and potentially addictive. The prevailing view is that people consume drugs for their rewarding properties, which are mediated by this system. Drugs, including alcohol, enhance DA release, resulting in feelings of pleasure, euphoria, and well-being. The level of DA release by some drugs of abuse can be 10 times that produced by natural rewarding behaviors such as eating, drinking, and sex. However, the onslaught of DA release is transient and often results in adaptations including progressive, compensatory lowering of baseline DA levels during withdrawal. Addicts continue their cycle of abuse, in part, as a result of maladapted and depleted DA levels, resulting in feelings of anxiety and dysphoria that drives subsequent drug-seeking behavior. Enhancement of DA release during drug taking, and progressive, protracted decreases in DA release during withdrawal represent a persuasive neural correlate of the Opponent-process and Allostatic theories of addiction, wherein tolerance accrues to repeated drug use, resulting ultimately in compensatory lowering of DA release in the NAc. Although addiction begins as a personal choice to consume a drug or other reinforcer, the motivation to continue to seek the reinforcing stimulus is influenced greatly by genetic, environmental and experiential factors, leading to a spiraling dysregulation of brain DA with chronic intermittent exposure to the reinforcer, in particular alcohol.

As humans are driven to fulfill motivation and feel pleasure, it is easy to see how this naturally occurring reward system can be abused. What becomes dangerous is when this reward system is abused through drugs. Research has concluded that both the DA response to a drug and baseline DA levels decrease with repeated use of drugs of abuse. This results in a non-intoxicated person never being able to reach baseline mood without the use of the substance. Initially the addict will be chasing the first dopamine high that was produced from the drug; however, over time, they will abuse the drug simply to feel normal. For this reason, the mechanism of DA release during intoxication in the brain must be understood as to counteract and potentially reverse the devastating effects of addiction. The main goal in this project is to determine if there are effects of the hormones during the phases of menstruation that would influence dopamine release when alcohol is injected.
Previous work has been done in Dr. Steffensen’s lab surrounding ethanol effects on dopamine release in this area of the brain. Using behavioral tests, voltammetry, microdialysis, and ex vivo experiments, a variety of agonist and antagonists have been studied that may increase or decrease the DA response in an intoxicated rat. Preliminary studies have been performed in male vs female rats regarding differences in their response to ethanol. Figure 1 shows that while males show enhanced DA release by ethanol females are characterized by a biphasic response consisting of enhancement followed by inhibition. These preliminary studies were performed in female rats that were likely in the same phase of their cycle as they were group housed. Typically, group housing induces a synchronization of the cycle. No effort was made to determine which phase of the cycle the females were in. Regardless, these results underscore that there are differences between males and females and provide the justification for evaluating ethanol effects on DA release across the cycle in the same rats. My focus will be with microdialysis trials in which a guide cannula (MD-2250, BASI Instruments, West Lafayette, IN, USA) will be implanted in the NAc (+1.7 AP, +0.8 ML, -6.0 DV). A microdialysis probe (MD-2200, BASI Instruments, West Lafayette, IN, USA) will be inserted into the guide cannula and samples will be taken by flowing artificial cerebral spinal fluid (aCSF) through the probe at a rate of 2.0 μL/s. Dialysate samples will be collected every twenty minutes for two hours to establish baseline DA values at which point ethanol will be injected. Samples will then be collected every twenty minutes for the next three hours to determine the effects of ethanol on DA release in the NAc. Samples will be evaluated via high pressure liquid chromatography and electrochemical detection (HPLC-ECD) to determine the levels of DA. This analysis will occur using an Ultimate 3000 HPLC system (Thermo Fisher Scientific, Waltham, MA, USA) connected to a Coulochem III electrochemical detector (Thermo Fisher Scientific, Waltham, MA, USA) with a HR-80 catecholamine column attached to perform electrochemical separations (68-0100, Thermo Fisher Scientific, Waltham, MA, USA). Alcohol doses of 0.25 g/kg, 0.5 g/kg, 1.0 g/kg, 2.0 g/kg, and 4.0 g/kg will be administered in each phase of the menstrual cycle to allow me to compute a dose response curve for alcohol for each phase of proestrus, estrus, and diestrus. Comparing dose response curves throughout each phase of the menstrual cycle will determine if there is any change in alcohol mediated DA release based upon phase of the menstrual cycle.

In freely-behaving rats implanted with cannulae and microdialysis probes we will test the effects of ethanol on DA release in the NAc. We will evaluate this profound effect in males, ovariectomized (OVX) females, and non-OVX females during diestrus, proestrus and estrus. Fortunately, estrus cycle studies can be done serially in the same female rats separated by at least one day. The phase of the estrus cycle will be evaluated in each animal daily following the microdialysis session with vaginal swab and histology. Blood samples will be taken for evaluation of blood alcohol levels. Eight to rats each for males, OVX females, and non-OVX
females during diestrus, proestrus and estrus will be needed in this experiment for a total of 30 rats. This experiment will be a randomized, repeated measures, within-subjects design.

We will also explore the effects of alcohol on DA response in rats that have had OVX preformed. This will be particularly informative as we observe the menstrual cycle in an animal with simulated menopause.

This project has the potential to become more expansive as we begin to explore the interactions between addiction and the menstrual cycle. Other possible avenues of research include methamphetamine, the effects of which are currently also under study in Dr. Steffensen’s lab.

Qualifications

I am qualified to conduct research in this area because of my educational background and training in neuroscience. I have learned much of the brain’s anatomy from neurobiology and neuropsychology courses and plan to take neuroanatomy next semester. I am currently taking a psychology class called Drugs, Reward, and Addiction which is adding to my knowledge of current drug research and how the brain responds to addiction. I have worked in Dr. Steffensen’s addiction research lab for a year and a half and have conducted many voltammetry and microdialysis experiments that have yielded good data. Dr. Steffensen and the PhD students who work in his lab have taught me how to run many types of experiments on rats that have aided in the understanding addiction.

Dr. Steffensen is the faculty advisor for this project and has been researching addiction at BYU for over 20 years and has been funded by grants from the National Institutes of Health for the past twenty-eight years and has published many peer reviewed articles on the subject. He is more than qualified to aid and guide on this project because of his multiple degrees and his experiences as professor in the psychology department who specializes in neuroscience areas.

Jordan Yorgason is a postdoctoral fellow working within Dr. Steffensen’s lab that will serve as my faculty reader. He is trained as an electrochemist and received his Bachelor’s degree in Neuroscience from Brigham Young University where he studied the neural circuits underlying alcohol addiction in Scott Steffensen’s laboratory. During his doctoral studies, he developed widely used software for performing cyclic voltammetry and analyzing the effects of alcohol and other drugs within the dopamine reward system. His efforts in alcohol research qualifies him to aid in my research as he is well informed in current dogma surrounding the topic of my honor’s thesis and is readily available as he is working in the same lab that I will be conducting my research.

Project Timetable

We have begun preliminary research and will conduct animal trials from now until the end of Winter 2018. Analysis and conclusions will be explored during the Spring/Summer of 2018. This project should be completed by Fall 2018.

Anticipated Academic Outcome
The results and progress report of this research will be presented at the Utah Conference of Undergraduate Research (UCUR) held in February 2018 and the annual meeting of the Society for Neuroscience. We will publish the results of this study in a peer reviewed journal.

**IACUC Approvals**

Protocol number: 160301

**Funding**

We are requesting $1,000 in research funds from the Honors Program to support our research. We hope to also obtain an ORCA grant of $750 to work towards our financial needs as well.

Surgery supplies (guide cannulas, isoflurane, gauze, q-tips, sterile gloves, etc.) $500

Travel to and from UCUR (gas, hotel accommodations, poster printing, conference fee) $500

**Scholarly Sources**


