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SEX DIFFERENCES IN ETHANOL MODULATION OF DOPAMINE RELEASE IN
THE MESOLIMBIC REWARD SYSTEM

by
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ABSTRACT

SEX DIFFERENCES IN ETHANOL MODULATION OF DOPAMINE RELEASE IN THE MESOLIMBIC REWARD SYSTEM

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Bachelor of Science

This thesis seeks to synthesize previous studies that have shown sex differences in response to drugs of abuse, specifically cocaine and alcohol. These differences have been noted through the study of behavior, nitric oxide levels in the medial amygdala, and dopamine release within the mesolimbic system. Importantly, it has been consistently found that these differences seem to correlate with the changing hormonal environment produced by the estrus cycle in females. Furthermore, this thesis examines new research on how the estrus cycle modulates dopamine release within the reward circuit through the utilization of fast scan cyclic voltammetry and microdialysis techniques. A dosage response curve was created for several clinically relevant doses of alcohol of basal dopamine response in a male rat and differential effects at each dose were observed. Additionally, it has been found that blood catecholamine levels rise in response to ethanol, evidence of a peripheral mechanism modulating a central nervous system process within the mesolimbic system. Finally, dopamine response in the reward circuit in response to ethanol has been analyzed both between male and female rats and across the estrus cycle.
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REVIEW OF CRITICAL LITERATURE

The Effects of Drug Abuse on Society

The consequences of drug abuse are widespread and extreme including loss of productivity, squandered earnings, deteriorating physical health, job loss, violence, and premature death (Sacks, Gonzales, Bouchery, Tomedi, & Brewer, 2015). Furthermore, drug addiction is a problem that impacts family, friends, coworkers, and anyone who is close to the abuser just as much as the abuser. Alcohol in particular is the most abused legal drug in the world and, according to the same report, the direct costs of alcoholism to the US in 2006 was $249 billion. Additionally, the Alcohol-related Disease Impact report found that nearly 88,000 people (approximately 62,000 men and 26,000 women) die annually from alcohol-related deaths, making it the third leading preventable cause of death in the United States (CDC, 2015). Because of differences in hormonal chemistry and body fat density, alcohol absorption rates tend to be higher for women than men. This translates to a more immediate and long-lasting alcoholic effect (Ashley et al., 1977). In fact, about 12% of adult women report that they binge drink, drinking an average of 5 drinks per binge resulting in excessive brain damage such as shrinkage and memory loss (Hommer, Momenan, Kaiser, & Rawlings, 2001). New research indicates that low dose ethanol’s effect, like similar stimulants, is different in females due to the way the estrus cycle modulates dopamine release in the reward circuit. Due to the prevalence and history of alcoholism, the effects of ethanol on the human body have been a topic of many research endeavors.
Dopamine Release as a Function of Reward

Drugs, including alcohol, have been found to hijack the body’s natural reward processing system resulting in feelings of pleasure, euphoria, and well-being (Nestler, 2001). This system is known to be involved in reward from natural behaviors such as feeding (Agmo, Galvan, & Talamantes, 1995), quenching thirst (Agmo, Federman, Navarro, Padua, & Velazquez, 1993), and other rewards such as intracranial self-stimulation (Gratton & Wise, 1983). Specifically, the mesocorticolimbic pathway located in the striatum has been implicated in reward processing. Current dogma maintains that dopamine (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 a scalar index of reward (Wise, 2008). The dogma is that any drug or behavior that increases mesolimbic DA neuron activity and release will be reinforcing, and potentially addictive (Kalivas & Volkow, 2005). 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. The prevailing view is that people consume drugs for their rewarding properties, which are mediated by this system.

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 drive 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-processing and Allostasis theories of addiction, wherein tolerance accrues to
repeated drug use, resulting ultimately in compensatory lowering of DA release in the NAc (Koob, 2003; Solomon & Corbit, 1974). 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. Sex hormones may also contribute to these effects variably across the estrus cycle as ethanol modulates dopamine levels within the VTA, changing the addiction response.

*Amygdala Components of Addiction*

Aside from dopamine release, the amygdala has also been implicated recently in the reward circuitry. Research done in the amygdala is scarce and is only beginning to indicate that it is a key player in reward processing. Despite the scarcity of research, the amygdala’s role in emotional response is a compelling argument for its involvement in addiction. The shell of the nucleus accumbens is continuous with the basolateral and central amygdala through glutamate projections, giving the amygdala excitatory control of dopamine release in the striatum (Ahn & Phillips, 2002). It seems that there is an amygdalar control of the reward system that parallels the mesocorticoclimbic pathway for motivated behavior in the nucleus accumbens and medial prefrontal cortex (Phillips, Ahn, & Howland, 2003). This research is ongoing, but concludes that the processes of addiction are modulated through multiple mechanisms that activate the reward circuitry to motivate behavior.
Sex Differences in the Addiction Model

Historically, there have been a dearth of studies in female animals in regards to the neurobiology of addiction. This trend may be due to the complications arising from the cycling of variously concentrated hormones throughout the estrus cycle. As such, results and patterns previously discovered in male animal models may not apply to females specifically because of their unique hormone cycle. The estrus cycle in both rats and mice lasts about four days and is broken up into four phases: proestrus, estrus, metestrus, and diestrus unless disrupted by pregnancy, drugs, or anestrus. Researchers are about to determine which phase an animal is in by visualizing cell types in a vaginal swab. These phases are driven primarily by the changing hormonal levels of estrogen, but levels of progesterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) also fluctuates. During the first part of proestrus, the estrogen levels rise dramatically followed shortly by an increase in progesterone, LH, and FSH signaling the beginning of estrus. The estrus phase is when ovulation occurs and the reproductive organs prepare for pregnancy. If a pregnancy does not occur, the metestrus phase sloughs off the excess cells lining the uterus and hormone levels drop back to baseline. The human estrus cycle is similar to this except it is 28-days long and the surge of progesterone happens right before menses during metestrus. Additionally, the human cycle is traditionally broken up into two phases: the follicular phase which is comparable to the diestrus and proestrus phases in rodents; and the luteal phase which encompasses the estrus and metestrus phases. Because of the similarities between rodent and human estrus cycles, animal models are ideal to elucidate the effects of drugs on the female cycle.
Research in stimulant addiction has been dominated by studies involving male animals and cocaine exposure. However, in recent years the National Institute of Health has required researchers to consider sex as a variable in their studies in order to elucidate any key sex related differences. The majority of these studies have been done using cocaine as a model drug of abuse, but there are several key studies performed with ethanol showing similar results. Ethanol is biphasic in its addiction pathology as it is a stimulant at low doses and a depressant at higher doses. It is therefore justified to use cocaine as a model for how ethanol would behave in addiction because these studies only deal with the stimulant doses of ethanol. Furthermore, because of their similar addiction pathology, it is reasonable to believe that both drugs affect individual sexes in similar ways. These effects can be seen through behavioral, hormonal, mesolimbic, and amygdala studies.

The following four studies discuss sex differences that have been found in behavior aspects of drug abuse. One key study performed by Cunningham et al used conditioned place preference (CPP) and a dosage curve of ethanol to study the rewarding effects of ethanol while comparing the two sexes (Cunningham & Shields, 2018). Their most important finding was that females were more active in their movement than males during every dosage of ethanol (p<.001). This demonstrates a greater variability in preference for alcohol than males, a result that could potentially be explained due to the variability of the female rat’s estrus phases. In another study by Calipari et al, CPP was used to test conditioning comparing the estrus and the diestrus phases of the estrus cycle (Calipari et al., 2017). Importantly, an increased CPP was observed during estrus (P<.001). Later, using voltammetry to measure invoked dopamine release in the
mesolimbic system, there was an increase in Nucleus Accumbens dopamine per one pulse tonic stimulation in estrus females as compared to diestrus females.

Another important addiction-related behavior involves the rate of consumption of alcohol. In another study performed by Ford et al, the consumption of alcohol was tracked across the estrus cycle through “licking” (Ford, Eldridge, & Samson, 2002). These rats were all female and vaginal swabs indicated the phase of estrus. Importantly, there was a distinctive shift in licking patterns between estrus and proestrus phases (P<.001). Researchers concluded that the different consumption patterns must indicate altered sensitivities to the intoxicating and reward effects of alcohol, mediated by the changing hormone cycle. To further compound the evidence of sex differences in the addiction model, several studies have definitively concluded that females have a propensity to self-administer alcohol and cocaine more than males (Jill B. Becker & Koob, 2016). These studies only tested the differences between males and females and not how administering rates changed throughout the estrus cycle in female subjects, something that requires more research to be conducted. Additionally, female rats generally showed less motivational withdrawal, a result that could be related to emotional changes reflected by changing hormones. This will be discussed as a possibility when studies involving the amygdala are addressed.

It is clear that there are distinct sex differences in the use and effects of ethanol and cocaine abuse. There are also hormonal changes in the estrus cycle that alter the addiction response in females as compared to males. These hormonal changes are explored through the manipulation of estradiol, a key naturally occurring estrogen in the estrus cycle. Three studies specifically explored these hormonal changes in the estrus
cycle and how it affected addiction response. Cummings et al performed an experiment involving ovariectomized (OVX) females and estradiol restoration of function (Cummings, Jagannathan, Jackson, & Becker, 2014). Because the rats’ ovaries were removed, their hormone fluctuation mimicked menopause with anestrus. Treatment with estradiol restored a small part of the estrus cycle while controlling for other hormonal factors that are present in a normal cycling female. They found that treatment of OVX females with estradiol increased cocaine-induced release of dopamine in the VTA, but not in males treated with the same estradiol injection. This indicates that rising estrogen levels affect dopamine release in females but not in males. Additionally, Becker et al showed that estradiol treatment greatly enhances addiction behaviors in chronic cocaine-fed female rats (J. B. Becker & Hu, 2008). Taken together, increased estrogen seems to prime dopamine mediation of addiction behavior.

The hormonal cycling of estrus is very sensitive to the duration of alcohol exposure. In a study with alcohol, Emanuele et al compared short chronic ethanol exposure to long chronic ethanol exposure while measuring the consistency of the estrus cycle in rats. Researchers found that both types of chronic exposure to ethanol saw a disruption in cycling, but the longer the rats were exposed to ethanol, the more significant the disruptions were. Uniquely, it found that ethanol significantly increased estradiol which led to a decrease in proestrus and an elongation of the diestrus phase (Emanuele, LaPaglia, Steiner, Kirsteins, & Emanuele, 2001). Additionally, four of the ethanol-fed rats experienced complete cessation of the estrus cycle, showing that alcohol has a major influence on the ability of the reproductive system to perform at normal levels.
Estradiol is not the only hormone that is affected by chronic ethanol exposure. Sanchis et al studied the effects of chronic alcohol on cycling hormones as well. They specifically showed that luteinizing hormone (LH) levels were depressed while prolactin levels were dramatically increased in chronically alcoholic rats (Sanchis, Esquifino, & Guerri, 1985). Much like Emanuele et al, they found that there was a decrease in frequency of estrus and proestrus phases with a marked increase in frequencies of the diestrous phase of these alcoholic rats (P<.001). In a corroborating study, Chuffa et al studied the morphological changes in ovaries of chronic ethanol exposed mice (Chuffa, Padovani, & Martinez, 2009). Researchers observed marked irregularities in the estrus cycle that were reflective of injury of the ovaries due to this chronic ethanol consumption. Overall, these studies show that all estrus cycle hormones are impacted by chronic ethanol exposure in predictable and noticeable ways.

**Sex Differences in Amygdala Control**

These previous studies focused on dopamine release within the mesolimbic pathway as an indicator that the animals were being rewarded by cocaine and ethanol. However, it is important to note that dopamine is not the only gauge of addiction. Because of its close association with the nucleus accumbens, the amygdala may have influence over the addiction cycle as well. Regarding the amygdala’s implication in addiction, Martini et al conducted a study involving cocaine addiction in specific phases of estrus (Martini, Pinto, & Valverde, 2014). Researchers theorized that nitric oxide levels in the anteroventral part of the medial amygdala would be linked to a response to cocaine in wild type mice. The results showed that acute cocaine administration resulted in significant differences in nitric oxide release in the medial amygdala and an increase in
behavioral changes associated with anxiety in females. Thus, females have less motivation to stop the self-administration of cocaine and presumably similar stimulants.

Sex differences in Human Addiction Studies

Human research involving addiction is scarce and difficult to perform. This is primarily due to the inability to perform invasive procedures to measure the same hormonal and neurotransmitter levels that researchers can in animals. Additionally, common methods of human studies involve self-reporting measures that are inherently subjective and difficult to track. Evans et al performed a study involving women and how they responded to alcohol throughout the estrus cycle (Evans & Levin, 2011).

Specifically, researchers were curious if there was a varied response in specific phases and if a paternal family history of ethanol affected the results. This study is significant because it involved women tracking their alcohol consumption across their menstrual cycle and rating their experience for how rewarding the alcohol was. Ultimately it was found that the difference across the estrus cycle were subtle, but that alcohol was found to be more pleasurable (rewarding) during the luteal phase. Additionally, researchers were surprised to note that there was no significant differences between woman with a history of alcoholism and those without alcoholism in their family. This human study corroborates with the animal model of alcohol addiction and the variable reward sensitivity throughout the different phases of estrus.

Discussion

Although there are several brain regions regulating responses to addiction, low dose ethanol, like other stimulants, modulates dopamine within the mesolimbic system variably across the estrus cycle. These changes across the estrus cycle have been seen
behaviorally in Cunningham’s study where there was great variability in the CPP of female mice exposed to chronic low dose ethanol as compared to their male counterparts. This difference indicates a sex dimorphism that is variable, yet predictable. Calipari’s study showed a marked increase of CPP during the estrus phase in chronically exposed animals. This suggests a variable sensitivity to ethanol and other drugs’ rewarding effects across the estrus cycle with an increase in reward during the estrus phase. This finding is supported by Evans et al who determined that women found alcohol more pleasurable during the luteal phase of their cycle, a part that the estrus phase belongs in. Additionally, Cunningham et al found that alcohol and cocaine both significantly affect the normal rhythm of the estrus cycle. Phase changes are further seen in Emanuele’s study showing that there is a marked prolongation of diestrus phase due to chronic alcohol exposure. Sanchis agreed that there was a distinct prolongation of the diestrus phase and found that LH levels were depressed while prolactin levels were substantially increased. It seems that both cocaine and ethanol interact with the release of estradiol, altering and disrupting the duration of both proestrus and diestrus. This finding is poignant as it shows that ethanol has a particular, noticeable impact on the female’s reproductive system. Additional research in this area can further elucidate negative impacts of drugs and alcohol on the human body in specific ways.

In addition to changes in hormone levels, there are sex differences in dopamine levels of alcoholic animals. This has been demonstrated through Calipari’s voltammetry experiments showing that VTA dopamine levels were enhanced during the estrus phase and that this observation was mediated by estradiol fluctuation. Importantly, Ford found that there was a distinct change in consumption rates of alcohol by noting a progressive
shift in licking rates between estrus and proestrus. Such a change supports that there may be an increased sensitivity to the neurobiological rewarding effects of alcohol at that phase of the cycle. These irregularities were also shown through a decrease in ovary mass and morphological changes reflected by unnatural prolongation and cessation of certain phases of the normal female cycle.

Finally, Martini discovered changes in nitric oxide levels within the medial amygdala that differed between male and female rats. Most notably, diestrus females and males were the most susceptible to changes in nitric oxide and were also shown with increased addiction-like behaviors. Given that the amygdala is an extension of the shell of the nucleus accumbens, these findings show that the reward circuitry is a highly connected, incredibly complex system and the amygdala has similar responses to drug abuse. More research should be conducted involving the amygdala and how its connections to the mesolimbic circuitry aid in the formation of a reward response that transforms into addictions. Specifically, new research in these sex differences could be further compounded by female’s enhanced anxiety response to withdrawal.

Conclusion

Overall, the animal model of female addiction is complex and is actively studied. These findings are observed in mice and rats and seen through chronic cocaine and alcohol abuse and are therefore fairly generalizable. It is important to note that though there are several key sex studies involving chronic alcohol exposure, many of these studies were conducted using cocaine to commandeer the brain’s natural reward system. Since ethanol is a stimulant at low doses, many of these findings with cocaine could be observed when studying alcohol addiction as well. In particular, there is a dearth of
studies involving tonic release of dopamine within the nucleus accumbens in regards to alcohol. Calipari conducted important studies involving phasic response of dopamine utilizing in-vivo voltammetry experiments of cocaine response that could be complemented using freely-moving microdialysis experiments involving alcohol. This type of research is important in order to continue to elucidate the differences within the estrus cycle when alcoholism is a factor.

PRELIMINARY WORK

Previous work has been done in Dr. Steffensen’s lab surrounding ethanol effects on DA release in the mesolimbic DA system implicated in drug reward, including alcohol. 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. In preliminary studies, work in Dr. Steffensen’s lab has shown clear sex-related differences in DA release in the NAc. 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 rationale for evaluating ethanol effects on DA release across the cycle in the same rats. The core hypothesis is that ethanol effects on DA release will vary across the estrus cycle.

METHODS

**Microdialysis.** Survival surgeries were performed in male and female rats with stereotaxic implantation of a guide cannula (MD-2250, BASI Instruments, West Lafayette, IN, USA) in the NAc (+1.7 AP, +0.8 ML, -6.0 DV). A microdialysis probe (MD-2200, BASI Instruments, West Lafayette, IN, USA) was inserted into the guide cannula on experimentation days and samples were taken by flowing artificial cerebral spinal fluid (aCSF) through the probe at a rate of 2.0 µL/s. Dialysate samples were collected every 20 min for 2 hours to establish baseline DA values at which point ethanol was injected. Samples were then collected every twenty minutes for the next three hours to determine the effects of ethanol on DA release in the NAc. Samples were evaluated via high pressure liquid chromatography with electrochemical detection (HPLC-ECD) to determine the levels of DA 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 0.5 g/kg, 1.0 g/kg, 2.0 g/kg, and 4.0g/kg were administered.

**FSCV.** Subjects were placed in a stereotactic rig (Kopf Instruments, Tujunga, CA, USA) under isoflurane anesthesia (1.5% - 2.0%). The skull of the rat was then
exposed by making an incision on the top of the head and carefully drawing back the tissue. Measurements were taken and holes were drilled at -2.5 AP, +1.9 ML and +1.6 AP, +1.9 ML for insertion of the stimulating and recording electrodes, respectively. The stimulating electrode was inserted to a depth of -8.0 to -8.2 DV while the recording electrode was inserted to a depth of -6.5 to -6.8 DV. A reference electrode was placed in the tissue at the back of the skull. The MFB was stimulated with 60 biphasic pulses at 60 hertz once every 120 seconds to evoke DA release in the NAc. The electrode potential was linearly scanned from -0.4 V to +1.3 V and back versus an Ag/AgCl at a scan rate of 400 V/s. The sampling rate of these scans was 10 hertz. A ChemClamp voltage clamp amplifier (Dagan Corporation, Minneapolis, MN, USA) connected to a Windows-PC compatible computer running Demon Voltammetry was used to record the data. Demon Voltammetry was used to analyze the data. The alcohol doses of 0.5 g/kg, 1.0 g/kg, 2 g/kg, and 4 g/kg was injected respectively for each trial after the signal had been stable for 5 successive collections (all collections within 10% of each other).

**Vaginal Lavage.** Female Wistar rats (*Rattus norvergicus*), weighing between 200 and 300 g on a reversed 12 h light cycle were used. On the day microdialysis or voltammetry is performed, vaginal secretion was collected. The secretion was collected with a latex bulb on the end of a plastic pipette filled with deionized water by placing the tip at the opening of the vaginal canal without penetrating. The vaginal fluid was placed on a glass slide and then, after being allowed to dry, were stained with crystal violet. Cell type was determined using a light microscope with 10 and 40 x objective lenses (Figure 2). Cell types were analyzed using indicators described by McLean et al (McLean, Valenzuela, Fai, & Bennett, 2012).
Data Analysis. Data analysis will be carried out using Stata (StataCorp LLC, College Station, TX, USA) after all the data is gathered. Appropriate mixed model or repeated measure analysis of variance (ANOVA) will be used when the data involves a within subjects comparison. When the comparisons involve between subjects variables appropriate one-way or two-way ANOVA will be used. All data will be checked for normality and outliers. Additionally, a Greenhouse-Geisser correction for sphericity will be used for analyses of within-subjects data. Tukey’s honestly significant difference test (HSD) will be used in any post-hoc analysis performed. A Bonferroni correction for multiple comparisons will be applied to tests of a priori hypotheses where appropriate.

RESULTS

Effects of Ethanol on Basal Dopamine Release in the Nucleus Accumbens of Male Rats

We first needed to evaluate the dose-response effects of acute ethanol on DA release in the NAc of male rats. Figure 3 shows the effects of IP administration of ethanol (0.5-4.0 g/kg) on basal DA release in the NAc of male rats, which represents a physiologically-relevant range of ethanol doses. The lowest dose of 1.0 g/kg is mildly intoxicating, akin to what alcohol drinkers describe as a “buzz”. The 2.0 g/kg dosage is fairly close to the legal blood alcohol limit, while the highest dose is severely intoxicating, with rats exhibiting profound sedation and loss of righting reflex. Interestingly, note that the three lowest doses of ethanol enhanced DA release at 20 min after ethanol injection, but the highest dose of ethanol produced an inhibition of DA release followed by a latent increase at 2 hr. Although 4.0 g/kg ethanol is often used to model binge alcohol drinking, it is near the LD$_{50}$ for ethanol.

Figure 3. Effect of ethanol on basal extracellular DA release in the NAc of males. (A) Ethanol enhances DA release in the NAc in a dose-dependent manner.
Effects of Ethanol on Blood Catecholamine Levels of Male Rats

As part of this project, it was important to evaluate not just the effects of ethanol on brain DA levels, but on blood DA levels, and compare them to other catecholamines including norepinephrine (NE) and epinephrine (EPI). The rationale for these experiments is to gain insight into how the peripheral effects of ethanol might affect central processes, an ongoing interest in the Steffensen lab. In particular, we are interested in the ability of the sympathetic nervous system to modulate inflammatory profiles in response to ethanol through both direct effects at dopaminergic and adrenergic receptors on leukocytes (pro-inflammatory) as well as through suppression of peripheral cholinergic tone (pro-inflammatory) and prolactin release (anti-inflammatory) (Devins, Miller, Herndon, O'Toole, & Reisz, 1992; Kohm & Sanders, 2001; Rosas-Ballina & Tracey, 2009; Van den Berghe & de Zegher, 1996)

Figure 4 demonstrates that shortly after administration of IP ethanol at the 0.5 g/kg dose level there are marked increases in DA and NE levels in the blood plasma while EPI levels are not affected. This time course is somewhat consistent with ethanol effects on DA release in the brain (Figure 3).
Effects of Ethanol on Evoked Dopamine Release in the Nucleus Accumbens of Male and Female Rats

While microdialysis measures basal DA release, fast scan cyclic voltammetry (FSCV), otherwise known as voltammetry, measures evoked DA release. Both are important indices of DA release and reflect different processes. Although it is clear that DA is involved in ethanol reinforcement, ethanol’s mechanism of action in the NAc is complicated. Microdialysis studies have shown that basal DA release in the NAc is increased by ethanol and voltammetry studies have reported increases in DA transient frequency after ethanol (Cheer et al., 2007; Robinson, Howard, McConnell, Gonzales, & Wightman, 2009). However, numerous voltammetry studies from our lab (Schilaty et al., 2014) and others have shown that ethanol ex vivo and in vivo (Budygin, Phillips, Robinson, et al., 2001; Robinson, Venton, Heien, & Wightman, 2003; Robinson, Volz, Schenk, & Wightman, 2005; Yorgason, Ferris, Steffensen, & Jones, 2013) decreases evoked DA release, a model of phasic release thought to emulate VTA DA neuron bursting activity by ethanol. The inhibitory effects of ethanol on electrically-evoked DA release in vivo appears to be localized to terminals, as ex vivo voltammetry studies in NAc slices lacking the influence of DA cell bodies evince ethanol inhibition of electrically-evoked DA release (Budygin, Phillips, Wightman, & Jones, 2001; Jones, Mathews, & Budygin, 2006; Yavich & Tiihonen, 2000). We evaluated the effects of 2.0 g/kg ethanol on evoked DA release in the NAc. This dose was chosen because it is near the IC₅₀ determined in previous studies.

Figure 5. Sex Effects on Ethanol Inhibition of DA in the NAc. Acute ethanol (2.0 g/kg, IP) reduces evoked DA release in both males and females 20 min after injection.
In both males and females, ethanol inhibited evoked DA release as measured by voltammetry (Fig. 5)

*Effects of Ethanol on Evoked Dopamine Release in Female Rats across the Estrus Cycle*

In order to evaluate the changes of evoked release of DA in the NAc due to ethanol exposure across the estrus cycle, preliminary voltammetry data has been collected in each of the four phases of estrus: estrus, metestrus, proestrus, and diestrus. Ethanol inhibition of evoked DA release does not appear to be affected by phase of the cycle at this point in our data collection (Fig. 6). However, this is still preliminary given the low sample numbers of subjects to date. Data collection is ongoing in order to elucidate any differential effects of the estrus cycle. The main objective of this project was to evaluate ethanol effects on basal DA release across the estrus cycle. We have only recently begun these studies due to a 14 month delay associated with the remodel of the KMBL vivarium wherein we could not do any behavioral experiments. In addition, we have had problems with our HPLC machine for measuring basal DA release. Although we have collected dialysates from multiple freely-behaving female rats across the estrus cycle, we have not been able to analyze them for DA levels. Currently we are in the process of continually collecting data across the estrus cycle reflecting exposure to 4 g/kg, 2 g/kg, 1 g/kg, and 0.5 g/kg ethanol.

![Figure 6. Effects of Ethanol on Evoked DA Release in NAC across Estrus Cycle. Acute ethanol (2.0 g/kg, IP) reduces evoked DA release but with no apparent differences across phases of the estrus cycle.](image-url)
CONCLUSION

The sex differences in the release of DA in the NAc due to alcohol exposure is a gap in our understanding of addiction. This project has begun the process of filling this gap as we compared evoked and basal DA levels between male and female rats. The dosage response curve of basal DA in male rats shows a differential effect of varying alcohol concentrations on dopamine release in the reward circuitry (Figure 3). As microdialysis trials continue and data is analyzed, this dosage response curve will act as a control to determine sex differences with various relevant ethanol doses. Additionally, it has been found that there may be an immune response to ethanol in the peripheral nervous system which has mediating effects within the central nervous system, progressing our current model of addiction (Figure 4).

Although the evoked and basal dopamine release experiments measuring in female rats across the estrus cycle have shown no effect, they are still in their preliminary stages (Figures 5 and 6). As sample sizes increase, it is possible that a differential effect will be elucidated within the phases of estrus. Understanding how the reward circuit handles alcohol in the various hormonal phases experienced by females can have widespread implications into how alcohol abuse disorder is treated in humans. If sex differences are understood, treatment plans can be tailored to the individual and increase in efficacy as we attempt to help those ensnared in the clutches of alcohol addiction. Ultimately even conclusions of no differential effect between males and females will aid researchers as they continue to understand sex differences and findings will benefit the field greatly as it complies with recent NIH sex studies guidelines.
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