2021

# PHARMACOKINETICS AND REWARD-RELATED BEHAVIORS OF ETHANOL IN MALE AND FEMALE JAPANESE QUAIL (*COTURNIX JAPONICA*)

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Digital Object Identifier: https://doi.org/10.13023/etd.2021.253

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# PHARMACOKINETICS AND REWARD-RELATED BEHAVIORS OF ETHANOL IN MALE AND FEMALE JAPANESE QUAIL (*COTURNIX JAPONICA*)

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DISSERTATION

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A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
College of Arts and Sciences
at the University of Kentucky

By
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ABSTRACT OF DISSERTATION

PHARMACOKINETICS AND REWARD-RELATED BEHAVIORS OF ETHANOL IN MALE AND FEMALE JAPANESE QUAIL (*COTURNIX JAPONICA*)

Ethanol is one of the most widely used and abused drugs. Problem use is associated with many different health problems and the economic burden is in the billions of dollars. Additionally many people have difficulty controlling their ethanol consumption and about 5% of adults end up with an alcohol use disorder (AUD). Many people with an AUD often find themselves in a cycle of binge, remission, and relapse.

Following ethanol consumption ethanol enters the bloodstream from the small intestine where it gets distributed to peripheral tissues. Ethanol in the bloodstream is cleared from the system by the liver. The primary metabolism of ethanol uses alcohol dehydrogenase (ADH). Previous research indicates a significant sex difference in ADH activity in males and females depending on the tissue sample examined. Females tend to have higher ADH activity in liver samples than males. The purpose of the current study was to analyze sex differences in ADH levels following 12 days of ethanol administration (i.e., water or 2 g/kg) in male and female quail. Following the last daily treatment of ethanol, quail were euthanized and their livers were extracted and ADH was analyzed in liver homogenate samples. Females had higher ADH levels, heavier livers, and a greater liver to body weight ration than males. In a second experiment, we aimed to develop a blood ethanol concentration (BEC) profile for both male and female quail. Quail were administered 0.75 or 2 g/kg and blood was collected at 0.5, 1, 2, 4, 6, 8, 12, 24 hours after gavage. Blood ethanol concentration was analyzed using an Analox. We found that quail had a fairly rapid increase in BECs followed by a steady and slow disappearance of ethanol from the blood samples. Female quail had a lower peak and a smaller area under the curve (AUC) than male quail. Female quail have higher ADH levels, which may be responsible for the metabolism of ethanol and therefore it follows that female quail would have less overall exposure to ethanol than male quail.

The conditioned place preference (CPP) paradigm uses a Pavlovian conditioning procedure to assess the reward or aversion of a conditioned stimulus. This method is often used to test the abuse potential of drugs in rodents. However, many of the cues associated with AUD tend to be visual in people, therefore, a visual species such as Japanese quail may be a better model to examine visual cues associated with AUD. Additionally, rodents often find an oral gavage to be aversive, whereas it is a typical administration for birds. The aim of the current study was to examine the rewarding

properties of ethanol in Japanese quail using the CPP paradigm. Male and female quail received an ethanol (0, 0.75, or 2.0 g/kg) gavage and confined to their initially least preferred side based on a pretest (i.e. biased design) of the three-chamber CPP apparatus for 30 min on every other conditioning day. On alternate days, quail received a gavage of water and confined to the opposite side for 30 min. Quail received a total of 4 ethanol and 4 water conditioning trials on alternating days and locomotor activity was collected during conditioning trials. Twenty-four hours after the last conditioning trial, quail were allowed to freely roam the entire apparatus for 15 min. A difference score was calculated between time spent in the drug paired chamber (i.e. posttest) and time initially spent in the least preferred side (i.e. pretest). An ANOVA analysis revealed a significant effect of treatment. Post hocs revealed quail that received the 0.75 g/kg developed a CPP compared to the other groups, whereas quail treated with 2 g/kg developed an aversion to their drug paired side. Additionally, during conditioning days there was a significant interaction between time and treatment on locomotion. Specifically, animals that were treated with the 2 g/kg dose of ethanol had significantly less movement over the 30 min conditioning trials. Our results indicate that quail may be a good model to study AUD because they acquire both a CPA and CPP to an oral gavage of ethanol suggesting both the rewarding and aversive properties of ethanol are observable in this model.

KEYWORDS: Alcohol, pharmacokinetics, Japanese quail, conditioned place preference, alcohol dehydrogenase

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06/24/2021

Date

# PHARMACOKINETICS AND REWARD-RELATED BEHAVIORS OF ETHANOL IN MALE AND FEMALE JAPANESE QUAIL (*COTURNIX JAPONICA*)

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DEDICATION

To everyone that has supported me over the years and made me into the scientist I am today, and to my dogs that have helped get me through my past year and kept me company during lockdown.

ACKNOWLEDGMENTS

I would like to acknowledge the long hours and tireless work of my mentor Chana K. Akins, PhD. Without her help over the last six years, I would not be where I am today. She has always supported my goals and offered encouragement and supported when most needed. This past year with COVID, she always made sure to check on my mental health and provided much needed support in person to finish my research projects.

In addition to Chana, I would like to also acknowledge the rest of my committee, Mark A. Prendergast, PhD., Mike Bardo, PhD, Jessica Santollo, PhD for their advice, support, and suggestions along the way. I would also like to thank Jim R. Pauly, PhD for his time, input, and suggestions as my outside examiner.

I would also like to acknowledge those that have helped me along the way. Specifically, I would like to thank Julia E. Jagielo-Miller for always helping me run blood samples on the Analox. I would not have made it through those days when the Analox was not working properly if not for your wit and friendship. I would like to thank all previous and current lab mates including, Karin Gill, PhD who always was there to help me think through issues; Beth Ann Rice, PhD for her help adjusting to UK; Mia E. Radevski for help running the lab and taking over this last year.

Lastly, I would like to thank my family. The hours spent editing my writing, the times you talked me through problems, and the general company you've provided throughout my grad student career has been so helpful. I could not have made it to this point without your help. Morgan and Bacardi perhaps have been the most supportive and always knew just when I needed snuggles.

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# CHAPTER 1. INTRODUCTION

## 1.1 BACKGROUND

Alcohol is consumed worldwide and is one of the most frequently used recreational drugs. Over 85% of Americans reported drinking alcohol at some point in their lives (NSDUH, 2019). Over half of those surveyed reported consuming an alcoholic beverage in the last month (NSDUH, 2019). The CDC (2010) reports that the economic impact of alcohol misuse costs 249 billion dollars annually. It is not just the US with this problem. Worldwide, alcohol is responsible for more than 3 million deaths annually (WHO, 2018). Health risks result from both acute alcohol use (i.e., binge drinking) and chronic use. Specifically, acute use may lead to alcohol poisonings and injuries, whereas chronic alcohol use leads to cirrhosis of the liver, cardiovascular disease, and alcohol-related cancers (Rehm, 2011). Furthermore, in individuals with preexisting conditions such as diabetes, the predicted outcomes are much worse in individuals with heavy alcohol use (Carlsson et al., 2005).

While many are able to consume alcohol responsibly, this is not the case for everyone. More than 5% of adults in the United States reported having an alcohol use disorder (AUD) (NSDUH, 2019). This disorder is especially problematic because even though between 8-16% enter treatment (Mekonen et al., 2020; NSDUH, 2018), treatment s frequently unsuccessful and within a few months individuals often relapse. The rates for relapse are around 60% at three months following treatment and between 60-70% at 12 months following treatment (Hunt et al., 1971; Miller et al., 2001). Therefore,

understanding this disorder and developing successful treatment methods are of critical importance.

There are notable gender differences in the diagnosis of AUD. Males are more likely to be heavy users and diagnosed with an AUD compared to women (Dawson et al., 2014; Grant et al., 2017; Haberstick et al., 2014). However, women tend to exhibit a "telescoping" effect when it comes to alcohol use. Telescoping describes a phenomenon in which women have a more rapid progression from alcohol use to treatment entry than men (Diehl et al., 2007; Hernandez-Avila, 2004). In addition, in recent years the rate of women engaging in heavy drinking has increased faster than for men (Dawson et al., 2014; Grant et al., 2017). Diagnosis of AUD has also been increasing faster in women than in men. Between 2001 and 2013, the diagnosis of AUD in women increased by 83.7%, while in men, AUD diagnoses increased 34.7% during the same time (Grant et al., 2017). Therefore even though men are currently more likely to be diagnosed with AUD, women are catching up and may progress to problem drinking more rapidly.

In addition to having greater alcohol use and higher rates of diagnoses of AUD, women tend to suffer from worse health outcomes due to alcohol consumption. Women are at a greater risk of developing liver diseases (for review see, Eagon, 2010), alcohol-related cancers (for review see, Roswall & Weiderpass, 2015), cardiomyopathy (Fernández-Solà & Nicolás-Arfelis, 2002), and may have greater brain atrophy from alcohol use (for review see, Hommer, 2003). Although men tend to have higher alcohol-related mortality rates, women tend to die at younger ages due to alcohol compared to men (White et al., 2020; Haberman & Natarajan, 1989; John & Hanke, 2002).

Rodent studies indicate that women might be at a greater risk of alcohol use and abuse than men. Female Long-Evans rats freely drink more beer than males (Lancaster & Spiegel, 1992). Additionally, female rodents tend to have a greater intake of ethanol and may find ethanol more rewarding than males (Randall et al., 2017; Torres et al., 2014; Cunningham & Shields, 2018). Research has also found that females have greater dopamine (DA) release in the nucleus accumbens following low doses of ethanol compared to males (Blanchard et al., 1993). Thus, there may be a biological basis for a sex difference in alcohol reward.

## 1.2 ETHANOL

Ethanol is produced by fermentation and is typically consumed orally. As a recreational drug, ethanol acts as a central nervous system depressant, although it also has stimulant effects at low doses (Hendler et al., 2011; Martin et al., 1993). Consumption of ethanol leads to a wide range of behavioral and cognitive effects, including mood elevation, impulsivity, increased sociability, sedation, slurred speech, impairment of memory and cognition, and nausea. Repeated use of ethanol may cause tolerance, physical dependence, and withdrawal symptoms (Bayard et al., 2004 Kalant, 1988). There are two types of tolerance including pharmacokinetic or pharmacodynamic tolerance. Pharmacokinetic tolerance develops from an increase in the catabolism of ethanol, resulting in less ethanol reaching the bloodstream. Whereas pharmacodynamic tolerance results from an change in receptor expression (Hoffman & Tabakoff, 1994).

### 1.2.1 NEUROSCIENCE OF ALCOHOL USE AND ABUSE

Ethanol easily enters the bloodstream and readily crosses the blood-brain barrier. Once in the brain, ethanol is a promiscuous drug and affects various systems (Oscar-Berman et al., 1997). The effect ethanol has on the nervous system results from the direct interaction with receptors and second messenger systems, as well as by altering the fluid makeup of the cellular membranes (Kalant, 1975). These neurological effects result in widespread behavioral and cognitive alterations following the consumption of ethanol.

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#### 1.2.1.1 GLUTAMATE

Glutamate (Glu) is one of the primary amino acid neurotransmitters located throughout the central nervous system. Glu is responsible for many different functions including long-term potentiation, learning, and memory (Riedel et al., 2003). Glu is a non-essential amino acid synthesized from glutamine by glutaminase and is stored for release in synaptic vesicles. Following synaptic release, Glu may bind to eight types of metabotropic receptors and three types of ionotropic (for review, see Willard & Koochekpour, 2013). The metabotropic receptors are labeled mGluR1 through mGluR8. The mGluR1 and mGluR5 receptors are coupled to Gq second messengers that increase intracellular Ca2+, and mGluR2 and mGluR3 are coupled to Gi second messengers that

inhibit adenylyl cyclase. The remaining receptors are grouped together because they have different agonist preferences but are Gi protein-coupled. The ionotropic receptors are broken into subtypes and include the α-amino-3-hydroxy-5-methyl-4- isoxazolepropionic acid (AMPA) receptor, the kainic acid receptor, and the N-methyl-D-aspartate (NMDA receptor). When activated, these receptors allow Na+ ions into the cell. In addition to Na+, the NMDA receptor also allows Ca2+ ions into the cell, which triggers second messenger systems. However, the NMDA receptor requires both ligand activation in addition to membrane depolarization to activate the receptor. Glu is removed from the synapse by either reuptake into the presynaptic neuron or into surrounding glial cells. Within glial cells, Glu is converted back into glutamine and then released back into the extracellular space to be taken up by glutamatergic neurons (Hertz, 1979).

#### *1.2.1.1.1 GLUTAMATE AND ETHANOL*

Ethanol alters activity at the glutamatergic receptors and causes inhibition of Glu release. Ligand-induced activation of the mGluR5 receptor is attenuated by ethanol, perhaps through activation of the protein kinase c (PKC) site on the mGluR5 (Minami et al., 1998). At relatively low doses, ethanol inhibits the flow of Ca2+ ions following NMDA receptor activation (Wirkner et al., 1999). Additionally, the synaptic release of Glu is reduced in the presence of ethanol, therefore reducing the excitatory effect on postsynaptic neurons affecting the release of other neurotransmitters such as dopamine, norepinephrine, and acetylcholine (Hernandez et al., 2003). Both AMPA and NMDA receptors are inhibited by ethanol (Wirkner et al., 2000). As a result of less NMDA receptor activation, ethanol inhibits long-term potentiation in rodents and may affect

learning and memory. Additionally, NMDA receptors become upregulated due to long-term ethanol use (Haugbøl et al., 2005; Hu & Ticku, 1995). Due to this increase in receptors, Glu mediated excitotoxicity becomes disrupted resulting in seizures when ethanol is withheld (Haugbøl et al., 2005; Tsai et al., 1995).

#### 1.2.1.2 γ-AMINOBUTYRIC ACID

One of the primary inhibitory neurotransmitters in the central nervous system is γ-aminobutyric acid (GABA)(Krnjevic, 1997). It is found ubiquitously throughout the brain. GABA is an amino acid neurotransmitter. GABA is synthesized from GLU by glutamic acid decarboxylase and binds to both ionotropic (i.e., GABAA) and metabotropic (i.e., GABAB) receptors. Activation of the GABAA receptor allows for an influx of Cl- ions resulting in a hyperpolarization of the neuron and most of the fast inhibitory neurotransmission in the CNS (Krnjevic, 1997). GABAB is a metabotropic receptor and is coupled to K+ and Ca2+ channels through a second messenger G-protein (Misgeld et al., 1995). GABAB is located both pre- and post-synaptically. GABAB receptors act as autoreceptors on the presynaptic neuron, and may regulate GABA release (Misgeld et al., 1995).

##### *1.2.1.2.1 GABA AND ETHANOL*

Ethanol affects the GABAergic system in a few different ways. GABA turnover is altered in a dose-dependent manner. At low doses of ethanol, GABA turnover is increased, whereas at high doses, GABA turnover is decreased (Hunt & Majchrowicz, 1983). In addition to turnover, ethanol also facilitates the vesicular release of GABA by

the presynaptic neuron (Roberto et al., 2003). At GABAA receptors, ethanol modulates the receptor to allow for increased activation, resulting in enhanced depolarization of the postsynaptic neuron (Grobin et al., 1998). Ethanol also enhances the function of presynaptic GABAB in hippocampal slices, perhaps due to increased synaptic GABA release (Ariwodola & Weiner, 2004). This alteration at the GABAB autoreceptor may limit the amount of ethanol-induced GABA release.

#### 1.2.1.3 SEROTONIN

Serotonin (5-HT) is a ubiquitous monoamine neurotransmitter that originates within the brainstem in the raphe nuclei (Steinbusch, 1981). Due to the fairly unrestricted and widespread pathways of serotonergic neurons in the central nervous system (CNS), 5-HT has been thought to have widespread control over many functions, including mood (Ruhé et al., 2007), memory (Schmitt et al., 2000), sleep (Moore et al., 1998), and appetite (Blundell, 1984).

5-HT is derived from L-tryptophan, an essential amino acid (Côté et al., 2003; Schaechter & Wurtman, 1990). L-tryptophan is converted to 5-hydroxy-L-tryptophan (5-HTP) via the enzyme tryptophan hydroxylase (Ichiyama et al., 1970). 5-HTP is then converted to 5-HT via the enzyme aromatic amino acid decarboxylase (Ichiyama et al., 1970). The rate-limiting step in the biosynthesis of 5-HT is tryptophan hydroxylase activity (Fitzpatrick, 1999). After 5-HT is released into the synaptic cleft, the primary method of inactivation is through the 5-HT reuptake transporter (SERT) (Blakely et al., 1994). SERT is a protein located on the presynaptic membrane that actively removes 5-HT from the synaptic cleft and pumps it back into the presynaptic neuron (Blakely et al.,

1994). 5-HT is metabolized intracellularly by enzymatic degradation by the enzyme monoamine oxidase- A (MAO-A) (Pizzinat et al., 1999). MAO-A binds to monoamines, including 5-HT, and catalyzes the oxidation of amines (Gaweska & Fitzpatrick, 2011).

5-HT has 14 different receptors classified into seven different families numbered 5-HT1 through 5-HT7 (Frazer & Hensler, 1999). Six of the families couple to a G-protein, and one to an ion channel (Rho & Storey, 2001). The serotonergic G-protein coupled second messenger receptors mediate both excitatory and inhibitory transmission (Frazer & Hensler, 1999), whereas the ligand-gated sodium and potassium channel is responsible for excitatory transmission (Frazer & Hensler, 1999).

When activated, the 5-HT1 and 5-HT5 receptors decrease intracellular levels of cAMP (Frazer & Hensler, 1999). Five receptors belong to the 5-HT1 family; 5-HT1A, 5-HT1B, 5-HT1D, 5-HT1E, and 5-HT1F. The 5-HT2 family consists of three receptor subtypes, including 5-HT2A, 5-HT2B, and 5-HT2C. The 5-HT2 receptor is coupled to a Gq protein and, when activated, increases intracellular levels of inositol trisphosphate (IP3) and diacylglycerol (DAG). The 5-HT3 receptor is the only ligand-gated cation channel. Upon activation, the structure goes through a conformational change and allows cations to pass through the pore, leading to an excitatory response in the postsynaptic cell. The remaining families of receptors, 5-HT4, 5-HT6, and 5-HT7, all increase intracellular levels of cAMP when ligands bind to these receptors (Frazer & Hensler, 1999; Hoyer et al., 1994; Rho & Storey, 2001).

#### *1.2.1.3.1 SEROTONIN AND ETHANOL*

There is growing evidence of the role of serotonin in the use and abuse of ethanol. Systemic administration of ethanol increases the release of 5-HT, but only with a high dose (Yoshimoto et al., 1992). Ethanol has also been shown to inhibit the clearance of 5-HT from the synapse (Daws et al., 2006), and cause changes in the synthesis and metabolism of 5-HT (Dundon et al., 2004; Helander et al., 1993). Heavy alcohol users have less SERT density in their cerebral cortex compared to controls (Mantere et al., 2002). After consumption of a single dose of ethanol people have more exreated 5-HT metabolites (LeMarquand et al., 1994). Taken together, these studies indicate that ethanol may elevate synaptic 5-HT levels.

Ethanol may also interact with serotonergic receptors. Specifically, the 5-HT3 ionotropic receptor has received a lot of attention (for review, see Lovinger, 1999). Ethanol has been shown to enhance activity at the 5-HT3 receptor and, as a result, may facilitate the release of GABA (Jun et al., 2021; Sung et al., 2000). Additionally, over-expression of the 5-HT3 receptor has been shown to reduce ethanol self-administration and enhance ethanol sensitivity in mice (Engel et al., 1998; Engel & Allan, 1999). This receptor seems to play a role in intoxication and ethanol seeking behaviors.

### 1.2.1.4 DOPAMINE

Dopamine (DA) is the neurotransmitter most associated with reward. DA is one of the catecholamine neurotransmitters and is mainly produced in the ventral tegmental area (VTA) and the substantia nigra (see Kalivas, 1993 for review). The synthesis of DA

takes several steps. First, tyrosine is converted to L-dihydroxyphenylalanine (L-DOPA) by tyrosine hydroxylase and then L-DOPA gets converted by aromatic amino acid decarboxylase into DA (Elsworth & Roth, 1997). DA is transported and stored in synaptic vesicles and is released via calcium-dependent exocytosis. Following the release of DA, the main method of inactivation is by reuptake by the dopamine transporter (DAT). Several enzymes, monoamine oxidases (MAO) and catechol methyltransferase (COMT) are responsible for the degradation of DA.

Dopamine is localized to four distinct pathways, the tuberoinfundibular, nigrostriatal, the mesocortical, mesolimbic pathways. The tuberoinfundibular pathway is primarily involved in hormone regulation and has projections from the hypothalamus to the anterior pituitary (Björklund et al., 1973). The nigrostriatal pathway originates in the substantia nigra and terminates in the striatum. This pathway is responsible for motor movement. Both the mesocortical pathway and mesolimbic pathway originate in the VTA (Swanson, 1982). However, the mesocortical pathway has projections to the prefrontal cortex and is involved in the regulation of motivation, whereas the mesolimbic pathway projects to the nucleus accumbens (for review, see Koob, 1992). The mesolimbic pathway is often associated with reward and drug-taking behaviors.

DA exerts its effect by binding to, and activating, DA receptors. There are a total of five different dopamine receptors that are all G-protein coupled receptors (GPCR) (for review, see Vallone et al., 2000). DA receptors are grouped into two families based on the type of G-protein the receptor is coupled to. The D1 and D5 receptors are coupled to a second messenger that increases adenylyl cyclase. Whereas the D2, D3, and D4 receptors are coupled to a second messenger that inhibits adenylyl cyclase.

#### *1.2.1.4.1 DOPAMINE AND ETHANOL*

Ethanol appears to facilitate both the direct and indirect release of DA. Ethanol directly activates DA neurons in the VTA in brain slices (Brodie et al., 1990). Additionally, ethanol administered both systemically and directly infused in the nucleus accumbens (NAcc) appears to cause a dose-dependent increase in DA (Imperato & Di Chiara, 1986; Yoshimoto et al., 1992). Research in rodents suggests that DA levels in the NAcc are elevated indirectly as an effect of ethanol on cholinergic (Ericson et al., 2003), GABAergic, and opioidergic neurotransmission (Cowen & Lawrence, 1999). Additionally, after repeated ethanol administration, an injection of vehicle also caused an increase in DA (Philpot & Kirstein, 1998). This indicates that expectancy alone may increase DA release (Philpot & Kirstein, 1998).In addition to increasing DA release, ethanol also affects DA receptors. Ethanol-induced activity may be mediated by DA receptors (Cohen et al., 1997). For example, administration of a D3 antagonist attenuates ethanol consumption in mice, and D3 deficient mice consume less than wild type (Leggio et al., 2014). The ethanol-induced DAergic activity may be associated with ethanol reward.

### 1.2.1.5 OPIOID SYSTEM

The endogenous opioid system has been implicated in the rewarding effects of drugs of abuse, including ethanol. Endogenous opioids are peptides. There are three different types of opioid peptides associated with reward cognition, including the endorphins, enkephalins, and dynorphins (Froehlich, 1997). These peptides are derived

from three distinct pro-peptide precursor proteins, proopiomelanocortin (POMC), pro-enkephalin, and pro-dynorphin, respectively. When cleaved, POMC creates β-endorphin, pro-enkephalin becomes leu-enkephalin and met-enkephalin, and pro-dynorphin creates dynorphin-A, dynorphin-B, and neo-endorphin.

There are at least three primary opioid receptors (μ and δ and κ) (Waldhoer et al., 2004). All opioid receptors are coupled to Gi proteins, but each is associated with very different subjective effects (Williams et al., 2001). The μ-opioid receptors (MOP) are located throughout the brain both pre-and post-synaptically and are found in areas associated with reward, such as the mesolimbic and mesocortical regions. When activated in the VTA, MOP inhibits GABA and thus downstream disinhibits DA (Waldhoer et al., 2004). β-endorphin is the primary endogenous ligand for MOP, although leu-enkephalin and met-enkephalin both have low affinities for MOP. Activation of MOP is often associated with euphoric feelings. The δ-opioid receptor (DOP) is more restricted in the regions it is found in, but DOP is also found readily throughout the limbic system and may be involved in nociception (Vanderah, 2010). The enkephalins and β-endorphin have a high affinity for DOP and when this receptor is activated, intercellular signaling is initiated. The κ-opioid receptor (KOP) is found fairly ubiquitously throughout the brain, including the mesolimbic and mesocortical regions where KOP is thought to act in opposition to MOP. The dynorphins have a high affinity for KOP, and activation of these receptors tends to be unpleasant and associated with dysphoric feelings.

#### *1.2.1.5.1 ETHANOL AND OPIOIDS*

Opioid levels, synthesis, and release are altered following chronic ethanol administration. Ethanol may affect the binding properties of opioid receptors. However, the effect of ethanol within the opioidergic system appears to be region and dose-dependent. Overall, ethanol consumption appears to increase the release of β-endorphin, and activation of MOP is required for rodent self-administration of ethanol (Roberts et al., 2000). MOP knockouts do not self-administer ethanol and may even develop an aversion to ethanol (Roberts et al., 2000). Further, the administration of MOP antagonists results in a decrease in ethanol responding and consumption in rodents (Samson & Doyle, 1985; Ripley et al., 2015). Conversely, DOP knockouts have been shown to administer more ethanol than wild type mice (Van Rijn & Whistler, 2009). This may be due to the interaction of ethanol in the paraventricular nucleus (PVN) since local infusions of DOP agonists have been shown to increase ethanol consumption (Barson et al., 2009). Additionally, genetic variations in the genes for pro-dynorphins and KOP have been shown to be associated with an increased risk of alcohol dependence (Xuei et al., 2006).

### 1.2.1.6 ACETYLCHOLINE

Acetylcholine (ACh) is the primary neurotransmitter used at neuromuscular junctions and has many different functions within the CNS. ACh is synthesized from acetyl coenzyme A and choline by choline acetyltransferase. Within the CNS, ACh has widespread projections to the cortex and works as a neuromodulator by influencing synaptic transmission and excitability (Picciotto et al., 2012).

The cholinergic system is the target of multiple drugs of abuse, most notably, nicotine. Cholinergic receptors are named for the drugs that activate them and grouped into the ionotropic nicotinic (nAChR) and metabotropic muscarinic (mAChR) families (Wess et al., 2003). Five types of mAChRs have been identified. The M1, M3 and M5 receptors are couples with Gq proteins and activate phospholipase C. The M2 and M4 receptors are coupled to a Gi protein which inhibits adenylyl cyclase and may be located both pre- and post- synaptically (Wess et al., 2003). The nAChRs are cation channels and are identified by the composition of their α and β protein subunits. The α4β2 and α7 receptor subtypes are the most numerous nAChRs in the brain (Davis et al., 2006). Following activation of the α4β2 receptor by a ligand, the receptor allows for both Na+ and Ca2+ to enter the neuron whereas α7 receptors primarily allow for Ca2+ to enter the cell following activation. The α7 are expressed on Glu terminals and the α4β2 receptors are expressed on GABAergic terminals (Mansvelder et al., 2002), the primary neurotransmitters affected by ethanol.

#### *1.2.1.6.1 ACETYLCHOLINE AND ETHANOL*

Ethanol directly acts on some nAChRs. Ethanol enhances the function of the α4β2 receptor, but it inhibits the function of the α7 receptor (Cardoso et al., 1999). Ethanol can also alter number of nAChRs. In cultured cells exposed to ethanol for 48 hours, ethanol exposure decreased nAChRs, however, by 96 hours of exposure to ethanol nAChRs were elevated compared to controls (Dohrman & Reiter, 2003). Additionally, a partial agonist for the a4b2 nicotinic receptor, varenicline, may reduce ethanol consumption. Varenicline has been shown to reduce ethanol consumption in smokers who were heavy drinkers

(Mitchell et al., 2012), and has been shown to reduce ethanol consumption in rodents (Chandler et al., 2020; Hendrickson et al., 2010), and nonhuman primates (Kaminski & Weerts, 2013). However, varenicline failed to attenuate an ethanol-induced place preference in mice but it did reduce the stimulant locomotor effects of ethanol (Gubner at al., 2014).

### 1.2.2 PHARMACOKINETICS OF ETHANOL

When ethanol is consumed orally, it gets absorbed into the bloodstream through the small intestine. The primary method of elimination is by hepatic alcohol dehydrogenase (ADH) which catabolizes ethanol. Approximately 90% of ethanol is catabolized by the enzyme ADH in a zero-order fashion, specifically a constant amount of ethanol is eliminated per time independently of the dose of ethanol. About 0.11% of ethanol is excreted unchanged through breath, urine, and sweat (Holford, 2012). ADH is mostly located in the liver, but it is also found in other tissues as well including in the stomach lining. ADH catalyzes the oxidation of ethanol. ADH along with the cofactors nicotinamide adenine dinucleotide (NAD+) and zinc process ethanol into acetaldehyde. Acetaldehyde is then further processed into acetate by aldehyde dehydrogenase (ALDH). Acetate is then processed by acetyl synthetase into acetyl CoA. The remaining ethanol is metabolized by the microsomal ethanol oxidizing system (MEOS) by a CYP2E1 enzyme into acetaldehyde. Repeated consumption of ethanol results in the upregulation of MEOS (for review see, Lieber, 2004; Shigeta et al., 1983). Upregulation is results in increased catabolism of ethanol in alcohols and may contribute to increased rates of liver diseases because of the creation of free radicals during the metabolism process (Lieber, 1999).

Although less ethanol metabolism occurs in the stomach, ADH located in the stomach lining is involved in some of the first-pass metabolism of ethanol following consumption. Research by Frezza et al. (1990) indicates a sex difference in the first-pass metabolism of ethanol. In gastric biopsy samples, women under 50 had less ADH activity than age-matched men (Steiz et al., 1993). However, Parlesak and colleagues (2002) found this difference was reversed in older subjects, specifically gastric biopsy samples from middle-aged women had greater ADH activity than age-matched men. In addition to sex differences, there is a negative relationship between the number of daily drinks and ADH activity (Parlesak et al., 2002). Therefore, gastric ADH is dependent on sex, age, and ethanol use.

Hepatic liver ADH is the same when alcoholics are compared to non-alcoholics, but ADH does become reduced in individuals with liver disease (Vidal et al., 1990). Additionally, the risk of alcohol use disorder may be somewhat related to ADH activity. There are genetic variations of ADH that result in faster or slower metabolism of ethanol (Bosron & Li, 1986). Subjects with the slow ADH genotype (i.e., ADH1B 1/1) tend to drink more than subjects who have the fast ADH genotype (i.e., ADH1B 1/2) due to those with the faster ADH possibly resulting in elevated acetaldehyde build up (Tolstrup et al., 2008). Therefore, ADH activity is related to ethanol consumption and perhaps the development of an AUD.

#### 1.2.2.1 ALCOHOL DEHYDROGENASE IN RODENTS

Similar to humans, research has found that male mice have more gastric ADH activity than female mice (Desroches et al., 1995). In the liver, hepatic ADH activity is

higher in female mice compared to male mice (Kishimoto et al., 2002). In rats however, research has found that gastric metabolism of ethanol is negligible, and most first-pass metabolism is done by the liver (Smith et al., 1992). The role of hormones on ADH activity is still under debate. In male and female rats, neither castration nor ovariectomy had any effect on ADH activity in some studies (Mezey et al., 1992), but others have found that castration increases ADH activity (Quintanilla et al., 2007). However, estradiol-treated rats had increased hepatic ADH activity when not exposed to alcohol, but when these animals were maintained on an alcohol diet, there was no effect of estradiol on ADH (Teschke et al., 1986). Female hamsters have twice as much hepatic ADH as male hamsters and recover their righting response faster than males (Moss et al., 1987). Although the literature is inconclusive on the effect of hormones on ADH activity, it appears as though metabolism differences are related to observed behavioral sex differences. Further, sex differences in ADH appear to be species-dependent.

#### 1.2.2.2 ALCOHOL DEHYDROGENASE IN BIRDS

In birds ADH activity can vary significantly by species. Waxwings have about 15 times more ADH activity than starlings and six times more activity compared to greenfinch (Eriksson & Nummi, 1982). Researchers have characterized avian ADH enzymes in both quail and quail-chick hybrids (Castro-Sierra & Ohno, 1968; Ohno et al., 1969). Specifically, quail have four classes of ADH enzymes that share similarities with mammalian ADH enzymes (Nussrallah et al., 1989). ADH expression in quail livers is dependent on both age and sex (Nussrallah et al., 1989). Young female quail have ADH1, ADH2, and ADH3 expressed in liver tissue, but as they mature ADH3 becomes the only

detectable ADH. Male quail exhibit the reverse when they are young. Males only express ADH3, but as they age ADH1, ADH2, and ADH3 are all expressed in liver tissue. Both ADH2 and ADH3 are similar to mammalian ADH enzymes. The elimination rate of quail ADH2 and ADH3 is similar to the human class 1 ADH enzyme, and both human and quail ADH are similarly inhibited by fomepizole, an ADH inhibitor (Nussrallah et al., 1989). However, despite these findings no research has examined ADH enzymes in Japanese quail in vivo.

### 1.2.3 BLOOD ETHANOL CONCENTRATION

Blood ethanol concentrations (BECs) may influence behavioral and cognitive effects following ethanol consumption. Low BECs are often associated with reduced inhibition and relaxation (Koob & LeMoal, 2005). Higher BECs are related to memory loss, loss of coordination, and loss of consciousness, and risk of death. Additionally, individuals may develop an acute tolerance to ethanol within a single administration. Specifically, the effects of ethanol tend to be greater while BECs are rising than when BECs are falling, even if BECs are the same due to an acute tolerance to ethanol (Portans et al., 1989). BECs on the increasing side of the curve tend to be associated with hedonic effects, and the same BEC on the descending limb may be associated with adverse subjective effects (Evens & Leven, 2004). Various factors affect BECs in both humans and animals.

#### 1.2.3.1 FACTORS THAT INFLUENCE BECS IN HUMANS

Blood ethanol concentrations (BECs) can be affected by the route of administration, previous experience with ethanol, and sex. For example, BECs are higher when alcohol is administered systemically compared to orally (Frezza et al., 1990). These differences between systemically and orally administered ethanol are a result of gastric ADH activity and the first-pass metabolism of alcohol when consumed orally (Julkunen et al., 1985). Furthermore, repeated alcohol use causes an increase in CYPE450 enzymes, thus increasing the efficiency of ethanol metabolism by the MEOS system (Lieber, 1999). Therefore, individuals who use ethanol frequently will metabolize ethanol faster and therefore have lower BECs than individuals who do not use ethanol as frequently.

There are known sex differences in the pharmacokinetics of alcohol including, peak levels, absorption rates, and elimination rates (Baraona et al., 2001). These sex differences may be due to many different factors, including differences in body water (Goist Jr, & Sutker, 1985), gastric emptying (Datz et al., 1987; Wald et al., 1981), liver weight (Dettling et al., 2007), and enzymatic activity (for review see; Crabb, Bosron, & Li, 1987; Frezza et al., 1990). At a given dose of alcohol, women achieve higher BECs than men (Frezza et al., 1990; Jones & Jones, 1976), but women also have faster elimination rates (Baraona et al., 2001; Dettling et al., 2007). Differences in peak levels appear to be affected by the increased role of gastric ADH in men (Frezza et al., 1990; Seitz et al., 1993). Men have increased gastric ADH activity in the stomach compared to women (Seitz et al., 1993). However, the research is inconsistent with regards to the role of gonadal hormones in these metabolism differences. Although alcohol affects estradiol and progesterone levels in women (Sarkola et al., 1999), the effects of sex hormones on alcohol metabolism are less clear. Some research has shown that there are alcohol

metabolism differences across the menstrual cycle (Sutker, Goist, & King, 1987), while other researchers have not found BEC differences across the menstrual cycle (Dettling et al., 2010) or in individuals taking birth control compared to freely cycling individuals (Hay et al., 1984).

#### 1.2.3.2 FACTORS THAT INFLUENCE BECS IN RODENTS

In rodent models, BECs are affected by the route of administration, sex, and strain. Mice tend to have a faster rise to peak BECs and faster metabolism of ethanol than rats (Livy et al., 2003). Both rats and mice administered ethanol via intragastric gavage tend to have a lower peak than intraperitoneal treated rodents (Livy et al., 2003). In rodents, the research is mixed with regard to the effect of sex on BECs. Some research found no sex difference in BECs (Livy et al., 2003; Torres et al., 2014), while others have observed a difference between males and females (Crippens et al., 1999; Desroches et al., 1995; Moss et al., 1987). Specifically, male rats injected (ip) with alcohol had higher BECs than females, and females had faster elimination rates and smaller area under the curve (AUC) than males (Crippens et al., 1999). Similarly, Desroches and colleagues (1994) found that male mice have higher BECs following ip injections; however, they also found that following a gavage, females have higher BECs. Sprague Dawley rats exposed to ethanol vapor achieve lower BECs than Long Evans rats (Glover et al., 2021). These sex differences are thought to result from differences in ADH activity.

#### 1.2.3.3 FACTORS THAT INFLUENCE BECS IN BIRDS

To our knowledge, no one to date, has examined BECs in quail. In general, there have been relatively few studies investigating BECs in birds. Olsen et al. (2013) administered 2 or 3 g/kg ethanol (i.e., ip) to zebra finch and collected blood samples at various time points. They found that at 30 min following injection, BECs were elevated and at 210 min following injection the BECs had not decreased significantly (Olsen et al., 2013). One other study examined BECs in a few different species of birds that consume berries (Eriksson & Nummi, 1982). Following this, they examined the rate of elimination in waxwings, starlings, and bullfinches. They injected each species with either 1 or 2 g/kg ethanol and measured the ethanol elimination at various time points for each species. Waxwings eliminated ethanol faster than the other species, at a rate of 900 mg/kg/h, followed by starlings (i.e., 270 mg/kg/h), and bullfinches were the slowest at eliminating ethanol at 130 mg/kg/h (Eriksson & Nummi, 1982). Taken together, there appears to be considerable variability in the rate of ethanol elimination dependent on species of bird.

### 1.2.4 CONDITIONED PLACE PREFERENCE AND AVERSION

Conditioned place preference (CPP) is a Pavlovian conditioning paradigm that assesses whether a stimulus is rewarding or aversive. Conditioning occurs when a specific environment is repeatedly paired with a stimulus, such as a drug of abuse. Following repeated drug and environment pairings, the rewarding or aversive properties of the drug are assessed in a drug-free state by measuring the time spent in the drug-paired environment (for review see, Bardo & Bevins, 2000). If a drug is rewarding, the animal tends to spend more time in the drug paired chamber and if the animal finds the drug aversive, they spend less time in the drug paired chamber.

#### 1.2.4.1 PLACE PREFERENCE AND AVERSION IN RODENTS

In rodents, the rewarding qualities of ethanol have been difficult to assess due to ethanol both having rewarding and aversive qualities. There is more evidence for an ethanol-induced CPP in mice than rats in the literature. The Cunningham lab has examined ethanol-induced CPP in different strains of mice and found that DBA2/J mice are more sensitive to ethanol reward compared to C57BL6/J (Cunningham & Shields, 2018). They also have examined various administration routes and have found that CPP is more readily acquired when ethanol is administered by ip (Cunningham et al., 1993) or intragastric catheter (Ciccocioppo et al., 1999; Cunningham et al., 2002), but these effects may be strain-dependent (see Fidler et al., 2004). Furthermore, conditioning trial duration affects the strength of conditioning. In mice, shorter (i.e., 5 min) trials resulted in stronger preference than longer (i.e., 30 min) trials (Cunningham & Prather, 1992). This change in preference is thought to be due to the shift in the hedonic effects of alcohol. Initially, alcohol has rewarding effects, but over time, alcohol produces aversive effects (Risinger & Cunningham, 1992).

In addition to the route of administration and trial duration, many other factors alter the expression of an ethanol-induced CPP, including sex and hormones (Hilderbrand & Lasek, 2018; Roger-Sánchez et al., 2012). In mice both young and late adolescent females developed an ethanol-induced CPP but only young adolescent males developed a CPP (Roger-Sánchez et al., 2012). One reason for enhanced female sensitivity may be due to estradiol as Hilderbrand & Lasek (2018) found that in mice estradiol enhanced an

ethanol CPP through activation of ERα and ERβ. Additionally in rats, research has found that only intact females develop a place preference following ethanol conditioning and that neither males nor ovariectomized females develop a CPP (Torres et al., 2013).

The rewarding and aversive effects of ethanol in rats are much less clear. There has been an abundance of studies showing that rats find ethanol aversive (Bormann & Cunningham, 1997; Cunningham et al., 1981; Gauvin & Holloway, 1992; Stewart & Grupp, 1989; Van der Kooy et al.,1983) or develop no conditioning to the drug paired chamber (Bienkowski et al.,1996; Bormann & Cunningham, 1997; Ciccocioppo et al., 1999; Van der Kooy et al.,1983) and a few demonstrating a CPP (Ciccocioppo et al., 1999; Stewart & Grupp, 1989; van der Kooy et al.,1983). Many different factors may influence whether ethanol treatment results in a place preference or aversion in rats (for review see, Fiddler et al., 2004).

## 1.3 QUAIL MODEL

Even though no research has examined ethanol's rewarding and aversive effects in birds, ethanol is not expected to be aversive in birds because they consume ethanol in the wild in the form of fermented fruits (Kinde et al., 2012). A variety of species, including quail, have also been shown to consume ethanol in a laboratory setting (Bashir & Javed, 2005; Eriksson & Nummi, 1982; Olson et al., 2014). Ethanol is provided in either food or juice and birds consume enough to exhibit behaviors associated with intoxication, often sitting on the floor of the cage or stumbling (Bashir & Javed, 2005; Olson et al., 2014).

Quail also have good color vision and acuity (Kovach, 1974), and within the CPP paradigm, this allows for visual manipulation of the environmental cues associated with drugs. Specifically, previous research has found that quail can distinguish either a yellow or green drug paired environment and develop a CPP to a psychostimulant paired environment. In several studies, repeated pairings of either cocaine or nicotine paired chamber resulted in the development of a CPP to the drug-paired chamber (Bolin et al., 2012; Gill et al., 2016; Akins et al., 2004). A similar cue-based phenomenon is thought to be involved in drug-taking and relapse behaviors in humans (Witteman et al., 2015). Within the framework of classical conditioning, ethanol often serves as the unconditioned stimulus (US), and the conditioned stimuli (CS) paired are ethanol-related cues or neutral cues. Following the pairing of ethanol with the CS, various conditioned responses are then assessed. For example, in a laboratory setting, Mayo and De Wit (2016) paired a neutral cue (i.e., landscape) with alcohol in social drinkers. These participants have increased attention and subjective ratings of a neutral cue paired with ethanol (Mayo & De Wit, 2016). Taken together, quail readily acquire a CPP to rewarding stimuli and thus may be a good model to study visual cues associated with ethanol reward.

## 1.4 CURRENT EXPERIMENTS

Alcohol abuse is a worldwide problem resulting in multiple health risks and great socioeconomic costs. Current rodent research has found mixed results regarding the rewarding or aversive effects of ethanol (for a summary see, Fidler et al., 2004). These effects seem to vary significantly by strain and may only be evident in mice and rat strains that have greater sensation seeking (Dickson et al., 2008; Manzo et al., 2014).

This may be due to an evolutionary trait (Crabbe et al., 2010) to avoid ethanol and therefore rodents might not be the best model to study AUD. Birds may provide a useful model to examine AUD because they will consume fermented fruits in the wild and ethanol may not be aversive. Furthermore, a controlled oral administration (i.e., gavage) may be easier to administer in a bird model than in rodents due to differences in esophagus and trachea placement. However, to develop a quail model of AUD, there should be an understanding of both ethanol pharmacokinetics and ethanol associated reward behaviors.

The present set of experiments examines pharmacokinetics and behaviors associated with ethanol. In the current experiment, ADH levels were measured in male and female livers because ADH is the primary enzyme involved in ethanol metabolism, and the level of ADH is related to observed BECs. (Haseba et al., 2012). Additionally, ADH levels may be related to observed behavioral differences (Moss et al., 1987). For example, female hamsters have twice as much hepatic ADH as males and recover their righting response faster than males, presumably due to faster clearance of ethanol from their system (Moss et al., 1987). Both subjective and behavioral responses are dependent on BEC. At low BECs, an individual may experience elevated mood and stimulating effects of ethanol, moderate BECs are associated with exaggerated behaviors and loss of coordination, and high BECs are accompanied by memory trouble and heavy sedation (Koob & LeMoal, 2005). Due to the biphasic effects of ethanol, most research aims to assess the behaviors associated with the ascending limb of the BEC curve. A BEC profile for quail, similar to that in rodents, will account for BECs across time following administration of a high and low dose. Along with BECs across time, ethanol absorption

and disappearance, and peak BECs are assessed. Peak BECs may be a variable that is easily compared across species to determine the ethanol exposure when absorption and metabolism vary (Livy et al., 2003). Lastly, we examine the rewarding and aversive properties of a low and high dose of ethanol in male and female quail.

# CHAPTER 2. SEX DIFFERENCES OF ETHANOL PHARMACOKINETICS IN JAPANESE QUAIL

## 2.1 INTRODUCTION

Ethanol is a central nervous system depressant and it is consumed for its psychoactive effects (Hendler et al., 2011). When consumed, ethanol is readily absorbed into the bloodstream from the small intestine and is distributed throughout the body into peripheral tissues (Levitt et al., 1997). There are a few factors that influence the rate at which ethanol is absorbed, including the amount of ethanol consumed, body composition, gastric emptying, and enzymatic activity (for review see, Crabb et al., 1987; Frezza et al., 1990). The amount of ethanol consumed affects absorption as higher doses diffuse across membranes more readily. Additionally, the rate at which the stomach is emptied affects the rate at which ethanol is absorbed such that faster emptying results in higher blood ethanol concentration (BEC) (Holt, 1981). The distribution of ethanol may also be influenced by body composition, specifically by total body water. The same dose of ethanol may vary drastically due to fat and water variations in the bodies, even in individuals who weigh the same amount (Davies & Bowen, 1999).

Following absorption, ethanol is mainly metabolized in the liver by two pathways (Cederbaum, 2012). The primary pathway uses alcohol dehydrogenase (ADH) and breaks down ethanol by catalyzing the oxidation of ethanol into acetaldehyde, a toxic metabolite. Acetaldehyde causes many unpleasant effects including facial flushing and nausea.

Acetaldehyde is further broken down by aldehyde dehydrogenase (ALDH) into acetate, which can be further broken down into acetyl CoA. These pathways account for approximately 90% of the metabolism of ethanol (Cederbaum, 2012). The main site for ethanol metabolism is the liver and ADH levels are highest in the liver compared to other tissues (Boleda et al., 1989).

In both humans and animals, there appears to be a sex difference in ADH levels that may affect BEC. In humans, men have higher gastric ADH activity than women resulting in men having lower peak BECs when alcohol is consumed orally (Frezza et al., 1990; Seitz et al., 1993). However, women have higher ADH activity in the liver (Mezey, 2000) which may result in faster elimination of ethanol (Dettling et al., 2007). Although Dettling et al. (2007) found women eliminate ethanol more rapidly, however, this effect was diminished when liver weight was considered. Similarly, male mice have more gastric ADH activity than female mice (Desroches et al., 1995). However, compared to males hepatic ADH activity is higher in female mice and rats (Kishimoto et al., 2002; Simon et al., 2002). This difference in ADH activity may be related to differences in ethanol elimination. In mice, the research has been mixed as to the sex difference in the elimination of ethanol. Kushimoto and colleagues (2002) found a sex difference in the disappearance of ethanol but other research has not (Livy et al., 2003; Lopez et al., 2003). Rats have a similar pattern where females have more ADH (Quintanilla et al., 2007; Simon et al., 2002) and faster ethanol elimination compared to males (Robinson et al., 2002). Taken together, it follows, that sex differences observed in ADH activity may contribute to a faster ethanol disappearance rate from the blood.

ADH may also be affected by ethanol administration. Drosophila larvae fed an ethanol diet had a two-fold increase in ADH levels compared to larvae fed a control diet (McKechnie & Geer, 1984). In zebrafish, ADH activity following acute ethanol exposure follows an inverse U pattern based on the dose (Tran et al., 2015). Similarly, in mice, both ADH activity and content are affected by dose and follow a similar inverse U pattern (Haseba et al., 2012). Chronic administration of ethanol to rats results in a gradual increase of ADH, peaking at 26 weeks before decreasing (Dajani et al., 1963). Taken together, ADH appears to be higher following treatment with ethanol.

The research on ADH in quail has been limited to the development of ADH classes and their expression in quail, and in vitro studies. Quail have four classes of ADH enzymes that share some similarities with mammalian ADH enzymes (Nussrallah et al., 1989). ADH develops similarly between males and females early in ontogeny, but adult levels mainly differ in the class that is dominantly expressed, with males expressing the ADH1, ADH2, and ADH3 classes whereas females predominantly express the ADH3 class. The elimination rate of quail ADH is similar to the human class 1 ADH enzyme (Kaiser et al., 1990).

Research has previously established BECs over time in both rats and mice (Livy et al., 2003), however, there is relatively little known about the BEC profile in birds. One study examined BECs in fruit-eating birds (Eriksson & Nummi, 1982). Eriksson & Nummi, (1982) injected fruit-eating birds with 1 or 2 g/kg ethanol and observed the rate of elimination in waxwings, starlings, and bullfinches. They found that the rate of ethanol elimination varied greatly between these species (Eriksson & Nummi, 1982). Another study examined a more controlled approach by injecting ethanol ip (i.e., 2 and 3 g/kg) in

finches (Olson et al., 2014). They found that finch BECs rose rapidly in 30 min and remained elevated for at least 3 hours (Olson et al., 2014). However, no studies in birds have examined BECs across time following an oral gavage. Additionally no one has created a BEC profile in quail.

The purpose of the current research is to examine the ADH levels in male and female quail since these enzymes may play a role in the development of AUD, and develop a BEC profile in Japanese quail. Quail were chosen because previous research has shown that they may be a good model to study behaviors associated with drug use (Akins & Geary, 2008; Bolin et al., 2012; Mills et al., 1998; Rosine et al., 2008). However, there is currently no research examining ethanol pharmacokinetics in quail. We hypothesize that there will be a sex difference in ADH because, in unpublished studies, female quail appeared to metabolize ethanol more quickly than male quail. Therefore, we expect female quail to have higher ADH levels than males. We also hypothesize that ethanol treatment will have an effect on ADH levels. Similar to previous research that has created a BEC profile for rats and mice (Livy et al., 2003), the current research aimed to create BEC profiles of a high and low dose of ethanol. This BEC profile will allow for a better understanding of the pharmacokinetics of blood ethanol levels following an oral administration in quail. We predict a fairly rapid rise in BECs followed by a slow metabolism in our high dose. We also hypothesize a dose-dependent difference in peak BEC. Additionally, we predict females will metabolize ethanol faster than males. Therefore we expect a smaller area under the curve for females compared to males.

## 2.2 EXPERIMENT 1

### 2.2.1 METHODS

#### 2.2.1.1 SUBJECTS

Adult male (n = 18) and female (n = 23) quail were the subjects in this experiment. Fertilized eggs were supplied by GQF (Savannah, GA) and hatched and raised at the University of Kentucky. All quail were housed under a 16:8 L:D cycle and had *ad lib* access to food and water. Quail were kept in mixed-sex brooders until 28 days post-hatch, and then males were individually housed, and females were group-housed. Before starting the experiment, all quail were individually housed and habituated to the colony for at least two weeks. Quail were randomly assigned to receive water (n = 22) or ethanol (n = 19) repeatedly for 12 days. All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Kentucky.

#### 2.2.1.2 DRUGS

Water or ethanol (25% w/v) was administered by gavage daily for 12 days.

#### 2.2.1.3 ELISA PROCEDURE

Twenty-four hours after the last administration, quail were euthanized by rapid decapitation and trunk blood was collected in heparinized tubes. The tubes were then

centrifuged at 1500 RPM (21,890 x g) for 15 min, and plasma separated. Livers were extracted and washed in PBS before being frozen in isopentane, cooled by dry ice. All samples were stored at - 80℃ until assayed. In preparation for the ELISA, livers were thawed, rinsed, and weighed. Then 200 mg of the liver was added to 500 ul of phosphate-buffered saline (PBS) and homogenized in a glass tube homogenizer on ice. The liver homogenate was then sonicated. The samples were centrifuged at 1500 RPM (21,890 x g) for 10 min, the lipid layer was then removed, and the samples respun at 1500 RPM (21,890 x g) for 15 min before the supernatant was collected.

Alcohol dehydrogenase (ADH) levels were measured in duplicate via an enzyme-linked immunoassay (ELISA) kit (MyBioSource; MBS743834) according to the manufacturer's instructions. Briefly, 100 ul of samples and standards were transferred to assigned wells, followed by 50 ul of enzyme conjugate. The plate was then allowed to incubate for 1 hour at 37℃. After incubation, the wells were washed five times, and 50 ul of each substrate was added and allowed to incubate for 20 min at 37℃. A stop solution was then added and optic densities were immediately analyzed at 450 nm using a Beckman Coulter DTX 880 Multimodal Detector (Lagerhausstrasse, Austria) and Beckman Coulter Multimode Detection Software (v.20.0.12). Results were determined using a four-parameter logistic standard curve analysis within SigmaPlot version 14 (Systat Software, Inc., San Jose, CA).

#### 2.2.1.4 STATISTICAL ANALYSIS

Liver weights were measured as a percent of total body weight (liver weight/total body weight). Sex differences and the effect of ethanol on ADH levels and liver weights

were analyzed by a 2 x 2 (sex x treatment) analysis of variance (ANOVA) using SPSS (IBM Corp., version 27).

### 2.2.2 RESULTS

#### 2.2.2.1 ADH LEVELS IN QUAIL LIVERS

ADH levels (ng/mL) in male and female livers are shown in Figure 2.1. Female quail ($M$ =20.436, $SEM$ = 3.413) had higher ADH levels than male quail ($M$ = 8.99, $SEM$ = 3.114) as revealed by a main effect of sex, $F(1,27)= 6.137$, $p=0.020$, $\eta 2 = 0.166$. There was no main effect of treatment $F(1,27)= 0.354$, $p=0.557$, $\eta 2 = 0.013$, and no significant interaction between sex and treatment $F(1,27)= 2.476$, $p=0.127$, $\eta 2 = 0.084$.

#### 2.2.2.2 LIVER WEIGHTS

Figure 2.2 shows the overall weight of the livers in grams. An ANOVA revealed a significant main effect of sex of liver weight, $F(1,37)= 29.747$, $p< 0.001$, $\eta 2 = 0.446$. Female quail ($M$ = 479.688, $SEM$ = 24.037) had significantly heavier livers than male quail ($M$ = 266.158, $SEM$ = 30.902). However there was no main effect of treatment, $F(1,37)= 0.120$, $p=0.731$, $\eta 2 = 0.003$, nor an interaction between sex and treatment, $F(1,37)= 0.0$, $p= 0.999$, $\eta 2 = 0.0$.

#### *2.2.2.3* RATIO OF LIVER WEIGHT TO TOTAL BODY WEIGHT

Figure 2.3 shows the liver weights as a ratio of total body weight. An ANOVA revealed a significant main effect of sex in the ratio of liver weight to body weight, $F(1,37)= 34.439$, $p< 0.001$, $\eta 2 = 0.482$. Female quail ($M = 0.031$, $SEM = 0.001$) had a significantly greater liver weight to body weight ratio than male quail ($M = 0.017$, $SEM = 0.002$). However, there was no main effect of treatment, $F(1,37)= 0.595$, $p=0.445$, $\eta 2 = 0.016$, nor an interaction between sex and treatment, $F(1,37)= 0.0$, $p= 0.984$, $\eta 2 = 0.0$.

## 2.3 EXPERIMENT 2

### 2.3.1 METHODS

#### 2.3.1.1 SUBJECTS

The subjects in this experiment were male (n = 23) and female (n = 25) quail. Fertilized eggs were purchased from GQF manufacturing (Savannah, GA) and hatched and raised at the University of Kentucky (7 birds were provided as adults by Centre College, Danville, KY). Following hatch, quail chicks were kept in mixed-sex brooders until approximately 28 days post-hatch. Male quail were then individually housed, and female quail were group-housed. All quail were maintained on a 16:8 L:D cycle. Prior to the day of the experiment, quail had ad lib access to food and water. On the day of the experiment, their feed was removed 1 hour before gavage, and they did not have access to food again until 4 hours following the gavage. All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of

Laboratory Animals, and experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Kentucky.

#### 2.3.1.2 BLOOD ALCOHOL CONCENTRATION

Based on previous research (Gauvin et al., 2000), quail were randomly assigned to receive a 0.75 or 2.0 g/kg dose of ethanol by gavage (25% w/v alcohol in tap water). Blood was collected from the brachial (i.e., wing) vein at 30 min, 1, 2, 4, 6, 8, 12, and 24 hours after gavage. These time points were chosen because they would help create a complete metabolism curve. Previous research suggests that the metabolism and elimination of ethanol may be substantially longer in birds than in rodents (Olson et al., 2014). Blood samples were centrifuged at 1500 RPM (21,890 x g) for 5 min, and plasma separated and stored at - 80℃ until assayed.

Plasma was used to measure BECs using an Analox AM1 instrument (Analox Instruments, London, England). BEC analysis was used to determine various pharmacokinetics, including peak levels and area under the curve in quail.

#### 2.3.1.3 STATISTICAL ANALYSIS

. To analyze the pharmacokinetics of alcohol, absorption and elimination were analyzed as the slope to or from peak using a simple slope analysis in GraphPad (version 8.0.0 for Windows, GraphPad Software, San Diego, California USA). The other measures were analyzed using a repeated-measures ANOVA with sex (male and female) and ethanol dose (0.75 and 2.0 g/kg) as between factors and time (30 min, 1, 2, 4, 6, 8,

12, and 24 hours) as the within-subjects factor to determine any differences in BECs. When the assumption of sphericity was violated, the Greenhouse-Geisser correction was used, with α set at < 0.05. Additionally, the area under the curve was analyzed using GraphPad assessed and compared between groups. Post hoc analyses were done using Tukey tests.

### 2.3.2 RESULTS

#### 2.3.2.1 BEC PROFILE

Figure 2.4 shows the average BEC at each time point following a gavage of either 0.75 or 2 g/kg ethanol. BECs rose quickly reaching peak levels around 1 (0.75 g/kg) or 2 hours (2 g/kg) after gavage, followed by a slow reduction in BECs for the next few hours depending on the dose. A RM ANOVA revealed a main effect of time, [$F(3,36) = 25.08$, $p < .001$, $\eta 2 = 0.695$], a main effect of treatment [$F(1,11) = 16.702$, $p = 0.002$, $\eta 2 = 0.603$] and a time by treatment interaction [$F(3,36) = 5.472$, $p = 0.003$, $\eta 2 = 0.332$]. Post hoc analyses revealed quail treated with 0.75 g/kg had BECs that were significantly different from the first time point (i.e., 30 min) at 8, 12, and 24 hours, $p<.05$. They also had higher BECs at 60 min compared to 6, 8, 12, and 24 hours following ethanol administration, $p<.05$. Quail treated with 2 g/kg had significant differences in BECs for 30 min and 2, 4, 8, 12 and 24 hours, BECs at 60 min were significantly different from time points 6-24 hours, and their BECs at 2 hours were significantly greater than 30 min and 4-24 hours, $ps<.05$. No main effect of sex or an interaction with sex was evident.

#### 2.3.2.2 PEAK LEVELS

Figure 2.5 shows the average peak BEC reached by male and female quail treated with 0.75 or 2 g/kg ethanol. A 2 x 2 ANOVA revealed a main effect of sex [$F(1,44)= 4.032$, $p=0.049$, $\eta 2 = 0.060$] on peak BEC levels. However there was a main effect of dose [$F(1,44)= 262.276$, $p<0.001$, $\eta 2 = 0.856$] on peak BEC levels. Quail treated with 0.75 g/kg ethanol ($M = 69.878$, $SEM = 5.443$) had lower peak BECs than quail treated with 2 g/kg ethanol ($M = 191.051$, $SEM = 5.133$). There was no interaction between sex and ethanol dose [$F(1,44)= 0.026$, $p=0.872$, $\eta 2 = 0.001$].

#### 2.3.2.3 AREA UNDER THE CURVE

Figure 2.6 shows the average area under the curve (AUC) for male and female quail treated with 0.75 and 2 g/kg. Student's t-test revealed a sex difference for quail treated with 0.75 g/kg [$t(144) = 2.004$, $p= 0.047$, $R^2 = 0.0271$]. When treated with 0.75 g/kg female quail ($M = 122.1$, $SEM = 17.2$) had a smaller AUC than male quail ($M = 198.3$, $SEM = 39.25$). Additionally there was a sex difference for quail treated with 2 g/kg [$t(96) = 2.107$, $p = 0.038$, $R^2 = 0.0442$]. Specifically female quail ($M = 544.2$, $SEM = 68.0$) had a smaller AUC than male quail ($M = 741.2$, $SEM = 64.2$).

#### 2.3.2.4 ETHANOL ABSORPTION

Ethanol absorption was measured as the slope of the BECs from the first time point (i.e., 30 min) to 60 min. The average amount of change in BEC was 42.91 for males and 58.59 for females treated with 0.75 g/kg ethanol. For quail treated with 2 g/kg

ethanol the average amount of change in BEC to peak was 99.82 for males and 63.34 for females. A comparison of the simple slopes revealed that there was no main effect of sex for 0.75 g/kg ethanol-treated quail, [$F(1,36) = 0.6360$, $p = 0.4304$] nor an effect of sex for 2 g/kg ethanol-treated quail [$F(1,38) = 0.4334$, $p = 0.5143$].

#### 2.3.2.5 ETHANOL DISAPPEARANCE

Ethanol disappearance was measured as the slope from peak to the first point following the peak BEC. The average amount of change in slope for BECs following the peak was -28.48 for males and -36.22 for females treated with 0.75 g/kg ethanol. For quail treated with 2 g/kg ethanol, the average amount of change in BEC following peak was -14.36 for males and -21.61 for females. A comparison of the simple slopes failed to reveal an effect of sex for 0.75 g/kg ethanol-treated quail, [$F(1,36) = 0.3679$, $p = 0.5480$] nor an effect of sex for 2 g/kg ethanol-treated quail [$F(1,24) = 1.541$, $p = 0.2264$].

## 2.4 DISCUSSION

The current studies examined ADH levels and BECs in male and female quail following repeated in vivo ethanol treatment. We observed an overall sex difference, however, we did not find an effect of repeated ethanol treatment. Specifically, female quail had greater ADH levels, greater overall liver weights, and a greater liver to body weight ratio than male quail. Additionally, we created a BEC profile for male and female quail. Female quail had lower peak BECs and a smaller AUC compared to male quail. However, there were no sex differences in the slope from the first time point to peak nor from peak to the next following time point.

The current study extends previous work that examined ADH in Japanese quail (Nussrallah et al., 1989) by quantifying the ADH levels present in the male and female liver. However, the previous work was mainly focused on the development of ADH throughout development. They observed sex differences in adult types of ADH present. Adult female quail primarily expressed ADH3, whereas adult males expressed ADH1, ADH2, and ADH3 (Nussrallah et al., 1989). An overall sex difference in ADH was not reported by Nussrallah and colleagues. Additionally, they pooled together livers to purify quail ADH and did not examine sex differences in ethanol kinetics (Nussrallah et al., 1989). Although the current study did not examine activity or ADH kinetics in liver samples, we found that ADH levels were greater in female quail compared to male quail. Therefore it would follow that female quail would metabolize ethanol faster than male quail.

Previous research has shown that females across species have more hepatic ADH compared to males. Female mice have higher ADH activities in liver samples than male mice (Kishimoto et al., 2002). Similarly, female rats have higher hepatic ADH activity compared to males (Quintanilla et al., 2007). This sex difference is found in human liver biopsy samples as well. Young (<50) women have higher ADH activity levels compared to young (<53) men. However, older individuals (i.e., postmenopausal) do not exhibit this sex difference (Maly & Sasse, 1991). Similarly the current findings were that female quail had higher ADH levels than males. Taken together, it this sex difference in ADH is apparent in a variety of species including human, rodent, and bird.

The current results did not show an increase in ADH levels following repeated ethanol treatment (i.e., 12 days). Perhaps no ethanol effects were observed due to the

length of time required to observe any change in ADH levels. ADH studies that found an increase in ADH levels following ethanol treatment found an increase in ADH activity following chronic or continuous exposure (Mirone, 1965; Tran et al., 2015). For example, ADH activity was elevated in rats pretreated with ethanol for 21 days compared to non-pretreated controls (Mirone, 1965). However, not all rodent studies found an increase in ADH activity. Eight weeks of exposure to ethanol vapor decreased ADH expression in the livers of rats (Mouton et al., 2016). Thus, the route of administration may also affect ADH activity. Zebrafish exposed to ethanol continuously for 22 days had an increase in ADH activity, but fish that only received ten days of repeated exposure did not show a change in ADH activity compared to controls (Tran et al., 2015). Therefore, even though we did not find a difference in ADH levels in our ethanol-treated quail, this may be in line with previous findings. In order to observe a difference in ADH levels in quail, an extended period of ethanol administration may be required.

Similar to previous research, we found a sex difference in both liver weights and liver weights relative to body weights. Female quail had much heavier livers than male quail. These findings are in agreement with most previous quail research that has found females have heavier livers than male quail (Salem et al., 2006; Toelle et al., 1991; Tserveni-Gousi & Yannakopoulos, 1986). In quail, liver weight is more highly correlated with muscle mass than with other internal organ weights (Toelle et al., 1991). However, Tarhyel et al. (2012) did not observe sex differences in liver weights. This may be due to the age at which the current study and previous studies euthanized the quail. The current study euthanized adult birds (i.e., over 90 days old) whereas Tarhyel et al. (2012) examined the organ weights of quail that were 52 days old. Similarly, previous rodent

research has found XX mice have heavier livers than XY mice when fed a high-fat diet (Chen et al., 2012). However, in people, no gender difference in calculated liver volumes is found (Kwo et al., 1998). This may just be due to humans not being as sexually dimorphic in relation to size as birds. Female quail also had a greater liver to total body weight ratio compared to male quail. Liver weight to body weight ratios in birds vary across species, and those with higher liver weight to body weight also have faster ethanol disappearance (Eriksson & Nummi, 1982).

Additionally, we did not see any changes in liver weight or the ratio to total body weight in the ethanol-treated group. This may be due to the same reason we did not observe changes in ADH levels. Perhaps the length of ethanol treatment needed to be longer to observe these differences. Rats pretreated with ethanol for 21 days had heavier liver weights compared to non-pretreated rats (Mirone, 1965). Furthermore, mice that were allowed one month *ad lib.* access to ethanol as the sole drinking fluid had heavier livers and a greater liver to body weight ratio (Okuda et al., 2018). Liver volume is strongly correlated to the elimination rate of ethanol in rats (Lumeng et al., 1978).

Our findings extend previous ethanol pharmacokinetics research into a bird model. Prior to the current study, no BEC profile for Japanese quail existed. In a previous finch study, researchers administered ethanol (2 or 3 g/kg) ip and found that BECs remained high 3 hours later (Olsen et al., 2013). Furthermore, Eriksson & Nummi (1982) found there was wide variability across species in the rates at which they eliminated ethanol. Specifically, they found that a 2 g/kg dose of ethanol administered ip was eliminated in about 2 hours in waxwings, starlings took about 3 hours, and greenfinches took about 13 hours (Eriksson & Nummi, 1982). The current study found that quail may

be similar to greenfinches as BECs were low around 12 hours after administration of 2 g/kg ethanol and ethanol was not detectable following 24 hours.

Both of these previous studies administered ethanol via an ip injection (Eriksson & Nummi, 1982; Olsen et al., 2014). The current study captured absorption, peak levels, and elimination following an ethanol gavage. Ethanol is typically consumed orally and there may be a first-pass metabolism of ethanol following an oral administration. Thus creating a BEC profile of ethanol following this type of administration is essential to account for the possibility of the first-pass metabolism.

BECs can vary widely across rodent species. Mice eliminate ethanol much faster than rats (Livy et al., 2003). In both rats and mice, BECs rose rapidly following ethanol administration. However, mice reached peak BECs at one hour after administration whereas rats did not reach peak until 2 hours after administration (Livy et al., 2003). The quail in the current study similarly reached peak levels at 1or 2 hours after ethanol administration dependent on dose. However, in quail, this dose-dependent change in peak may be due to a larger volume of ethanol being slowly released by the crop into the gastrointestinal tract. Other previous research has found that female rats reach peak levels more rapidly than males following an ip administration of ethanol (Crippens et al., 1999). In quail we did not observe a shift or a difference in slope to peak between males and females, perhaps due to differences in ethanol administration (i.e., ip vs. gavage). Due to differences in rates of absorption and metabolism between species, peak BECs may be a valuable measure allowing for comparison across species when examining the exposure to ethanol (Livy et al., 2003).

We did find a sex difference in peak BECs reached following an ethanol gavage. Male quail reached a higher peak BEC compared to females. Previous rodent research found that sex differences in peak BECs may be dependent on age. Livy et al. (2003) did not observe any sex difference in peak BEC levels, however, they used adolescent rats and mice, and therefore sex differences in ethanol pharmacokinetics may not be as apparent as in adults. Adults often exhibit a sex difference in BECs (Desroches et al., 1995; Middaugh et al., 1992) but not always (Torres et al., 2014). Contrary to the current experiment, female rodents often have greater peak BECs, however, this may be dependent on the route of administration and a sex difference in ADH activity (Desroches et al., 1995; Frezza et al., 1990).

Similar to previous research, female quail had a smaller AUC compared to male quail, and thus female quail had less overall exposure to ethanol compared to males. Previous rodent research similarly observed a smaller AUC for female rats compared to male rats (Crippens et al., 1999). In rats a larger AUC observed in males may depend on tissue measured, the AUC of BECs was 14% smaller in females but ethanol content was 16% smaller in brain tissue (Robinson et al., 2002). In contrast, most of the research in people has found that women have greater AUCs than men (Frezza et al., 1990; Lucey et al., 1999). This effect in women may be driven by the larger peak BECs they reach compared to males (Frezza et al., 1990), as they tend to have faster ethanol disappearance perhaps due to higher hepatic ADH activity (Vidal et al., 1990).

Contrary to our hypothesis, we did not observe any sex difference in ethanol disappearance (i.e., the slope from peak to the first following time point). Both human and rat studies found a higher rate of ethanol disappearance in females compared to

males. In humans, it has been typically observed that women have a faster elimination (Cole-Harding & Wilson, 1987; Mumenthaler et al., 1999). Specifically, it may be that women have a faster rate of disappearance per volume of blood per hour (for review see, Mumenthaler et al., 1999). Crippens et al. (1999) found that female rats had a faster elimination rate from tail blood. Although they observed more rapid elimination in blood samples in females, they did not see a corresponding difference in brain ethanol levels. Therefore, although female rats had faster ethanol elimination from their blood than males, this effect may not be throughout all tissues.

In sum, female quail had more ADH than male quail and also had a greater liver to body weight ratio. The current research found female quail had less overall ethanol exposure as indicated by a smaller AUC. Female quail also had lower peak BECs than males perhaps resulting in an increased first-pass metabolism because of their higher ADH levels and liver weights.

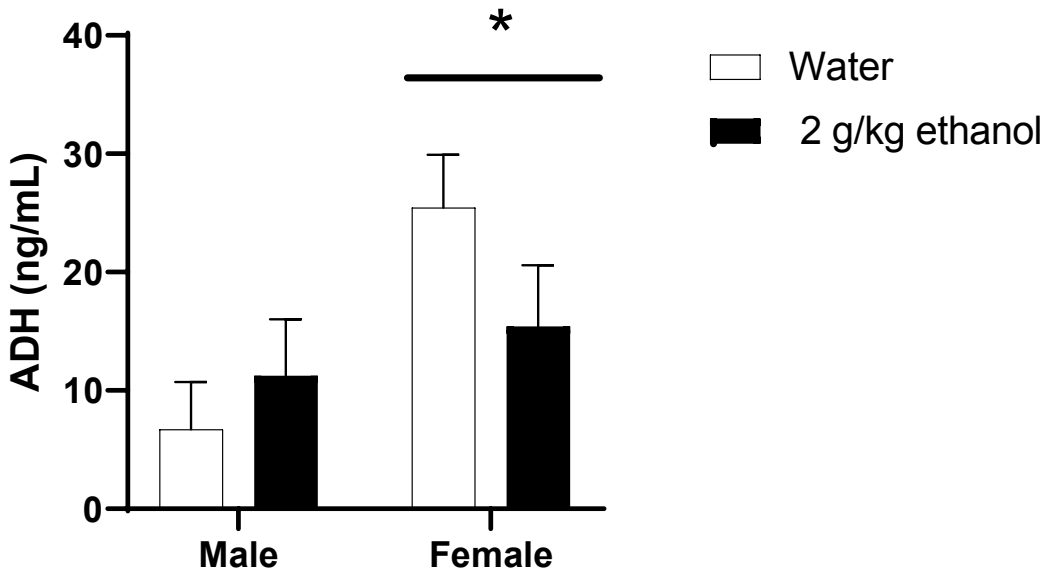


Figure 2-1 Alcohol dehydrogenase levels in male and female quail

Average (±SEM) alcohol dehydrogenase (ADH) levels (ng/ml) for male and female quail that received water or 2 g/kg ethanol for 12 days. * indicates a significant difference from males, $p<0.05$.

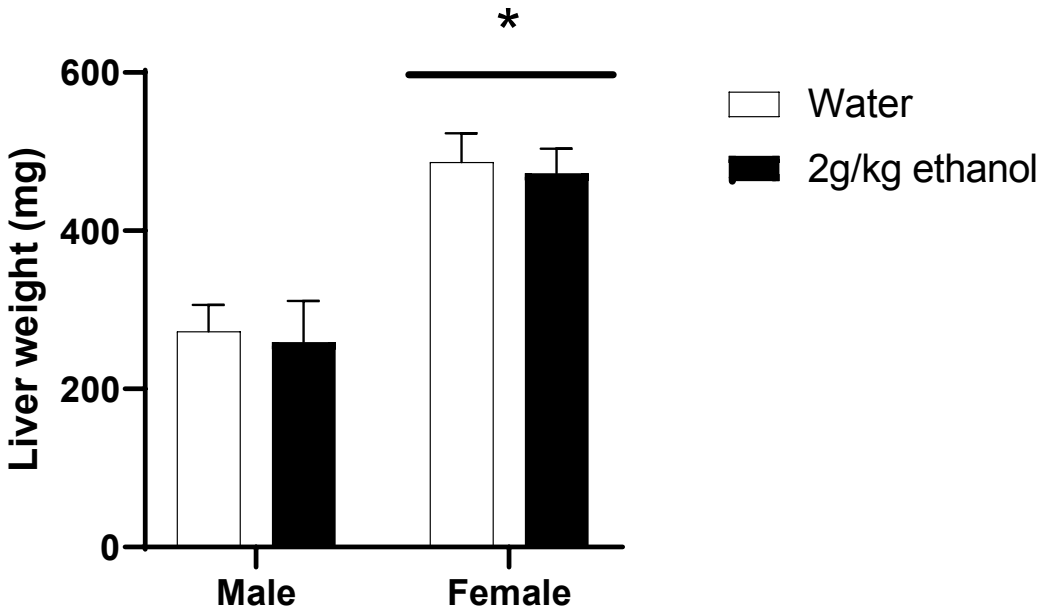


Figure 2-2 Average liver weights of male and female quail

Average liver weights (±SEM) for male and female quail that received water or 2 g/kg ethanol for 12 days. * indicates a significant difference from males, $p<0.05$

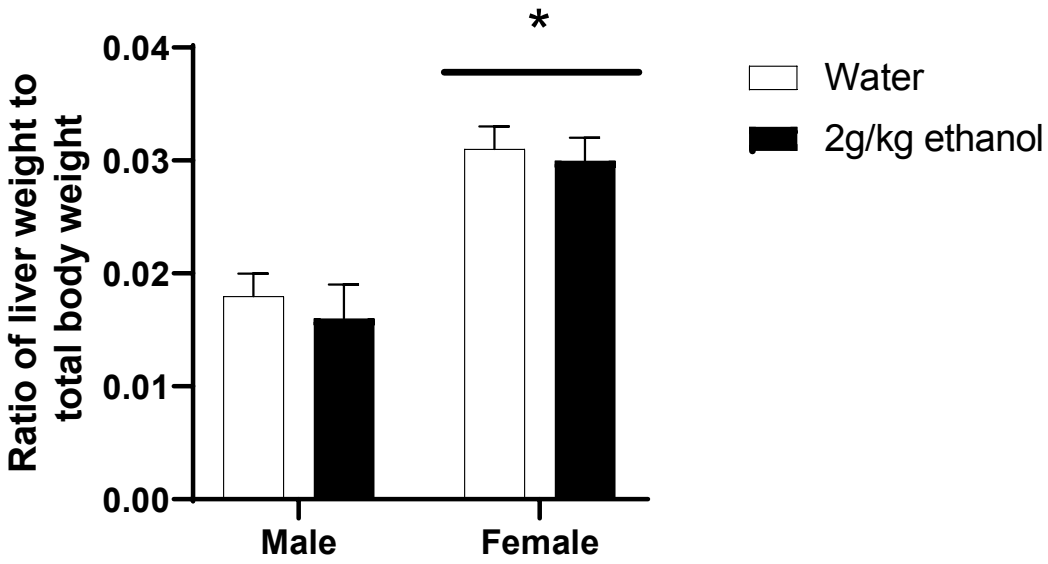


Figure 2-3 Ratio of liver weight to total body weight in male and female quail

Liver to total body weight averages (±SEM) for male and female quail that received water or 2 g/kg ethanol for 12 days. * indicates a significant difference from males, $p<0.05$.

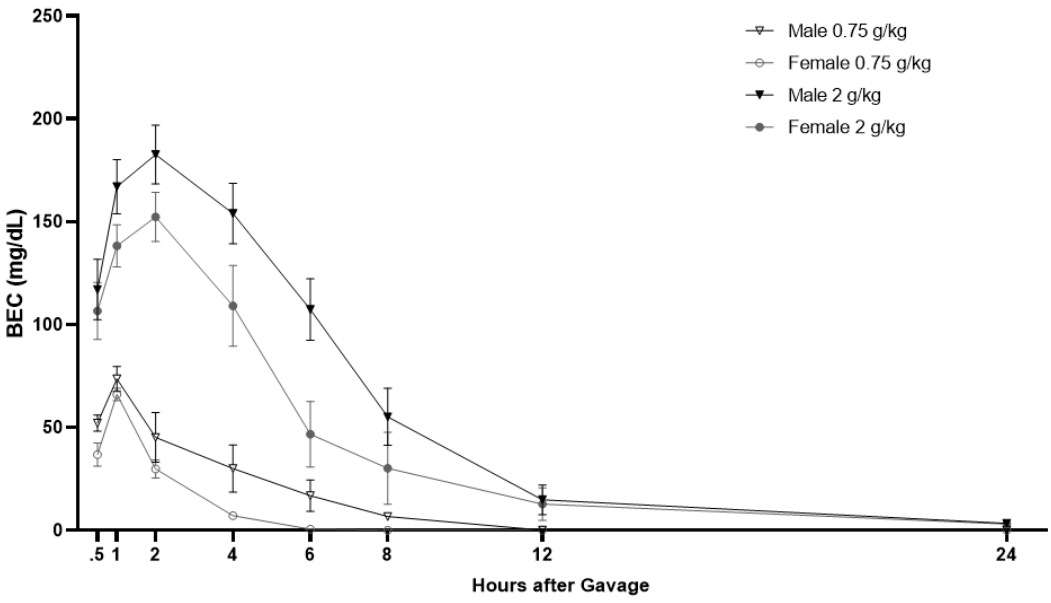


Figure 2-4 Blood ethanol content for male and female quail treated with 075 g/kg or 2 g/kg

Average blood ethanol content (BEC) over 24 hours for male and female quail following a gavage of 0.75 g/kg or 2 g/kg ethanol.

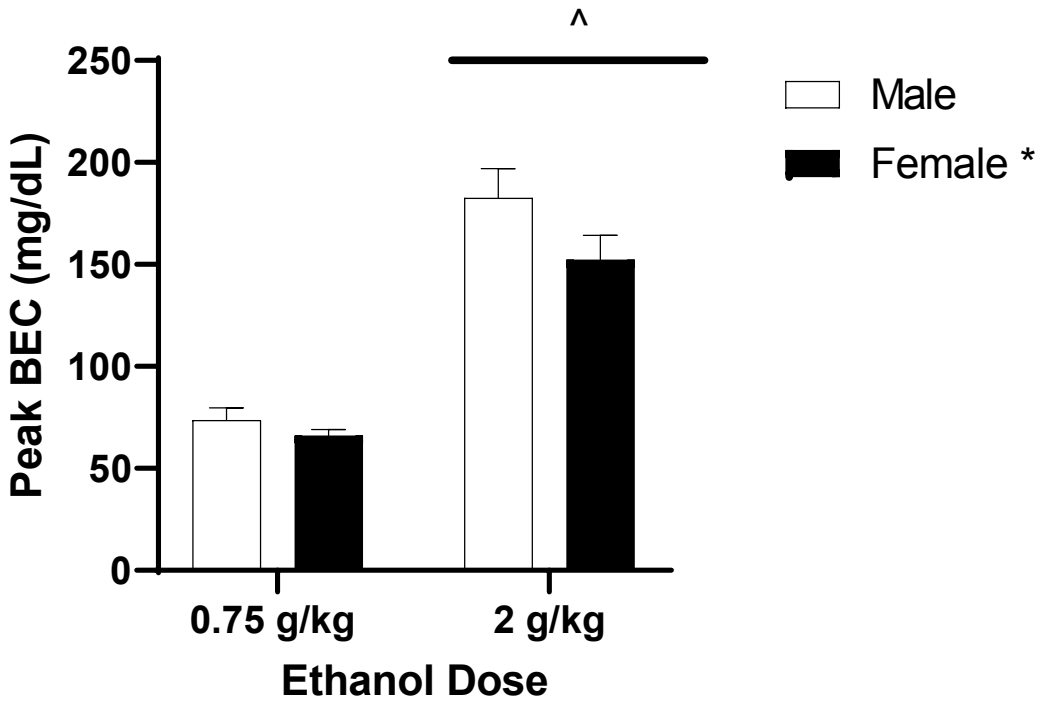


Figure 2-5 Peak blood ethanol content of male and female quail

Average peak blood ethanol content (BEC) for male and female quail gavaged with 0.75 g/kg or 2 g/kg ethanol. ^ indicates a significant difference from 0.75 g/kg. * indicates a significant difference from males

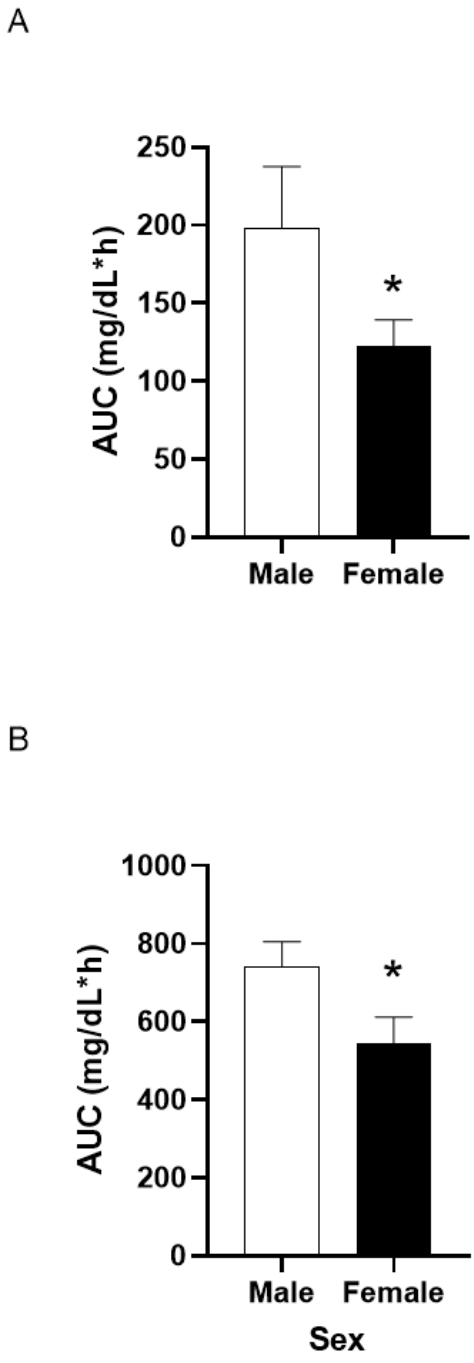


Figure 2-6 Area under the curve for male and female quail

Average area under the curve (AUC) for male and female quail gavaged with 0.75 g/kg (A) or 2 g/kg (B) * indicates a significant difference from male quail.

# CHAPTER 3. ETHANOL ELICITS A DOSE-DEPENDENT CONDITIONED PLACE PREFERENCE AND PLACE AVERSION IN JAPANESE QUAIL

## 3.1 INTRODUCTION

Alcohol use disorder (AUD) is characterized by an impaired ability to control alcohol use despite negative health, social, or personal consequences (DSM-5, 2013). Although there are different treatments, from therapeutics to behavioral, many people with an AUD still relapse. A proposed reason for relapse is that environmental contexts and alcohol-related cues cause craving (Witteman et al., 2015). Drug use often occurs in conjunction with specific environmental and drug-related stimuli resulting in Pavlovian conditioning. Specifically, the rewarding or aversive effects of a drug become associated with the environmental and drug-related cues. In alcohol users, alcohol-related cue increases salivation (Rubonis et al., 1994) and craving (Thomas et al., 2005). Furthermore, alcohol-related cues and craving are positively correlated (Witteman et al., 2015). Thus, alcohol-related cues appear to affect both the physiological and subjective responses of alcohol users.

Conditioned place preference (CPP) is a Pavlovian conditioning paradigm that assesses the rewarding and aversive properties of a stimulus such as drugs of abuse (for review, see Bardo & Bevens, 2000). Typically, a drug is administered to an animal before being placed in a distinct environment during conditioning. On alternate days, vehicle is administered and the animal is placed in another distinct environment. The rewarding or

aversive properties are assessed in a drug-free state by examining time spent in each environment.

Much of the ethanol CPP research in rodents has been mixed (Roma et al., 2006). Species type appears to affect the development of an ethanol-induced CPP. Compared to rats, mice develop a place preference when injected with ethanol (Cunningham et al., 1993). An ethanol-induced place preference in mice can be elicited at various doses (for review see, Tzschentke, 2007). However, in rats, the acquisition of an ethanol CPP does not seem to be as robust. Research indicates that sex (Torres et al., 2014), dose (Torres et al., 2014), strain (Roma et al., 2006; Torres et al., 2014), and delivery method (Ciccocioppo et al., 1999) might factor into the development of a CPP. Female Wistar rats have been shown to develop a CPP following an ip injection of 1 g/kg, but a conditioned place aversion (CPA) to higher ethanol doses (Torres et al., 2014). Yet other researchers have found that the same dose did not affect Sprague-Dawley rats (Reid et al., 1985). Route of administration also seems to play a role in developing an ethanol CPP, and an intragavage catheter delivery method resulted in CPP, but gavage did not (Ciccocioppo et al., 1999). Taken together, there are many inconsistencies in alcohol-induced CPP research with rodents developing a CPP, CPA, or neither (for review see, FIdler et al., 2004; Tzschentke, 2007).

Most current research demonstrates that rodents find alcohol aversive, and researchers must overcome this aversion with various methodologies (for review, see Bell et al., 2006). Additionally, rodents may find gavage and intubation aversive (Ciccocioppo et al., 1999). This administration technique may not be as aversive in birds and, as a result, is one of the more common methods of drug administration in birds (for review,

see Dorrestein & Miert, 1988). Therefore, quail may provide a unique insight into the rewarding and aversive aspects of alcohol due to their natural affinity for alcohol and because researchers are able to administer ethanol using a non-aversive direct oral.

Quail may also help expand our knowledge of visual drug-related cues. Unlike rodents, which have poor color vision (Jacobs et al., 2001), quail have good color vision and high visual acuity similar to humans (Fidura & Gray, 1966). Previous research has found that quail have a strong preference for green and yellow colors, followed by blue and lastly red (Duecker & Schulze, 1977). Because cues involved in human relapse behaviors are often visual in nature (Van Dyke & Fillmore, 2015; Witteman et al., 2015), the use of visual cues may elucidate the nature of these behaviors.

Although no research has been conducted to examine the rewarding and aversive effects of ethanol in quail, previous research has shown that quail develop a CPP for rewarding psychostimulants, including cocaine and nicotine (Bolin et al., 2012; Gill et al., 2016; Akins et al., 2004). Specifically, quail showed a place preference for a nicotine-paired chamber over one paired with saline (Bolin et al., 2012). Similarly, multiple studies demonstrated that cocaine produces a place preference (Akins et al., 2004; Gill et al., 2016; Levens & Akins, 2001). The cocaine-induced CPP effect was shown to be attenuated with a dopamine receptor antagonist (Akins et al., 2004).

The purpose of the current study is to assess whether an ethanol gavage would elicit a place preference or aversion in male and female Japanese quail. We predict that there would be a dose-dependent place preference. Specifically, we hypothesize that quail might develop a place preference for a low dose of ethanol and a place aversion to a high

dose of ethanol. We predict that similar to Torres et al. (2014) female quail would be more sensitive to the rewarding effects of ethanol. Additionally, we expect that ethanol administration would reduce locomotor activity compared to water-teated quail. The activity suppressing effect would be most evident at the high dose.

## 3.2 METHODS

### 3.2.1 SUBJECTS

Fifty adult male (n = 25) and female (n = 25) quail were housed under a 16:8 L:D throughout the experiment. Fertilized eggs were purchased from GQF Manufacturing (Savannah, GA) and hatched and raised at the University of Kentucky (Centre College, Danville, KY, provided 7 quail). Following hatch, quail were kept in mixed-sex brooders until post-hatch day 28 when they were separated by sex. Males were single housed in wire mesh cages (GQF Manufacturing, Savannah, GA) starting on post-hatch day 28. Females were group-housed in pens until two weeks before the start of the experiment, at which point they were single-housed in the wire mesh cages. All quail had free access to food and water prior to the start of the experiment. Starting during habituation and continuing through the post-test, their feed was removed 1 hour before the start of the experiment to ensure that their crop was not full before the gavage. Subjects were drug-naive at the start of the experiment. All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Kentucky.

### 3.2.2 DRUGS

Ethanol was diluted in tap water (25% w/v alcohol) and gavaged at a dose of either 0.75 or 2 g/kg. These doses were selected based on previous studies (Gauvin et al., 2000) and preliminary data that indicated that 2 g/kg was a relatively high dose for quail. Tap water was used as a vehicle and gavaged at a similar volume as the ethanol based on weight.

### 3.2.3 PROCEDURE

Quail were randomly assigned to receive a gavage (25% w/v alcohol in tap water) of water (n = 18) or 0.75 (n = 16), or 2.0 g/kg (n = 16) of ethanol. A biased design was used for this experiment, similar to previous quail research (Akins et al., 2004; Gill et al., 2016; Levens & Akins, 2001). A biased design accounts for individual differences by taking into account animals that have an initial preference for one chamber over the other. Quail were assigned to the same box for the entirety of the experiment (i.e., habituation, pre-test, conditioning, and post-test). Throughout the experiment, white noise was used to reduce any extraneous noise.

#### 3.2.3.1 APPARATUS

The apparatus used was a three-chamber visual place preference apparatus (ENV-013; Med Associates Inc., St. Albans, VT). One side of the apparatus had green walls,

and the other side had red walls, and there was a gray middle chamber. These colors were selected based on research that found quail strongly prefer wavelengths in the green and yellow range and have less of a preference for wavelengths associated with red (Duecker & Schulze, 1977). The preferred and nonpreferred colors were selected to mimic the rodent CPP paradigm that uses black (i.e., preferred in rodents) and white (i.e., not preferred in rodents) chambers. The flooring of the apparatus was covered in paper so that the texture is the same in the two outer chambers. The conditioning chambers had the same floor cues, such that the walls (visual cues) were the only cues that differed. The two outer chambers (28.6 cm long × 21.2 cm wide × 21.2 cm deep) of the apparatus were each equipped with six photobeams. The grey central chamber (10.8 cm long × 21.2 cm wide × 21.2 cm deep) had three photobeams. Removable doors separated all chambers. In all three chambers, the photobeams were approximately 6.4 cm and 3.2 cm from the apparatus floor.

#### 3.2.3.2 HABITUATION

Before the start of the experiment, quail were habituated to the CPP apparatus. During habituation, quail had access to the middle chamber and one of the outer chambers. The bird was placed in the center chamber and confined to one side of the box for 60 min (i.e., red or green). On the following day, the quail was confined to the other side for 60 min. Quail habituated for a total of four days to alternating sides of the apparatus (Gill et al., 2016).

#### 3.2.3.3 PRE-TEST FOR BIASED DESIGN

Prior to conditioning, quail were given a pre-test to determine their initial preference (i.e., biased design). Quail were placed in the center chamber and had 15 min to freely explore the entire apparatus (i.e., all three chambers). Time spent in each region of the apparatus was measured. Quail that did not explore all chambers of the apparatus had a second pre-test the following day. Initial preference was determined by spending more time in one chamber (e.g., green) compared to the others (e.g., red or grey).

#### 3.2.3.4 CONDITIONING

On the first day of conditioning, quail were gavaged with a 0, 0.75, or 2.0 g/kg dose of alcohol and immediately placed into their least preferred chamber (as determined by the pre-test) for 30 min. On alternate days, quail were gavaged with water and placed in the opposite chamber for 30 min. There were a total of eight conditioning days, for a total of four ethanol and four vehicle sessions. Locomotor activity was collected as photobeam breaks in 5 min time bins on all conditioning days.

#### 3.2.3.5 POST-TEST

The post-test occurred 24 h after the eighth day of conditioning trials. Similar to the pre-test, the doors between the chambers were removed, and quail were placed in the center chamber. The quail had free access to the entire apparatus in a drug-free state for 15 min and time spent in each chamber was recorded.

### 3.2.4 STATISTICAL ANALYSIS

Place preference was assessed using a difference score. The difference score was calculated by subtracting the time spent in the least preferred chamber during the pre-test from the time spent in the drug paired chamber during the post-test. For those quail that had a second pre-test, the time spent in each chamber on the second pre-test was averaged with the first pre-test day, and that was used to determine initial preference and the difference score. Prior to analysis of the difference score, a Grubb's test was conducted and one female quail was removed from the analysis as a result. A 2 x 3 ANOVA with sex (male and female) and ethanol dose (0, 0.75, and 2.0 g/kg ethanol) as between factors was used to determine significant differences in the difference score. Additionally, locomotion (i.e., beam breaks) was analyzed on conditioning days using a 2 x 3 x 4 x 6 (sex x ethanol dose x day x time bin) repeated measures ANOVA (RM ANOVA) with sex and ethanol dose as between-subject factors and day and time bin as within-subject factors. When the assumption of sphericity was violated, the Greenhouse-Geisser correction was used, with $\alpha$ set to $< 0.05$. Post hoc analyses, when needed, were done using Bonferroni corrected pairwise comparisons and Tukey post hoc tests.

## 3.3 RESULTS

### 3.3.1 DIFFERENCE SCORE

Figure 3.1 shows CPP difference scores for male and female birds treated with ethanol (0, 0.75, 2 g/kg). An ANOVA revealed a significant change in difference score based on treatment, $F(2,44) = 14.178$, $p < 0.001$, $\eta 2 = 0.392$. Tukey's post hoc analysis revealed that quail gavaged with 0.75 g/kg ($M = 197.212$, $SEM = 41.196$) ethanol had a significantly greater difference score than quail gavaged with water ($M = 44.24$, $SEM = 38.84$) or 2 g/kg ($M = -113.011$, $SEM = 41.096$), $ps < .05$. Thus, a greater positive difference score (CPP) to the ethanol-paired chamber was observed in the 0.75 g/kg dose compared to water-treated controls. Additionally, quail gavaged with 2 g/kg had a significantly lower difference score than quail gavaged with water or 0.75 g/kg, $ps < 0.05$. A significant negative shift in preference (CPA) was observed in quail gavaged with 2 g/kg compared to water-treated controls. There was no main effect of sex [$F(1,44) = 0.00$, $p = 0.986$, $\eta 2 = 0.00$], or an interaction between sex and treatment, [$F(2,44) = 1.648$, $p = 0.204$, $\eta 2 = 0.070$].

When separated by sex, males have a significant effect of treatment, $F(2,23) = 10.464$, $p = 0.001$, $\eta 2 = 0.476$. A Tukey's post hoc reveals a CPP as male quail treated with 0.75 g/kg ethanol ($M = 257.453$, $SEM = 63.569$) have a greater difference score than those treated with water ($M = 20.715$, $SEM = 56.858$), $p < 0.05$. However, no CPA is evident as there is no difference between the difference score of male quail treated with 2

g/kg ($M$ = -151.883, $SEM$ = 63.569) and those treated with water, $p$=0.164. Female quail similarly have a significant effect of treatment, $F(2,25) = 4.134$, $p$= 0.028, $\eta 2 = 0.249$. However, a Tukey's post hoc reveals only a difference between 0.75 g/kg ($M$ = 127.061, $SEM$ = 41.193) and 2 g/kg treated quail ($M$ = -53.719, $SEM$ = 47.566), $p< 0.05$.

### 3.3.2 LOCOMOTOR ACTIVITY

Figure 3.2 shows the average number of beam breaks in 5 min time bins for male and female quail treated with water, 0.75, or 2 g/kg ethanol on drug conditioning days. There was a main effect of sex [$F(1,44) = 13.17$, $p = 0.001$, $\eta 2= 0.230$]; male quail ($M$ = 389.54, $SEM$ = 22.91) tended to have greater beam breaks than female quail ($M$ = 274.01, $SEM$ = 22.01). There was also a main effect of treatment [$F(2,44) = 4.45$, $p = 0.017$, $\eta 2 = 0.168$]. Tukey's post hoc analysis revealed that quail treated with water ($M$ = 380.83, $SEM$ = 25.65) were not different than those treated with 0.75 g/kg ethanol ($M$ = 347.61, $SEM$ = 27.91), $p$ = ns. But those treated with 2 g/kg ethanol had fewer beam breaks ($M$ = 267.26, $SEM$ = 28.89) than water treated quail $p < 0.05$. Additionally, the ANOVA revealed a main effect of time [$F(4,167) = 10.31$, $p< 0.001$, $\eta 2 = 0.19$]. Post hoc analysis revealed that quail had more beam breaks in the first five min (i.e., time bin 1) than in min 10-15, 20-25, and 25-30 (i.e., time bin 3, 5, & 6), $p< 0.05$. They also had more beam brakes in the second time bin (i.e., 5-10 min) than in the last two time bins (i.e., 20-25 and 25-30 min), $p< 0.05$. Lastly the RM ANOVA revealed a significant treatment x time interaction [$F(8,167) = 2.55$, $p = 0.014$, $\eta 2 = 0.104$]. Post hoc analysis revealed that there was no difference in beak breaks across the time bins in water-treated birds. In quail treated with 0.75 g/kg, the only difference was that quail had more beam breaks at the

second time bin (i.e., 5-10 min) than at time bin 5 (i.e., 20-25 min), $p < 0.05$. In quail treated with 2 g/kg, beam breaks in the first time bin were significantly different from time bins 3, 5, and 6, $p < 0.05$. However, no other main effects or interactions were evident, *ps* = ns.

## 3.4 DISCUSSION

The present study investigated the rewarding effects of ethanol in male and female Japanese quail. The results indicated that quail developed a CPP to a 0.75 g/kg dose of ethanol compared to those treated with water. However, quail treated with 2 g/kg developed a CPA. Additionally, quail treated with 0.75 g/kg did not differ in locomotion compared to water treated quail on ethanol conditioning, while quail administered 2 g/kg had less locomotor activity than those treated with either water or 0.75 g/kg.

The current study extends the previous literature and demonstrates that quail find a low dose of ethanol rewarding. Similar to some previous rodent studies, quail developed a place preference for a chamber paired with a dose (i.e., 0.75 g/kg) of ethanol. Most of the rodent studies demonstrated that ethanol was rewarding were conducted in mice with doses ranging from 1.5 to 4 g/kg (Boyce-Rustay & Holmes, 2006; Cunningham et al., 1991; Cunningham et al., 1992a; Cunningham et al., 1992b; Mcgeehan & Olive, 2003). However, research in rats has been more varied. Rats administered 1.5 g/kg ethanol or less occasionally developed a CPP (Black et al., 1973; Ciccocioppo et al., 1999; Torres et al., 2014) but usually showed no preference for the ethanol paired chamber (Bienkowski et al., 1996; Bormann & Cunningham, 1997; Ciccocioppo et al., 1999; Van der Kooy et al.,1983). When rats were administered doses above 1.5 g/kg, a CPA was usually

observed (Bedingfield et al., 1999; Cunningham et al., 1992b; Gauvin et al., 1994; Gauvin & Holloway, 1992; Sherman et al., 1983). A study by Cunningham and colleagues (1993) compared mice and rats directly and used the same methodologies in both species. They found that mice developed a CPP similar to previous studies, but rats developed a CPA (Cunningham et al., 1993). Some research has found that rats will develop a CPP to ethanol, but it appears that many different factors are involved in the development of an ethanol-induced CPP.

In addition to the hedonic effects of a low dose of ethanol demonstrated in quail, the current study also found that a high dose elicited a CPA. Although mouse studies tend to find CPP across a wide range of doses (Boyce-Rustay & Holmes, 2006; Cunningham et al., 1991; Cunningham et al., 1992a; Cunningham et al., 1992b; Mcgeehan & Olive, 2003), rat studies may be more similar to the current study. If a CPP is observed in rats, it is usually at lower doses, and higher doses tend to elicit a CPA (see Fidler et al., 2004; Torres et al., 2014). Future research should examine a range of ethanol doses to determine what doses are maximally rewarding and what doses are aversive in quail.

In rodents, the route of administration also has an effect on the development of a CPP. Previous findings in rats have been mixed when ethanol is administered orally or via a gavage. Rats that consumed ethanol orally in the CPP apparatus have been found to develop both a CPP (Gauvin & Halloway, 1991) and a CPA (Stewart & Grupp, 1989). Similarly, when ethanol has been administered by gavage to rats the findings have been mixed [(CPA, Gauvin & Halloway, 1991), (CPP, Gauvin et al., 2000), (No conditioning, Ciccocioppo et al., 1998; Gauvin & Halloway, 1991)]. As explained by Ciccocioppo et al. (1998), a gavage may cause the animal discomfort and influence the acquisition of

CPP. However, this route may not be as aversive to birds. An oral gavage is one of the most common routes used to administer a variety of drugs to birds in veterinary offices and laboratory settings (Evans, 2007). Therefore, quail may be a good model to study the rewarding and aversive effects of ethanol following this type of administration which may mimic the typical method of ethanol consumption while still controlling dosing.

Contrary to our hypothesis, we did not observe any sex differences in difference scores for place preference. Our findings may be similar to many mice studies that have not found a sex difference in the acquisition of an ethanol-induced CPP. However, one mouse study did find a sex difference in adolescent animals (Roger-Sanchez et al., 2012). In young adolescent mice, males developed a CPP to two doses (i.e., 1.25 and 2.5 g/kg) of ethanol, but females only developed a CPP at the higher dose (Roger-Sanchez et al., 2012). Thus young male mice may be more sensitive to the rewarding effect compared to young females. In rats, Torres et al. (2014) found that only females treated with 1 g/kg ethanol developed a CPP, whereas males did not develop a CPP to any dose of ethanol. Although the current study did not find any sex difference between male and female quail in CPP, this could have resulted from low statistical power. Further research may be needed to assess sex differences in ethanol sensitivity.

Previous research examining cocaine-induced behavioral sensitization has shown a locomotor activity difference in male and female quail across all treatment groups including saline-treated quail (Gill et al., 2015). Therefore, the observed overall sex effect in locomotor activity was expected in the current study. The locomotor activity differences may have been greater because quail were raised and maintained in long light conditions. These conditions mimic the breeding season for quail and as a result of high

estradiol female quail have less activity allowing for them to be sexually receptive, nest, and lay eggs (see Mills et al., 1997 for review). While speculative, the observed sex difference in locomotor activity may therefore be driven by the lighting conditions.

The current study found a dose-dependent effect of ethanol on locomotor activity. Specifically we found that the activity-suppressing effect of ethanol was most evident at the 2 g/kg dose. Similarly the administration of ethanol typically reduces locomotor activity in rats at similar doses (Cunningham et al., 1993; Fidler et al., 2004). Fidler et al. (2004) found a dose-dependent decrease in activity counts in rats following ethanol administration. Specifically, a .7 g/kg dose did not change locomotor activity counts but a 1.5 g/kg dose did (Fidler et al., 2004). There may be some stimulant-like effects following ethanol administration in rats, however, this increase appeared to be strain dependent (Erickson & Kochhar, 1985). Locomotor activating and suppressing effects of ethanol have not been found to be related to motivational effects (i.e., CPP or CPA) in rats (Fidler et al., 2004) or mice (Cunningham, 1995; Risinger et al., 1993).

Visual cues may become associated with the rewarding effects of drug-taking behaviors. In individuals with an AUD, ethanol cues elicit increased skin conductance (Kaplan et al., 1985; Laberg et al., 1992). Field & Duka (2002) found that individuals with no history of AUD also had increased skin conductance and feelings of craving were higher following the representation of a cue that had been paired with ethanol. Other research has paired novel cues with ethanol, producing conditioned heart rate responses and skin temperatures (Staiger & White, 1988). Additionally, ethanol paired cues elicit greater attention, and individuals report more enjoyment when consuming an ethanol beverage when exposed to the ethanol paired cue (Mayo & de Wit, 2016). Along with

enjoyment, previous studies have shown stimuli that are paired with alcohol-induced craving (Carter & Tiffany, 1999) and cue-induced craving has been associated with increased brain activity and relapse behaviors (Sinha & Li, 2007). Although visual cues were not directly manipulated in the current study, the only difference between the two outer chambers was the colors of the walls, as the flooring and everything else was the same between the two chambers. The visual cues presented in the current study can easily be manipulated to examine the role of these visual environmental cues in a CPP paradigm. Therefore, the model of a visual CPP may allow for further understanding and extend the research on ethanol-paired visual cues and their role in addiction and relapse.

In sum, we have demonstrated, for the first time to date, both dose-dependent ethanol-induced CPP and CPA in Japanese quail. The results suggest that a low dose of ethanol may be rewarding whereas a high dose may be aversive in quail. Further investigation of a range of doses and sex may further elucidate the motivational and locomotor effects of ethanol in quail, as the current experiment may have been underpowered to detect any sex differences.

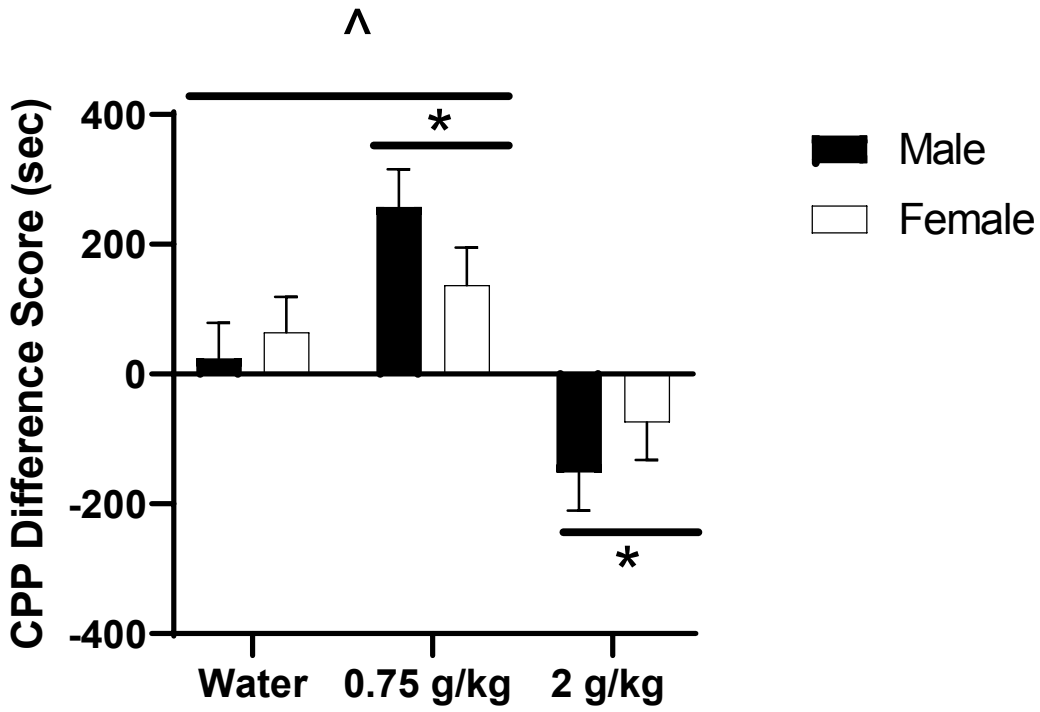


Figure 3-1 Conditioned place preference difference score for male and female quail

Mean (± SEM) CPP difference scores for male and female quail treated with water, 0.75, or 2 g/kg ethanol. * indicates a significant difference from water-treated quail. ^ indicates a significant difference from 2 g/kg ethanol-treated quail.

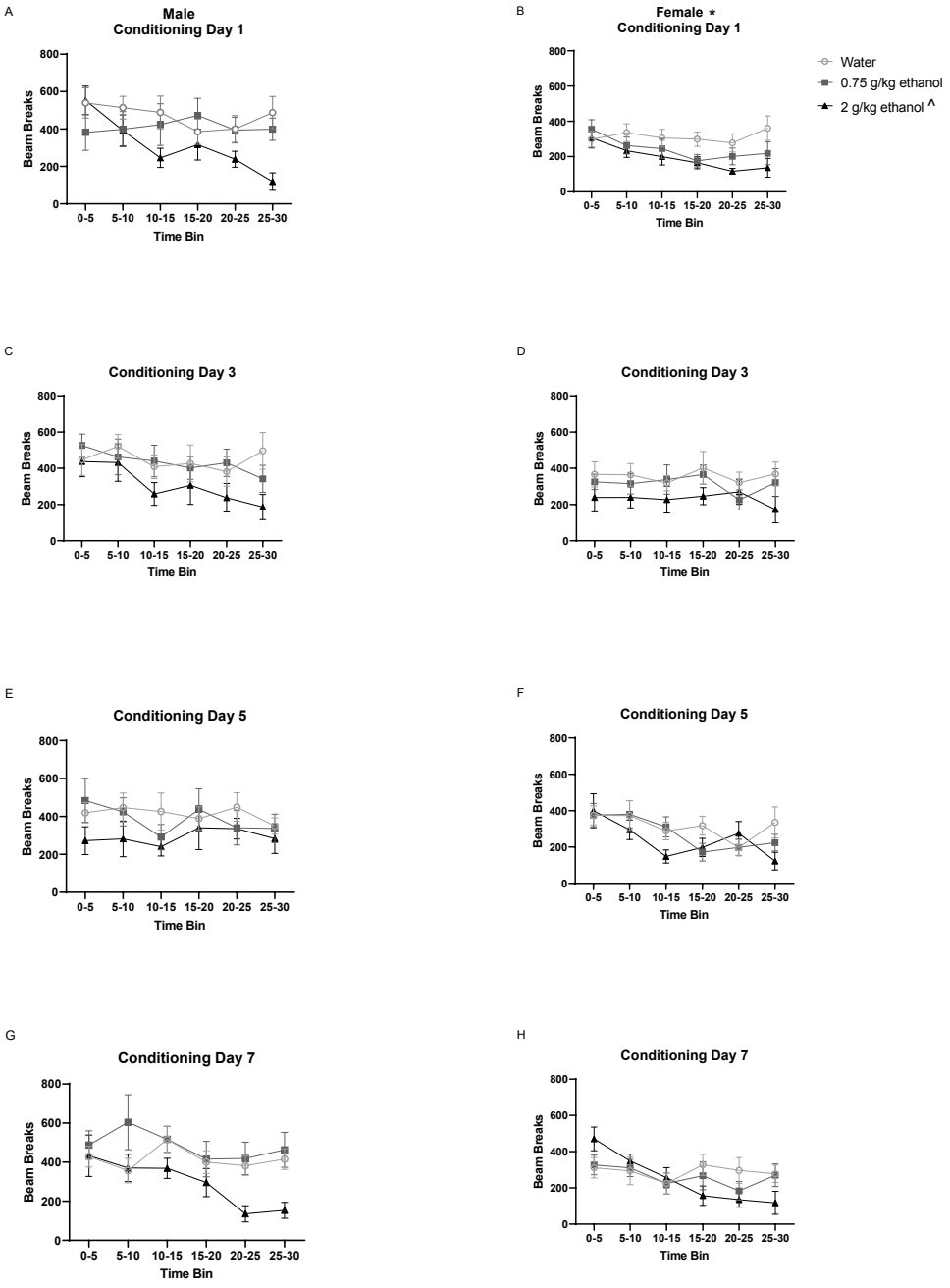


Figure 3-2 Locomotor activity for male and female quail on ethanol conditioning days

Mean (± SEM) beam breaks across 30 min of conditioning for males (A, C, E, G) and females (B, D, F, H) on ethanol conditioning days. * indicates a significant difference from males, $p<0.05$. ^ indicates a significant difference from water-treated quail, $p<0.05$.

# CHAPTER 4. GENERAL DISCUSSION

We have examined ethanol pharmacokinetics for male and female Japanese quail by measuring the ADH levels in their livers. Female quail had greater ADH levels and larger livers. Perhaps the higher ADH levels in females may contribute to a greater first pass metabolism of ethanol. This contributes to the current literature by extending research that examined the development of ADH in Japanese quail and other bird research that examined the elimination of ethanol from the blood of a few different bird species (Eriksson & Nummi, 1982; Nussrallah et al., 1989).

Another phamroacokentic measure we took was the concentration of ethanol in the blood of male and female quail over time. Similar to previous rodent research we reported BECs over time to create a BEC profile (Livy et al., 2003) we found that females had lower peak levels and a smaller AUC compared to male quail. The BEC profile created can be used by researchers wanting to capture the ascending and not the descending limb of the BEC curve since there has been evidence of acute tolerance (Martin & Moss, 1993; LeBlanc et al., 1975) and a biphasic effect of ethanol (Holdstock & de Wit, 1998). The BEC profile also allows for comparisons across species that may have different rates of absorption and elimination. Peak BECs are an important factor when considering the damage to neural development (Bonthius & West, 1988).

We also examined the rewarding and aversive properties of ethanol in Japanese quail using a CPP paradigm. Similar to some rat studies, we found that quail may find a

low dose of ethanol rewarding as indicated by the development of a CPP to an ethanol paired chamber (Torres et al., 2013). Additionally, a high dose appeared to be aversive as indicated by the development of a CPA. Similarly, when people consume large amounts of ethanol they sometimes develop a conditioned taste aversion. Ethanol-induced CTA has long been examined as a treatment for heavy ethanol use (Addolorato et al., 2002; Sinclair, 1984). Similar to people, quail appear to model the dose-dependent rewarding and aversive effect of ethanol.

Together we have shown that female quail have higher levels of ADH compared to males and although the disappearance rate was not different between the sexes, females may have an increased first-pass metabolism as indicated by the lower peak BECs and smaller AUC. Although the current study failed to find a significant sex effect in place preference and aversion it may be worth further examination. Because females have less overall exposure to ethanol (i.e., smaller AUC) and have lower peak BECs, they may find the same dose of ethanol slightly less rewarding than males. Perhaps females never reach the same "rewarding" and "aversive" BEC as males due to these pharmacokinetic differences but the current study may have been underpowered to test for sex differences. It may be worth controlling dosing so that males and females reach the same peak levels and assessing if there are differences when BEC is controlled.

Taken together the current studies add to the literature by examining the underlying pharmacokinetics and reward-related behavior of ethanol in an animal model that has not been previously used in ethanol research. Currently, quail are used to examine other drug use disorders. Specifically, quail have been used to study the effects of cocaine, methamphetamine, and nicotine (Bolin et al., 2012; Gill et al., 2015; Rosine et

al., 2009) and given the current findings they may also be a good model to examine AUD. Quail may provide additional insight to alcohol research because they have a good visual system, and an oral gavage may not be as aversive as in rodents. Further pharmacokinetic research should examine possible sex differences in ALDH because the presence of acetaldehyde may be aversive. Additionally, further behavioral research should aim to further establish quail as a model for AUD by examining more doses of ethanol and the role of cue-induced relapse. This may lead to a better understanding of the role of visual cues in AUD.

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# VITA

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## Educational Experience

| | |
|---|---|
| 2015-2021 | Doctoral Candidate, Experimental Psychology<br>Department of Psychology, Experimental Psychology (Cognitive Neuroscience)<br>University of Kentucky, Lexington, KY<br>Mentor: Chana K. Akins, PhD |
| 2018 | M.S., Experimental Psychology<br>Department of Psychology, Experimental Psychology (Behavioral Neuroscience and Psychopharmacology)<br>University of Kentucky, Lexington, KY<br>Mentor: Chana K. Akins, PhD |
| 2009 | B.A., Psychology, with Distinction, Cum Laude<br>California State University, Channel Islands, Camarillo, CA |

## Academic Positions

| | |
|---|---|
| 2020 | Adjunct Professor, Basic Statistics<br>Department of Psychology<br>St. Norbert College, De Pere, WI |
| 2020 | Adjunct Professor, Learning and Motivation<br>Department of Psychology<br>Transylvania University, Lexington, KY |

2019 Instructor, Brain and Behavior

Department of Psychology

University of Kentucky, Lexington, KY

2015- 2019 Lab Instructor*

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**Honors**

| | |
|---|---|
| 2019 | Travel Award 2019, Research Society on Alcoholism |
| 2015-2019 | Research Challenge Trust Fund Travel Award, University of Kentucky |
| 2016 | Graduate Student Congress Travel Award, University of Kentucky |
| 2013-2014 | Student Research & Travel Award, Cal State University, San Bernardino |
| 2010 | SfN 2013 Lay Summary, *Effect of adolescent and adult nicotine exposure on methamphetamine self-administration in male rats* |
| 2009 | Graduated *Cum Laude,* California State University, Channel Islands |
| 2008-2012 | Dean's List, California State University, Channel Islands |
| 2004-2008 | Dean's Honors, Pasadena City College |

**Publications**

*Manuscripts in preparation:*

1. **Eaton, S. E**., Hopkins, D. M., Pauly, J. R. & Akins, C. K., Localization of the norepinephrine transporter in male and female quail brains (*submitted-Journal of Basic and Applied Zoology*)
2. **Eaton, S. E.,** Dzhala, S., Robinson, L. E., Radevski, M.E. & Akins, C. K. Ethanol elicits a dose- dependent conditioned place preference and aversion in male and female Japanese Quail *(in preparation)*
3. **Eaton, S. E.,** Jagielo-Miller, J. E., Dzhala, S. Prendergast, M.A. & Akins, C. K. Ethanol pharmacokinetics in male and female Japanese quail (*in preparation)*

*Peer-reviewed publications:*

1. Crawford, C. A., Teran, A., Ramirez, G. I., Katz, C. G., Mohd-Yusof, A., **Eaton, S. E.,** ... & McDougall, S. A. (2019). Age-dependent effects of dopamine receptor inactivation on cocaine-induced behaviors in male rats: Evidence of dorsal striatal D2 receptor supersensitivity. *Journal of Neuroscience Research*, *97*(12), 1546-1558.

2. Rice, B. A., **Eaton, S. E.,** Prendergast, M. A., & Akins, C. K. (2018). A glucocorticoid receptor antagonist reduces sign-tracking behavior in male Japanese quail. *Experimental and Clinical Psychopharmacology*, *26*(4), 329.
3. Amodeo, L. R., Greenfield, V. Y., Humphrey, D. E., Varela, V., Pipkin, J. A., **Eaton, S. E.**, ... & Crawford, C. A. (2015). Effects of acute or repeated paroxetine and fluoxetine treatment on affective behavior in male and female adolescent rats. *Psychopharmacology*, *232*(19), 3515-3528.
4. S. A. McDougall, **S. E. Eaton**, A. Mohd-Yusof, C. A. Crawford (2015). Age-dependent changes in cocaine sensitivity across early ontogeny in male and female rats: Possible role of dorsal striatal $D2^{High}$ receptors. *Psychopharmacology,* 1-15.
5. Pipkin, J. A., Kaplan, G. J., Plant, C. P., **Eaton, S. E**., Gil, S. M., Zavala, A. R., & Crawford, C. A. (2014). Nicotine exposure beginning in adolescence enhances the acquisition of methamphetamine self-administration, but not methamphetamine-primed reinstatement in male rats. *Drug and Alcohol Dependence*, *142*, 341-344.

*Book chapters:*

1. Akins, C. K. **Eaton, S. E.**, & Bolin, B. L. (2017). Conditioned Place Preference. In: Vonk, J. & Shackelford, T. (eds), *Encyclopedia of Animal Cognition and Behavior* (p. 1-8). Springer, Cham.