ABSTRACT

The Effects of Acute Alcohol on Motor Impairments
in Adolescent, Adult, and Aged Rats

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Acute alcohol exposure has been shown to produce differential motor impairments between younger and older rodents. However, the effects of acute alcohol exposure among adolescent, adult, and aged rats have yet to be systematically investigated within the same project using a dose-dependent analysis. We sought to determine the age- and dose-dependent effects of acute alcohol exposure on gross and coordinated motor performance across the rodent lifespan. In addition, an ethanol clearance curve was generated to examine blood ethanol concentrations (BEC) across age groups following a high dose of ethanol. Aged animals performed worse on gross and coordinated motor tasks compared to younger animals. Older rodents were more sensitive to the sedative/hypnotic effects of acute ethanol compared to younger rodents. With the inclusion of three different developmental age groups, the current study provides a comprehensive view of age-dependent alcohol-induced motor impairments during the rodent lifespan.

The Effects of Acute Alcohol on Motor Impairments in Adolescent, Adult, and Aged Rats

by

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A Thesis

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ADH	Alcohol dehydrogenase
ARR	Aerial righting reflex
BEC	Blood ethanol concentration
LORR	Loss of righting reflex
PD	Postnatal day
RR	Accelerating rotarod

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To my parents Benito and Maria Ornelas.

Throughout my life you have instilled in me the confidence and integrity needed to be successful in life. Your continuous drive and motivation for the success of your children is what makes you the best parents any child could ask for. I thank you for always believing in me and having faith that through God, all things are possible

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Thank you for all your love and support throughout my life

I love you.

CHAPTER ONE

Introduction and Background

Introduction

Research investigating the effects of ethanol consumption on the elderly population is limited throughout the field of alcohol research. As individual's age, they become significantly susceptible to impairments in locomotor behavior due to reduction in bone mineral density, skeletal mass (Janssen, Heymsfield, & Ross, 2002) and an aging cerebellum (Nadkarni et al., 2013). In addition, ethanol administration can elicit further impairments in motor functioning including motor coordination, balance, and posture (Weafer & Fillmore, 2011; Mukamal, Robbins, Cauley, Kern, & Siscovick, 2007). Understanding the effects of ethanol on the biology of aging, specifically in physical impairments is imperative due to the risk factors associated with age-and ethanol-dependent motor impairments. Furthermore, the effects of ethanol intoxication are age-dependent; different age groups throughout the human lifespan experience differential effects of ethanol. To further understand the detrimental age-dependent effects of alcohol, it is important to investigate these impairments and compare the effects by ages. Experimentally investigating these impairments is difficult when using human participants due to ethical guidelines. Animal models in research allow scientists to systematically investigate the effects of drugs and behavior, specifically to a greater and more advantageous extent than would be possible with humans. Therefore, the current study included three age groups of male Sprague-Dawley rats (i.e., aged, adults, and adolescents) to examine the age-and dose-dependent effects of acute ethanol on motor

performance and sedation. Furthermore, a dose-response procedure provided evidence as to the variance in effects of acute ethanol administration.

The Senescence Period

The average age of the world's population is rapidly rising (Lutz, Sanderson, & Scherbov, 2008). With-in the last century, the number of elderly persons has significantly increased as a result of worldwide improvement of socio-economic conditions (Solana et al., 2012). Within the past century, the average lifespan of a human has increased at an approximate rate of three months per year in both males and females (Oeppen & Vaupel, 2002). According to the United States Census Bureau, individuals aged 65 and older are projected to total approximately 72 million people and represent nearly 20% of the total U.S. population in the year 2030 (He, Sengupta, Velkoff, & DeBarros, 2005). As a result, with-in the last few years there has been an exponential growth in research on aging (Martin, 2011). Specifically, researchers are interested in age-related diseases and the progressive decline of multiple physiological processes that occur within the human body during aging (Hayflick, 2007; Kirkwood, 2005).

The aging process can be defined as a deterioration of multiple physiological functions that will ultimately lead to an increased probability of death (Wheeler & Kim, 2014). Such physiological functions can vary from deterioration of the physical body including muscle mass (Lauretani et al., 2003), bone density (Delconico et al., 2009), and skeletal muscle (Siu et al., 2005). The senescence period also includes major risk factors for human diseases that affect organ function including infectious diseases, cardiovascular diseases, and cancer (Heidenreich et al., 2011). Additionally, age changes include psychological and neurological functions such as neurodegenerative disorders

and impairments in cognitive processes (Brockmann et al., 2013; Theies & Bleiler, 2013; Noyes et al., 2006). Therefore, considering how susceptible elderly individuals are to age-related impairments to the brain and body, it is important to further understand the biology of aging and how changes to the human system can ultimately affect an individual's health and behavior.

As the proportion of individuals aged 65 years and older continues to increase, it is expected that common acute and chronic conditions and other aged-related infectious diseases will increase. Elderly individuals undergo immunosenescence, which affects both the innate and adaptive immune system, making elderly individuals more prone to reduced functioning and alterations to their biological internal defense mechanisms (Castelo-Branco & Soveral, 2014; Solana, Pawelec & Tarazona, 2006). Furthermore, chronic impairments in the immune system could lead to an increased prevalence of cancer (Siegel, Naishadham, & Jemal, 2012; Derhovanessian, Solana, Larbi & Pawelec, 2008; Ries, Reichman, Lewis, Hankey, & Edwards, 2003), autoimmune disorders, and other chronic diseases (Tonet & Nóbrega, 2008).

According to a recent study, approximately 92.2 percent of Americans age 65 and older suffered from at least one chronic disease in 2008 (Hung, Ross, Boockvar & Siu, 2011). Cardiovascular diseases such as hypertension, coronary heart disease, and heart failure are the most prevalent; however, diabetes, stroke, and arthritis are also common in the elderly population (Heidenreich et al., 2011). Prevalence rates of neurodegenerative diseases such as Parkinson's, Alzheimer's, and other forms of dementia are also high in older populations. Parkinson's disease affects approximately 1% to 2% of individuals

over the age of 65 years (Brockmann et al., 2013) and increases to 4% to 5% for persons over the age of 85 (Noyes et al., 2006).

Individuals age 65 years and older suffer from physiological changes in their body, including a decline in muscle and bone mineral density (Delconico et al., 2009; Baumgartner, 2000). Age-related muscle atrophy, also known as sarcopenia, produces a decline in the functional ability of muscles which contributes to motor functioning impairments (Rolland et al., 2008; Visser et al., 2005). These motor impairments include walking, climbing stairs, lifting or carrying heavy objects, crouching or kneeling, and standing up from a chair (Jannsen, Heymsfield & Ross, 2002). Furthermore, individual's 50 years and older will lose approximately 1-2% of their muscle mass each year (Lauretani et al., 2003). Therefore, as individuals age they are at an increased risk for suffering from a dangerous or potentially life-threatening situation which may occur due to impairments in their locomotor behavior.

As muscles atrophy, individual's will suffer from decreased muscle strength, power, and mass which could lead to frailty and other forms of disabilities in elderly individuals (Fried & Guralnik, 1997). Lauretani et al. (2003) extensively examined muscle strength and power in a wide range of subjects from young adults to old age individuals (ages ranged from 20-102 years old). Muscle strength was examined in both lower and upper muscle extremities using a variety of weight training assessment tools. Weight-adjusted muscle power was measured in upper and lower extremities and locomotor ability was assessed by walking distance and speed. Results indicated that in both males and females, low muscle strength and power is correlated with poor mobility (Lauretani et al., 2003). The percentage of muscle power significantly decreased by year

compared to muscle strength which was a significant indication of poor mobility (i.e., walking speed and distance).

The morphological changes that occur in muscles as individual's age are associated significantly with impaired mobility and locomotor activity. Muscle fibers undergo changes in size and fiber type grouping, which are known symptoms of sarcopenia (Edström & Ulfhake, 2005). Furthermore, muscle atrophy in aging is associated with profound changes in mature muscle-specific proteins, transcription factors and energy producing enzymes (Edström et al., 2007). There are many factors that are associated with degeneration of muscles leading to impairments in muscle strength and functioning, as well as muscle mass. Although studies have suggested ways muscle attenuation is reduced, the progressive decline in muscle mass and strength are physiological impairments that ultimately result from an individual's continuous physiological decline in age.

In addition to muscle atrophy, elderly individuals also suffer from a decrease in bone loss and bone mineral density which may result in fragility and fractures in the elderly (Janssen, Heymsfield, & Ross, 2002). Clinical research has ranked osteoporosis as one of the top detrimental diseases for elderly people along with diabetes, hyperlipidemia, hypertension and heart disease. Consequently elderly persons who suffer from osteoporosis have an increased susceptibility to bone fragility and fracture. According to the National Hospital Discharge Survey, in 2010 approximately 258,000 individual's ages 65 and older in the United States were hospitalized for hip fractures. Similar to other degenerative diseases, symptom severity of osteoporosis increases with age, as well as the incidence rate of the disease (Robert & Cosman, 2008). In 2008,

according to the National Osteoporosis Foundation, approximately 19.3% of men and 30.8% of women aged 50 years or older in the United Sates met the treatment thresholds for osteoporosis (Dawson-Huges et al., 2010). Hannan et al. (2000) assessed the reduction in bone mineral density in elderly individual's age 67-92 years old for a 4-year time span. Bone loss was measured in a variety of skeletal structures including femur, radial shaft, lumbar spine, and ultradistal radius. Results indicated that women lost approximately 0.86% to 1.12% of mean bone loss and men averaged about 0.05% to 0.90% bone loss. In addition, bone mineral density decreased by approximately 3% to 5% in women and 0.1% to 4% in men (Hannan et al., 2000).

Current aging research is interested in observing functional and pathological similarities between sarcopenia and osteoporosis. Both disorders are age-dependent, as they are more pronounced and prevalent in elderly populations (DiGirolamo, Kiel & Esser, 2013). In addition, sarcopenia and osteoporosis are characterized by progressive loss in tissue mass and can produce physical detriments including falls and bone fractures (Nguyen et al., 1993). Recent evidence suggests that low muscle mass and strength is positively correlated to low levels of bone mineral density (Kim et al., 2013). Therefore, with the similarities and associations between sarcopenia and osteoporosis, aging individuals are increasingly susceptible to physiological impairments that can be sustained from decreased muscle mass and bone density. As individuals enter the senescence period, understanding these types of impairments is essential for maintaining a healthy and safe lifestyle. Furthermore, detrimental lifestyle behaviors, specifically heavy alcohol consumption, can significantly affect individual's health and make them increasingly susceptible to neurological and physiological impairments.

Ethanol and Aging

As individuals enter the senescence period, they become more susceptible to degenerative disorders such as osteoporosis (Robert & Cosman, 2008), sarcopenia (Rolland et al., 2008; Visser et al., 2005) and motor dysfunction such as ataxia (Odenheimer et al., 1994). It is important for elderly individuals to modify certain types of behaviors to reduce the risk of symptom severity in these types' of physiological impairments. As the percentage of elderly individuals continues to rise throughout the world, as well as the average lifespan of an individual, elderly alcohol consumption and misuse is becoming an increasing focus for epidemiological studies (Trevisan, 2014). Approximately 50% of the elderly population (aged over 65) and almost 25% of individuals over 85 years old currently drink alcohol (Caputo et al., 2012). In addition, nearly 13% of men and 8% of women over the age of 65 consume alcohol in a binge drinking manner (Blazer & Wu, 2009). According to the National Institute on Alcohol Abuse and Alcoholism (NIAAA), individuals 65 years of age and older who consume more than seven drinks per week or more than three drinks on a single day are drinking at a risk like manner (NIAAA, 2005). According to previous research on elderly Medicare recipients, approximately 9% of elderly Medicare recipients reported drinking more than 30 drinks per month or greater than 3 drinks per day (Merrick et al., 2008). In the United States, prevalence of alcohol use disorders among the elderly population is approximately 1-3% (Caputo et al., 2012). As the global population continues to increase, substantial alcohol consumption and its consequences in the elderly is an important but understudied public health concern (Babor, 2010).

Individuals aged 65 years and older are especially susceptible to the risk factors associated with alcohol consumption including cognitive deficits, dementia, and hypertension (Thomas & Rockwood, 2001; Coulton et al., 2008; Rosen et al., 2011). A previous study that examined the association between heavy drinking patterns and chronic disorders in elderly individuals (i.e., 65 years or older) reported hypertension (6.9%) and diabetes (4.5%) to be the most prevalent chronic conditions associated with heavy drinking (Ryan et al., 2013). The prevalence and occurrence rates of these disorders are age-dependent. Therefore, elderly individuals who are already susceptible to these disorders and who also consume high quantities of alcohol may suffer from significant neurological and physiological impairments.

The relationship between alcohol and cognitive functioning has been extensively studied in the field of alcohol research. Rather than producing a linear relationship, the effects of alcohol consumption on cognition produces a "U" or "J" shaped relationship (Mukamal et al., 2004). For example, low to moderate amounts of alcohol (i.e., 1 to 6 drinks per week) consumed revealed individual's had a lower risk of dementia compared to subjects who consumed higher quantities of alcohol (i.e., 14 or more drinks per week). A recent study examined the association between alcohol-related problems and cognition in elderly patients (Lopes et al., 2010). Elderly subjects (mean age of 70.9 years) exhibited greater impairments in cognition including memory and functional performance including dementia with heavy alcohol use. Interestingly, results also indicated significant impairments in cognition in association with heavy alcohol consumption was only found in elderly women participants and not men.

Heavy and chronic administration of alcohol can produce neurotoxic effects on the brain including a reduction in cerebral blood flow, neurological atrophy, cell death and loss of synapses (Brun & Anderson, 2001; Harper & Matsumoto, 2005). Neurodegenerative disorders such as dementia and Alzheimer's are both associated with loss or degeneration of neuronal tissue as of result of aging. Heavy and chronic consumption of alcohol produces neuronal atrophy, which is associated with a decline in cognitive functioning resulting in disorders such as dementia, including Alzheimer's disease (Ryan et al., 2013). Neuroimaging studies have examined the association between chronic alcohol consumption and brain atrophy (Cardenas, Studholme, Gazdzinski, Durazzo & Meyerhoff, 2006). Chronic alcohol-induced brain tissue loss was observed in recovering alcoholic older adults and elderly individuals (Cardenas et al., 2006). Brain atrophy was observed in the frontal and temporal lobes in abstinent alcoholics compared to normal aging individuals. Frontal and temporal lobe volumes were inversely related to the amount of alcohol consumed chronically, suggesting that the greater amount of alcohol consumed the smaller the volume size of frontal and temporal lobes (Cardenas et al., 2006). According to Thompson et al. (2003), cortical gray matter loss in temporal and frontal regions of the brain was found in patients who suffered from Alzheimer's disease. Furthermore, impairments in these regions of the brain were associated with declining cognitive functioning. Recently, Ryan et al. (2013) investigated the association between prevalence rates of alcohol consumption and chronic conditions using elderly individuals ages 65 years and older. Approximately 3.4% of elderly subjects who reported drinking patterns that exceeded the NIAAA drinking guidelines suffered from Alzheimer's disease and related disorders of senile dementia (Ryan et al., 2013).

Additionally, it has been well established that alcohol consumption can have physical impairments to older individual's motor coordination, bone density, and increased falls (Weafer & Fillmore, 2011; Mukamal, et al., 2007). Chronic effects of heavy alcohol consumption have been associated with osteoporosis and osteoporotic fractures (Diamond et al., 1989). High consumption of alcohol significantly produces an increase in risk of two types of fractures including osteoporotic fractures and hip fractures (Kanis et al., 2005). In relation to bone mineral density (BMD), low to moderate levels of alcohol have been shown to increase BMD; however, higher levels of alcohol intake may produce detrimental effects on BMD (Ganry, Baudoin & Fardellone, 2000). Furthermore, a decrease in BMD has been observed in the forearm, spine, iliac crest and trochanter after chronic heavy alcohol consumption (Alvisa-Degrin et al., 2009; Diamond et al., 1989).

In accordance with elderly individuals having decreased bone density, research has shown that alcohol consumption is highly correlated with increased falls and hip fractures in elderly individuals (Kim et al., 2013; Mukamal et al., 2007). Hip fractures are one of the leading causes of impairments in mobility and mortality among the elderly community (Cummings & Melton, 2002). In addition, approximately 90% of hip fractures occur directly from elderly individuals falling (Parkkari et al., 1999). A previous study examined different lifestyle behavioral factors that are associated with fall-related hip fractures in individuals 65 years and older (Feel, McClure & Hendrikz, 2006). Low to moderate alcohol consumption was considered a protective effect on the risk of falling-related hip fractures in older age. Results suggest that lower levels of alcohol consumed decreased risk of fall-related hip fracture in older age (Feel et al., 2006). Factors that may

contribute to alcohol-associated bone mass reduction include poor nutrition due to chronic alcohol consumption and toxicity of alcohol on bone. Alcoholics suffer from poor nutrition and diet, resulting in individuals not receiving certain vitamins and minerals needed for optimal health (Gariballa & Sinclair, 1998). Deficiencies in these vitamins and minerals could lead to detrimental changes in bone mineral density. Heavy alcohol consumption produces a direct toxic effect on osteoblast function by significantly reducing osteosarcoma cell proliferation, as well as inhibiting bone mineralization (Vignesh et al., 2006). Falls and fractures are direct effects of heavy alcohol consumption in both acute and chronic administration. In addition to falls and fractures, heavy consumption of alcohol can produce ataxia.

Gait ataxia is characterized as a disorder of the cerebellum and produces locomotor impairments including deficits in balance and motor coordination, walking, and irregular joint movement and limb placements (Morton & Bastian, 2002; Morton & Bastian, 2007). Individuals who suffer from gait ataxia are more likely to experience impairments in physical stance and fall-related injuries (Schniepp et al., 2014; Fonteyn et al., 2012). In addition, consumption of alcohol can exacerbate motor impairments associated with ataxia and cerebellar damage (Schniepp et al., 2014; Fein & Greenstein, 2013). The prevalence and symptom severity of ataxia and other motor impairments rise with age (Odenheimer et al., 1994). Therefore, elderly individuals who are more susceptible to ataxia and other motor impairments while consuming large quantities of alcohol may be at high risk for fall-related injuries, bone disorders and significant motor disabilities.

Alcohol-induced ataxia which may be increased in elderly drinkers. For example, low to moderate doses of alcohol have been shown to significantly impair balance in women between the ages of 70 and 79, as measured by absent or distorted vision and proprioception (Jones et al., 1994). In addition to impaired balance, the effect of high alcohol consumption by elderly individuals and associated risk of hip fractures has been investigated (Høidrup, Grønbæk, Gottschau, Lauritzen & Schroll, 1999). Elderly males who consumed 27 or more drinks per week had a higher increase of hip fractures compared to subjects in the same age group who consumed less than 27 drinks per week. Women, approximately 70 years or older, who drank between 14-27 drinks per week also had an increase in hip fractures compared to women in the same age group who drank less than 14 drinks per week. In relation to hip fractures, Mukamal et al. (2004) found that individuals ages 65 years or older who consumed 14 or more drinks per week had a higher risk of falls. Smith and Fein (2011) observed the effects of short and long-term abstinence on alcohol-dependent individuals' gait and balance impairments. Results revealed alcohol-dependent individuals who were abstinent from alcohol short-term had more impaired gait and balance performance compared to individuals undergoing long-term abstinence (Smith & Fein, 2011). Weakening motor coordination or movement could be attributable to a decline in cerebellar functioning, which plays a key role in the regulation of posture, balance, and motor control (Piguet et al., 2006; Carpenter, 1976). In addition, ethanol produces multiple types of motor impairments including decline in motor coordination and balance; therefore, to further understand impairments in motor behavior, it is important to investigate areas of the brain associated with these functions, as well as the role of ethanol intoxication has on the brain.

The Cerebellum

The primary functioning of the cerebellum includes the maintenance of balance and posture, coordination of voluntary movements, postural balance, and shifting and orienting attention (Carpenter, 1976). In addition to motor functioning, the cerebellum plays an important role in cognition including associate learning, verbal memory, spatial memory, episodic memory, and language (Cabeza & Nyberg, 2000). Damage to the cerebellum can produce significant impairments in motor functioning and cognition. Lateral and midline cerebellar lesions in rat's causes damage the cerebellar vermis, fastigal nucleus, and cerebellar cortex leading to impairments in equilibrium and posture (Joyal et al., 1996). In addition, damage to the cerebellar vermis, a primary structure of the cerebellum involved with coordination of movement, equilibrium, balance and posture (Piguet et al., 2006) can produce multiple types of cerebellar dysfunction including gait ataxia and impairments in stance (Thach & Bastian, 2004). Localized focal ischemic lesions to the deep cerebellar nuclei (i.e., interpose, dentate, and fastigial nuclei) produce motor impairments including ataxia of posture and gait abnormalities that are associated with irregularity in walking and taking staggering steps with a wide-based stance (Blumenfeld, 2010; Schoch, Dimitrova, Gizewski & Timmann, 2006). In addition to cerebellar lesions, chronic neuronal atrophy of cerebellar tissue plays an important role in motor dysfunction.

Current research has focused on the role of the aging cerebellum in motor impairments such as gait and truncal ataxia due to long-term neuronal atrophy. Age-associated decline in gait is related to neuronal atrophy of both gray and white matter cortical regions of the cerebellum in older adults (Nadkarni et al., 2013). According to

Nadkarni et al. (2013), total cerebellar gray matter volume in older adults was associated with reduced impairments in gait speed. Interestingly, when controlling for small vessel disease in the cerebellum, total gray matter volume in the cerebellum was still significantly associated with impairments in gait, suggesting that neuronal atrophy of the cerebellum play a major role in motor functioning. Atrophy studies reveal that specific areas of the cerebellum are more susceptible to neuronal atrophy than other regions. Degeneration of the cerebellar vermis is primarily age-dependent (Koller et al., 1981). Furthermore, the aging superior lobules of the vermis show increased cell loss (Torvik, Torp & Lindboe, 1986). Wu et al. (2012) indicated cerebellar atrophy in older age individual's using MRI scans, resulting from loss of gray matter in the posterior vermis and anterior lobe, both of which are involved with stance and gait movement (Wu et al., 2012; Thach & Bastian, 2004). Cerebellar peduncles and the posterior lobe exhibited a reduction in white matter tissue (Wu et al., 2012). In addition to atrophy of neuronal tissue in the cerebellum due to aging, other behaviors during an individual's lifetime can produce similar cerebellar atrophy.

It is well known that alcohol can cause neurotoxic effects on neuronal tissue in the brain. Alcohol is both hydrophilic and lipophilic, allowing the drug to diffuse easily though biological membranes and readily enter bodily and neural tissue (Julien, 2001). The blood brain barrier is a protective barrier between the brain and peripheral blood that blocks out harmful and toxic substances from the brain that could cause inflammation or neurodegeneration (Singh, Jiang, Gupta & Benlhabib, 2007). However, alcohol is able to penetrate lipid endothelial cells in the brain, making the brain susceptible to the

detrimental effects of alcohol (Julien, 2001). Therefore, alcohol can produce extensive altering neuroanatomical and neurochemical effects in the brain.

The effects of alcohol on the central nervous system have been well established. Alcohol produces a wide variety of motor impairments, which are dependent on cerebellar functioning; therefore, the cerebellum is an important neurological structure to target for investigation. Purkinje cells are the sole output neuron from the cerebellar cortex to deep cerebellar nuclei, which produce inhibitory effects on multiple extracellular nuclei and vestibular nuclei in the brain to coordinate an individual's movement. Stellate and basket cell, two types of cerebellar interneurons, relay inhibitory GABAergic input to Purkinje cells, producing an overall inhibitory response (Dizon & Khodakhah, 2011). Alcohol enhances GABA-mediated synaptic transmission, both directly via $GABA_A$ receptors and indirectly by stimulating GABA release. Therefore, alcohol can produce significant inhibitory effects of cerebellar interneurons by modulating GABA functioning (for review see Kumar, Fleming & Morrow, 2004). Furthermore, ethanol mediated GABAergic inhibition of Purkinje neurons directly impairs complex motor tasks (Wulff et al., 2007) and cause detrimental motor impairments (Van Skike et al., 2010).

Previous evidence using animal models, has demonstrated the acute and chronic effects of ethanol on GABAergic interneurons and Purkinje cells. Using patch-clamp electrophysiological techniques, Mameli et al. (2008) examined the acute effects of ethanol on GABAergic interneurons and Purkinje cell firing in the cerebellar vermis. Results indicated ethanol increased spontaneous GABA release at the presynaptic level producing miniature inhibitory synaptic currents (mIPSCs) and spontaneous inhibitory

synaptic currents (sIPSCs) in Purkinje cells. Ming, Criswell, Yu & Breese (2006), also examined the effects of ethanol on neuronal firing of cerebellar Purkinje neurons. Similar to Mameli et al. (2008), results indicated ethanol increased presynaptic release of GABA to Purkinje neurons producing GABAergic inhibition (Ming et al., 2006). Furthermore, acute ethanol exposure increased the frequency of $GABA_A$ mIPSCs in the cerebellar vermis in a dose-dependent manner (Ming et al., 2006). Overall, ethanol can increase presynaptic GABAergic inhibition of Purkinje neurons resulting in an inhibitory effect on cerebellar functioning and motor activity.

In addition to neuronal inhibition in the cerebellum, long-term heavy alcohol consumption has been shown to produce cerebellar atrophy in neural tissue located in the vermis, flocculonodular lobe, and central white matter (Victor, Adams, & Mancall, 1959; Baker, Harding, Halliday, Kril, & Harper, 1999). The cerebellar vermis has been shown to be the region of the cerebellum most greatly impaired by ethanol consumption, producing cerebellar atrophy (Victor et al.,1959). Cerebellar atrophy of the vermis is potentially due to the cell death and degeneration of Purkinje neurons in the vermis (Andersen, 2004: Karhunen, Erkinjuntti, Laippala, 1994; Allsop & Turner, 1966). Furthermore, aged individuals are increasingly susceptible to neurotoxic effects of heavy alcohol consumption.

Alcohol injures the cerebellar vermis through decreasing cell density, resulting in gait ataxia (Piguet et al., 2006; Johnson-Greene et al., 1997; Phillips, Harper, & Kril, 1990). Piguet et al., (2006) reported that higher levels of daily alcohol consumption in subjects age 75 and over were found to be associated with smaller cerebellar vermis sizes. There was also a strong correlation between large daily alcohol consumption in

males and smaller cerebellar volume. Sullivan et al. (2000) reported alcoholic older aged individuals (mean age range 46-65 years old) exhibited cerebellar tissue volume loss in the cerebellar hemispheres, anterior, superior and middle vermis as a result of normal aging and chronic alcohol consumption. Patients exhibited greater gray matter loss compared to white matter in the cerebellar hemispheres. Volume loss in these cerebellar vermal regions was functionally significant with impairments in ataxia. Therefore, the aging cerebellum paired with heavy and chronic alcohol consumption could lead to increased motor impairments in the elderly. Research focused on alcohol and elderly individual's has increased. However, the effects of alcohol consumption during the adolescent developmental period, specifically in a heavy and binge manner is well known and well established.

Adolescence

Adolescence is defined as the transition state between childhood and adulthood, where young individuals experience a variety of biological changes that could potentially lead to new social, behavioral, and emotional occurrences in the adolescent's life (Crone & Dahl, 2012). Through the occurrence of pubertal maturation and rapid secretion of hormones including adrenal androgens, gonadal steroids, and growth hormones, adolescent individuals will start to experience substantial physiological and psychological changes (Blakemore, Burnett, & Dahl, 2010). In humans, the beginning of the age period is defined by a variety of maturational changes that occur during puberty. Furthermore, the end of adolescence is culturally defined to occur towards the individual's early twenties (Arnett, 2004). However, in rodents, adolescence has been more clearly defined. In mice, the adolescent time period occurs from postnatal day PD 22 to PD 60 (Laviola,

Macri, Morley-Fletcher, & Adriani, 2003). The adolescence time period in rats is controversial; however, adolescence has been shown to begin on PD 30 and extends until approximately PD 55 (Odell, 1990). Therefore, with the extensive physiological and psychological changes that occurs during adolescence, consumption of alcohol by adolescent individuals can produce a variety of neurological and behavioral effects.

The adolescence period is a critical period in which adolescents start to establish a sense of autonomy, in which they develop their own personal identity (Temple, 2012). In addition, adolescents are exposed to new environments, situations, and behaviors that could potentially be beneficial or harmful to their development. Peer influence and social behavior are two significant factors that facilitate adolescent's identity exploration and new learning (Balsa, Homer, French & Norton, 2011). During the adolescent period, individuals can conduct risky behaviors to conform to social norms and peer pressure (Allen, Porter & McFarland, 2006). Furthermore, adolescents are particularly invested in gaining recognition and maintaining their social status amongst peers (Michell & Amos, 1997). According to Bandura (1973), social learning theory states that individuals conform to behaviors that they believe will earn levels of high peer status. Risky behaviors including alcohol use and substance abuse are strongly associated with maintaining social status (Killeya-Jones, Nakajima ad Costanzo, 2007). Therefore, adolescents may be increasingly motivated to consume alcohol, specifically in a heavy or binge-like manner, to engage in peer social norms and maintain high social status (Reboussin, Song & Wolfson, 2012; Balsa et al., 2011).

The Adolescent Brain

The adolescent period is a critical period of brain development, in which individuals utilize a variety of resources such as the environment and social situations to assist in the growth and developmental process into adulthood. During adolescence the brain undergoes substantial neural development due to biological changes associated with puberty, environmental stimuli and social learning (Blakemore et al., 2010). According to Klein et al. (2014), major modifications of cortical circuits occur during adolescence including reductions in gyrification across large areas of the cerebral cortex, in particular the precentral, frontal, and temporal regions. Modifications of these regions of the cerebral cortex during late brain maturation may be associated with cognition and experience-dependent plasticity (Klein et al., 2014). Myelination and development of white matter extends into the forebrain regions of humans through adolescence and into early adult life (Giedd, 2004). Impairments in white matter development or incomplete myelination may be associated with detriments in cognitive ability including poor decision-making skills and IQ, as well as increased risk taking in adolescents (Beckman, 2004). According to Giedd (2004), many of the subcortical gray matter changes that occur in adolescents are gender dependent. For example, the caudate nucleus of the basal ganglia decreased in volume during teenage years and is relatively larger in females compared to males. Furthermore, regions of the limbic system are also sexually dimorphic. The amygdala volume increased significantly only in males, while the hippocampus volume increased significantly with age only in females, which may be influenced by increases in hormones during adolescence (Giedd et al., 1996). Structural changes and decreasing synaptic connectivity in the adolescent brain is associated with

reorganization of neural activity and prepare for neurological maturation into an adult brain (Gogtay et al., 2004: Andersen, 2003).

The volume of specific brain regions undergo developmental maturation from childhood to adolescence and into adulthood. For example, frontal cortical regions in adolescent brains develop in an inverted "U" shaped pattern across adolescence (Giedd, 2004; Gogtay et al., 2004; Giedd et al., 1999), suggesting an increase in brain volume into adolescence then decreasing volume towards adulthood. Possible factors associated with larger brain volumes include increases in neuronal count and white matter during adolescence. Growth of white matter continues into the frontal cortical regions (Giedd, 2004), as well as into the cerebellum, limbic regions, and internal capsule through adolescence into early adulthood (Østby et al., 2009; Juraska & Markham, 2004). Therefore, the numerous neurological changes that occur during adolescence compared to adulthood may be associated with differential detrimental effects of ethanol consumption in both age groups.

Ethanol and Adolescence

During the adolescent period, adolescents consume heavy and binge-like amounts of alcohol at increasingly higher rates compared to adults (Ehlers et al., 2011). In addition, the rate at which adolescents are consuming binge amounts of alcohol peaks during the adolescent period (Ehlers et al., 2011). According to Duncan, Duncan and Strycker (2006), the onset of intermittent binge drinking in pre-adolescence can occur as early as 9 through 12 years of age, and continue into the adolescent period. In the United States, the average age of adolescent individual's onset of drinking is 16 years of age (SAMHSA, 2012). Furthermore, early age of onset for alcohol consumption is a major

risk factor for hazardous drinking in college students (Morean et al., 2014; Hingson & Zha, 2009; Hingson, Heeren, & Winter, 2006). In addition to early onset, Johnston, O'Malley, Bachman, & Schulenberg (2013) reported adolescents' rate of alcohol use to have increased from 24% to 64% in 2012. Therefore, with the extensive neuronal maturation that occurs in the adolescent period, heavy alcohol consumption during adolescence may affect these individuals into adulthood and beyond the period of intoxication.

There are many factors that may be associated with increased alcohol consumption during the adolescent period compared to adulthood. Parental influence on adolescent drinking behavior is strongly correlated with consumption of alcohol during adolescence (Mathijssen et al., 2014). A positive and higher quality of parent-child relationship is significantly associated with reduced alcohol use during adolescence, as well as, throughout the adolescent's lifetime. Furthermore, parents' attitude towards drinking alcohol plays an important role in adolescent alcohol use (Mathijssen et al., 2014). Adolescents' social and personal motivations to consume alcohol also play an important role in alcohol use and binge drinking. During adolescence, an individual's social environment may either positively or negatively influence an adolescent's initiation to consume alcohol, as well as the rate and amount of alcohol consumed. In addition, peer pressure and social norm expectations may further influence risk taking and consumption of alcohol (Beck & Treiman, 1996). Finally, a major factor associated with increased alcohol consumption during adolescence is reduced sensitivity to the impairing effects of alcohol. Reductions in sensitivity to the impairing effects of ethanol are major risk factors associated with the development of alcoholism (Schuckitt, 1994).

Adolescents are significantly less sensitive to the impairing effects of ethanol compared to adult rats, specifically in motor impairments (Novier, Van Skike, Chin, Diaz-Granados, Matthews, 2012; Van Skike et al., 2010; White et al., 2002) and sedation (Little et al., 1996). Reduced sensitivity to the acute effects of alcohol consumption may be a potential risk factor for binge-like consumption of alcohol. Adolescents may associate this type of behavioral insensitivity to alcohol as a positive moderating cue to continue to consume alcohol in a binge-like manner (Hefner & Holmes, 2007; Spear & Varlinskaya, 2005). Reports of reduced sensitivity of alcohol during adolescence is troublesome due to the increasing rate of alcohol consumption in the United States (Johnston et al., 2013) and age of onset for drinking (SAMSHA, 2012). Furthermore, previous research reports the adolescent brain is vulnerable to chronic and binge-pattern effects of alcohol intoxication which produce behavioral and cognitive impairments (Squeglia et al., 2014; Fleming et al., 2013; Van Skike et al., 2012).

Neurological changes in brains of adolescent heavy drinkers have also been examined in addition to behavioral and cognitive impairments. Squeglia et al. (2014) investigated brain volume reductions in adolescent heavy drinkers between the ages of 12 to 17. MRI results indicated heavy drinking during adolescence is associated with volume reductions in subcortical structures including the diencephalon and caudate nucleus. Binge drinking during adolescence is also associated with decreased cerebellar volumes (Lisdahl, Thayer, Squeglia, McQueeny & Tapert, 2013). MRI imaging from adolescent binge drinkers ages 16 to 19 exhibited both grey and white matter reductions in cerebellar hemispheres, as well as reduced white matter in the superior cerebellar peduncles. Lisdahl et al. (2013) provides further evidence as to the detrimental effects of alcohol on

GABAergic inhibition on Purkinje neurons in the cerebellum and decreasing neuronal density. In addition, previous research has reported adolescents with alcohol use disorders (AUD) show reductions in left hippocampal volume densities (Nagel et al., 2005: De Bellis et al., 2000). Furthermore, Medina et al. (2008) investigated the effects of AUDs on adolescent prefrontal cortex (PFC) volumes, while controlling for conduct disorders, gender, and intracranial volume. Results indicated that alcohol consumption during adolescence is associated with smaller PFC volume and gender-dependent reduction in PFC white matter volume.

Therefore, alcohol can produce significant impairments throughout the adolescent period that can persist into adulthood. In addition, alcohol consumption produces significant detrimental physiological and neurological effects in aged individuals 65 years and older. To further understand the effects of alcohol on the brain and body, it is necessary to investigate the dose-dependent effects of alcohol across age groups. Specifically, a comparison of adolescent, adult and aged impairments associated with alcohol administration.

Ethanol and the Rodent Lifespan

In concordance with age-related effects of alcohol in humans, research using animal models has shown that many of the effects of alcohol are age-dependent (Chin et al., 2010). Adolescent rats are less sensitive to acute ethanol effects including sedation as measured by loss of righting reflex (Little, Kuhn, Wilson, & Swartzwelder, 1996), anxiogenic effects (Doremus, Brunell, Varlinskaya, & Spear, 2003), hypnotic (Silveri & Spear, 1998; Matthews, Tinsley, Diaz-Granados, Tokunaga, & Silvers, 2008) and motor impairments (Van Skike et al., 2010; Ramirez & Spear, 2010; White et al., 2002)

compared to adult rats. However, chronic exposure to ethanol produces increased motor impairments. Recently, Forbes et al. (2013) investigated the effects of intermittent binge exposure of 3.0 g/kg ethanol on long-term motor functioning throughout the adolescent age period. Using a variety of motor assessing tasks, adolescent animals exhibited significant impairments in motor functioning. In addition, administration of at least four 3.0 g/kg injections in adolescent animals can produce significant degeneration of cerebellar Purkinje cells, shown three weeks after injection.

Adolescent rats are also less sensitive to hang over impairments as seen through a decrease in exploratory or social behavior (Doremus-Fitzwater & Spears, 2007; Varlinskaya & Spear, 2004). Research observing acute tolerance in adolescent and adult rats found that adolescent rats often exhibit significantly more acute tolerance compared to adult rats (Draski et al., 2001; Grieve and Littleton, 1979; Silveri and Spear, 1998, 2002; Varlinskaya and Spear, 2006). Furthermore, adolescent rodents exhibit less sensitivity to alcohol-induced hypothermia (Ristuccia & Spear, 2008). However, chronic administration of ethanol produces altering effects compared to acute alcohol. Crews et al. (2000) examined the effect of a 4-day binge ethanol exposure during adolescence. Results indicated that chronic ethanol exposure produced brain damage to specific areas in the adolescent brain but not to the adult brain.

Research indicates that older rodents are more sensitive to acute alcohol exposure compared to adolescent and adult rats (Novier, Van Skike, Diaz-Granados, Mittleman, & Matthews, 2013; Van Skike et al., 2010). Older rodents are more sensitive to the hypnotic (Ott, Hunter, & Walker, 1985) and hypothermic effects of acute alcohol (Wood & Armbrecht, 1982), as well as the severity of alcohol withdrawal (Wood, Armbrecht, &

Wise, 1982) compared to younger rodents. White et al. (2002) demonstrated that adult rats produce greater motor coordination impairments compared to adolescent rats following alcohol administration. To explore the effects of alcohol across the lifespan, Van Skike et al. (2010) examined the impact of a single acute alcohol dose on motor impairments in four rodent age groups. Aged rats showed significantly more impairment in motor performance compared to periadolescent and adolescent rats. Furthermore, Novier et al. (2013) investigated the motor and memory impairing effects of acute alcohol between adult and aged rats. Similarly, results indicate that aged animals performed significantly worse in all behavioral measures compared to adult rats.

Primary Investigative Goal

Although current research has recognized differential motor impairments between adolescent and aged rats (White et al., 2002; Van Skike et al., 2010) and adult and aged rats (Novier et al., 2013), research has yet to systematically investigate the effects of acute ethanol on two types of motor impairments using multiple motor assessment tasks across the rodent lifespan using a dose-dependent analysis. In addition, the effect of age on a high dose of ethanol-induced hypnosis has yet to be investigated. Therefore, we sought to determine the age-and dose-dependent effects of 1.0 g/kg and 2.0 g/kg acute alcohol exposure on gross and coordinated motor performance in adolescent, adult, and aged rats using the accelerated rotarod (RR) and aerial righting reflex (ARR). In addition, the effect of 3.0 g/kg on ethanol-induced loss of righting reflex (LORR) was determined. Finally, blood ethanol concentrations (BEC) were determined in separate group of animals at 7 different time points following a 3.0 g/kg ethanol injection to better understand how BEC impacts LORR at three different ages. We present evidence that

alcohol produces greater motor impairments in older rats when compared to adolescent and adult rats and these motor impairments are not completely explained by blood ethanol concentrations.

CHAPTER TWO

Materials and Methods

Subjects and Drug Administration

Twelve adolescent (postnatal day (PD) 28), twelve young adult (approximately PD 70), and twelve aged (approximately 18 months) male Sprague-Dawley rats were obtained from Harlan Laboratories (Indianapolis, IN). These ages were selected because PD 30 is a developmental period during early adolescence based on evidence that mature sperm is not yet found, while all sperm are mature at PD 70 indicating this age is the beginning of adulthood (O'Dell, 1990). Aged animals were 18 months to be consistent with our previous work (Novier et al., 2013). Animal care procedures were approved by the Institutional Animal Care and Use Committee of Baylor University. Animals were individually housed and given ad libitum access to food and water throughout the experiment. Following previously published methods, all rats acclimated to the colony room for 2 days before the start of any experimental procedures (Novier et al., 2013, 2012; Van Skike et al., 2012; Chin et al., 2011; Silvers et al., 2006; Tokunaga et al., 2006).

To investigate the dose-dependent effects of acute exposure in the same animals and therefore reduce subject number, animals were involved in a repeated measures design with experimental doses of 1.0 g/kg or 2.0 g/kg (10% w/v) ethanol, or a saline dose equivalent to the volume of a 1.0 g/kg dose of ethanol. Three separate trials were conducted three days apart to minimize carry over effects. The first trial session occurred

twenty-four hours after the last RR training trial. Animals were randomly assigned to each of the three different drug orders and counterbalanced by age and dose for all three testing trials.

Experiment 1: Aerial Righting Reflex (ARR)

The effect of acute alcohol exposure on gross motor impairment was assessed 20 minutes after saline or alcohol administration by ARR as previously described (Novier et al., 2013; Van Skike et al., 2010). Six adolescent rats, six adult rats, and six aged rats received an i.p. injection of 1.0 g/kg or 2.0 g/kg (10% w/v) ethanol, or a saline dose equivalent. A ruler was vertically taped above a 10-inch foam pad. Animals were initially released 5 inches (12.7 cm) above the foam pad in a supine position. An animal's righting reflex was considered successful if three out of four paws made direct contact with the foam pad on two out of three releases. If righting was not successful, the height of release was increased in 5-inch (12.7 cm) increments up to a maximum height of 25 inches (63.5 cm). Subjects who failed to achieve successful righting reflex at 25 inches (63.5 cm) were given a score of 30 inches (76.2 cm) for statistical analysis.

Experiment 2: Accelerating Rotarod (RR)

Apparatus: RR was used to investigate the effects of alcohol exposure on motor coordination. Motor activity was tested on a four station Rota-Rod treadmill (Model ENV 575, Med Associates, St. Albans, VT). The apparatus was located in a behavioral room isolated from animal caging and housing.

Training: Adolescent, adults and aged animals received five consecutive training trials on the RR, as previously described (Novier et al., 2013). The rod accelerated from 4

rpm to 40 rpm over a 5-min period. The RR is covered with fine grit sandpaper to provide a uniform surface and to reduce slipping (Rustay, Wahlsten, & Crabbe, 2003). The RR was interfaced to a computer that collected the time each subject remained on the rod up to a maximum time of 360 seconds. After the five training trials were complete, averages of the last three trials were calculated and animals who failed to meet a criterion of 7 seconds were given additional training trials until their final three training trials averaged 7 seconds.

Testing: Six adolescent rats, six adult rats, and six aged rats received an i.p. injection of 1.0 g/kg or 2.0 g/kg (10% w/v) ethanol, or a saline dose equivalent. Motor activity on the RR was recorded 10 minutes after alcohol or saline administration. At the start of trial 1, adolescent rats were PD 31, adult rats were PD 73 and aged animals were 18 months.

Experiment 3: Loss of Righting Reflex (LORR)

Procedure: The sedative/hypnotic effect of alcohol was assessed using the loss of righting reflex paradigm. One week after the last testing trial (PD 46 for adolescents, PD 88 for adult, and 18 months for aged rats), a subset of previously tested animals (n = 4) received an i.p. injection of 3.0 g/kg alcohol. Animals were monitored throughout LORR. Latency to regain righting reflex was assessed. Recovery of reflex was defined as successfully righting three times in one minute.

Experiment 4: Blood Ethanol Concentration

Procedure: To better understand how age impacts blood ethanol levels, six adolescent rats, six adult rats, and six aged rats were injected with 3.0 g/kg ethanol i.p.

before collection of blood via the tail. Animals were the same age as those previously assessed for LORR to allow for a comparison of how BEC influences righting reflex. Following injection, the tail was nicked and blood collection at 7 different time points (30 min, 60 min, 120 min, 180 min, 240 min, 300 min and 360 min) and BEC was assessed via the AM1 Analox system following manufactures recommendations.

CHAPTER THREE

Results

Experiment 1: Aerial Righting Reflex

A two-way repeated measure ANOVA revealed a significant Age x Dose interaction, $F(4,15) = 6.79$, $p < 0.05$. To further investigate the significant interaction, one-way ANOVAs for age were performed at each of the three doses tested. A significant effect was found when animals were tested with 1.0 g/kg alcohol, $F(2,15) = 9.80$, $p < 0.05$. Bonferroni post-hoc tests revealed that aged rats were more sensitive to the effects of 1.0 g/kg alcohol compared to adolescents, $t = 3.84$, $p < 0.05$ and adults, $t = 3.84$, $p < 0.05$ (see Fig. 1a). Similarly, a significant effect was found when rats were tested with 2.0 g/kg alcohol, $F(2,15) = 10.19$, $p < 0.05$. Specifically, aged animals performed worse on ARR compared to adolescent, $t = 4.37$, $p < 0.05$, and adult rats, $t = 3.17$, $p < 0.05$ following the 2.0 g/kg dose (see Fig. 1b).

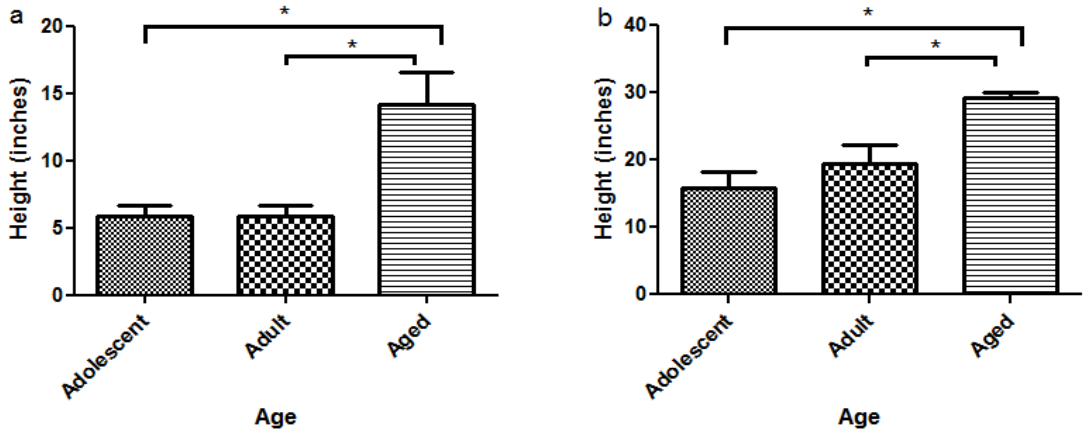

Figure 1. Aged animals are more sensitive to the gross motor effects of acute ethanol compared to adolescent and adult rats. (a) ARR height achieved after 1.0 g/kg ethanol (b) ARR height achieved after 2.0 g.kg ethanol, *$p < 0.05$, error bar denote SEM.

Importantly, no significant differences by age were found when subjects were tested with saline, $F(2,15) = 1.00$, $p = 0.39$, indicating no baseline or carry over effects in performance (data not shown). Finally, adolescent and adult rats did not significantly differ in ARR performance when given 1.0 g/kg alcohol, $t = 0.00$, $p > 0.05$ or 2.0 g/kg alcohol, $t = 1.20$, $p > 0.05$.

Experiment 2: Accelerating Rotarod

There was a significant main effect of age during RR training, $F(2,15) = 7.57$, $p < 0.05$. Bonferroni post-hoc tests revealed a significant difference between adolescent and aged rats, $t = 3.50$, $p < 0.05$ and between adolescent and adult rats in training performance, $t = 3.22$, $p < 0.05$ (see Figure 2).

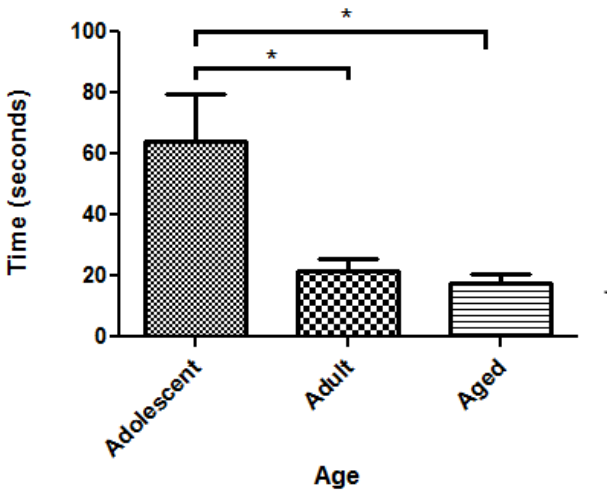

Figure 2. Adolescents spent more time on the RR during training compared to adult and aged rats, *$p < 0.05$, error bars denote SEM.

However, adult and aged animals did not significantly differ from one another on RR training performance, $t = 0.29$, $p > 0.05$. To equate for differential baseline

performance, the effect of acute alcohol on RR performance was analyzed by percent change from each animal's baseline performance.

A two-way repeated measures ANOVA revealed a significant Age x Dose interaction during RR testing, $F(4,15) = 11.76$, $p < 0.05$. One-way ANOVAs were performed to assess the significant differences between each individual dose tested. A significant effect was found when animals were tested with 1.0 g/kg alcohol, $F(2,15) = 6.51$, $p < 0.05$. Bonferroni post-hoc tests showed aged animals performed worse on RR compared to adult rats when administered 1.0 g/kg alcohol, $t = 3.46$, $p < 0.05$ (see Fig. 3a). A significant effect was found when animals were tested with 2.0 g/kg alcohol indicating motor performance impairments increased with age, $F(2,15) = 4.28$, $p < 0.05$ (however, post-hoc test revealed no significant effects between ages) (see Fig. 3b). A one-way ANOVA revealed no significant differences between ages when tested with saline, $F(2,15) = 2.80$, $p > 0.05$.

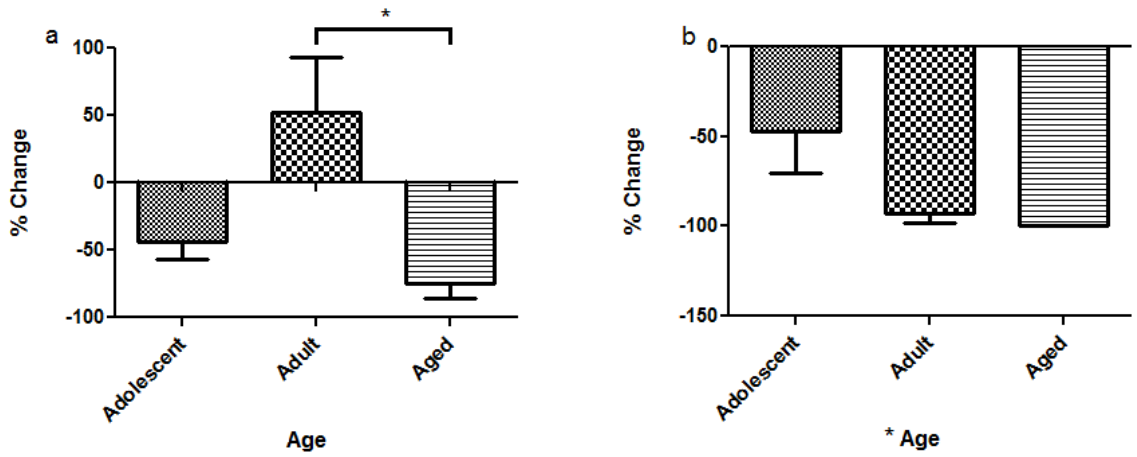

Figure *3*. Coordinated motor impairments on RR increased with age following acute ethanol administration. (a) RR performance following acute ethanol administration of 1.0 g/kg ethanol (b) RR performance following acute ethanol administration of 2.0 g/kg ethanol, *$p < 0.05$, error bars denote SEM (Post-hoc test revealed no significant effects between ages).

Experiment 3: Loss of Righting Reflex

A one-way ANOVA revealed a significant difference in sleep time, $F(2,9) = 8.02$, $p < 0.05$ (See Figure 4). Bonferroni post-hoc analysis showed aged animals slept longer (mean sleep time of 157.0 min and a standard error of the mean of 53.0 minutes) compared to adult (mean sleep time of 0 min and a standard error of the mean of 0 minutes), $t = 3.56$, $p < 0.05$, and adolescent rats (mean sleep time of 8.25 min and a standard error of the mean of 8 minutes), $t = 3.37$, $p < 0.05$. No significant difference in sleep time was found between adolescent and adult rats, $t = 0.19$, $p > 0.05$.

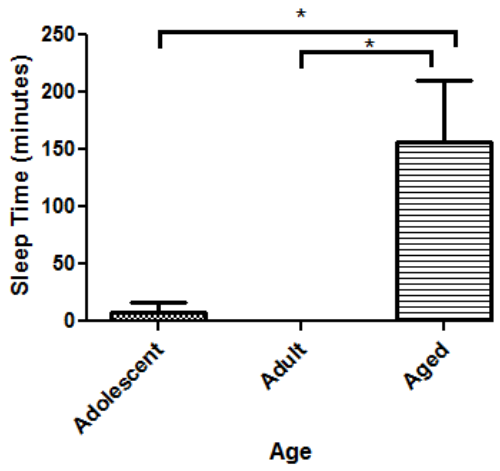

Figure *4*. Aged animals are more sensitive to the sedative/hypnotic effects of ethanol compared to adolescent and adult rats. Duration of LORR sleep time after 3.0 g/kg ethanol. *$p < 0.05$, error bars denote SEM

Experiment 4: Blood Ethanol Concentration

A two-way ANOVA revealed a significant Age x Time interaction on blood ethanol concentration, $F(12,90) = 23.41$, $p < 0.001$. One-way ANOVAs were performed to assess the significant differences at each time tested. Adolescent blood ethanol levels were significantly greater 30 minutes post-injection compared to both adult and aged

animals (Tukey post hoc comparisons, $p < 0.05$). However, adolescent blood ethanol concentrations were significantly less than adults ($p < 0.05$) and aged animals ($p < 0.001$) at the 240, 300 and 360 minute time point. Finally, adult blood ethanol concentrations were significantly different than aged animals 360 minutes post ethanol injection ($p < 0.001$) (see Table 1).

Table 1. *Mean blood ethanol concentration (BEC). SEM shown in parentheses*

Time (min)	Adolescent (mg/dL)	Adult (mg/dL)	Aged (mg/dL)
30	331 (8.52)	256 (30.77)	255 (11.41)
60	304 (12.49)	259 (20.60)	256 (13.36)
120	255 (14.20)	271 (14.91)	266 (18.26)
180	244 (14.57)	273 (9.68)	269 (15.07)
240	209 (11.37)	257 (7.72)	269 (13.03)
300	185 (13.87)	263 (12.39)	275 (13.39)
360	95 (9.61)	174 (7.18)	272 (9.44)

CHAPTER FOUR

Discussion

The current study examined the age-and dose-dependent effects of acute alcohol exposure on gross and coordinated motor performance across the rodent lifespan and provides a partial ethanol clearance curve following a high dose ethanol administration. In ARR and RR tasks, aged rats exhibited deficits in motor performance compared to both adolescent and adult rats when exposed to 2.0 g/kg and 1.0 g/kg ethanol. Although aged animals had impaired motor coordination on both motor tasks, aged animals performed consistently worse on the ARR task. Aged animals exhibited greater ethanol induced ataxia when administered both 1.0 g/kg and 2.0 g/kg ethanol compared to both adolescents and adults as evidenced by increased righting height on the ARR tasks. In addition, there were no significant motor performance deficits compared to both adolescent and adult rats. While aged rats produced consistent performance impairments on ARR, only RR task produced deficits in motor coordination in aged rats compared to adults rats when administered 1.0 g/kg ethanol. No differences in performance were found between aged and adolescent rats or adults and adolescent rats.

The present study provides evidence that the ARR paradigm appears to be a better assessment of motor performance and produces more consistent results compared to the RR task. In addition to assessing motor activity, LORR paradigm indicated that aged animals were more sensitive to the sedative-hypnotic effects of ethanol exposure compared to both adult and adolescent rats. To our knowledge, this study is the first to report a difference in motor impairments and sedative/hypnotic effects of acute alcohol

among adolescent, adult and aged rats and provides novel data concerning blood ethanol levels following a high dose ethanol exposure.

Research from our laboratory has investigated the age-dependent effects of acute alcohol exposure on motor impairments (Novier et al., 2013; Van Skike et al., 2010). The results of the present study add to previous research that shows age-dependent impairments in ARR performance after acute alcohol administration (Van Skike et al., 2010). Van Skike et al. (2010) reported adult and aged rats did not significantly differ from each other during the ARR paradigm; however in the present set of studies, aged animals were significantly more impaired relative to adults. A possible explanation for this discrepancy is the age difference between the adult groups among the two studies. In the present study, adult rats were tested between PD 73 and PD 88, while Van Skike et al. (2010) tested adult rats at PD 120. In addition, Van Skike et al. (2010) assessed gross motor performance using only the ARR task. The current study included the RR as an additional measure of motor skill learning to investigate the age-dependent differences in motor performance associated with acute alcohol exposure prior to the ARR task. Therefore, results from the current study provide evidence for differential motor impairments between adult and aged animals during both ARR and RR motor assessments. However, it is possible that the sequential nature of the testing procedure in the current set of studies altered ARR performance and may have produced greater ARR deficits in aged subjects. Future research using multiple measures of behavior within a single ethanol dose needs to address potential effects of sequential testing.

Novier et al. (2013) established that aged animals exhibited greater alcohol induced ataxia on the RR compared to adult rats when animals were tested with moderate

to high alcohol doses. However, Novier et al. (2012) found that adolescent and adult animals had facilitated RR performance following administration of a low (1.0 g/kg) dose of alcohol, a result that is replicated for adult animals but not adolescent animals in the current work. Furthermore, the current work is the first to investigate both low (1.0 g/kg) and moderately high (2.0 g/kg) doses of ethanol on the rotarod across the full age range. The current work extends previous findings to demonstrate that aged animals are still impaired on RR at a low dose of ethanol and this impairment is magnified as the dose of ethanol increases. Additionally, in agreement with results from the present study, Novier et al. (2013) reported no significant differences between adult and aged rats in RR training performance. It should be noted that the experimental procedures for RR training in the present study were slightly different compared to our previous work (Novier et al., 2013). Due to a small sample size, animals who failed to meet criterion at 7 seconds in the present study were given additional training trials until their final three training trials averaged 7 seconds, rather than being excluded from RR testing as was done previously. Such a training procedure may bias our results and confound a potential impairment due to a floor effect. Future studies should use a slower acceleration speed or a fixed speed task to determine if aged animals are indeed more impaired by acute ethanol on this task.

Finally, aged animals were more sensitive to the sedative/hypnotic effects of acute alcohol compared to adolescent rats during LORR. These results are consistent with previous studies showing adolescent animals to be less sensitive to alcohol-induced sedative/hypnotic effects (Little et al., 1996; Silver & Spear, 1999; Matthews et al., 2008). BECs were collected in a separate group of same-aged adolescent, adult, and aged animals following a 3.0 g/kg ethanol i.p. injection. Initially, adolescent animals

demonstrate higher BEC levels compared to adult or aged animals but adolescents have lower BEC levels compared to the other two age groups following the 240 minute time point until the last blood collection time. This age-dependent effect in BEC is intriguing and suggests that behaviors impacted by alcohol may also show an age-dependent effect when different ages are tested at different times in the BEC curve. Furthermore, the BEC data provide some important insight into the LORR data as it relates to the question of whether aged animals are more sensitive to a high dose of alcohol than adolescent or adult animals. Specifically, aged rats slept approximately 150 minutes following administration of 3.0 g/kg ethanol, a time point when the BEC clearance study suggests blood ethanol levels among the three ages should be similar (no significant difference in BEC at either the 120 min or 180 min time point). However, without determining BEC in subjects undergoing the LORR experiment future research will need to determine if aged animals are indeed more sensitive to acute ethanol on the LORR test. Furthermore, BEC in aged animals remained high throughout the experiment even though collection times occurred six hours after injection. This suggests that the aged system is impaired in clearance of the drug and future studies should address this issue. Finally, the impact of sex and age on ethanol's effects is understudied, particularly for aged animals. Future studies should include female subjects to better understand how sex and age interact.

Previous research has shown that adolescent rats perform significantly better on the ARR task compared to adult rats in response to ethanol (Van Skike et al., 2010; Linsenbardt et al., 2009; Hefner & Holmes, 2007). Interestingly, in the present study, adolescent and adult rat's motor performance on ARR did not significantly differ from each other. In contrast to the previous published studies, there were only 40 days

separating the adolescent and adult age groups (i.e., adolescent animals were 30 days old while the adult animals were 70 days old). The closeness in age could be a possible explanation as to why there were no differences in motor performance between adolescent and adult rats. Importantly, aged animals displayed greater ethanol induced ataxia compared to adult and adolescents animals on ARR, which is consistent with previous research that has demonstrated age-related impairments on ARR (Novier et al., 2013).

Results from the RR indicated age-dependent impairments in motor coordination using a dose-dependent procedure. Previous research in the field has shown that motor coordination and performance using RR and other motor tasks decreases in aged animals (Novier et al,. 2013; Shukitt-Hale, Mouzakis, & Joseph, 1998; Wallace, Krauter, & Campbell, 1980). Furthermore, to the best of our knowledge, this is the first study to show motor impairments using the RR in three different rodent age groups while incorporating an acute ethanol dose-response procedure. Although results were inconclusive as to which age groups significantly differed from each other when administered 2.0 g/kg ethanol, graphical analysis suggests RR performance decreases in an age-dependent manner.

The continuous decline of motor function is a widespread feature of aging (Ranganathan, Siemionow, Sahgal, & Yue, 2001; Smith et al., 1999; Desrosiers, Hébert, Bravo & Dutil, 1995). Age-related deficits in motor function may be the consequence of age-related muscle deterioration, or sarcopenia (Herndon et al., 2002), leading to impaired mobility, gait abnormalities, and increased risk of falls (Hunter, White, & Thompson, 1998). Deficits in motor movement found in rats may be associated with age-

dependent decline in strength and muscle mass (Hepple, Ross, & Rempfer, 2004). Rodent models of age-associated atrophy in skeletal muscles show an increase in pro-apototic proteins involved in the mitochondria-associated apopotic pathway in skeletal muscles as a result of aging (Siu et al., 2005). Previous research has indicated that aged rats have deficits in motor coordination, balance, and reflexes on the RR and other tasks of motor performance (Shukitt-Hale et al., 1998; Wallace et al., 1980). These deficits may be associated with age-dependent decline in strength and muscle mass (Hepple et al., 2004)

In addition, alcohol metabolism changes with age, which may result in aged humans being more sensitive to the toxic effects of alcohol (Seitz et al., 1993). Seitz et al. (1993) reported gastric alcohol dehydrogenase (ADH) activity was significantly lower in elderly male subjects. Decreased ADH may contribute to reduced first pass metabolism leading to an increase in blood alcohol concentrations (BECs) as well as impairments in motor coordination (Seitz et al., 1993). This raises the possibility that aged animals in the current project are more impaired by acute alcohol due to differential BECs (see Table 1). Although we did not measure BECs following the lower injection amounts of 1.0 g/kg and 2.0 g/kg ethanol, we do not think differential BEC levels explain the current data for two reasons. First, alcohol was administered i.p. and therefore different ADH activity in the gut would not alter BEC levels. Second, previous work from our laboratory has shown, using identical ages and the same lower alcohol doses, that while alcohol produces greater cognitive and motor impairment in aged animals compared to adult animals, BEC did not predict greater alcohol impairment in aged animals (Novier et al., 2013). However, when feasible, future projects should continue to monitor BEC in studies investigating the effect of alcohol across different ages.

The present study provides evidence that the ARR paradigm appears to be a better assessment of motor performance compared to the RR task. Animals' performance on the ARR task was consistent throughout each testing trial and provided less variability in motor performance compared to RR. In addition, there is no significant baseline difference. A possible explanation as to why the RR produced differences in motor impairments compared to the ARR could be the specific design of the RR. The size of the cylindrical rotating rod was the same size in diameter for all three age groups tested. In addition, the acceleration of the rod might not be the best procedure to test performance in this task. Future studies which use the RR to assess motor performance on aged animals should adjust RR parameters to accommodate changes in body weight and size of the animals. In addition, another possible adjustment to the RR to account for body weight and size of animals would be to reduce the RR acceleration speed. The RR in the current study accelerated from 4 rpm to 40 rpm over a 5-min period. Future research using aged rats may want to start the rotating speed below 4 rpm and reduce the accelerating speed over a longer period of time.

It is important to note that age-and dose-dependent effects of acute ethanol were only assessed at one time point for RR and ARR during testing sessions. ARR performance was assessed 20 minutes after injection and RR was assessed 10 minutes after injection. Previous studies in our lab have tested animals on ARR at 10, 20, 50, 70, and 90 minutes and RR performance at 10, 30, and 60 minutes following ethanol injection (Novier et al., 2013). Inclusion of additional test points on ARR and RR may elucidate further age-and dose-dependent effects of ethanol on motor behavior between

adolescent, adult and aged rats. Therefore, future studies should include additional testing time points on ARR and RR to further investigate the effects of acute ethanol.

Conclusion

In conclusion, the results of the present study demonstration that acute alcohol administration produces age- and dose-dependent effects on motor performance. A goal of the current study was to further improve previous research from our laboratory on the age-dependent effects of ethanol. With the inclusion of three developmental age groups (adolescence, adult, and aged), the current study provides a more comprehensive view of age-dependent motor impairments during the rodent lifespan. Furthermore, the current study included a dose-response procedure with two differential doses of ethanol (i.e., 1.0 g/kg and 2.0 g/kg) as well as a control saline injection. Using multiple doses of ethanol provided further knowledge on the motor impairing effects of low to moderate doses of ethanol. In addition, two different motor performance tasks were conducted in the current study. The ARR assessed impairments in gross motor performance, while the RR assessed impairments in motor coordination. Inclusion of two separate motor assessment tests allowed for examination of different types of motor impairments as the result of differential doses of acute ethanol administration. Finally, the sedative/hypnotic effects of acute ethanol were measured using the LORR task. To our knowledge, this is the first study to not only examine LORR in aged animals but also across different rodent age groups.

The present study adds to the growing body of research that indicates alcohol produces age-dependent impairments in motor performance. Overall, aged animals exhibited greater motor impairments in both motor coordination and gross motor

performance compared to younger animals in response to acute ethanol administration. Also, aged animals are more sensitive to the sedative/hypnotic effects of acute ethanol compared to adolescent and adult rats. These findings add to the growing body of research stating that adolescent animals are less sensitive to the impairing effects of ethanol. It is important to examine the effects of ethanol during the senescence period given how susceptible individuals aged 65 years and older are to the risk factors associated with alcohol consumption. Aged individuals are more sensitive to the motor impairing effects of alcohol consumption compared to younger drinkers (Weafer & Fillmore, 2012; Mukamal et al., 2007). With the increasing population of aged individuals and rising alcohol consumption rates in this age group, it is necessary to investigate the detrimental effects of ethanol on the brain and body to further educate aged individuals on their increased susceptibility to these impairments.

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