CHAPTER THREE

I can't drink what I used to: The interaction between ethanol and the aging brain

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Abstract

The population of most countries is increasing and the United Nations predicts that by the year 2050 those over the age of 60 years old will increase from 900 million individuals to approximately 2.1 billion individuals (United Nations, 2015). The increase in the number of older individuals will place a strain on many national health care systems making it important to investigate behaviors in the aged that may negatively impact general health in this demographic. Recent work has shown that older adults consume alcohol, often at levels that exceed the legal limit of intoxication. Unfortunately, consumption of high levels of ethanol in the older population is associated with many health consequences and may negatively impact the brain. Given ethical constraints found in many biomedical studies, animal models are needed to investigate the possible negative impact of high ethanol use in aged populations. However, few studies have investigated the effect of ethanol exposure in aged animals compared to ethanol exposure in younger animals and consequently the impact of ethanol in the aged population is not well understood.

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The current review summarizes initial work establishing the impact of ethanol in aged animals. The reviewed research studies support the working hypothesis that ethanol exposure produces significantly greater effects in aged animals compared to younger animals on many, if not all, behavioral tasks. In addition, the review proposes several initial, promising avenues of research to explore the neurobiological mechanisms that underly greater effects on ethanol-induced ataxia, cognition and sleep time. It is hoped that this effort will not only lead to a better understanding of behaviors impacted by ethanol in aged animals, but also improve the understanding brain mechanisms of the reported increased sensitivity to ethanol in the aged population.

In Genesis, Chapter 9 Verse 21 we read how a man named Noah planted a vineyard after he had lived over 600 years, consumed ethanol made from the grapes of the vineyard and passed out. If this account is accurate, several important points can be made. First, ethanol has been in existence, and used by humans, for a very long time. Second, this story is quite likely one of the first, if not the first, written account of ethanol-induced loss of righting reflex (see McClearn, 1963 for an early description in mice). Third, Noah must have consumed a great amount of ethanol. Fourth, and critical in the present context; Noah, being of old age when this occurred, likely was significantly more sensitive to the effects of the drug than when he was a younger man.

Little has changed in the intervening thousands of years. Humans still consume ethanol, and unfortunately at times lose consciousness from excessive consumption and the cognitive and ataxic impairments produced by ethanol seems to increase with age. What is changing, though, is the understanding of the impact of ethanol in the aged and the development of suitable animal studies to understand this important health interaction. This paper seeks to highlight some of our initial work investigating the impact of ethanol on the aging brain.

The world's population is graying. The average population age in most countries, including both more developed countries and lesser developed countries, has increased due to a variety of factors such as fertility decline, dietary advances and development of more effective medicine and sanitation (Kinsella, 2000). When investigating the world population as a single unit, the United Nations estimates there are over 900 million people aged 60 or greater and estimates are that by the year 2050 this age demographic will increase to over 2.1 billion people (United Nations, 2015). More specifically, in the United States alone, it has been predicted that the total number of people over the age of 65 years will likely double from approximately 46 million people to 98 million people by the year 2060, potentially straining

the health care system (Ortman, Velkoff, & Hogan, 2014). A captivating analogy (Tampi, Tampi, & Durning, 2015) suggests that one effect of the rapidly aging population will be a "silver tsunami" that overwhelms the country's social and health care systems. It is therefore critical to understand how such factors like behavioral choices, cognitive and attentional abilities, genetic, economic and dietary factors impact the health and well-being of the aging population.

One particular behavioral pattern that increases health risk factors for the elderly is alcohol consumption. While the elderly consume less ethanol than people of younger ages (Molander, Yonker, & Krahn, 2010), research has revealed that the level of alcohol consumption in the aged is increasing over time, particularly in women (Ahlner et al., 2018). For example, over 10% of adults >65 years of age report binge alcohol consumption (Han, Moore, Ferris, & Palamar, 2019), while adults aged 55 and older with self-reported depression are significantly more likely to have an alcohol use disorder (Laborde-Lahoz et al., 2015). Alcohol use in aging is a leading factor influencing the development of cancer and deaths associated with cancer (Boffetta, Hashibe, La Vecchia, Zatonski, & Rehm, 2006; Schutze et al., 2011), as well as suicidal behavior (Morin et al., 2013). Furthermore, alcohol produces a variety of behavioral effects including impaired movement and balance which can lead to increased falls in older adults (Mukamal et al., 2004), impaired cognition (for a recent review see Van Skike, Goodlett, & Matthews, 2019), and at high doses, loss of consciousness (McClearn, 1963). Consequently, it is important to understand the levels of ethanol consumption in the aged population and the potential negative consequences of such consumption.

In the United States, health data demonstrate that 43% of people over the age of 65 continue to engage in alcohol consumption (Blazer & Wu, 2009). Specifically, 33% of this age demographic consume alcohol with 10% of males reporting heavy alcohol drinking, 14% of males and 5% of females reporting binge alcohol drinking, and up to 3% of responders meeting the diagnosis of an alcohol use disorder (Blazer & Wu, 2009; Breslow, Castle, Chen, & Graubard, 2017; Breslow, Faden, & Smothers, 2003; Caputo et al., 2012). Logistic regression analyses reveal that higher education and income level, coupled with being single, predicted unhealthy drinking in the aged population (Merrick et al., 2008). Similar data is reported for a wide variety of countries around the world (Ahlner et al., 2018; Topiwala & Ebmeier, 2018). Increases in alcohol consumption can be exceedingly problematic considering that alcohol use in the aged can

increase the risk for other health issues such as high blood pressure, diabetes, congestive heart failure, impaired cognitive functions and increased risk for dementia (Stevenson, 2005; Thomas & Rockwood, 2001; see Rehm et al., 2009 and Topiwala & Ebmeier, 2018 for review). It should be apparent that understanding the impact of alcohol consumption in the aged is a critical health concern.

Alcohol produces more pronounced effects in older individuals compared to younger people (see Novier, Diaz-Granados, & Matthews, 2015 and other papers in this volume for detailed review). For example, alcohol produces greater impairments in ataxia as measured by body-sway and hand tremor in older adults compared to younger adults (Jones & Neri, 1994) and causes greater neurobehavioral deficits in older adults compared to younger adults (Boissoneault, Sklar, Prather, & Nixon, 2014; Gilbertson, Ceballos, Prather, & Nixon, 2009; Lewis, Boissoneault, Frazier, & Nixon, 2016). The increased effects of alcohol in older people (see previous citations and Nixon via this volume) dramatically highlight the need to investigate the behavioral, genetic and neurobiological consequences of alcohol use in the elderly. However, such investigations have been hampered by a lack of detailed neurobehavioral studies that compare the effect of ethanol in aged animals to the effect of ethanol in adult and/or adolescent animals (cross-sectional studies), or, the effect of ethanol across the lifespan in the same animal (longitudinal studies).

Our laboratory has been investigating the effect of ethanol in aged animals for almost a decade; primarily focused on understanding how aging alters the sensitivity of aged animals compared to younger animals to ethanol. Our goal was to effectively lay the ground work for future neurobiological investigations into mechanism(s) underlying the differential effects of ethanol in the older population. The current brief review seeks to organize our existing work, highlight areas in need of additional behavioral research and propose potential first steps for the neurobiological research.

1. Aged animals have greater sensitivity to acute ethanol administration compared to younger animals

Research has demonstrated that many of ethanol's effects on ataxia, cognition, hypothermia and self-administration are age-dependent (see Novier et al., 2015 and Wood & Armbrecht, 1982 for review). However, the bulk of this work has focused on differential sensitivity of ethanol between adolescent and adult rats. This work has demonstrated that

adolescent animals are less sensitive to some of the effects of ethanol compared to adult animals (e.g., ataxia or hypothermia), while being more sensitive to other effects of ethanol (e.g., rewarding properties potentially leading to greater ethanol intake). Specifically, adolescent rodents are less sensitive, compared to adult rodents, to ethanol-induced motor ataxia as measure by the aerial righting reflex (Van Skike et al., 2010; White et al., 2002), tilting plane (White et al., 2002) and accelerating roto-rod (Hefner & Holmes, 2007), loss of righting reflex to a high dose ethanol challenge (Silveri & Spear, 1998), the anxiolytic effects of acute ethanol (Doremus, Brunell, Varlinskaya, & Spear, 2003) and ethanol-induced hypothermia (Ristuccia, Hernandez, Wilmouth, & Spear, 2007; Ristuccia & Spear, 2004, 2008; Watson, James, Mittleman, & Matthews, 2019). However, the reduced sensitivity to ethanol is not universally observed in that adolescent animals have similar hippocampal-dependent cognitive impairments compared to adult animals (Chin et al., 2011; however, see Markwiese, Acheson, Levin, Wilson, & Swartzwelder, 1998; see Chin, Van Skike, & Matthews, 2010 and Novier et al., 2015 for review). Based on this extensive literature demonstrating differential sensitivity to ethanol by age, we first sought to investigate if aged rats are more, less, or equally sensitive to acute ethanol compared to both adolescent and adult rats.

Comparison of ethanol's effect across the lifespan require defining the age range used for specific life stage categories. Adolescence in rodents has been defined as spanning between 28 days postnatal (PD) and ~50 PD yet this broad definition can miss important changes in the neurobehavioral development of animals. For example, Spear (2015) has argued that differences exist between early adolescence and late adolescence. With this in mind, it is important for researchers to define the specific age ranges used in their work. For the current review, our aged animals are at least 18 months of age, adult rats are between 70 PD and 120 PD, while adolescent rats were between PD 30 and PD 48. Finally, all animals used in the Matthews' laboratory are Sprague-Dawley animals obtained from Envigo (Indianapolis, IN).

Aged rats demonstrate a profound sensitivity to ethanol compared to rats of younger ages. Our first investigation in this area utilized the aerial righting reflex test; a simple, yet powerful behavioral method to investigate the effect of ethanol on gross body control. In this task, animals are held upside down and released at various heights in 5-in. increments where they fall onto a 10-in. soft foam pad. The dependent variable is the height needed for the animal to right itself in the air and land with three of four paws on the foam

at the same time. The greater the motor impairment the greater height needed above the foam for the animal to "right." While simple, the aerial righting reflex is a powerful task due to several properties: 1. The fast, high throughput nature of the task, 2. The lack of a behavioral "carryover" effect in that animals do not improve performance over time due to previous experience with the task, even within the same session (Matthews & Mittleman, 2017) and 3. Animals have similar pre-drug baseline performance between rats ranging from 26 days of age to 19 months old (Van Skike et al., 2010).

Using this task, we discovered that aged animals (19 months of age) were very sensitive to the motor impairing effects of a 2.0 g/kg acute ethanol injection (i.p.) compared to adult (120 PD), adolescent (43 PD) or peri-adolescent (28 PD) rats and this increased effect lasted throughout a 40-min experimental session. Importantly, the increased sensitivity in aged rats was not due to differential blood ethanol levels between the ages (Van Skike et al., 2010). The **similar** pre-drug baseline effect in the height needed to right themselves between ages and **similar** blood ethanol levels between ages coupled with the **divergent** behavioral effects where aged rats require significantly greater height to right themselves compared to younger animals is important because this pattern suggests that central neurobiological mechanisms underlie the differential effect and not just other potential confounds such as muscular coordination, ethanol uptake or ethanol clearance between rats of different ages. However, animals were only tested every 10 min for 40 min after the ethanol injection. During this limited testing, the aged animals never began to recover their aerial righting reflex and therefore the magnitude of the effect was not determined.

We next followed this preliminary finding with an additional study designed to confirm and extend these findings with a longer time frame (i.e., testing out to 90 min post-ethanol injection). Once again, we found that aged animals were significantly more impaired on the aerial righting reflex compared to adult animals, and this effect lasted at least 90 min following the ethanol injection (Novier, Van Skike, Diaz-Granados, Mittleman, & Matthews, 2013). This experiment demonstrated that aged rats are not only more sensitive to the effects of acute ethanol exposure, but also that the effects are extremely long lasting. Finally, we explored if the increased sensitivity was only found at high ethanol doses or could also be observed at lower doses of the drug. Importantly, we found that following a low, 1.0 g/kg ethanol injection, only the aged animals were impaired in the aerial righting reflex compared to adult or adolescent animals demonstrating an age-dependent effect in the sensitivity of ethanol. Once again we demonstrated that blood ethanol levels did not account for the differential behavioral

effect (Ornelas, Novier, Van Skike, Diaz-Granados, & Matthews, 2015). The increased sensitivity at the low dose, 1.0 g/kg ethanol, has potentially important ramifications on complex human behaviors such as driving behavior. Specifically, blood ethanol concentrations from low dose ethanol exposure can easily be reached by people in a social setting and yet might produce differential behavioral effects in the aged compared to younger adults. For example, low dose ethanol exposure can alter driving characteristics in older adults compared to younger adults, particularly when cognitive load is high (Price, Lewis, Boissoneault, Frazier, & Nixon, 2018). The potential overlap of findings in animal and human studies should be a guide as research investigating the effect of ethanol in aged populations continues to develop. Specifically, it will be important to focus on translational work so that results from animal and human studies inform the development of future research and the impact of alcohol in the aged.

A related task of ethanol-induced motor impairments in the accelerating roto-rod where the subjects' ability to walk at increasing speeds on a rotating rod is ascertained. Given the impairment in gross movement found in the aerial righting reflex we predicted that aged rats would also be impaired on performance in the accelerating roto-rod task. As predicted, aged rats were more impaired (i.e., they fell off the roto-rod earlier) than adult rats following a range of ethanol doses: 1.0, 1.5 or 2.0 g/kg i.p. injections. Once again, we found blood ethanol concentrations (see Table 1; Novier et al., 2013 for BEC values) did not differ between ages nor did baseline performance on the roto-rod (Novier et al., 2013). To support our previous work demonstrating that acute ethanol produces greater sensitivity in aged rats, compared to adult or adolescent subjects, we conducted a separate study comparing all three ages on the accelerating roto-rod following acute ethanol exposure. We found that aged animals were significantly more impaired than either adult or adolescent animals (Ornelas et al., 2015). However, we also found a significant baseline performance difference. Similar to our previous work (Novier et al., 2013), adult rats and aged rats did not differ in baseline performance. However, in this study we found that adolescent rats performed significantly better than both the adult rats and the aged rats, which limits the overall strength of the conclusions of this study. The baseline difference highlights an important experimental concern when investigating the effect of any drug, such as ethanol, at vastly different ages. Experimental apparati are designed and developed for specific ages and body size and can impact overall results. Therefore, based on these results we do not recommend the accelerating roto-rod as a suitable tool to investigate the effect of ethanol in aged rats compared to adolescent rats.

Table 1 Increased sensitivity to acute ethanol in aged animals on many neurobehavioral effects.

Neurobehavioral effect	Technique used	Effect compared to adults	Dose used (g/kg)
Gross body control	Aerial righting reflex	↓	1.0 and 2.0
Ataxia	Accelerating roto-rod	↓	1.0 and 2.0
Spatial memory	Morris water maze	↓	1.0, 1.5 and 2.0
Hypothermia	Core body temperatures	↓	2.0 and 3.0
Loss of righting reflex	Sleep time/BAC upon waking	↓	3.0 and 3.5

The size of the down arrow represents the magnitude of the effect produced by ethanol in aged animals compared to adult animals.

As previously mentioned, loss of righting reflex is a common behavioral screen to assess sensitivity to high dose ethanol (McClearn, 1963). We sought to replicate and extend previous work (Abel & York, 1979; Wood & Armbrecht, 1982; York, 1982). We first investigated 19-month-old male rats compared to young adult (PD 88) and adolescent (PD 46) male rats on loss of righting reflex following a 3.0 g/kg ethanol injection. Consistent with the hypothesis that aged rats are significantly more sensitive to ethanol relative to younger animals, we found that aged rats slept on average 157 min, whereas none of the adults rats went to sleep at this dose and the average sleep time for the adolescent animals was only 8 min (Ornelas et al., 2015). This is a striking effect and highlights the potentially dangerous health issues associated with ethanol consumption in the aged. This work was extended and several important questions answered as it relates to the sensitivity of high dose ethanol on loss of righting reflex (Perkins, Vore, Lovelock, Varlinskaya, & Deak, 2018). Specifically, it was confirmed that aged rats slept significantly longer following a high ethanol dose challenge supporting the hypothesis that aged animals are more sensitive to the acute effects of ethanol. Furthermore, it was found that initiation of sleep was not different between animals and,

importantly, aged animals had significantly lower blood ethanol levels upon waking, supporting the hypothesis that aged animals have increased sensitivity to acute ethanol. Finally, the Perkins et al. (2018) study was conducted in Fisher 344 rats and the similarity of the data with Sprague-Dawley rats from our studies suggest the increased sensitivity to acute ethanol in aged rats are not strain dependent. These data from two laboratories support the hypothesis that aged rats are significantly more sensitive to the effects of acute ethanol compared to both adult and adolescent rats when the doses used are low (1.0 g/kg), moderate (2.0 g/kg) or high (3.0–4.0 g/kg).

We, and others, have demonstrated that one effect of acute ethanol exposure is to selectively impair hippocampal-dependent spatial memory compared to hippocampal-independent nonspatial memory (Berry & Matthews, 2004; Chin et al., 2011; Hoffmann & Matthews, 2001; Matthews, Ilgen, White, & Best, 1999; Matthews, Morrow, Tokunaga, & McDaniel, 2002; Matthews, Simson, & Best, 1995; see Van Skike et al., 2019 for a recent review). Therefore, it seems reasonable to predict that acute ethanol administration will produce greater spatial cognitive impairments in aged rats compared to younger animals. To our knowledge only one study to date investigated whether acute ethanol increased spatial memory impairments in aged rats compared to younger animals (Novier et al., 2013). Specifically, that study found that acute ethanol administration did produce a significantly greater spatial memory impairment in aged rats, compared to adult animals, as determined by both swim latency and pathlength. However, this study did not examine the selective effects of acute ethanol as a nonspatial task was not included in the assessment battery. See Table 1 for a summary of the current work.

1.1 Preliminary conclusions and future needs

To date, all of our current work indicates that aged rats are significantly more sensitive to the effects of acute ethanol administration, compared to either adult and/or adolescent animals. In addition, our laboratory recently confirmed previous work demonstrating that aged animals show significantly greater ethanol-induced hypothermia compared to either adult or adolescent rats (Abel & York, 1979; Watson et al., 2019). Given the dearth of research comparing the effect of acute ethanol in aged animals compared to adult and/or adolescent animals and the overwhelming societal need to understand the effect of ethanol in the aged population, it is important that research continue to establish a foundation of behavioral studies on the effects of ethanol in aged animals. These include tests on

ethanol self-administration, conditioned place preference/aversion, ethanol induced anxiolysis, changes in heart rate and sleep-wake cycles in response to acute ethanol administration.

As previously mentioned, the effect of acute ethanol on cognition is selective in that impairment on hippocampal-dependent tasks were observed, whereas performance on hippocampal-independent cognitive tasks were not affected or even improved (for review see Van Skike et al., 2019). To date, no studies to our knowledge have investigated if acute ethanol is selective in the type of cognitive impairment produced in aged rats, or whether acute ethanol exposure simply impairs all cognitive tasks. Understanding the potential selective nature of the cognitive impairment produced in aged animals is important because reduced cognitive function in the aging human population could impact healthy life decisions. In addition, if selective or reduced impairments are found, important insights into how ethanol interacts with the aging brain, and specific brain regions (e.g., the hippocampus vs the striatum), will be obtained.

2. Potential mechanisms that increase sensitivity to acute ethanol in aged animals

We have argued here, and in our published work, that blood ethanol levels cannot explain the differential effect of ethanol in aged animals compared to those of younger ages. However, that leaves open the question: "What mechanism(s) explain the differential effects of ethanol in the various ages?" One exciting study has been published demonstrating that ethanol can differentially increase cytokine expression in the hippocampus of aged rats (Gano, Doremus-Fitzwater, & Deak, 2017) suggesting one possible mechanism. Additionally, it has been shown in adult and adolescent rats that acute ethanol increases concentrations of allopregnanolone, a potent $GABA_A$ receptor modulator, in some brain regions such as the hippocampus and cortex, but not other brain regions such as the cerebellum. Importantly, allopregnanolone enhances many of the effects of acute ethanol exposure (Balan, Beattie, O'Buckley, Aurelian, & Morrow, 2019; Chin et al., 2011; Maldonado-Devincci et al., 2014; VanDoren et al., 2000). Consequently, it would be important to understand if ethanol differentially increases allopregnanolone levels in aged animals compared to younger animals. Finally, we have previously demonstrated that lower levels of PKCγ expression correlate with reduced ethanol ataxia in adolescent animals (Van Skike et al., 2010), whereas others have shown that PKA expression may underlie

the reduced effect in adolescents (Gigante, Santerre, Carter, & Werner, 2014). Considering aged animals show a greater ataxic effect following acute ethanol exposure compared to adults while adolescent animals show a reduced ataxic effect following acute ethanol, it would be promising to investigate if aged animals have opposite PKCγ and PKA expression patterns compared to adolescent animals.

Previously, when investigating the effect of ethanol in adolescent animals compared to adult animals, we sought to identify in vivo electrophysiological correlates that mirrored the differential behavioral effects reported. One such effect was reduced inhibition of spontaneously active cerebellar Purkinje neurons in adolescent animals compared to the inhibition produced in Purkinje neurons from adult animals (Van Skike et al., 2010). While similar studies in aged animals remain to be done, we predict that acute ethanol may produce greater inhibition of spontaneously active cerebellar Purkinje neurons in aged animals compared to adult animals.

3. Impact of chronic ethanol exposure in aged animals compared to younger animals

Previous work demonstrated that aged animals are more sensitive to the ataxic, cognitive and hypothermic effects of acute ethanol compared to younger animals. While informative, the face validity of this research is lacking. Specifically, it is not often that an older, perhaps retired person, decides suddenly to consume a large amount of ethanol for the first time. To address this concern and begin to explore the impact of chronic ethanol in aged animals compared to younger animals, we have initiated a focused set of studies comparing chronic ethanol in aged animals compared to younger animals.

One of our first investigations into the effect of chronic ethanol exposure attempted to leverage the extensive use of liquid diet methods to maintain a chronic ethanol consumption pattern (Lieber, DeCarli, & Sorrell, 1989). While this exposure procedure has the advantage of mimicking the typical route of ethanol absorption through the gastrointestinal tract, we encountered a confounding variable in relation to the study design that limited the effectiveness of the use of ethanol containing liquid diet. Specifically, aged rats were found to consume more fluid than adult rats over the 8-week exposure period but, due to their greater body weight and different body composition between muscle and fat, the aged animals had consistently lower blood ethanol concentrations than the adult rats (Novier, Ornelas,

Diaz-Granados, & Matthews, 2016). The resultant difference in blood ethanol levels across various ages limited the validity of directly comparing the resultant behavioral data from the aged and adult animals. Once again, this work highlights some hard-earned methodological wisdom when working with aged and younger animals in the same studies. While it may be difficult to overcome the methodological limitation of differential ethanol consumption levels between different ages, future studies should investigate if a more limited ethanol consumption period may lead to closer resultant blood ethanol levels between aged and adult rats. Although the differential blood ethanol levels did limit the strength of this study, it is still possible to draw some tentative conclusions from the data. For example, aged rats showed minimal ethanol withdrawal scores using the Majowitz rating scale but increased anxiety-like behavior on the elevated plus maze following removal of ethanol, and a modest spatial memory impairment when tested in the Morris Water Maze (Novier et al., 2016).

Based on the experimental control needed by equating blood ethanol levels in subjects of different ages, the next set of projects used our previous chronic intermittent ethanol procedure via intraperitoneal (i.p.) injections (Matthews, Tinsley, Diaz-Granados, Tokunaga, & Silvers, 2008; Silvers et al., 2006; Silvers, Tokunaga, Mittleman, & Matthews, 2003; Tokunaga, Silvers, & Matthews, 2006) in aged, adult and adolescent rats. It was first found that chronic intermittent ethanol exposure for 20 days in an every other day fashion (a total of 10 1.5 or 2.5 g/kg ethanol exposures) significantly impacted body weights in aged and adult animals compared to adolescent animals, and this was not due to differential blood ethanol levels (Matthews & Mittleman, 2017). Specifically, chronic intermittent ethanol exposure significantly reduced body weights in aged animals compared to the younger animals at low doses. Such an effect in a global measure of health like body weight can be very problematic for the aging human population and health care providers in general. Specifically, unintended body weight reductions in the aging population can be a significant marker for underlying disease states (Miller & Wolfe, 2008). Furthermore, the previous data was replicated demonstrating that aged animals were significantly more impaired than younger animals on the aerial righting reflex and this measure did not demonstrate tolerance due to the previous chronic intermittent ethanol exposure (Matthews & Mittleman, 2017).

Based on this success in equating blood ethanol levels, we conducted a more extensive project investigating the effect of low dose (1.0 and 2.0 g/kg ethanol) chronic intermittent ethanol exposure in aged animals compared to adult and adolescent animals. Once again, we replicated the reduced body

weights in aged animals treated with chronic intermittent ethanol, but did not see changes in anxiety-like behaviors in the elevated plus-maze, depressive-like behavior in the forced swim test, spatial or non-spatial learning in the Morris Water Mze due to the ethanol treatment (Matthews et al., 2019). However, we did find a reduction in sleep time to a high dose ethanol challenge (3.0 g/kg) in a loss of righting reflex test following the chronic ethanol treatment compared to animals of other ages (significance level $P < 0.051$) (Matthews et al., 2019). This work highlights the importance of understanding the impact of chronic binge-like alcohol exposure in aged rats and how such chronic use can impact general health and potentially produce differential tolerance in aged animals compared to younger animals.

3.1 Preliminary conclusions and future needs

Understanding the impact of chronic ethanol use in aged animals compared to younger animals is an understudied research field and can provide insights into the approximately 33% of individuals that develop alcoholism later in life, usually after the age of 25 and following a long period of alcohol consumption (Adams & Waskel, 1991; Cloninger, Sigvardsson & Bohman, 1996; Menninger, 2002). Previous work in adult and adolescent animals on this topic are numerous and have demonstrated changes in receptor number, receptor composition and electrophysiological changes (see Chandrasekar, 2013; Kumar et al., 2009; Zorumski, Mennerick, & Izumi, 2014 for review). For example, chronic ethanol exposure can both increase (e.g., $GABA_A$ receptor α4 subunit peptide and Glutamate NMDA sensitive NR1 and NR2B receptor subunit peptide; Devaud, Fritschy, Sieghart, & Morrow, 1997; Kumari & Anji, 2005; Matthews, Devaud, Fritschy, Sieghart, & Morrow, 1998) and decrease (e.g., $GABA_A$ receptor α1 subunit peptide; Devaud et al., 1997; Matthews et al., 1998) specific isoforms of various neurotransmitter receptor systems that are sensitive to ethanol. Furthermore, chronic ethanol can reduce the induction of hippocampal long term potentiation (Durand & Carlen, 1984; Tremwel & Hunter, 1994). Given the differences between aged animals and younger animals in terms of the sensitivity to ethanol it seems quite likely important findings with chronic ethanol exposure await discovery in relation to neurotransmitter receptor number, receptor subunit composition and electrophysiological properties of neuronal circuits. Without these baseline studies, pharmacotherapies to treat alcohol abuse will likely not be advantageously designed for the aging population.

4. The impact of adolescent ethanol exposure in aged animals

While studies investigating the impact of chronic ethanol exposure in aged animals more closely approximates ethanol intake histories in humans, the face validity of these studies is still lacking. Specifically, the majority of people who consume ethanol later in life actually start consuming ethanol in the early years of their life (Haighton et al., 2016), a behavior that might convey a health risk (Kendler, Ohlsson, Sundquist, & Sundquist, 2016). To fully understand how ethanol impacts behavior and neurobiology during aging, studies are needed where animals are exposed to ethanol during adolescence then studied over the course of their lifespan.

While little research has investigated the impact of earlier life ethanol exposure later in life, a small subset of studies has shown that ethanol during adolescence can alter spatial memory, ethanol-induced ataxia, loss of righting reflex, BDNF expression, novel object recognition and hippocampal neurogenesis during adulthood (Crews et al., 2015; Matthews et al., 2008; Scheidt et al., 2015; Vetreno & Crews, 2015; White et al., 2002; White, Ghia, Levin, & Swartzwelder, 2000). However, these studies leave open the question of what is the impact of ethanol in later stages of life, such as those stages classified as aged. Note that the Chapter included in this volume by Toledo Nunes et al. discusses chronic ethanol exposure effects in several rodent models and their association with alcohol-related brain pathology.

We have recently published our first longitudinal study investigating the effect of adolescent ethanol exposure into early aging (~18 months of age) (Matthews et al., 2017). In this study we exposed adolescent rats (PD 30 to PD 48) to either 5.0 ethanol or saline every other day for a total of 10 ethanol exposures and then tested them every 4 months until postnatal day 532 (approximately 18 months of age). The testing procedure included tests of their response to high dose ethanol via loss of righting reflex, ethanol-induced impairments in hippocampal dependent spatial memory, and body control via the aerial righting reflex. Perhaps the two most striking findings from the work were that ethanol exposure during adolescence produced tolerance to high dose ethanol approximately 530 days later. Specifically, animals exposed to ethanol during adolescence slept significantly less to the high dose treatment when they were 18 months old compared to animals that were not treated with ethanol during adolescence. In addition, animals

exposed to ethanol during adolescence performed significantly worse on hippocampal dependent spatial memory tasks 18 months later. These data suggest that ethanol exposure during adolescence can produce long lasting, perhaps permanent, changes that can alter responses to the drug in late aging.

The potential health implications of such effects are large. For example, most people begin their ethanol consumption as adolescents and if this causes tolerance later in life to high dose ethanol, then it is expected aged individuals would then consume more ethanol to accommodate the tolerance. Such an increase could be dangerous although additional research is needed to better understand the lifetime impact of adolescent ethanol exposure.

4.1 Preliminary conclusions and future needs

Research into the effect of ethanol across the lifespan is challenging, time consuming, expensive and very much needed. To best understand how ethanol interacts with the human aging brain and general physiology researchers need to model, as closely as possible, the ethanol exposure patterns in non-human animal models. To this end, multi-year longitudinal studies are needed.

Our initial study investigating the impact of adolescent ethanol exposure over the subsequent 18 months' of the animals life coupled with other work which studied animals into adulthood demonstrate important findings will likely arise from these types of procedures. However much work needs to be accomplished. First, Matthews et al. (2017) only used male subjects, therefore how such a longitudinal approach will impact female animals is unknown. Second, a large number of relevant behavioral measures such as anxiety, fine motor ataxia, non-hippocampal cognitive measures and depressive-like behavior to name a few, were not investigated. Third, a tremendous amount of effort is used to generate subjects for studies such as these. However, no secondary studies were conducted on the resultant brain tissue. Thus, longitudinal studies of alcohol exposure should be collaborative and multi-disciplinary in nature in order to capitalize on time invested, tissue availability, and to accelerate progress in the field.

5. Conclusion

It has been shown that aged animals are significantly more sensitive to many of the acute effects of ethanol including ataxia, hypothermia, hippocampal dependent memory and the hypnotic effects of the drug (loss of righting reflex). Furthermore, chronic intermittent ethanol exposure during

aging can led to impaired general physical health and ethanol tolerance at moderate ethanol doses, doses that do not produce tolerance in younger animals. Finally, ethanol exposure early in life profoundly impacts ethanol responsiveness late in life.

The developing animal models suggest that much work is needed to understand how ethanol impacts the aged brain. Given the current and predicted increase in the aged population combined with the number of elderly that consume ethanol it is important to understand the health implications of this behavior. Progress can be made understanding the impact of ethanol and the aged best by leveraging the excellent research from human laboratories (see chapter "Clarifying the neurobehavioral sequelae of moderate drinking lifestyles and acute alcohol effects with aging" by Nixon and Lewis in this volume) with research from preclinical laboratories that are developing appropriate animal models to study the neurobiology and physiological impact of ethanol in the aged.

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