ABSTRACT

Taurine: A Novel Approach to Reducing the Reinforcing Properties of Ethanol in Adolescents

Rebecca S. Helfand, Ph.D.

Mentor: Jaime L. Diaz-Granados, Ph.D.

Adolescent ethanol use continues to be a societal problem with ethanol drinking beginning as early as 11 years old. Early initiation of drinking behavior is indicative of an increased risk for future substance abuse problems. This may stem from ethanol-induced changes in the brain that could potentially increase the rewarding properties of ethanol, making a person more likely to drink in the future. The neuroprotective amino acid taurine may attenuate ethanol-induced changes in the brain, potentially reducing the reinforcing properties of ethanol.

In this study, three experiments were conducted using behavioral tests and tissue analysis to investigate the effects of taurine treatment on ethanol self-administration in C57BL/6J mice. Experiment 1 measured ethanol consumption in adolescent mice with the two-bottle choice test resulting in reduced ethanol preference, but not consumption. Experiment 2 utilized the drinking in the dark protocol, in adolescents, revealing a 20% decrease in ethanol intake in the taurine-treated group. This effect was ethanol specific, as consumption of a sucrose solution was not similarly decreased by taurine treatment. Upon completion of drinking in the dark testing, two tissues within the mesolimbic

dopamine system, the VTA and NAc, were extracted and analyzed for amino acid and dopamine content. Amino acid analysis revealed that taurine treatment effectively increased taurine concentrations in both the VTA and NAc. Dopamine turnover in the NAc of the taurine-treated/ethanol exposed group was significantly lower than their water-treated counterparts. Turnover of dopamine in the NAc increases in response to reinforcing stimuli. A reduction in turnover therefore indicates a decrease in the reward associated with ethanol consumption. The reduction in ethanol consumption, in the taurine-treated group, can therefore be explained, at least in part, by the decrease in dopamine turnover in the NAc. Experiment 3 investigated the efficacy of taurine treatment in adults using the drinking in the dark procedure. Treatment did not significantly change ethanol intake, revealing the effect to be adolescent specific.

Given the efficacy of taurine treatment in reducing ethanol consumption in an adolescent population, it may be a potential new direction for the investigation into therapeutic mechanisms to reduce drinking behavior.

Taurine: A Novel Approach to Reducing the Reinforcing Properties of Ethanol in Adolescents

by

Rebecca S. Helfand, B.A., M.A.

A Dissertation

Approved by the Department of Psychology and Neuroscience

_____
Jaime L. Diaz-Granados, Ph.D., Chairperson

Submitted to the Graduate Faculty of
Baylor University in Partial Fulfillment of the
Requirements for the Degree
of
Doctor of Philosophy

Approved by the Dissertation Committee

_____
Jaime L. Diaz-Granados, Ph.D., Chairperson

_____
Jim H. Patton, Ph.D.

_____
N. Bradley Keele, Ph.D.

_____
Charles A. Weaver III, Ph.D.

_____
Eric L. Robinson, Ph.D.

Accepted by the Graduate School
May 2011

_____
J. Larry Lyon, Ph.D., Dean

Page bearing signatures is kept on file in the Graduate School.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGMENTS

There are many people I want to thank for helping me through this process and making this dissertation possible. First I want to thank my advisor Dr. Jaime Diaz-Granados for his help and support throughout my graduate career. Without his willingness to indulge my drive to try new methods, and his faith in my ability, this accomplishment would not be possible. I would also like to thank my committee members for their advice and support. In particular, I want to thank Dr. Jim Patton for his wise counsel and for pushing me to be the best scientist I can be. Dr. Charles Weaver, for his constant support and open door policy, he has helped me more times than I can count. In addition, I want to thank Dr. Bradley Keele and Dr. Eric Robinson for serving on all of my committees and seeing me through this process.

My family also deserves thanks, for their unending support, understanding, and encouragement for the last 8 years. They have provided me with every opportunity and instilled in me the knowledge that I can do anything if I try hard enough.

Finally, I want to thank Dr. André Zalud. Words cannot express how much I appreciate everything he has done. He has helped guide me through this journey, been a constant source of support, and believed in me when I didn't believe in myself. I need to thank him for all the work he did, in and out of the lab, to make this project possible, for tirelessly editing, and keeping me going. None of this would have been possible without him. André, I think I finally get it and I can't wait for our next adventure.

To André and the rest of my family, without your support and understanding this accomplishment would not have been possible. Thank you, I love you all.

CHAPTER ONE

Literature Review

Introduction

Adolescent ethanol use and abuse are continuing societal problems, with an average age of twelve years for drinking-behavior initiation in the United States (Johnston, O'Malley, Bachman, & Schulenberg, 2008). According to the Monitoring the Future Study (Johnston et al., 2008), 43% of students graduating from high school in America admitted to consuming ethanol in the 30 days prior to the survey. Such early use of ethanol is particularly problematic as the brain continues to develop throughout adolescence, evidenced by both significant neural pruning (Huttenlocher & de Courten, 1987) and different neurochemical composition, numbers, and functions of receptors than those found in adults (Lidow, Goldman-Rakic, & Rakic, 1991). Moreover, early initiation of ethanol consumption leads to an increased likelihood of future substance-abuse problems (Schramm-Sapyta et al., 2008; Windle et al., 2008). This increased likelihood may be due to ethanol-induced changes in the adolescent brain (De Bellis et al., 2000; White, Bae et al., 2002), some of which may enhance the reinforcing properties of ethanol and, therefore, the likelihood of future ethanol abuse. This study aims to examine the potential of the neuroprotective amino acid taurine in altering the reinforcing properties of ethanol in the adolescent mouse.

Adolescence

The terms adolescence and puberty are often used interchangeably, and although their ranges do overlap, the terms are not synonymous (Sisk & Foster, 2004; Spear,

2000). Puberty refers to gonadal maturation. Adolescence, however, refers to a gradual period of maturation of social and cognitive behaviors during which organisms gain the necessary skills for independence (Powell, 2006; Sisk & Foster, 2004; Spear, 2000). This period is loosely defined, however, and has no specific boundaries. Human adolescence spans the period between ages 10 and 19 ("Improving the Health of Adolescents and Young Adults: A Guide for States and Communities," 2004) and, in mice, spans the period from postnatal day (PD) 22 to PD 60 (Laviola, Macri, Morley-Fletcher, & Adriani, 2003). Across species, the brain continues to develop during this transitional period (Hua & Smith, 2004; Powell, 2006; Sisk & Foster, 2004). Specifically, the adolescent brain undergoes significant pruning of neocortical neurons and changes in neuroanatomical makeup. Some researchers estimate that as many as 30,000 synapses may be lost each second throughout the entire cortex during the pubertal/adolescent period in primate brains (Rakic, Bourgeois, & Goldman-Rakic, 1994). Electron microscopy studies show that the synaptic density of the cortex decreases during late childhood and adolescence (Hua & Smith, 2004; Huttenlocher & de Courten, 1987; McGee, Yang, Fisher, Daw, & Strittmatter, 2005).

In addition to decreases in the number of synapses, the cortex changes its composition throughout adolescence. The maturation of both white and gray matter accelerates during adolescence (Giedd et al., 1999; Sowell et al., 1999) in a sequence that follows developmental milestones (Gogtay et al., 2004). The prefrontal cortex, temporal and occipital regions show the most prominent increases in gray-matter between the ages of five and 11 years in humans (Sowell et al., 2004). Gray-matter volume peaks during late childhood and early adolescence, although the specific timing varies among brain

regions (Giedd et al., 2009; Shaw et al., 2008). While gray-matter volume decreases, white matter volume increases linearly with age through the first 25 years of life (Giedd et al., 1999; Giedd et al., 2009). The maturation of frontal lobe gray and white matter accelerates during adolescence (Sowell et al., 1999). However, this shift from gray matter to white is most prominent in adolescents between the ages of 12 and 16, and is seen primarily in the parietal lobe, home to language centers. Decreased gray matter is correlated with improved performance on verbal tasks (Sowell et al., 2004). This adolescent cortical reorganization allows for the emergence of adult-like connections between brain regions (Andersen, 2003; Hua & Smith, 2004; Powell, 2006; Spear, 2000). Recent evidence suggests that this reorganization is experience-dependent (Gogtay et al., 2004; Hua & Smith, 2004; Powell, 2006; Shaw et al., 2006), leading to the proposal of a second "critical period for development" during adolescence (Gogtay et al., 2004; Shaw et al., 2006).

During the adolescent period, in addition to substantial neuronal pruning and changes in brain constitution, the brains of adolescents exhibit a different neurochemical makeup and different receptor numbers and functions than adult brains (Bolanos, Glatt, & Jackson, 1998; Lidow & Wang, 1995; Lyss, Andersen, LeBlanc, & Teicher, 1999; Malosio, Marquèze-Pouey, Kuhse, & Betz, 1991). During the early stages of development, dopamine, serotonin, acetylcholine, and γ-aminobutyric acid (GABA) receptor levels are nearly twice as high in the primate cortex, and then decline from infancy through adolescence (Fritschy, Paysan, Enna, & Mohler, 1994; Lidow et al., 1991; Lidow & Rakic, 1992). Adolescent brains also have different concentrations of catecholaminergic and amino acid neurotransmitters than adults (Benedetti, Russo,

Marrari, & Dostert, 1991; Miranda-Contreras, Mendoza-Briceño, & Palacios-Pru, 1998; Rassin, Sturman, & Guall, 1978; Saransaari & Oja, 2003). Levels of dopamine, epinephrine, and norepinephrine peak during the first week of postnatal life before gradually decreasing, over several months, to adult levels, yet serotonin levels show no significant variations, remaining at low levels until young adulthood (Miranda-Contreras et al., 1998). In addition, the brains of adolescents have higher tissue concentrations of taurine and glutamate than any other amino acids (Gregory, Sovetts, Clow, & Scriver, 1986; Miller, Hanson, & Yancey, 2000; Miranda-Contreras et al., 1998). Taurine-mediated reductions in neuronal membrane hyperexcitability represents one aspect of neuroprotection during adolescent development in the mouse (Lima, Obregon, Cubillos, Fazzino, & Jaimes, 2001; Yoshida, Fukuda, Tozuka, Miyamoto, & Hisatsune, 2004) and human brain (El Idrissi, Harris, & Trenkner, 1998; H. Wu et al., 2005). Given the continuation of brain development throughout adolescence, exposure to drugs of abuse during this period, particularly exposure to ethanol, may be detrimental.

Ethanol exposure during adolescence alters normal brain development and impacts distinct brain regions. Four days of binge-like ethanol exposure produces significant tissue loss in the frontal cortex of adolescent rats, but produces damage predominantly to the entorhinal cortex in adults (Crews, Mdzinarishvili, Kim, He, & Nixon, 2006). Rats exposed to ethanol during adolescence exhibit less powerful low-frequency EEG signals compared to controls when tested in adulthood (Slawecki, 2002; Slawecki, Betancourt, Cole, & Ehlers, 2001). This suggests that ethanol exposure during adolescence causes long-lasting functional changes in brain activity (Slawecki et al., 2001). Similar effects occur in humans who initiate ethanol consumption during

adolescence. Adolescents with "adolescent-onset alcohol use disorders," (meeting DSM IV criteria for alcohol dependence or abuse), have smaller hippocampal volumes bilaterally compared to matched controls; age of initiation of consumption correlated with volume of consumption (De Bellis et al., 2000). Functional MRI brain scans of adolescents with alcohol-use disorders show less activation of the frontal cortex and cerebellum but more activation of the inferior parietal and temporal regions during a spatial working-memory task, as compared to adolescents without this disorder (Caldwell et al., 2005). Working-memory performance did not differ, suggesting that ethanol may induce neuronal reorganization in the brains of these adolescents. To perform adequately on the task, additional brain regions became activated to compensate for the areas of decreased activation in adolescents with an alcohol-use disorder (Caldwell et al., 2005). Altogether, the cumulative data on the immediate and long-term effects of adolescent ethanol exposure provide a picture of an adolescent brain that responds differently to ethanol than the adult brain.

Ethanol and Adolescence

Ethanol intoxication and withdrawal differs between adolescents and adults, as sensitivity to ethanol increases during ontogeny (Land & Spear, 2004; Smith, 2003; Spear & Varlinskaya, 2005). Specifically, adolescent rats exhibit less sedation (significantly shorter sleep times) (Silveri & Spear, 1998), are less sensitive to ethanol-induced hypothermia (Silveri & Spear, 2000), and exhibit less severe motor impairment following ethanol exposure (Ramirez & Spear, 2010; White, Truesdale et al., 2002). During ethanol withdrawal, similar results appear: adolescent rodents exhibited less motor impairment (White, Bae et al., 2002) and anxiety (Doremus, Brunell, Varlinskaya, & Spear, 2003;

Varlinskaya & Spear, 2004a). Repeated adolescent binge-drinking episodes attenuate the emergence of the normal ontogenetic increase in sensitivity to the motor-impairing effects of ethanol. White and collegues (2002) tested rats on a measure of motor coordination following chronic intermittent ethanol exposure (CIE) (injections of ethanol every two days for 20 days). CIE interfered with the normal increase in sensitivity to ethanol-induced motor-impairing effects that occurs between adolescence and adulthood (White, Bae et al., 2002). Repeated injections of ethanol during adolescence impaired cognitive function in adult rats, three weeks after the cessation of ethanol exposure (Pascual, Blanco, Cauli, Miñarro, & Guerri, 2007). Conditional discrimination and object recognition is also impaired by adolescent ethanol exposure (Pascual et al., 2007) as is spatial memory (Farr, Scherrer, Banks, Flood, & Morley, 2005; Land & Spear, 2004; Markwiese, Acheson, Levin, Wilson, & Swartzwelder, 1998; Yttri, Burk, & Hunt, 2004). Although adolescents appear somewhat resistant to the negative physiological consequences of ethanol withdrawal, considerable evidence suggests that sensitization occurs to the social-facilitation and cognitive-impairing effects of ethanol (Varlinskaya & Spear, 2004b, 2009; Varlinskaya & Spear, 2010). The lack of withdrawal-induced symptomology in adolescents, including hypothermia, motor impairment, and anxiety in adolescents positively reinforces ethanol consumption, potentially increasing the likelihood of future episodes of intoxication (Brasser & Spear, 2002; Doremus, Varlinskaya, & Spear, 2004). The cause of these differences remains unknown. The lack of negative effects of ethanol, as well as increased sensitivity to the pleasurable effects in the adolescent brain, increases the reinforcing properties of ethanol in adolescents (Doremus-Fitzwater, Varlinskaya, & Spear, 2010), potentially increasing the likelihood

for future substance abuse problems. It may be possible then, that a neuroprotective agent might be able prevent these ethanol-induced changes from occurring and may also potentially decrease the risk of adolescents developing substance-abuse problems.

Taurine

Taurine (2-aminoethanesulfonic acid), a small sulfur-containing β-amino acid, is the most abundant free intracellular amino acid in humans (Lourenco & Camilo, 2002) and present in nearly all cells in the mammalian CNS (Dawson, Liu, Eppler, & Patterson, 1999; Huxtable, 1989, 1992; Lourenco & Camilo, 2002; Palkovits, Elekes, Lang, & Patthy, 1986). While not utilized in protein production, CNS taurine does provide a key role in neuroprotection (W. Q. Chen et al., 2001; El Idrissi & Trenkner, 1999, 2004) and osmoregulation (Franco, Quesada, & Pasantes-Morales, 2000; Pasantes-Morales, Franco, Torres-Marquez, Hernandez-Fonseca, & Ortega, 2000). Classification of taurine as a conditionally essential amino acid stems from the dietary necessity for this amino acid in infants and newborns, given incomplete synthesis ability early in life (Aerts & Van Assche, 2002; Chesney et al., 1998; Huxtable, 1989, 1992; Rassin et al., 1978). The majority of taurine synthesis occurs in the liver, with only minimal amounts produced in the brain (Tang et al., 1997; Tappaz, Almarghini, Legay, & Remy, 1992). In fact, adult levels of cysteine sulfinic acid decarboxylase (CSAD), the rate-limiting enzyme for taurine synthesis, are 2.5 fold higher in the liver than in the brain, where neonatal levels are even 50% less than adults (Tappaz, Reymond, Bitoun, & Sergeant, 1998). In general, dietary taurine intake represents the primary source of tissue taurine reserves, with only small amounts derived from endogenous production (Chesney et al., 1998; Huxtable, 1989, 1992, 1993).

Taurine functions primarily as an osmoregulator and as a calcium modulator, which enables this amino acid to exert its varied effects such as: facilitating normal neurodevelopment, membrane stabilization, neuroinhibitory actions, and neuroprotection (El Idrissi et al., 1998; Huxtable, 1989; J. Y. Wu et al., 2000). The highest concentrations of taurine are found in the developing brain (Huxtable, 1989, 1993; Sturman, 1977). These initially high taurine levels slowly decrease over the individual's lifetime, with adult brains containing approximately one-third the taurine concentration found in neonatal brains (Banay-Schwartz, DeGuzman, Lajtha, & Plakovits, 1996; Huxtable, 1992). The observed decrease in brain tissue taurine content may result from either reduced endogenous taurine production (Banay-Schwartz et al., 1996; Benedetti et al., 1991; Lourenco & Camilo, 2002; Miller et al., 2000) or increased physiological demands not met by dietary taurine intake (Dawson, Liu et al., 1999). This pattern of age-dependent decrease in taurine levels is found across many species, including humans, monkeys, mice, rabbits, rats, and insects (Huxtable, 1989). Later in life, such age-associated declines in brain taurine levels can relate to reduced striatal dopamine content and corresponding learning deficits (Dawson, Pelleymounter, Cullen, Gollub, & Liu, 1999).

Taurine levels are high during development because the amino acid is necessary for normal neural development (Franconi et al., 2004; Miller et al., 2000; Rentería, Johnson, & Copenhagen, 2004; Saransaari & Oja, 2000; Sturman, 1977). A deficiency of taurine during development increased rates of epilepsy, and learning disabilities, retarded growth, impaired cerebellar development, and caused retinal degeneration in animals (Huxtable, 1993; Rentería et al., 2004; Sturman, 1993; Sturman & Chesney, 1995). In

vitro studies of cultured human fetal neuronal cells show that taurine promotes increases in neuronal cell proliferation, differentiation, improves neuronal survival, and also promotes dendritic extensions (X. C. Chen, Pan, Liu, & Han, 1998; Chesney et al., 1998). In addition, taurine plays a role in normal neuronal migration during early development (Maar, Moran, Schousboe, & Pasantes-Morales, 1995) and facilitates the development of normal neuronal transmission (Franconi et al., 2004; Lourenco & Camilo, 2002). Taurine, as a calcium modulator, can stabilize neuronal membranes in the face of increasing levels of glutamate (Lourenco & Camilo, 2002). This may also be accomplished through taurine's established agonistic actions on GABA (del Olmo, Bustamante, del Rio, & Solio, 2000; Hussy, Deleuze, Pantaloni, Desarmenien, & Moos, 1997; McCool & Botting, 2000) and glycine receptors (Flint, Liu, & Kriegstein, 1998; Han, Haddrill, & Lynch, 2001; Sergeeva & Haas, 2001). The high endogenous levels of taurine found in adolescents may not only facilitate normal development but also protect against potential perturbations of the brain that could impede normal growth. Moreover, taurine's modulation of both osmolarity and glutamate-induced excitability makes this amino acid a likely candidate for countering ethanol-induced pathologies, particularly within vulnerable neural tissues.

Taurine and Ethanol

Taurine's action during ethanol intoxication may represent a protective response of neural cells to the toxic effects produced by ethanol. In rats, acute ethanol injections increases taurine release from the nucleus accumbens (Dachour, Quertemont, & De Witte, 1996; Quertemont, Devitgh, & De Witte, 2003) and hippocampus (Lallemand, Dachour, Ward, & De Witte, 2000) as measured *in vivo*. Additionally, taurine levels

increase in astrocytes of the hippocampus following short-term exposure to ethanol in drinking water (Sakurai et al., 2003). Since taurine functions as a partial $GABA_A$ receptor agonist (del Olmo et al., 2000; Hussy et al., 1997; McCool & Botting, 2000), it may reduce the hypnotic effects induced by ethanol-enhanced $GABA_A$ receptor activation (Mihic & Harris, 1996; Stahl, 2000). Behavioral studies support this theory: as mice treated for 10 days with taurine show no memory or motor-coordination deficits when challenged with ethanol 30 minutes before testing (Vohra & Hui, 2000).

As previously discussed ethanol causes different responses in adolescents and adults. A potential explanation for the age-dependent response to ethanol intoxication is the high taurine concentrations of the brains of adolescents as compared to adults (Hayes & Sturman, 1981). Given that taurine levels decrease with age (Banay-Schwartz et al., 1996; Dawson, Pelleymounter et al., 1999; Miller et al., 2000), the moderating effects of taurine on ethanol intoxication in adults may be amplified in adolescents. Adolescent rats are more sensitive to taurine's analgesic effects than adults (Serrano et al., 2002). Researchers hypothesize that the higher endogenous levels of taurine enhance the effects of taurine administration. In a similar manner, endogenous taurine may protect adolescents from the negative physiological effects of ethanol.

Taurine Supplementation

In addition to the neuroprotective effects of endogenous taurine levels, increasing taurine concentrations via supplementation provides additional benefits. Dietary supplementation with taurine is beneficial in a variety of situations. In aged rats, 18 months old, adding taurine to the drinking water corrected age-related deficits and brought the taurine content to levels higher than 12 month old controls in the spleen,

kidney, eyes, cerebellum, and serum (Dawson, Liu et al., 1999). Additionally, taurine supplementation, added to the drinking water, in aged mice has been shown to prevent natural age-related declines in cognitive function in a passive avoidance test (El Idrissi et al., 1998). Taurine supplementation benefits young rodents too, as increasing taurine intake, by adding it to the drinking water, during the early post-weaning period in rodents improves operant conditioning learning in C57Bl/6J mice (Suge et al., 2007). Further supporting taurine's role as a neuroprotective agent, taurine supplementation during the early postnatal period (via the mother's milk), prevents lead-induced impairments of synaptic plasticity and reduces lead deposits in the hippocampus (Zhu, Wang, She, Yu, & Ruan, 2005). Long-term treatment (Yu et al., 2007) produces similar effects. Given the important role of taurine in development and the benefits of supplementation, particularly its ability to prevent lead-induced damage, taurine supplementation may have other benefits. One such benefit may be modulation of the reward pathways within the brain. A new drug, acamprosate, is on the market, and is a taurine derivative, which prevents relapse in recovering alcoholics (Niederhofer & Staffen, 2003; Whitworth et al., 1996).

The current study aims to investigate the effects of taurine supplementation on ethanol reward and thus a review of the literature on acamprosate (calcium acetylhomotaurine), a taurine derivative, recently approved by the FDA for prevention of ethanol addiction relapse seems warranted. Rodents show a reduction in ethanol self-administration in both the two-bottle choice (Rimondini, Arlinde, Sommer, & Heilig, 2002) and drinking in the dark procedures (Gupta et al., 2008) after acamprosate treatment. Similar studies have found that acamprosate decreased ethanol-induced dopamine release (Olive, Nannini, Ou, Koenig, & Hodge, 2002) and the motivational

salience of an ethanol cue (Cowen, Adams, Kraehenbuehl, Vengeliene, & Lawrence, 2005). Clinical studies with acamprosate show a reduction in relapse episodes of recovering adult alcoholics (Whitworth et al., 1996) as well as ethanol-dependent adolescents (Niederhofer & Staffen, 2003). While these studies with acamprosate show promising results, no studies have looked at the potential for taurine, in its pure form, for reducing the reinforcing properties of ethanol. Given taurine's impressive neuroprotective properties and the benefits gained from dietary supplementation with additional taurine, this paradigm may be useful in potentially reducing ethanol's reinforcing properties. Especially given the limited long-term effectiveness of the current pharmacological agents (Wright & Myrick, 2006) warranting continued exploration of new therapeutic approaches.

Reward Pathway / Reinforcement

Drugs of addiction, such as ethanol, function as rewarding stimuli, or reinforcers, contributing to continued use. Across all age groups, ethanol intoxication results in anxiolysis contributing one element to its reinforcing properties and subsequent widespread recreational use (Krystal & Tabakoff, 2002). Identification of a reward system within the brain began in 1954, with the discovery of areas in the brain that are more sensitive to intracranial stimulation than others (J. Olds & Milner, 1954). Expansion of this work continued and researchers later found that lesions in or near the medial forebrain bundle impaired or blocked electrical self-stimulation in rodents, leading to the conclusion that tissues in and around this area are necessary components of the reward pathway (M. Olds & Olds, 1969). This system was later neuroanatomically mapped (Corbett & Wise, 1979, 1980) and these studies, in addition to lesion studies (Colle &

Wise, 1987; Fibiger, LePiane, Jakubovic, & Phillips, 1987), formed the basis for the conclusion that the mesolimbic dopamine (DA) system is an important pathway in the processing of rewarding stimuli. While other systems may play an ancillary role in the reinforcing properties of ethanol (Roberts, Cole, & Koob, 1996; Thiele, Marsh, Ste Marie, Bernstein, & Palmiter, 1998), evidence continues to suggest a major role for the mesolimbic dopamine system in the mediation and processing of ethanol as well as other reinforcing drugs.

DA, a monoamine neurotransmitter synthesized in the cell bodies of DA neurons, is primarily known for its roles in the facilitation of movement (Siegel, Agranoff, Albers, Fisher, & Uhler, 1999) and reinforcement (Koob & Volkow, 2010). The DA hypothesis of drug reinforcement originated in the late 1970s with pharmacological studies (Wise, 1978) adding to the body of neuroanatomical and lesion studies. Within the reward pathway, activation of DA has multiple roles: to facilitate the association of motivational salience on reward associated cues (Ikemoto & Panksepp, 1999), give incentive salience to environmental stimuli (Robinson & Berridge, 1993), to promote performance of goal-directed behavior (Salamone, Correa, Farrar, & Mingote, 2007), and general activation (Le Moal & Simon, 1991). DA therefore modulates the processing of sensorimotor information across an array of neural circuits to increase the likelihood of the organism focusing on reward availability.

While a full review of the neuroanatomical make-up of the DA system is beyond the scope of this paper (for a review, see Ikemoto, 2007) it is important to mention three critical pathways. Dopaminergic neurons project rostrally in three main overlapping pathways: 1 – the nigrostriatal pathway originates in the substantia nigra and projects to

the caudate and putamen, 2 – the mesocortical pathway, primarily associated with movement initiation originates in the ventral tegmental area (VTA) and projects to a variety of forebrain cortical regions, and 3 – the mesolimbic pathway, primarily associated with reward and reinforcement, originates in the VTA and projects to cortical and limbic structures (Nolte, 2009).

Rewarding stimuli activate DA containing neurons within the VTA leading to the release of DA within the nucleus accumbens (NAc) (Pierce & Kumaresan, 2006). Of particular relevance to this study, ethanol exposure increases DA release (Howard, Schier, Wetzel, Duvauchelle, & Gonzales, 2008) and increases DA uptake in the NAc (Carroll, Rodd, Murphy, & Simon, 2006). Local administration of ethanol onto VTA DA neurons increases spontaneous firing (Brodie & Appel, 1998) and has been shown to directly excite these neurons as isolation from surrounding neurons does not mediate this effect (Brodie, Pesold, & Appel, 1999). Once released DA can bind to DA receptors on the postsynaptic neuron or presynaptic autoreceptors, be cleared from the synapse by the dopamine transporter, or broken down by catecholamine-O-methyl-transferase (COMT) or monoamine oxidase (MAO) into homovanillic acid (HVA) or dihydroxyphenylacetic acid (DOPAC) (Nestler, Hyman, & Malenka, 2009). Measurement of metabolites expressed as a ratio of their levels over DA (HVA/DA) and (DOPAC/DA), are indicative of DA usage within the tissue (Bacopoulos, Hattox, & Roth, 1979) and can often provide more useful information regarding action within the tissue (Broersen et al., 2000; Molinoff & Axelrod, 1971). This surge of dopaminergic activity within the NAc underlies the pleasurable and rewarding sensations of ethanol consumption increasing the likelihood of future use.

Reward System and Adolescence

While this DA system plays an integral part in the rewarding properties of ethanol, the adolescent brain differs biochemically from adults with respect to DA. Animal models of the adolescent brain, exhibit higher basal DA levels in the NAc (Pascual, Boix, Felipo, & Guerri, 2009) and increased DA neurotransmission (Bolanos et al., 1998) than adults. Although there are differences in the mesolimbic DA system between adolescents and adults, both systems responds similarly to ethanol, with an increase in NAc DA levels in response to ethanol in adolescents (Philpot & Kirstein, 1998). Repeated exposure to ethanol injections during adolescence, in rats, leads to increased basal NAc DA levels (Philpot & Kirstein, 2004) and these changes persist into adulthood (Badanich, Maldonado, & Kirstein, 2007). Chronic exposure to ethanol in a free-choice paradigm also significantly increases DA release in the NAc when measured in adulthood (Sahr, Thielen, Lumeng, Li, & McBride, 2004). The long-term changes induced by ethanol consumption alter the natural development of the mesolimbic dopaminergic system. This may make the brain more sensitive to the rewarding properties of ethanol in the future, and may account for the increased risk of developing substance-abuse problems as an adult after early ethanol exposure (Chassin, Pitts, & Prost, 2002; McGue & Iacono, 2008).

Taurine levels may also influence ethanol preference. Extracellular taurine levels, within the NAc, increase after acute ethanol exposure in rats (Dachour et al., 1996; Quertemont, Linotte, & De Witte, 2002). These findings reflect an endogenous response to ethanol mediated by taurine. Additionally, rats from the Sardinian alcohol-preferring line, bred for ethanol preference, release less taurine from their NAc in response to an

ethanol injection than rats in the non-preferring line (Quertemont, Lallemand, Colombo, & De Witte, 2000). Together, these points suggest that that taurine release, within tissues of the mesolimbic system, may modulate reactions to ethanol. Increased tissue taurine content, via exogenous supplementation, might mitigate the rewarding properties of ethanol and thereby reduce the likelihood of abuse.

Primary Investigative Goal

In the current study I investigated the potential for taurine to reduce ethanol self-administration in the adolescent mouse. Analysis of the VTA and NAc for taurine and DA levels provided a picture of changes in neurochemical make-up within the mesolimbic DA system. The hypothesis is that taurine supplementation will reduce the reinforcing properties of ethanol as measured via ethanol self-administration in the adolescent mouse. These changes in behavior should be accompanied by alterations in DA and amino acid levels in both the NAc and VTA.

CHAPTER TWO

Materials and Methods

Animal Gender and Strain

Male C57BL/6J mice were obtained from Jackson Laboratories (Bar Harbor, ME). This strain was chosen as they typically exhibit a preference for an ethanol solution over water in a variety of drinking models (Ho, Chin, & Dole, 1989; Shelton & Grant, 2002). This trait was necessary for the efficacy of the ethanol self-administration studies. Immediately upon arrival the mice were randomly assigned to one of two initial treatment groups (taurine treated or water treated) and housed four to a cage by group. Mice were allowed to habituate to the IACUC-approved vivarium for six days. Rodent lab chow and liquid (either DI or taurine supplemented water) were available continuously. On the sixth day the animals were moved to a pretreatment room and individually housed in Plexiglas cages (8.5" x 5.25" x 5") (Techniplast, Italy) with wire lids and *ad lib* access to food and their assigned liquids. Behavioral testing started the third week the animals were in the facility. Three days before the experiment began, the standard water bottles were replaced with 15ml graduated water tubes fitted with rubber stoppers and metal sipper tube with ball bearings. The animals' established drinking liquid were placed in the new tubes to allow the mice to acclimate.

Taurine Supplementation – Experiments 1 & 2

Taurine (Sigma-Aldrich, St. Louis, MO) was dissolved in DI water to form a 0.23% (w/v) solution (Suge et al., 2007) resulting in mean consumption of 517.19 ± 13.48 mg/kg/day of taurine; this solution was the primary source of liquid for the taurine

treated animals. Taurine supplementation began upon arrival in the facility and continued throughout the experiments.

Experiment 1: Two Bottle Choice

The mice were postnatal day (PD) 21 upon arrival and were allowed to habituate per protocol. Animals were maintained under a standard 12 hr light/dark cycle, lights on at 08:00 and off at 20:00.

Behavioral Testing

The two-bottle choice (2BC) protocol was used to measure the animal's voluntary ethanol intake when granted unlimited access to both a sweetened ethanol solution and their established non-ethanol containing liquid. The two treatment groups for this study were: 0.23% Taurine/Sweetened Ethanol Solution (n = 6), and the Water/Sweetened Ethanol Solution (n = 6). Testing began, for the 2BC procedure, on PD 34 and continued for 10 days (Garcia-Burgos, Gonzales, Manrique, & Gallo, 2009). Upon the initiation of 2BC testing each mouse was presented with two drinking bottles; one containing a sweetened ethanol solution (10% (v/v) ethanol; Pharmco-Aaper, Louisville, KY) and 0.1% (w/v) saccharin (Sigma-Aldrich, St. Louis, MO) (Vetter, Doremus-Fitzwater, & Spear, 2007) and the other containing the established drinking liquid. To control for any side bias, the bottle positions were counterbalanced, the left/right position of the bottles switched every two days and the solutions were refreshed daily. Consumption of both liquids were measured daily by reading the meniscus on the graduated tubes. Evaporation was controlled for by measuring changes in the liquid levels of bottles placed on an empty cage. Animal weights were recorded daily to monitor the health of the animal and standard rodent chow was available *ad lib* throughout the experiment.

Experiment 2: Drinking in the Dark

Mice were PD 21 upon arrival and housed four to a cage, by group, under a reversed 12 hr light/dark cycle, lights off at 08:00 and on at 20:00.

Behavioral Testing

The drinking in the dark (DID) protocol was used to measure the animal's voluntary ethanol intake during its dark phase. The two initial treatment groups were subdivided into ethanol exposed and non-ethanol exposed groups, creating a total of four treatment groups: taurine treated and ethanol exposed - 0.23% Taurine/20% Ethanol (n = 10), water treated and ethanol exposed - Water/20% Ethanol (n = 10), taurine treated with no ethanol exposure - 0.23% Taurine/No-Ethanol (n = 12), water treated with no ethanol exposure - Water/No-Ethanol (n = 12). The DID procedure began on PD 34 and followed the established four day protocol (Rhodes et al., 2007). Under this protocol, 180 minutes after the lights were turned off, for the ethanol exposed groups, the regular liquid tubes were removed and replaced with similar tubes containing a 20% (v/v) ethanol (Pharmco-Aaper, Louisville, KY) solution. Intake of the initially assigned liquids was measured by reading the meniscus levels on the graduated tubes when those tubes were removed from the cages. Likewise, ethanol intake amounts were measured upon removal of the ethanol tubes. For the first three days of the DID procedure, the ethanol solution was presented for two hours before it was removed and the initially assigned liquid bottles were replaced. The mice were weighed 3 hours after removal of the ethanol tubes to monitor the health of the animal. On the fourth day of the procedure, testing day, the ethanol solution was present for four hours. Consumption amounts were measured at two separate points on testing day: after two and four hours. On testing day, the animals were

weighed immediately after the ethanol bottles were removed before blood and tissues were extracted.

Blood and Tissue Collection

Immediately following the conclusion of behavioral testing, each animal was sacrificed, via decapitation, and trunk blood collected for blood ethanol concentration analysis, as well as the extraction of VTA and NAc tissues. Using an acrylic mouse-brain matrix (Kopf Instruments, Tujunga, CA), 2mm coronal slices were cut around the VTA and NAc and the slices were placed on a glass Petri dish on dry ice. Bilateral removal of the target tissues was accomplished via 1mm tissue punch (Fine Scientific Tools, Foster City, CA). The tissues were placed in pre-weighed, 1.5 ml Eppendorf microcentrifuge tubes, and immediately stored at -80° C (Ultima II Cryo Vault; REVCO) for later processing.

Blood Ethanol Concentration

For the analysis of blood ethanol concentrations (BEC), trunk blood was collected in heparinized tubes and diluted 50:1 with chilled perchloric acid (3.4% v/v). The samples were then vortexed and centrifuged (4° C) at 12,000 x g. The resultant supernatant was then used in a modified enzymatic assay, derived from the Calbiochem-Behring method (La Jolla, CA), based upon the conversion of alcohol to acetaldehyde by alcohol dehydrogenase in the presence of the cofactor nicotinamide adenine dinucleotide (NAD). NAD is reduced stoichiometrically during the reaction to NADH that is detectable by UV spectrophotometry at 340 nm. Blood ethanol levels were determined on an Eppendorf BioPhotometer Plus (Hauppauge, NY) and were expressed as g/dl.

Amino Acid and Monoamine Quantification

After thawing at 4° C, extracted tissues were homogenized in ice-cold 0.1 N HCl, using an Eppendorf micropestle, and then spun at 28,000 g for 25 minutes at 4° C (Allegra 64R; Beckman-Coulter). The resulting supernatants were filtered through 0.2µ GHP Acrodisc syringe filters (Pall, East Hills, NY). Each filtered sample was divided into aliquots for two separate high-performance liquid chromatography (HPLC) methods, monoamine and amino acid analysis. Tissue monoamine and amino acid levels were both measured via isocratic reversed-phase HPLC, using a BAS HPLC system comprised of an LC-4C electrochemical detector (BAS, West Lafayette, IN).

For the quantification of monoamines, samples were injected into the HPLC system via a 10 µl sample loop (Rheodyne, Rohnert Park, CA) and analytes separated by a C_{18}-ZORBAX StableBond analytical column (50 x 4.6 mm I.D., 1.8 µm particle size; Agilent Technologies, Santa Clara, CA) maintained at 34º C. An isocratic mobile phase, delivered at a constant flow rate of 1.7 ml/min, consisted of: 7% (v/v) acetonitrile, 0.5% (v/v) tetrahydrofuran, 30 mM sodium citrate, 30 mM NaH_2PO_4, 0.1 mM Na_2EDTA, and 2.00 mM 1-octanesulfonic acid (adjusted to pH 3.20 via *o*-phosphoric acid). Analytes were detected on a glassy carbon electrode (with full scale output set at 2.0 nA) against a Ag/AgCl reference electrode set at +750 mV (BAS, West Lafayette, IN). Complete separation of monoamines (NE, DOPAC, DA, 5-HIAA, HVA, and 5-HT) was achieved in 4.5 min. In all tissues, monoamine concentrations are expressed as pg / mg tissue weight.

For the quantification of amino acids, samples required pre-column derivatization with an *o*-phthalaldehyde (OPA) / β-mercaptoethanol (BME) reagent. Fresh stock reagent

solution (stored at 4º C) was prepared every four days by dissolving 20 mg OPA (Sigma-Aldrich, St. Louis, MO) in 1 ml HPLC-grade methanol, adding 10 µl BME (Sigma-Aldrich, St. Louis, MO), and diluting with 9 ml of 0.1 M sodium tetraborate buffer (pH 9.3). To prepare the working reagent solution, a 1 ml aliquot of stock reagent was diluted with 9 ml of 0.1 M sodium tetraborate buffer and stored on ice during analysis. A 20 µl aliquot of purified sample was mixed with 80 µl of deriving reagent, allowed to react for exactly 60 seconds, and injected into the HPLC system via a 5 µl sample loop (Rheodyne, Rohnert Park, CA). Derivitized analytes were separated by a C_{18}-ZORBAX Eclipse analytical column (50 x 4.6 mm I.D., 3.5 µm particle size; Agilent Technologies, Santa Clara, CA) maintained at 28º C. An isocratic mobile phase, delivered at a constant flow rate of 2.4 ml/min, consisted of: 22.5% (v/v) methanol, 6% (v/v) tetrahydrofuran, 30 mM Na_2HPO_4, 20 mM NaH_2PO_4, and 0.1 mM Na_2EDTA (adjusted to pH 6.55 via *o*-phosphoric acid). Analytes were detected on a glassy carbon electrode (with full scale output set at 50 nA) against a Ag/AgCl reference electrode set at +700 mV (BAS, West Lafayette, IN). Complete separation of amino acids (taurine and GABA) was achieved in 3 min. In all tissues, taurine and GABA concentrations are expressed as nmol / mg tissue weight.

Experiment 2a: Sucrose Controls

To ensure that any treatment effects of the DID protocol were ethanol specific the study was replicated with a separate set of animals. In this study a 10% (w/v) sucrose (Sigma-Aldrich, St. Louis, MO) solution was used in place of ethanol (Hendrickson, Zhao-Shea, & Tapper, 2009). Mice were PD 21 upon arrival, maintained on the established reversed light/dark cycle, and were divided into two groups: taurine treated

and sucrose exposed - 0.23% Taurine/10% Sucrose (n = 8) or water treated and sucrose exposed - Water/10% Sucrose (n = 7). All other parameters, housing, light cycle, and the timing of liquid and weight measurements were held constant.

Experiment 3: Efficacy in Adults

Replication of Experiment 2 was conducted to examine the efficacy of the same taurine treatment on DID in adult mice. Mice arrived at 10 weeks old (PD 70) and were allowed to habituate to the animal care facility and housed as established. Adolescence in the mouse spans the period between PD 21 and PD 60 (Crawley, 2000), thus the age of PD 70 was chosen to ensure that testing would occur beyond the bounds of adolescence. Mice were maintained under the established reversed 12 hr light/dark cycle, lights off at 08:00 and on at 20:00.

Taurine Supplementation

The taurine concentration was adjusted from 0.23% (w/v) to 0.33% (w/v), to create a dose equivalent to the adolescents, and resulted in a mean consumption of 552.51 ± 27.14 mg/kg/day of taurine in order to account for the increased weight and liquid consumption of the adult mouse. Taurine supplementation began upon arrival in the facility and continued throughout the study.

Behavioral Testing

The DID protocol was used to measure the animal's voluntary ethanol intake during their dark phase. The two initial treatment groups were subdivided into ethanol exposed and non-ethanol groups, creating a total of four treatment groups: taurine treated and ethanol exposed - 0.33% Taurine/20% Ethanol (n = 8), water treated and ethanol

exposed - Water/20% Ethanol (n = 8), taurine treated with no ethanol exposure - 0.33% Taurine/No-Ethanol (n = 7), water treated with no ethanol exposure - Water/No-Ethanol (n = 7). The DID procedure began on PD 83 and followed the established four day protocol (Rhodes et al., 2007) established above.

Blood and Tissue Collection

Immediately following the conclusion of behavioral testing, each animal was sacrificed, via decapitation, and trunk blood collected for blood ethanol concentration analysis, as well as the extraction of VTA and NAc tissues. Using an acrylic mouse-brain matrix (Kopf Instruments, Tujunga, CA), 2mm coronal slices were cut around the VTA and NAc and the slices were placed on a glass Petri dish on dry ice. Bilateral removal of the target tissues was accomplished via 1mm tissue punch (Fine Scientific Tools, Foster City, CA). The tissues were placed in pre-weighed, 1.5 ml Eppendorf microcentrifuge tubes, and immediately stored at -80° C (Ultima II Cryo Vault; REVCO) for later processing.

Blood Ethanol Concentration

For the analysis of BEC, trunk blood was collected in heparinized tubes and diluted 50:1 with chilled perchloric acid (3.4% v/v). This procedure was identical to that described in the previous section.

Amino Acid and Monoamine Quantification

Amino acid and monoamine levels, in tissues extracted from adult animals, were measured via the same procedure as described in the previous section.

Statistical Analysis

Blood ethanol concentration, ethanol intake (g/kg), and preference [(ethanol intake / total intake)*100], and initial tissue taurine concentrations were analyzed with t-tests. The effect of taurine and ethanol exposure on tissue levels of monoamines and amino acids were analyzed by two-way analysis of variance (ANOVA), and post hoc analysis was conducted with Tukey-Kramer HSD. Significance was set at $p < 0.05$ throughout. All statistical analysis were conducted using JMP (v. 7.0) statistical software for PC (SAS Institute Inc.).

CHAPTER THREE

Results

Experiment 1: Two-Bottle Choice

Consumption and Preference for a Sweetened Ethanol Solution

Consumption of the sweetened ethanol solution (g/kg) was not significantly reduced by taurine supplementation, $t(10) = 1.39$, $p = .196$ (Figure 1).

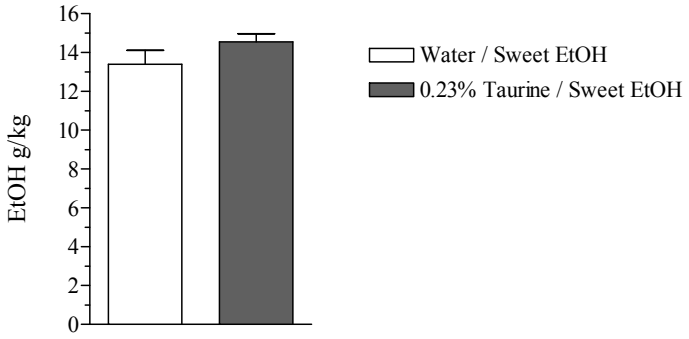

Figure 1. Mean ethanol consumption (g/kg) (+*SEM*) for taurine-treated (0.23% Taurine) and water-treated (Water) groups.

Taurine treatment significantly reduced preference for the sweetened ethanol solution, $t(10) = 2.93$, $p = .015$ (Figure 2).

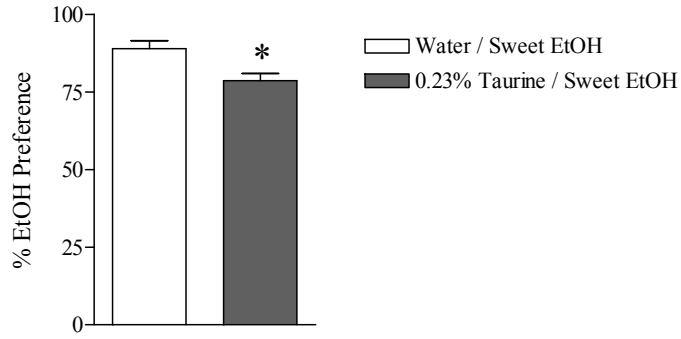

Figure 2. Mean ethanol preference (+*SEM*) for taurine treated (0.23% Taurine) and water treated (Water) groups. *Significantly different from water group ($p < .05$).

Experiment 2: Drinking in the Dark

Baseline Liquid Consumption and Weights

Baseline liquid consumption was measured in the non-ethanol exposed mice. Consumption did not differ between treatment groups (taurine or water), $t(22) = -0.51$, $p = .615$, nor did weight groups, $t(22) = -1.86$, $p = .079$ (Table 1).

Table 1. Experiment 2 Treatment Group Descriptive Statistics

Treatment Group	Liquid Consumption (ml/g/day)	Mean Weight (g)
Taurine	0.25 ± 0.004	20.54 ± 0.327
Water	0.25 ± 0.005	19.93 ± 0.233

Tissue Taurine Concentrations

Tissue taurine concentrations in taurine-treated animals were compared to water-treated animals to establish the effectiveness of taurine supplementation. Taurine supplementation significantly increased tissue concentrations in both the NAc, $t(22) = 2.65$, $p = .015$, and VTA, $t(22) = 2.69$, $p = .013$, Figure 3a displays mean taurine levels in the NAc and Figure 3b displays mean VTA taurine levels.

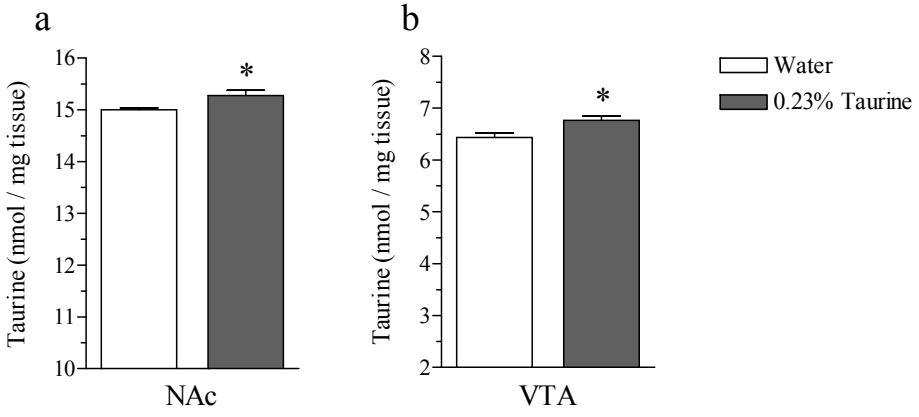

Figure 3. Mean tissue taurine concentration (nmol / mg tissue) (+*SEM*) in NAc (a) and VTA (b) for non-ethanol exposed taurine and water treated groups. *Significantly different from water group ($p < .05$)

Ethanol Consumption

Mean ethanol consumption, expressed as (g/kg), was significantly decreased during the final ethanol exposure period of four hours in the taurine-treated, $t(18) = 3.48$, $p = .003$ (Figure 4).

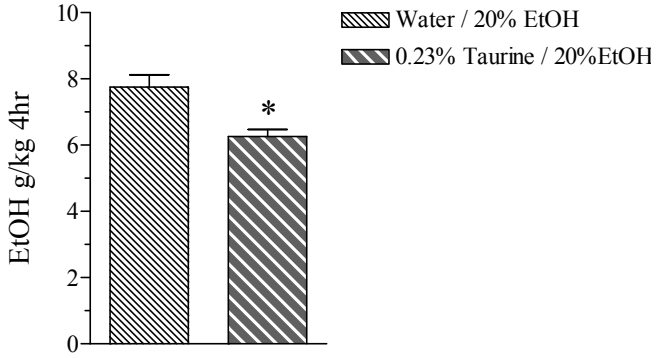

Figure 4. Mean ethanol consumption (g/kg) (+*SEM*) for taurine treated-ethanol exposed (0.23% Taurine/20% EtOH), water-ethanol exposed (Water/20% EtOH) groups. *Significantly different than water treated group ($p < .05$).

Blood Ethanol Concentration

Taurine treatment and the resulting changes in ethanol consumption significantly reduced blood ethanol concentrations (BEC) in the taurine treated group $t(18) = 2.55$, $p = .020$ (Figure 5).

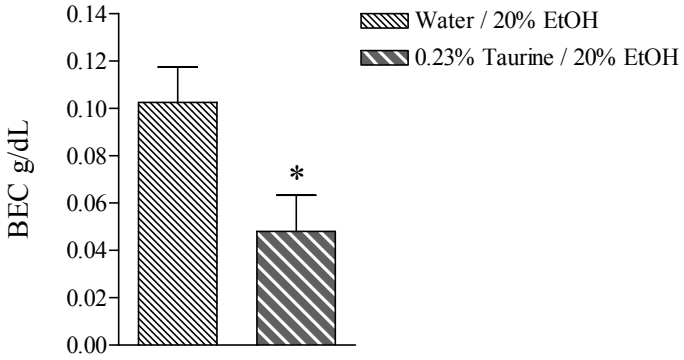

Figure 5. Mean BEC (+ *SEM*) for taurine treated-ethanol exposed (0.23% Taurine/20% EtOH) and water-ethanol exposed (Water/20% EtOH) groups. *Significantly different than water treated group ($p < .05$).

Nucleus Accumbens

Dopamine and turnover. DA levels and turnover (HVA/DA and DOPAC/DA) in the NAc are illustrated in Figures 6a, 6b, 6c. A two-way ANOVA, comparing the effects of taurine treatment and ethanol exposure on DA levels was conducted (Figure 6a). Analysis found no significant main effect of taurine treatment, $F(1,40) = .04$, $p = .839$, $\omega^2 = .07\%$. A significant main effect of ethanol exposure was found, $F(1,40) = 12.56$, $p = .001$, $\omega^2 = 21.25\%$, in that the ethanol exposed groups had lower DA levels than the non-ethanol exposed groups. There was also a significant taurine-treatment by ethanol exposure interaction, $F(1,40) = 6.37$, $p = .016$, $\omega^2 = 10.78\%$, wherein the difference in DA levels was larger between the non-ethanol exposed groups compared to the ethanol treated groups. Post-hoc analysis with Tukey-Kramer HSD revealed significantly lower DA levels in the water-treated/ethanol-exposed group than the water-treated/non-ethanol exposed group (Figure 6a). HVA/DA turnover is illustrated in Figure 6b. A two-way ANOVA, comparing the effects of taurine treatment and ethanol exposure, revealed a significant main effect of taurine treatment, $F(1,40) = 12.42$, $p = .001$, $\omega^2 = 9.38\%$, with the taurine treated groups having lower HVA/DA turnover than the water treated groups. There was also a significant main effect of ethanol exposure, $F(1,40) = 59.79$, $p < .0001$, $\omega^2 = 45.15\%$, in that ethanol exposure increased HVA/DA turnover compared to the non-ethanol exposed groups. A significant taurine treatment by ethanol interaction was found, $F(1,40) = 23.02$, $p < .0001$, $\omega^2 = 17.38\%$, wherein there was a larger between group difference in the ethanol exposed groups than the non-ethanol exposed groups. Post-hoc analysis with Tukey-Kramer HSD revealed significantly higher HVA/DA levels in the water-treated/ethanol exposed group than the water-treated/non-ethanol exposed group

($p < .05$) and that the taurine-treated/ethanol exposed group had significantly lower HVA/DA turnover than the water-treated/ethanol exposed group ($p < .05$). DOPAC/DA turnover in the NAc is illustrated in Figure 6c. A two-way ANOVA was conducted comparing the effects of taurine treatment and ethanol exposure on DOPAC/DA turnover. Analysis revealed no significant main effects of taurine treatment $F(1,40) = 1.41$, $p = .241$, $\omega^2 = 3.33\%$, ethanol exposure, $F(1,40) = .92$, $p = .343$, $\omega^2 = 2.17\%$, or taurine treatment by ethanol interaction, $F(1,40) = .11$, $p = .741$, $\omega^2 = .26\%$.

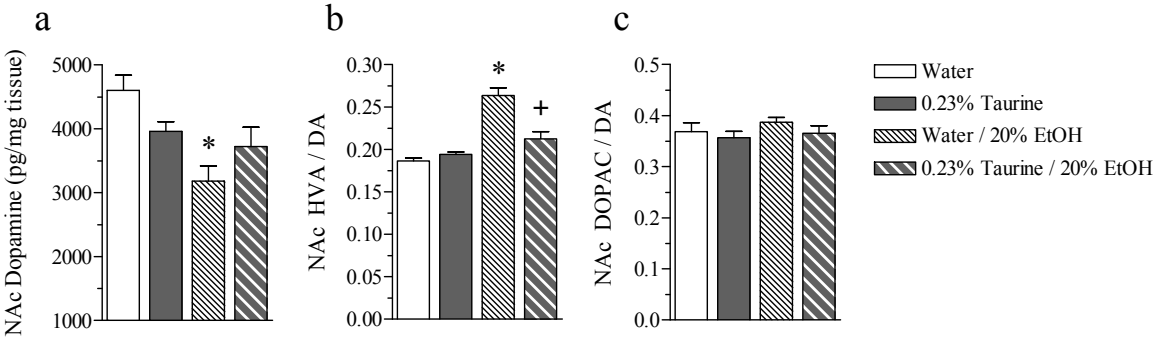

Figure 6. (a) Mean NAc DA (pg/mg tissue) (+*SEM*). * Significantly different from water-treated/non-ethanol exposed group ($p < .05$). *(b)* Mean NAc HVA/DA (+*SEM*). *Significantly different from water-treated/non-ethanol exposed group ($p < .05$). +Significantly different from water-treated/ethanol exposed group ($p < .05$). *(c)* Mean NAC DOPAC/DA (+*SEM*).

Amino Acid Analysis. Figure 7a illustrates taurine levels in the NAc. Analysis of taurine levels with a two-way ANOVA revealed a significant main effect of taurine treatment, $F(1,40) = 10.38$, $p = .003$, $\omega^2 = 17.6\%$, as previously noted, taurine treatment increased tissue taurine levels. No significant main effect of ethanol exposure was found, $F(1,40) = .62$, $p = .436$, $\omega^2 = 1.05\%$. A significant taurine treatment by ethanol exposure interaction was found, $F(1,40) = 6.35$, $p = .016$, $\omega^2 = 10.77\%$, wherein the between group differences were greater in the non-ethanol exposed groups than the ethanol-exposed groups. Post-hoc analysis with Tukey-Kramer HSD revealed significantly higher taurine levels in the taurine-treated/non-ethanol exposed group than the water-treated/non-

ethanol exposed group ($p < .05$). GABA levels in the NAc are illustrated in Figure 7b. A two-way ANOVA was conducted and revealed no significant main effect of taurine treatment, $F(1,40) = .54$, $p = .468$, $\omega^2 = 1.06\%$. A significant main effect of ethanol was found, $F(1,40) = 10.15$, $p = .003$, $\omega^2 = 20.0\%$, ethanol exposure increased GABA levels compared to the non-ethanol exposed groups . The taurine treatment by ethanol exposure interaction was not significant, $F(1,40) = .10$, $p = .756$, $\omega^2 = .19\%$. Post-hoc analysis with Tukey-Kramer HSD revealed significantly higher GABA levels in the taurine-treated/ethanol-exposed group compared to the water-treated/non-ethanol exposed group ($p < .05$).

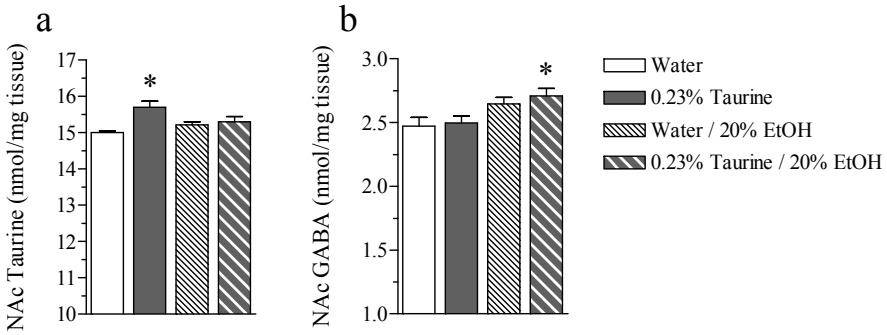

Figure 7. (a) Mean NAc Taurine (nmol/mg tissue) (+*SEM*). *Significantly different than water-treated/non-ethanol exposed group ($p < .05$). *(b)* Mean NAc GABA (nmol/mg tissue) (+*SEM*). *Significantly different than water-treated/non-ethanol exposed group ($p < .05$).

Ventral Tegmental Area

D*opamine and turnover*. Figures 8a, 8b, and 8c illustrate DA levels and turnover (HVA/DA and DOPAC/DA) in the VTA. Two-way ANOVA of DA levels, comparing the effects of taurine treatment and ethanol exposure resulted in a significant main effect of taurine treatment, $F(1,40) = 55.37$, $p < .0001$, $\omega^2 = 48.60\%$, taurine treatment increased DA levels compared to water-treated groups. A significant main effect was also found for ethanol exposure, $F(1,40) = 16.43$, $p = .0002$, $\omega^2 = 14.42\%$, wherein ethanol exposure

decreased DA levels compared to non-ethanol exposed groups. No significant taurine treatment by ethanol exposure interaction was found $F(1,40) = .60$, $p = .444$, $\omega^2 = .52\%$. Post-hoc analysis with Tukey-Kramer HSD revealed significantly higher DA levels in the taurine-treated/non-ethanol exposed group than the water-treated/ethanol exposed group ($p < .05$) and significantly higher DA levels in the taurine-treated/ethanol-exposed group than the water-treated/ethanol-exposed group ($p < .05$) (Figure 8a). Figure 8b illustrates HVA/DA turnover in the VTA. A two-way ANOVA was conducted comparing the effects of taurine treatment and ethanol exposure and revealed a significant main effect of taurine treatment, $F(1,40) = 4.17$, $p = .048$, $\omega^2 = 9.34\%$, taurine treatment reduced HVA/DA turnover. No significant main effect of ethanol exposure was found, $F(1,40) = .26$, $p = .611$, $\omega^2 = .59\%$, and the interaction was not significant, $F(1,40) = .06$, $p = .803$, $\omega^2 = .14\%$. DOPAC/DA turnover in the VTA are illustrated in Figure 8c. Two-way ANOVA comparing the effects of taurine treatment and ethanol exposure revealed no significant main effects of taurine treatment, $F(1,40) = 1.64$, $p = .208$, $\omega^2 = 3.83\%$, ethanol exposure, $F(1,40) = .70$, $p = .408$, $\omega^2 = 1.64\%$, or taurine treatment by ethanol interaction, $F(1,40) = .34$, $p = .565$, $\omega^2 = .78\%$.

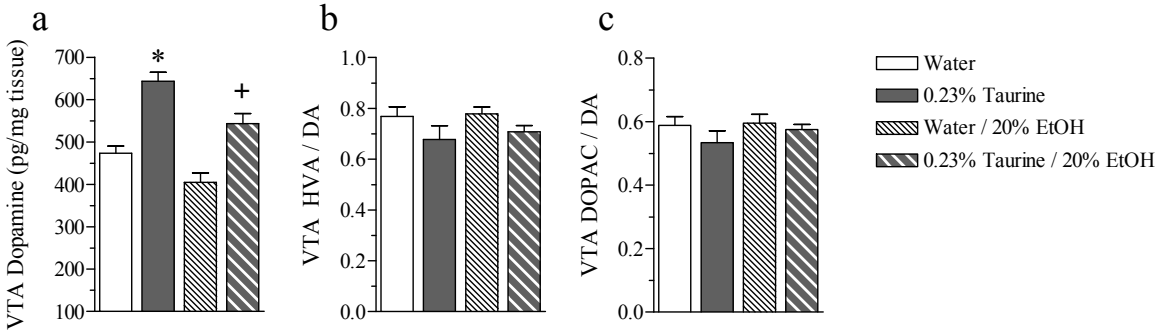

Figure 8. (a) Mean VTA DA (pg/mg tissue) (+*SEM*). * Significantly different than water-treated/non-ethanol exposed group ($p < .05$). + Significantly different than water-treated/ethanol exposed group ($p < .05$). *(b)* Mean VTA HVA/DA (+*SEM*). *(c)* Mean VTA DOPAC/DA (+*SEM*).

Amino Acid Analysis. Figures 9a and 9b illustrate taurine levels in the VTA. A two-way ANOVA of taurine levels was conducted, comparing the effects of taurine treatment and ethanol exposure. A significant main effect of taurine treatment was found, $F(1,40) = 13.93$, $p < .001$, $\omega^2 = 22.77\%$, as previously noted, taurine treatment increased tissue concentrations. No significant main effect was found for ethanol exposure, $F(1,40) = 2.21$, $p = .145$, $\omega^2 = 3.61\%$, and the taurine treatment by ethanol exposure interaction was also not significant, $F(1,40) = 3.59$, $p = .065$, $\omega^2 = 5.87\%$. Post-hoc analysis with Tukey-Kramer HSD revealed significantly higher taurine levels in the taurine-treated/non-ethanol exposed group than the water-treated/non-ethanol exposed group ($p < .05$) (Figure 9a). GABA levels in the VTA are illustrated in Figure 9b. Two-way ANOVA comparing the effects of taurine treatment and ethanol exposure revealed no significant main effects of taurine treatment, $F(1,40) = 1.41$, $p = .242$, $\omega^2 = 3.29\%$, or ethanol exposure, $F(1,40) = .19$, $p = .668$, $\omega^2 = .44\%$. The taurine treatment by ethanol interaction was also not significant, $F(1,40) = 1.00$, $p = .323$, $\omega^2 = 2.34\%$.

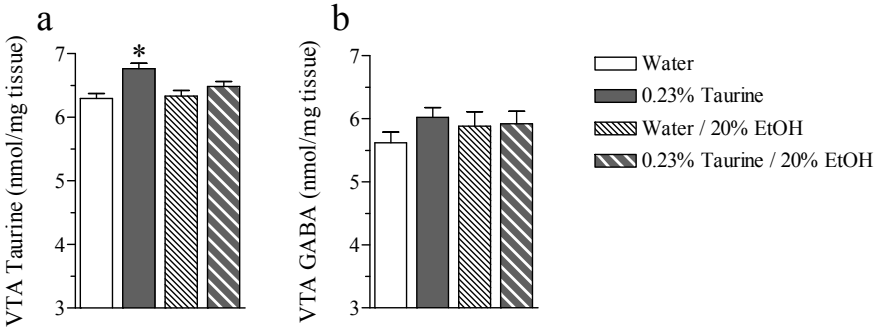

Figure 9. (a) Mean VTA Taurine (nmol/mg tissue) (+*SEM*). *Significantly different than water-treated/non-ethanol exposed group ($p < .05$). *(b)* Mean VTA GABA (nmol/mg tissue) (+*SEM*).

Experiment 2a: Sucrose Controls

Figure 10 illustrates the effect of taurine treatment on consumption of a 10% sucrose solution. Taurine treatment significantly increased consumption of the sucrose solution, $t(13) = 2.39$, $p = .032$.

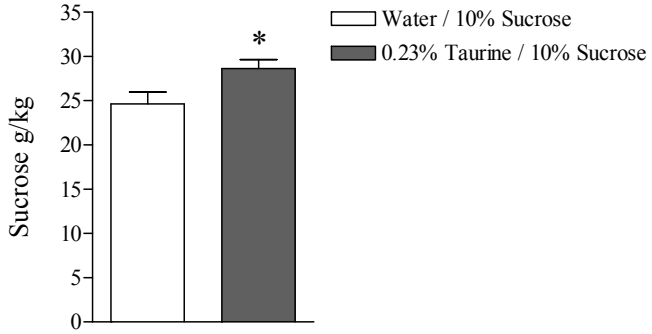

Figure 10. Mean sucrose consumption (g/kg) (+*SEM*) for taurine-treated/sucrose exposed (0.23% Taurine/10% sucrose) and water-treated/sucrose exposed (Water/10% sucrose) groups. *Significantly different from water treated group.

Experiment 3: Efficacy in Adults

Taurine Dose – Adults

The adjusted taurine dose (0.23% for adolescents vs. 0.33% for adults) was not significantly different from adolescent dose, $t(18) = 1.29$, $p = .215$.

Baseline Liquid Consumption and Weights - Adult

Liquid consumption was measured in the baseline, non-ethanol exposed mice. Taurine supplementation did not significantly alter liquid consumption in adult mice (Table 2). There was no significant difference in liquid consumption between groups, $t(12) = 0.01$, $p = .992$. There was no significant difference in weights between the water treated baseline group and the taurine-treated baseline group, $t(12) = 1.72$, $p = .112$.

Table 2. Experiment 3 Treatment Group Descriptive Statistics

Treatment Group	Liquid Consumption (ml/g/day)	Mean Weight (g)
Taurine	0.23 ± 0.021	27.98 ± 0.245
Water	0.23 ± 0.014	28.67 ± 0.321

Ethanol Consumption

Mean ethanol consumption in adult mice, expressed as (g/kg), during the final ethanol exposure period (4hr) in adult mice is illustrated in Figure 11. Taurine supplementation did not significantly affect ethanol consumption, $t(14) = .12$, $p = .907$.

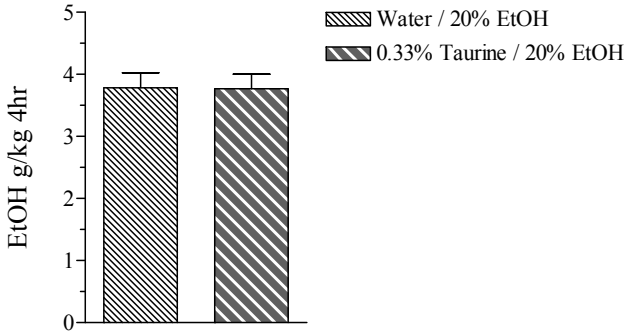

Figure 11. Mean Ethanol Consumption (g/kg) (+*SEM*) for taurine-treated/ethanol exposed (0.33% Taurine/20% ethanol) and water-treated/ethanol exposed (Water/20% ethanol) groups.

Blood Ethanol Concentration

Taurine treatment did not significantly affect BECs. There was no significant difference in BECs between treatment groups, $t(14) = 1.189$, $p = .254$ (Figure 12).

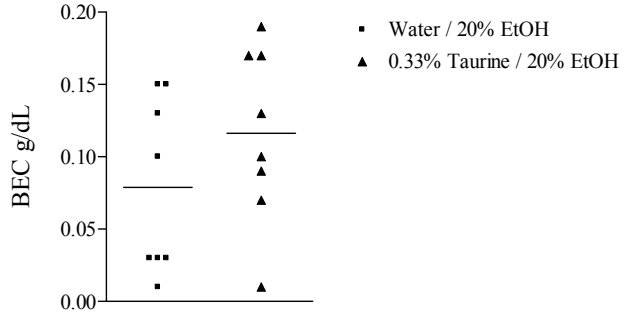

Figure 12. Mean BEC (+*SEM*) for 0.33% Taurine / 20% EtOH group and Water / 20% EtOH group

Nucleus Accumbens

Dopamine and turnover. DA levels and turnover (HVA/DA and DOPAC/DA) in the NAc are illustrated in Figures 13a, 13b, 13c. A two-way ANOVA, comparing the effects of taurine treatment and ethanol exposure on DA levels was conducted (Figure 13a). Analysis found no significant main effects of taurine treatment, $F(1,26) = .66$, $p = .426$, $\omega^2 = 2.39\%$, ethanol exposure, $F(1,26) = .53$, $p = .474$, $\omega^2 = 1.39\%$, or taurine-treatment by ethanol exposure interaction, $F(1,26) = .25$, $p = .618$, $\omega^2 = .93\%$. HVA/DA turnover is illustrated in Figure 13b. A two-way ANOVA, comparing the effects of taurine treatment and ethanol exposure, revealed no significant main effect of taurine treatment, $F(1,26) = 2.93$, $p = .099$, $\omega^2 = 6.96\%$. There was a significant main effect of ethanol exposure, $F(1,26) = 12.96$, $p = .001$, $\omega^2 = 30.78\%$, in that ethanol exposure increased HVA/DA turnover compared to the non-ethanol exposed groups. No significant taurine treatment by ethanol interaction was found, $F(1,26) = .11$, $p = .738$, $\omega^2 = .27\%$. Post-hoc analysis with Tukey-Kramer HSD revealed significantly higher HVA/DA levels in the taurine-treated/ethanol exposed group than the water-treated/non-ethanol exposed group ($p < .05$). DOPAC/DA turnover in the NAc is illustrated in Figure 13c. A two-way ANOVA was conducted comparing the effects of taurine treatment and ethanol exposure on DOPAC/DA turnover. Analysis revealed no significant main effect of taurine treatment $F(1,26) = .75$, $p = .395$, $\omega^2 = 2.07\%$. There was a significant main effect of ethanol exposure, $F(1,26) = 8.89$, $p = .006$, $\omega^2 = 24.55\%$, ethanol exposure increased DOPAC/DA turnover compared to the non-ethanol exposed groups. No significant taurine treatment by ethanol interaction was found, $F(1,26) = .65$, $p = .427$, $\omega^2 = 1.80\%$.

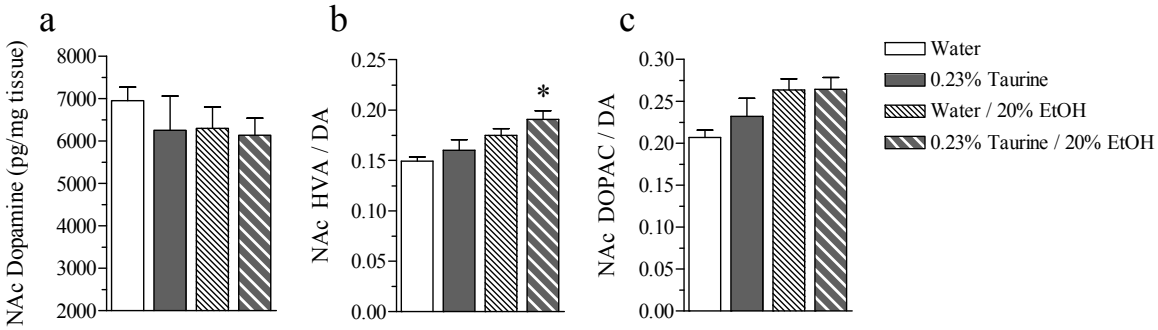

Figure 13. (a) Mean NAc DA (pg/mg tissue) (+*SEM*). *(b)* Mean NAc HVA/DA (+*SEM*). *Significantly different from water-treated/non-ethanol exposed group ($p < .05$). *(c)* Mean NAC DOPAC/DA (+*SEM*).

Amino acid analysis. Figure 14a illustrates taurine levels in the NAc. Analysis of taurine levels with a two-way ANOVA revealed a significant main effect of taurine treatment, $F(1,26) = 21.68$, $p < .0001$, $\omega^2 = 30.05\%$, taurine treatment increased tissue taurine levels. A significant main effect of ethanol exposure was found, $F(1,26) = 11.61$, $p = .002$, $\omega^2 = 16.10\%$, ethanol exposure decreased taurine levels compared to the non-ethanol exposed groups. A significant taurine treatment by ethanol exposure interaction was found, $F(1,26) = 10.66$, $p = .003$, $\omega^2 = 14.78\%$, wherein the between group differences were greater in the ethanol exposed groups than the non-ethanol exposed groups. Post-hoc analysis with Tukey-Kramer HSD revealed significantly lower taurine levels in the water-treated/ ethanol exposed group than the water-treated/non-ethanol exposed group ($p < .05$). GABA levels in the NAc are illustrated in Figure 14b. A two-way ANOVA was conducted and revealed no significant main effects of taurine treatment, $F(1,26) = .40$, $p = .531$, $\omega^2 = 1.37\%$, ethanol exposure, $F(1,26) = .24$, $p = .627$, $\omega^2 = .82\%$, or taurine treatment by ethanol exposure interaction, $F(1,26) = 2.64$, $p = .116$, $\omega^2 = 8.98\%$.

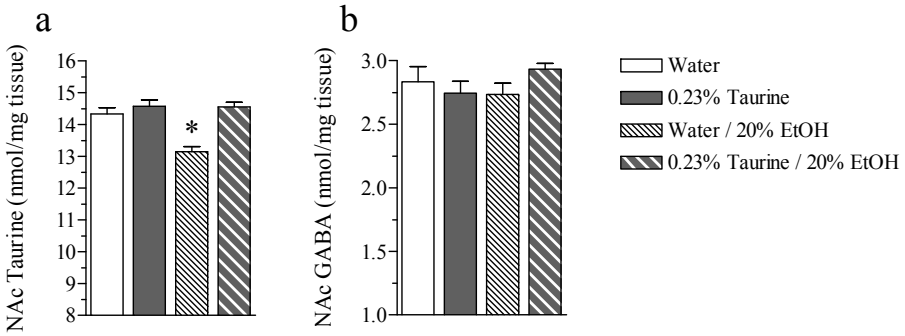

Figure 14. (a) Mean NAc Taurine (nmol/mg tissue) (+*SEM*). *Significantly different than water-treated/non-ethanol exposed group ($p < .05$). *(b)* Mean NAc GABA (nmol/mg tissue) (+*SEM*).

Ventral Tegmental Area

Dopamine and turnover. Figures 15a, 15b, and 15c illustrate DA levels and turnover (HVA/DA and DOPAC/DA) in the VTA. Two-way ANOVA of DA levels, comparing the effects of taurine treatment and ethanol exposure revealed no significant main effects of taurine treatment, $F(1,26) = 1.23$, $p = .278$, $\omega^2 = 4.19\%$, ethanol exposure, $F(1,26) = 1.99$, $p = .171$, $\omega^2 = 6.77\%$, or taurine treatment by ethanol exposure interaction $F(1,26) = .07$, $p = .800$, $\omega^2 = .23\%$ (Figure 15a). Figure 15b illustrates HVA/DA turnover in the VTA. A two-way ANOVA was conducted comparing the effects of taurine treatment and ethanol exposure and revealed no significant main effects of taurine treatment, $F(1,26) = .06$, $p = .816$, $\omega^2 = .20\%$, ethanol exposure, $F(1,26) = 2.07$, $p = .162$, $\omega^2 = 7.31\%$, or the taurine treatment by ethanol interaction, $F(1,26) = .17$, $p = .683$, $\omega^2 = .60\%$. DOPAC/DA turnover in the VTA are illustrated in Figure 15c. Two-way ANOVA comparing the effects of taurine treatment and ethanol exposure revealed no significant main effect of taurine treatment, $F(1,26) = .36$, $p = .553$, $\omega^2 = .87\%$. There was a significant main effect of ethanol exposure, $F(1,26) = 15.20$, $p < .001$, $\omega^2 = 36.54\%$, ethanol exposure significantly increased DOPAC/DA turnover compared to the non-ethanol exposed groups. There was no significant taurine treatment by ethanol

interaction, $F(1,26) = .02$, $p = .886$, $\omega^2 = .05\%$. Post-hoc analysis with Tukey-Kramer HSD found significantly higher turnover in the water-treated/ethanol exposed group than the water-treated/non-ethanol exposed group ($p < .05$).

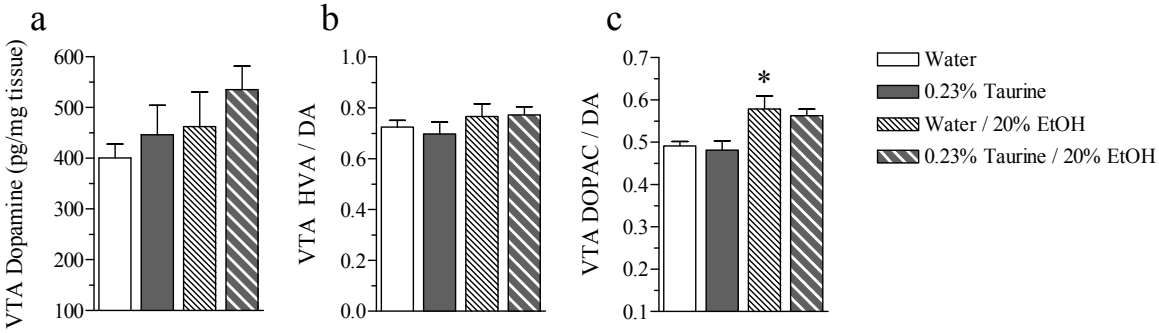

Figure 15. (a) Mean VTA DA (pg/mg tissue) (+*SEM*). *(b)* Mean VTA HVA/DA (+*SEM*). *(c)* Mean VTA DOPAC/DA (+*SEM*). *Significantly different than water-treated/non-ethanol exposed group ($p < .05$).

Amino acid analysis. Figures 16a and 16b illustrate taurine levels in the VTA. A two-way ANOVA of taurine levels was conducted, comparing the effects of taurine treatment and ethanol exposure. No significant main effects were found of taurine treatment, $F(1,26) = .67$, $p = .421$, $\omega^2 = 2.48\%$, ethanol exposure, $F(1,26) = .08$, $p = .779$, $\omega^2 = .30\%$, or the taurine treatment by ethanol exposure interaction, $F(1,26) = .18$, $p = .674$, $\omega^2 = .67\%$ (Figure 16a). GABA levels in the VTA are illustrated in Figure 16b.

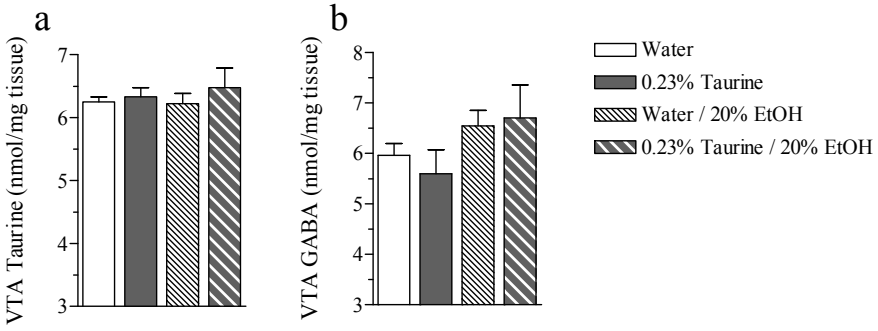

Figure 16. (a) Mean VTA Taurine (nmol/mg tissue) (+*SEM*). *(b)* Mean VTA GABA (nmol/mg tissue) (+*SEM*).

Two-way ANOVA comparing the effects of taurine treatment and ethanol exposure revealed no significant main effects of taurine treatment, $F(1,26) = .05$, $p = .822$, $\omega^2 = .17\%$, ethanol exposure, $F(1,26) = 3.39$, $p = .077$, $\omega^2 = 11.41\%$, or the taurine treatment by ethanol interaction, $F(1,26) = .33$, $p = .571$, $\omega^2 = 1.11\%$.

CHAPTER FOUR

Discussion

Ethanol Self- Administration

Ethanol self-administration has long been understood to measure the reinforcing properties of ethanol (Myers, Veale, & Yaksh, 1972), specifically consumption in a free-access test is indicative of the rewarding properties of a substance (Green & Grahame, 2008). The current project used two different behavioral tests to assess the efficacy of taurine treatment in reducing the reinforcing properties of ethanol, measured primarily by consumption of an ethanol solution. In behavioral testing, while the key variable being measured (ethanol consumption g/kg) may be the same between two tests, seemingly small procedural changes can significantly alter the validity of a test.

Two-Bottle Choice

The 2BC test is a well-established behavioral test, where mice are given two bottles one containing an ethanol solution and the other containing water. Consumption from both bottles is then measured over several days to assess their relative preference for ethanol over water (Belknap, Crabbe, & Young, 1993; Metten et al., 1998). Experiment 1 of the current study used the 2BC method to test the effectiveness of taurine treatment in reducing ethanol self-administration in adolescent mice. The results of this experiment were mixed as taurine supplementation did not significantly change ethanol consumption (g/kg) (Figure 1) but reduced ethanol preference (Figure 2). While unexpected, it is possible for one measure (consumption or preference) to change independently of the other (Simms et al., 2008). Both groups (taurine-treated and water-treated) drank similar

amounts of ethanol, therefore the decrease in ethanol preference derives from the taurine-treated group drinking more of the non-ethanol-containing solution than the water-treated group. C57BL/6 mice, when given unlimited access to both an ethanol solution and water, will drink more ethanol than water (Dole & Gentry, 1984), leading to a decrease in water consumption, as seen in the current study. The addition of taurine to drinking water does not alter liquid consumption compared to water alone, evidenced by consistent liquid consumption between animals drinking either water or taurine solution in the absence of an ethanol solution (Table 1). This suggests that increased consumption of the non-ethanol containing solution in the taurine-treated group does not reflect treatment-induced changes in liquid taste or intake needs. While the reduction in ethanol preference in the taurine-treated group was encouraging, further investigation attempted to understand why the 2BC test produced such inconclusive results. This uncovered several potential problems with the test that, while not problematic in some testing situations, may have reduced our ability to detect treatment effects.

Two- Bottle Choice vs. Drinking in the Dark

The first problem with the 2BC method is that the mice had unlimited access to both the ethanol solution and non-ethanol containing solution (water or taurine solution). While C57BL/6J mice voluntarily drink ethanol, their drinking is sporadic throughout a 24 hour period (Dole & Gentry, 1984). Specifically, adolescents consume ethanol in discrete bouts throughout the dark period of the light / dark cycle, typically lasting no more than 12 minutes at a time, and occurring for a total of approximately one hour per night (Bell et al., 2006). This sporadic consumption may lead to periods of intoxication, that would likely be short-lasting and unlikely to produce significant BECs or behavioral

intoxication (Bell et al., 2006). Mice also limit ethanol drinking to amounts that can be readily metabolized, possibly in an attempt to avoid reaching intoxicating BECs (Murphy et al., 1986). If consumption of ethanol is rewarding due to its activation of the mesolimbic DA system (Koob, 2009; Koob & Volkow, 2010), then the intermittent nature of ethanol consumption in the 2BC, combined with the unlikelihood of the animal drinking to behavioral intoxication, complicate the interpretation of the motivation behind ethanol consumption in this paradigm.

The second problem with the 2BC test (Experiment 1) is the addition of saccharin to the ethanol solution. Adding sweetener to an ethanol solution increases the rewarding properties of the solution, above that of ethanol alone (Heyman, 1997). Furthermore, the amount of ethanol consumed in a 2BC test is significantly increased when presented in a sweetened solution (Blednov et al., 2008; Linseman, 1989). These studies provided the rationale for the addition of sweetener to the ethanol solution in Experiment 1. However, while sweetening the solution increases the likelihood of ethanol consumption in the 2BC method, a problem arises when one considers that rodents find a sweet solution more rewarding than water alone (Heyman, 1997). Given the rodent's preference for sweetened substances the challenge of interpretation, in the current experiment, lies in whether ethanol consumption occurred because of the interoceptive cues / pharmacological properties of ethanol or because of the sweetener added to the solution. The combination of unlimited access and sweetened ethanol solution may have contributed to the ambiguous results of Experiment 1.

Throughout the past several decades different types of testing procedures have been designed to increase ethanol consumption, prolong the period of intoxication, and to

more closely mirror the effects of human drinking behavior (Grahame & Grose, 2003; Lê, Ko, Chow, & Quan, 1994; Middaugh, Kelley, Bandy, & McGroarty, 1999). One variation limits the availability of an ethanol solution, creating a 30 minute version of the 2BC test, in the home cage (Grahame & Grose, 2003). Restricting access to a period of 30-minutes increased ethanol consumption compared to unlimited access (Grahame & Grose, 2003), which greatly improves the likelihood of reaching behavioral intoxication. Another variant of the procedure was developed wherein mice are transferred from their home cages to a wire-mesh cage for one hour per day and given access to two bottles, one with water the other with ethanol escalating in strength over days (Lê et al., 1994). This procedure also increases ethanol consumption above that of an unlimited access testing procedure, illustrating that making a change, as seemingly simple as limiting access to ethanol, can alter the an animal's behavior. Following this work, Middaugh et al. (1999) designed a procedure where C57BL/6 mice drink significantly more ethanol in a 30 minute period than in other studies. This procedure requires a 35 day acclimation period, significant food restriction (to 80% of initial body weight), and periodic water deprivation (Middaugh et al., 1999), making testing a difficult and lengthy process. Operant conditioning paradigms have also been used to test ethanol self-administration in rodents. This procedure results in behavioral intoxication and offers high face validity (Cunningham, Fidler, & Hill, 2000) in that animals perform a behavior, in order to obtain access to ethanol. This more closely mirrors human drinking, wherein a person is typically required to do something to acquire ethanol. Although the operant conditioning paradigm effectively induces behavioral intoxication and mirrors human drinking behavior, the procedure requires significant training and implementation can be

complicated (Roberts, Heyser, & Koob, 1999). Given the complications associated with the discussed behavioral tests and in light of the findings that limiting ethanol access increased consumption and induced behavioral intoxication Rhodes and colleagues (2005) developed a new test.

Drinking in the Dark Benefits

The new method, DID, also known as 'drinking to intoxication' restricts ethanol access to early in the dark phase of the light / dark cycle (Rhodes, Best, Belknap, Finn, & Crabbe, 2005; Rhodes et al., 2007) and increases ethanol consumption compared to unlimited access procedures (Rhodes et al., 2005). The test also results in significant BECs and behavioral intoxication, evidenced by impaired performance on the rotarod and balance beam (Crabbe et al., 2009). The efficacy of this test stems primarily from the timing of the limited ethanol access. Mice, being nocturnal animals, eat and drink primarily during the dark phase of their light / dark cycle. The highest level of food intake in mice occurs during the first four hours of the dark period (Bell et al., 2006; Tabarin et al., 2007). Additionally, Dole and Gentry (1984) found that most ethanol consumption also occurs during the dark phase of the light / dark cycle. These data combine to highlight the dark phase as the ideal time for granting access to ethanol in a limited-access paradigm.

A criticism of the DID method questions the motivation behind ethanol consumption in this paradigm. By limiting ethanol access to the dark phase, a time of increased consumption for dietary needs and caloric intake, ethanol consumption during this period may reflect increased caloric need, and not the reinforcing properties of ethanol. However, as shown in a thorough study by Lyons et al. (2008) limiting the

availability of food around the period of DID testing does not significantly change ethanol consumption (Lyons, Lowery, Sparta, & Thiele, 2008). An injection of a known effective dose, of ghrelin, a peptide that stimulates food intake, fails to increase ethanol consumption; similarly an injection of leptin, a protein that attenuates feeding, fails to decrease ethanol consumption during this period (Lyons et al., 2008). This suggests that the high level of ethanol consumption during the DID procedure occurs independently of caloric need, as modulating factors that affect caloric intake does not change results. Furthermore, during this time period, consumption of saccharin, a sweet non-caloric substance, tends to increase (Lyons et al., 2008). This suggests that the time period during which DID testing occurs is a period of increased consumption of rewarding substances, independent of caloric need (Lyons et al., 2008).

The DID test may not be 'better' than other tests, but it is useful in the development of medication, as it produces results with predictive validity. Naltrexone, a drug known to reduce ethanol craving (Czachowski & Delory, 2009), decreases ethanol consumption when tested with the DID procedure as did acamprosate (Kamdar et al., 2007). Thus the DID protocol is ideal for testing the efficacy of drugs for reducing ethanol consumption (Rhodes et al., 2007).

DID Testing During Adolescence – Effects of Taurine Supplementation

Employing the DID procedure, with the aforementioned benefits it provides, resulted in marked improvements over the 2BC test. The taurine-treated animals drank 20% less ethanol than the water-treated group during the test period on the final day of the DID procedure (Figure 4). This supports the hypothesis that taurine supplementation does, in fact, reduce ethanol consumption. Given that a taurine derivative, acamprosate

reduces ethanol consumption in humans, adolescents (Niederhofer & Staffen, 2003) and adults (Whitworth et al., 1996), as well as rats (Rimondini et al., 2002) and mice (Gupta et al., 2008) it seems logical that treatment with taurine would produce similar results. Interestingly, the 20% reduction in ethanol consumption induced by taurine-treatment in the current study were remarkably consistent with the results of acamprosate treatment in the same strain of mice, which also lead to a 20% reduction in consumption using the DID procedure (Gupta et al., 2008). As a result of decreased ethanol drinking, understandably, BECs were significantly lower in the taurine-treated group than the water-treated group (Figure 5). Taurine treatment was consistent between the 2BC and DID experiments, thus treatment effects found using the DID paradigm (Experiment 2) likely reflect the established benefits of the test. Ethanol consumption and BECs for the water-treated group in the DID test were similar to those reported for adolescent C57BL/6J mice in the literature (Moore, Mariani, Linsenbardt, Melon, & Boehm, 2010), corroborating the efficacy of the testing procedure.

Sucrose Study – Experiment 2a. Sucrose replaced ethanol in the second part of the DID test in adolescents (Experiment 2). This enabled the differentiation between a taurine-induced reduction in consumption of other rewarding substances (e.g. sweetened water), or if the effect was ethanol-specific. Sucrose was chosen instead of saccharin because saccharin is a non-caloric substance and the caloric content of sucrose more closely matches that of ethanol. The adolescent mice treated with taurine drank significantly more of the sucrose solution than the water-treated animals on the test day of the DID procedure (Figure 10). Although this was an unexpected result, it is not without precedent as C57BL/6J mice, treated with low doses of acamprosate show trends

for increasing consumption of sugar water (Gupta et al., 2008). Interestingly, other studies have also investigated the interaction of taurine and diabetes. In isolated pancreatic cells from hamsters, chronic taurine administration induced hypoglycemia (L'Amoreaux et al., 2010) and in rats fed a high fat diet, taurine supplementation improves blood lipid profiles compared to rats without added taurine (Du et al., 2010). These data combine to suggest that taurine may, in some way, facilitate the processing of glucose. This may explain the increase in sucrose consumption by the taurine-treated mice in Experiment 2, as they may have processed the glucose faster than the water-treated group, enabling them to drink more sweetened water without significantly increasing blood glucose levels. This result is important as it shows that the taurine-induced decrease in ethanol consumption is specific to ethanol, as treatment did not decrease consumption of another rewarding substance.

DID Testing During Adulthood – Effects of Taurine Supplementation

In the third experiment of this study, the effects of taurine treatment were investigated in adults using the DID procedure. The adult study was conducted to examine the efficacy of taurine supplementation in reducing ethanol consumption in an adult population. In Experiment 3, both adult ethanol-exposed groups (taurine-treated and water-treated) drank similar amounts of ethanol (g/kg) during the test period of the DID procedure (Figure 11). It is uncertain why taurine supplementation reduced ethanol consumption in adolescents but not adults. A potential explanation for this difference may be a decrease in endogenous taurine levels that occurs with age (Benedetti et al., 1991). The water-treated adolescent mice (Experiment 2) had slightly higher endogenous tissue taurine concentrations in both the NAc and VTA than water-treated adult mice

(Experiment 3). Taurine supplementation produced a significant increase in tissue concentrations, in both the NAc and VTA, of the taurine-treated/non-ethanol-exposed group of adolescents compared to their water-treated counterparts (Figure 3). This change was not observed in the adult taurine-treated/non-ethanol-exposed group and the adult water-treated/non-ethanol-exposed group (Figure 16a). The lack of a significant difference in tissue taurine levels between the adult taurine-treated/ethanol-exposed group and the adult water-treated/ethanol-exposed group in either the NAc or VTA, may account for the lack of behavioral changes seen in Experiment 3. Given that taurine treatment did not significantly change ethanol consumption, BECs were not significantly different between the taurine-treated and water-treated adults (Figure 14). Surprisingly, although the adolescent ethanol consumption in the water-treated/ethanol-exposed (Experiment 2) was consistent with that reported in the literature, the adult water-treated/ethanol-exposed mice (Experiment 3) drank less ethanol (Mean = 3.78 g/kg) than previously reported for the DID procedure in the same strain of mice (Mean = 6.25 g/kg) (Rhodes et al., 2007). Small changes in rearing, handling, or cage position, introduce variations that can cause differences in behavior within a genetically homogenous sample such as the C57BL/6J strain (Rhodes et al., 2005). The ability of small changes to influence behavioral outcome in the DID test may explain the differences in consumption seen between the current experiment and the data reported in the literature. Although not the focus of the current study, it is important to note that the adolescent mice drank more ethanol than the adults during the testing period of the DID procedure (Figures 4 & 11 respectively). This result confirms findings of other studies that show increased ethanol

consumption during adolescence compared to adults (Moore et al., 2010; Vetter et al., 2007) and was therefore an expected result.

Brain Tissue Analysis

Using an ethanol self-administration paradigm, the current study investigated the efficacy of taurine supplementation on reducing the rewarding properties of ethanol. Behavioral data illustrated a treatment-associated decrease in drinking behavior, additional data collected from mesolimbic tissues attempted to identify any corresponding changes to the DA system.

Thus, after behavioral testing, the NAc and VTA were extracted and analyzed for monoamine and amino acid levels. These tissues were chosen because of their integral roles in the reward pathway. Monoamine analysis examined the effects of treatment (taurine/water and ethanol/no ethanol) on levels of DA, and its metabolites (HVA and DOPAC). In the analysis of tissue extracts, the calculations of monoamine turnover provides a reasonable estimate of usage (Molinoff & Axelrod, 1971), rather than monoamine levels alone (Bacopoulos et al., 1979). Amino acid analysis examined the effects of treatment on tissue taurine and GABA levels. Exploration of these results will begin with the NAc, as it is primarily considered the 'pleasure center' of the brain and then examine the VTA, the origin of the pathway. Importantly, this collected data intended to provide some general explanation for the observed behavioral effects, while not attempting to characterize a specific mechanism of action.

Nucleus Accumbens

Dopamine. Ethanol intake, the focus of the current investigation, promotes the release of DA in the NAc, an effect often associated with drugs of abuse including amphetamine, cocaine, opiates, and nicotine (Di Chiara & Imperato, 1988). In adolescent water-treated groups (Experiment 2), ethanol-exposed animals showed significantly lower NAc DA than their non-ethanol-exposed counterpart (Figure 6a). This decline in tissue DA content agrees, in part, with reported increases in extracellular NAc DA after ethanol exposure (Doyon, Anders, Ramachandra, Czachowski, & Gonzales, 2005). While DA levels alone suggest some degree of output, measures of turnover provide a better representation of usage. Ethanol self-administration typically increases NAc DA levels, an effect corroborated in the current study as reductions in DA levels were accompanied by increased DA turnover (HVA/DA). The water-treated/ethanol-exposed group had significantly higher NAc HVA/DA ratios than their non-ethanol-exposed counterpart (Figure 6b). Ethanol exposure (Gil-Martín, Colado, Fernández-López, Fernández-Briera, & Calvo, 1996) and other reinforcers such as food and electrical stimulation of the VTA (Fiorino, Coury, Fibiger, & Phillips, 1993; Joseph & Hodges, 1990) increase NAc HVA levels and HVA/DA ratios (Martel & Fantino, 1996). Between water-treated groups, ethanol exposure increased NAc DA turnover (HVA/DA), representing an elevation in DA use that likely corresponds to ethanol-related reinforcement. Interestingly, between the two ethanol-exposed groups, those treated with taurine showed significantly less NAc DA turnover (HVA/DA) than those treated with water. This reduction in NAc HVA/DA ratios, in animals treated with taurine, indicates a decrease in DA usage that may reflect a reduction in the reward associated with ethanol intake. Despite significant ethanol-related

changes in NAc DA levels and HVA/DA ratios, the ratio of DOPAC/DA was not significantly altered by either ethanol exposure or taurine treatment (Figure 6c). Turnover of one DA metabolite can occur without altering turnover of the other metabolite (Gil-Martín et al., 1996) or DA levels (Birgner, Kindlundh-Högberg, Nyberg, & Bergström, 2007), so the effect seen in this study appears to be consistent with other published reports.

In contrast to adolescent groups, adult animals (Experiment 3) consumed less ethanol and did not show any taurine-related effects on overall ethanol intake (Figure 11). Accordingly, NAc DA levels did not significantly differ across all adult treatment groups (Figure 13a). However, ethanol exposure did promote a significant increase in NAc DA turnover (HVA/DA) in adult animals treated with taurine, compared to the water-treated/non-ethanol exposed group (Figure 13b). A similar ethanol-related increase in HVA/DA was also seen in adolescents treated with taurine (Experiment 2), which remained significantly less than the water-treated/ethanol exposed group (Figure 6b). Exposure to ethanol increases DA turnover (Gil-Martín et al., 1996). This established effect may then explain the higher level of DA turnover (HVA/DA) in the adult taurine-treated/ethanol exposed compared to the water-treated/non-ethanol exposed group. Given that the adult ethanol exposed groups did not differ in ethanol consumption or DA turnover, the similarities in HVA/DA ratio are understandable. However, an exact cause for the disparate pattern of DA turnover compared to adolescents remains unclear. Additionally, an overall effect of ethanol exposure caused an increase in NAc DA turnover, in terms of DOPAC/DA ratios, within both ethanol-exposed adult groups (Figure 13c). Partial explanation of this main effect of ethanol may lie in the higher

ethanol-induced DA turnover within the NAc of adults, compared to adolescents (Philpot & Kirstein, 2004). Another potential explanation may stem from the rapid, non rate-limited metabolism of DOPAC into HVA (Siegel et al., 1999), this fast breakdown may contribute to the overall similarities in DOPAC levels.

Amino acids. In adolescent groups not exposed to ethanol, taurine treatment resulted in significantly higher taurine concentrations within the NAc (Figure 7a). While these taurine-treated animals showed an increase in NAc taurine, compared to the water-treated group, these levels did not differ from the taurine-treated/ethanol exposed group. In general, this finding simply represents the efficacy of exogenous treatment to increase taurine levels in discrete brain regions (NAc), in congruence with the established literature (Yang et al., 2009). Taurine-treated/ethanol exposed animals showed significantly higher NAc GABA levels, compared to the water-treated/non-ethanol exposed group, but this increase was not different from the corresponding water-treated/ethanol exposed group (Figure 7b). Given that these ethanol groups did not differ in NAc GABA concentrations, it seems possible that elevated NAc GABA resulted from ethanol-mediated effects. In support of this, other investigators report that repeated injections of ethanol does, in fact, increase GABA levels within the NAc (Kapasova & Szumlinski, 2008; Szumlinski et al., 2007). Additionally, the slight increase in NAc GABA levels, within the taurine-treated/ethanol exposed group, may have resulted from a taurine-related enhancement of brain GABA levels. Accordingly, mice fed with taurine in drinking water (0.05% for 4 weeks) show significantly elevated GABA levels in the brain (El Idrissi & Trenkner, 2004).

In ethanol exposed adults (Experiment 3), water-treated animals showed significantly lower taurine levels than those treated with taurine (Figure 14a). Although ethanol typically stimulates the release of taurine (Yang, Chen, Chiu, & Huang, 2006), increased taurine availability, in taurine-treated animals, may have preserved tissue concentrations in the face of ethanol-induced release. In adult groups, treatment did not significantly change GABA levels (Figure 14b), however a slight increase occurred in the taurine-treated/ethanol exposed group, similar to the effects on taurine levels.

Ventral Tegmental Area

Dopamine. The VTA functions to mediate, at least partly, the rewarding effects of nicotine, opiates, and ethanol (Ikemoto, 2007). In adolescents not exposed to ethanol (Experiment 2), taurine treatment caused a significant increase in VTA DA levels, compared to the water-treated group (Figure 8a). This increase in VTA DA may be attributed to taurine-mediated regulation of dopaminergic output from the VTA, given that taurine locally infused into the VTA modulates DA levels in the striatum (Routsalainen, Majasaari, Salimaki, & Ahtee, 1998). Similarly, in adolescents exposed to ethanol, taurine-treated mice revealed significantly higher DA levels than water-treated counterparts (Figure 8a). This may once again represent taurine-mediated regulation of dopaminergic output that could, in turn, alter the reinforcing actions of ethanol. Considering that rewarding substances, such as ethanol activate the mesolimbic pathway by stimulating DA neurons in the VTA (Brodie, Shefner, & Dunwiddie, 1990) and facilitating DA release in the NAc (Fiorino et al., 1993), taurine may potentially attenuate ethanol-induced activation of DA neurons. This dampening of VTA DA neuron activation may reduce the associated release of DA in the NAc, thereby, lowering the

rewarding effects of ethanol. In further support of this, no significant changes occurred in VTA DA turnover, HVA/DA (Figure 8b) or DOPAC/DA (Figure 8c), suggesting that VTA DA declines resulted from DA-related movement from the VTA and not local use. This could provide some explanation for the reduction in ethanol consumption in the taurine-treated/ethanol exposed group, compared to the water-treated/ethanol exposed group.

In adult groups (Experiment 3), treatment did not significantly alter DA levels (Figure 15a) or turnover (HVA/DA) in the VTA (Figure 15b). Similar to findings in the NAc, these VTA measures likely reflect the lack of ethanol consumption and / or treatment differences. However, in adults not treated with taurine, ethanol exposed animals showed increased DA turnover (DOPAC/DA) (Figure 15c), compared to their non-ethanol exposed counterparts. This likely reflects an ethanol-induced increase in DA usage (Beart & Gundlach, 1980) that potentially involves a positive feedback loop related to ethanol-mediated effects within the VTA (Xiao et al., 2009). Since the ethanol exposed adult groups were not different from each other this effect does not appear to be mediated by taurine.

Amino acids. In the non-ethanol exposed adolescents, taurine-treatment significantly increased VTA taurine concentrations (Figure 9a), an effect similarly found in the NAc. Taurine concentrations were increased in the taurine-treated/non-ethanol exposed group compared to its water-treated counterpart; however, the taurine-treated groups did not differ from each other. Once again, this illustrates the efficacy of taurine supplementation to increase taurine concentrations in discrete brain tissues, in this case the VTA. In adolescents (Experiment 2), GABA levels in the VTA were not affected by

treatment (Figure 9b). Although adolescent ethanol-exposed mice showed an increase in NAc GABA levels, the same did not hold true in the VTA. This may represent a tissue-specific response to ethanol and is in accordance with the finding that systemic ethanol exposure does not increase VTA GABA levels (Yan, Zheng, Feng, & Yan, 2005). In contrast to adolescents, treatment did not change VTA taurine levels (Figure 16a). The cause for this is unclear, but given that ethanol exposure induces the release of taurine from the NAc (Yang et al., 2006) as seen in Figure 14a, the lack of effect in the VTA may represent a tissue specific effect. GABA levels were unchanged by treatment in adults (Figure 16b) which is consistent with the adolescent data.

Conclusion

The primary finding from these studies is that taurine supplementation reduced ethanol consumption and, by extension, the reinforcing properties of ethanol in adolescent mice. Using the DID method, taurine supplementation produced a 20% reduction in ethanol consumption compared to the water-treated group (Figure 4). This finding was supported by changes in DA and amino acid levels within the NAc and VTA. In the ethanol-exposed adolescent groups (Experiment 2), taurine treatment mitigated the elevations in NAc DA turnover (HVA/DA) caused by ethanol (Figure 6b). However, in the VTA of the same animals, the pattern is reversed: the water-treated group had less VTA DA (Figure 8a). The lower levels of VTA DA in the water-treated/ethanol exposed group likely indicate the release of DA from the VTA to the NAc, where DA output is increased. Together, these findings illustrate DA-related changes characteristic of mesolimbic reinforcement responses, particularly in light of the NAc HVA/DA ratio in the water-treated/ethanol exposed group compared to their taurine-treated counterparts.

The reciprocal nature of DA and turnover (HVA/DA) in the VTA and NAc of the water-treated/ethanol exposed adolescents exemplifies the changes one would expect to see in response to a reinforcing substance. It follows then, that the water-treated adolescents consumed more ethanol than their taurine-treated counterparts. Taken together, the tissue concentrations of DA and turnover (HVA/DA) in the VTA and NAc, supports the assertion that the water-treated/ethanol exposed adolescent group showed a greater reinforcement response to ethanol than taurine-treated counterparts. The reduction of the NAc HVA/DA ratio in the taurine-treated/ethanol-exposed group and the corresponding decrease in ethanol consumption provide compelling evidence for a taurine-associated reduction in ethanol-related reinforcement.

Another important finding of this study is that taurine supplementation successfully increased NAc and VTA taurine concentrations in adolescent mice, (Experiment 2). In both tissues, the taurine-treated/non-ethanol exposed adolescent mice had significantly higher taurine levels than their water-treated counterparts (Figure 3). In adult mice (Experiment 3), tissue taurine concentrations were not significantly higher, but did trend slightly higher in the NAc and VTA of the taurine-treated/non-ethanol exposed group compared to the water-treated/non-ethanol exposed group (Figures 14a and 16a respectively). These increases in taurine concentrations illustrate the efficacy of supplementing dietary taurine intake by simply adding it to the drinking water, a non-invasive route of administration.

One potentially limiting factor should be noted. Both the NAc and VTA can be subdivided into different parts. The NAc is comprised of two parts, the core and shell. Of these parts, the shell appears more responsive to drugs of reward than the core (Ikemoto,

2007), as ethanol-induces greater DA release in the shell than core following i.v. administration of ethanol (Howard et al., 2008). The two components of the VTA are the anterior and posterior portions (Ikemoto, 2007). As in the NAc, the different regions of the VTA respond differently to ethanol. The posterior portion is more sensitive to rewarding stimuli than the anterior portion (Ikemoto, 2007) as rats will self-administer ethanol into the posterior but not anterior portion of the VTA (Rodd-Henricks, McKinzie, Crile, Murphy, & McBride, 2000). In the current investigation the tissues were extracted as a whole tissue, and the different portions were not analyzed separately. This may have influenced the results as the changes in either the intra or extracellular concentrations may have been overshadowed when the tissue was analyzed as a whole (Broersen et al., 2000).

Ethanol consumption in the DID procedure shifted significantly following taurine treatment in the adolescent mice. Animals treated with taurine displayed a 20% reduction in ethanol intake. This change corresponded with decreased NAc DA turnover. DA turnover in this tissue increases in response to rewarding stimuli; therefore the decrease seen in the taurine-treated group likely indicates a reduction in the rewarding properties of ethanol. Taurine treatment did not reduce consumption of a sucrose solution, indicating that this effect is ethanol-specific. This is important, as an effective treatment for reducing ethanol consumption would ideally change just the desired behavior and not reduce the rewarding properties of other substances. This investigation illustrates the efficacy of taurine supplementation in reducing the reinforcing properties of ethanol in adolescent mice. These findings implicate the potential use of taurine in combating ethanol abuse in adolescents. The apparent capacity of taurine to curb the reinforcement

of ethanol in the adolescent population may also help reduce the probability of abuse later in life.

LITERATURE CITED

Aerts, L., & Van Assche, F. A. (2002). Taurine and taurine-deficiency in the perinatal period. *Journal of Perinatal Medicine, 30*, 281-286.

Andersen, S. L. (2003). Trajectories of brain development: point of vulnerability or window of opportunity? *Neuroscience and Biobehavioral Reviews, 27*, 3-18.

Bacopoulos, N. G., Hattox, S. E., & Roth, R. H. (1979). 3,4-Dihydroxyphenylacetic acid and homovanillic acid in rat plasma: Possible indicators of central dopaminergic activity. *European Journal of Pharmacology, 56*, 225-236.

Badanich, K. A., Maldonado, A. M., & Kirstein, C. L. (2007). Chronic ethanol exposure during adolescence increases basal dopamine in the nucleus accumbens septi during adulthood. *Alcoholism: Clinical and Experimental Research, 31*, 895-900.

Banay-Schwartz, M., DeGuzman, T., Lajtha, A., & Plakovits, M. (1996). Amino Acid distribution in immature rat brain. *Neurobiology, 4*, 393-403.

Beart, P. M., & Gundlach, A. L. (1980). 3,-4 Dihydroxyphenylacetic acid (DOPAC) and the rat meso-limbic dopaminergic pathway: Drug effects and evidence for somatodendritic mechanisms. *British Journal of Pharmacology, 69*, 241-247.

Belknap, J. K., Crabbe, J. C., & Young, E. R. (1993). Voluntary consumption of ethanol in 15 inbred mouse strains. *Psychopharmacology, 112*, 503-510.

Bell, R. L., Rodd, Z. A., Sable, H. J., Schultz, J. A., Hsu, C. C., Lumeng, L. (2006). Daily patterns of ethanol drinking in peri-adolescent and adult alcohol-preferring (P) rats. *Pharmacology Biochemistry and Behavior, 83*(1), 35-46.

Benedetti, M. S., Russo, A., Marrari, P., & Dostert, P. (1991). Effects of ageing on the content in sulfur-containing amino acids in rat brain. *Journal of Neural Transmission, 86*, 191-203.

Birgner, C., Kindlundh-Högberg, A. M. S., Nyberg, F., & Bergström, L. (2007). Altered extracellular levels of DOPAC and HVA in the rat nucleus accumbens shell in response to sub-chronic nandrolone administration and a subsequent amphetamine challenge. *Neuroscience Letters, 412*, 168-172.

Blednov, Y. A., Walker, D., Martinez, M., Levine, M., Damak, S., & Margolskee, R. F. (2008). Perception of sweet taste is important for voluntary alcohol consumption in mice. *Genes, Brain, and Behavior, 7*, 1-13.

Bolanos, C., A., Glatt, S., J., & Jackson, D. (1998). Subsensitivity to dopaminergic drugs in periadolescent rats: a behavioral and neurochemical analysis. *Developmental Brain Research, 111*, 25-33.

Brasser, S. M., & Spear, N. E. (2002). Physiological and behavioral effects of acute ethanol hangover in juvenile, adolescent, and adult rats. *Behavioral Neuroscience, 116*(2), 305-320.

Brodie, M. S., & Appel, S. B. (1998). The effects of ethanol on dopaminergic neurons of the ventral tegmental area studies with intracellular recording in brain slices. *Alcoholism: Clinical and Experimental Research, 22*, 236-244.

Brodie, M. S., Pesold, C., & Appel, S. B. (1999). Ethanol directly excites dopaminergic ventral tegmental area reward neurons. *Alcoholism: Clinical and Experimental Research, 23*, 1848-1852.

Brodie, M. S., Shefner, S. A., & Dunwiddie, T. V. (1990). Ethanol increases the firing rate of dopamine neurons of the rat ventral tegmental area in vitro. *Brain Research, 508*(1), 65-69.

Broersen, L. M., Abbate, F., Feenstra, M. G. P., de Bruin, J., P. C., Heinsbroek, R. P. W., & Olivier, B. (2000). Prefrontal dopamine is directly involved in the anxiogenic interoceptive cue of pentylenetetrazol but not in the interoceptive cute of chlordiazepoxide in the rat. *Psychopharmacology, 149*, 366-376.

Caldwell, L. C., Schweinsburg, A. D., Nagel, B. J., Bartlett, V. C., Brown, S. A., & Tapert, S. F. (2005). Gender and adolescent alcohol use disorders on BOLD (blood oxygen level dependent) response to spatial working memory. *Alcohol & Alcoholism, 40*, 194-200.

Carroll, M. R., Rodd, Z. A., Murphy, J. M., & Simon, J. R. (2006). Chronic ethanol consumption increases dopamine uptake in the nucleus accumbens of high alcohol drinking rats. *Alcohol, 40*, 103-109.

Chassin, L., Pitts, S., C., & Prost, J. (2002). Binge drinking trajectories from adolescence to emerging adulthood in a high-risk sample: predictors and substance abuse outcomes. *Journal of Consulting and Clinical Psychology, 70*(1), 67-78.

Chen, W. Q., Jin, H., Nguyen, M., Carr, J., Lee, Y. J., Hsu, C. C. (2001). Role of taurine in regulation of intracellular calcium level and neuro-protective function in cultured neurons. *Journal of Neuroscience Research, 66*, 612-619.

Chen, X. C., Pan, Z. L., Liu, D. S., & Han, X. (1998). Effect of taurine on human fetal neuron cells: Proliferation and differentiation. *Advances in Experimental Medicine and Biology, 442*, 397-403.

Chesney, R. W., Helms, R. A., Christensen, M., Burdreau, A. M., Han, X., & Sturman, J. A. (1998). The role of taurine in infant nutrition. *Advances in Experimental Medicine and Biology, 442*, 463-476.

Colle, L. M., & Wise, R. A. (1987). Opposite effects of unilateral forebrain ablations on ipsilateral and contralateral hypothalamic self-stimulation. *Brain Research, 407*, 285-293.

Corbett, D., & Wise, R. A. (1979). Intracranial self-stimulation in relation to the ascending noradrenergic fiber systems of the pontine tegmentum and caudal midbrain: a moveable electrode mapping. *Brain Research, 177*, 423-426.

Corbett, D., & Wise, R. A. (1980). Intracranial self-stimulation in relation to the ascending dopaminergic systems of the midbrain: a moveable electrode mapping. *Brain Research, 185*, 1-15.

Cowen, M. S., Adams, C., Kraehenbuehl, T., Vengeliene, V., & Lawrence, A. J. (2005). The acute anti-craving effect of acamprosate in alcohol-preferring rats is associated with modulation of the mesolimbic dopamine system. *Addiction Biology, 10*, 233-242.

Crabbe, J. C., Metten, P., Rhodes, J. S., Yu, C.-H., Brown, L. L., Phillips, T. J. (2009). A line of mice selected for high blood ethanol concentrations shows drinking in the dark to intoxication. *Biological Psychiatry, 65*, 662-670.

Crawley, J. N. (2000). *What's Wrong With My Mouse? Behavioral Phenotyping of Transgenic and Knockout Mice*. New York: Wiley-Liss.

Crews, F. T., Mdzinarishvili, A., Kim, D., He, J., & Nixon, K. (2006). Neurogenesis in adolescent brain is potently inhibited by ethanol. *Journal of Neuroscience, 137*, 437-445.

Cunningham, C. L., Fidler, T. L., & Hill, K. G. (2000). Animal models of alcohol's motivational effects. *Alcohol Research & Health: The Journal Of The National Institute On Alcohol Abuse And Alcoholism, 24*, 85-92.

Czachowski, C. L., & Delory, M. J. (2009). Acamprosate and naltrexone treatment effects on ethanol and sucrose seeking and intake in ethanol-dependent and nondependent rats. *Psychopharmacology, 204*, 335-348.

Dachour, A., Quertemont, E., & De Witte, P. (1996). Taurine increases in the nucleus accumbens microdialysate after acute ethanol administration to naive and chronically alcoholised rats. *Brain Research, 735*, 9-19.

Dawson, R., Liu, S., Eppler, B., & Patterson, T. (1999). Effects of dietary taurine supplementation or deprivation in aged male Fischer 344 rats. *Mechanisms of Ageing and Development, 107*, 73-91.

Dawson, R., Pelleymounter, M. A., Cullen, M. J., Gollub, M., & Liu, S. (1999). An age-related decline in striatal taurine is correlated with a loss of dopaminergic markers. *Brain Research Bulletin, 48*(3), 319-324.

De Bellis, M. D., Clark, D. B., Beers, S. R., Soloff, P. H., Boring, A. M., Hall, J. (2000). Hippocampal volume in adolescent-onset alcohol use disorders. *American Journal of Psychiatry, 157*, 737-744.

del Olmo, N., Bustamante, J., del Rio, R. F., & Solio, J. M. (2000). Taurine activates $GABA_A$ but not $GABA_B$ receptors in rat hippocampal CA1 area. *Brain Research, 864*, 298-307.

Di Chiara, G., & Imperato, A. (1988). Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proceedings of the National Academy of Sciences of the United States of America, 85*, 5274-5278.

Dole, V. P., & Gentry, R. T. (1984). Toward an analogue of alcoholism in mice: Scale factors in the model. *Proceedings of the National Academy of Sciences of the United States of America, 81*, 3543-3546.

Doremus-Fitzwater, T. L., Varlinskaya, E. I., & Spear, L. P. (2010). Motivational systems in adolescence: Possible implications for age differences in substance abuse and other risk-taking behaviors. *Brain and Cognition, 72*(1), 114-123.

Doremus, T. L., Brunell, S. C., Varlinskaya, E. I., & Spear, L. P. (2003). Anxiogenic effects during withdrawal from acute ethanol in adolescent and adult rats. *Pharmacology Biochemistry and Behavior, 75*(2), 411-418.

Doremus, T. L., Varlinskaya, E. I., & Spear, L. P. (2004). Age-related differences in elevated plus maze behavior between adolescent and adult rats. *Annals of the New York Academy of Sciences, 1021*, 427-430.

Doyon, W. M., Anders, S. K., Ramachandra, V., Czachowski, C. L., & Gonzales, R. A. (2005). Effect of operant self-administration of 10% ethanol plus 10% sucrose on dopamine and ethanol concentrations in the nucleus accumbens. *Journal of Neurochemistry, 93*, 1469-1481.

Du, H., You, J.-S., Zhao, X., Park, J.-Y., Kim, S.-H., & Chang, K.-J. (2010). Antiobesity and hypolipidemic effects of lotus leaf hot water extract with taurine supplementation in rats fed a high fat diet. *Journal of Biomedical Science, 17*(Suppl 1), S42.

El Idrissi, A., Harris, C., & Trenkner, E. (1998). Taurine modulates glutamate- and growth factors- mediated signaling mechanisms. *Advances in Experimental Medicine and Biology, 442*, 385-396.

El Idrissi, A., & Trenkner, E. (1999). Growth factors and taurine protect against excitotoxicity by stabilizing calcium homeostasis and energy metabolism. *Journal of Neuroscience, 19*, 9459-9468.

El Idrissi, A., & Trenkner, E. (2004). Taurine as a modulator of excitatory and inhibitory neurotransmission. *Neurochemical Research, 29*(1), 189-197.

Farr, S. A., Scherrer, J. F., Banks, W. A., Flood, J. F., & Morley, J. E. (2005). Chronic ethanol consumption impairs learning and memory after cessation of ethanol. *Alcoholism: Clinical and Experimental Research, 29*, 971-982.

Fibiger, H. C., LePiane, F. G., Jakubovic, A., & Phillips, A. G. (1987). The role of dopamine in intracranial self-stimulation of the ventral tegmental area. *Journal of Neuroscience, 7*, 3888-3896.

Fiorino, D. F., Coury, A., Fibiger, H. C., & Phillips, A. G. (1993). Electrical stimulation of reward sites in the ventral tegmental area increases dopamine transmission in the nucleus accumbens of the rat. *Behavioural Brain Research, 55*, 131-141.

Flint, A. C., Liu, X., & Kriegstein, A. R. (1998). Nonsynaptic glycine receptor activation during early neocortical development. *Neuron, 20*, 43-53.

Franco, R., Quesada, O., & Pasantes-Morales, H. (2000). Efflux of osmolyte amino acids during isovolumic regulation in hippocampal slices. *Journal of Neuroscience, 61*, 701-711.

Franconi, F., Diana, G., Fortuna, A., Galietta, G., Trombetta, G., Valentini, G. (2004). Taurine administration during lactation modifies hippocampal CA1 neurotransmission and behavioural programming in adult male mice. *Brain Research Bulletin, 63*, 491-497.

Fritschy, J. M., Paysan, J., Enna, A., & Mohler, H. (1994). Switch in the expression of rat $GABA_A$-receptor subtypes during postnatal development: an immunohistochemical study. *Journal of Neuroscience, 14*(9), 5302-5324.

Garcia-Burgos, D., Gonzales, F., Manrique, T., & Gallo, M. (2009). Patterns of ethanol intake in preadolescent, adolescent, and adult wistar rats under acquisition, maintenance, and relapse-like conditions. *Alcoholism: Clinical and Experimental Research, 33*, 722-728.

Giedd, J. N., Blumenthal, J., Jeffries, N. O., Castellanos, F. X., Liu, H., Zijdenbos, A. (1999). Brain development during childhood and adolescence: a longitudinal MRI study. [Article]. *Nature Neuroscience, 2*(10), 861.

Giedd, J. N., Lalonde, F. M., Celano, M. J., White, S. L., Wallace, G. L., Lee, N. R. (2009). Anatomical brain magnetic resonance imaging of typically developing children and adolescents. *Journal of the American Academy of Child and Adolescent Psychiatry, 48*, 465-470.

Gil-Martín, E., Colado, I., Fernández-López, A., Fernández-Briera, A., & Calvo, P. (1996). Effects of an acute dose of ethanol on dopaminergic and serotonergic systems from rat cerebral cortex and striatum. *Comparative Biochemistry and Physiology Part C: Comparative Pharmacology and Toxicology, 113*, 399-402.

Gogtay, N., Giedd, J. N., Lusk, L., Hayashi, K. M., Greenstein, D., Vaituzis, A. C. (2004). Dynamic mapping of human cortical development during childhood through early adulthood. *Proceedings of the National Academy of Sciences of the United States of America, 101*, 8147-8179.

Grahame, N. J., & Grose, A. M. (2003). Blood alcohol concentrations after scheduled access in high-alcohol-preferring mice. *Alcohol, 31*, 99-104.

Green, A. S., & Grahame, N. J. (2008). Ethanol drinking in rodents: is free-choice drinking related to the reinforcing effects of ethanol? *Alcohol, 42*, 1-11.

Gregory, D. M., Sovetts, D., Clow, C. L., & Scriver, C. R. (1986). Plasma free amino acid values in normal children and adolescents. *Metabolism, 35*(1), 967-969.

Gupta, T., Syed, Y. M., Revis, A. A., Miller, S. A., Martinez, M., Cohn, K. A. (2008). Acute effects of acamprosate and MPEP on ethanol drinking-in-the-dark in male C57BL/6J mice. *Alcoholism: Clinical and Experimental Research, 32*, 1992-1998.

Han, N. L., Haddrill, J. L., & Lynch, J. W. (2001). Characterization of a glycine receptor domain that controls the binding and gating mechanisms of the beta-amino acid agonist, taurine. *Journal of Neurochemistry, 79*, 636-647.

Hayes, K. C., & Sturman, J. A. (1981). Taurine in metabolism. *Annual Review of Nutrition, 1*, 401-425.

Hendrickson, L. M., Zhao-Shea, R., & Tapper, A. R. (2009). Modulation of ethanol drinking-in-the-dark by mecamylamine and nicotinic acetylcholine receptor agonists in C57BL/6J mice. *Psychopharmacology, 204*, 563-572.

Heyman, G. M. (1997). Preference for saccharin-sweetened alcohol relative to isocaloric sucrose. *Psychopharmacology, 129*, 72-78.

Ho, A., Chin, A. J., & Dole, V. P. (1989). Early experience and the consumption of alcohol by adult C57BL/6J Mice. *Alcohol, 6*, 511-515.

Howard, E. C., Schier, C. J., Wetzel, J. S., Duvauchelle, C. L., & Gonzales, R. A. (2008). The shell of the nucleus accumbens has a higher dopamine response compared with the core after non-contingent intravenous ethanol administration. *Neuroscience, 154*, 1042-1053.

Hua, J. Y., & Smith, S. J. (2004). Neural activity and the dynamics of central nervous system development. *Nature Neuroscience, 7*(4), 327-332.

Hussy, N., Deleuze, C., Pantaloni, A., Desarmenien, M. G., & Moos, F. (1997). Agonist action of taurine on glycine receptors in rat supraoptic magnocellular neurones: possible role in osmoregulation. *Journal of Physiology, 502*(3), 609-621.

Huttenlocher, P. R., & de Courten, C. (1987). The development of synapses in striate cortex of man. *Human Neurobiology, 6*(1), 1-9.

Huxtable, R. J. (1989). Taurine in the central nervous system and the mammalian actions of taurine. *Progress in Neurobiology, 32*, 471-533.

Huxtable, R. J. (1992). Physiological actions of taurine. *Physiological Reviews, 72*, 101-163.

Huxtable, R. J. (1993). Taurine in nutrition and development. *Nutrizione, 1*, 1-7.

Ikemoto, S. (2007). Dopamine reward circuitry: Two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex. [doi: DOI: 10.1016/j.brainresrev.2007.05.004]. *Brain Research Reviews, 56*(1), 27-78.

Ikemoto, S., & Panksepp, J. (1999). The role of nucleus accumbens dopamine in motivated behavior: a unifying interpretation with special reference to reward-seeking. [doi: DOI: 10.1016/S0165-0173(99)00023-5]. *Brain Research Reviews, 31*(1), 6-41.

Improving the Health of Adolescents and Young Adults: A Guide for States and Communities. (2004). Atlanta, GA: Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Division of Adolescent and School Health, Health Resources and Services Administration, Maternal and Child Health Bureau, Office of Adolescent Health, National Adolescent Health Information Center, University of California, San Francisco.

Johnston, L. D., O'Malley, P. M., Bachman, J. G., & Schulenberg, J. E. (2008). Monitoring the Future national results on adolescent drug use: Overview of key findings, 2007 *(NIH Publication No. 08-6418)*. Bethesda, MD: National Institute on Drug Abuse.

Joseph, M. H., & Hodges, H. (1990). Lever pressing for food reward and changes in dopamine turnover and uric acid in rat caudate and nucleus accumbens studied chronically by in vivo voltammetry. *Journal of Neuroscience Methods, 34*, 143-149.

Kamdar, N. K., Miller, S. A., Syed, Y. M., Bhayana, R., Gupta, T., & Rhodes, J. S. (2007). Acute effects of Naltrexone and GBR 12909 on ethanol drinking in the dark in C57BL/6J mice. *Psychopharmacology, 192*, 207-217.

Kapasova, Z., & Szumlinski, K. K. (2008). Strain differences in alcohol-induced neurochemical plasticity: a role for accumbens glutamate in alcohol intake. *Alcoholism, Clinical And Experimental Research, 32*, 617-631.

Koob, G. F. (2009). Dynamics of neuronal circuits in addiction: Reward, antireward, and emotional memory. *Pharmacopsychiatry, 42 (Suppl. 1)*, 532-541.

Koob, G. F., & Volkow, N. D. (2010). Neurocircuitry of addiction *Neuropsychopharmacology, 35*, 217-238.

Krystal, J. H., & Tabakoff, B. (2002). Ethanol Abuse, Dependence, and Withdrawal: Neurobiology and Clinical Implications. In K. L. Davis, D. Charney, J. T. Coyle & C. Nemeroff (Eds.), *Neuropsychopharmacology the Fifth Generation of Progress* (pp. 1425-1443). Philadelphia: Lippincott Williams & Wilkins.

L'Amoreaux, W., Cuttitta, C., Santora, A., Blaize, J., Tachjadi, J., & El Idrissi, A. (2010). Taurine regulates insulin release from pancreatic beta cell lines. *Journal of Biomedical Science, 17*(Suppl 1), S11.

Lallemand, F., Dachour, A., Ward, R. J., & De Witte, P. (2000). Does taurine play an osmolarity role during ethanol intoxication? *Advances in Experimental Medicine and Biology, 483*, 203-212.

Land, C., & Spear, N. E. (2004). Ethanol impairs memory of a single discrimination in adolescent rats at doses that leave adult memory unaffected. *Neurobiology of Learning and Memory, 81*, 75-81.

Laviola, G., Macri, S., Morley-Fletcher, S., & Adriani, W. (2003). Risk-taking behavior in adolescent mice: psychobiological determinants and early epigenetic influence. [review]. *Neuroscience and Biobehavioral Reviews, 27*, 19-31.

Lê, A. D., Ko, J., Chow, S., & Quan, B. (1994). Alcohol consumption by C57BL/6, BALB/c, and DBA/2 mice in a limited access paradigm. *Pharmacology Biochemistry and Behavior, 47*, 375-378.

Le Moal, M., & Simon, H. (1991). Mesocorticolimbic dopaminergic network: functional and regulatory roles. *Physiological Reviews, 71*, 155-234.

Lidow, M. S., Goldman-Rakic, P. S., & Rakic, P. (1991). Synchronized overproduction of neurotransmitter receptors in diverse regions of the primate cerebral cortex. *Proceedings of the National Academy of Sciences of the United States of America, 88*(22), 10218-10221.

Lidow, M. S., & Rakic, P. (1992). Scheduling of monoaminergic neurotransmitter receptor expression in the primate neocortex during postnatal development. *Cerebral Cortex, 2*(5), 401-416.

Lidow, M. S., & Wang, F. (1995). Neurotransmitter receptors in the developing cerebral cortex. *Critical Reviews in Neurobiology, 9*(4), 395-418.

Lima, L., Obregon, F., Cubillos, S., Fazzino, F., & Jaimes, I. (2001). Taurine as a micronutrient in development and regeneration of the central nervous system. *Nutritional Neuroscience, 4*(6), 439-443.

Linseman, M. A. (1989). Effects of weight restriction and palatability on the apparent pharmacological regulation of alcohol consumption by rats in a limited access paradigm. *Appetite, 12*, 153-159.

Lourenco, R., & Camilo, M. E. (2002). Taurine: a conditionally essential amino acid in humans? An overview in health and disease. *Nutricion Hospitalaria, 6*, 262-270.

Lyons, A. M., Lowery, E. G., Sparta, D. R., & Thiele, T. E. (2008). Effects of food availability and administration of orexigenic and anorectic agents on elevated ethanol drinking associated with drinking in the dark procedures. *Alcoholism, Clinical And Experimental Research, 32*, 1962-1968.

Lyss, P. J., Andersen, S. L., LeBlanc, C. J., & Teicher, M. H. (1999). Degree of neuronal activation following FG-7142 changes across regions during development. *Developmental Brain Research, 116*, 201-203.

Maar, T., Moran, J., Schousboe, A., & Pasantes-Morales, H. (1995). Taurine deficiency in dissociated mouse cerebellar cultures affects neuronal migration. *International Journal of Developmental Neuroscience, 13*(5), 491-502.

Malosio, M. L., Marquèze-Pouey, B., Kuhse, J., & Betz, H. (1991). Widespread expression of glycine receptor subunit mRNAs in the adult and developing rat brain. *The EMBO Journal, 10*(9), 2401-2409.

Markwiese, B. J., Acheson, S. K., Levin, E. D., Wilson, W. A., & Swartzwelder, H. S. (1998). Differential effects of ethanol on memory in adolescent and adult rats. *Alcoholism: Clinical and Experimental Research, 22*, 416-421.

Martel, P., & Fantino, M. (1996). Influence of the amount of food ingested on mesolimbic dopaminergic system activity: A microdialysis study. *Pharmacology Biochemistry and Behavior, 55*, 297-302.

McCool, B. A., & Botting, S. K. (2000). Characterizationos oof strychnine-sensitive glycine receptors in acutely isolated adult rat basolateral amygdale neurons. *Brain Research, 859*, 341-351.

McGee, A. W., Yang, Y., Fisher, Q. S., Daw, N. W., & Strittmatter, S. M. (2005). Experience-driven plasticity of visual cortex limited by myelin and nogo receptor. *Science, 309*, 2222-2225.

McGue, M., & Iacono, W. G. (2008). The adolescent origins of substance use disorders. *International Journal of Methods in Psychiatric Research, 17*(SI), S30-S38.

Metten, P., Phillips, T. J., Crabbe, J. C., Tarantino, L. M., McClearn, G. E., Plomin, R. (1998). High genetic susceptibility to ethanol withdrawal predicts low ethanol consumption. *Mammalian Genome, 9*, 983-990.

Middaugh, L. D., Kelley, B. M., Bandy, A.-L. E., & McGroarty, K. K. (1999). Ethanol consumption by C57BL/6 mice: Influence of gender and procedural variables. *Alcohol, 17*, 175-183.

Mihic, J. S., & Harris, A. R. (1996). Alcohol actions at the $GABA_A$ receptor/chloride channel complex In R. A. Deitrich & V. G. Erwin (Eds.), *Pharmacological Effects of Ethanol on the Nervous System* (pp. 51-72). Boca Raton: CRC Press.

Miller, T. J., Hanson, R. D., & Yancey, P. H. (2000). Developmental changes in organic osmolytes in prenatal and postnatal rat tissues. *Comparative Biochemistry and Physiology, Part A*(125), 45-56.

Miranda-Contreras, L., Mendoza-Briceño, R. V., & Palacios-Pru, E. L. (1998). Levels of monoamine and amino acid neurotransmitters in the developing male mouse hypothalamus and in histotypic hypothalamic cultures. *International Journal of Developmental Neuroscience, 16*(5), 403-412.

Molinoff, P. B., & Axelrod, J. (1971). Biochemistry of catecholamines. *Annual Review of Biochemistry, 40*, 465-500.

Moore, E. M., Mariani, J. N., Linsenbardt, D. N., Melon, L. C., & Boehm, S. L. I. (2010). Adolescent C57BL/6J (but not DBA/2J) mice consume greater amounts of limited-access ethanol compared to adults and display continued elevated ethanol intake into adulthood. *Alcoholism: Clinical and Experimental Research, 34*, 734-742.

Murphy, J. M., Gatto, G. J., Waller, M. B., McBride, W. J., Lumeng, L., & Li, T. K. (1986). Effects of scheduled access on ethanol intake by the alcohol-preferring (P) line of rats. *Alcohol, 3*, 331-336.

Myers, R. D., Veale, W. L., & Yaksh, T. L. (1972). Preference for ethanol in the rhesus monkey following chronic infusion of ethanol into the cerebral ventricles. *Physiology & Behavior, 8*, 431-435.

Nestler, E. J., Hyman, S. E., & Malenka, R. C. (2009). *Molecular Neuropharmacology: A Foundation for Clinical Neuroscience* (Second Edition ed.). New York: McGraw-Hill Companies.

Niederhofer, H., & Staffen, W. (2003). Acamprosate and its efficacy in treating alcohol dependent adolescents. *European Child & Adolescent Psychiatry, 12*, 144-148.

Nolte, J. (2009). *The Human Brain an Introduction to its Functional Anatomy* (Vol. 6th Edition). Philadelphia: Mosby Elsevier.

Olds, J., & Milner, P. (1954). Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. *Journal of Comparative Physiology and Psychology, 47*, 419-427.

Olds, M., & Olds, J. (1969). Effects of lesions in medial forebrain bundle on self-stimulation behavior. *Americal 217*, 1253-1264.

Olive, M. F., Nannini, M. A., Ou, C. J., Koenig, H. N., & Hodge, C. W. (2002). Effects of acute acamprosate and homotaurine on ethanol intake and ethanol-stimulated mesolimbic dopamine release. *European Journal of Pharmacology, 437*, 55-61.

Palkovits, M., Elekes, I., Lang, T., & Patthy, A. (1986). Taurine levels in discrete brain nuclei of rats. *Journal of Neurochemistry, 47*, 1333-1335.

Pasantes-Morales, H., Franco, R., Torres-Marquez, M. E., Hernandez-Fonseca, K., & Ortega, A. (2000). Amino acid osmolytes in regulatory volume decrease and isovolumetric regulation in brain cells. Contribution and mechansims. *Cellular Physiology and Biochemistry, 10*, 361-370.

Pascual, M., Blanco, A. M., Cauli, O., Miñarro, J., & Guerri, C. (2007). Intermittent ethanol exposure induces inflammatory brain damage and causes long-term behavioural changes in adolescent rats. *European Journal of Neuroscience, 25*, 541-550.

Pascual, M., Boix, J., Felipo, V., & Guerri, C. (2009). Repeated alcohol administration during adolescence causes changes in the mesolimbic dopaminergic and glutamatergic systems and promotes alcohol intake in the adult rat. *Journal of Neurochemistry, 108*, 920-931.

Philpot, R. M., & Kirstein, C. L. (1998). The effects of repeated alcohol exposure on the neurochemistry of the periadolescent nucleus accumbens septi. *Neuroreport, 9*(7), 1359-1363.

Philpot, R. M., & Kirstein, C. L. (2004). Developmental differences in the accumbal dopaminergic response to repeated ethanol exposure. *Annals of New York Academy of Sciences, 1021*, 422-426.

Pierce, R. C., & Kumaresan, V. (2006). The mesolimbic dopamine system: The final common pathway for the reinforcing effect of drugs of abuse? *Neuroscience and Biobehavioral Reviews, 30*, 215-238.

Powell, K. (2006). How does the teenage brain work? *Nature, 442*(24), 865-867.

Quertemont, E., Devitgh, A., & De Witte, P. (2003). Systemic osmotic manipulations modulate ethanol-induced taurine release: a brain microdialysis study. *Alcohol, 29*, 11-19.

Quertemont, E., Lallemand, F., Colombo, G., & De Witte, P. (2000). Taurine and ethanol preference: a microdialysis study using Sardinian alcohol-preferring and non-preferring rats. *European Neuropsychopharmacology, 10*, 377-383.

Quertemont, E., Linotte, S., & De Witte, P. (2002). Differential taurine responsiveness to ethanol in high- and low-alcohol sensitive rats: a brain microdialysis study. *European Journal of Pharmacology, 444*, 143-150.

Rakic, P., Bourgeois, J. P., & Goldman-Rakic, P. S. (1994). Synaptic development of the cerebral cortex: implications for learning, memory, and mental illness. *Progress in Brain Research, 102*, 227-243.

Ramirez, R. L., & Spear, L. P. (2010). Ontogeny of ethanol-induced motor impairment following acute ethanol: Assessment via the negative geotaxis revlex in adolescent and adult rats. *Pharmacology Biochemistry and Behavior, 95*, 242-248.

Rassin, D. K., Sturman, J. A., & Guall, G. E. (1978). Taurine and other free amino acids in milk of man and other mammals. *Early Human Development, 2*(1), 1-13.

Rentería, R. C., Johnson, J., & Copenhagen, D. R. (2004). Need rods? Get glycine receptors and taurine. *Neuron, 41*, 839-844.

Rhodes, J. S., Best, K., Belknap, J. K., Finn, D. A., & Crabbe, J. C. (2005). Evaluation of a simple model of ethanol drinking to intoxication in C57BL/6J mice. *Physiology & Behavior, 84*, 53-63.

Rhodes, J. S., Ford, M. M., Yu, C.-H., Brown, L. L., Finn, D. A., Garland Jr, T. (2007). Mouse inbred strain differences in ethanol drinking to intoxication. *Genes, Brain, and Behavior, 6*, 1-18.

Rimondini, R., Arlinde, C., Sommer, W., & Heilig, M. (2002). Long-lasting increase in voluntary ethanol consumption and transcriptional regulation in the rat brain after intermittent exposure to alcohol. *Federation of American Societies for Experimental Biology Journal, 16*, 27-35.

Roberts, A. J., Cole, M., & Koob, G. F. (1996). Intra-amygdala muscimol decreases operant ethanol self-administration in dependent rats. *Alcoholism: Clinical and Experimental Research, 20*, 1289-1298.

Roberts, A. J., Heyser, C. J., & Koob, G. F. (1999). Operant self-administration of sweetened versus unsweetened ethanol: effects on blood alcohol levels. *Alcoholism, Clinical And Experimental Research, 23*, 1151-1157.

Robinson, T. E., & Berridge, K. C. (1993). The neural basis of drug craving: An incentive-sensitization theory of addiction. *Brain Research Reviews, 18*, 247-291.

Rodd-Henricks, Z. A., McKinzie, D. L., Crile, R. S., Murphy, J. M., & McBride, W. J. (2000). Regional heterogeneity for the intracranial self-administration of ethanol within the ventral tegmental area of female Wistar rats. *Psychopharmacology, 149*, 217-224.

Routsalainen, M., Majasaari, M., Salimaki, J., & Ahtee, L. (1998). Locally infused taurine, GABA, and homotaurine alter differentially the striatal extracellular concentrations of dopamine and its metabolites in rats. *Amino Acids, 15*, 117-134.

Sahr, A. E., Thielen, R. J., Lumeng, L., Li, T. K., & McBride, W. J. (2004). Long-lasting alterations of the mesolimbic dopamine system after periadolescent ethanol drinking by alcohol-preferring rats. *Alcoholism: Clinical and Experimental Research, 28*(5), 702-711.

Sakurai, T., Miki, T., Li, H. P., Miyatake, A., Satriotomo, I., & Takeuchi, Y. (2003). Colocalization of taurine and glial fibrillary acidic protein immunoreactivity in mouse hippocampus induced by short-term ethanol exposure. *Brain Research, 959*, 160-164.

Salamone, J. D., Correa, M., Farrar, A., & Mingote, S. M. (2007). Effort-related functions of nucleus accumbens dopamine and associated forebrain circuits. *Psychopharmacology, 191*(3), 461-482.

Saransaari, P., & Oja, S. S. (2000). Modulation of the ischemia-induced taurine release by adenosine receptors in the developing and adult mouse hippocampus. *Neuroscience, 97*(3), 425-430.

Saransaari, P., & Oja, S. S. (2003). Characterization of N-methyl-D-aspartate-evoked taurine release in the developing and adult mouse hippocampus. *Amino Acids, 24*, 213-221.

Schramm-Sapyta, N. L., Kingsley, M. A., Rezvani, A. H., Propst, K., Swartzwelder, H. S., & Kuhn, C. M. (2008). Early ethanol consumption predicts relapse-like behavior in adolescent male rats. *Alcoholism: Clinical and Experimental Research, 32*(5), 754-762.

Sergeeva, O. A., & Haas, H. L. (2001). Expression and function of glycine receptors in striatal cholinergic interneurons from rat and mouse. *Neuroscience, 104*, 1043-1055.

Serrano, M. I., Goicoechea, C., Serrano, J. S., Serrano-Martino, M. C., Sanchez, E., & Martin, M. I. (2002). Age-related changes in the antinociception induced by taurine in mice. *Pharmacology Biochemistry and Behavior, 73*, 863-867.

Shaw, P., Greenstein, D., Lerch, J., Lenroot, R., Gogtay, N., Evans, A. (2006). Intellectual ability and cortical development in children and adolescents. *Nature, 440*, 676-679.

Shaw, P., Kabani, N. J., Lerch, J. P., Eckstrand, K., Lenroot, R., Gogtay, N. (2008). Neurodevelopmental Trajectories of the Human Cerebral Cortex. *Journal of Neuroscience, 28*(14), 3586-3594. doi: 10.1523/jneurosci.5309-07.2008

Shelton, K. L., & Grant, K. A. (2002). Discriminative stimulus effects of ethanol in C57BL/6 and DBA/2J mice. *Alcoholism: Clinical and Experimental Research, 26*(6), 747-757.

Siegel, G. J., Agranoff, B. W., Albers, R. W., Fisher, S. K., & Uhler, M. D. (Eds.). (1999). *Basic Neurochemistry Molecular, Cellular, and Medical Aspects* (6 ed.). Philadelphia: Lipincott Williams & Wilkins.

Silveri, M. M., & Spear, L. P. (1998). Decreased sensitivity to the hypnotic effects of ethanol early in ontogeny. *Alcoholism: Clinical and Experimental Research, 22*, 670-676.

Silveri, M. M., & Spear, L. P. (2000). Ontogeny of ethanol elimination and ethanol-induced hypothermia. *Alcohol, 20*, 45-53.

Simms, J. A., Steensland, P., Medina, B., Abernathy, K. E., Chandler, L. J., Wise, R. A. (2008). Intermittent access to 20% ethanol induces high ethanol consumption in Long-Evans and Wistar rats. *Alcoholism, Clinical And Experimental Research, 32*, 1816-1823.

Sisk, C. L., & Foster, D. L. (2004). The neural basis of puberty and adolescence. *Nature Neuroscience, 7*(10), 1040-1047.

Slawecki, C. J. (2002). Altered EEG responses to ethanol in adult rats exposed to ethanol during adolescence. *Alcoholism: Clinical and Experimental Research, 26*, 246-254.

Slawecki, C. J., Betancourt, M., Cole, M., & Ehlers, C. L. (2001). Periadolescent alcohol exposure has lasting effects on adult neurophysiological function in rats. *Developmental Brain Research, 128*, 63-72.

Smith, R. F. (2003). Animal models of periadolescent substance abuse. *Neurotoxicology and Teratology, 25*, 291-301.

Sowell, E. R., Thompson, P. M., Holmes, C. J., Batth, R., Jernigan, T. L., & Toga, A. W. (1999). Localizing Age-Related Changes in Brain Structure between Childhood and Adolescence Using Statistical Parametric Mapping. *NeuroImage, 9*(6), 587-597.

Sowell, E. R., Thompson, P. M., Leonard, C. M., Welcome, S. E., Kan, E., & Toga, A. W. (2004). Longitudinal Mapping of Cortical Thickness and Brain Growth in Normal Children. *Journal of Neuroscience, 24*(38), 8223-8231. doi: 10.1523/jneurosci.1798-04.2004

Spear, L. P. (2000). The adolescent brain and age-related behavioral manifestations. *Neuroscience and Biobehavioral Reviews, 24*, 417-463.

Spear, L. P., & Varlinskaya, E. I. (2005). Adolescence. Alcohol sensitivity, tolerance, and intake. *Recent Developments in Alcoholism, 17*, 143-159.

Stahl, S. M. (2000). *Essential Psycopharmacology Neuroscientific Basis and Practical Applications* (2 ed.). Cambridge: Cambridge University Press.

Sturman, J. A. (1977). Taurine in developing rat brain: transfer of [35S]Taurine to pups via the milk. *Pediatric Research, 11*, 28.

Sturman, J. A. (1993). Taurine in development. *Physiological Reviews*(73), 119-147.

Sturman, J. A., & Chesney, R. W. (1995). Taurine in pediatric nutrition. *Pediatric Clinician North America, 42*(4), 879-897.

Suge, R., Hosoe, N., Furube, M., Yamamoto, T., Hirayama, A., Hirano, S. (2007). Specific timing of taurine supplementation affects learning ability in mice. *Life Sciences, 81*, 1228-1234.

Szumlinski, K. K., Diab, M. E., Friedman, R., Henze, L. M., Lominac, K. D., & Bowers, M. S. (2007). Accumbens neurochemical adaptations produced by binge-like alcohol consumption. *Psychopharmacology, 190*, 415-431.

Tabarin, A., Diz-Chaves, Y., Consoli, D., Monsaingeon, M., Bale, T. L., Culler, M. D. (2007). Role of the corticotropin-releasing factor receptor type 2 in the control of food intake in mice: a meal pattern analysis. *European Journal of Neuroscience, 26*, 2303-2314.

Tang, X. W., Hsu, C. C., Schloss, J. V., Faiman, M. D., Wu, E., Yang, C. Y. (1997). Protein phosphorylation and taurine biosynthesis in vivo and in vitro. *Journal of Neuroscience, 17*(18), 6947-6951.

Tappaz, M., Almarghini, K., Legay, F., & Remy, A. (1992). Taurine biosynthesis enzyme cysteine sulfinate decarboxylase (CSD) from brain: the long and tricky trail to identification. *Neurochem Res, 17*(9), 849-859.

Tappaz, M., Reymond, I., Bitoun, M., & Sergeant, A. (1998). Cysteine sulfinate decarboxylase (CSD): molecular cloning, sequence and genomic expression in brain. *Adv Exp Med Biol, 442*, 25-32.

Thiele, T. E., Marsh, D. J., Ste Marie, L., Bernstein, I. L., & Palmiter, R. D. (1998). Ethanol consumption and resistance are inversely related to neuropeptide Y levels. *Nature, 396*, 366-369.

Varlinskaya, E. I., & Spear, L. P. (2004a). Acute ethanol withdrawal (hangover) and social behavior in adolescent and adult male and female Sprague-Dawley rats. *Alcoholism, Clinical And Experimental Research, 28*, 40-50.

Varlinskaya, E. I., & Spear, L. P. (2004b). Changes in sensitivity to ethanol-induced social facilitation and social inhibition from early to late adolescence. *Annals of New York Academy of Sciences, 1021*, 459-461.

Varlinskaya, E. I., & Spear, L. P. (2009). Ethanol-induced social facilitation in adolescent rats: role of endogenous activity at mu opoid receptors. *Alcoholism: Clinical and Experimental Research, 33*, 991-1000.

Varlinskaya, E. I., & Spear, L. P. (2010). Sensitization to social anxiolytic effects of ethanol in adolescent and adult Sprague-Dawley rats after repeated ethanol exposure. *Alcohol, 44*(1), 99-110.

Vetter, C. S., Doremus-Fitzwater, T. L., & Spear, L. P. (2007). Time course of elevated ethanol intake in adolescent relative to adult rats under continuous, voluntary-access conditions. *Alcoholism: Clinical and Experimental Research, 31*(7), 1159-1168.

Vohra, B. P. S., & Hui, X. (2000). Improvement of impaired memory in mice by taurine. *Neural Plasticity, 7*(245-259).

White, A. M., Bae, J. G., Truesdale, M. C., Ahmad, S., Wilson, W. A., & Swartzwelder, H. S. (2002). Chronic-intermittent ethanol exposure during adolescence prevents normal developmental changes in sensitivity to ethanol-induced motor impairments. *Alcoholism, Clinical And Experimental Research, 26*(7), 960-968.

White, A. M., Truesdale, M. C., Bae, J. G., Ahmad, S., Wilson, W. A., Best, P. J. (2002). Differential effects of ethanol on motor coordination in adolescent and adult rats. *Pharmacology Biochemistry and Behavior, 73*, 673-677.

Whitworth, A. B., Fischer, F., Lesch, O. M., Nimmerrichter, A., Oberbauer, H., Platz, T. (1996). Comparison of acamprosate and placebo in long-term treatment of alcohol dependence. *Lancet, 347*, 1438-1442.

Windle, M., Spear, L. P., Fuligni, A. J., Angold, A., Brown, J. D., Pine, D. (2008). Transitions into underage and problem drinking: developmental processes and mechanisms between 10 and 15 years of age. *Pediatrics, 141*(Supplement 4), S273-S289.

Wise, R. A. (1978). Catecholamine theories of reward: a critical review. *Brain Research Reviews, 152*, 215-247.

Wright, T. M., & Myrick, H. (2006). Acamprosate: a new tool in the battle against alcohol dependence. [Journal Article]. *Neuropsychiatr Dis Treat, 2*(4), 445-453.

Wu, H., Jin, Y., Wei, J., Jin, H., Sha, D., & Wu, J.-Y. (2005). Mode of action of taurine as a neuroprotector. *Brain Research, 1038*, 123-131.

Wu, J. Y., Chen, W., Tang, X. W., Jin, H., Foos, T., Schloss, J. V. (2000). Mode of action of taurine and regulation dynamics of its synthesis in the CNS. *Advances in Experimental Medicine and Biology, 483*, 35-44.

Xiao, C., Max Shao, X., Olive, M. F., Griffin III, W. C., Li, K. Y., Krnjevic', K. (2009). Ethanol facilitates glutamatergic transmission to dopamine neurons in the ventral tegmental area. *Neuropsychopharmacology, 34*, 307-318.

Yan, Q.-S., Zheng, S.-Z., Feng, M.-J., & Yan, S.-E. (2005). Involvement of 5-HT1B receptors within the ventral tegmental area in ethanol-induced increases in mesolimbic dopaminergic transmission. *Brain Research, 1060*, 126-137.

Yang, H.-T., Chen, Y.-H., Chiu, W.-C., & Huang, S.-Y. (2006). Effects of consecutive high-dose alcohol administration on the utilization of sulfur-containing amino acids by rats. *Journal of Nutritional Biochemistry, 17*, 45-50.

Yang, H.-T., Chien, Y.-W., Tsen, J.-H., Chang, C.-C., Chang, J.-H., & Huang, S.-Y. (2009). Taurine supplementation improves the utilization of sulfur-containing amino acids in rats continually administrated alcohol. *The Journal of Nutritional Biochemistry, 20*, 132-139.

Yoshida, M., Fukuda, S., Tozuka, Y., Miyamoto, Y., & Hisatsune, T. (2004). Developmental shift in bidirectional functions of taurine-sensitive chloride channels during cortical circuit formation in postnatal mouse brain. *Journal of Neurobiology, 60*, 166-175.

Yttri, E. A., Burk, J. A., & Hunt, P. S. (2004). Intermittent ethanol exposure in adolescent rats: dose-dependent impairments in trace conditioning. *Alcoholism: Clinical and Experimental Research, 28*, 1433-1436.

Yu, S.-S., Wang, M., Li, X.-M., Chen, W.-H., Chen, J.-T., Wang, H.-L. (2007). Influences of different developmental periods of taurine supplements on synaptic plasticity in hippocampal CA1 area of rats following prenatal and perinatal lead exposure. *BMC Developmental Biology, 7*, 51-63.

Zhu, D. M., Wang, M., She, J. Q., Yu, K., & Ruan, D. Y. (2005). Protection by a taurine supplemented diet from lead-induced deficits of long-term potentiation/depotentiation in dentate gyrus of rats in vivo. *Neuroscience, 134*(1), 215-224.