ABSTRACT

Taurine Depletion in Adolescent Mice and Implications
for Ethanol Withdrawal-Induced Anxiety

Rebecca S. Helfand, M.A.

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

Adolescents exhibit few negative neurobehavioral effects of ethanol withdrawal and have higher levels of taurine, an inhibitory amino acid, than adults. Given taurine's neuroprotective role and abundance during adolescence, it is possible that taurine is acting to attenuate adolescents' negative response to ethanol withdrawal. The current study examined the effects of 7 days of taurine depletion with GES on withdrawal-induced anxiety as measured on the elevated plus maze (EPM) and plasma corticosterone levels. Plasma corticosterone levels were significantly higher in the GES and ethanol exposed group than any of the other groups. The results indicate that while taurine depletion did not decrease open arm time as expected, the combination of exposure to GES and ethanol significantly increased plasma corticosterone levels. The major finding of this study is that the depletion of taurine during adolescence affected biochemical but not behavioral measures of anxiety further implicating taurine's protective role during ethanol withdrawal.

Taurine Depletion in Adolescent Mice and Implications
for Ethanol Withdrawal-Induced Anxiety

by

Rebecca S. Helfand, B.A.

A Thesis

Approved by 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
Master of Arts

Approved by the Thesis Committee

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

_____
Jim H. Patton, Ph.D.

_____
N. Bradley Keele, Ph.D.

_____
Eric L. Robinson, Ph.D.

Accepted by the Graduate School
May 2007

_____
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

I would first like to thank Dr. Jaime Diaz-Granados for his unfailing support and encouragement. I credit him with the success and continuation of my graduate career. Dr. Diaz-Granados' faith in my ability to succeed and willingness to do whatever it takes to help have made my academic success possible and I will always be grateful for the opportunities he has provided.

I would like to thank my committee members; Dr. Jim Patton, Dr. N. Bradley Keele, and Dr. Eric Robinson for their support and contributions. Their unique perspectives and input were immensely valuable. It was an honor to work with each of them.

Additionally, I would like to thank André W. Zalud without whom this project would not have been possible. His dedication and effort in all aspects of this project, from the design and construction of the elevated plus maze, to his expert biochemical analysis have enabled the completion of this thesis. André's continued support, wisdom and foresight were truly a blessing and has fostered in me, an understanding and respect for the research process. I could not have done this without him.

I dedicate my thesis to my grandfather, Theodore Helfand, (1926-2007), a man of great honor and integrity. His limitless love and support have helped me through many trials and was a constant source of encouragement. His inquisitive and meticulous natures are traits I hope to exemplify. I will miss him greatly and hope I have made him proud. May his soul be bound up in the bonds of eternal life.

I also dedicate my thesis to my family, without their love, support, and understanding this accomplishment would not have been possible. Thank you, I love you all.

CHAPTER ONE

Literature Review

Adolescence

The terms adolescence and puberty are often used interchangeably, while their timing overlaps, 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 (Powell, 2006; Sisk & Foster, 2004; Spear, 2000). This period is loosely defined however and has no specific boundaries. In mice, adolescence spans the period from postnatal day (PD) 22 to PD 60 (Laviola, Macri, Morley-Fletcher, & Adriani, 2003). During this transitional period the brain continues to develop (Hua & Smith, 2004; Powell, 2006; Sisk & Foster, 2004). While definitive causality, between specific behavioral and neurological changes cannot be absolutely determined, the phenomena do occur concurrently. Thus, adolescence is a time of great change and therefore a period of great risk for perturbations by alcohol.

Adolescent Behavior

In order to successfully navigate the developmental transition from childhood to adulthood, organisms across species, must gain the necessary skills for independence. Accordingly, behavioral changes developed during this time period are conserved across species and likely play an adaptive role in helping the organism to fit into a specific niche (Spear, 2000). In particular, because they facilitate niche exploration and new learning, especially developing independence, an adolescent-associated increase in risk-taking behavior as well as an increase in novelty seeking is seen across species (Bina, Graziano,

& Bonino, 2006; Gardner & Steinberg, 2005; Laviola et al., 2003; Rupp, Rosenthal, & Middleman, 2006). When surveyed in high school, 38% of seniors do not view alcohol consumption, even heavy daily drinking as a great risk, evidence of a propensity for risky behavior (Johnston, O'Malley, Bachman, & Schulenberg, 2006). In rodents, a marked preference for novel environments emerges (Adriani, Chiarotti, & Laviola, 1998; Douglas, Varlinskaya, & Spear, 2003; Laviola et al., 2003; Stansfield, Philpot, & Kirstein, 2004) as well as an increase in the drive for social interactions with individuals outside the family unit (Gardner & Steinberg, 2005; Varlinskaya & Spear, 2007). This drive to expand interactions beyond the family may facilitate independence and prevent inbreeding, yet is a risky behavior.

Adolescent Neural Alterations

During adolescence the brain undergoes significant pruning of neocortical neurons. Some estimate that as many as 30,000 synapses may be lost per second over 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). This synaptic loss is believed to reduce by half, the average number of synapses per cortical neuron compared to the preadolescent period (Hua & Smith, 2004; Sisk & Foster, 2004). 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) indicating

adolescence as a second "critical period for development" one in which the brain should be stimulated and challenged just as recommended for the first five years of life (Gogtay et al., 2004; Shaw et al., 2006).

In addition to substantial neuronal pruning during the adolescent period, the brains of adolescents exhibit both a different neurochemical makeup and different numbers and functions of receptors than adults (Bolanos, Glatt, & Jackson, 1998; Lidow & Wang, 1995; Lyss, Andersen, LeBlanc, & Teicher, 1999; Malosio, Marquèze-Pouey, Kuhse, & Betz, 1991). In particular, loci for dopamine, serotonin, acetylcholine, and γ-aminobutyric acid (GABA) receptors are overproduced and pruned from infancy through adolescence in the primate cortex (Fritschy, Paysan, Enna, & Mohler, 1994; Lidow, Goldman-Rakic, & Rakic, 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 decreasing to adult levels yet serotonin levels show no significant variations, remaining at low levels until young adulthood (Lidow & Rakic, 1992; 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; T. J. Miller, Hanson, & Yancey, 2000; Miranda-Contreras et al., 1998). These high levels of taurine have been shown to reduce neuronal membrane hyperexcitability, allowing this amino acid to function as a neuroprotector during adolescent development in the mouse brain (Lima, Obregon, Cubillos, Fazzino, &

Jaimes, 2001; Yoshida, Fukuda, Tozuka, Miyamoto, & Hisatsune, 2004). The differences in adolescent and adult brains may contribute to the ontogenetic differences in the effects of ethanol.

Chronic Ethanol Exposure

Given the preponderance of ethanol use and abuse by adolescents, further investigations into the effects of early exposure are clearly warranted. Ethanol is a CNS depressant that exerts sedative and hypnotic effects (Nestler, Hyman, & Malenka, 2001; Wolf, 2006). Symptoms of intoxication vary in a dose dependent manner, ranging from stimulation, with an initial low dose, to sleepiness, with increasing doses (Wolf, 2006). At intoxicating doses, memory and judgment are impaired, reaction time is slowed, coordination is impaired, and self-control is reduced (Krystal & Tabakoff, 2002; Nestler et al., 2001; Wolf, 2006). Chronic consumption of ethanol results in both adaptation and neurotoxicity within the brain which account for the development of tolerance and dependence (Krystal & Tabakoff, 2002; Wolf, 2006). These persistent neurochemical changes can lead to increasing, cyclical patterns of abuse (Ballenger & Post, 1978; Becker, Diaz-Granados, & Hale, 1997; Becker, Veatch, & Diaz-Granados, 1998). During periods of ethanol withdrawal, the aversive conditions such as anxiety, tremors, and seizures, experienced by alcoholics most likely promote further episodes of ethanol abuse as a means of abolishing withdrawal-related symptoms (Krystal & Tabakoff, 2002; Wolf, 2006). Researchers recognize disruptions in glutamate, and GABA homeostasis, within various brain regions, as primary facilitators of ethanol withdrawal pathophysiologies, such as hyperexcitability, seizures, and anxiety (Gatch & Lal, 2001; Tsai & Coyle, 1998). Ethanol functions as an effective anxiolytic agent and continued use often produces

marked dysfunction within the hypothalamic-pituitary-adrenocortical (HPA) stress-response axis (Overstreet, Knapp, & Breese, 2004; Pruett, Collier, & Wu, 1998; Rasmussen et al., 2000; Rivier, 1996), possibly explaining, at a neuroendocrine level, the anxiogenesis of withdrawal.

Prolonged exposure to ethanol significantly disrupts the synaptic activities mediated by both GABA and glutamate (Faingold, Li, & Evans, 2000), the predominant inhibitory and excitatory amino acids within the CNS, respectively. Although no definitive evidence currently exists to explain the precise mechanism(s) by which ethanol influences neural function, studies over the past two decades provide compelling data that delineate ethanol-related behaviors as a function of ethanol's action at GABA and glutamate receptors. The anxiolytic, ataxic, and reinforcing properties of short-term ethanol exposure demonstrate a few behavioral components that correspond to ethanol's enhancement of $GABA_A$ receptor ($GABA_A$)-mediated chloride currents (Mihic & Harris, 1996). In support of ethanol's interaction with glutamate transmission, glutamate receptor antagonists fully mimic ethanol-associated effects on glutamatergic function, as demonstrated by interference with memory formation, disruption in neuronal development, and withdrawal-related seizures/hyperexcitability (Tabakoff & Hoffman, 1996). The combination of glutamatergic up-regulation and GABAergic down-regulation are suggested to be responsible for the increase in seizure susceptibility during withdrawal from ethanol (Faingold et al., 2000).

Ethanol & Adolescence

Ethanol intoxication and withdrawal result in different effects in adolescents and adults. Researchers have shown that sensitivity to ethanol increases during ontogeny

(Land & Spear, 2004; R. F. Smith, 2003; Spear & Varlinskaya, 2005). Specifically, adolescent rats exhibit less sedation, exhibiting significantly shorter sleep times (Silveri & Spear, 1998), and are less sensitive to ethanol-induced hypothermia (Silveri & Spear, 2000) compared to adults. In addition, adolescent rats exhibit less severe motor impairment following ethanol exposure (Spear & Varlinskaya, 2005; White, Bae et al., 2002). In particular, adolescents exhibit significant acute tolerance to ethanol and a decrease in brain sensitivity to ethanol's sedative effects compared to adults (Silveri & Spear, 1998). This acute tolerance may account for the lack of sedative effects as well as the lack of significant negative effects of ethanol intake which may increase the likelihood of future consumption in adolescents. Across age groups, ethanol intoxication results in anxiolysis contributing to its reinforcing properties and subsequent widespread recreational use (Krystal & Tabakoff, 2002). Early ontogeny is marked by increased sensitivity to the anxiolytic effects of ethanol intoxication (Varlinskaya & Spear, 2004) and, when combined with the lack of negative effects, likely contributes to continued use of the drug among adolescents.

Ethanol withdrawal in adolescents results in few immediate negative physiological effects. During adolescence, rodents exhibit less withdrawal-induced hypothermia (Silveri & Spear, 2000), motor impairment (White, Bae et al., 2002) and anxiety (Varlinskaya & Spear, 2004). Following five or 10 days of ethanol exposure, during adolescence, rats exhibit altered EEGs and event-related potentials (Slawecki, Betancourt, Cole, & Ehlers, 2001), as well as persistent morphological alterations (Evrard et al., 2006), illustrating a long-lasting change in functional brain activity without concurrent behavioral changes. Throughout ontogeny a natural increase in sensitivity to

the motor impairing effects of ethanol between adolescence and adulthood emerges (Silveri & Spear, 1998). Repeated adolescent binge-drinking episodes prevent the emergence of this normal developmental change (White, Bae et al., 2002). Further investigations into the long-term effects of adolescent ethanol exposure have revealed a persistent tolerance to the sedative effects of ethanol, resulting in continued attenuation of the animal's loss of righting reflex (Silveri & Spear, 1998; Silvers, Tokunaga, Mittleman, & Matthews, 2003). In addition, animal research has found that adolescent binge-drinking impairs spatial memory when tested during adulthood (Farr, Scherrer, Banks, Flood, & Morley, 2005; Land & Spear, 2004; Markwiese, Acheson, Levin, Wilson, & Swartzwelder, 1998; Yttri, Burk, & Hunt, 2004), illustrating long-term effects of early ethanol exposure. Despite the lack of immediate negative effects of ethanol, consumption during adolescence induces damage to the brain that persists into adulthood.

Ethanol affects different brain regions in adolescents and adults. Four days of binge-like exposure, in rats, produces significant tissue loss in the frontal cortex of adolescent animals and produces damage predominantly to the entorhinal cortex in adults (Crews, Braun, Hoplight, & Switzer III, 2000). Rats exposed to ethanol during adolescence exhibit less powerful low-frequency EEG signals in the hippocampus and parietal lobes compared to controls, (Slawecki, 2002; Slawecki et al., 2001). Similar results occur in humans who initiated ethanol consumption during adolescence. A study of adolescents with "adolescent-onset alcohol use disorders," found bilaterally smaller hippocampal volumes compared to matched controls, with a positive correlation between age of initiation of consumption and volume (De Bellis et al., 2000). Functional MRI brain scans of adolescents with an alcohol use disorder show less activation of the frontal

cortex and cerebellum and increased activation of the inferior parietal and temporal regions during a spatial working memory task, compared to adolescents without the disorder (Caldwell et al., 2005). 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. While 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. The lack of withdrawal-induced symptomology; hypothermia, motor impairment, or 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 and deserves further investigation.

Hypothalamic – Pituitary – Adrenal Axis

The hypothalamic – pituitary – adrenal (HPA) axis is activated in response to differing forms of stress: hunger, social, and withdrawal from ethanol and other drugs of abuse (Hadley, 2000; Prendergast & Little, 2007; Rasmussen et al., 2000). Researchers believe that this pathway, composed of the hypothalamus, pituitary gland, and adrenal glands (Carrasco & Van de Kar, 2003; Rivier, 1996), plays an integral role in facilitating drug dependencies, sensitivities, and withdrawal symptoms (Nestler & Aghajanian, 1997). Stimulation of this pathway activates the hypothalamic release of corticotropin releasing factor (CRF) and arginine vasopressin (AVP), which act upon the anterior portion of the pituitary gland (adenohypophysis), in a synergistic manner (Gillies, Linton, & Lowry, 1982; Rivier & Vale, 1983), to promote the release of adrenocorticotropic

hormone (ACTH) into systemic circulation (Mason, Hassan, Chacko, & Thompson, 2002; Rasmussen et al., 2000; Rivier, 1996). ACTH, through activation of the adrenal glands, stimulates the synthesis and secretion of glucocorticoids, most notably cortisol (corticosterone in rodents) (Pruett et al., 1998; Rivier, 1996; Stenzel-Poore, Heinrichs, Rivest, Koob, & Vale, 1994). This hormone, acting upon target tissues, carries out the final portion of the stress response by inducing physiological changes (release of energy stores, up/down-regulation of gene transcription, increasing cardiovascular output, and redistribution of blood flow) meant to counteract a perceived threat and increase the organism's survival (Carrasco & Van de Kar, 2003; Millan, 2003) Additionally, glucocorticoids exert a negative feedback on the activity of the HPA, attenuating the hypothalamic formation of CRF and decreasing the formation of ACTH in the adenohypophysis, aiding in the return to homeostasis following a stressor (Aguilera & Rabadan-Diehl, 2000; Guyton & Hall, 1996). The release of corticosterone increases prior to waking or prior to the onset of the dark cycle in nocturnal animals (McEwen, 1999). Plasma corticosterone levels are significantly increased by environmental or behavioral stress (Patel, Roelke, Rademacher, Cullinan, & HIllard, 2004; Shepard, Barron, & Myers, 2003). Forced swimming, open field and inescapable novel environment exposure, induce increases in increase AVP in the suprachiasmatic nucleus (Engelmann, Ebner, Landgraf, & Wotjak, 1998), CRF and AVP gene transcription (Jiang et al., 2004), and corticosterone levels (Mikics, Barsy, Barsvári, & Haller, 2005; Robertson, Beattie, Reid, & Balfour, 2005). Prior exposure to stressors potentiates plasma corticosterone levels in response to new stressful situations (Storey et al., 2006),

possibly due to sensitization of the HPA by the initial stressor (Dallman, 1993; Stam, van Laar, Akkermans, & Wiegant, 2002).

Within the hypothalamus, the paraventricular nucleus (PVN) and the supraoptic nucleus (SON) are of interest with regard to neuroendocrine regulation of the HPA axis. In particular, parvocellular neurons of the PVN constitute the primary source of CRF synthesis, and to a lesser degree AVP, whereas magnocellular neurons of the SON exist as the primary source of AVP synthesis (Amaya, Tanaka, Hayashi, & Tanaka, 2001; Carrasco & Van de Kar, 2003; Madiera & Paula-Barbosa, 1999; Ogilvie, Lee, & Rivier, 1997). Since the hypothalamus maintains control over HPA activation, via AVP and CRF release, ethanol-induced disruptions in hypothalamic function would undoubtedly change the normal regulatory state of the HPA axis. Furthermore, since ethanol inhibits both cellular (Aschner, Mutkus, & Allen, 2001; Kanli & Terreros, 1990) and systemic osmoregulation (Carney, Gillies, & Ray, 1995; Taivainen, Laitinen, Tähtelä, Kiianamaa, & Välimäki, 1995; X. M. Wang, Dayanithi, Lemos, Nordmann, & Treistman, 1991), the highly osmosensitive hypothalamus appears particularly susceptible to the effects mediated by chronic ethanol intoxication.

During adolescence, the neuroendocrine response to acute and chronic stress is dampened (Laviola, Adriani, Morley-Fletcher, & Terranova, 2002). Adolescent mice exhibit lower basal levels of corticosterone than adults (Laviola, Adriani, Terranova, & Gerra, 1999). When exposed to a novel environment, adolescents exhibit less locomotor activity than adults, indicating a reduction in the behavioral stress-response or reduced perception of the stressful condition (Adriani & Laviola, 2000). When corticosterone levels are analyzed in adolescents, only a slight elevation was found in response a novel

environment, a situation where adults exhibit a significant increase in corticosterone levels. Thus, the adolescent HPA axis may be hyporesponsive compared to adults (Laviola et al., 1999; Prendergast & Little, 2007). This may be adaptive as adolescents begin to seek out and explore novel environments (Adriani et al., 1998) and this behavior is supported by a lower corticosterone response to stress (Adriani & Laviola, 2000).

Consequences of Chronic Ethanol on HPA Axis Regulation

Ethanol exposure and withdrawal alters the overall activity of the HPA axis, resulting in significant long-term changes that disrupt normal homeostatic regulation of this system (Lovallo, 2006; Madiera & Paula-Barbosa, 1999; Rasmussen et al., 2000; Silva, Paula-Barbosa, & Madeira, 2002). Investigators demonstrate that ethanol exposure significantly downregulates the HPA axis altering the set point (Aguilera & Rabadan-Diehl, 2000; Madiera & Paula-Barbosa, 1999; Rivier, 1996). This ethanol-induced alteration in the set point then subsequently results in persistent stress-like responses, in the absence of ethanol (Goldman & Barr, 2002).

Neurons of the hypothalamus exhibit detrimental effects from chronic ethanol exposure, directly via morphological evidence (Carmona-Calero et al., 1995; Harding, Halliday, Ng, Harper, & Kril, 1996) and indirectly via abnormal levels of AVP release (Eisenhofer, Lambie, Whiteside, & Johnson, 1985; László et al., 2001; Taivainen et al., 1995). Prolonged ethanol exposure alters the firing patterns and normal endocrine secretions of hypothalamic neurons, resulting in the loss of circadian rhythmic control (Adinoff et al., 1991; Hadley, 2000; Tabakoff, Jafee, & Ritzmann, 1978), relating to a progressive degeneration of vasopressinergic neurons (Harding et al., 1996; Rivier, Imaki, & Vale, 1990). These alterations of AVP synthesis and movement parallel edema-

induced neural damage induced by episodic ethanol exposure (Collins, Zou, & Neafsey, 1998). Analysis of hypothalamic tissue, isolated from mice chronically exposed to ethanol, shows substantial accumulation of AVP within SON neurons, along with notable increases in nuclear area size (Carmona-Calero et al., 1995). These findings illustrate ethanol's inhibitory effect on the transport of AVP from the SON to the neurohypophysis, where ethanol also blocks the release of AVP into systemic circulation (X. M. Wang et al., 1991), resulting in prevalent states of dehydration. Additionally, prolonged ethanol exposure (seven days of vapor inhalation) has been shown to blunt the expression of CRF and AVP in the PVN, decreasing activation of the HPA (Rivier et al., 1990; Silva et al., 2002). Both AVP and CRF are necessary in the adenohypophysis to elicit maximal ACTH release and full activation of the HPA axis (Gillies et al., 1982; Ogilvie et al., 1997; Rivier & Vale, 1983). Therefore, by altering the release of either AVP or CRF, ethanol effects changes within the HPA axis (Aguilera & Rabadan-Diehl, 2000; Tanoue et al., 2004).

Interestingly, osmotic conditions, in the absence of typical external stressors, can elicit physiological activation similar to that of fight-or-flight stress responses. Dehydrated (salt-loaded) rats demonstrate significant decreases in hypothalamic CRF mRNA levels, corresponding with reduced plasma corticosterone concentrations (Chowdrey, Jessop, Patel, & Lightman, 1991; Madiera & Paula-Barbosa, 1999; Zemo & McCabe, 2002). Surprisingly, rehydration induces significant increases in CRF mRNA levels, above that of acutely-stressed groups, and substantially elevated plasma corticosterone concentrations, which show no decline for two weeks after rehydration and require four weeks for basal level reestablishment (Amaya et al., 2001). These results

depict the powerful effect of osmotic states on hypothalamic and pituitary neurons, both seemingly unresponsive to the normal negative feedback of increased plasma glucocorticoids. Thus, continuous cycles of ethanol intake / withdrawal may cause significant decrements in the osmoregulatory capacity of the hypothalamus, which, in turn, leads to more pronounced rebound rehydration effects and a dysfunctional HPA axis.

Ethanol clearance can elicit an overactivated, compensatory AVP response (overhydration) that results in significant, withdrawal-induced cerebral swelling, corresponding to hyperexcitable states, cell damage, and seizure episodes (Collins et al., 1998; Eisenhofer et al., 1985; Madiera & Paula-Barbosa, 1999). Investigators report that the symptoms of ethanol withdrawal, such as tremors, anxiety, delirium, and seizures, appear qualitatively similar to states of hyponatremia / water intoxication, indicating that increased AVP secretion, during withdrawal (Taivainen et al., 1995), may induce cerebral edema as well as (Collins et al., 1998; Lambie, 1985; Mander, Weppner, Chick, Morton, & Best, 1988). Given that AVP potentiates the release of CRF during osmotic alterations (Aguilera & Rabadan-Diehl, 2000), the ethanol withdrawal-induced increase in AVP likely results in elevated ACTH release and activation of the HPA axis and anxious behaviors (Wotjak et al., 2002). During withdrawal, peak CRF levels occur concurrently with peak behavioral expression of anxiety (Menzaghi et al., 1994; Overstreet et al., 2004; Pich et al., 1995) and induce persistent changes (Rasmussen et al., 2000).

In addition to osmotic changes, ethanol withdrawal induces perturbations of calcium-dependent activity. Glucocorticoids stimulate depolarization-induced calcium influxes (Sze, 1996). The molecular events underlying glucocorticoid excitatory actions

are not well understood at this time; however, radioimmunoassays from slices of rat hypothalamic tissue containing the PVN and SON have been analyzed to investigate the mechanism of glucocorticoids on AVP release. The researchers found that after 20 minutes, corticosterone inhibited AVP release in a dose-dependent manner (X. Liu, Wang, & Chen, 1995). In addition, the same group found that increases in extracellular calcium levels increased, AVP levels and corticosterone's inhibitory effect was enhanced (X. Liu et al., 1995). Extracellular calcium levels may play a role in the rapid effect of corticosterone on AVP release and is another way in which ethanol withdrawal affects the HPA axis, inducing anxious behavior.

Adults, not adolescents, exhibit ethanol withdrawal-induced anxiety (Doremus, Brunell, Varlinskaya, & Spear, 2003), in fact, corticosterone responses to ethanol withdrawal increase in an ontogenetic manner (Silveri & Spear, 2004). This increase in response to ethanol with age mirrors those seen in sensitivity to motor-impairing, sedative, and hypothermic effects of ethanol (Silveri & Spear, 1998; White, Truesdale et al., 2002). A family history of alcoholism, in particular, a paternal history of abuse increases the likelihood that the children will suffer from an alcohol abuse disorder (Adinoff et al., 1991; Chassin, Pitts, & Prost, 2002; Dai, Thavundayil, & Gianoulakis, 2002). Adolescent boys with a familial history (father with alcohol use disorders) have lower salivary cortisol levels (Moss, Vanyukov, & Martin, 1995), lower basal ACTH, and less stress-induced increases in ACTH release than those without a family history (Dai et al., 2002). Sons of alcoholic fathers exhibit reduced startle reflexes (Zimmermann, Spring, Wittchen, & Holsboer, 2004) and increased AVP response when the HPA axis is activated than in those without a family history of alcoholism

(Zimmermann, Spring, Wittchen, Himmerich et al., 2004). Hyporeactivity to stress in this group predicts future substance abuse (Moss, Vanyukov, Yao, & Kirillova, 1999). Taken together, these data highlights adolescence as a period during which few negative symptoms of ethanol withdrawal are experienced and underlines the fact that a family history of alcohol abuse may begin to predict the adolescents' behavior. In light of the relationship between HPA axis function and varying levels of osmoregulation, the abundance of taurine, a powerful osmoregulator, in the adolescent system may attenuate the negative withdrawal symptoms experienced in adulthood.

Taurine

Taurine (2-aminoethanesulfonic acid), a small sulfur-containing β-amino acid, is 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) and is the most abundant intracellular amino acid in humans (Lourenco & Camilo, 2002). Taurine is a semi-essential amino acid for adults, but is essential for infants and newborns who cannot fully synthesize the amino acid before weaning (Aerts & Van Assche, 2002; Chesney et al., 1998; Huxtable, 1989, 1992; Rassin et al., 1978). In adults, dietary taurine intake provides the majority of circulating taurine and is supplemented by 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 effects such as; facilitating normal neurodevelopment, membrane stabilization, antiarrhythmic actions, and neuroprotection (El Idrissi, Harris, & Trenkner, 1998; J. Y. Wu et al., 2000). Given taurine's protective roles within the body, the higher levels of taurine found in highly excitable tissues, such

as the brain and heart, seem logical (Lourenco & Camilo, 2002). 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 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 taurine tissue content results from the slowing of endogenous taurine production in adults (Banay-Schwartz et al., 1996; Benedetti et al., 1991; Dawson, Liu et al., 1999; Lourenco & Camilo, 2002; T. J. Miller et al., 2000) with concentrations continuing to decrease into old age (Dawson, Liu et al., 1999). This pattern of age dependent decrease in taurine levels is found across many species; humans, monkeys, mice, rabbits, rats, and insects (Huxtable, 1989).

Taurine levels are high during development because it is necessary for normal neural development (Franconi et al., 2004; T. J. Miller et al., 2000; Rentería, Johnson, & Copenhagen, 2004; Saransaari & Oja, 2000; Sturman, 1977). A deficiency of taurine during development in animal models results in increased rates of epilepsy, learning disabilities, retarded growth, impaired cerebellar development, and retinal degeneration (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). When neurons were incubated in a medium containing higher levels of taurine than controls, neuronal proliferation was greatly increased (X. C. Chen 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, by virtue of its ability to modulate the flow of calcium ions during depolarization, functions to stabilize the neuronal membranes in the face of increasing levels of glutamate (Lourenco & Camilo, 2002). It is possible that the high endogenous levels of taurine found in adolescents acts not only to facilitate normal development, but also to protect them from potential perturbations of the brain that would impede normal growth. Taurine's modulation of both osmolarity and glutamate-induced excitability makes this amino acid a likely candidate for countering ethanol-induced pathologies, particularly within highly osmosensitive neural tissues. Taurine's action, during ethanol intoxication, may represent a protective response of neural cells to the toxic effects produced by ethanol.

Taurine and Ethanol

Acute ethanol injections result in significant taurine release from the rat nucleus accumbens (Dachour, Quertemont, & De Witte, 1996; Quertemont, Devitgh, & De Witte, 2003) and hippocampus (Lallemand, Dachour, Ward, & De Witte, 2000) as measured i*n vivo*. Although the release of taurine in response to acute ethanol exposure seems to function as an osmotic or excitable countermeasure, extracellular taurine could potentially protect against some of the potent pharmacological effects of alcohol intoxication. Since taurine functions as a weak $GABA_A$ receptor agonist (del Olmo, Bustamante, del Rio, & Solio, 2000; Hussy, Deleuze, Pantaloni, Desarmenien, & Moos, 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, in that mice treated for 10 days with taurine show no memory or motor coordination deficits when treated with ethanol 30 minutes before testing (Vohra & Hui, 2000).

During ethanol withdrawal, taurine release co-occurs with the steady rise in extracellular glutamate within the rat nucleus accumbens (Dachour et al., 1996) and from astrocytes (Allen, Mutkus, & Aschner, 2002). Subsequent episodes of ethanol withdrawal exacerbate the increase in both taurine and glutamate release (Dachour & De Witte, 2002), in agreement with the kindling effect of repeated withdrawal episodes (Becker et al., 1997; Becker et al., 1998). Remarkably, an intraperitoneal (i.p.) taurine injection administered after ethanol removal completely alleviates the elevation of nucleus accumbens glutamate release during chronic ethanol withdrawal (Dachour & De Witte, 2000). By inhibiting glutamate-induced increased intracellular calcium levels, taurine attenuates glutamate-induced membrane depolarization (Sakurai et al., 2003; H. Wu et al., 2005), protecting the brain from excitotoxic insults (Saransaari & Oja, 2000). Experimentally-induced systemic hypertonicity dampens the ethanol withdrawal-induced taurine release in the brain (Quertemont et al., 2003) illustrating that taurine's effects are not entirely the result of changes in neurotransmitter function, but also involve osmotic changes. Taurine attenuates ethanol withdrawal-induced increases in extracellular glutamate by (1) modulating intracellular calcium levels (Molchanova, Oja, & Saransaari, 2005; Zhao, Huang, & Cheng, 1999), (2) being released in response to glutamate (Dachour & De Witte, 2000; El Idrissi et al., 1998), and (3) regulating osmolarity (Mongin, Cai, & Kimelberg, 1999; Olson & Li, 2000; Quertemont et al., 2003). These findings suggest that although the excitation associated with ethanol exposure alone can

elicit a taurine response, systemic and cellular osmolarities also seem to influence this taurine mechanism.

A potential explanation for the age-dependent response to ethanol intoxication is the high adolescent brain taurine concentrations compared to adults (Hayes & Sturman, 1981). Given that taurine levels decrease with age (Banay-Schwartz et al., 1996; Dawson, Pelleymounter, Cullen, Gollub, & Liu, 1999; T. J. Miller et al., 2000) it is possible, that the effects of taurine on ethanol intoxication in adults are amplified in adolescents. Adolescent rats have been found to be more sensitive to taurine's analgesic effects than adults (Serrano et al., 2002), the 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. In adults, acquisition of a passive avoidance response is impaired with oral administration of taurine (Sanberg & Fibiger, 1979). This may be due to taurine's anxiolytic effects which reduce the stress response necessary for this type of learning. It is likely then, that taurine's attenuation of ethanol withdrawal symptoms in combination with the increased levels of taurine found in the developing brain lends support to its role in protecting adolescents from ethanol withdrawal-induced pathologies.

Taurine and the HPA axis

Withdrawal from chronic ethanol induces pervasive alterations in the HPA axis which likely originate in an area that contains abundant intracellular taurine pools, the hypothalamic-neurohypophysial system (Decavel & Hatton, 1995; Palkovits et al., 1986; Vellan, Gjessing, & Stalsberg, 1970). Within the osmosensitive hypothalamus, persistent ethanol-induced dehydration (during intoxication) potentiates future AVP responses and

HPA axis activation by: inducing increases in extracellular osmolarity (Ludwig, Callahan, Neumann, Landgraf, & Morris, 1994) and/or dehydration-based up-regulation of glutamate receptors (Decavel & Curras, 1996; Meeker, 2002). Ethanol exerts additional dehydrating effects through its inhibition of calcium channels within neurohypophysial nerve terminals which results in reduced plasma AVP release (Taivainen et al., 1995; X. M. Wang et al., 1991). As a result of ethanol clearance (removal of inhibition), an overactivated, compensatory AVP response (overhydration) can manifest as withdrawal-induced cerebral swelling, corresponding to hyperexcitable states (Collins et al., 1998; Eisenhofer et al., 1985; Mander et al., 1988) and anxious behavior (Amaya et al., 2001; Gillies et al., 1982; Silva et al., 2002).

Taurine exerts neuroprotective effects on the HPA axis in much the same manner as it protects against ethanol effects, via osmoregulation and calcium modulation. Within SON and hypophysial tissues, taurine's high concentration (Miyata, Matsushima, & Hatton, 1997), localization within glial cells (Decavel & Hatton, 1995; Miyata et al., 1997) , and responsiveness to osmolar fluctuations (Hussy et al., 1997; Ludwig et al., 1994) indicate taurine's role in modulating systemic osmolarity. Taurine released from glial cells in the SON, acts upon neuronal glycine receptors, and functions as a primary regulator of vasopressinergic signals (Hussy et al., 1997; Voisin & Bourque, 2002). In addition to its regulatory capacity in the SON, taurine responds quickly to osmotic changes within the neurohypophysis (Miyata et al., 1997), with only small reductions in osmolarities sufficient to provoke significant increases in extracellular taurine levels (Hussy et al., 2001). Taurine's regulation of AVP levels aids in the regulation of the HPA axis given that the release of ACTH is greatly potentiated by AVP (Gillies et al.,

1982; Rivier & Vale, 1983). Evidence for taurine's participation in modulating anxiety shows that ten minutes of forced swimming induces both a significant release of plasma corticosterone (Jiang et al., 2004) as well as a significant increase in taurine release from the SON (Engelmann et al., 2001). This response of taurine to osmotic changes may prevent ethanol withdrawal-induced hyperactivation of the HPA axis by reducing the amount of AVP acting on the adenohypophysis and ultimately reducing the amount of ACTH being released.

Recent studies provide compelling evidence of taurine's role in pituitary neuroendocrine control in that endogenously released AVP stimulates taurine efflux from pituicytes (Rosso, Peteri-Brunback, Poujeol, Hussy, & Mienville, 2004). This release of taurine functions as negative feedback to inhibit further AVP release through the modulation of calcium influx (Hussy et al., 2001). Vasopressin has been shown to increase calcium signals in the pituitary which result in taurine release from pituicytes (Rosso et al., 2004). The concurrent release of AVP and taurine may reflect taurine's role in regulating vasopressin-induced increases in calcium activity. Glucocorticoids delay the release of intracellular calcium and increase the levels of extracellular glutamate, resulting in excitotoxicity during withdrawal from ethanol (Mulholland et al., 2005; Prendergast & Little, 2007). This hyperexcitability may be attenuated by taurine, which helps prevent increases in intracellular calcium (Dachour & De Witte, 2000; Sakurai et al., 2003). Further inhibition of the overactive HPA axis may be the result of taurine's role as a partial agonist for both $GABA_A$ and glycine receptors (Ghavanini, Mathers, & Puil, 2005; Paula-Lima, De Felice, Brito-Moreira, & Ferreira, 2005) which may inhibit further release of CRF and AVP (Engelmann et al., 2001; Welt et al., 2006).

Behavioral support for taurine's role in attenuating anxiety results from the efficacy of exogenous taurine administration to decrease anxious behavior on the elevated plus maze (EPM) (S. W. Chen et al., 2004; Helfand, Zalud, & Diaz-Granados, 2006) as well as attenuating anxious behavior on the open field, hole-board, and social behavior tests (Kong et al., 2006).

Adolescents have higher endogenous levels of taurine than adults which may result in the magnification of the above described attenuation of ethanol-withdrawal-induced anxiety. Testing has revealed an age-related shift in anxious behavior on the EPM, with aged rats displaying significantly more anxious behaviors than young rats (Bessa, Oliveira, Cerqueira, Almeida, & Sousa, 2005; Doremus et al., 2004). Furthermore, in humans the predominant population receiving anxiolytic medication is the elderly (Craig et al., 2003), a population with the lowest endogenous taurine concentrations. Thus, the age-dependent shift in behavior may be due to the naturally lower levels of taurine found in adults.

Given the evidence of taurine's ability to reduce anxiety, it seems likely that the high level of taurine present in adolescence may prevent the behavioral expression of ethanol withdrawal-induced anxiety. To test this hypothesis, guanidinoethane sulfonate (GES), a specific taurine transporter (TauT) antagonist, which is frequently used to examine potential physiological decrements that result from taurine deficiencies (Izumi et al., 1985; Maar et al., 1995; Nilsson, Lehmann, & Hansson, 1989) was administered. The specific sodium-dependent TauT is widely distributed in mouse (Q.-R. Liu, López-Corcuera, Nelson, Mandiyan, & Nelson, 1992), rat (K. E. Smith et al., 1992), dog (Uchida et al., 1992) and man (Jhiang et al., 1993). Osmosensitive areas, such as cardiac

tissue (Chapman, Suleiman, & Earm, 1993) and glial cells (Tchoumkeu-Nzouessa & Rebel, 1995) express the TauT. Hypertonic conditions increase TauT mRNA levels (Uchida et al., 1992), indicating taurine's mechanism of action in exerting its osmoregulatory function. There are two isoforms of the transporter, TauT-1 and TauT-2 (Pow, Sullivan, Reye, & Hermanussen, 2002). Using specific antibodies designed against the two subtypes, Pow and colleagues (2002) demonstrated distinct immunoreactivity for TauT within the brain. TauT-1 is localized primarily within the cerebellum, and TauT-2 shows wider distribution, with heavier staining in the hippocampus, cerebellum, and olfactory bulb. During development, the cells rely solely on the taurine transporter to bring in taurine (Fujita, Shimada, Wada, Miyakawa, & Yamamoto, 2006). GES, by competing with uptake sites on the TauT, produces a significant reduction in tissue taurine levels, while not affecting the uptake of other amino acids (Huxtable, 1989). In mice, administration of 1% GES in drinking water decreases the anti-epileptic actions of phenobarbital and phenytoin in response to electroshock seizures (Izumi et al., 1985). In rodent studies, investigators widely use 1% GES solution in drinking water as an effective method to deplete tissue taurine pools (De Luca, Pierno, & Camerino, 1996; Hiramatsu, Edamatsu, Kabuto, Higashihara, & Mori, 1989; Huxtable, 1989; Yan & Huxtable, 1996). Likewise, this protocol was followed by adding 1% GES (Toronto Research Chemicals) to the drinking water of animals assigned to the GES experimental groups (GE & GC).

Primary Investigative Goal

The current study is designed to investigate the role of taurine in the attenuation of ethanol withdrawal-induced anxiety in adolescence. In light of taurine's primary role

as an osmoregulator, and calcium modulator (Huxtable, 1992) and the large accumulations in the hypothalamus and pituitary (Decavel & Hatton, 1995; Miyata et al., 1997; Vellan et al., 1970), it is reasonable to suggest that this amino acid functions as a neuroprotector. It is therefore likely that the high endogenous levels of taurine found in adolescents would likely increase taurine's ability to protect the brain, in particular from the anxiety induced by withdrawal from chronic ethanol exposure. Therefore, the overarching hypothesis is that lowered levels of taurine (depleted via GES treatment) in adolescent mice will result in a greater expression of ethanol withdrawal-induced anxiety as measured behaviorally (on the EPM) and physiologically (plasma corticosterone levels).

CHAPTER TWO

Materials and Methods

Animal Model and Experimental Design

This study utilized 64 male C3H/HeJ mice (Jackson Laboratories, Bar Harbor, ME). Animals arrived at postnatal day (PD) 21 and were allowed to habituate to the Baylor University vivarium for six days. Mice were housed four to a cage under a 12 hr dark/light cycle, lights on at 08:00 and off at 20:00, with continuous access to rodent lab chow and water. On the sixth day, PD 27, 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 water. The following day, PD 28, the animals were randomly assigned to one of six treatment groups. Animals assigned to the GE-0 hr group were pretreated with GES, exposed to ethanol, and sacrificed via decapitation for the collection of trunk blood, upon removal from the ethanol chamber, (for the assessment of blood ethanol concentrations (BEC)). Animals assigned to the CE-0 hr group were pretreated with water, exposed to ethanol, and sacrificed via decapitation for the collection of trunk blood, upon removal from the ethanol chamber, (for the assessment of BEC). The GES and ethanol exposed group (GE) was pretreated with GES, exposed to ethanol, and tested on the elevated plus maze (EPM). The control ethanol group (CE) was pretreated with water, exposed to ethanol, and tested on the EPM. The GES control group (GC) was pretreated with GES, not exposed to ethanol, and tested on the EPM. The control-control group (CC) was pretreated with water, not exposed to ethanol, and tested on the EPM. Animals exposed to GES were given ad lib

access to a 1% GES and de-ionized (DI) water solution and the non-GES exposed groups had ad lib access to DI water throughout pretreatment, ethanol exposure, and testing. All subjects were weighed and their liquid consumption was measured daily through both pretreatment and ethanol exposure periods.

Route of Continuous Ethanol Administration

Individually housed mice were placed in a Plexiglas® chamber (28" x 38" x 13") (Plas Labs, Lansing, MI) modified after Goldstein (1972). Ethanol was sent to a volatilizing flask by an Agilent HPLC pump (154 μl/min) (Agilent Technologies, Santa Clara, CA) and delivered to the chambers via air pumps at a rate of 7 L/min. These parameters resulted in an ethanol concentration of 12.0 ± 0.5 mg/L air which yielded BECs between 53 and 166 mg/dl. At the initiation of ethanol exposure, animals were given a 1.6 g/kg loading dose of ethanol (8% w/v; IP) combined with a 1mmol/kg dose of the alcohol dehydrogenase inhibitor pyrazole to stabilize BECs. During the 64 hours of continuous ethanol exposure, animals were given a pyrazole booster (1mmol/kg) at hour 24 and 48. Concurrently, control animals were housed individually in the same room with access to untreated air and received identical treatment to that of the ethanol exposed animals. In these groups however, the initial ethanol dose was substituted with physiological saline and pyrazole, in this manner, the possible pyrazole-related effects were controlled for (Crabbe *et al.*, 1981). Body weights and water intake were recorded for all experimental groups prior to the administration of pyrazole and/or ethanol in order to monitor the overall health of the animals. During ethanol exposure and withdrawal, animals had free access to food and liquid as described above.

Testing Apparatus: Elevated Plus Maze

The EPM was comprised of two opposing open arms (30 x 5 cm) and two closed arms (30 x 5 x 15 cm), which join at a square central area (5 x 5 cm) to form a plus sign. The apparatus was elevated to a height of 50 cm above the floor. The maze floor and arms are constructed from white acrylic. Testing was conducted during the animals' dark cycle under indirect dim red light (S. W. Chen et al., 2004; Rodgers, Cole, Aboualfa, & Stephenson, 1995; Rodgers, Johnson, Carr, & Hodgson, 1997).

Elevated Plus Maze Testing Procedure

Upon the completion of ethanol exposure the animals were moved to the testing room and allowed to habituate to the new room in order to minimize the stress of movement immediately prior to testing. Anxiety testing was conducted at eleven hours post withdrawal beginning at 17:00 pm, during early – middark phase (Mikics et al., 2005; Rasmussen, Mitton, Green, & Puchalski, 2001; Rodgers et al., 1996). The testing period lasted five minutes. The testing time point corresponds to the previously reported peak withdrawal (Becker et al., 1998; Pich et al., 1995). All testing sessions were video-taped by an overhead camera and later analyzed by a researcher blind to the treatment conditions (the same researcher scored all testing sessions) to ensure the accurate recording of movement and behavior. After the testing session, the animal was returned to its home cage and placed outside the testing room. Immediately after being placed outside the testing room, the animals were sacrificed and trunk blood collected for the analysis of corticosterone (Koob, 1999; Menzaghi et al., 1994). Following each testing session the EPM was thoroughly cleaned with water and dried with clean paper towels.

Elevated Plus Maze Behavioral Analysis

The videotapes were scored for standard spatiotemporal measures as well as a number of ethologically derived behavioral measures. The standard parameters are open and closed arm entries (all four paws onto an arm) and time spent on each arm. Ethologically derived behavioral measures, as described and verified by Rodgers and Johnson (1995), are comprised of exploratory or risk assessment behavior, specifically protected head dips. Protected head dips are defined as head and shoulder movement over the edge of the maze from the relative safety of the center square or around the wall of a closed arm (S. W. Chen et al., 2004). The ethological data was recorded while the animal was on the maze and confirmed via video analysis.

Sampling and Determination of Ethanol Concentrations

Blood and chamber air ethanol concentrations were determined in each study to ensure that the level of intoxication remained constant and appropriate through each study. Chamber air samples were collected and analyzed daily, to ensure consistency in ethanol vapor saturation over the 64 hour exposure. Monitoring began one day prior to the initiation of exposure and was done on each subsequent day, before the pyrazole booster, and prior to the removal of the animals from the chamber. Upon removal of the animals from the exposure chamber, the GES-0 hr animals and the CE-0 hr animals were sacrificed via decapitation and trunk blood was collected in Becton, Dickinson (BD) Microtainer plasma separating tubes (PST) tubes with Lithium Heparin kept at 4° C for analysis of BEC. Blood samples (10 µl) were removed and diluted 50:1 with chilled perchloric acid (3.4% v/v). The samples were vortexed and centrifuged (4° C) at 12,000 x g. The 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 a Beckman DU-530 spectrophotometer and were expressed as g/dl.

Corticosterone Assay

Corticosterone levels were determined for all animals. For the 0 hr animals (GE-0 hr, CE-0hr), plasma separated from the previously collected blood was used for the corticosterone assay. Immediately following each EPM testing session, the animal was removed from the testing apparatus, placed in its cage, and the individual cage was placed outside the testing room. The animal was moved to another part of the laboratory and sacrificed via decapitation for the collection of trunk blood in BD Microtainer PST tubes with Lithium Heparin. Corticosterone was measured in duplicate plasma samples by using an enzyme immunoassay kit (Assay Designs, Ann Arbor, MI) and the protocol used was in accordance with the manufacturer's specifications. The sensitivity of the assay was 27 pg/ml; all samples were run on microplates from the same batch. The optical density was measured at 405 nm with a Benchmark Plus Microplate Reader (Bio-Rad, Hercules, CA).

Statistical Analysis

An a priori power analysis was conducted, to determine the number of subjects needed. For this study, with six treatment groups, a desired power of 0.8, and an *f* of 0.40, the use of at least 54 animals total, nine per treatment group (minimum) was necessary (Kirk, 1995). Behavioral data were reported as percent time spent on the open arm (open arm time/300 sec x 100; %), percent of open arm entries (open arm entries/total entries x

100; %), the number of closed arm entries and the total number of arm entries made. Data was analyzed by one and two-way analysis of variance (ANOVA) using JMP (v. 6.0) statistical software for PC (SAS Institute Inc.). Post hoc analysis was done with Tukey's HSD. Reported data are expressed as mean ± standard error of the mean (*SEM*).

CHAPTER THREE

Results

Pretreatment

Data for pretreatment comparisons were analyzed based on the type of liquid administered (GES or water). Given that pretreatment data was collected before ethanol exposure the type of liquid consumed is the only between group difference.

Liquid Consumption

Liquid consumption was measured daily throughout the seven day pretreatment period. The mean daily intake was normalized to animal weight and is expressed as ml/g/day (Table 1). There was no significant difference in liquid consumption between pretreatment groups GES or water ($t(62) = 0.704$, $p = 0.405$).

Weight Gain

The animals were divided into treatment groups and weighed at the initiation of pretreatment. There was no difference in initial weights between groups ($t(62) = 0.0002$, $p = 0.988$). Throughout pretreatment the GES treated group gained significantly less weight than water treated controls ($t(62) = 8.722$, $p < 0.005$) (Table 1).

Table 1. Pretreatment Group Descriptive Statistics

Pretreatment Group	Liquid Consumption (ml/g/day)	Initial Weight (g)	% Weight Gain (g)
GES	0.263 ± 0.007	17.59 ± 0.289	11.37 ± 0.774
Water	0.257 ± 0.004	17.59 ± 0.280	14.83 ± 0.882

Ethanol Exposure

Liquid Consumption

Figure 1 illustrates the mean liquid consumption expressed as (ml/g/day) throughout the 64 hours of exposure to ethanol. A two-way ANOVA revealed significant main effects of both the type of liquid consumed ($F(1,60) = 4.77$, $p < 0.05$, $\omega^2 = 4.5\%$) and ethanol exposure ($F(1,60) = 40.25$, $p < 0.0001$, $\omega^2 = 38.26\%$) indicating that both variables had an effect on mean liquid consumption. Ethanol exposure resulted in significantly less liquid consumption in both ethanol exposed groups, compared to air exposed counterparts. While there was not a statistically significant difference between means of the GC and CC groups or between the GE and, CE groups, treatment with GES resulted in slightly higher mean liquid consumptions than their water treated counterparts. No significant interaction between the type of liquid consumed and ethanol exposure was found ($F(1, 60) = 1.49$, $p = 0.227$, $\omega^2 = 1.42\%$).

Table 2. Ethanol Exposure Descriptive Statistics

Treatment Groups	Liquid Consumption (ml/g/day)	% Weight Loss (g)	N
GE	0.109 ± 0.003	3.754 ± 0.449	22
CE	0.097 ± 0.017	2.973 ± 0.520	22
GC	0.199 ± 0.025	5.902 ± 1.035	10
CC	0.159 ± 0.024	1.479 ± 1.289	10

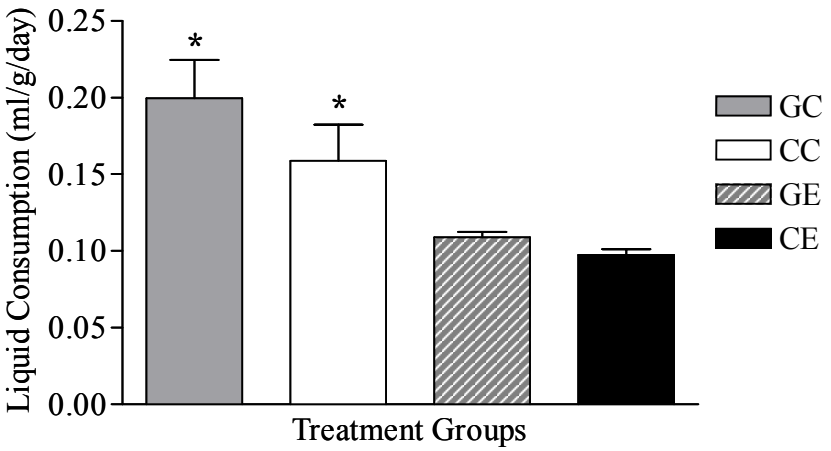

Figure 1. Mean liquid consumption (ml/g/day) (+*SEM*) for GES and ethanol exposed (GE) (n=22), water and ethanol exposed (CE) (n=22), GES and air exposed (GC) (n=10), water and air exposed (CC) (n=10). *Significantly different (p<0.05) from GC and CC groups

Weight Loss

A two-way ANOVA revealed a significant main effect of the type of liquid consumed ($F(1,60) = 11.49$, $p < 0.001$, $\omega^2 = 15.72\%$) indicating, treatment with GES increased the percent of weight lost. There was a significant interaction between exposure to ethanol and the type of liquid consumed ($F(1,60) = 5.62$, $p < 0.05$, $\omega^2 = 2.1\%$). Given this interaction, uncertainty still exists as to whether or not GES alone accounted for the increased weight loss in the GES-treated groups. The presence of this interaction signals that the ANOVA provides only a partial explanation of the results and that the increased weight loss may be due to chance sampling error (Kirk, 1995). Exposure to ethanol did not significantly effect the percent weight lost ($F(1,60) = 0.18$, $p = 0.672$, $\omega^2 = 0.25\%$).

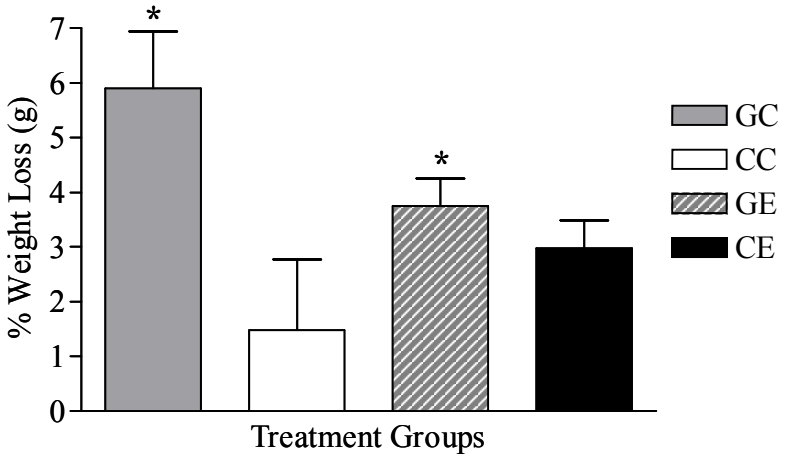

Figure 2. Mean percent weight loss (+*SEM*) for GES and ethanol exposed (n=22), water and ethanol exposed (n=22), GES and air exposed (n=10), water and air exposed (n=10). *Significant main effect of the type of liquid consumed ($p<0.05$) GES-treatment (GC, GE) increased percent weight lost compared to water-treated groups (CC, CE).

Blood Ethanol Concentration

Treatment with GES significantly affected blood ethanol concentration (BEC). For this measure, in order to minimize the potential for type 2 error, failure to reject a false null hypothesis, a more conservative level of significance was adopted and set at $p < 0.01$ (Kirk, 1995). Within the ethanol-exposed animals, those treated with GES had significantly lower BECs (0.093 ± 0.006) than those treated with water (0.116 ± 0.009) ($t(16) = 4.17$, $p = 0.058$).

Behavioral Analysis

Percent Open Arm Time

The percent of the testing period that each group spent on the open arms of the EPM are depicted in Figure 3. Each animal's individual score on the EPM was calculated as follows [open time (s) / total time on EPM (300 sec)]. A two-way ANOVA comparing the effects of the type of liquid consumed and ethanol exposure on the percent of time spent on the open arms revealed a nearly significant effect of the type of liquid

consumed ($F(1,42) = 3.99$, $p = 0.052$, $\omega^2 = 2.8\%$), treatment with GES modestly reduced the percent time spent on the open arms in the GE and GC groups compared to their water treated counterparts, CE and CC groups. A significant main effect of ethanol exposure was found ($F(1,42) = 96.55$, $p < 0.001$, $\omega^2 = 67.66\%$), as animals in both the ethanol exposed groups (GE, CE) spent significantly more time on the open arm than animals in the air exposed groups (GC, CC) ($p < 0.05$). No significant interaction was found between the type of liquid consumed and ethanol exposure ($F(1,42) = 0.44$, $p = 0.509$, $\omega^2 = 0.31\%$).

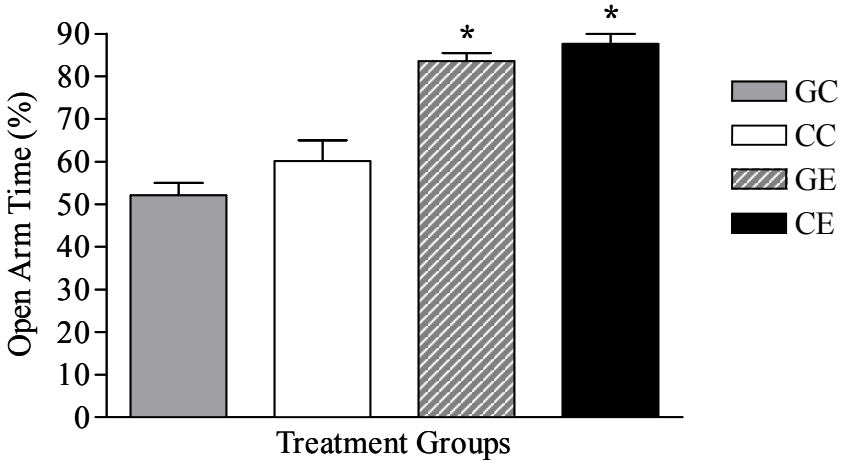

Figure 3. Percent open arm time (%) (+*SEM*) for GES and ethanol exposed (GE) (n=13), water and ethanol exposed (CE) (n=13), GES and air exposed (GC) (n=10), water and air exposed (CC) (n=10). *Significantly different ($p<0.05$) from GC and CC groups.

Percent Open Entries

A two-way ANOVA comparing the influence of the type of liquid consumed and exposure to ethanol revealed no significant influence of the type of liquid consumed on the percent of open arm entries ($F(1,42) = 1.43$, $p = 0.238$, $\omega^2 = 2.96\%$) data shown in Figure 4. There was however a significant main effect of ethanol exposure ($F(1,42) = 4.79$, $p < 0.05$, $\omega^2 = 9.9\%$) confirmed by the moderate increase in open arm entries

performed by the ethanol exposed groups (GE, CE) compared to the air exposed groups (GC, CC). No significant interaction between the type of liquid consumed and ethanol exposure was found ($F(1,42) = 0.33$, $p = 0.568$, $\omega^2 = 0.68\%$).

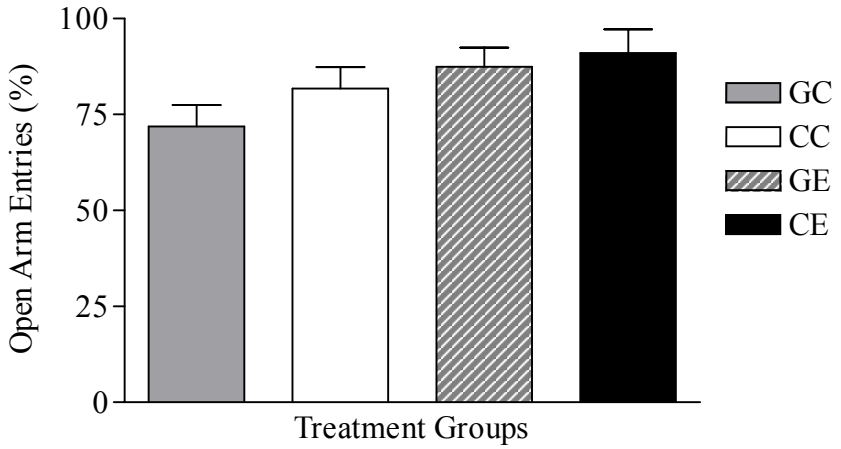

Figure 4. Percent open arm entries (%) (+*SEM*) for GES and ethanol exposed (GE) (n=13), water and ethanol exposed (CE) (n=13), GES and air exposed (GC) (n=10), water and air exposed (CC) (n=10). No significant between group differences.

Number of Closed Arm Entries

A two-way ANOVA comparing the effects of type of liquid consumed and ethanol exposure on the number of closed arm entries performed revealed no significant effect of the type of liquid consumed ($F(1,42) = 2.04$, $p = 0.161$, $\omega^2 = 3.05\%$) data shown in Figure 5. A significant main effect of ethanol exposure was found ($F(1,42) = 22.21$, $p < 0.001$, $\omega^2 = 33.19\%$) in that the ethanol exposed groups (GE, CE) performed fewer closed arm entries than the air exposed groups (GC, CC). No significant interaction between the type of liquid consumed and ethanol exposure was found ($F(1,42) = 0.99$, $p = 0.324$, $\omega^2 = 1.49\%$).

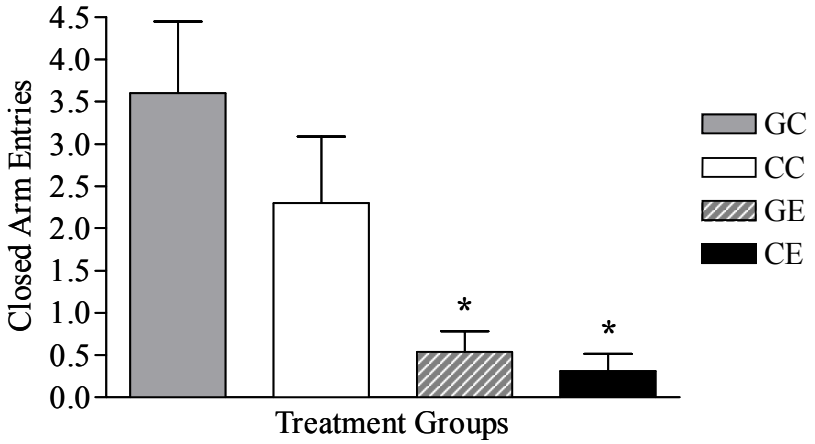

Figure 5. Number of closed arm entries (+*SEM*) for GES and ethanol exposed (GE) (n=13), water and ethanol exposed (CE) (n=13), GES and air exposed (GC) (n=10), water and air exposed (CC) (n=10). *Significantly different (p<0.05) from air exposed groups GC and CC groups.

Total Entries

Figure 6 represents the total number of both open and closed arm entries made by each group. A two-way ANOVA comparing the effects of the type of liquid consumed and ethanol exposure revealed a significant main effect of ethanol exposure ($F(1,42) = 134.70$, $p < 0.0001$, $\omega^2 = 75.29\%$) with the ethanol exposed groups (GE, CE) performing fewer arm entries that the air exposed groups (GC, CC). No significant effect was found of the type of liquid consumed ($F(1,42) = 2.04$, $p = 0.161$, $\omega^2 = 1.14\%$) and no significant interaction effect was found ($F(1,42) = 0.05$, $p = 0.826$, $\omega^2 < 0.1\%$).

Percent Protected Head Dips

The percent of protected head dips performed during testing is illustrated in Figure 7. A two-way ANOVA comparing the effects of type of liquid consumed and exposure to ethanol on the percent protected head dips performed revealed no significant effect of type of liquid consumed ($F(1,42) = 0.08$, $p = 0.160$, $\omega^2 = 0.13\%$). A significant

main effect of ethanol exposure was found ($F(1,42) = 15.64$, $p < 0.001$, $\omega^2 = 26.1\%$), as the ethanol exposed groups (GE, CE) performed fewer head dips overall than the air exposed groups (GC, CC). No significant interaction between the type of liquid consumed and ethanol exposure was found ($F(1,42) = 2.04$, $p = 0.160$, $\omega^2 = 3.41\%$).

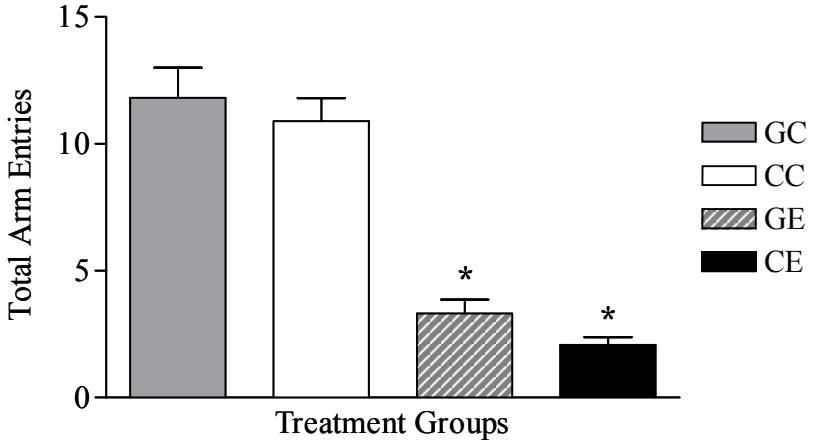

Figure 6. Total number of entries (+*SEM*) for GES and ethanol exposed (GE) (n=13), water and ethanol exposed (CE) (n=22), GES and air exposed (GC) (n=10), water and air exposed (CC) (n=10). *Significantly different (p<0.05) from air exposed groups GC and CC.

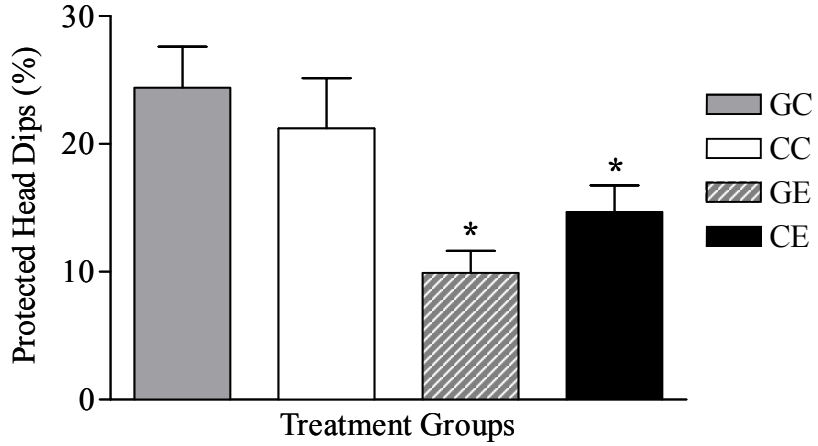

Figure 7. Percent protected head dips (%) (+*SEM*) for GES and ethanol exposed (GE) (n=22), water and ethanol exposed (CE) (n=22), GES and air exposed (GC) (n=10), water and air exposed (CC) (n=10). *Significantly different (p<0.05) air exposed groups GC and CC.

Plasma Corticosterone

Figure 8 shows the plasma corticosterone levels for each behaviorally tested group; 0 hr, although not behaviorally tested were added for comparison. A one-way ANOVA revealed a significant difference in plasma corticosterone levels between groups, ($F(5,58) = 13.798$, $p < 0.001$). *Post-hoc* analysis, using Tukey-Kramer HSD, showed that the GE group had significantly higher plasma corticosterone levels than any of the other groups ($p < 0.05$). No statistically significant difference was found between the GE-0 hr and CE-0 hr groups; however, both were significantly lower than the GE and CE groups ($p < 0.05$). No statistically significant differences were found in the plasma corticosterone levels between CE, GC, and CC groups. A two-way ANOVA comparing the effects of the type of liquid consumed and ethanol exposure on plasma corticosterone levels revealed a significant effect of the type of liquid consumed ($F(1,58) = 9.09$, $p < 0.05$, $\omega^2 = 7.16\%$), indicating that GES treatment elevated plasma corticosterone concentrations. Ethanol exposure also had a significant effect on plasma corticosterone levels ($F(2,58) = 27.69$, $p < 0.001$, $\omega^2 = 43.62\%$). The effect of ethanol depended upon the time point of plasma collection; the hour zero groups (GE-0 hr, CE-0 hr) had the lowest corticosterone levels, while the ethanol exposed groups that went through withdrawal (GE, CE) had the highest levels. No significant interaction between the type of liquid consumed and ethanol exposure was found ($F(2,58) = 1.35$, $p = 0.267$, $\omega^2 = 2.13\%$).

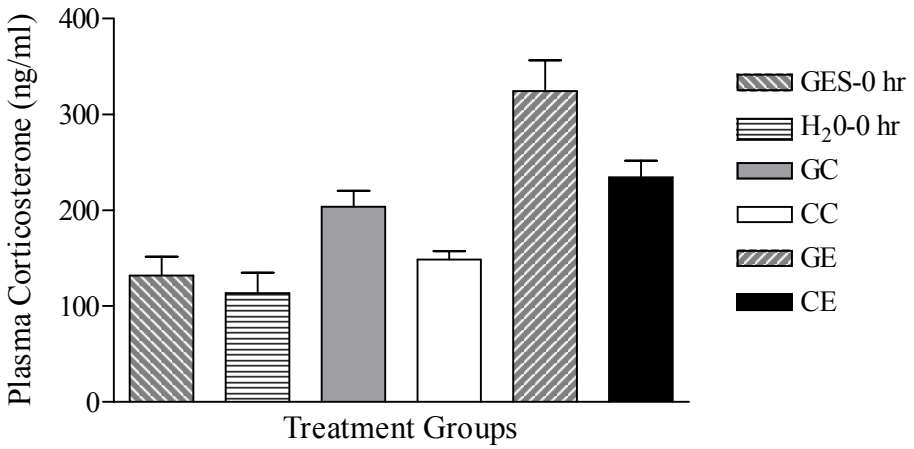

Figure 8. Plasma corticosterone (ng/ml) (+*SEM*) for GES and ethanol exposed (GE) (n=13), water and ethanol exposed (CE) (n=13), GES and air exposed (GC) (n=10), water and air exposed (CC) (n=10), GES and ethanol 0 hr. (GE-0hr) (n=9), water and ethanol 0 hr.(CE-0hr) (n=9). *Significantly different ($p<0.05$) from all other groups (CE, GC, CC, GE-0hr, CE-0hr). **Significantly different ($p<0.05$) from GE and 0 hr (GE-0hr and CE-0hr) groups.

CHAPTER FOUR

Discussion

Pretreatment Liquid Consumption and Weight

There was no difference in the amount of liquid consumed between groups (GES or water treated), indicating that the presence of GES did not influence liquid intake. Given the successful use of a 1% GES solution by other researchers (De Luca et al., 1996; Hiramatsu et al., 1989; Izumi et al., 1985; Nilsson et al., 1989) this was a predicted outcome. Despite equivalent weights prior to pretreatment (Table 1), the GES treated group showed a significant reduction in weight gain during pretreatment compared to their water treated counterparts. The absence of taurine during the critical period of development may provide an explanation for this finding; given that taurine promotes normal development (Chesney et al., 1998; Huxtable, 1993; Sturman, 1993), maintains osmolarities and hydrational states (Hussy, Deleuze, Desarmenien, & Moos, 2000; T. J. Miller et al., 2000; Olson & Li, 2000; Pasantes-Morales, Quesada, & Moran, 1998), and modulates stress levels (S. W. Chen et al., 2004; Engelmann, Landgraf, & Wotjak, 2003; Hayes & Sturman, 1981). Overall, a decrease in taurine's regulatory control in any one of these factors may account for the observed differences in weight gain.

Ethanol Exposure

Animals exposed to 64 hours of ethanol (GE, CE) showed significantly reduced liquid consumption, as compared to those exposed to air only (GC, CC). This decrease in liquid intake, in the ethanol exposed groups most likely results from ethanol-induced sedation. During the 64 hour exposure, the GC showed significantly greater weight loss

and both GES-treated groups (GE, GC) showed modestly elevated liquid intake (not significant) than those treated with water. Similar to the effects seen during pretreatment, GES-treated animals showed a greater decrease in body weight than those that consumed water. While chronic ethanol exposure typically results in decreases in body weight (Krystal & Tabakoff, 2002; Silvers et al., 2003; Wolf, 2006), the GC group, (exposed to GES and air), unexpectedly showed the largest decrease in body weight over the 64 hour period. With a reduction in taurine levels, the animals in GC group may have experienced an elevated state of anxiety when faced with the stress-inducing event of daily injections (S. W. Chen et al., 2004; Engelmann et al., 2003; Sanberg & Fibiger, 1979). In contrast, the GE group may have been protected from this elevated stress response by the sedative effects of ethanol. The importance of taurine is highlighted by the significant weight loss in the GC group without the influence of ethanol. Complicating the interpretation of results however, is the significant interaction between GES-treatment and ethanol exposure. This interaction signals that GES-treatment may account for the increased weight loss in the GE and GC groups, or that this difference may be the result of sampling error (Kirk, 1995), further studies to investigate this interaction are therefore warranted.

Blood ethanol concentration was negatively influenced by GES treatment, which likely stems from osmotic imbalance. This difference in BEC could result from the lack of regulatory control by taurine on systemic water balance, resulting in an increased susceptibility to ethanol-related disruptions in osmoregulation. Given that ethanol distributes within the body in proportion to body water content, low body water, due to dehydration, would result in lower BECs (Jones, Hahn, & Stalberg, 1990, 1992; M. Q.

Wang, Nicholson, Jones, Fitzhugh, & Westerfield, 1992; Winek & Carfagna, 1987). Vasopressin, released from the pituitary, acts in the kidneys where it induces the reabsorption of water (Guyton & Hall, 1996), however, ethanol inhibits vasopressin release, resulting in dehydration (Guyton & Hall, 1996). Glial cells take up taurine during periods of dehydration (Allen et al., 2002; Dachour et al., 1996; Kreisman & Olson, 2003; Quertemont et al., 2003) helping to maintain normal osmotic states (Beetsch & Olson, 1996; Hussy et al., 2000). The uptake of taurine relies on the taurine transporter (Bitoun & Tappaz, 2000; Huxtable, 1989, 1992) and given that GES inhibits the transporter (Kim, Lee, Kim, & Kim, 2000; Mellor, Gunthorpe, & Randall, 2000); glial cells are unable to bring in taurine (Mellor et al., 2000; Trenkner, Gargano, Scala, & Sturman, 1992). This inability to increase taurine concentration, which protects against ethanol-induced dehydration likely leads to an exacerbation of ethanol-induced dehydration. While the difference in BECs may have influenced the results, researchers have found that BECs fluctuate throughout the 64 hours of ethanol exposure (Metten & Crabbe, 2005). In addition, the level of intoxication upon withdrawal from the chamber does not necessarily result in a strong correlation with withdrawal severity (Crabbe, 1998; Metten & Crabbe, 2005) as BECs are determined from a single sampling of blood and does not therefore represent the fluctuations. These findings indicate that despite the differences reported in this study, behavior may not have been significantly influenced by BECs.

Behavioral Analysis

The EPM measures several aspects of behavior, namely anxiety, activity, and exploration/risk assessment (File, 2001; Rodgers & Johnson, 1995). Both the percent of open arm time and the percent of open arm entries measure anxiety, with the assumption that less anxious animals will spend more time on the open arms and make more open arm entries than anxious animals (File, 2001; Hogg, 1996; Pellow, Chopin, File, & Briley, 1985). The number of closed arm entries and the total number of arm entries measure activity, specifically locomotor function (Lamberty & Gower, 1996; Rodgers & Johnson, 1995; Schmitt & Hiemke, 1998). Finally, the percent of protected head dips performed measure both exploratory and risk-assessment behavior, which are positively correlated with anxiety (Fernandes & File, 1996; Hogg, 1996; Lister, 1987; Pellow et al., 1985). Anxious animals have been found to perform more protected head dips than animals that are less anxious (Rodgers & Johnson, 1995).

Measures of Anxiety

Adolescent rodents do not exhibit anxious behaviors on the EPM (decreased open arm time) during ethanol withdrawal as found in adults (Doremus et al., 2003; Laviola et al., 2003). In contrast, when compared to air exposed adolescents, animals exposed to ethanol and withdrawal display increased open arm time (Bessa et al., 2005; Cole, Littleton, & Little, 2000; Doremus et al., 2004). The reported lack of anxious behavior during ethanol withdrawal in adolescence likely results from the adolescent drive toward risk-taking behavior (Adriani et al., 1998). Taken together, responses to ethanol withdrawal exhibited during adolescence explains the increase in time spent on the open arms in the ethanol exposed groups (GE, CE) compared to the air exposed (GC, CC)

found in this study. The GES-treated groups (GE, GC) spent slightly less time on the open arms (not significant) than the water-treated groups (CE, CC). While not significant, this decrease in the percent of time spent on the open arms after GES consumption may indicate that there is an effect of GES on anxiogenesis and points to a potential threshold effect. It seems possible that lower taurine levels did induce anxiety and that a more anxiogenic stimulus (foot shock, open field test) may elicit more reliable anxious behaviors from the GES-treated groups. There were no significant between group differences in the number of open arm entries performed. However, the moderate increase in the number of open arm entries performed (not significant) in the ethanol exposed groups (GE, CE) compared to the air exposed groups (GC, CC) provides further evidence of the adolescent lack of ethanol withdrawal-induced anxiety, consistent with other studies. Overall, the adolescent mice in this study performed as expected based on previous work. The slight trend toward more anxious behaviors in the GES groups provides modest support for the theory that the depletion of taurine would increase ethanol withdrawal-induced anxiety.

Measures of Activity

When compared to adults, adolescents display less motor impairment during ethanol-withdrawal (Criswell, Knapp, Overstreet, & Breese, 1994; Rasmussen et al., 2001; White, Bae et al., 2002); however, motor impairment becomes evident when ethanol exposed adolescents are compared to adolescents not exposed to ethanol (Acheson, Richardson, & Swartzwelder, 1999; Doremus et al., 2003; Doremus et al., 2004). Consistent with these findings, in this study, ethanol exposure decreased the number of closed arm entries and the total number of entries performed by the ethanol

exposed (GE, CE) groups compared to those exposed only to air (GC, CC). Surprisingly, despite no significant effect of the type of liquid consumed, the GES-treated, air exposed group (GC) performed the most closed arm entries. Additionally, both GES-treated groups (GE, GC) exhibited more closed arm entries (not significant) than their water counterparts (CE, CC). This trend toward a GES-induced increase in locomotor activity indicates that GES-treatment did not negatively affect locomotor activity. As the reciprocal of open arm entries, high numbers of closed arm entries may be indicative of increased anxiety levels (Bessa et al., 2005; Rasmussen et al., 2001). Given that taurine modulates stress levels (S. W. Chen et al., 2004; Kong et al., 2006), the depletion of endogenous taurine levels would increase anxiety; and, as found in this study, resulted in an increase in closed arm time and entries (GE, GC), than animals treated with water (CE, CC). Future investigations focused on taurine's effect on motor activity during ethanol withdrawal in adolescents are warranted.

Measure of Exploration / Risk Assessment

Protected head dips are an exploratory behavior performed from a protected area (closed arm or center square) on the EPM. While a positive correlation has been found between anxiety and protected head dips in adult rodents (Kliethermes, Cronise, & Crabbe, 2004; Rodgers et al., 1999; Rodgers et al., 1997), different results are found during ethanol withdrawal in the adolescent population (Laviola et al., 2003). In the current study, the combination of GES-treatment and ethanol exposure negatively affected the percent of protected head dips performed, as animals in the GE group performed fewer protected head dips than the air exposed (GC, CC) groups, consistent with the findings of other researchers (Doremus et al., 2003; Doremus et al., 2004).

Given that in this study, ethanol exposed groups spent less time and made fewer entries onto protected areas, the performance of significantly fewer protected head dips in the GE group was an expected outcome. The adolescent tendency toward risk-taking (Laviola et al., 2003; Steinberg, 2004) and exploratory behavior (Darmani, Shaddy, & Gerdes, 1996; Hogg, 1996; Schmitt & Hiemke, 1998), as well as the significant increase in open arm time found in the current study, and others (Adriani et al., 1998; Doremus et al., 2003), indicates that adolescents exhibit altered responses to ethanol withdrawal than adults, in particular, a decrease in the percent of protected head dips performed. The ontogenetic increase in exploratory and risk-taking may explain the increase in protected head dips in the air exposed groups (GC, CC).

Plasma Corticosterone

During periods of dehydration / ethanol intoxication, the HPA axis is downregulated (Chowdrey et al., 1991) due to the inhibition of AVP (László et al., 2001; Tanoue et al., 2004) resulting in lower plasma corticosterone levels during intoxication (Aguilera & Rabadan-Diehl, 2000; Dai et al., 2002; Dallman, 1993). Specifically, decreased AVP levels during dehydration reduces ACTH release, as AVP and CRF act synergistically within the (adenohypophysis) (Timpl et al., 1998) to elicit the release of ACTH (Aguilera & Rabadan-Diehl, 2000; Madiera & Paula-Barbosa, 1999; Ogilvie et al., 1997). Blood samples for corticosterone analysis in the 0 hr. animals (GE-0hr, CE-0hr) were collected immediately upon removal from ethanol exposure, while the animals were intoxicated. The 0 hr animals had the lowest plasma corticosterone concentration illustrating the anxiolytic effect of ethanol intoxication (Burghardt & Wilson, 2006; Krystal & Tabakoff, 2002; M. W. Miller & Spear, 2006; R. F. Smith, 2003). There was

no difference between the 0 hr animals (GES or water-treated) indicating that GES treatment did not significantly alter intoxication-induced HPA-hypoactivity.

In contrast to the downregulation of the HPA axis during periods of dehydration, rehydration, which occurs during ethanol withdrawal, induces hyperactivity of the HPA axis (Amaya et al., 2001; Zemo & McCabe, 2002; Zimmermann, Hundt, Spring, Grabner, & Holsboer, 2003). In particular, peak ethanol withdrawal is correlated with the largest levels of CRF release and the onset of anxious behaviors (Pich et al., 1995). In the current study, the highest plasma corticosterone concentration was found in the GE group which was treated with GES, and exposed to ethanol withdrawal. The higher levels of taurine in the water-treated and ethanol exposed animals (CE) may have helped prevent the rehydration-induced increase in HPA axis activity. Following ethanol administration, astrocytes surrounding capillaries take-up taurine (Sakurai et al., 2003). The presence of taurine in astrocytes facilitates taurine's regulation of osmolarity (Lallemand et al., 2000; Pasantes-Morales, Franco, Torres-Marquez, Hernandez-Fonseca, & Ortega, 2000; Schaffer, Takahashi, & Azuma, 2000), enabling astrocytes to attenuate the extreme states of dehydration and rebound rehydration (Franco, Quesada, & Pasantes-Morales, 2000; Miyata et al., 1997; Olson & Li, 2000). Taurine also functions to modulate calcium concentrations (W. Q. Chen et al., 2001; Simasko, Boydjieva, De, & Sarkar, 1999). During ethanol withdrawal intracellular taurine is released (Dachour & De Witte, 2003) in response to calcium influx (Trenkner et al., 1992; Z.-Y. Wu & Xu, 2003; Zungo et al., 2004) attenuating calcium-induced hyperexcitability (H. Wu et al., 2005; Zhao et al., 1999). This effective neuroprotective measure is dependent upon the movement of taurine across the membrane and relies on the taurine transporter (Molchanova et al.,

2005). In the GES-treated animals, the transporter is blocked, inhibiting taurine's neuroprotection (Beetsch & Olson, 1996; Bitoun & Tappaz, 2000; Huang et al., 2006), exacerbating withdrawal symptoms, including anxiety as seen in the GE group.

There was no significant difference in plasma corticosterone levels between the air exposed groups (GC, CC). However, there was also no significant difference between the GC group and the CE group (water and ethanol withdrawal); indicating that treatment with GES alone was sufficient to increase plasma corticosterone levels to those of the CE group. Other groups have found that concentrations of plasma corticosterone are increased in air exposed animals that receive daily injections, as necessary in the vapor inhalation method of ethanol exposure (D. A. Finn, Gallaher, & Crabbe, 2000; Keith & Crabbe, 1992). In accordance with this finding, the plasma corticosterone concentrations were higher in the air exposed animals (GC, CC) than the 0 hr animals (GE-0hr, CE-0hr). Given that plasma corticosterone levels are increased in response to daily injections, it would follow, that the depletion of taurine would exacerbate this increase in stress as seen in the GC group. This supports the previously asserted explanation, that during the 64 hour period, the GES-treated, air exposed group (GC) were stressed, likely causing the significant increase in weight loss. Furthermore, the increased levels of plasma corticosterone in the GE and GC groups compared to the water-treated groups (CE, CC), supports the theory that GES-treatment may have increased anxiety levels in both groups, but below a behavioral threshold. This is a plausible explanation as during ethanol withdrawal, elevated corticosterone levels have been found in the absence of behavioral expressions of anxiety (D. A. Finn et al., 2000). These results indicate that despite a non-significant effect of GES on behavior, the depletion of taurine did increase plasma

corticosterone levels, a biochemical measure of anxiety. Ethanol withdrawal-induced alterations in HPA activation persist for up to four weeks following the cessation of chronic daily consumption and withdrawal in rats (Rasmussen et al., 2001), thus, long-term studies into the effects of taurine depletion on plasma corticosterone levels would be interesting.

Conclusion

Adolescence represents a unique period of development during which experimentation with ethanol is often initiated. Contributing to this is the increase in risk-taking behavior that occurs across species during adolescence (Spear, 2000). This exploratory behavior often encourages independence and interactions with members of the opposite sex outside the family unit, which are adaptive and likely enable experiences that facilitate normal neuronal pruning (Beckman, 2004). However, this increased risk-taking behavior, in some cases may manifest as substance abuse problems (Brasser & Spear, 2002; CDC, 2004; Chassin et al., 2002; Gardner & Steinberg, 2005).

Although no causal relationship can be asserted, the continued development of the brain through adolescence may contribute to the increase in risk-taking behavior and experimentation with ethanol (Andersen, 2003; Beckman, 2004; Gogtay et al., 2004; Powell, 2006; Shaw et al., 2006; Tamminga & Benes, 1998). In particular, neural pruning (Andersen, 2003; Hua & Smith, 2004; Huttenlocher & de Courten, 1987; Rakic et al., 1994) and neurochemical alterations (Bolanos et al., 1998; Lidow & Wang, 1995; Lyss et al., 1999; Malosio et al., 1991) have been shown to continue until early adulthood (Steinberg, 2004; Tapert & Schweinsburg, 2005; Zeigler et al., 2005). Despite, or possibly due to the continued development of the brain, adolescents do not experience the

negative physiological effects of ethanol consumption or withdrawal the way adults do (Doremus et al., 2003; Spear, 2000; Spear & Varlinskaya, 2005). In contrast, adolescents are more sensitive than adults to the cognitive impairing effects of ethanol exposure and withdrawal (Farr et al., 2005; Land & Spear, 2004; Markwiese et al., 1998; Yttri et al., 2004), making early ethanol exposure particularly detrimental. When taken together, the continued brain development, lack of negative symptoms of withdrawal and increased memory impairing effect of ethanol highlight adolescence as a uniquely sensitive developmental phase.

Taurine, the most abundant intracellular amino acid in mammals (Lourenco & Camilo, 2002), is present in higher concentrations in adolescents than adults (Franconi et al., 2004; T. J. Miller et al., 2000; Rentería et al., 2004). This amino acid is primarily involved in osmoregulation and calcium modulation, enabling it to facilitate normal neurodevelopment (Aerts & Van Assche, 2002; Huxtable, 1993; Sturman, 1993) and function as a neuroprotector (W. Q. Chen et al., 2001; Lima et al., 2001; H. Wu et al., 2005). Taurine has been shown to protect the brain from excitotoxic insults that can result in seizures (Ferraz et al., 2002; Franconi et al., 2004; Idrissi, Messing, Scalia, & Trenkner, 2003; Mellor et al., 2000; Saransaari & Oja, 2003; Zalud, Riley, & Diaz-Granados, 2003), cerebral edema (Kreisman & Olson, 2003; Mander et al., 1988; Pasantes-Morales et al., 1998; Schaffer et al., 2000), as well as attenuating anxious behaviors (Adinoff et al., 1991; S. W. Chen et al., 2004; Cole et al., 2000; Engelmann et al., 2003; Kong et al., 2006) illustrating its neuroprotective properties. Taurine's role as an osmoregulator and calcium modulator help to mediate the alterations in the HPA induced by ethanol withdrawal (Engelmann et al., 2003; Gillies et al., 1982; László et al.,

2001) and make this amino acid a likely candidate for protecting the adolescent brain from ethanol induced damage.

The previously reported lack of negative symptoms of ethanol withdrawal (Acheson et al., 1999; P. R. Finn, Mazas, Justus, & Steinmetz, 2002; McBride, Bell, Rodd, Strother, & Murphy, 2005), and in particular the anxiety related to withdrawal (Doremus et al., 2003; Prendergast & Little, 2007; Slawecki et al., 2001), corroborate the results found in this study, as neither of the ethanol exposed groups (GE, CE) displayed adult-like withdrawal-induced anxiety. However, in the GES-treated groups, a tendency toward adult-like anxious behavior was seen on measures of anxiety and activity. Further supporting the hypothesis that reduced taurine levels would increase anxiety is found in the plasma corticosterone levels which were higher in the GES-treated groups (GE, GC) than the water-treated groups (CE, CC). The significantly elevated plasma corticosterone levels in the GE group who went through ethanol withdrawal illustrates the elevation of adolescent anxiety with lower levels of taurine. Future investigations will focus on taurine's protective effects on the expression of anxious behaviors in alternate settings.

REFERENCES

Acheson, S. K., Richardson, R., & Swartzwelder, H. S. (1999). Developmental changes in seizure susceptibility during ethanol withdrawal. *Alcohol, 18*(1), 23-26.

Adinoff, B., Risher-Flowers, D., De Jong, J., Ravitz, B., Bone, G. H., Nutt, D. J., et al. (1991). Disturbances of hypothalamic-pituitary-adrenal axis functioning during ethanol withdrawal in six men. *American Journal of Psychiatry, 148*(8), 1023-1025.

Adriani, W., Chiarotti, F., & Laviola, G. (1998). Elevated novelty seeking and peculiar *d*-amphetamine sensitization in periadolescent mice compared with adult mice. *Behavioral Neuroscience, 112*(5), 1152-1166.

Adriani, W., & Laviola, G. (2000). A unique hormonal and behavioral hyporesponsivity to both forced novelty and d-amphetamine in periadolescent mice. *Neuropharmacology, 39*, 334-346.

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

Aguilera, G., & Rabadan-Diehl, C. (2000). Vasopressinergic regulation of the hypothalamic-pituitary-adrenal axis: Implications for stress adaptation. *Regulatory Peptides, 96*, 23-29.

Allen, J. W., Mutkus, L. A., & Aschner, M. (2002). Chronic ethanol produces increased taurine transport and efflux in cultured astrocytes. *NeuroToxicology, 23*, 693-700.

Amaya, F., Tanaka, M., Hayashi, S., & Tanaka, Y. (2001). Hypothalamo-pituitary-adrenal axis sensitization after chronic salt loading. *Neuroendocrinology, 73*, 185-193.

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

Aschner, M., Mutkus, L. A., & Allen, J. W. (2001). Aspartate and glutamate transport in acutely and chronically ethanol exposed neonatal rat primary astrocyte cultures. *NeuroToxicology, 22*, 601-605.

Ballenger, J. C., & Post, R. M. (1978). Kindling as a model for alcohool withdrawal syndromes. *British Journal of Psychiatry, 133*, 1-14.

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

Becker, H. C., Diaz-Granados, J. L., & Hale, R. L. (1997). Exacerbation of ethanol withdrawal seizures in mice with a history of multiple withdrawal experience. *Pharmacology Biochemistry and Behavior, 57*, 179-183.

Becker, H. C., Veatch, L. M., & Diaz-Granados, J. L. (1998). Repeated ethanol withdrawal experience selectively alters sensitivity to different chemoconvulsant drugs in mice. *Psychopharmacology, 139*, 145-153.

Beckman, M. (2004). Crime, culpability, and the adolescent brain. *Science, 305*, 596-599.

Beetsch, J. W., & Olson, J. E. (1996). Hyperosmotic exposure alters total taurine quantity and cellular transport in rat astrocyte cultures. *Biochimica et Biophysica Acta, 1290*, 141-148.

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.

Bessa, J. M., Oliveira, M., Cerqueira, J. J., Almeida, O. F. X., & Sousa, N. (2005). Age-related qualitative shift in emotional behavior: paradoxical findings after re-exposure of rats in the elevated-plus maze. *Behavioural Brain Research, 162*, 135-142.

Bina, M., Graziano, F., & Bonino, S. (2006). Risky driving and lifestyles in adolescence. *Accident Analysis & Prevention, 38*, 472-481.

Bitoun, M., & Tappaz, M. (2000). Gene expression of taurine transporter and taurine biosynthetic enzymes in hyperosmotic states. *Advances in Experimental Medicine and Biology, 483*, 239-248.

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.

Burghardt, P. R., & Wilson, M. A. (2006). Microinjection of naltrexone into the central, but not the basolateral, amygdala blocks the anxiolytic effects of diazepam in the plus maze. *Neuropsychopharmacology, 31*, 1227-1240.

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.

Carmona-Calero, E., del Mar Pérez-Delgado, M., Bañuelos-Pineda, J., Marrero-Gordillo, N., Ferres-Torres, R., & Castañeyra-Perdomo, A. (1995). Effects of chronic alcohol intake on the vasopressin content in the hypothalamic paraventricular and supraoptic nuclei of the mouse. *Drug and Alcohol Dependence, 38*, 19-24.

Carney, S. L., Gillies, A. H. B., & Ray, C. D. (1995). Acute effect of ethanol on renal electrolyte transport in the rat. *Clinical and Experimental Pharmacology and Physiology, 22*, 629-634.

Carrasco, G. A., & Van de Kar, L. D. (2003). Neuroendocrine pharmacology of stress. *European Journal of Pharmacology, 463*, 235-272.

CDC. (2004). National Youth Risk Behavior Survey. *MMWR, 53*, SS-2.

Chapman, R. A., Suleiman, M. S., & Earm, Y. E. (1993). Taurine and the heart. *Cardiovascular Research, 27*, 358-363.

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, S. W., Kong, W. X., Zhang, Y. J., Li, Y. L., Mi, X. J., & Mu, X. S. (2004). Possible anxiolytic effects of taurine in the mouse elevated plus-maze. *Life Sciences, 75*, 232-240.

Chen, W. Q., Jin, H., Nguyen, M., Carr, J., Lee, Y. J., Hsu, C. C., et al. (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.

Chowdrey, H. P., Jessop, D. S., Patel, H., & Lightman, S. L. (1991). Altered adrenocorticotropin, corticosterone and oxytocin responses to stress during chronic salt load. *Neuroendocrinology, 54*(6), 635-638.

Cole, J. C., Littleton, J. M., & Little, H. J. (2000). Acamprosate, but not naltrexone, inhibits conditioned abstinence behaviour associated with repeated ethanol administration and exposure to a plus-maze. *Psychopharmacology, 147*, 403-411.

Collins, M. A., Zou, J.-Y., & Neafsey, E. J. (1998). Brain damage due to episodic alcohol exposure *in vivo* and *in vitro*: Furosemide neuroprotection implicates edema-based mechanism. *Journal of FASEB, 12*, 221-230.

Crabbe, J. C. (1998). Provisional mapping of quantitative trait loci for chronic ethanol withdrawal severity in BXD recombinant inbred mice. *Journal of Pharmacology and Experimental Therapeutics, 286*, 263-271.

Craig, D., Passmore, A. P., Fullerton, K. J., Beringer, T. R. O., Gilmore, D. H., Crawford, V. L. S., et al. (2003). Factors influencing prescription of CNS medications in different elderly populations. *Pharmacoepidemiology and Drug Safety, 12*, 383-387.

Crews, F. T., Braun, C. J., Hoplight, B., & Switzer III, R. C. (2000). Binge ethanol consumption causes differential brain damage in young adolescent rats compared with adult rats. *Alcoholism: Clinical and Experimental Research, 24*, 1712-1723.

Criswell, H. E., Knapp, D. J., Overstreet, D. H., & Breese, G. R. (1994). Effects of ethanol, chlordiazepoxide, and MK-801 on performance in the elevated-plus maze and on locomotor activity. *Alcoholism: Clinical and Experimental Research, 18*(3), 596-601.

Dachour, A., & De Witte, P. (2000). Taurine bloks the glutamate increase in the nucleus accumbens microdialysate of ethanol-dependent rats. *Pharmacology Biochemistry and Behavior, 65*(2), 345-350.

Dachour, A., & De Witte, P. (2002). Excitatory and inhibitory amino acid changes during repeated episodes of ethanol withdrawal: an *in vivo* microdialysis study. *European Journal of Pharmacology, 609*, 1-8.

Dachour, A., & De Witte, P. (2003). Excitatory and inhibitory amino acid changes during repeated episodes of ethanol withdrawal: an in vivo microdialysis study. *European Journal of Pharmacology, 459*, 171-178.

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.

Dai, X., Thavundayil, J., & Gianoulakis, C. (2002). Response of the hypothalamic-pituitary-adrenal axis to stress in the absence and presence of ethanol in subjects at high and low risk of alcoholism. *Neuropsychopharmacology, 27*(3), 442-452.

Dallman, M. F. (1993). Adaptation of the hypothalamic-pituitary-adrenal axis to chronic stress. *Trends in Endocrinology and Metabolism, 4*(2), 62-69.

Darmani, N. A., Shaddy, J., & Gerdes, C. F. (1996). Differential ontogenesis of three DOI-induced behaviors in mice. *Physiology & Behavior, 60*, 1495-1500.

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., et al. (2000). Hippocampal volume in adolescent-onset alcohol use disorders. *American Journal of Psychiatry, 157*, 737-744.

De Luca, A., Pierno, S., & Camerino, D. C. (1996). Effect of taurine depletion on excitation-contraction coupling and Cl^- conductance of rat skeletal muscle. *European Journal of Pharmacology, 296*, 215-222.

Decavel, C., & Curras, M. C. (1996). Increased expression of the *N*-methyl-$_D$-aspartate receptor subunit, NR1, in immunohistochemically identified magnocellular hypothalamic neurons during rehydration. *Neuroscience, 78*, 191-202.

Decavel, C., & Hatton, G. I. (1995). Taurine immunoreactivity in the rat supraoptic nucleus: Prominent localization in glial cells. *Journal of Comparative Neurology, 354*, 13-26.

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.

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*, 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.

Douglas, L. A., Varlinskaya, E. I., & Spear, L. P. (2003). Novel-object place conditioning in adolescent and adult male and female rats: effects of social isolation. *Physiology & Behavior, 80*, 317-325.

Eisenhofer, G., Lambie, D. G., Whiteside, E. A., & Johnson, R. H. (1985). Vasopressin concentration during alcohol withdrawal. *British Journal of Addiction, 80*, 195-199.

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.

Engelmann, M., Ebner, K., Landgraf, R., & Wotjak, C. T. (1998). Swim stress triggers the release of vasopressin within the suprachiasmatic nucleus of male rats. *Brain Research, 792*, 343-347.

Engelmann, M., Landgraf, R., & Wotjak, C. T. (2003). Taurine regulates corticotropin secretion at the level of the supraoptic nucleus during stress in rats. *Neuroscience Letters, 348*, 120-122.

Engelmann, M., Ludwig, M., Singewald, N., Ebner, K., Sabatier, N., Lubec, G., et al. (2001). Taurine selectively modulates the secretory activity of vasopressin neurons in conscious rats. *European Journal of Neuroscience, 14*, 1047-1055.

Evrard, S. G., Duhalde-Vega, M., Tagliaferro, P., Mirochnic, S., Caltana, L. R., & Brusco, A. (2006). A low chronic ethanol exposure induces morphological changes in the adolescent rat brain that are not fully recovered even after a long abstinence: an immunohistochemical study. *Experimental Neurology, 200*, 438-459.

Faingold, C., Li, Y. L., & Evans, M. S. (2000). Decreased GABA and increased glutamate receptor-mediated activity on inferior colliculus neurons in vitro are associated with susceptibility to ethanol withdrawal seizures. *Brain Research, 868*, 287-295.

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.

Fernandes, C., & File, S. E. (1996). The influence of open arm ledges and maze experience in the elevated plus-maze. *Pharmacology Biochemistry and Behavior, 54*(1), 31-40.

Ferraz, A. C., Anselmo-Franci, J. A., Perosa, S. R., De Castro-Neto, E. F., Bellissimo, M. I., De Oliveira, B. H., et al. (2002). Amino acid and monoamine alterations in the cerebral cortex and hippocampus of mice submitted to ricinine-induced seizures. *Pharmacology Biochemistry and Behavior, 72*, 779-786.

File, S. E. (2001). Factors controlling measures of anxiety and responses to novelty in the mouse. *Behavioural Brain Research, 125*, 151-157.

Finn, D. A., Gallaher, E. J., & Crabbe, J. C. (2000). Differential change in neuroactive steroid sensitivity during ethanol withdrawal. *Journal of Pharmacology and Experimental Therapeutics, 292*, 394-405.

Finn, P. R., Mazas, C. A., Justus, A. N., & Steinmetz, J. (2002). Early-onset alcoholism with conduct disorder: Go/No go learning deficits, working memory capacity, and personality. *Alcoholism: Clinical and Experimental Research, 26*(2), 186-206.

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., et al. (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.

Fujita, T., Shimada, A., Wada, M., Miyakawa, S., & Yamamoto, A. (2006). Functional expression of taurine transporter and its up-regulation in developing neurons from mouse cerebral cortex. *Pharmaceutical Research 23*(4), 689-696.

Gardner, M., & Steinberg, L. (2005). Peer influence on risk taking, risk preference, and risky decision making in adolescence and adulthood: an experimental study. *Developmental Psychology, 41*(4), 625-635.

Gatch, M. B., & Lal, H. (2001). Animal models of the anxiogenic effects of ethanol withdrawal. *Drug Development Research, 54*, 95-115.

Ghavanini, A., Mathers, D. A., & Puil, E. (2005). Glycinergic inhibition in thalamus revealed by synaptic receptor blockade. *Neuropharmacology, 49*, 338-349.

Gillies, G. E., Linton, E. A., & Lowry, P. J. (1982). Corticotrpin releasing activity of the new CRF is potentiated several times by vasopressin. *Nature, 299*(5881), 355-357.

Gogtay, N., Giedd, J. N., Lusk, L., Hayashi, K. M., Greenstein, D., Vaituzis, A. C., et al. (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.

Goldman, D., & Barr, C. S. (2002). Restoring the addicted brain. *New England Journal of Medicine, 347*(843-845).

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.

Guyton, A. C., & Hall, J. E. (1996). *Textbook of Medical Physiology* (9 ed.). Philadelphia: W. B. Saunders Company.

Hadley, M. E. (2000). *Endocrinology* (5 ed.). Upper Saddle River: Prentice Hall.

Harding, A. J., Halliday, G. M., Ng, J. L. F., Harper, C. G., & Kril, J. J. (1996). Loss of vasopressin-immunoreactive neurons in alcoholics is dose-related and time-dependent. *Journal of Neuroscience, 72*, 699-708.

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

Helfand, R. S., Zalud, A. W., & Diaz-Granados, J. L. (2006). Taurine attenuates ethanol withdrawal-induced anxiety on the elevated plus maze. *Alcoholism: Clinical and Experimental Research, 30*(6), 87A.

Hiramatsu, M., Edamatsu, R., Kabuto, H., Higashihara, Y., & Mori, A. (1989). Increased seizure susceptibility induced by guanidinoethane sulfonate in E1 mice and its relation to glutamatergic neurons. *Neurochemical Research, 14*(1), 85-89.

Hogg, S. (1996). A review of the validity and variability of the elevated plus-maze as an animal model of anxiety. *Pharmacology Biochemistry and Behavior, 54*(1), 21-30.

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

Huang, D. A., Boini, K. M., Lang, P. A., Grahammer, F., Duszenko, M., Heller-Stilb, B., et al. (2006). Impaired ability to increase water excretion in mice lacking the taurine transporter gene TAUT. *European Journal of Physiology, 451*, 668-677.

Hussy, N., Bres, V., Rochette, M., Dugoid, A., Alonso, G., Dayanithi, G., et al. (2001). Osmoregulation of vasopressin secretion via activation of neurohypophysial nerve terminals glycine receptors by glial taurine. *Journal of Neuroscience, 21*, 7110-7116.

Hussy, N., Deleuze, C., Desarmenien, M. G., & Moos, F. C. (2000). Osmotic regulation of neuronal activity: a new role for taurine and glial cells in a hypothalamic neuroendocrine structure. *Progress in Neurobiology, 62*, 113-134.

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.

Idrissi, A. E., Messing, J., Scalia, J., & Trenkner, E. (2003). Prevention of epileptic seizures by taurine. *Advances in Experimental Medicine and Biology, 526*, 515-525.

Izumi, K., Kishita, C., Nakaawa, K., Huxtable, R. J., Shimizu, T., Koja, T., et al. (1985). Modification of the antiepileptic actions of phenobarbital and phenytoin by the taurine transport inhibitor, guanidinoethane sulfonate. *European Journal of Pharmacology, 110*(2), 219-224.

Jhiang, S. M., Fithian, L., Smanik, P., McGill, J., Tong, Q., & Mazzaferri, E. L. (1993). Cloning of the human taurine transporter and characterization of taurine uptake in thyroid cells. *FEBS Letters, 318*(139-144).

Jiang, Y.-Q., Kawashima, H., Iwasaki, Y., Uchida, K., Sugimoto, K., & Itoi, K. (2004). Differential effects of forced swim-stress on the corticotropin-releasing hormone and vasoppressin gene transcription in the parvocellular division of the paraventricular nucleus of rat hypothalamus. *Neuroscience Letters, 358*, 201-204.

Johnston, L. D., O'Malley, P. M., Bachman, J. G., & Schulenberg, J. E. (2006). Monitoring the Future national survey results on drug use, 1975-2005: Volume 1, Secondary school students, *(NIH Publication No. 06-5883)*. Bethesda, MD: National Institute on Drug Abuse.

Jones, A. W., Hahn, R. G., & Stalberg, H. P. (1990). Distribution of ethanol and water between plasma and whole blood; inter- and intra-individual variations after administration of ethanol by intravenous infusion. *Scandanavian Journal of Clinical and Laboratory Investigation, 50*(7), 775-780.

Jones, A. W., Hahn, R. G., & Stalberg, H. P. (1992). Pharmacokinetics of ethanol in plasma and whole blood: estimation of total body water by the dilution principle. *European Journal of Clinical Pharmacology, 42*(4), 445-448.

Kanli, H., & Terreros, D. A. (1990). Acute ethanol effects on cell volume regulation. *Annals of Clinical and Laboratory Sciences, 20*, 205-213.

Keith, L. D., & Crabbe, J. C. (1992). Specific and nonspecific effects of ethanol vapor on plasma corticosterone in mice. *Alcohol, 9*, 529-533.

Kim, H. W., Lee, E. J., Kim, W. B., & Kim, B. K. (2000). Ionomycin restores taurine transporter activity in cyclosporin-A treated macrophages. *Advances in Experimental Medicine and Biology, 483*, 127-135.

Kirk, R. E. (1995). *Experimental Design Procedures for the Behavioral Sciences* (3rd ed.). Pacific Grove: Brooks/Cole Publishing Company.

Kliethermes, C. L., Cronise, K., & Crabbe, J. C. (2004). Anxiety-like behavior in mice in two apparatuses during withdrawal from chronic ethanol vapor inhalation. *Alcoholism: Clinical and Experimental Research, 28*, 1012-1019.

Kong, W. X., Chen, S. W., Li, Y. L., Zhang, Y. J., Wang, R., Min, L., et al. (2006). Effects of taurine on rat behaviors in three anxiety models. *Pharmacology Biochemistry and Behavior, 83*(271-276).

Koob, G. F. (1999). Corticotropin-releasing factor, norepinephrine, and stress. *Biological Psychiatry, 46*, 1167-1180.

Kreisman, N. R., & Olson, J. E. (2003). Taurine enhances volume regulation in hippocampal slices swollen osmotically. *Neuroscience, 120*, 635-642.

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.

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.

Lamberty, Y., & Gower, A. J. (1996). Arm width and brightness modulation of spontaneous behavior of two strains of mice tested in the elevated plus-maze. *Physiology & Behavior, 59*(3), 439-444.

Lambie, D. G. (1985). Alcoholic brain damage and neurological symptoms of alcohol withdrawal - Manifestations of overhydration. *Medical Hypotheses, 16*, 377-388.

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.

László, F. A., Varga, C., Pávó, I., Gardi, J., Vecsernyés, M., Gálfi, M., et al. (2001). Vasopressin pressor receptor-mediated activation of HPA axis by acute ethanol stress in rats. *American Journal of Physiology, 280*, R458-R465.

Laviola, G., Adriani, W., Morley-Fletcher, S., & Terranova, M. L. (2002). Peculiar response of adolescent mice to acute and chronic stress and to amphetamine: evidence of sex differences. *Behavioural Brain Research, 130*, 117-125.

Laviola, G., Adriani, W., Terranova, M. L., & Gerra, G. (1999). Psychobiological risk factors for vulnerability to psychostimulants in human adolescents and animal models. *Neuroscience and Biobehavioral Reviews, 23*(7), 993-1010.

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

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.

Lister, R. G. (1987). The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacology, 92*, 180-185.

Liu, Q.-R., López-Corcuera, B., Nelson, H., Mandiyan, S., & Nelson, N. (1992). Cloning and expression of a cDNA encoding the transporter of taurine and β-alanine in mouse brain. *Proceedings of the National Academy of Sciences of the United States of America, 89*, 12145-12149.

Liu, X., Wang, C. A., & Chen, Y. Z. (1995). Nongenomic effect of glucocorticoid on the release of arginine vasopressin from hypothalamic slices in rats. *Neuroendocrinology, 62*(3), 628-633.

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.

Lovallo, W. R. (2006). Cortisol secretion patterns in addiction and addiction risk. *International Journal of Psychophysiology, 59*, 195-202.

Ludwig, M., Callahan, M. F., Neumann, I., Landgraf, R., & Morris, M. (1994). Systemic osmotic stimulation increases vasopressin and oxytocin release within the supraoptic nucleus. *Journal of Neuroendocrinology, 6*, 369-373.

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.

Madiera, M. D., & Paula-Barbosa, M. M. (1999). Effects of alcohol on the synthesis and expression of hypothalamic peptides. *Brain Research Bulletin, 48*(1), 3-22.

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.

Mander, A. J., Weppner, G. J., Chick, J. D., Morton, J. J., & Best, J. J. K. (1988). An NMR study of cerebran oedema and its biological correlates during withdrawal from alcohol. *Alcohol and Alcoholism, 23*, 97-102.

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.

Mason, D., Hassan, A., Chacko, S., & Thompson, P. (2002). Acute and chronic regulation of pituitary receptors for vasopressin and corticotropin releasing hormone. *Archives of Physiology and Biochemistry, 110*(1), 74-89.

McBride, W. J., Bell, R. L., Rodd, Z. A., Strother, W. N., & Murphy, J. M. (2005). *Adolescent alcohol drinking and its long-range consequences studies with animal models* (Vol. 17). New York: Kluwer Academic/Plenum Publishers.

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.

McEwen, B. S. (1999). Endocrine effects on the brain and their relationship to behavior. In G. J. Siegel, B. W. Agranoff, R. W. Albers, S. K. Fisher & M. D. Uhler (Eds.), *Basic Neurochemistry* (6th ed., pp. 1007-1026). Philadelphia: Lippincott Williams & Wilkins.

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.

Meeker, R. B. (2002). Metabotropic and NMDA glutamate receptor interactions with osmotic stimuli in supraoptic neurons. *Pharmacology Biochemistry and Behavior, 73*, 475-484.

Mellor, J. R., Gunthorpe, M. J., & Randall, A. D. (2000). The taurine uptake inhibitor guanidinoethyl sulphonate is an agonist at γ-aminobutyric $acid_A$ receptors in cultured murine cerebellar granule cells. *Neuroscience Letters, 286*, 25-28.

Menzaghi, F., Rassnick, S., Heinrichs, S., Baldwin, H., Pich, E. M., Weiss, F., et al. (1994). The role of corticotropin-releasing factor in the anxiogenic effects of ethanol withdrawal. *Annals of the New York Academy of Sciences, 739*, 176-184.

Metten, P., & Crabbe, J. C. (2005). Alcohol withdrawal severity in inbred mouse (mus musculus) strains. *Behavioral Neuroscience, 1199*(4), 911-925.

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.

Mikics, É., Barsy, B., Barsvári, B., & Haller, J. (2005). Behavioral specificity of non-genomic glucocorticoid effects in rats: effects on risk assessment in the elevated plus-maze and the open-field. *Hormones and Behavior, 48*, 152-162.

Millan, M. J. (2003). The neurobiology and control of anxious states. *Progress in Neurobiology, 70*, 83-244.

Miller, M. W., & Spear, L. P. (2006). The Alcoholism Generator. *Alcoholism: Clinical and Experimental Research, 30*(9), 1466-1469.

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.

Miyata, S., Matsushima, O., & Hatton, G. I. (1997). Taurine in rat posterior pituitary: Localization in astrocytes and selective release by hypoosmotic stimulation. *Journal of Comparative Neurology, 381*, 513-523.

Molchanova, S. M., Oja, S. S., & Saransaari, P. (2005). Mechanisms of ethanol taurine release under Ca^{2+} depletion. *Neurochemistry International, 47*, 343-349.

Mongin, A. A., Cai, Z., & Kimelberg, H. K. (1999). Volume-dependent taurine release from cultured astrocytes requires permissive ($[Ca^{2+}]_i$) and calmodulin. *American Journal of Physiology, 277*, C823-832.

Moss, H. B., Vanyukov, M. M., & Martin, C. S. (1995). Salivary cortisol responses and the risk for substance abuse in prepubertal boys. *Biological Psychiatry, 38*, 547-555.

Moss, H. B., Vanyukov, M. M., Yao, J. K., & Kirillova, G. P. (1999). Salivary cortisol responses in prepubertal boys: the effects of parental substance abuse and association with drug use behavior during adolescence. *Biological Psychiatry, 45*, 1293-1299.

Mulholland, P. J., Self, R. L., Hensley, A. K., Little, H. J., Littleton, J. M., & Prendergast, M. A. (2005). A 24 h corticosterone exposure exacerbates excitotoxic insult in rat hippocampal slice cultures independently of glucocorticoid receptor activation. *Brain Research, 1082*, 165-172.

Nestler, E. J., & Aghajanian, G. K. (1997). Molecular and cellular bassis of addiction. *Science, 278*, 58-63.

Nestler, E. J., Hyman, S. E., & Malenka, R. C. (2001). *Molecular Neuropharmacology: A Foundation for Clinical Neuroscience*. New York: McGraw-Hill Companies.

Nilsson, M., Lehmann, A., & Hansson, E. (1989). Effects of 2-guanidinoethane sulfonate on glutamate uptake in primary astroglial cultures from the rat cerebral cortex. *Neuropharmacology, 28*(12), 1415-1418.

Ogilvie, K. M., Lee, S., & Rivier, C. (1997). Role of arginine vasopressin and corticotropin-releasing factor in mediating alcohol-induced adrenocorticotropin and vasopressin secretion in male rats bearing lesions of the paraventricular nuclei. *Brain Research, 744*, 83-95.

Olson, J. E., & Li, G. (2000). Osmotic sensitivity of taurine release from hippocampal neuronal and glial cells. *Advances in Experimental Medicine and Biology, 483*, 213-218.

Overstreet, D. H., Knapp, D. J., & Breese, G. R. (2004). Modulation of multiple ethanol withdrawal-induced anxiety-like behavior by CRF and CRF_1 receptors. *Pharmacology Biochemistry and Behavior, 77*, 405-413.

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.

Pasantes-Morales, H., Quesada, O., & Moran, J. (1998). Taurine: an osmolyte in mammalian tissues. *Advances in Experimental Medicine and Biology, 442*, 209-217.

Patel, S., Roelke, C. T., Rademacher, D. J., Cullinan, W. E., & HIllard, C. J. (2004). Endocannabinoid signaling negatively modulates stress-induced activation of the hypothalamic-pituitary-adrenal axis. *Endocrinology, 145*(12), 5431-5438.

Paula-Lima, A. C., De Felice, F., Brito-Moreira, J., & Ferreira, S. T. (2005). Activation of $GABA_A$ receptors by taurine and muscimol blocks the neurotoxicity of β-amyloid in rat hippocampal and cortical neurons. *Neuropharmacology, 49*, 1140-1148.

Pellow, S., Chopin, P., File, S. E., & Briley, M. (1985). Validation of open : closed arm entries in an elevate plus-maze as a measure of anxiety in the rat. *Journal of Neuroscience Methods, 14*, 149-167.

Pich, E. M., Lorang, M., Yeganeh, M., de Fonseca, F. R., Raber, J., Koob, G. F., et al. (1995). Increase of extracellular corticotropin-releasing factor-like immunoreactivity levels in the amygdala of awake rats during restraint stress and ethanol withdrawal as measured by microdialysis. *Journal of Neuroscience, 15*(8), 5439-5447.

Pow, D. V., Sullivan, R., Reye, P., & Hermanussen, S. (2002). Localization of taurine transporters, taurine and ^3H taurine accumulation in the rat retina, pituitary, and brain. *Glia, 37*, 153-168.

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

Prendergast, M. A., & Little, H. J. (2007). Adolescence, glucocorticoids and alcohol. *Pharmacology Biochemistry and Behavior, 86*, 234-245.

Pruett, S. B., Collier, S. D., & Wu, W. J. (1998). Ethanol-induced activation of the hypothalamic-pituitary-adrenal axis in a mouse model for binge-drinking: role of Ro15-4513-sensitive gamma-aminobutyric acid receptors, tolerance, and relevance to humans. *Life Sciences, 63*(13), 1137-1146.

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

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.

Rasmussen, D. D., Boldt, B. M., Bryant, C. A., Mitton, D. R., Larsen, S. A., & Wilkinson, C. W. (2000). Chronic daily ethanol and withdrawal: 1. Long-term changes in the hypothalamo-pituitary-adrenal axis. *Alcoholism: Clinical and Experimental Research, 24*, 1836-1849.

Rasmussen, D. D., Mitton, D. R., Green, J., & Puchalski, S. (2001). Chronic daily ethanol and withdrawal: 2. Behavioral changes during prolonged abstinence. *Alcoholism: Clinical and Experimental Research, 25*, 999-1005.

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.

Rivier, C. (1996). Alcohol stimulates ACTH secretion in the rat: mechanisms of action and interactions with other stimuli. *Alcoholism: Clinical and Experimental Research, 20*(2), 240-254.

Rivier, C., Imaki, T., & Vale, W. (1990). Prolonged exposure to alcohol: effect on CRF mRNA levels and CRF-and stress-induced ACTH secretion in the rat. *Brain Research, 520*, 1-5.

Rivier, C., & Vale, W. (1983). Interaction of corticotropin-releasing factor and arginine vasopressin on adrenocorticotropin secretion in vivo. *Endocrinology, 113*(3), 939-942.

Robertson, D. A. F., Beattie, J. E., Reid, I. C., & Balfour, D. J. K. (2005). Regulation of corticosteroid receptors in the rat brain: the role of serotonin and stress. *European Journal of Neuroscience, 21*, 1511-1520.

Rodgers, R. J., Cole, J. C., Aboualfa, K., & Stephenson, L. H. (1995). Ethopharmacological analysis of the effects of putative 'anxiogenic' agents in to mouse elevated plus-maze. *Pharmacology Biochemistry and Behavior, 52*, 805-813.

Rodgers, R. J., Haller, J., Holmes, A., Halasz, J., Walton, T. J., & Brain, P. F. (1999). Corticosterone response to the plus-maze: High correlation with risk assessment in rats and mice. *Physiology & Behavior, 68*, 47-53.

Rodgers, R. J., & Johnson, N. J. T. (1995). Factor analysis of spatiotemporal and ethological measuresin the murine elevated plus-maze test of anxiety. *Pharmacology Biochemistry and Behavior, 52*(2), 297-303.

Rodgers, R. J., Johnson, N. J. T., Carr, J., & Hodgson, T. P. (1997). Resistance of experimentally-induced changes in murine plus-maze behaviour to altered retest conditions. *Behavioural Brain Research, 86*, 71-77.

Rodgers, R. J., Johnson, N. J. T., Cole, J. C., Dewar, C. V., Kidd, G. R., & Kimpson, P. H. (1996). Plus-maze retest profile in mice: importance of initial stages of Trial 1 and response to post-trial cholinergic receptor blockade. *Pharmacology Biochemistry and Behavior, 54*(1), 41-50.

Rosso, L., Peteri-Brunback, B., Poujeol, P., Hussy, N., & Mienville, J. M. (2004). Vasopressin-induced taurine efflux from rat pituicytes: A potential negative feedback for hormone secretion. *Journal of Physiology, 554*, 731-742.

Rupp, R., Rosenthal, S. L., & Middleman, A. B. (2006). Vaccination: an opportunity to enhance early adolescent preventative services. *Journal of Adolescent Health, 39*, 461-464.

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.

Sanberg, P. R., & Fibiger, H. C. (1979). Impaired acquisition and retention of a passive avoidance response after chronic ingestion of taurine. *Psychopharmacology, 62*, 97-99.

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.

Schaffer, S., Takahashi, K., & Azuma, J. (2000). Role of osmoregulation in the actions of taurine. *Amino Acids, 19*, 527-846.

Schmitt, U., & Hiemke, C. (1998). Combination of open field and elevated plus-maze: A suitable test battery o assess strain as well as treatment differences in rat behavior. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 22*, 1197-1215.

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., et al. (2006). Intellectual ability and cortical development in children and adolescents. *Nature, 440*, 676-679.

Shepard, J. D., Barron, K. W., & Myers, D. A. (2003). Stereotaxic localization of corticosterone to the amygdala enhances hypothalamo-pituitary-adrenal responses to behavioral stress. *Brain Research, 963*, 203-213.

Silva, S. M., Paula-Barbosa, M. M., & Madeira, M. D. (2002). Prolonged alcohol intake leads to reversible depression of corticotropin-releasing hormone and vasopressin immunoreactivity and mRNA levels in the parvocellular neurons of the paraventricular nucleus. *Brain Research, 954*, 82-93.

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.

Silveri, M. M., & Spear, L. P. (2004). Characterizing the ontogeny of ethanol-associated increases in corticosterone. *Alcohol, 32*, 145-155.

Silvers, J. M., Tokunaga, S., Mittleman, G., & Matthews, D. B. (2003). Chronic intermittent injections of high-does ethanol during adolescence produce metabolic, hypnotic, and cognitive tolerance in rats. *Alcoholism: Clinical and Experimental Research, 27*, 1606-1612.

Simasko, S. M., Boydjieva, N., De, A., & Sarkar, D. K. (1999). Effect of ethanol on calcium regulation in rat fetal hypothalamic cells in culture. *Brain Research, 824*, 89-96.

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, K. E., Borden, L. A., Wang, D. C., Harting, P. R., Branchek, T. A., & Weinshank, R. L. (1992). Cloning and expression of a high affinity taurine transporter from rat brain. *Molecular Pharmacology, 42*, 563-569.

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

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.

Stam, R., van Laar, T.-J., Akkermans, L. M. A., & Wiegant, V. M. (2002). Variability factors in the expression of stress-induced behavioral sensitisation. *Behavioural Brain Research, 132*, 69-76.

Stansfield, K. H., Philpot, R. M., & Kirstein, C. L. (2004). An animal model of sensation seeking: the adolescent rat. *Annals of the New York Academy of Sciences, 1021*, 453-458.

Steinberg, L. (2004). Risk taking in adolescence what changes, and why? *Annals of the New York Academy of Sciences, 1021*, 51-58.

Stenzel-Poore, M. P., Heinrichs, S. C., Rivest, S., Koob, G. F., & Vale, W. W. (1994). Overproduction of corticotropin-releasing factor in transgenic mice: a genetic model of anxiogenic behavior. *Journal of Neuroscience, 14*(5), 2579-2584.

Storey, J. D., Robertson, D. A. F., Beattie, J. E., Reid, I. C., Mitchell, S. N., & Balfour, D. J. K. (2006). Behavioral and neurochemical responses evoked by repeated exposure to an elevated open platform. *Behavioural Brain Research, 166*, 220-229.

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.

Sze, P. Y. (1996). Glucocorticoid action on depolarization-dependent calcium influx in brain synaptosomes. *Neuroendocrinology, 59*, 457-465.

Tabakoff, B., & Hoffman, P. L. (1996). Ethanol and glutamate receptors. In R. A. Deitrich & V. G. Erwin (Eds.), *Pharmacological Effects of Ethanol on the Nervous System* (pp. 73-93). Boca Raton: CRC Press.

Tabakoff, B., Jafee, R. C., & Ritzmann, R. F. (1978). Corticosterone concentrations in mice during ethanol drinking and withdrawal. *Journal of Pharmacy and Pharmacology, 30*(6), 371-374.

Taivainen, H., Laitinen, K., Tähtelä, R., Kiianamaa, K., & Välimäki, M. J. (1995). Role of plasma vasopressin in changes of water balance accompanying acute alcohol intoxication. *Alcoholism: Clinical and Experimental Research, 19*, 759-762.

Tamminga, C., A., & Benes, F., M. (1998). Human brain growth spans decades. *American Journal of Psychiatry, 155*(11), 1489.

Tanoue, A., Itoi, K., Honda, K., Oshikawa, S., Kitagawa, Y., Koshimizu, T.-a., et al. (2004). The vasopressin V1b receptor critically regulates hypothalamic-pituitary-adrenal axis activity under both stress and resting conditions. *Journal of Clinical Investigation, 113*, 2.

Tapert, S. F., & Schweinsburg, A. D. (2005). The human adolescent brain and alcohol use disorders. *Recent Developments in Alcoholism, 17*, 177-197.

Tchoumkeu-Nzouessa, G. C., & Rebel, G. (1995). Characterization of taurine transport in human glioma GL15 cell line: Regulation by protein kinase C. *Neuropharmacology, 35*(1), 37-44.

Timpl, P., Spanagel, R., Sillaber, I., Kresse, A., Reul, J. M. H. M., Stall, G. K., et al. (1998). Impaired stress response and reduced anxiety in mice lacking a functional corticotropin-releaseing hormone receptor 1. *Nature, 19*, 162-166.

Trenkner, E., Gargano, A., Scala, P., & Sturman, J. A. (1992). Taurine synthesis in cat and mouse *in vivo* and *in vitro*. *Advances in Experimental Medicine and Biology, 315*, 7-14.

Tsai, G., & Coyle, J. T. (1998). The role of glutamatergic neurotransmission in the pathophysiology of alcoholism. *Annual Review of Medicine, 49*, 173-184.

Uchida, S., Kwon, H. M., Yamauchi, A., Preston, A. S., Marumo, F., & Handler, j. S. (1992). Molecular cloning of the cDNA for an MDCK cell Na^+ and Cl^- dependent taurine transporter that is regulated by hypertonicity. *Proceedings of the National Academy of Sciences of the United States of America, 89*, 8230-8234.

Varlinskaya, E. I., & Spear, L. P. (2004). 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. (2007). Chronic tolerance to the social consequences of ethanol in adolescent and adult Sprague-Dawley rats. *Neurotoxicology and Teratology, 2007*, 23-30.

Vellan, E. J., Gjessing, L. R., & Stalsberg, H. (1970). Free amino acids in the pineal and pituitary glands of human brain. *Journal of Neurochemistry, 17*, 699-701.

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

Voisin, D. L., & Bourque, C. W. (2002). Integration of sodium and osmosensory signals in vasopressin neurons. *Trends in Neuroscience, 25*, 199-205.

Wang, M. Q., Nicholson, M. E., Jones, C. S., Fitzhugh, E. C., & Westerfield, C. R. (1992). Acute alcohol intoxication, body composition, and pharmacokinetics. *Pharmacology Biochemistry and Behavior, 43*(2), 641-643.

Wang, X. M., Dayanithi, G., Lemos, J. R., Nordmann, J. J., & Treistman, S. N. (1991). Calcium currents and peptide release from neurohypophysial terminals are inhibited by ethanol. *Journal of Pharmacology and Experimental Therapeutics, 259*, 705-711.

Welt, T., Engelmann, M., Renner, U., Erhardt, A., Müller, M. B., Landgraf, R., et al. (2006). Temazepam triggers the release of vasopressin into the rat hypothalamic paraventricular nucleus: novel insight into benzodiazepine action on hypothalamic-pituitary-adrenocortical system activity during stress. *Neuropsychopharmacology, 31*, 2573-2579.

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*, 960-968.

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

Winek, C. L., & Carfagna, M. (1987). Comparison of plasma, serum, and whole blood ethanol concentrations. *Journal of Analytical Toxicology, 11*(6), 267-268.

Wolf, M. E. (2006). Addiction. In G. J. Siegel, R. W. Albers, S. T. Brady & D. L. Price (Eds.), *Basic Neurochemistry Molecular, Cellular, and Medical Aspects* (Vol. 7, pp. 911-926). Amsterdam: Elsevier.

Wotjak, C. T., Ludwig, M., Ebner, K., Russell, J. A., Singewald, N., Landgraf, R., et al. (2002). Vasopressin from hypothalamic magnocellular neurons has opposite actions at the adenohypophysis and in the supraoptic nucleus on ACTH secretion. *European Journal of Neuroscience, 16*, 477-485.

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., et al. (2000). Mode of action of taurine and regulation dynamics of its synthesis in the CNS. *Advances in Experimental Medicine and Biology, 483*, 35-44.

Wu, Z.-Y., & Xu, T.-L. (2003). Taurine-evoked chloride current and its potentiation by intracellular Ca^{2+} in immature rat hippocampal CA1 neurons. *Amino Acids, 24*, 155-161.

Yan, C. C., & Huxtable, R. J. (1996). Effects of taurine and guanidinoethane sulfonate on toxicity of the pyrrolizidine alkaloid monocrotaline. *Biochemical Pharmacology, 51*, 321-329.

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.

Zalud, A. W., Riley, H. H., & Diaz-Granados, J. L. (2003). Attenuation of ethanol withdrawal seizures via taurine treatment. *Alcoholism: Clinical and Experimental Research, 27*(5), 99A.

Zeigler, D. W., Wang, C. C., Yoast, R. A., Dickinson, B. D., McCaffree, M. A., Robinowitz, C. B., et al. (2005). The neurocognitive effects of alcohol on adolescents and college students. *Preventive Medicine, 40*, 23-32.

Zemo, D. A., & McCabe, J. T. (2002). Transcriptional responses of the rat vasopressin gene to acute and repeated acute osmotic stress. *Neuroscience Research, 44*, 45-50.

Zhao, P., Huang, Y.-L., & Cheng, J. S. (1999). Taurine antagonizes calcium overload induced by glutamate or chemical hypoxia in cultured rat hippocampal neurons. *Neuroscience Letters, 268*, 25-28.

Zimmermann, U., Hundt, W., Spring, K., Grabner, A., & Holsboer, F. (2003). Hypothalamic-pituitary-adrenal system adaptation to detoxification in alcoholic-dependent patients is affected by family history of alcoholism. *Biological Psychiatry, 53*, 75-84.

Zimmermann, U., Spring, K., Wittchen, H.-U., Himmerich, H., Landgraf, R., Uhr, M., et al. (2004). Arginine vasopressin and adrenocorticotropin secretion in response to psychosocial stress is attenuated by ethanol in sons of alcohol-dependent fathers. *Journal of Psychiatric Research, 38*, 385-393.

Zimmermann, U., Spring, K., Wittchen, H.-U., & Holsboer, F. (2004). Effects of ethanol administration and induction of anxiety-related affective states on the acoustic startle reflex in sons of alcohol-dependent fathers. *Alcoholism: Clinical and Experimental Research, 28*(4), 424-432.

Zungo, A. I., Franzon, R., Chiarani, F., Bavaresco, C. S., Wannmacher, C. M. D., Wajner, M., et al. (2004). Evaluation of the mechanism underlying the inhibitory effect of guanidinoacetate on brain Na^+, K^+-ATPase activity. *International Journal of Developmental Neuroscience, 22*, 191-196.